FACTORS WHICH INFLUENCE THE PERFORMANCE OF ALKYLAMMONIUM COMPOUNDS AS WOOD PRESERVATIVES

by

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Abstract.

There has been an ongoing debate into the reasons behind the unexpected failure of alkylammonium compound (AAC) treated wood in field trials. The main objectives of this thesis were to investigate wood and fungal interactions with AAC wood preservatives, in order to help answer the question regarding lack of performance.

Fixation studies found that ion exchange plays a minor role in the adsorption of AACs in the outer regions of the wood. Both fixation and cell wall distribution studies indicated that AACs preferentially bind onto the lignin. Cellular distribution studies using SEM-EDX showed that several factors influenced the retention and distribution of AACs. These included cell type, wood species, sample size, solution pH and treating concentration. The above work suggests that AAC distribution in the wood has a single weakness. AACs in the earlywood tracheids are prone to leaching and decline rapidly from the outer surface. These characteristics would result in areas without protection against invading organisms. Mobility studies indicated that DDAC depletion in a flooded soil bed study was a combination of both physical leaching and biological effects.

Bioassay analysis indicated that standard deteriorating fungi were sensitive to AACs. Field isolations yielded DDAC tolerant *Verticillium* spp., an *Acremonium* sp. and *Gliocladium roseum*. Further studies found that these tolerant organisms could degrade AACs in a wood matrix. This highlights an area of concern where the presence of non-decay fungi, could colonize the AAC-treated wood and may degrade the AACs in the wood to levels inadequate to control wood decay fungi.

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List of Acronyms.

AAC(s)	Alkylammonium compound(s).
ACA	Ammoniacal Copper Arsenate.
ACC	Ammoniacal Copper Carboxylate.
ACQ	Ammoniacal Copper Quaternary Ammonium Compounds.
ACZA	Ammoniacal Copper Zinc Arsenate.
AmCC	Ammoniacal Copper Citrate.
Amm AAC Cu	Ammoniacal Copper Benzalkonium Chloride.
Amm Iodo AAC	Ammoniacal Iodo-Benzalkonium Chloride.
Amm Iodo AAC Cu	Ammoniacal Copper Iodo-Benzalkonium Chloride.
ANOVA	Analysis of Variance.
CAC	Trimethylcoco Ammonium Chloride.
CCA	Copper Chromated Arsenic.
CDDC	Ammoniacal Copper Dithiocarbonate.
CML	Compound Middle Lamella.
DDAB	Didecyl Dimethyl Ammonium Bromide.
DDAC	Didecyl Dimethyl Ammonium Chloride.
DIY	Do It Yourself.
DMBC	n-alkyl Dimethyl Benzyl Ammonium Chloride.
EDX	Energy Dispersive X-ray Analyzer.
EPA	Environmental Protection Agency.
F2™	Mixture of DDAC and Sodium Octaborate.
FT	Field Trial Isolate.
HPLC	High Performance Liquid Chromatography.
IPBC	3-Iodo-2-Propyl Butyl Carbamate.
LD_{50}	Lethal Dose (dose required to kill 50%).
NP-1™	Mixture of DDAC and IPBC.
NP	Non-Pasteurized.
P/B Ratio	Peak to Background Ratio.

PCP	Pentachlorophenol.
S	Secondary wall region.
SB	Soil Bed Isolate.
SEM	Scanning Electron Microscope.
TC	Timbercote™.
TCMTB	2-(thiocyanomethylthio)-benzathiazole.
TEM	Transmission Electron Microscope.
TFA	Trifluroacetic Acid.
TPA	Timber Preservation Authority.
WDX	Wavelength Dispersive X-ray Analyzer.

List of Fungal Synonyms.

Coniophora puteana (Schum. : Fr.) P. Karsten

Syn. *Coniophora cerebella* Per.

Thelephora puteana Schum. : Fr.

Coniophora membranecea DC.

Coniophora piceae Cerný

Gloephyllum trabeum (Per. : Fr.)

Syn. *Lenzites trabea* (Per. : Fr.)

Trametes trabea (Per. : Fr.) Bres

Lenzites thermophila R Falck

Postia Placenta (Fr.) M. Lars. et Lomb

Syn. *Oligoporus placentus* (Fr.) Gilbn. et Ryv.

Poria carnicolor Baxter

Poria microspora Overh.

Poria monticola Murr.

Poria placenta (Fr.) Cke.

Tyromyces placenta (Fr.) Ryv.

Ceriporia incarnata Parm. nom. nud

Trametes versicolor (l. : Fr.) Pilát

Syn. *Coriolus versicolor* (L. : Fr.) Quélet

Polyporus versicolor L. : Fr.

Polystrictus versicolor (L. : Fr.)

Daedalea lobata Velen.

Polyporus vitellinus Velen.

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1. Introduction.

1.1. Development of Wood Preservation.

Wood has been used by man as a building material since the early iron age. The one distinguishing feature of wood over other modern day construction materials is that it comes from a renewable resource. If managed properly, wood will be with us for many years to come. As a construction material wood has a number of unique qualities that make it useful in many applications. These include characteristics such as high strength to weight ratio, non-corrosiveness, good thermal insulation, chemical inertness and steady state combustion. Wood has other qualities such as the aesthetic beauty, low machining costs, batch or single construction options and adaptability, which make it the most popular construction material used today.

The principal disadvantage of wood based construction, can also be considered one of its redeeming features in today's environmentally conscious society, is the susceptibility to biodeterioration. Experts estimate that the failure to control wood deteriorating insects and fungi in the USA causes the additional cutting of over 300 thousand acres of forests yearly (Barnes, 1993). The treatment of wood with chemicals to prevent this biodeterioration should extend the service life and reduce unnecessary forest consumption for replacing damaged wood. Not all of the wood used in construction is at risk and only the portion that is subject to biodeterioration requires treatment.

Clark (1868), first compared wood decay to slow combustion with both resulting in "striking similar" features in the wood. It was not until 1894 that Hartig first associated the fungal

mycelium/fruiting bodies with the decay process of trees. Today it is recognized that wood biodeterioration is a result of a number of organisms including: insects, marine borers, fungi and bacteria. In specific world regions this can be attributed to one or more specific organisms, as in temperate countries such as Canada and Britain where the main agents limiting woods' service life are decay fungi.

In order to evaluate effective preservation techniques, the primary requirements of decay fungi need to be determined. In general the basic requirements of deteriorating fungi are: oxygen availability, a suitable temperature range (15-30°C), a minimal amount of water (~20 % in the wood) and a source of nutrition (wood). The exact requirements are dependent on the individual organism or species. If one or more of the basic requirements were to be controlled, then wood decay may be prevented. Under normal exposure conditions the oxygen availability and environmental temperature are not controllable. One of the simplest approaches is to use construction techniques that keep the wood dry, thus eliminating water from the growth equation. However, good building techniques cannot eliminate all of the situations where wood is at risk from decay. Examples of situations where the wood is at risk from decay are in basements, steps, ledges and decks. The nutrient source can also be chemically altered to prevent the utilization of the wood by any deteriorating organism. Such treatments with chemicals would make the food substrate, in this instance wood, biostatic or biocidal to the deteriorating organisms.

The history of wood preservation chemicals began with early civilizations such as the Egyptians. These early civilizations used natural oils, salt water and embalming fluids to protect wood from decay. They also identified that construction design and wood species

played an important role in the durability of wood in service, while also recognizing that wood stored by ponding was less susceptible to decay. The introduction of new chemicals into wood preservation has grown exponentially in the last two centuries. There are two classes of modern day preservation chemicals, these being waterborne and oilborne preservative systems. There are many examples of both systems, which will be discussed separately.

In 1838, John Bethell laid the foundation of modern day preservation with a patent on impregnation process for tar oils using vacuum and pressure. The active ingredient was a mixture of neutral aromatics, bases and acids obtained from coal tar distillates, which was referred to as creosote. Creosote was the first commercially successful preservative, and it is still in use today. The treating of wood with creosote was carried out at high temperatures, which resulted in sterilization and excellent penetration. The product obtained was highly durable, but had several major drawbacks. It was a very dirty product that bled easily and could not be glued or painted. Market trends in the creosote consumption reflected the limitations imposed by these characteristics going from a 90% treated wood market share in 1950 to 16% in 1990. Considering the above negative aspects, the use of creosote treated wood remained about 50% of the market until 1977. The subsequent reduction resulted from a change in the treated wood market, going from being industrial based to consumer based. In 1955 the industrial treated wood products accounted for 186.2 million cubic feet, 75% of the total market. The consumer treated wood products produce in 1955 was 59.4 million cubic feet (Stahl, 1992). However, by 1990 the consumer treated wood products had increased to 433.5 million cubic feet, 74 % of the total market. Creosote treated wood was

not suitable for this commodity market due to its dirty appearance and toxicity to humans. Commodity markets were dominated by waterborne inorganic preservatives that were cleaner and could be handled safely.

Pentachlorophenol (PCP), the second generation oilborne preservative, was developed in 1940 to replace creosote in protecting utility poles. Subsequently many other markets were developed for the use of PCP in light oils and the salt in water (i.e. window joinery, anti-sapstain treatment). The PCP market share of pressure treated wood went from 5% in 1953 to a high of 23% in 1967. In 1990 the use of PCP treated wood declined to ~8% due to concerns over dioxin related toxicity (Sterling, *et al.*, 1986) and like creosote it could not be used in consumer products, such as decking.

Other examples of oilborne preservatives are copper/zinc naphthenates and copper-8-quinolinolate. Copper naphthenates have been used successfully in light oils for Do It Yourself (DIY) products such as brush-on preservatives. These wood preservatives purchased by retail consumers relates to 5% of the industrial market (Stahl, 1992). Later, in Canada and the US, standards for copper naphthenate treatment with heavy oils were introduced for ground contact commodities. Copper-8-quinolinolate has been used in anti-sapstain and above ground applications. This pesticide is approved for use on wood in contact with food. Both chemicals have low toxicity with LD_{50} values of 3,000 mg/kg and 10,000 mg/kg (oral rats) respectively. Their use in wood preservation has been restricted, due to high costs and limited effectiveness against copper tolerant organisms.

The other class of chemicals are waterborne preservatives and the first examples were simple salts, introduced between 1800-1850. However, simple salts were doomed to be unsuccessful

in most circumstances, due to lack of permanency in wood. This was overcome with the introduction of multi-component formulations, which were more effective. The first patented salt system, acid copper chromate, was introduced in 1929. This was shortly followed by copper chromated arsenic (CCA). There have been other salt mixtures introduced, but CCA has been the most successful system used in North America to date.

The first CCA formulation proposed under the trade name Greensalt® was by Baechler in 1949 and the reason behind the success of CCA are CCA stability in the wood and effectiveness against a wide range of organisms. Treatment with CCA and other waterborne systems results in certain changes in the wood including increased surface checking, hardness, corrosiveness and a green colouration of the wood. Despite these minor drawbacks CCA has become the most popular preservative used. The first significant use of CCA in the USA was reported in 1970 with 48.8 million cubic feet of treated lumber and timber being produced. Since then the market has grown to 359 million cubic feet of CCA treated lumber and timber in 1990 (Stahl, 1992).

Since the introduction of CCA many multi-component systems have been introduced such as ammoniacal copper arsenate (ACA) and ammoniacal copper zinc arsenate (ACZA). The above systems were not as successful due to patchy colour of treated wood, corrosiveness, difficulty in handling ammonia and reduced effectiveness when compared with CCA.

Over the last 20 years the wood preservation industry has identified certain crucial properties, which are required in the next generation of wood preservative chemicals. One area of concern is safety with respect to the wood treating process and the environment. The preservative has to be user friendly and the final treated product must have low

environmental impact. The demands on wood preservatives have grown over the years, they must be more effective against fungi, insects, marine borers and bacteria. It is also important that retention and penetration of the preservative be appropriate to protect the wood from decay. Any chemicals proposed must be cost effective in order to provide an affordable end-product. In the past ten years, wood preservation research has intensified and diversified into several areas. These areas include biological control, wood modification and new chemicals for the future.

Novel preservatives have been developed in both oilborne and waterborne system and other treating systems are under development including emulsion and microemulsion systems, presently used in agriculture. Examples of new oilborne systems proposed for the wood preservation industry include isothiazolones, chlorothalonil, 2-(thiocyanomethylthio)-benzothiazole (TCMTB) and 3-iodo-2-propyl butyl carbamate (IPBC). Examples of new waterborne systems include alkylammonium compounds (AAC), ammoniacal copper carboxylates (ACC), ammoniacal copper-quaternary ammonium compounds (ACQ), ammoniacal copper dithiocarbamate (CDDC) and ammoniacal copper citrate (AmCC).

In 1990 585.6 million cubic feet of treated wood was produced in the USA with 75% being treated with CCA (Stahl, 1992). However, in 1984 the USA Environmental Protection Agency (EPA) announced plans to cancel all non-wood preservative and retail uses of creosote, PCP and inorganic arsenicals. The EPA cited these three chemicals as being potential carcinogens. In September 1985 the EPA and the wood preservation industry agreed on regulatory measures covering the use of pesticides for preserving wood, including creosote, PCP and inorganic arsenicals. Due to the presence of arsenic in CCA, the future use

of CCA as a wood preserving agent is uncertain and as a consequence intensive research on new preservative systems has occurred.

Alkylammonium compounds (AACs) are one class of chemicals, which have been studied intensively as wood preservative agents in the last 25 years. Initial research into AACs as wood preservatives showed great promise, to the extent that approval for commercial use was passed in New Zealand (Butcher, 1980). In the early 1980's the effectiveness of AACs as wood preservatives was questioned by many researchers and following research found that AACs did not provide the expected field results. This has lead to an ongoing debate into the reasons behind their lack of field performance.

1.2. Thesis Objectives.

The main objective of this research was to help answer one question that is "why do AACs fail to protect wood against decay exposed under field conditions?". The research was split into two areas, the interactions of AACs with the wood and the interactions of AACs with wood inhabiting fungi. Under each area of investigation several questions have to be answered.

Several fundamental areas need to be addressed with respect to AACs interaction with wood. The distribution of AACs at a cellular and subcellular level has to be identified along with factors influencing their distribution. AAC fixation mechanisms and interaction sites need to be determined. Permanency of AACs within the wood during soil exposure.

The interaction of AACs with wood inhibiting fungi was the second area under review in this thesis. The main question to be addressed is whether certain fungi, which inhabit the wood, have the ability to detoxify AACs.

The results of the above research will be related to how they may affect the performance of AACs as wood preservatives. The findings may influence the evaluation process for new preservatives in the future.

2. Literature Review.

During the late seventies, a group of chemicals, which received considerable attention as possible wood preservative agents were alkylammonium compounds (AACs). This group included quaternary ammonium compounds and their related tertiary amine salts. AACs are derived from ammonia by successive replacement of hydrogen by alkyl chains of various lengths. Multiple alkyl chains can be formed on the nitrogen with various chain lengths resulting in numerous compounds. For example, didecyldimethylammonium chloride (DDAC) is one AAC that has considerable potential as a wood preservative (Figure 2.1.). What is the attraction of AACs as wood preservatives?

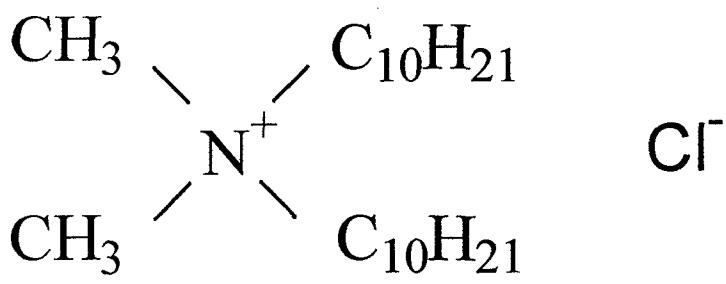


Figure 2.1.: Didecyldimethylammonium chloride (DDAC).

AACs have been used as general purpose microbiocides in household disinfectants, nappy sanitizers, eyedrops and as paint fungicides. They are also used as lubricants, antistatic agents, fabric softeners, hair conditioners and dye additives due to their surfactant properties. Their use as fungicides in paints may have initially suggested their potential as a wood preservative. The first published data on the potential of AACs as wood preservatives was by Oertel in 1965. AACs were very attractive to this industry because their uses have been wide spread over many years and they are considered to be environmentally acceptable. The oral and dermal toxicity of AACs are generally very low. For example, DDAC has an oral LD_{50} value of 600-700 mg/kg (mouse) and an acute dermal LD_{50} value of 4,300 mg/kg compared with PCP's oral LD_{50} value of 50-140 mg/kg (rat) (Drysdale & Preston, 1982; and Sterling *et al.*, 1986). More importantly is that they do not bioaccumulate in the tissue of fish like PCP, which makes them less harmful to the aquatic environment (Knezovich, Lawton & Inouye, 1989). The widespread commercial use of AACs ensures that they are readily available and economical for use in the preservation industry. However, further work was required to assess AACs effectiveness as wood preservatives.

Wood preservation is usually considered to be a process that protects the wood in service over several decades. However, in wood protection, DDAC has been also used against mould and sapstain during storage and transit. This is referred to as anti-sapstain control of lumber. In the case of long term protection of wood in service, wood can be classified into different biological hazards including: high hazard situations such as ground contact; moderate hazard situations occurring above ground; and minor hazards (protected from direct rainwater) where little or no preservation required. The amount of preservative required in the different

hazard situations will vary with each preservative. The relative importance of specific organisms, which cause wood deterioration is dependent on those present in the particular world region in question. For example in temperate regions, fungi are the primary concern, while in warmer climates termites or insects tend to be the major concern. Therefore, to ensure that the service life of the treated wood meets all the needs of the intended customer, toxic threshold values have to be established for each preservative against the different organisms.

Ground Contact Application.

Initial Laboratory Trial.

Initial laboratory screening trials of various AACs in *Pinus radiata* D. Don against several types of wood destroying organisms including *Postia placenta* (Fries) M. Larson *et* Lombard, *Fomes gilvus* (Fr.) Lloyd and *Chaetomium globosum* Kunze : Fries generated toxic thresholds of the order of 3.2-6.4 kg/m^3 for the majority of the chemicals (Butcher, Hedley & Drysdale, 1977; and Butcher, Preston & Drysdale, 1977). The AACs, which were tested included: dimethyl-lauryl amine and alkyltrimethylammonium chloride with threshold values of <1.6 kg/m^3 and 1.6-3.2 kg/m^3 respectively. Further laboratory work with AACs against soft rot confirmed the initial finding and two AAC-treated hardwood species gave equal or better control than the CCA treated wood (Butcher, Hedley & Drysdale, 1977). The same level of protection was obtained against several decay organisms when using AACs (1-2 kg/m^3) at approximately half of the CCA (2-4 kg/m^3) retention (Butcher, 1979[a]; Preston & Chittenden, 1982; Preston & Nicholas, 1982; Tsunoda & Nishimoto, 1983; and Hedley,

Tsunoda & Nishimoto, 1982). Studies using a range of wood-destroying basidiomycetes found that dialkyldimethylammonium chloride had toxic threshold values of 2.5-6.4 kg/m^3, which were similar to CCA values (2.7-5.2 kg/m^3) (Butcher & Drysdale, 1977). Soil block tests on dialkyldimethylammonium chlorides and alkyltrimethylammonium chlorides with a range of alkyl chain lengths indicated those with chain lengths of C_{10}/C_{12} were the most effective (Preston, 1983). A similar trial with benzyldialkyldimethylammonium chlorides suggested that C_{16}/C_{18} were most effective (Tsunoda, 1990). Several researchers found that DDAC was the most effective AAC tested with threshold values in the order of <1-2 kg/m^3 (Preston & Chittenden, 1982; Preston & Nicholas, 1982; Preston, 1983; Hedley, Tsunoda & Nishimoto, 1982; and Tsunoda & Nishimoto, 1987[a]).

Field Trials of AAC-Treated Wood.

Parallel field trials did not reflect the initial laboratory observations. After a 30 month field exposure, unmodified AACs did not perform as well as CCA (Butcher, Preston & Drysdale, 1979). In the above study the AAC-treated stakes showed signs of surface degradation soon after being placed in the field. In the eighties, further field trials of AAC-treated stakes did not look very promising. Tillott and Coggins (1981) detected decay and failure of stakes treated with dialkyldimethyl amine chloride (14.6 kg/m^3) after only one year in the field. The above findings were confirmed by Ruddick (1983), who found that DDAC-treated stakes (3.2 kg/m^3) failed after two years in the field. Subsequently it was recorded that unmodified AAC-treated wood with retentions as high as 11 kg/m^3 had noticeable decay after six years (Ruddick, 1987; Ruddick & Ingram, 1987 and Morris & Ingram, 1988). Repeated trials by Drysdale (1983[a]) found that dialkyldimethylammonium chloride-treated (6.2 kg/m^3) and

benzalkonium chloride-treated (10.6 kg/m^3) P. *radiata* stakes were colonized and attacked by brown rot, soft rot and white rot fungi after four years in the field.

Later Laboratory Trials.

Subsequent laboratory trials found the performance of AAC-treated wood to be variable against similar fungi. A serial exposure of AAC-treated blocks in the laboratory indicated that at least 6.4 kg/m^3 of the AAC was required to provide adequate protection after three subsequent soil jar evaluations (Butcher, 1979[b]). By pre-exposing AAC-treated wood blocks to staining fungi it was found that the toxic threshold values went from 0.7-5 kg/m^3 (unexposed) to 5-10 kg/m^3 (exposed) for the same decay fungi (Ruddick, 1986). Later studies suggested that 6-8 kg/m^3 benzalkonium chloride was required to protect the wood against decay in the laboratory (Preston, *et al.*, 1987). Researchers in Japan found that *Cyptomeria japonica* D. Don treated with DDAC (2.5 kg/m^3) failed to prevent decay by *Tyromyces palustris* (Berkeley *et* Curtis) Murrill and *Coriolus versicolor* (Fries) Quelet (Tsunoda & Nishimoto, 1987[a]). At the same time, DDAC-treated wood (4 kg/m^3) gave very poor protection against seven decay fungi in an Australian laboratory trial (Greaves, Cookson & Tighe, 1988).

Formulation of AACs with Secondary Biocides.

As a result of the poor field performance of AACs, focus was turned to formulating AACs with other biocides to improve their performance. Many workers found that the addition of acidic copper salts to the AAC improved the effectiveness in both laboratory and field trials (Butcher, Preston & Drysdale 1979; and Drysdale, 1983[a]). Tsunoda and Nishimoto (1987[b]) found that copper-amended DDAC (1 kg/m^3) had an improved effectiveness against T.

palustris and C. *versicolor*. However, in the same study the addition of zinc did not improve the performance of AACs. Tillott and Coggins (1981) found that acidic copper used in the various formulations did not undergo any apparent chemical fixation in the wood. In order to fix the copper in the wood, Sundman (1984) used ammoniacal copper oxide. This formulation appeared to give better protection than the acid copper modified AACs. Addition of ammoniacal copper oxide (3.2 kg/m^3) to octyldecyldimethylammonium chloride (8 kg/m^3) resulted in superior protection during a severe fungal cellar exposure when compared to CCA (24 kg/m^3) or ammoniacal-copper arsenate (ACA- 10 kg/m^3) (Sundman, 1984; and Wallace, 1986). The effect of each component in the above formulation was evaluated by Hedley, Tsunoda and Nishimoto (1982). The increasing order of effectiveness was unmodified AACs, ammonium hydroxide amended AACs, copper modified AACs and the ammoniacal-copper-AACs formula that gave the best protection. These results were confirmed by five year field trials, where it was shown that the most successful combination: ammoniacal copper oxide and alkyl(C_8,C_{10})dimethylbenzylammonium chloride (7.1 kg/m^3) (Ruddick, 1987; Ruddick & Ingram, 1987; and Morris & Ingram, 1988). Another system that showed promise was alkyl(C_8,C_{10})dimethylbenzylammonium chloride (4.8 kg/m^3) amended with tributyltin chloride. The treated stakes were still sound after six year in field trials (Ruddick, 1983; and Morris & Ingram, 1988). Ruddick (1987) found the combination of sodium tribromophenate and an AAC (~10 kg/m^3) gave reasonable stake protection in a four year field exposure. Even though unmodified AACs appeared to have no potential as wood preservatives, formulations of AACs with a cobiocide showed potential as effective preservatives. Recent field research has suggested that ammoniacal-copper-AAC systems can

perform as well as CCA-treated wood (Preston, Walcheski & M^{c}Kaig, 1985; Jin & Archer, 1991; and Jin, Archer & Preston, 1992). As a consequence of improved field performance, the ammoniacal-copper modified AACs such as ACQ have been approved for use in several countries including Scandinavia, Japan and USA (Jin & Preston, 1992).

Above Ground Application.

As a consequence of the early laboratory results and other available information, alkyl-dimethylbenzylammonium chloride and alkyldimethyl amine acetate were approved in 1978 by the New Zealand Timber Preservation Authority (TPA). The approval was limited to P. *radiata* that would be treated for commodities used out of ground contact, at recommended retention of 2.5 kg/m^3 for the AAC (Butcher, 1980; and Butcher & Greaves, 1982). Early above ground field trials of AAC-treated wood agreed with the laboratory results. Barnes, Buchanan and Amburgry (1985), found that southern yellow pine (*Pinus* sp.) shakes treated with DDAC were still sound after 28 months of exposure. AAC-treated L-joints, which were in field trials for five years, did not give rise to any decay fungi under mycological examination, while untreated controls did produce decay fungi (Morris, 1987^{b}). P. *radiata* shingles treated with 1.39% alkyl(C_{12},C_{14},C_{16})dimethylbenzylammonium chloride showed no sign of decay after a seven year field trial (Placket, Chittenden & Preston, 1984). Later studies found that western hemlock, pacific silver fir and western white pine treated with 4.8 kg/m^3 DDAC were highly durable (Degroot, Woodward & LeVan, 1992). Highley (1990 & 1984) indicated that 5% AAC treatment of maple did not provide adequate protection over five years in the field, while AAC-treated pine did not show any signs of decay. Both

laboratory and field trial results indicated that AACs had potential as a wood preservative especially in above ground commodities such as decking. It would be unlikely that use of AACs would become wide spread in the world, since their initial approval in New Zealand was strictly for above ground treated products and not for the more abundant ground contact treated products. However, in August 1984 the use of AACs as above ground wood application was restricted by TPA after some instances of decay. It was suggested at the time that most of the decay was a direct consequence of inadequate treatment or misuse of the treated wood. However, there were some cases of preservative loss from timber in bulk storage (Plackett, Chittenden & Preston, 1984).

Insecticide Application.

Fungi are not the only biological agents responsible for deterioration of wood products, the impact of insects such as termites can not be ignored. Therefore, numerous studies have been carried out on the insecticidal activity of AACs. A 2 kg/m^3 retention of cocodimethyl-benzylammonium chloride used to treated *Dacrycarpus dacrydioides* was found to eradicate *Anobium punctum* Dr Geer and *Ambeodontus trists* (F.) within 12 months (Cross, 1979). Benzalkonium chloride (6.2 kg/m^3) was shown to be effective against various insects including *Mastotermes darwiniensis, Nasutitermes exitiosus* and *Coptotermes acinaciformes* in P. *radiata* (Butcher & Greaves, 1982; and Howick, *et al.*, 1983). Preston and Nicholas (1982) found that dialkyl(C_{12},C_{14})dimethylammonium chloride (1.6 kg/m^3) prevented attack by *Reticulitermes flavipes* (Kollar) (*Termes*) in southern yellow pine. Laboratory trials in Japan showed that DDAC (2.2 kg/m^3) did control the attack of *Coptotermes formosanus*

Shiraki attack in *Tsuga heterophylla* Sarg. and *Pinus densiflora* Sieb. *et* Zucc. (Tsunoda & Nishimoto, 1983). However, later studies found DDAC (1.76 kg/m^3) did not prevent *lyctus brunneus* (Stephens) attack in *Quercus serrata* Thunb. (Tsunoda & Nishimoto, 1987[a]). This failure may be a direct result of using too low a retention of DDAC. Termite tests found that 4 kg/m^3 protected the wood, but did not cause complete mortality of C. *formosanus* (Tsunoda & Nishimoto, 1983). Overall unmodified AACs appeared to have good potential as an insecticide. Therefore, the incorporation of AACs into wood preservative formulations will aid insecticidal properties.

Anti-sapstain Application.

The scope of modern preservatives is not just limited to wood-destroying fungi. One other area of concern is mould and sapstain control, where superficial deterioration cause loss in value rather than significant structural damage. Nevertheless these organisms are of major concern since they discolour the wood and make it unmarketable, especially for export (Byrne & Smith, 1987). Butcher and Drysdale, (1978[a]) found that a 1% solution of an AAC was effective in preventing sapstain. However, other researchers found the above concentration was not adequate in preventing sapstain (Cserjesi & Roff, 1975). Like the work undertaken with wood destroying fungi these initial findings were not confirmed in subsequent field trials. In the early eighties it was found that unmodified AACs reduced mould and sapstain, but did not completely protect the wood (Cassens & Eslyn, 1981; Cserjesi & Johnston, 1982; and Hayward, Rae & Duff, 1984). However, when a 0.5% solution of trimethylcocoammonium chloride (CAC) was combined with 5% sodium

thiosulphate or sodium sulphite, it provided complete protection to *Pinus strobus* from mould and sapstain colonization (Hulme & Thomas, 1979 & 1983). The addition of sodium carbonate to CAC also increased its' effectiveness against both mould and sapstain (Drysdale & Preston, 1982; Hulme & Thomas 1979 & 1983; and Linderborg, 1984). Therefore, unmodified AACs were not to be recommended for mould and sapstain control, but modified AACs had potential. In recent years AACs have been successfully combined with cobiocides such as IPBC to provide an excellent sapstain and mould treatment (Ward, 1990). At present four anti-sapstain formulations containing AAC biocides have been registered in Canada (Byrne, 1991).

Factors which Influence the Performance of Treated Wood.

In above ground field and laboratory studies AACs appeared to render sufficient protection to justify their approval for above ground use in New Zealand in 1978. However, in 1984 their use was restricted due to incidences of decay. It was suggested that their poor in vivo performance could be a consequence of the recommended retention levels being too low (~3 kg/m^3). However, field trials with higher retentions also showed signs of decay after four years (Ruddick, 1983). General hypotheses for failure of AACs which have been circulated, include poor treatment, preservative leaching, uneven distribution and interference of other micro-organisms.

Fixation and Treatment.

An understanding of fixation mechanisms is essential in the investigation of the long term potential of AACs. Factors, which influence the treatment, are important in optimizing the

wood coverage of AACs. The first studies on the treatment of P. *radiata* with AACs found that they fixed rapidly and the solution uptake was affected by the wood density (Vinden & McQuire, 1979). Due to the rapid fixation a pressure treating process was required and it was found that the Lowry process gave better penetration (Vinden & McQuire, 1979) than the Bethell process in heartwood (Vinden, 1982 & 1984). Treatment studies suggest that there are only a limited number of adsorption sites available, which may reduce AACs overall effectiveness (Vinden, 1984). As for incorporation of AACs into existing treating plants, there appear to be no foreseeable problem and the temporary approval of AACs in New Zealand did not indicate any problems in their commercial use either.

Due to the nature of AACs one possible mechanism for their fixation in wood is ion exchange (Rosen, 1975). The influence of the treating solution pH on fixation was initially discussed by Butcher and Drysdale (1978[b]) with respect to ion exchange. It was suggested that under slightly acidic treating solution conditions, the proton would compete with the AAC cation for binding sites in the wood. This would retard fixation and result in a more even distribution, providing better protection against basidiomycetes and soft-rot fungi. An alkaline treating solution would create more negative binding sites and increase the fixation rate, resulting in higher loadings especially in the peripheral regions. Later studies confirmed that an increase in treating solution pH increased retentions of DDAC in the wood (Jin & Preston, 1991; and Doyle & Ruddick, 1994). It was proposed that the primary mode of DDAC fixation was by cation exchange on the carboxyl and phenolic hydroxyl groups on the lignin. Other studies agreed with this theory, where cation adsorption was not matched by anion uptake in the wood (Loubinoux & Malek, 1992). The AAC chemical structure, wood

type and extractive content influenced the amount of AAC retained (Loubinoux, *et al.*, 1992).

Distribution.

Poor distribution of AACs in wood has been suggested to be a factor that could contribute to the failure of AAC-treated wood in field trials. A few researchers have looked at the distribution of AACs indirectly. Vinden (1982) first questioned the distribution of AACs in the P. *radiata* during treatment trials. Ruddick and Sam (1982) found that AACs distribution in four Canadian softwoods did not appear to be particularly uneven and could not be blamed for the failure of AAC-treated wood. Analysis of the AAC-treated wood by Drysdale (1983[b]) did not detect uneven distribution in the outer 6 mm of P. *radiata*. Analysis of the AAC distribution in L-joints suggested that end grain penetration was excellent, but lateral penetration was very poor (Morris, 1987[a]). Later studies on the different wood regions found that earlywood retained more AACs than the latewood regions (Nicholas, *et al.*, 1991; and Doyle & Ruddick, 1994). Uneven distribution of the AAC may influence the performance of the stakes, but results have not been conclusive.

Leachability.

Leaching of AACs has been proposed as one of the reasons why AACs fail in field trials. Initial AACs leachability studies using four softwoods found 10-20% loss of AACs. The rate of depletion was dependent on the surface to volume ratio, but was not significant enough to account for the failure AAC-treated wood (Ruddick & Sam, 1982). Drysdale (1983[b]) found that 30% of the AAC leached from the outer 2 mm in a 18 week leaching regime. This was suggested as the explanation for the superficial degrade noted during field exposure

(Drysdale, 1983[b]). The amount of AAC leached was found to be dependent on the initial retention, with the higher retention the greater the leaching (Loubinoux, *et al.*, 1992). Water leaching studies found that the AAC leaching was independent of the solution conditions (Sundman 1984; and Ruddick & Lum, 1991). A laboratory evaluation on the effect of preleaching AAC-treated blocks in water found little influence on the performance of the treated blocks (Hedley, Tsunoda & Nishimoto, 1982; and Ruddick, 1986). Later studies using soil as a leaching medium found that AAC leaching went increased to 30-40 compared with negligible amount in water alone (Nicholas, *et al.*, 1991; and Ruddick & Lum, 1991). In the same study the loss of DDAC from the earlywood (35%) region was greater than the latewood (20%) region (Nicholas, *et al.*, 1991; and Ruddick & Lum, 1991). All the laboratory trials undertaken to date appear to suggest that AACs do not excessively leach from the wood. However, the effect of leaching will depend on the initial retention of AACs in the wood. If the initial retention is close to the toxic threshold values then the amount of leaching may result in the remaining AACs retention being inadequate to protect the wood from decay.

Influence of Non-Decay Fungi.

Many researchers report that stakes in the field studies became heavily stained soon after being placed in the trial (Ruddick & Sam, 1982). These observations lead to a study on the effect of staining fungi on performance of AAC-treated wood in a standard soil block test (Ruddick, 1986). The toxic threshold value for two out of the three fungi, more than doubled when first exposed to the staining fungi. Later studies found that non-decay fungi isolated from DDAC treated wood were able to decrease AACs in solid media (Doyle & Ruddick,

1993). Therefore, staining fungi may have the ability to suppress the effectiveness of the AAC as wood preservatives.

The history of AAC development as wood preservatives has demonstrated that laboratory work is unable to mimic in vivo situations. Therefore, results obtained from laboratory can only be a rough guide to what actually happens in the field. For more accurate information, field studies are necessary. In the case of AACs the amine salts appeared to be more active in the laboratory studies, but the quaternary ammonium salts were superior in field studies. This phenomenon has been demonstrated in many areas and indicates that field study of any product is essential.

Even though unmodified AACs do not appear to have potential as wood preservatives, their performance can be improved by addition of certain chemicals such as copper. However, before AAC containing formulations can become accepted further work has to be done to ensure their effectiveness without loss of any of the desirable properties of AACs. An understanding of the reasons behind their failure will clearly aid in identifying how improved formulations can be developed. The successful formulation of AACs will need to have many specific qualities, which are not found in today's' preservatives in order to be accepted by the wood preservation industry of the future. However, AACs appear to have many of these qualities and with fine tuning may become as widely acceptable as CCA is today.

3. AACs Interactions with Wood.

3.1. Introduction

In order to maximize a wood preservatives' performance, a fundamental understanding of the preservative interaction with the wood is essential. Alkylammonium compounds (AACs) are no exception to this rule, in fact the lack of understanding of the wood-chemical interaction may have contributed to their disappointing field performance (Ruddick, 1983 & 1987; and Preston, *et al.*, 1987). The fundamental aspects can include fixation, identification of the binding sites, micro- and macro-distribution and mobility.

A knowledge of the mechanisms, which are involved in the fixation of AACs on to the wood, is important to determine if the chemicals have any real potential as wood preserving agents. A great deal of information on AACs interaction with various substrates, including cellulose, is available due to their universal use (White, 1970). In aqueous solutions, surfactants can adsorb onto a solid substrate by several mechanisms including: ion exchange, ion pairing, physical adsorption by polarization or dispersion forces (Rosen, 1975). The actual mechanism depends on the type of surfactant and the solid substrate. The amount and type of fixation are two critical factors in the development of a wood preservative.

The term decay can be split into several different types. The two types of decay, which are of primary importance in wood preservation are white-rot and brown-rot. These types of decay are caused by basidiomycete fungi and their definition is based on the wood components they attack. The white-rot fungi attack both the cellulosic and lignin components in the wood, while the brown-rot fungi degrade exclusively cellulosic material (Nilsson, 1985).

Therefore, an understanding of the AAC interaction with components such as the lignin and cellulose would provide information on the effectiveness against these two types of decay. This type of information becomes more essential with the move away from single component to multi-component wood preservative systems. An understanding of the strengths and weaknesses of the individual components will help formulators design multi-component systems with a better range of protection against organisms responsible for wood biodeterioration.

Due to the heterogeneous nature of wood, which can result in uneven distribution of the preservative. An adequate preservative treatment needs to provide the wood with a continuous shell of protection around an unprotected inner core. Therefore, an understanding of the distribution and factors influencing the distribution of AACs within the wood are vital. It is possible to use this knowledge, to develop a treatment that would provide the best possible distribution of a preservative. It is known that wood destroying organisms attack various sites within the wood cell wall. Therefore, information on the AAC microdistribution within the wood cell wall could highlight any potential weaknesses. An understanding of the bulk, cellular and microscopic distributions of the AAC and influencing factors are of importance.

When wood is exposed to water the preservative can be redistributed or released from the wood. This loss of chemical from treated wood during exposed to water is referred to as leaching. A knowledge of the factors, which affect the depletion and redistribution of a preservative is essential to determine the most suitable application and expected service life for a given treatment.

In this chapter some of the fundamental areas and factors, which influence these interactions, were determined. The importance of AAC fixation by ion exchange was estimated for wood, cellulose and lignin. As the bulk distribution of AACs has been studied by numerous researchers (Nicholas, *et al.*, 1991) the following research will focus on cellular and microscopic distribution. The influence of sample size, treating solution pH and the presence of copper ions, on the distribution of the AAC in the wood will also be investigated. The mobility of AACs has also seen a great deal of research in the recent past, with soil leaching showing the greatest effect. Therefore, the soil depletion of AACs will be studied to determine if the decline was caused solely by a leaching process or whether biological factors contributed to the loss of AACs.

3.2. Methodology

3.2.1. DDAC Fixation Mechanism and Adsorption by the Wood Components.

Test Chemical.

The alkylammonium compound used in this investigation was commercial grade Bardac® 2280 (Lonza Inc.) contained 80% didecyl(C_{10},C_{10})dimethylammonium chloride (DDAC) active ingredient, 10% ethyl alcohol and 10% water.

Wood Species and Wood Components.

The wood species used in this experiment was *Pinus ponderosa* Laws. sapwood, which had a moisture content of 8.2%. The wood was cut into small sticks with the approximate dimension of 50 x 3 x 3 mm. The wood sawdust was obtained by milling P. *ponderosa* sapwood through a Wiley mill fitted with a 20 mesh (850 μm) screen. The wood components were represented by two commercial cellulose powders (Avicel® & Whatman® cellulose) and two lignins prepared in the laboratory. The two types of lignin were Klason lignin and trifluoroacetic acid (TFA) lignin (Browning, 1967 ; Fengel & Wegener, 1989).

DDAC Treatment.

A 1% DDAC solution was used for the sawdust, cellulose and lignin adsorption studies. Concentrations of 0.001%, 0.01%, 0.1% and 1% DDAC were used with the small wood sticks.

The weight ratio of DDAC solution to wood or cellulose was 1:20, while the weight ratio of DDAC solution to lignin was 1:100. These ratios were used to make up the test samples in 200 ml flasks or beakers. Five replicates were prepared for wood stick experiments and four replicates for all other test materials. Two types of control samples were used: wood (component) in distilled water, as well as 100 ml of the various DDAC concentrations with no wood added. The wood sawdust and lignin samples were placed in an environmental incubator at 27.4°C for 96 hours, shaking at a rate of 160 rpm. The other samples were left stagnant on the bench for the 96 hour period.

The pH of each sample was measured, using a Accumet pH meter, at 24 hour intervals over a 96 hour period. The pH probe was allowed to stabilize for one minute before recording the value. The average value of three pH readings was determined and DDAC retention calculated from the pH changes based on a one to one exchange with hydrogen ions. DDAC and chloride analysis of the treating solution were performed at the end of the experiment on the wood sticks, cellulose and lignin samples. Aliquots of 2 mls removed, at 24 hour intervals from the wood sawdust samples. DDAC was extracted from the wood sawdust, cellulose and lignin for analysis at the end of the experiment. The extraction method used will be described in a separate section.

Analysis of DDAC.

DDAC was analyzed by a two-phase titration, which is a standard technique for the determination of anionic active detergents (Reid, Longman & Heinerth, 1967; and Zhi-ping & Rosen, 1981). The specific technique used was the AWPA standard method for the

determination of AACs (Ruddick, 1984), which involved the use of a standard sodium lauryl sulphate solution. The required neutralization prior to titration with hyamine was achieved by adding 0.05 M sodium hydroxide. The method involved the use of: 5 ml of approximately 0.004 M sodium lauryl sulphate solution, 20 ml of distilled water, 15 ml chloroform and 10 ml of the acid indicator. They were added (in the above order) to 250 ml flasks, which were shaken for one minute. The resulting solution was then titrated with a standard 0.004 M hyamine 1622 solution. Following each addition of hyamine, the flask was stoppered and carefully shaken. As the end point approached, the chloroform emulsion tended to breakdown more readily and the lower layer changed from pale pink to gray. This was repeated until three consistent values were obtained. Following the titration, the percentage weight of DDAC in each test solution was calculated then compared with DDAC content of the control solutions. The results gave the amount of DDAC adsorbed by wood and wood components, such as lignin and cellulose. The above titration procedure was also used to analyze the DDAC concentration in the extracting solutions used to remove DDAC from the wood, cellulose and lignin.

Analysis of Chloride.

The analysis was done using an argentometric method for chloride determination (Anonymous, 1976). A standard solution of sodium chloride (1.2 M) was titrated with silver nitrate (1.2 M) to determine the exact molarity of the silver nitrate solutions. A sample of 1 ml was removed from each test solution at completion of the experiment for all material. The wood sawdust had samples removed after every pH measurement. The sample pH was

adjusted to between 7 and 10 directly, using 0.05 M sodium hydroxide. One ml of potassium dichromate indicator solution was added to each sample, which was titrated with the standard 1.2 M silver nitrate solution. The amount of chloride present in each solution was calculated and was related to the amount of DDAC retained by the wood.

Extraction of DDAC from Test Material.

At the end of the experiment (~96 hours) the wood sawdust, cellulose and lignin samples were removed from the DDAC solutions and washed three times in distilled water, with a pH of 8 (to minimize leaching). The materials were dried at 102°C for one hour, after which they were divided into two sub-samples, which were placed in test tubes and weighed. The weight was recorded for each sample. A 10 ml aliquot of extracting solution (0.28 M HCl in ethanol) was added to each test tube. The tubes were labeled and ultrasonicated at room temperature for 2 hours at 40 Hz, after which they were allowed to settle overnight and then decanted into 50 ml flasks. The extracted solution was analyzed for DDAC as above. The extraction procedure was repeated using a fresh extracting solution for the wood sawdust samples.

3.2.2. Distribution of Alkylammonium Compounds in the Wood.

Sample Preparation.

P. *ponderosa* sapwood blocks, 19 x 19 x 19 mm, were impregnated with 1%, 3%, 5%, 6%, 10% and 13% solutions of N-N-dimethyl-N-dodecyl-N-(2-iodobenzyl) ammonium chloride (iodo-benzalkonium chloride). A second set of P. *ponderosa* sapwood blocks, 19 x 19 x 19 mm, was impregnated with 3% dimethyldodecylbenzyl ammonium chloride (benzalkonium chloride) and 3% didecyldimethyl ammonium chloride (DDAC). Earlywood and latewood regions were separated from a P. *ponderosa* sapwood sample before impregnation. The earlywood regions were treated with 6% iodo-benzalkonium chloride and latewood region treated with 12% iodo-benzalkonium chloride. A large P. *ponderosa* sapwood block with dimensions of 30 x 65 x 115 mm was treated with a 5% solution of iodo-benzalkonium chloride.

Supplemental treatments were performed using 1% and 3% solutions of iodo-benzalkonium chloride in which the pH was buffered to 10.6 and 2.7, with sodium carbonate and acetic acid solutions, respectively. Sodium carbonate was also used to amend a 10% solution to a pH of 11.

Additional treatments containing copper were carried out using P. *ponderosa* sapwood blocks, treatments including: ammoniacal iodo-benzalkonium chloride (Amm Iodo-AAC); ammoniacal copper carbonate (Amm Cu); ammoniacal copper carbonate iodo-benzalkonium chloride (Amm Iodo-AAC Cu); and ammoniacal copper carbonate benzalkonium chloride (Amm AAC Cu). The ratios used in the above treating solutions were CuO:AAC (2:1), plus

NH_4OH:CuO (2:1). A 3% iodo-benzalkonium chloride treating solution was formulated, with 6% CuO and 12% ammonia.

Two hardwood species were also treated with the iodo-benzalkonium chloride. Quaking aspen (*Populus tremuloides* MICHX) and yellow birch (*Betula alleghaniensis* BRITTON) blocks, 19 x 19 x 19 mm, were impregnated with 3% iodo-benzalkonium chloride.

All blocks/regions were oven dried and weighed before treatment. For each treatment, a number of labeled blocks/regions were placed in a beaker inside a vacuum desiccator and subjected to a 30 minute vacuum. The iodo-benzalkonium chloride solution (copper formulated or pH adjusted) was then added and the samples allowed to soak for one hour at atmospheric pressure. Upon removal from the desiccator the blocks/regions were carefully wiped to remove any excess solution, then weighed, wrapped in polythene, and left in the laboratory for seven days to allow for fixation. The polythene was then removed and the blocks/regions were air dried for one week.

The 19 x 19 x 19 mm blocks were split to separate the outer 5 mm section from each face from the inner core. The samples used in the analysis were prepared from the outer 5 mm sections, in the majority of the analysis. In the large block, a sample was removed from the outer 5 mm that was close to end grain (cross) plus samples from the mid way point (mid from either end). The mid point samples were removed at 0-5 mm, 5-10 mm, 10-20 mm and 20-30 mm from the surface of the block. The surface of the samples were smoothed using a sledge microtome. All cutting and microtoming was carried out on dry samples to minimize movement and loss of the chemical (Ryan 1986). All samples were placed with the smooth surface upper most on either aluminum stubs with a carbon disk or carbon stubs. The

samples were coated twice with carbon using a thermal evaporator to give a film thickness of 300-400 Å.

To examine the effect of soaking prior to microtoming, two end matched samples were taken from a block that was treated with 10% iodo-benzalkonium chloride. One sample was prepared dry as described previously, while the other was soaked in distilled/deionized water for 12 hours prior to microtoming.

Sample Analysis

The stubs were placed in a Hitachi S-570 Scanning Electron Microscope (SEM) fitted with a Kevex 8000 Energy Dispersive X-ray Analyzer (EDX). A 20 kV accelerating voltage and a 35 mm working distance were employed. In the EDX analysis software, windows for chlorine, iodine, copper and their background regions were created (Figure 3.2.2.1). Each X-ray analysis was recorded over a 200 second interval, using a reduced area analysis of two adjoining cell walls. Measurements were made on earlywood and latewood tracheids, and ray cells. All analyses were done with a double cell wall thickness of 5 to 10 μm (a typical example is shown in Figure 3.2.2.3.). Six analyses per cell type within each sample were collected and the results averaged. X-ray counts of iodine, chlorine, copper and their backgrounds were converted into peak-to-background (P/B) ratios, using the equations in Figure 3.2.2.2., which have found application in the semi-quantitative analysis of elements in biological samples (Goldstein, *et al.* 1981).

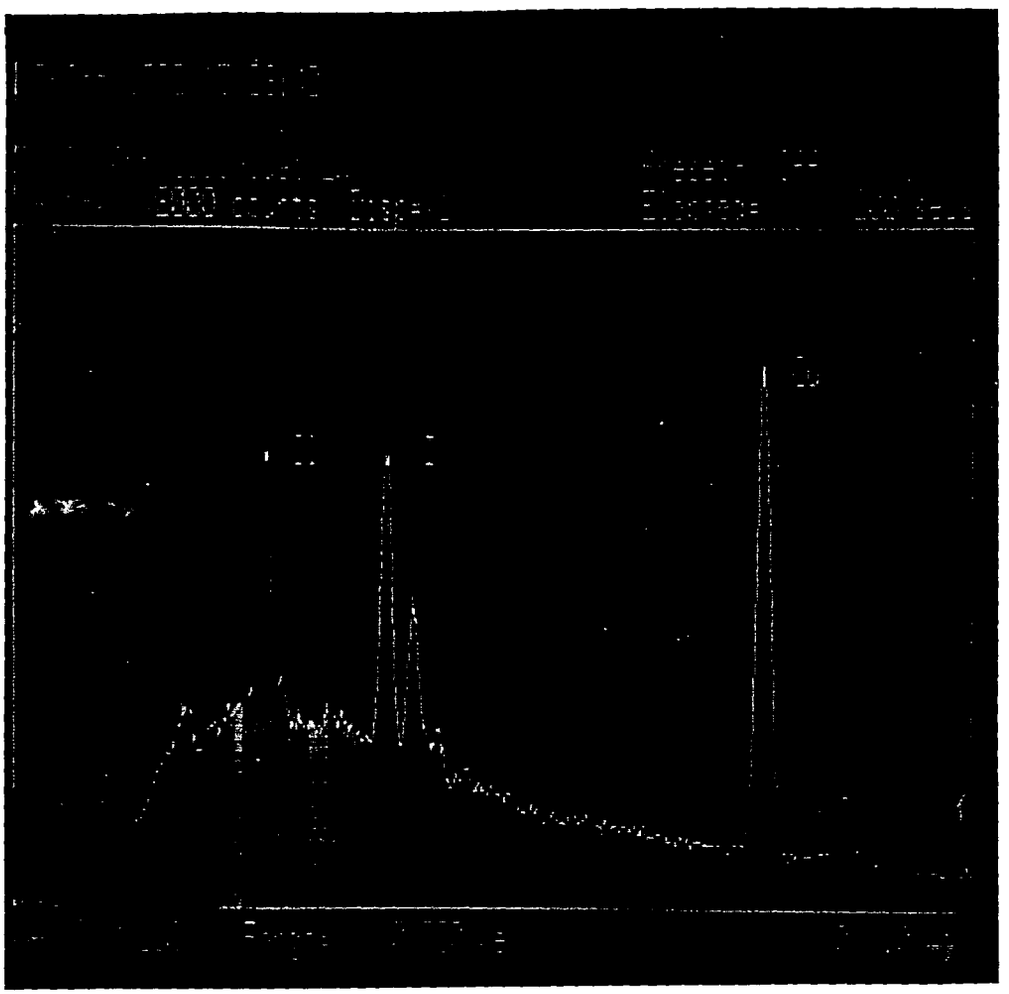


Figure 3.2.2.1: Spectral analysis windows for iodine, chlorine, copper with their respective background regions in the SEM-EDX.

Before any measurements were recorded on the experimental material, the iodine and chlorine counts produced by a reference sample were determined. The results for the

different samples were normalized using the K factor calculated from the variation in the reference sample as shown Figure 3.2.2.2. Analysis of variance (ANOVA) was carried out on paired sets of data to test their significant difference at 95% confidence interval. Iodine and chlorine line scans, across selected cell walls, were recorded at each iodo-benzalkonium chloride concentration. Spot analyses of the compound middle lamella (CML) and secondary wall region (S) within selected tracheids were carried out on the samples as illustrated in Figure 3.2.2.3.

$$P/B = \frac{\text{Element } \textit{Counts} - \text{Background } \textit{Counts}}{\text{Background } \textit{Counts}}$$

$$K = \frac{\text{Original P/B Ratio of the Control}}{\text{Present P/B Ratio of the Control}}$$

Figure 3.2.2.2.: Equations for determining the iodine, chlorine and copper P/B ratio and the correction factor for day to day variation in the SEM-EDX.

Iodo-benzalkonium Chloride Analysis.

Iodo-benzalkonium chloride analysis was carried out using high performance liquid chromatography (HPLC) combined with a modified Photometric Technique (Larson & Pfeiffer, 1983). The HPLC incorporated a Spectra Physics 1200 series HPLC pump equipped

with a 8 mm x 10 cm Partisil SCX Column (Water 85753) with 10 μm particles and a Spectra Physics variable wavelength UV detector set at 262 nanometers. A filtered (0.45 μm PTFE) mobile phase that was composed of 0.025 M of benzyltrimethylammonium chloride, 1% acetic acid and a water:methanol mixture (5:1) used at a 2.2 ml/min flow rate.

The wood samples were ground in a Wiley Mill through a 20 mesh screen (850 μm). Using approximately 0.2 grams of the wood sawdust and 2 ml of acidified ethanol (formic acid) the iodo-benzalkonium chloride was extracted by ultrasound at 40 Hz for 2 hours. The solution was allowed to settle for 12 hours. The extracted solutions were filtered through a PTFE filter (0.45 μm) and analyzed by HPLC for their iodo-benzalkonium chloride content, which was determined from a standard calibration curve prepared with standard solutions of iodo-benzalkonium chloride.

Copper Retention.

Measurement of the copper retention in the P. *ponderosa* sapwood was done using an ASOMA X-ray Fluorescence Analyzer. The copper content was expressed as copper oxide (kg) per unit volume (m^3) of wood. The wood samples were ground in a Wiley Mill through a 20 mesh filter (850 μm). The sample holder was filled with approximately one gram of sawdust and compacted with a hand press with a torque of 30 Newton meters (250 inch pounds). The sample holders were placed in the ASOMA X-ray analyzer and analyzed for copper retention based on a wood density of 410 kg/m^3 (AWPA, 1970).

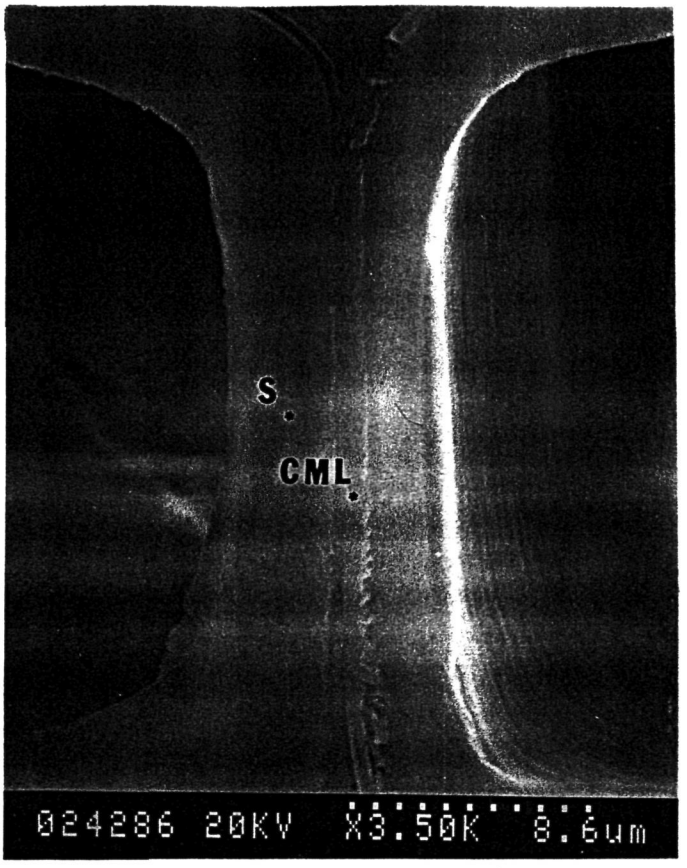


Figure 3.2.2.3: A typical tracheid cell wall showing the compound middle lamellae (CML) and secondary wall (S) regions.

3.2.3. Mobility of DDAC in P. *ponderosa* Sapwood.

Sample Preparation.

P. *ponderosa* sapwood mini stakes, 3 x 28 x 44 mm, were treated with 1% and 3%, didecyldimethylammonium chloride (DDAC). The samples were labeled, oven dried and weighed before treatment. For each treatment, a 30 labeled mini stakes were placed in a beaker inside a vacuum desiccator and subjected to a 30 minute vacuum. The DDAC solution was then added and the samples allowed to soak for one hour at atmospheric pressure. Upon removal from the desiccator and the mini stakes were carefully wiped to remove any excess solution and weighed. The samples were wrapped in polythene, and left in the laboratory for seven days to allow for fixation. The polythene was then removed and the mini stakes air dried for one week.

Soil Bed Exposure.

The mini stakes were oven dried and split along the long grain into two side matched sections with dimensions of 3 x14 x 44 mm. The weights of the matched samples were noted and one half was placed in the soil bed and other half being retained as a reference. The soil bed had been set up in a plastic sealable container, using soil obtained from the Forintek Canada Corp. field test site at Westham Island, in Ladner where AAC treated stakes had failed rapidly (Ruddick, 1983). The soil was first prepared to a 110% water holding capacity (AWPA E10-91, 1991), after which additional water was added until the water puddled on the surface. Water was added during the experiment to maintain the same water level in the

plastic container. The mini stake sub-samples were placed in the bed with 80% of the length being below the surface of the soil. Mini stakes were removed from the soil bed at 3, 13, 22 and 26 month intervals (replicates of seven). The moisture content, weight loss and the DDAC content were measure. The DDAC content of reference matched sections of the mini stakes were also measured.

Isolations from the Mini Stakes.

Isolations were carried out on three types of media: 4% nutrient agar with 100 ppm benomyl, 4% nutrient agar with 100 ppm of tetracycline and 4% potato dextrose extract agar. Isolations were carried out on the mini stake sub-samples, which had been in the soil bed for 3 months. Small sections were removed from each mini stake above the soil contact region (A), the region below the soil surface (B) and the region at the soil surface (C). The sections were split and a small unexposed sliver placed on the media. The plates were incubated and monitored for four weeks at 25^{o}C. Any fungal growth present was tentatively identified. Selected organisms were cultured and sent to the National Identification Service in Ottawa for identification.

Didecyldimethylammonium Chloride Analysis.

DDAC analysis was carried out using HPLC combined with an Indirect Photometric Technique (Larson & Pfeiffer, 1983). One system incorporated a Spectra Physics 1200 series HPLC pump with a Spectra Physics variable wavelength UV detector, while the second used a Waters HPLC pump with a Waters variable wavelength UV detector fitted with an

autosampler. Both HPLC systems were equipped with a 8 mm x 10 cm Partisil SCX Column (Water 85753) with 10 μm particles and the variable wavelength UV detector set at 262 nanometers. A mobile phase consisted of 0.025 M of benzyltrimethylammonium chloride, 1% acetic acid and water:methanol mixture (ratio of 5:1 used). The mobile phase was filtered (0.45 μm PTFE) and used at a flow rate 2.2 ml/min.

The mini stakes were ground in a Wiley Mill through a 20 mesh filter (850 μm). Approximately 0.2 grams of the wood and 5 ml of acidified ethanol (formic acid) were extracted by ultrasound at 40 Hz for 2 hours. The extracted solution was allowed to settle for 12 hours. An internal standard of 1,000 ppm of trimethyl(C_{12})dodecylammonium chloride had been added to the extracting solution. The extracted solutions were then filtered through a PTFE filter (0.45 μm) and analyzed in the HPLC for their DDAC content based on a standard calibration curve.

3.3. Results and Discussion.

3.3.1. DDAC Fixation Mechanism and Adsorption by the Wood Components.

Alkylammonium compounds (AACs) belong to a general class of organic substances, which are described as *cationic surfactants* (Resuggan, 1951). In aqueous solution, surfactants can adsorb onto a solid substrate by various mechanisms: ion exchange, ion pairing, physical adsorption by polarization or dispersion forces (Rosen, 1975). The actual mechanism depends on the type of surfactant and the solid substrate. Wood has been described as having 'strongly' charged sites. According to Rosen the mechanism for this type of material is a complex process during which the adsorption of the solute may occur successively by ion exchange, ion pairing and hydrophobic bonding. White (1970) found that adsorption of AACs occurred in two stages depending on the surfactant concentrations. All surfactants have what is referred to as the critical micelle concentration (CMC). Below the CMC, only the cation is adsorbed by ion exchange with the substrate to a measurable extent. Above the CMC both the cations and anions are adsorbed. The AAC concentrations used in early wood preservation research were close to the CMC of these surfactants, but later applications used concentrations well above the CMC. The mechanism by which AAC wood preservatives are adsorbed onto wood will most likely involve ion exchange when the concentration is close to or below, the CMC. Other mechanisms may play a significant role above the CMC. A typical adsorption isotherm shows an 'S' shaped curve where : the 'knee' corresponds to the AAC ion exchanging on a negatively charged surface - the extent of ion pairing is dependent on

the amount of ion exchanged AAC (Gotshal, Rebenfeld & Howard, 1959). As the concentration approaches the CMC hydrophobic interactions occur causing hemimicelle and admicelle formations (Rosen, 1972). Studies by Connor and Ottewill (1971) suggest that the bond formed by ion exchange is not a true ionic bond, but an electrostatic bond. This theory agrees with the poor AAC desorption observed and the easy displacement of highly charged ions such as calcium, by certain AACs (Chen, 1989). Butcher and Drysdale (1978) carried out a limited study that supports the importance of ion exchange in the fixation of AACs in wood. They showed that changing the pH of the treating solution resulted in different levels of protection against decay fungi. This would be expected as Weatherburn and Bayley (1952) found that the pH of the solution affected adsorption of surfactants by ion exchange on a cellulosic substrate. Accepting that cationic surfactants such as DDAC, will bind by ion exchange, a possible interaction with wood is shown in Figure 3.3.1.1.

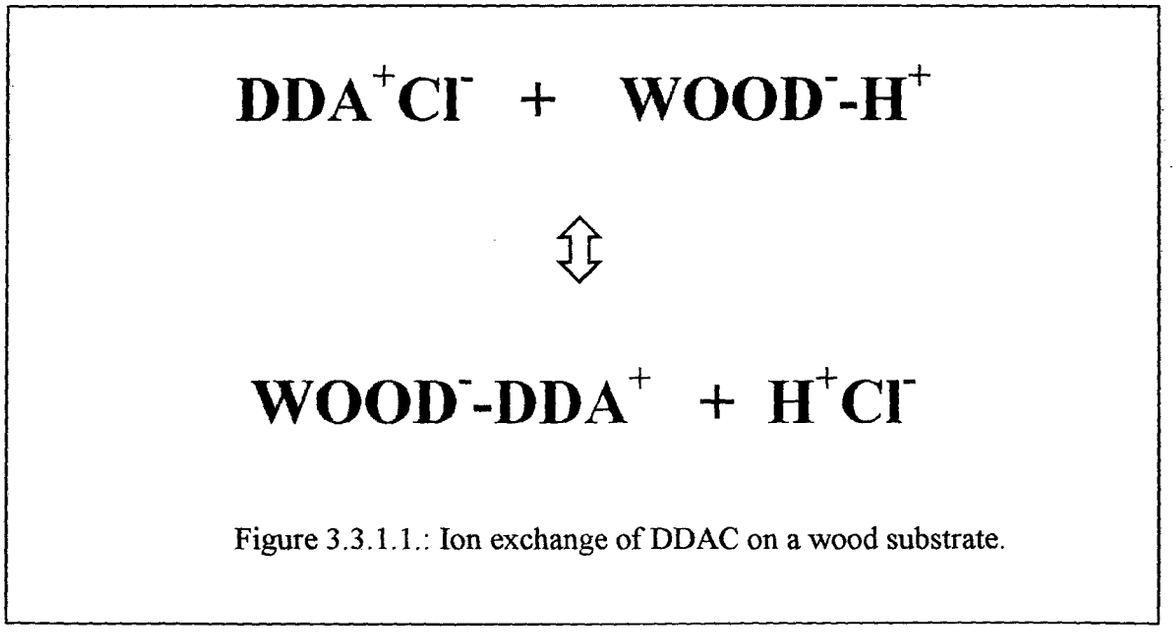


Figure 3.3.1.1.: Ion exchange of DDAC on a wood substrate.

The most likely functional groups on the wood that would participate in the interaction with cationic surfactants, are the negatively charged sites such as the carboxylic and phenolic groups on lignin (Brezny *et al.*, 1990). The interaction of AACs with holocellulose and lignin will contribute to the effectiveness of the preservative. Until recently, little information on the interaction of AACs with the wood components was known. However, current research found that DDAC adsorbed onto lignin preferentially, while small amounts were adsorbed onto the cellulose (Jin & Preston, 1991). That study also highlighted the importance of the treating solution pH, an increase in the pH resulted in higher DDAC adsorption especially on the lignin. The influence of pH on retention suggests that ion exchange adsorption may play a vital role in DDAC adsorption in the wood. A recent study found that the anion adsorption was not equivalent to the amount of cation adsorbed, suggesting that the benzalkonium chloride was adsorbed by ion exchange (Loubinoux & Malek, 1992).

If the DDAC binds by ion exchange as shown in Figure 3.3.1.1. then the solution pH would decline in response to the formation of HCl. Thus monitoring the DDAC cation and chloride ion concentrations by titration, will identify the amount of DDAC retained in the wood by ion exchange. The actual DDAC retention will be determined by extraction followed by titration analysis.

Wood Interaction.

Three analytical methods were used to determine the DDAC retention in the wood samples after 96 hours of soaking. The DDAC titration will indicate the true uptake of DDAC from

the solution by the wood. The chloride titration will indicate the amount of DDAC retained based on loss of chloride from the solution. However, if the DDAC adsorbed is by ion exchange then the chloride titration will not account for the cations adsorbed by the sample. The pH changes will indicate the amount of DDAC ion exchanged based on the formation of HCl. Figure 3.3.1.2. shows the DDAC adsorption based on all three methods. Initial measurements indicate that a large portion of DDAC adsorbs within the first half hour, based on the final retention based on extraction analysis, agreeing with the observations made by Vinden (1984). The analysis of DDAC cation titrations found an adsorption of 0.08 mmol/g DDAC on the wood sawdust, while chloride titration found an adsorption of 0.06 mmol/g DDAC after 30 minutes. Therefore, based on the difference in the amount of DDAC adsorbed by ion exchange would be 0.02 mmol/g. The DDAC retained by ion exchange based on solution pH changes resulted in a DDAC adsorption of 0.005 mmol/g. The titration results beyond the first 30 minutes had poor agreement, especially the chloride titrations. One possible reason for this discrepancy was cationic adsorption changed the surface charge and attracted the chloride from the solution. The pH technique has proven in the past to be only an indicator of chemical adsorbed and not a reliable analytical method (White, 1970). This observation was also seen by other workers studying AACs uptake in cellulose fibers (Rose, Weatherburn & Bayley, 1951).

The actual DDAC adsorption in the wood based on extraction and compared with pH/titration based DDAC adsorptions after the 96 hours are shown in Table 3.3.1.1. The DDAC adsorption determined by extraction was 0.108 mmol/g. The value was half way between the adsorptions based on DDAC and chloride titrations. This agrees with previous

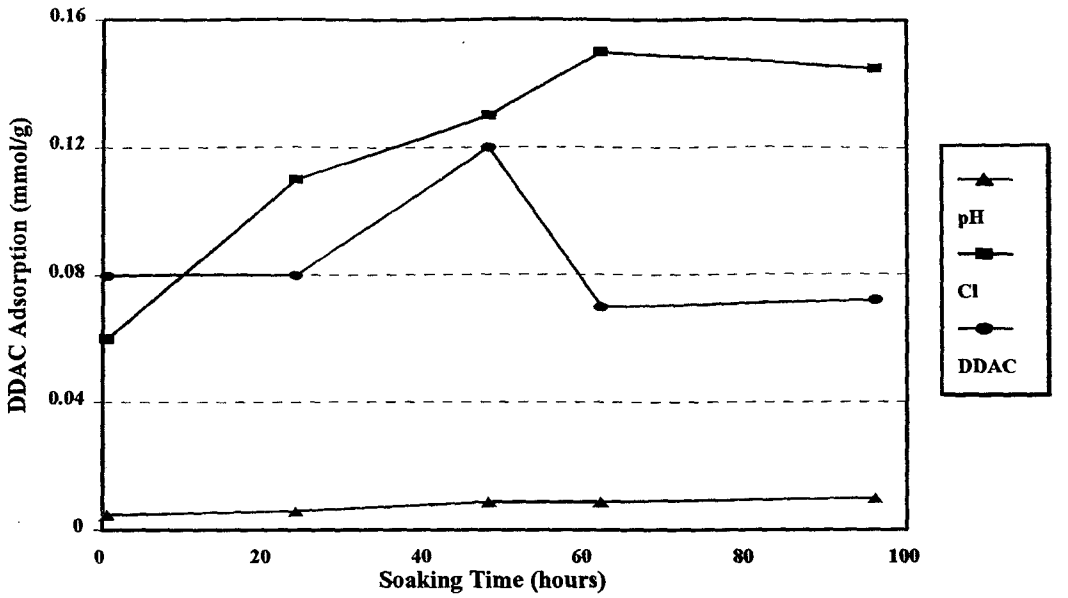


Figure 3.3.1.2.: DDAC adsorption in P. *ponderosa* sapwood.

research, where the maximum adsorption was 0.13 mmol/g of DDAC in southern yellow pine (Preston, *et al.*, 1987). In order to remove all the DDAC from the wood sawdust the extraction process had to be carried out twice. Approximately 0.014 mmol/g of DDAC was highly bound in the wood, and this may correspond to the DDAC adsorbed by ion exchange. This amount of DDAC is about twice the DDAC adsorbed based on pH measurements. Ion exchange of DDAC in the wood, would create an electrostatic bond that does not easily desorb (Connor & Ottewill, 1971). Therefore, approximately 13% of the DDAC adsorbed in the wood was fixed by ion exchange and the other 87% would be adsorbed by ion pairing, dispersion forces and hydrophobic interactions. Adsorption studies by Jin and Preston (1991)

found that southern yellow pine sawdust (passed through a 30 mesh filter -600 μm) adsorbed 0.216 mmol/g DDAC from a 0.5% solution concentration. The differences could be attributed to the surface area available for adsorption, wood species and the DDAC solution to wood ratio used. A study using beech sawdust found adsorption of benzalkonium chloride in the order of 0.25 mmol/g, for a similar concentration to the one used in this study (Loubinoux & Malek, 1992). Again the surface area, wood species and DDAC solution to wood ratio used could account for the variations observed.

The results of DDAC adsorption in the wood sticks compared to the sawdust were in reasonable agreement, with wood sticks having a slightly smaller adsorption. The difference could be attributed to the surface area available for adsorption in the wood sticks compared with wood sawdust. Again the retention based on the chloride analysis was on the high side and the actual retention would be between the two titration method values. Previous work on

Table 3.3.1.1.: DDAC retention in wood.

	DDAC Adsorption (mmol/g)*	
Method of Determination	Wood Sticks	Wood Sawdust
pH	0.006 (0.001)	0.010 (0.001)
Decrease in DDAC	0.070 (0.054)	0.072 (0.036)
Decrease in chloride	0.200 (0.028)	0.144 (0.047)
1st Extraction of DDAC	-	0.094 (0.009)
1st plus 2nd Extraction of DDAC	-	0.108 (0.013)

* Values in parentheses are the standard deviations.

wood indicated an equilibrium DDAC adsorption in the order of 0.1 to 0.2 mmol/g (Preston, *et al.*, 1987; and Jin & Preston, 1991).

The estimated DDAC adsorption by ion exchange in the wood sticks based on pH (Table 3.3.1.2.), was carried out with four DDAC concentrations. At the lowest concentration tested, 0.01 g/l, the adsorption accounted for 17% of the maximum possible adsorption. As the DDAC concentration went from 0.1 g/l to 1.0 g/l, the DDAC adsorption based on the maximum remains at about 6%. A large jump in adsorption was observed between 0.1 g/l and 1.0 g/l. This large increase in DDAC adsorption was in the region of the CMC, which is estimated to be in the region of 0.3 g/l (theoretical value) for DDAC. As the CMC point was approached, the retention of AACs on a cellulose substrate dramatically increases (White, 1970). Above the CMC retention of the AAC appear to reach a maximum. The amount of DDAC adsorbed by ion exchange was 17% of the maximum DDAC available at the lowest

Table 3.3.1.2.: DDAC retention in wood after exposure to various solution concentrations determined by changes in pH.

DDAC Solution Concentration	DDAC Adsorption (mmol/g)	
	Measured	Maximum
0.01g/l	0.10×10^{-3}	0.60×10^{-3}
0.1g/l	0.33×10^{-3}	6.00×10^{-3}
1.0g/l	4.10×10^{-3}	6.00×10^{-2}
10.0g/l	6.20×10^{-3}	6.00×10^{-3}

concentration. The lack of total adsorption may be a response of the equilibrium balance between the DDAC in solution and that in the wood.

Cellulose Interaction.

DDAC adsorption in the two commercial cellulose powders is shown in Table 3.3.1.3. The results indicate that cellulose has a very limited capacity for DDAC adsorption. The amount of DDAC retained by Avicel® was 0.01 mmol/g and none was detected on the Whatman® cellulose. The amount of ion exchanged onto cellulose was in the order of 0.1×10^{-3} mmol/g and 0.04×10^{-3} mmol/g respectively. The carboxyl content of cellulose was previously established to be 0.071 mmol/g (Jin & Preston, 1991). The amount actually retained under the treating conditions indicated that cellulose has a low affinity for DDAC. These results are in agreement with the order of adsorption of DDAC noted by Jin and Preston (1991), where cellulose has the lowest affinity with DDAC. However, the actual amount adsorbed by cellulose was measured to be 0.068 mmol/g using a 0.5% DDAC, while in this study only 0.01 mmol/g was actually retained. Considering these results it is unlikely that any significant amount of DDAC was adsorbed onto the wood would be associated with the cellulose component.

Lignin Interaction.

The DDAC adsorption by the two laboratory prepared lignin samples are shown in Table 3.3.1.4. The results suggested that lignin has a very high capacity for the adsorption of

DDAC. Determinations by either chloride anion or DDAC cation titrations indicated that both lignin's removed large amounts of the surfactant from solution. The differences

Table 3.3.1.3.: DDAC retention in two commercial cellulose powders.

	DDAC Adsorption (mmol/g)*	
Method of Determination	Avicel®	Whatman® Cellulose
pH	0.1×10^{-3}	0.04×10^{-3}
Decrease in DDAC	-	-
Decrease in chloride	-	-
Extraction of DDAC	0.010 (0.045)	**

* Results in parentheses are standard deviation values.

** no detectable DDAC present.

between DDAC adsorption for the two titrations techniques were extremely large, the amount was double the actual amount retained by the lignin (based on extraction). One possible explanation for this was surface build up of the surfactant that was easily desorbed during washing. However, DDAC retention based on extractions indicated that DDAC had a very high affinity for lignin. The DDAC adsorption based on the pH changes indicated that the Klason lignin and the TFA lignin have different ion exchange capacities. The difference in the adsorption by ion exchange almost certainly was a reflection of the two isolation methods. The pH of the control Klason lignin solution was about 8, while the control TFA lignin solution pH value was 3.8. The environment in the TFA lignin appears closer to the environment found in the wood, which has a solution pH of ~4. The amount of sites

available for ion exchange in HCl lignin used by Jin and Preston (1991) was 1.633 mmol/g and an adsorption of 0.100 mmol/g of DDAC was recorded, for a 0.5% DDAC solution. Both the Klason and TFA lignin adsorbed ~0.45 mmol/g DDAC from the 1% treating solution that was much higher than previously recorded. The isolation method does not appear to dramatically affect the actual amount adsorbed based on extraction. The amount of DDAC ion exchanged in TFA lignin was 0.108 mmol/g that is 23% of the DDAC retained by TFA lignin. The high capacity of lignin for DDAC adsorption is in agreement with the results found by Jin and Preston (1991) where lignin preferentially adsorbed DDAC.

Table 3.3.1.4.: DDAC retention in laboratory prepared lignin.

	DDAC Adsorption (mmol/g)*	
Method of Determination	Klason lignin	TFA lignin
pH	0.04×10^{-3}	0.108
Decrease in DDAC	0.832 (0.141)	1.456 (0.468)
Decrease in chloride	1.026 (0.658)	2.179**
Extraction of DDAC	0.423 (0.025)	0.478 (0.126)

* Results in parentheses are standard deviation values.
** only one sample available.

Wood and it's Components.

The adsorption of DDAC in the various components determined by pH and extraction is shown in Figure 3.3.1.3. The lignin and wood adsorbed relatively large quantities of DDAC. However, cellulose DDAC adsorption was very limited. The adsorption by ion exchange that

occurred in wood and lignin accounted for 13% to 23% of DDAC adsorbed respectively. The other 74% to 87% of the DDAC was adsorbed by a combination of ion pairing, dispersion forces and hydrophobic interactions. These observations are consistent with finding by Jin and Preston (1991), where lignin had the highest affinity with DDAC and cellulose had the lowest affinity. Pine softwoods generally contain between 27% to 30% lignin and based on complete adsorption of DDAC by lignin to the same order (0.45 mmol/g) as recorded on the extracted lignin (Table 3.3.1.4.). Using a value of 27% lignin the wood, the DDAC adsorption would be approximately be 0.12 mmol/g, which is very close to the DDAC adsorption of 0.108 mmol/g actually measure measured in the wood sawdust (Table 3.3.1.1). The only component not considered was hemicellulose. However, the heterogenious nature, high branched structure and low degree of polymerization of hemicelllulose, a greater reactivity than cellulose would be unlikely. Therefore, it can be postulated that hemicellulose retention of DDAC would be very small based on the results of cellulose adsorption. Coupled with an expected low DDAC adsorption on hemicellulose and the close agreement with actual adsorption and estimate adsorption based on retention by lignin, it can be concluded that hemicellulose does not participates in the adsorption of DDAC in the wood.

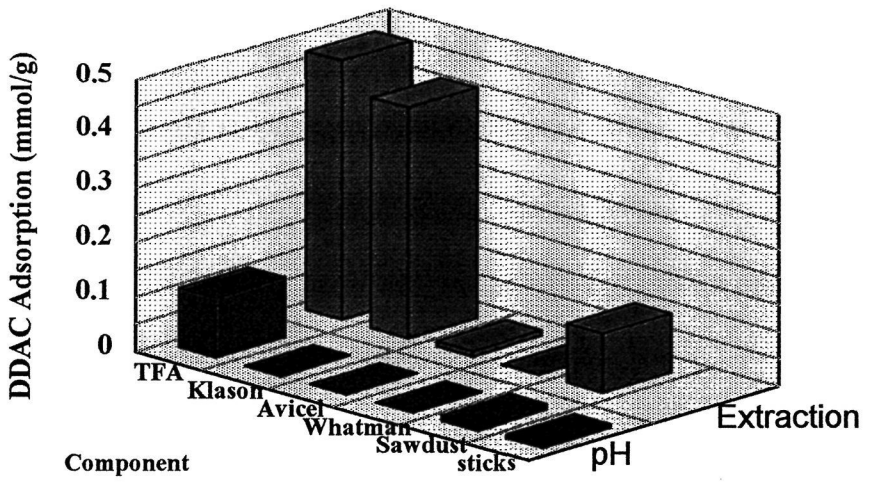


Figure 3.3.1.3.: DDAC retention within the various wood components based on pH and extraction techniques.

3.3.2. Distribution of Alkylammonium Compounds in the Wood.

An understanding of the distribution of alkylammonium compounds (AACs) at the bulk, cellular and microscopic may provide an insight into any weakness that resulted in the failure of AACs. One of the first theories on the poor field performance of AAC-treated wood was erratic distribution. In the first commercial treatment trials, one researcher found that core retentions of the AAC was variable in radiata pine treated with benzalkonium chloride using the Bethell process (Vinden, 1982). As expected the AAC retention in the outer zone was much higher than that of the core section (Butcher & Drysdale, 1978[b]; Ruddick & Sam, 1982; Drysdale, 1983[b]; and Nicholas, *et al.*, 1991). Initially it was believed that the earlywood and latewood interaction with the AAC were different (Butcher & Drysdale, 1978[b]). Chemical analysis of these regions found that earlywood (springwood) retained more chemical than the latewood (summerwood) (Ruddick & Sam, 1982; and Nicholas *et al.*, 1991). This observation was different from PCP distribution in softwoods, where latewood retained more chemical than earlywood, below a retention of 13 kg/m^3 (Gjovik, Roth & Lorenz, 1970). None of these studies found that the distribution pattern was sufficiently erratic to explain the failure of AAC-treated wood in field trials. While macrodistribution of AACs has been studied, the cellular and microdistribution remains unexplored. This distribution is very important as the organisms responsible for wood deterioration act at the cell wall level.

A number of treating parameters appear to influence the performance of AACs in the wood and these may alter the distribution of the AAC in the wood. These factors include treating

solution pH, the carrier solvent and the presence of competing ions, such as copper. As indicated earlier the pH of the treating solution has an influence on the retention and performance of AAC-treated wood (Butcher & Drysdale, 1978[b]; and Jin & Preston, 1991). The carrier solution for the preservative has also been shown to alter the preservatives ability to protect the wood. It has been demonstrated that when the aqueous carrier solution was changed to white spirits the AAC performance improved (Greaves, Cookson & Tighe, 1988; and Blow, 1986). The different carrier solutions may influence the distribution patterns of the AAC. To improve the performance of AACs, many scientists have added copper to the treating solution (Butcher, Preston & Drysdale, 1979; and Drysdale, 1983[a]). The formulation that has shown the best performance to date is an ammoniacal-copper-AAC formulation (Hedley, Tsunoda & Nishimoto, 1982). The reason for this success is easily recognized since copper is a known fungicide. It may also influence where the AAC interacts within the wood. The influence of the above treating parameters on the distribution of AACs may indicate the role they play in the performance of AACs.

Electron microscopy has proven a popular tool in studying microscopic objects in many research areas. Wood scientists have used electron microscopy to study: the cellular structure of wood; wood biodeterioration; the distribution of lignin within the wood cell wall; wood composites; and glue flow in wood composites (Greaves, 1974; Greaves & Nilsson, 1982; Bolton, Dinwoodie & Beele, 1985; Degroot & Kuster, 1986; and Saka & Goring, 1988). The application of electron microscopy can be split into two types, one being the study of the superficial features and the other the examination of the molecular structure of samples (Goodhew & Humphreys, 1988). These types are referred to as scanning electron

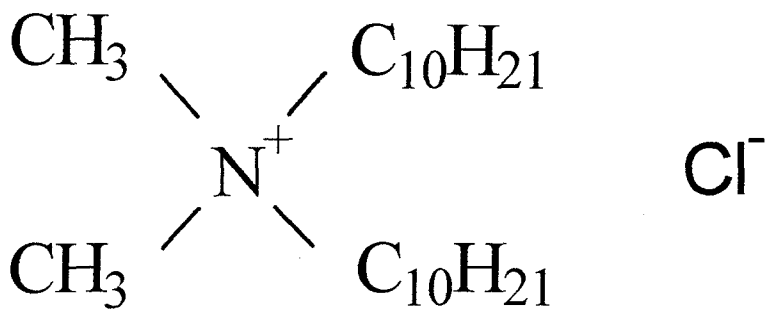
microscopy (SEM) and transmission electron microscopy (TEM). One of the main differences between SEM and TEM is the sample preparation techniques required. TEM tends to require complicated preparations, which are time consuming compared to the SEM sample preparation. In order to study the chemical composition of a sample, or to map an elements distribution the electron microscope must be coupled with an X-ray analyzer. This may be either an energy dispersive X-ray analyzer (EDX) or a wavelength dispersive X-ray analyzer (WDX). EDX has the advantage that it can study more than one element at a time, to provide the elemental composition profile of the sample. The WDX is more sensitive than EDX, but can only study one element at a time. If the microdistribution of preservatives is to be studied at the microscopic level, minimal sample preparation must be preferred in order to limit chemical redistribution and leaching, which may occur during sample preparation (Ryan, 1986). If the preservative formulation contains more than one element like CCA then an elemental profile would provide more information. Therefore, the SEM-EDX system would be a suitable technique for studying the distribution of AACs in wood.

SEM coupled with EDX has proven useful in understanding the microdistribution of traditional preservatives such as PCP and CCA in wood (Greaves & Levy 1978; Greaves, 1972 & 1974; and DeGroot & Kuster 1986). It is recognized that results obtained using this technique are only semi-quantitative even under optimum conditions, due to the heterogeneous nature of the material involved. EDX analysis of a sample requires that the element being studied has an atomic number greater than ten (often a metal or halogen) and a minimum concentration in the range of 0.1% to 0.3% w/w. The substrate (wood) to be analyzed should contain minimal levels of the element under study, so that the element can

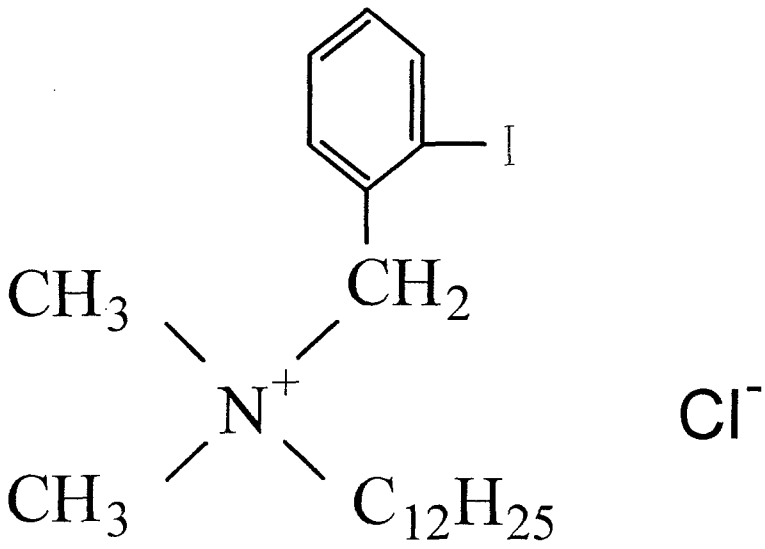
be distinguished from the substrate when treated. AACs are salts, composed of a cation and an anion (Figure 3.3.2.1.). The AACs, which have shown promise as wood preservative candidates, all have cationic portions with similar elemental composition to the wood substrate. DDAC is an example shown in Figure 3.3.2.1. that proved promising in early laboratory trials. The anionic portion can be either chloride or bromide, which are distinguishable from the wood substrate in the SEM-EDX. However, the anion can undergo ion exchange with the wood and its distribution does not confirm the distribution of the biocidal cation of the AAC. Distribution can be monitored if the cation portion has a chemically incorporated label. A custom AAC was prepared by Lonza Inc. that contained an iodo-substituted benzyl ring (Figure 3.3.2.1.). Since iodo-benzalkonium chloride has an iodo-labeled cation that can be distinguished from the wood substrate, its distribution can be monitored using SEM-EDX. The chlorine can also be monitored using the SEM-EDX. Therefore, this molecule could be used to monitor the cellular and microdistribution of the cation and the anion of this AAC in the wood.

Changes measured in iodine retentions accurately reflect the actual variation in the interaction of the iodo-benzalkonium chloride with the wood substrate. Under conditions favoring cationic exchange reactions as in wood with its negatively charged site, the chloride may be separated from the surfactant (Rosen, 1975). Therefore, chlorine distribution in the wood may not be completely associated with the surfactant. Nevertheless the application of SEM-EDX to wood treated with an iodo-substituted benzalkonium chloride will enable an understanding of the microdistribution of AACs in wood. Secondly it will also enable an

understanding of how this distribution was affected by wood cell type, sample depth, sample size, soaking samples after treatment, wood species and treating solution pH.



Didecyldimethylammonium Chloride (DDAC)



N-N-Dimethyl-N-dodecyl-N-(2-iodobenzyl)ammonium Chloride

Figure 3.3.2.1.: The structure of DDAC a wood preservative and the custom made iodo-benzalkonium chloride.

Comparison of iodine and chlorine counts in different cell types

The relative retentions of the iodo-benzalkonium chloride in the earlywood tracheids, latewood tracheids and ray cells of P. *ponderosa* sapwood, are indicated by the iodine (cation) and chlorine (anion) peak-to-background (P/B) ratios presented in Table 3.3.2.1. At each solution concentration the highest iodine retentions (P/B ratios) were observed in the ray cells. The exception being the samples treated with the highest concentration (13%), where a statistical analysis of the iodine counts for the ray cells and earlywood tracheids showed that they were the same. A line scan for iodine across several tracheids and through a ray cell indicated a concentrated pocket of iodine in the ray cells as shown in Figure 3.3.2.2. Penetration of AACs from ray cells into adjacent tracheids was not apparent from the line scan (shown in Figure 3.3.2.2.) suggesting rapid fixation within the ray cells.

Analysis of the iodine retentions in various cell types, showed that cell type has a significant effect on the AAC distribution. In all cases the ray cells have the highest iodine retentions, confirming that the main penetration pathway for AACs in softwoods was through the ray cells (Figure 3.3.2.2.). This is in agreement with similar studies using other waterborne chemicals (Greaves, 1974; Greaves & Levy, 1978; Bodner & Pekny, 1991).

Earlywood and latewood tracheids showed different absorption patterns of iodine and chlorine over the range of concentrations studied. Treatment of P. *ponderosa* sapwood blocks with 1% iodo-benzalkonium chloride produced similar iodine retentions in earlywood and latewood tracheids (Table 3.3.2.1.). Increasing the solution concentration to 3% significantly increased the earlywood retention of iodine, but not in the latewood tracheids. Above 3%, except for the 6% treatment where the retentions were statistically the same, the

iodine retention for the earlywood tracheids was significantly higher than recorded for latewood. Generally blocks, which were treated with 3% iodo-benzalkonium chloride and above showed twice the retention of iodine and chlorine in the earlywood tracheids compared with that in the latewood tracheids. Latewood tracheids showed significant increase when the solution concentration was increased to 5%. A maximum occurred at the 6% iodo-benzalkonium chloride concentration. These differences reflect the different structures of the tracheids, which is a response to conditions that prevail during their formation.

Table 3.3.2.1.: Peak-to-background ratio measured in P. *ponderosa* sapwood after treatment with iodo-benzalkonium chloride.[a]

			Cell Type (P/B Ratio)*		
Element	**Concentration**	**Retention (kg/m^3)**	**Ray Cells**	**Earlywood Tracheids**	**Latewood Tracheids**
Iodine					
	1%	10	2.75 (0.78)^{A}	0.79 (0.11)^{Ca}	0.78 (0.12)^{Fa}
	3%	25	2.08 (1.0)^{A}	0.98 (0.06)^{D}	0.78 (0.15)^{F}
	5%	50	4.68 (1.05)^{B}	2.91 (0.45)^{Eb}	1.34 (0.15)^{Gb}
	6%	49	4.63 (0.95)^{B}	3.03 (1.13)^{E}	3.00 (0.74)^{H}
	10%	85	5.03 (0.52)^{B}	4.02 (0.68)^{E}	1.96 (0.31)^{I}
	13%	182	4.55 (0.89)^{Bc}	3.56 (0.71)^{Ec}	1.59 (0.38)^{IG}
Chlorine					
	1%	10	0.34 (0.14)^{Aa}	0.49 (0.08)^{Db}	0.43 (0.06)^{Iab}
	3%	25	1.75 (0.24)^{B}	0.87 (0.12)^{E}	0.61 (0.11)^{J}
	5%	50	2.20 (0.93)^{BCc}	1.72 (0.20)^{Fc}	1.03 (0.13)^{K}
	6%	49	2.44 (0.41)^{Cd}	1.88 (0.43)^{FGd}	2.14 (0.28)^{Ld}
	10%	85	2.69 (0.69)^{Ce}	2.32 (0.16)^{Ge}	1.29 (0.19)^{M}
	13%	182	2.50 (0.52)^{C}	1.92 (0.23)^{F}	1.02 (0.20)^{K}

[a] Means are compared in columns within the element types. Means with the same capital letter are not significantly different at a 95% confidence level.
Means are compared in rows within the element types. Means with the same letter are not significantly different at a 95% confidence level.
*Values in parentheses are standard deviations

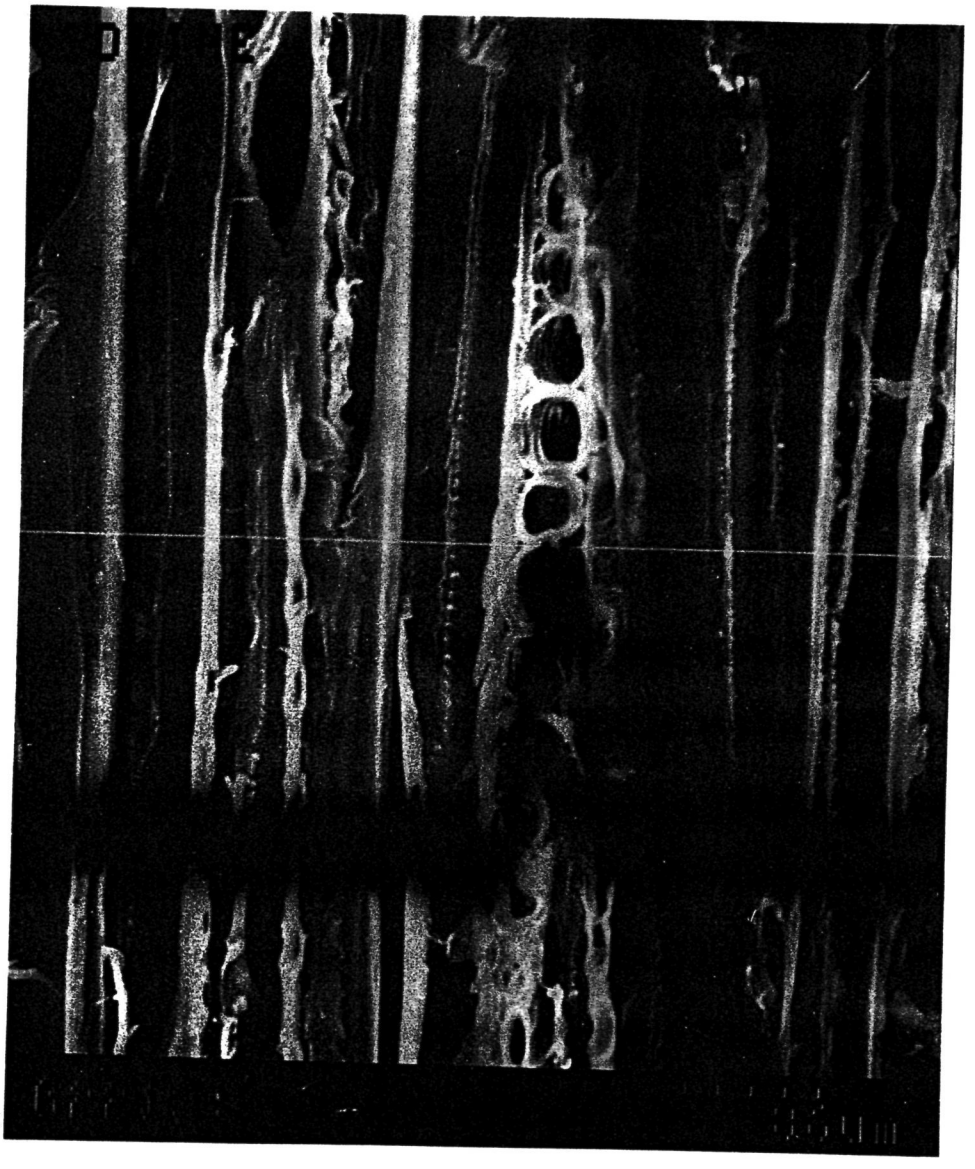


Figure 3.3.2.2.: The distribution of iodine across a tangential surface in the P. *ponderosa* sapwood.

Earlywood and latewood tracheids showed differences in their uptake of the preservative, similar to that previously reported (Ruddick & Sam, 1982; and Nicholas *et al.*, 1991). Using a 1% DDAC solution Nicholas *et al.* (1991) found that springwood (earlywood) retained one and a half times the DDAC taken up by the summerwood (latewood). The results of the current study clearly showed that when small P. *ponderosa* sapwood blocks were treated with iodo-benzalkonium chloride both the iodine (cation) and chloride (anion) retentions were generally greater in the earlywood tracheids than the latewood tracheids.

The corresponding changes in the chlorine retentions (i.e., P/B ratios) were less pronounced than those observed for iodine. The chlorine retentions in ray cells were similar to earlywood tracheids for the 5%, 6% and 10% iodo-benzalkonium chloride concentrations (Table 3.3.2.1.). As with iodine, the chlorine retentions for the earlywood tracheids were generally greater than those for the latewood. At the 3% treating solution the iodine and chlorine retentions were very similar in ray cells, latewood and earlywood tracheids. However, the sample treated with 1% iodo-benzalkonium chloride showed lower chlorine retentions compared with the iodine retentions (Table 3.3.2.1.). This indicated that adsorption by ion exchange was detectable at the 1% iodo-benzalkonium chloride solution. The degree of ion exchange is difficult to estimate using this procedure. However the trends suggested that between 10% and 20% was adsorbed by ion exchange. The reason for the more noticeable ion exchange at 1% treating solution could be a result of dilution of the AAC treating solution as it penetrates the wood block, causing a reduction in the AAC concentration. Dilution of the 1% solution may drop the concentration below the critical micelle concentration (CMC). Previous work found that ion exchange was the sole adsorption

mechanism on cellulosic materials at concentrations below the CMC (Sexsmith & White, 1959). This agrees with results reported by Loubinoux and Makel (1992) using 0.5% benzalkonium compounds, who found high levels of ion exchange. The observations reported here also suggest that at concentrations below 1% (close to CMC) ion exchange can be the primary mechanism for adsorption of AAC, while at higher concentrations other mechanisms predominate.

Influence of the iodo-benzalkonium concentration on retentions in each cell type.

The effect of increasing concentration on the retention of iodo-benzalkonium chloride by individual cell types, is shown in Figure 3.3.2.3. For ray cells a statistical analysis of the data revealed, that iodine retentions at 1% and 3%, were equivalent. At 5% and above a maximum retention of iodine was observed which remained unchanged with increasing concentrations, but were statistically greater than those recorded for solutions below 5%. Chlorine retentions for ray cells remained the increased upto 5%, after which it remained constant. The iodine retentions in earlywood tracheids increased with solution concentration up to 5%. A further increase in concentration resulted in the retention of iodo-benzalkonium chloride remaining constant. Chlorine showed similar responses. Iodine and chlorine retentions in latewood showed a steady increase to 6%, after which a slight decrease was observed.

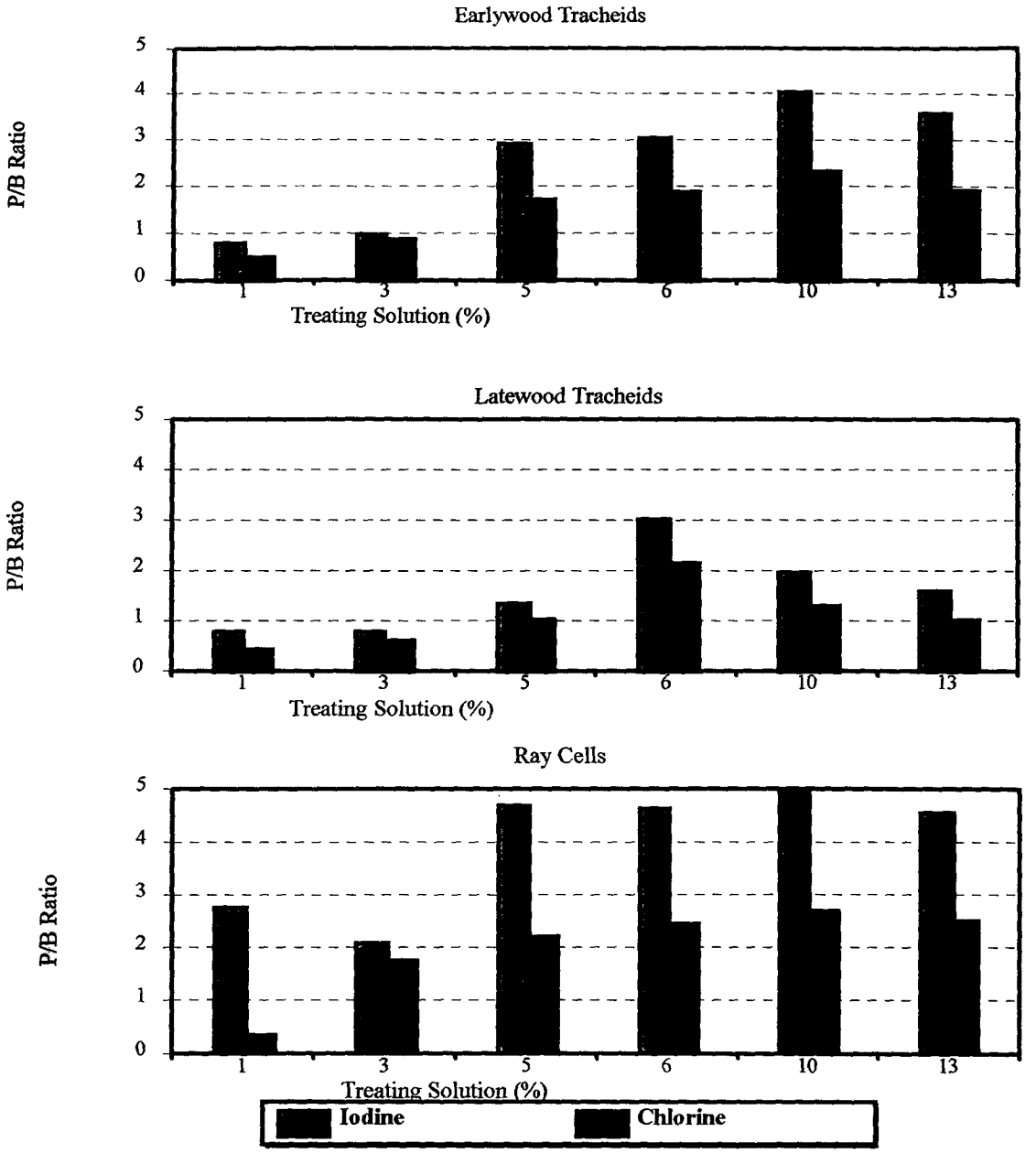


Figure 3.3.2.3.: Cell distribution of iodo-benzalkonium chloride in P. *ponderosa* sapwood as a response to increases in concentration.

Previous work found an equilibrium maximum adsorption of DDAC in southern yellow pine was obtained using a 10 mM (0.6%) treating solution (Preston *et al.*, 1987). All of the iodo-benzalkonium chloride concentrations used in these experiments were higher. However, the results indicated that under the conditions used, a maximum adsorption occurred in the ray cells, latewood and earlywood tracheids of wood blocks treated with 5%, 6% and 5% solutions, respectively. One possible reason for these differences could be the different physical condition of the wood-solution interaction in the two studies. Each cell type had a different maximum adsorption value, these may reflect the different structures and functions of the various wood cells. Once the maximum has been reached, increasing the concentration appears to inhibit further uptake of the preservative (Figure 3.3.2.3.). However, adsorption studies carried out on DDAC and DDAB also show a decline in adsorption above a maximum concentration (Preston *et al.*, 1987).

As the AAC concentration increased, the results suggested a significant change in the interaction occurred above 3% (Figure 3.3.2.3.), all three cell types showed a large increase in the iodine retention. This may be interpreted by the ability of the wood to buffer the pH of the treating solution. At the lower concentrations (1% and 3%) the wood may be able to buffer the equilibrium pH towards acidic conditions. Above 3%, the wood can no longer buffer the pH of the AAC solution resulting in a higher solution pH. It was noted that increasing the pH of the AAC solution caused the DDAC and DDAB retentions to double, even with 0.6% solution concentration (Preston, *et al.*, 1987).

Chlorine counts in two AACs, which are commonly used in wood preservation.

A second set of P. *ponderosa* sapwood blocks were treated with 3% DDAC and 3% benzalkonium chloride. The chlorine (anion) P/B ratios were measured in the ray cells, earlywood and latewood tracheids and the results are shown in Table 3.3.2.2. All three treatments had the same chlorine retentions within each cell type, which suggests that the absorption of all three AACs was similar. These results indicate that the iodo-benzalkonium chloride used in this study was a suitable model for AACs distribution within the wood substrate. The chlorine distribution in all three cells shows a similar pattern, with the greatest retention in the ray cells than the earlywood tracheids and finally latewood tracheids.

Table 3.3.2.2.: Comparison of chlorine (P/B ratio) retentions of two commercial AACs with the iodo-benzalkonium chloride in P. *ponderosa* sapwood[a].

AAC	Cell Type (P/B Ratio)*		
	Earlywood Tracheids	**Latewood Tracheids**	**Ray Cells**
Iodo-benzalkonium Chloride	0.87 (0.12)^{Aa}	0.61 (0.11)^{Ba}	1.75 (0.23)^{C}
Benzalkonium Chloride	0.95 (0.24)^{Ab}	0.84 (0.15)^{Bb}	1.18 (0.21)^{C}
Didecyldimethylammonium Chloride	1.37 (0.29)^{Ac}	0.81 (0.06)^{B}	2.02 (0.39)^{Cc}

[a] Means are compared in columns within the element types. Means with the same capital letter are not significantly different at a 95% confidence level.
Means are compared in rows within the element types. Means with the same letter are not significantly different at a 95% confidence level.
*Values in parentheses are standard.

Earlywood and latewood tracheid retentions of iodo-benzalkonium chloride.

Results indicate that the earlywood and latewood tracheids behave quite differently in the uptake of AACs over the various concentrations used. These different capacities of the earlywood and latewood tracheids were observed by several research groups (Ruddick & Sam, 1982; and Nicholas, *et al.* 1991). SEM-EDX results confirm previous findings where the earlywood tracheids retained more of the AAC than the latewood tracheids. One possible explanation is that the amount of solution absorbed by each wood region limits the amount of the AAC available for retention into the tracheid wall. Lumen size in the various wood region would influence the amount of water uptake in each region during treatment. The amount of chemical left in the lumen after treatment will be available for absorption into the wall of the tracheids. Absorption in this case refers to the penetration of AACs into the wood cell walls. The ability for absorption and transportation of water (treating solution) may reflect the conditions that prevail in the living tree when earlywood and latewood tracheids are formed. Earlywood tracheids are formed during the early part of the year when water is plentiful resulting in thin walled, large diameter lumen tracheids. The water availability is not the only factor influencing lumen size other factors include auxin and photosynthate availability. The latewood tracheids are formed in the later part of the year when water is limiting resulting in thick walled, small diameter lumen tracheids. Therefore, in a small block where the treating solution will be at saturation, the earlywood tracheids would absorb more of the treating solution due to the larger lumen surface area.

Earlywood and latewood regions were separated from a P. *ponderosa* sapwood block and treated with distilled water to determine their absorption capacities. Table 3.3.2.3. shows that

the earlywood regions retain approximately twice the water compared with the latewood regions. Based upon these findings earlywood regions were treated with 6% iodo-benzalkonium chloride and latewood regions were treated with 12% iodo-benzalkonium chloride solution. This should provide each region with the same amount of iodo-benzalkonium chloride for absorption into the cell wall. This would determine if the AAC absorption capacities of the earlywood tracheids and latewood tracheids were different. The results of the SEM-EDX analysis and the retentions based on HPLC analysis are shown in Table 3.3.2.3. Both iodine and chlorine ratio indicate that earlywood retention was statistically greater than the latewood retention. The differences are smaller than found under normal treating conditions, suggesting that the lumen size and absorption capacities will influence the retention in the various tracheids. The estimated retentions based on solution uptake during treatment and HPLC retention analysis found that the latewood region had a higher retention than the earlywood region. One possible explanation was the AAC built up on the lumen surface and did not penetrate the cell wall in the latewood tracheids. This agrees with later observations in latewood tracheids at high treating solution concentration where cell lumen exhibited a build up of the surfactant.

Penetration of iodo-benzalkonium chloride into P. ponderosa blocks from the outer surface. Most of the analyses of iodo-benzalkonium chloride cellular distributions were carried out in samples taken from the outer 5 mm of the blocks. Previous research found that the retention of AACs near the surface of the treated wood was extremely concentrated and decreased with an increased sampling distance from the wood surface (Ruddick & Sam, 1982;

Table 3.3.2.3.: The retention of iodo-benzalkonium chloride in earlywood and latewood tracheids[a].

Properties	Earlywood Tracheids	Latewood Tracheids
Water Uptake (%)	225 ±25	87 ±7
Treating Solution (%)	6	12
Density (kg/m^3)	425	641
Estimated Retention (kg/m^3)	51	88
Actual Retention (kg/m^3)	54	71
P/B Ratio		
Iodine	2.12 ±0.43^A	1.45 ±0.21^C
Chlorine	1.29 ±0.30^B	0.95 ±0.14^D

[a] Means are compared in columns within the element types. Means with the same capital letter are not significantly different at a 95% confidence level.

Drysdale, 1983[b]; Preston, *et al.*, 1987; and Nicholas, *et al.*, 1991). Samples were removed from the inner core, 5-10 mm from the outer surface in blocks treated with 1%, 3%, 6% and 10% iodo-benzalkonium chloride solution. These sections were analyzed in the SEM-EDX for the relative iodine and chlorine retentions (P/B ratios) in earlywood and latewood tracheids. The retentions of both iodine and chlorine were reduced as the analysis region changed from 0-5 mm to 5-10 mm (Table 3.3.2.4.). The block treated with a 1% iodo-benzalkonium chloride solution showed a 50% reduction in the P/B ratio of both ions in the earlywood and latewood tracheids compared with the sample from a region closer to the surface. Retention based on HPLC analysis agreed with the changes observed in the SEM-EDX, in that the outer region (0 to 5 mm) had an iodo-benzalkonium chloride retention of 10 kg/m^3. The corresponding inner assay (5 to 10 mm) had a retention of 6 kg/m^3. At the higher

treating solution concentrations, the earlywood tracheid P/B ratios declined by ~50% in samples removed from the inner core (5-10 mm assay zone) compared with those collected from the outer 5 mm sections. This decrease in retention in the earlywood tracheids reflects the differences in the tracheids and their ability to transport water. Earlywood tracheids tend to have more aspirated pits, which are not easily reversible making earlywood tracheids less readily treated than latewood tracheids.

Previous research found that with increasing distance from the outer surface, the AAC retention declined dramatically, which agrees with the above work (Drysdale, 1983[b]; and Preston, *et al.*, 1987). However, with the exception of the block treated with 6% iodo-benzalkonium chloride, the latewood retention does not show the same level of reduction in the inner sampling zone, as that noted for the earlywood. The difference in the amount of absorption and penetration of iodo-benzalkonium chloride in the earlywood and latewood tracheids may reflect differences in their physical characteristics. During treatment, the absorption in latewood tracheids appears to be more uniform within the wood sample, due to its ability to transport the treating solution further into the sample and its lower frequency of aspirated pits. The chlorine retention in the latewood tracheids also confirmed that at the lower concentrations (1% & 3%) the amount of ion exchange increased with distance from the surface. This increase most probably resulted from a dilution of the treating solution as it penetrated the wood block. The AAC treating solution concentration may the fall below the CMC of iodo-benzalkonium chloride, so that ion exchange adsorption was observed.

The small blocks (19 x 19 x 19 mm) used in the above experiments would tend to be saturated with treating solution and may not reflect the distribution at a cellular level in a

Table 3.3.2.4.: The penetration of iodo-benzalkonium chloride in the tracheids of the small P. *ponderosa* sapwood blocks[a].

Element	Treating Solution	Sample Location (mm)	Tracheid Type (P/B Ratio)* Earlywood Tracheids	Latewood Tracheids
Iodine	1%	0-5	0.79 (0.12)^{Aa}	0.78 (0.11)^{Ca}
		5-10	0.29 (0.10)^{Bb1}	0.34 (0.09)^{Cb}
	3%	0-5	0.97 (0.06)^{Dc}	0.78 (0.15)^{Ec}
		5-10	0.42 (0.21)^{Dd}	0.68 (0.12)^{Ed}
	6%	0-5	3.09 (1.09)^{Fe}	3.17 (0.79)^{He}
		5-10	1.56 (0.21)^{Gf}	1.47 (0.06)^{If}
	10%	0-5	4.02 (0.68)^{K}	1.96 (0.31)^{M}
		5-10	1.87 (0.58)^{Lg}	1.66 (0.15)^{Mg}
Chlorine	1%	0-5	0.49 (0.08)^{Aa}	0.13 (0.06)^{Ca1}
		5-10	0.07 (0.03)^{Bb1}	0.11 (0.01)^{Db}
	3%	0-5	0.87 (0.12)^{Ec}	0.61 (0.11)^{Gc}
		5-10	0.33 (0.22)^{Fd}	0.29 (0.06)^{Hd1}
	6%	0-5	1.96 (0.44)^{Ie}	2.15 (0.25)^{Je}
		5-10	1.01 (0.10)^{If}	0.99 (0.07)^{Kf}
	10%	0-5	2.32 (0.16)^{L}	1.29 (0.19)^{N}
		5-10	1.33 (0.16)^{Mg}	1.44 (0.12)^{Og}

[1] P/B ratio less than 0.3 indicates the amount of element present is below the limits of the detector

[a]Means are compared in columns within the element types. Means with the same capital letter are not significantly different at a 95% confidence level.

Means are compared in rows within the element types. Means with the same letter are not significantly different at a 95% confidence level.

* Values in parentheses are standard deviations.

larger block. Therefore, a larger block with dimensions of 30 x 65 x 115 mm was treated with 5% iodo-benzalkonium chloride and analyzed to determine if there was any major difference from the small block. Samples were removed from the outer 5 mm close to the

end grain (cross) and the mid point from the long dimension (mid). At the mid point assay zones of 5-10 mm, 10-15 mm and 20-30 mm from the surface were taken. The results of the analysis are shown in Table 3.3.2.5. and Figure 3.3.2.4. The results showed that the samples from the small block had a higher relative retention of iodine and chlorine in the earlywood tracheids. While, latewood tracheid differences are not so clear and statistical analysis does show a slight decline in iodine retentions for the samples taken from the outer 5 mm assay zone of the small and large blocks. HPLC analysis of these regions showed that the small sapwood block retained more than twice (50 kg/m^3) the iodo-benzalkonium chloride measured in the same outer 5 mm in the large block (22 kg/m^3). These retentions reflect the influence of surface area to volume ratio on the ability of the wood sample to absorb the AACs from the treating solution. This can be seen in retention differences observed by Nicholas, *et al.* (1991) where two samples with different dimension were used. Comparison of the tracheids taken from the outer 5 mm in the cross section and mid section showed the similar P/B ratio for both iodine and chlorine in latewood tracheids. Earlywood tracheids shows a drop in the iodine and chlorine values in the outer 5 mm sections from the cross and mid sections. Samples taken from the mid point at different depths showed a dramatic change in the relative earlywood retentions of iodine and chlorine. As the assay zone moves away from the outer surface the amount of iodine declined rapidly (Figure 3.3.2.4.). The 5-10 mm assay zone and beyond showed iodine and chlorine P/B ratios in the earlywood tracheids below the EDX detector limit. The corresponding data for latewood tracheids do not show the same reduction in the P/B ratios. In fact the retention based on P/B ratios of the iodine and chlorine remained fairly constant. A small decline occurs in samples

from the 5-10 mm assay zone and beyond. These results confirm the suggestion that the latewood tracheids have a greater capacity to transport the treating solution further into the sample providing a more uniform distribution of the AAC in the latewood regions. HPLC

Table 3.3.2.5.: Tracheid response due to different block sizes and sample location[a].

					Cell Type (P/B Ratio)*	
Element	Retention (kg/m^3)	Block size (mm)	Section	Depth (mm)	Earlywood Tracheids	Latewood Tracheids
Iodine						
	50	19x19x19	Cross	0-5	2.91 (0.45)^{A}	1.34 (0.15)^{E}
	-	30x65x115	Cross	0-5	1.31 (0.06)^{Ba}	1.06 (0.07)^{Fa}
	22	30x65x115	Mid	0-5	0.84 (0.28)^{Cb}	1.12 (0.32)^{Eb}
	15	30x65x115	Mid	5-10	0.16 (0.12)^{D1}	0.75 (0.30)^{F}
	15	30x65x115	Mid	10-20	0.16 (0.20)^{D1}	0.88 (0.33)^{F}
	16	30x65x115	Mid	20-30	-0.03 (0.00)^{D1}	0.94 (0.29)^{F}
Chlorine						
	50	19x19x19	Cross	0-5	1.72 (0.20)^{A}	1.03 (0.13)^{F}
	-	30x65x115	Cross	0-5	1.06 (0.11)^{B}	0.70 (0.10)^{G}
	22	30x65x115	Mid	0-5	0.49 (0.16)^{Ca}	0.63 (0.16)^{Ga}
	15	30x65x115	Mid	5-10	0.25 (0.07)^{Db1}	0.45 (0.19)^{Hb}
	15	30x65x115	Mid	10-20	0.19 (0.08)^{Ec1}	0.48 (0.18)^{Hc}
	16	30x65x115	Mid	20-30	0.15 (0.05)^{E1}	0.38 (0.13)^{H}

[1] P/B ratio less than 0.3 indicates the amount of element present is below the limits of the detector

[a] Means are compared in columns within the element types. Means with the same capital letter are not significantly different at a 95% confidence level.

Means are compared in rows within the element types. Means with the same letter are not significantly different at a 95% confidence level.

*Values in parentheses are standard deviation.

analysis of the samples showed that the retentions in the various samples showed an initial decline from 22 kg/m^3 to ~15 kg/m^3 after which it remained constant. A retention of 15

kg/m^3 should be sufficient to protect the wood against decay. However, cellular analyses show that the earlywood tracheids have almost no AAC and could be vulnerable to attack by decay organisms. These results agree with finding by Nicholas, *et al.* (1991) that earlywood retention reduced with distance from the wood surface. Earlywood tracheids retention of iodo-benzalkonium chloride rapidly decreased as the sections being assayed were more distant from the outer surface (Figure 3.3.2.4.). Latewood tracheids showed an even distribution of iodo-benzalkonium chloride throughout the large block. This rapid reduction in earlywood retention may be of concern with respect to protection of the wood during field exposure.

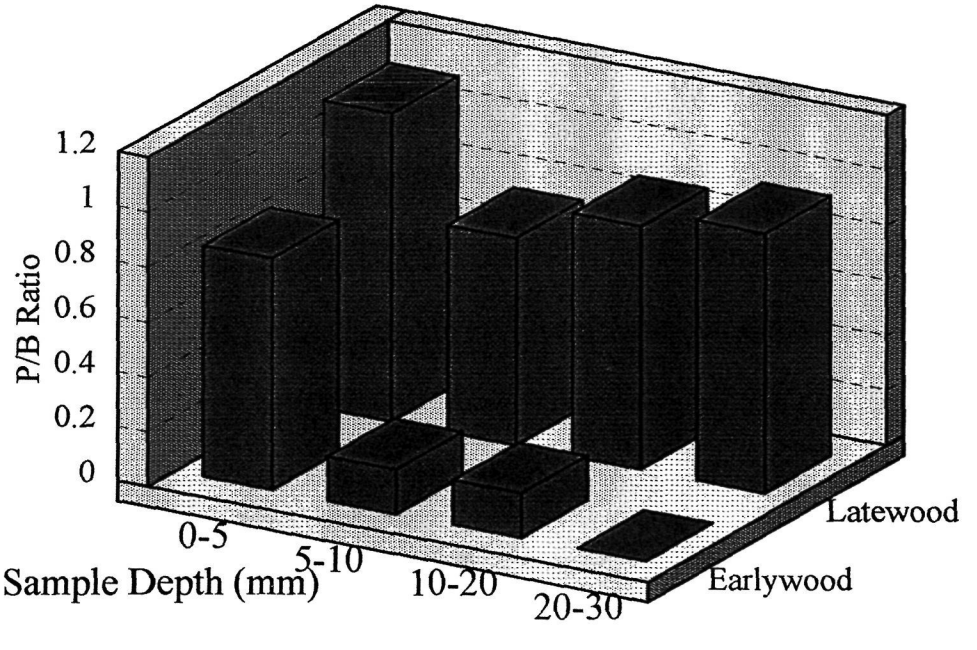


Figure 3.3.2.4: Cationic penetration of iodo-benzalkonium chloride in earlywood and latewood tracheids of a large sapwood block.

The influence of soaking on iodo-benzalkonium chloride in the tracheids.

Traditionally samples studied using the SEM are soaked prior to microtoming to improve the quality of the sections. Pre-soaking of the AAC treated samples for EDX analysis could result in either leaching or redistribution of the preservative, resulting in an inaccurate distribution pattern being recorded (Ryan , 1986). Microtoming the samples when dry produced poor quality sections with surfaces that were uneven or torn and left debris on the cell wall surface. However, the chemical distribution should remain undisturbed. A matched set of samples removed from the same block treated with 10% iodo-benzalkonium chloride was used to study the effect soaking on the preservative distribution. Two end matched sections were prepared with one of the sections being prepared dry, while the other section was soaked in distilled/deionized water for 12 hours before microtoming. The relative mobility of iodine labeled cation and chlorine anion will be identified when comparing the two end matched sections. The results are shown in Table 3.3.2.6 and Figure 3.3.2.5. Earlywood and latewood tracheids showed different responses to soaking. The earlywood tracheids lost 46% of iodine (cation) and chlorine (anion) retentions during the twelve hour soak. Latewood tracheids showed a very small loss in the iodine containing cation and a 46% reduction the chloride anion, the latter being similar to the earlywood tracheids.

Table 3.3.2.6.: The mobility of the cation and anion of iodo-benzalkonium chloride during a 12 hour soak[a].

Element	Soaking Period	Tracheid Type (P/B Ratio)*	
		Earlywood	Latewood
Iodine	-	4.13 (0.60)^{A}	2.95 (0.63)^{C}
	12 hours	2.32 (0.12)^{Ba}	2.15 (0.11)^{Da}
Chlorine	-	2.36 (0.29)^{A}	1.83 (0.23)^{C}
	12 hours	1.15 (0.14)^{Ba}	1.00 (0.09)^{Da}

[a] Means are compared in columns within the element types. Means with the same capital letter are not significantly different at a 95% confidence level.
Means are compared in rows within the element types. Means with the same letter are not significantly different at a 95% confidence level.
*Values in parentheses are standard deviations.

These results indicated that in the latewood tracheids, the iodo-benzalkonium chloride absorption was less prone to leaching than that absorbed in the earlywood tracheids. These results agree with the findings of Nicholas, *et al.*, (1991) where the earlywood regions leached more chemical than the latewood regions. HPLC determination showed that the actual retention decreased by at least 50% after the 12 hour soak, going from 85 kg/m^3 to 37 kg/m^3. The decline in the retention of AACs occurred mostly in the earlywood regions, but only to a limited extent in the latewood tracheids. Soaking of the sections appears to bring all the iodine and chlorine P/B ratios to a similar value. This suggests that there are a fixed number of adsorption sites to which the iodo-benzalkonium chloride can be fixed in a manner resistant to water leaching. This agrees with Vinden's (1984) conclusions on radiata pine adsorption of AACs. The rapid drop of AACs in the earlywood observed combined with

the shallow penetration, may prove to be vital in the poor field performance of the AAC treated wood.

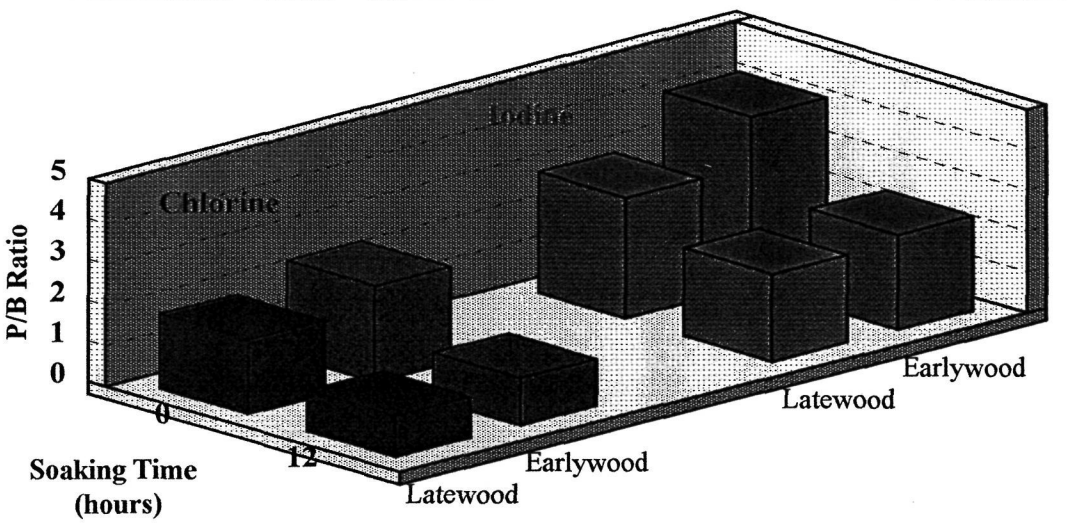


Figure 3.3.2.5: Permanency of iodine and chlorine in the earlywood and Latewood tracheids during a 12 hour soak.

The influence of solution pH on iodo-benzalkonium distribution

Previous research found that the treating solution pH plays a significant role in the distribution, retention and performance of AACs in the wood (Butcher & Drysdale, 1978[b]; Preston *et al.*, 1987; and Jin & Preston, 1991). Small pine sapwood blocks were treated with 1% and 3% iodo-benzalkonium chloride, with the solution pH adjusted by the addition of acetic acid or sodium carbonate. The influence of solution pH on the retention of the iodine (cation) and chlorine (anion) is presented in Table 3.3.2.7.

Reducing the pH by the addition of acetic acid did not have any affect on retention of either ion (Figure 3.3.2.6.) compared with the unbuffered treating solutions. The pH of the acetic acid modified treating solutions was approximately 2.7. Wood has an ability to buffer the solution pH to acid conditions with the lower concentrations of iodo-benzalkonium chloride. The unbuffered pH value of the 1% iodo-benzalkonium chloride treating solution with the wood was 3.7, while the 3% solution was 4.5. The buffering capacity of the wood results in slightly acid conditions in the 1% and 3% treating solution. This is reflected in the minor differences observed between the acetic acid modified treating solution and the unbuffered solution retention of iodine. These results agree with findings by Preston, *et al.* (1987) where changes in treating solution pH between 2-4 showed little change in adsorption of AACs. Increasing the iodo-benzalkonium chloride concentration overcame the buffering capacity of the wood with the pH tending to neutral. Increasing the treating solution pH by the addition of sodium carbonate resulted in higher retention of iodine (cation) in almost all of the cell types (Table 3.3.2.7.). The increase in iodine uptake was the most obvious at 3% (Figure 3.3.2.6.). Chlorine retentions were unaffected by treating solution pH at 1%. The retention of chlorine at the 3% treatment level increased by the addition of sodium carbonate in the earlywood and latewood tracheids. At 10% the addition of sodium carbonate did not affect the P/B ratios observed in the earlywood tracheids, but increased the P/B ratios of both ions in the latewood tracheids (Table 3.3.2.7.). HPLC analysis of AACs in the wood treated with 1% and 3% found that under acidic condition the retentions of iodo-benzalkonium chloride were 8 kg/m^3 and 17 kg/m^3, compared with 10 kg/m^3 and 25 kg/m^3 for wood impregnated with the corresponding unbuffered solutions. The retentions from the samples treated with an

Table 3.3.2.7.: The effect of treating solution pH on the retention of iodine and chlorine in P. *ponderosa* sapwood.[a]

Element	Concentration	Treating pH	Cell Type (P/B Ratio)*		
			Ray Cells	Earlywood Tracheids	Latewood Tracheids
Iodine					
	1%	unbuffered	2.75(0.78)^{Aa}	0.79(0.12)^{Ea}	0.78(0.11$^{)IKa}$
		acid	1.92(063)^{AC}	0.64(0.15)^{Eb}	0.69(0.07)^{Ib}
		alkaline	2.30(0.64)^{A}	1.24(0.22)^{F}	0.99(0.15)^{J}
	3%	unbuffered	2.08(1.00)^{B}	0.98(0.06)G	0.78(0.15)^{K}
		acid	2.00(0.29)^{C}	1.13(0.23)Gc	1.03(0.11)^{Lc}
		alkaline	4.30(1.08)^{D}	2.27(0.35)^{Hd}	2.14(0.50)^{Md}
	10%	unbuffered	-	4.02 (0.68)^{N}	1.96 (0.31)^{O}
		alkaline	-	3.53 (1.14)^{Ne}	3.22 (1.16)^{Pe}
Chlorine					
	1%	unbuffered	0.34(0.13)^{Aa}	0.49(0.08)^{F}	0.43(0.06)^{Ia}
		acid	0.55(0.19)^{Bb}	0.46(0.14)^{Fb}	0.38(0.07)^{I}
		alkaline	0.23(0.12)^{A1}	0.42(0.02)^{Fc}	0.50(0.09)^{Jc}
	3%	unbuffered	1.75(0.23)^{C}	0.87(0.12)^{G}	0.61(0.11)^{K}
		acid	0.48(0.09)^{D}	0.99(0.12)^{G}	0.79(0.07)^{L}
		alkaline	1.31(0.19)^{Ed}	1.22(0.04)^{Hd}	1.82(0.10)^{M}
	10%	unbuffered	-	2.32 (0.16)^{N}	1.29 (0.19)^{O}
		alkaline	-	2.56 (0.50)^{Ne}	1.98 (0.64)^{Pe}

[a] Means are compared in columns within the element types. Means with the same capital letter are not significantly different at a 95% confidence level.

Means are compared in rows within the element types. Means with the same letter are not significantly different at a 95% confidence level.

[1] P/B ratio less than 0.3 indicates the amount of element present is below the limits of the detector.

*Values in parentheses are standard deviations.

alkaline buffered solution of iodo-benzalkonium chloride showed retentions of 12 kg/m^3 and 30 kg/m^3 from the 1% and 3% iodo-benzalkonium chloride solution. These results agree with SEM-EDX observations.

Treating solution pH has been shown to affect the performance and retention of DDAC (Butcher and Drysdale, 1978[b]; and Preston, *et al.*, 1987). The influence of pH on the retention of iodine and chlorine can be clearly seen in Figure 3.3.2.6. Increasing the solution pH with sodium carbonate results in increased iodine retention in almost all three cell types at 1% and 3% iodo-benzalkonium chloride (Table 3.3.2.7.). This observation is consistent with previous findings that increasing the pH of the treating solution resulted in an increased retention (Preston, *et al.* 1987). Iodine retention increases in the earlywood and latewood tracheids treated with the alkaline 1% were not matched by chlorine increases indicating that the additional cation content was retained by ion exchange. Previous research has suggested that increasing the pH of the treating solution resulted in an increased retention in the outer regions of the wood at the expense of the AAC in the inner regions (Preston, *et al.*, 1987). SEM-EDX analyses of the inner core (5-10 mm assay zone) results from the outer surface are shown in Table 3.3.2.8. The results indicated that the increased retention of iodo-benzalkonium chloride in the outer regions at high treating solution pH did not occur at the expense of AACs absorbed in the inner zones of the sample. Iodine and chlorine values were slightly higher in earlywood and latewood tracheids treated with alkaline treating solution compared with unbuffered treating solutions. These observations are more obvious at the 3% iodo-benzalkonium chloride. HPLC analyses supported the conclusion that increased retentions in the blocks treated with alkaline buffered solution, were not at the expense of the

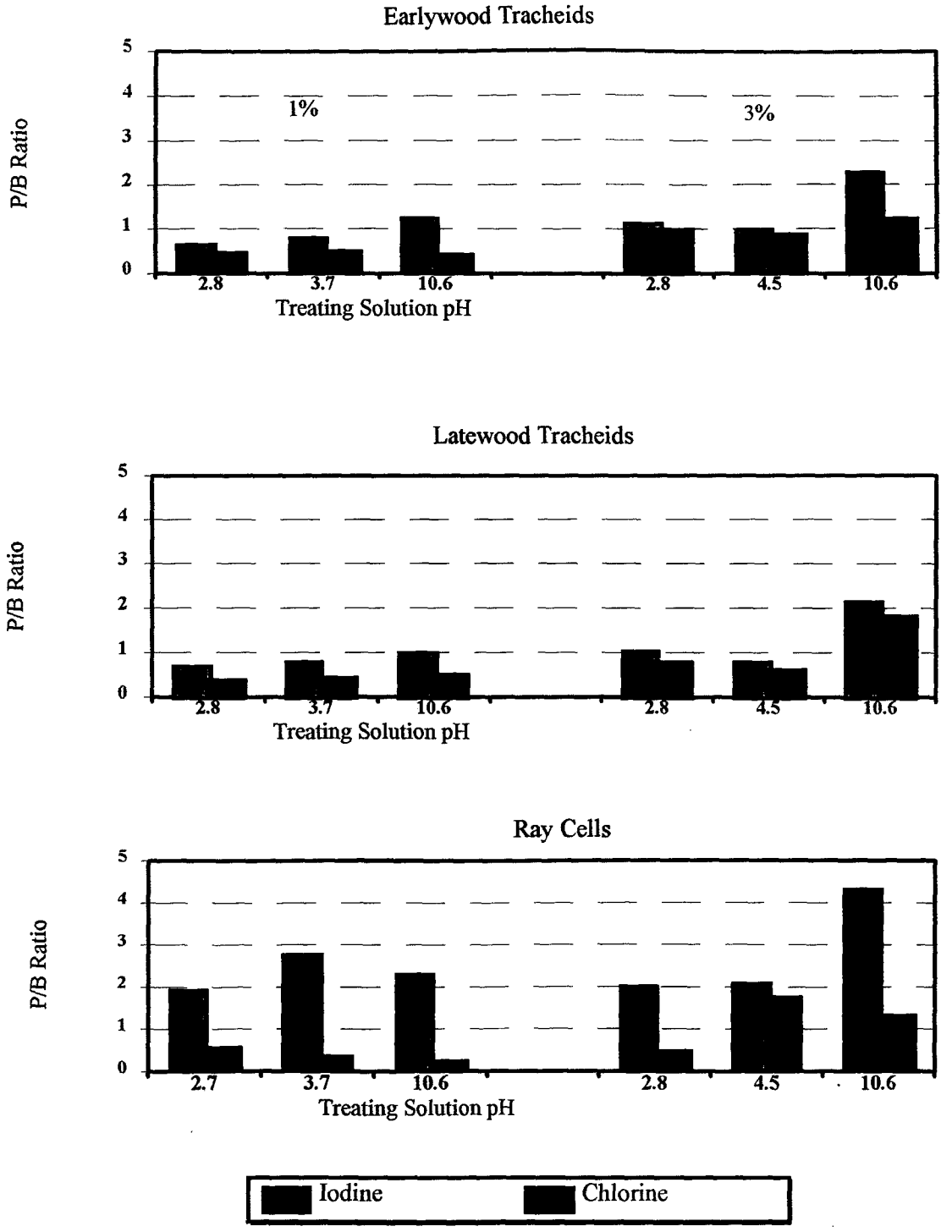


Figure 3.3.2.6.: Influence of treating solution pH on the retention of iodine and chlorine in the various cell types.

inner regions in these small P. *ponderosa* sapwood blocks. Alkaline buffered treated blocks had retentions of 5 kg/m^3 and 23 kg/m^3 compared with unbuffered treated block retentions of 6 kg/m^3 and 20 kg/m^3 in the 5-10 mm assay zone for the 1% and 3% iodo-benzalkonium chloride.

Table 3.3.2.8: Iodo-benzalkonium chloride retentions for 5-10 mm assay zone in blocks treated with alkaline amended and unbuffered treating solutions.[a]

Element	Treating Solution	Solution Buffer	Tracheid Type (P/B Ratio)*	
			Earlywood Tracheids	Latewood Tracheids
Iodine	1%	Alkaline Buffer	0.36 (0.14)^{Aa}	0.44 (0.45)^{Ba}
		Unbuffered	0.29 (0.10)^{Ab1}	0.34 (0.09)^{Bb}
	3%	Alkaline Buffer	0.71 (0.46)^{Ac}	0.82 (0.34)^{Bc}
		Unbuffered	0.42 (0.21)^{Ad}	0.68 (0.12)^{Bd}
Chlorine	1%	Alkaline Buffer	0.20 (0.18)^{Aa1}	0.11 (0.1)^{Ba1}
		Unbuffered	0.07 (0.03)^{Ab1}	0.11 (0.1)^{Bb1}
	3%	Alkaline Buffer	0.34 (0.03)^{Ac}	0.48 (0.03)^{Cc}
		Unbuffered	0.33 (0.22)^{Ad}	0.29 (0.06)^{Dd1}

[a] Means are compared in columns within the element types. Means with the same capital letter are not significantly different at a 95% confidence level.
Means are compared in rows within the element types. Means with the same capital letter are not significantly different at a 95% confidence level.
[1] P/B ratio less than 0.3 indicates the amount of element present is below the limits of the detector
*Values in parentheses are standard deviations.

Increasing the pH of the 10% treating solution did not increase the iodine or chlorine P/B ratios in the earlywood tracheids. However, the retention of both ions was increased in the latewood tracheids (Table 3.3.2.7.). This suggested that the earlywood tracheids had reached their maximum capacity for the AAC absorption under all pH treating conditions. The alkaline conditions increased retentions in the latewood tracheids to values similar to adsorption at 6% iodo-benzalkonium chloride.

The results of this study also indicated that 1% and 3% interacted quite differently with the wood substrate. At 1% the iodine retentions in the earlywood and latewood tracheids were increased by the addition of sodium carbonate. However, the chloride retentions remained unaffected. These results suggest that ion exchange was more noticeable at 1%. An increase in pH will result in higher cation retentions, as the sodium carbonate would create more negative sites on the wood, to which the iodine cation could be bound (Rosen, 1975). However, the presence of chlorine indicates ion exchange was not the sole mechanism for fixation, consistent with unpublished data on proton exchange accounting for 13% of the DDAC retained in wood sawdust using a 1% solution (Ruddick and Doyle, 1992).

The influence of copper on the retention and distribution of iodo-benzalkonium chloride.

Many researchers have developed preservative formulations containing an AAC together with a cobiocide to improve their performance. The most common cobiocides used have been copper salts (Butcher, Preston & Drysdale 1979; Drysdale, 1983[a]; and Tsunoda & Nishimoto, 1987[b]). The ammonical copper modified formulations of AACs prove to be one

of the most successful candidates to date (Wallace, 1986; and Sundman, 1984). The effect of copper on the distribution of AACs within the wood has not been determined. In this study,

Table 3.3.2.9.: The interaction of copper and alkylammonium compounds in P. *ponderosa* sapwood[a].

Treatment	Retention kg/m^3		P/B Ratio*		
	Cu	AAC	Iodine	Chlorine	Copper
Ray Cells					
Amm Cu	16	-	-	-	3.50 (2.34)^{Aa}
Amm Iodo-AAC	-	27	1.78 (0.60)^{Aa}	1.02 (0.08)^{Aa}	-
Amm Iodo-AAC Cu	15	27	2.13 (0.54)^{Ab}	0.64 (0.10)^{Bb}	2.67 (1.03)^{Ab}
Amm AAC Cu	15		-	-	-
Earlywood Tracheids					
Amm Cu	16	-	-	-	4.52 (0.59)^{Ba}
Amm Iodo-AAC	-	27	1.49 (0.35)^{Ba}	1.12 (0.24)^{Ca}	-
Amm Iodo-AAC Cu	15	27	1.56 (0.23)^{Bb}	0.67 (0.08)^{Db}	2.74 (0.45)^{BCb}
Amm AAC Cu	15		-	1.19 (0.21)^{Cc}	5.63 (0.94)^{Cc}
Latewood Tracheids					
Amm Cu	16	-	-	-	3.97 (0.32)^{Da}
Amm Iodo-AAC	-	27	0.78 (0.30)^{C}	0.37 (0.65)^{Ea}	-
Amm Iodo-AAC Cu	15	27	0.77 (0.36)^{C}	0.75 (0.12)^{Fb}	3.50 (0.34)^{Db}
Amm AAC Cu	15		-	1.00 (0.11)^{Fc}	4.43 (1.48)^{Ec}

[a] Means are compared in columns within the element types. Means with the same capital letter are not significantly different at a 95% confidence level.

Means are compared in columns within the treatments. Means with the same letter are not significantly different at a 95% confidence level.

*Values in parentheses are standard deviations.

P. *ponderosa* sapwood blocks were treated with an ammonical copper treating solution, ammonical iodo-benzalkonium chloride treating solution and the combination of both biocides. The iodine, chlorine and copper distribution in all three cell types are shown in Table 3.3.2.9. The analysis indicates that when the iodine, copper and chlorine are present, inter-element interference occurs which reduces copper and chlorine P/B ratio (Goodhew & Humphreys, 1988). However, upon the addition of copper to an AAC without the iodo-label AAC there is no change in the copper P/B ratios observed compared with the samples without the AAC present. Copper distribution under ammonical conditions showed an even distribution in all three cell types (Table 3.3.2.9.). The distribution of the iodo-benzalkonium chloride under ammonical conditions showed the expected pattern where ray cells retained the highest amount of iodine and chlorine, with smaller P/B ratios recorded for the earlywood and latewood tracheids. The results are slightly higher than the P/B ratios observed in wood impregnated with an unbuffered treating solution (Table 3.3.2.7.). The higher retention resulted from the increase in treating solution pH due to the presence of ammonia. The treatment containing copper and the iodo-labeled AAC solution did not alter the distribution patterns of either copper or iodine in the various cells. Secondly, measured retentions of either the AAC or the copper remained constant. These results suggested that the AAC and copper did not displace the total cellular retention of each biocide. Spot analyses of these tracheids suggested that the copper did not displace the AAC from the compound middle lamella. This would agree with the theory that AACs bind preferably to the lignin, while copper can complex with the cellulose or ion exchange with carboxylic acid

groups on the lignin or the hemicellulose (Jin & Preston, 1991). The distribution of copper and AAC appears to be more even, giving better protection to the wood than the AAC alone.

The retention of iodo-benzalkonium in two different hardwood species.

The distribution of CCA showed species to species variation and major difference between hardwoods and softwoods (Levy & Greaves, 1978). Quaking aspen and yellow birch were treated with 3% iodo-benzalkonium chloride solution and analyzed in the SEM-EDX. The results are shown in Table 3.3.2.10. Iodo-benzalkonium chloride distribution in the two

Table 3.3.2.10.: The distribution of iodo-benzalkonium chloride in hardwoods[a].

Wood Species	Cell Type (P/B Ratio)*		
	Ray Cells	**Vessels**	**Fibres**
Iodine			
Aspen	1.81 (0.30)^{Aa}	2.60 (0.22)^{Ba}	2.21 (0.07)^{Da}
Birch	1.86 (0.26)^{Ab}	2.11 (0.15)^{Cbc}	2.13 (0.07)^{Dc}
[Pine]**	[2.08 (1.00)]	[0.98 (0.06)]	[0.78 (0.15)]
Chlorine			
Aspen	1.50 (0.19)^{A}	1.81 (0.13)B	1.83 (0.05)^{D}
Birch	1.45 (0.11)^{Ab}	1.48 (0.08)Ca	1.59 (0.04)^{Ea}
[Pine]**	[1.75 (0.24)]	[0.87 (0.12)]	[0.61 (0.11)]

[a] Means are compared in columns within the element types. Means with the same capital letter are not significantly different at a 95% confidence level.
Means are compared in rows within the element types. Means with the same letter are not significantly different at a 95% confidence level.
*Values in parentheses are standard deviations.
**Included for comparison purposes.

hardwood species showed uniform distribution in all three cell types. These results indicate different preservative penetration pathways in the hardwood. These results show that hardwood have a more even cellular AAC distribution compared with softwoods, agree with research on CCA distribution in hardwoods by Levy and Greaves (1978). The structure of the hardwood results in a more even distribution and a higher retention than that observed in softwoods with the same treating concentration. The variation in the chlorine P/B ratios reflected that of the iodine, which supports the concept that ion exchange is not the sole mechanism for adsorption of iodo-benzalkonium chloride in the two hardwoods. Both hardwood species used show very similar P/B ratios of iodine and chlorine in all three cell types.

Distribution of ions across tracheid cell walls.

Iodine and chlorine X-ray line scans across adjacent cell walls were performed on samples treated with each solution concentration. For most treatments, chlorine showed a uniform distribution across the two tracheid cell walls (Figure 3.3.2.7). Iodine line scans also showed uniform distribution at the lower retentions, but at higher concentrations the accumulation of iodine occurred in the compound middle lamella. This build up of iodine in the compound middle lamella was particularly noticeable when the tracheids were treated with the 10% solution (Figure 3.3.2.8.). Latewood tracheids also showed an increased iodine retention in the secondary wall near the lumens in samples treated with a 13% solution (Figure 3.3.2.9.). The 3% treating solution was adjusted with sodium carbonate enhanced accumulation of iodine in the compound middle lamella region in earlywood tracheids.

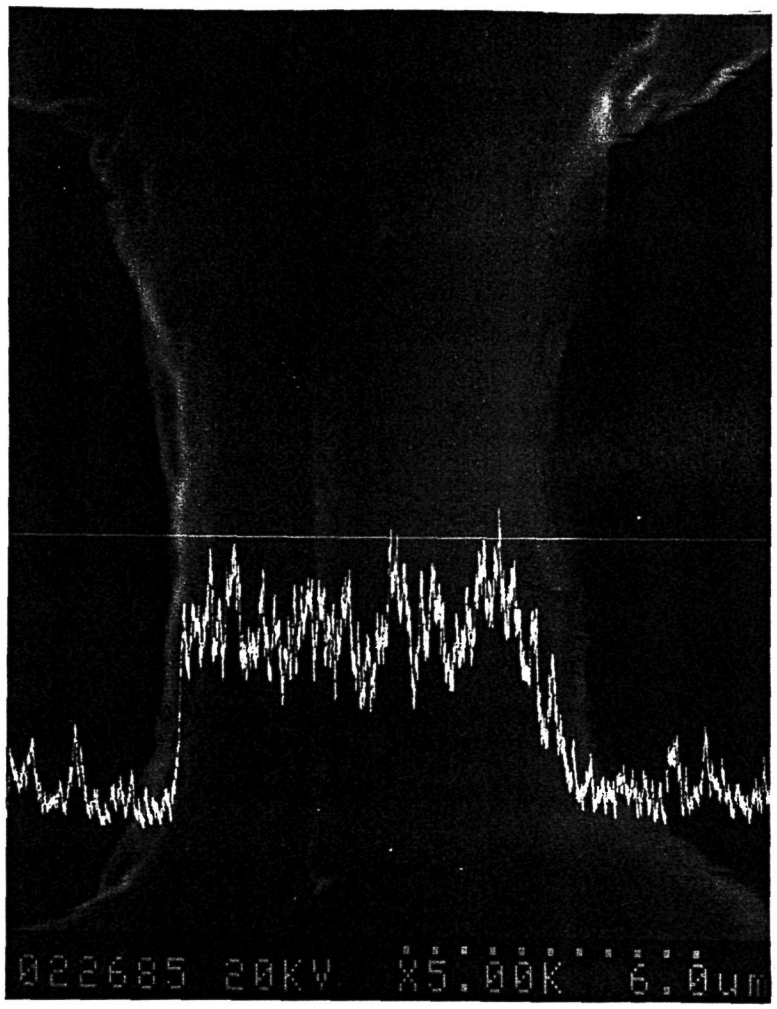


Figure 3.3.2.7.: Chlorine distribution across adjacent earlywood tracheids treated with 10% iodo-benzalkonium chloride.

To confirm these findings spot analyses of the compound middle lamella (CML) and secondary (S) wall were carried out as described in chapter 3.2.2., the results of which are

presented in Table 3.3.2.11. At higher treating solution concentrations (6%, 10% and 13%) earlywood tracheids showed higher P/B ratios for iodine in the compound middle lamella than in the secondary wall. This was also observed in the corresponding latewood, except for the sample treated at the highest concentration, where the greater amounts of iodine were found in the secondary wall next to the lumen.

Chlorine P/B ratios did not indicate any difference between the two cellular regions when treated with an unbuffered treating solution (Table 3.3.2.11.). Under alkaline conditions for the sample treated with 10% iodo-benzalkonium chloride the chlorine retentions were higher in the compound middle lamella of the latewood tracheids.

The line scans of iodine across a double cell wall shows accumulation in the compound middle lamella region (Figure 3.3.2.8.). The distribution patterns of iodo-benzalkonium chloride show similarities to that of lignin distribution (Saka & Goring, 1988), confirming that lignin plays an important role in the fixation of AACs, as suggested recently in studied involving model compounds to simulate wood component reactions with DDAC (Jin & Preston, 1991; Ruddick & Doyle, 1992). The build up of iodine in the compound middle lamella did not appear in tracheids under normal treating conditions until the solution reached 6 % (Table 3.3.2.11.). This may suggest that insufficient chemical is present at the lower concentrations to produce a higher retention in the compound middle lamella. Alternatively the buffering capacity of the wood treated with lower solution strengths may prevent the lignin from reacting with the chemical.

The latewood line scans also showed a large deposit in the secondary walls near the lumen indicating that the available surface area maybe a limiting factor in the retention of iodo-

benzalkonium chloride in the wood (Figure 3.3.2.9). This would be supported by higher levels of iodine in the secondary cell wall region of the sample treated with the highest concentration of the AAC. Under alkaline conditions earlywood tracheids showed an accumulation of iodine in the compound middle lamella region at 3% (Table 3.3.2.11.). This is consistent with recent observations using lignin model compounds (Jin & Preston, 1991). Increases in iodine P/B ratios in the compound middle lamella region were not accompanied by similar increases in the chloride P/B ratios suggesting that the cation was being adsorbed by ion exchange on the lignin. Latewood tracheids under alkaline conditions showed higher levels of chlorine in the middle lamella region, which suggested that the earlywood and latewood tracheids AAC adsorption mechanisms were different.

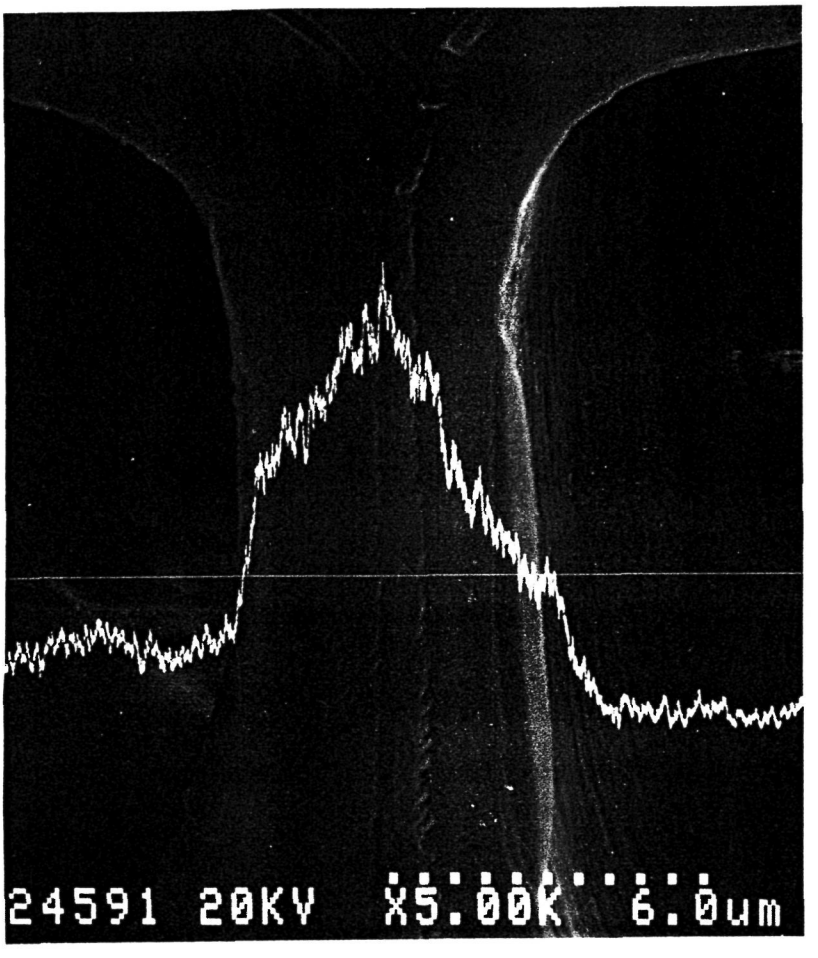


Figure 3.3.2.8.: Iodine distribution across adjacent earlywood tracheids treated with 10% iodo-benzalkonium chloride.

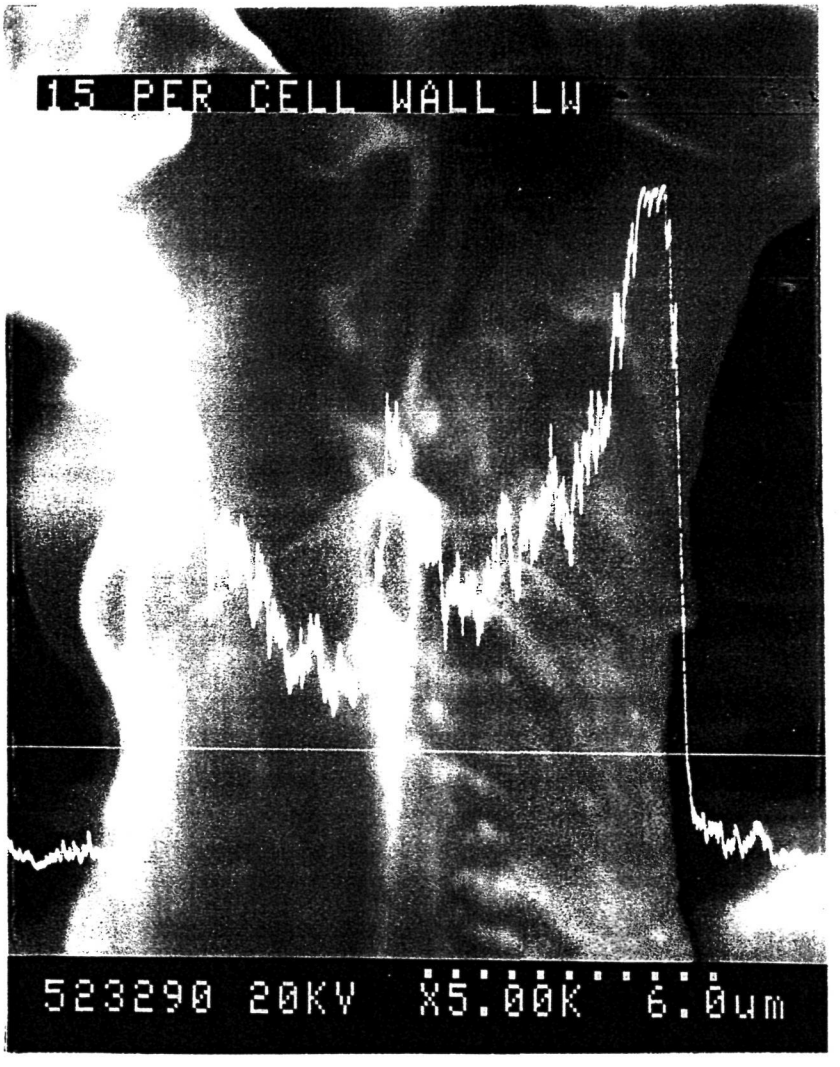


Figure 3.3.2.9.: Iodine distribution across adjacent latewood tracheids treated with 13% iodo-benzalkonium chloride.

Table 3.3.2.11.: The response of various cell wall regions to the treatment with iodo-benzalkonium chloride.

Element	Concentration	Treating pH	Tracheid (P/B Ratio) Earlywood CML*	Earlywood S**	Latewood CML*	Latewood S**
Iodine						
	1%	unbuffered	0.9	0.9	0.8	0.9
	3%	unbuffered	1.2	1.1	1.2	0.9
	3%	acidic	1.3	1.5	-	-
	3%	alkaline	2.4	1.8	1.6	1.2
	5%	unbuffered	2.3	2.6	1.4	1.7
	6%	unbuffered	4.8	3.9	4.0	3.0
	10%	unbuffered	5.5	3.6	3.4	1.5
	10%	alkaline	3.3	2.6	5.4	3.9
	13%	unbuffered	5.5	4.1	1.6	2.2
Chlorine	1%	unbuffered	0.4	0.5	0.4	0.7
	3%	unbuffered	0.9	0.9	0.6	0.8
	3%	acidic	1.3	1.4	-	-
	3%	alkaline	0.9	1.0	1.5	1.0
	5%	unbuffered	1.6	1.7	1.3	1.7
	6%	unbuffered	2.6	2.8	2.9	2.7
	10%	unbuffered	2.5	2.6	2.0	2.3
	10%	alkaline	2.0	2.1	2.7	2.4
	13%	unbuffered	2.7	2.3	1.4	1.6

*CML - Compound Middle Lamella
**S - Secondary wall region.

3.3.3. Mobility of DDAC in P. *ponderosa* Sapwood.

Mobility of the AAC in the wood can involve the bulk movement of chemical, which can be either be released from the wood or redistributed. The depletion of AACs from the wood can either be attributed to a physical process referred to as leaching or a complex process involving other factors. Results of leaching studies of AACs from the wood are conflicting, suggesting that the reason behind the failure of AAC-treated wood is a complex process. Leachability of AACs from treated wood has been the focus of many studies in the last 10 years. Initial research using water as a leaching medium found that AACs leaching was low and independent of the type of aqueous leaching solution used (Ruddick & Sam, 1982; Sundman, 1984; Nicholas, *et al.*, 1991; and Ruddick & Lum, 1991). Two factors identified as influencing water leaching of AACs were the surface area to volume ratio of the wood sample used and the initial retention of the AAC in the wood (Ruddick & Sam, 1982; and Loubinoux, *et al.*, 1992). Later research found that the AAC leaching occurred preferentially from the outer 2 mm, in the order of 30% of the initial concentration (Drysdale, 1983[a]). In recent years a new type of leaching technique has been investigated, namely the use of soil as a leaching medium. This resulted in greater loss of AACs from the wood compared to water alone (Jin & Archer, 1991). Comparisons of two AACs depletion studies found that wood exposed to sterile soil studies indicated a loss of 22% to 40%, while unsterile soils studies recorded losses of 40-70% AAC (Ruddick & Lum, 1991). A study by Nicholas, *et al.* (1991) found no significant difference between sterile and unsterile soil leaching. The leaching of AACs from the earlywood and latewood regions was found to be different, with a decrease

of 36% and 26% respectively (Nicholas, *et al.*, 1991). This agrees with results observed in the previous chapter on the loss of iodo-benzalkonium chloride after a 12 hour soak. A study by Ruddick and Lum (1991) using stakes with dimensions of 3 x 50 x 250 mm with retentions of 5.3 kg/m^3 and 9 kg/m^3 DDAC found 40% and 47% leaching over a 14 week exposure. While work by Nicholas, *et al.* (1991) found wafers of 2 x 25 x 150 mm dimensions of earlywood and latewood with DDAC retentions of ~7 kg/m^3 and ~9 kg/m^3 over 12 weeks, lost 35% and 26% respectively. These results suggest that there is a difference between the sterile and unsterile soil leaching abilities.

The level of AACs leaching from wood depends on the concentration of the preservative, sample size, wood species, the duration of the test and the type of medium used. The leaching of preservatives from wood exposed to natural field conditions can also be influenced through the wood contacting the soil.

A mobility study was initiated to determine the maximum loss of DDAC from mini stakes with high surface area to volume ratio, over an extended exposure period to a flooded unsterile soil bed.

Soil leaching of DDAC over twenty-six month period.

The moisture content and weight loss of the mini stakes were monitored during the twenty-six month soil bed exposure. The results are shown in Table 3.3.3.1. The DDAC treated wood weight loss and moisture contents did not show any significant difference from the untreated wood. The moisture conditions within the wood would prevent the growth of basidiomycetes, which are sensitive to high moisture content (Eaton & Hale, 1993). Ponding

is a method used to prevent deterioration by insects and fungi by limiting the oxygen availability.

Table 3.3.3.1: Weight loss and moisture content changes from mini stakes treated with 1% and 3% DDAC solutions, over the 26 month period in a flooded soil bed.

Exposure Period	Weight Loss (%)	Moisture Content (%)
1% Solution		
3 months	2.2%	145%
13 months	5.1%	187%
22 months	11%	247%
26 months	8%	194%
3% Solution		
3 months	1.6%	169%
13 months	5.6%	207%
22 months	9.8%	219%
26 months	10.9%	207%
Untreated		
3 months	5.0%	162%
13 months	-	-
22 months	10.6%	214%
26 months	11.9%	235%

The DDAC retentions were monitored during the twenty-six month exposure in the flooded soil bed, the results are shown in Table 3.3.3.2. The estimated retention based on treating solution uptake and the retention measured in the reference samples were significantly

different. The mini stakes treated with 1% DDAC solution had 3 to 4 kg/m^3 of DDAC greater retention than estimated. However, the estimated retention based on solution uptake during treatment and the actual retention measured in the mini stakes treated with 3% DDAC, were quite different (Table 3.3.3.2.). This highlights one problem that continually occurs with the treatment of wood using AACs, namely the variability in the AAC retentions. This was also noted by Nicholas *et al.* (1991) in two sets of wood samples, treated with the same solution concentration resulting in different retentions. The wood density of the mini stakes may influence the retentions. However, the calculated average densities were 524 kg/m^3 and 502 kg/m^3 for the mini stakes treated with 1% and 3% DDAC respectively. Therefore, the density difference was not large enough to account for the variation in retention obtained in the wood samples.

The level of DDAC lost over the 26 months appear to show a steady increase from 30% to 70%, as seen in Table 3.3.3.2. The mini stakes with ~12 kg/m^3 DDAC showed a decline of 36% after three months (Table 3.3.3.2.), which was similar to that observed by Nicholas, *et al.* (1991) for earlywood with ~7 kg/m^3 DDAC. Unlike the results obtained by Nicholas, *et al.* (1991), where following the initial loss of 35% DDAC no further loss was observed after an additional 3 months, the DDAC treated mini stakes in this study continued to lose DDAC, with a 51% reduction after 13 months and a maximum of 71% after 22 months. At the lower retention of ~10 kg/m^3 DDAC, a higher initial loss of 41% was recorded which agrees with previous finding of 40% for the same soil exposure reported by Ruddick & Lum (1991). However, the level of DDAC leached continued to increase.

Table 3.3.3.2.: DDAC loss from mini stakes during flooded soil bed exposure.

Exposure Period	DDAC Retention (kg/m³)*			DDAC Loss (%) (based on actual retention)
	Estimated	Actual	After Exposure	
1% Solution				
3 months	8.2 (0.7)	11.5 (1.4)	7.4 (0.8)	36%
13 months	8.6 (0.5)	12.3 (1.0)	6 (1.0)	51%
22 months	7.3 (1.2)	11.2 (2.5)	3.3 (0.8)	71%
26 months	9 (0.5)	13.5 (1.2)	5.4 (1.2)	60%
3% Solution				
3 months	26.4 (2.1)	9.9 (1.4)	5.8 (0.5)	41%
13 months	26 (3.0)	9.2 (1.2)	4.5 (1.3)	51%
22 months	27.3 (1.3)	10.3 (0.7)	3.6 (0.4)	65%
26 months	27 (0.8)	10.6 (1.0)	4 (1.2)	62%

*Values in parenthesis are standard deviations.

The amount of DDAC lost from the treated wood would only prove vital if the remaining levels declined below the toxic threshold for decay organisms. Initial research found that toxic threshold values for decay organisms were in the order of 1.6 to 4.8 kg/m³ for basidiomycetes and 5.8 to 7.9 kg/m³ for soft rot (Butcher & Drysdale 1978[a]). Later studies found that the toxic limits in soil contact were greater than 5 kg/m³ (Butcher & Greaves, 1982; and Preston, *et al.*, 1987). Even though a 51% reduction in DDAC was observed, the amount of DDAC remaining after 13 months was close to the toxic threshold limit and

should provide adequate protection. However, the continued decline of DDAC to levels below the toxic threshold value would result in a high risk of decay onset.

If the loss of DDAC was uniform throughout the wood, there would still be sufficient chemical present to provide protection of at least one year under the field trial conditions. The protection of wood treated with equivalent levels of DDAC in field trials should give enough protection for a longer period. However, field results suggest that 12 kg/m^3 did not protect the wood from rapid decay (Ruddick, 1983). If the depletion of DDAC from the mini stakes was uneven then pockets of wood would be prone to decay. The appearance of the mini stakes upon removal showed three distinct regions as shown in Figure 3.3.3.1. These included a region that was not exposed to the soil that was heavily stained; a highly intense band of stain at the soil contact region; and the region submerged in the soil was gray in colour. The mini stakes, which were removed after 26 months had the soil submerged regions and the upper regions (5 replicates) separated. These sections were analyzed for DDAC retention and the results are shown in Table 3.3.3.3. The results show that DDAC loss from the wood that had been submerged in the soil was of the order of 50% for both DDAC retentions, while the DDAC loss from the wood that had been above the ground level showed losses in the order of 73% and 78%. DDAC depletion measured below the ground line suggests that the continued loss observed in Table 3.3.3.2. may be a reflection on the upper regions. While, in the region below the ground line the DDAC depletion reached a maximum between the 3 to 13 month periods. This would agree with observation made by Nicholas, *et al.* (1991), that the loss after 3 months was completed and did not continue to decline. The 50% loss below the ground line

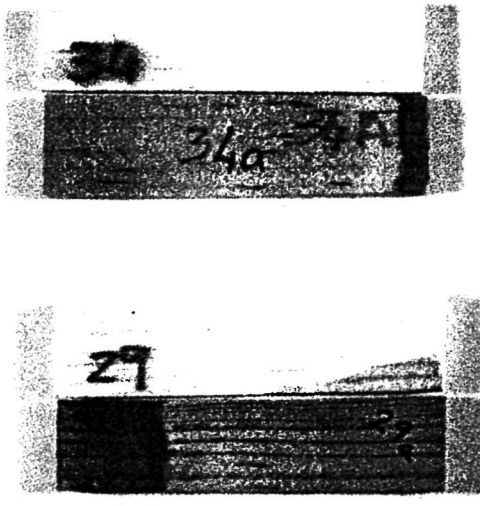


Figure 3.3.3.1.: The appearance of mini stakes after 13 months in the flooded soil bed exposure.

agrees with results observed by Ruddick and Lum (1991) for wood in the same soil bed and this may reflect the amount of DDAC lost by physical leaching and not by biological activity. The greater loss of DDAC was in the upper regions could be due to a wicking action of the water redistributing the AAC in the wood. However, research on wicking action on AAC treated wood with water did not find that the water readily redistributed the AAC in the wood (Hayward & Duff, 1987). Another possible explanation was related to fungal or

bacterial action. The wood that was submerged in the soil should not contain fungi due to the saturation of water limiting the oxygen available. However, the region that was above the soil contact zone would not suffer from oxygen starvation and the outer surface could be prone to fungal colonization of organisms such as moulds. These results indicate that the depletion of DDAC from the wood was uneven resulting in the upper regions, which had levels of DDAC too low to protect the wood from decay. The appearance of the mini stakes suggests that the earlywood sections were more prone to decay as a result of higher depletion of DDAC that agrees with work done by Nicholas, *et al.* (1991) and chapter 3.3.2.

Table 3.3.3.3.: DDAC retention within the various regions of the mini stakes after 26 months exposure.

Treating Solution	**DDAC Retention (kg/m^3) [DDAC Loss* (%)]**		
	Initial	**Below Soil Contact**	**Above Soil Contact**
1% Solution	13.1 (1.5)	6.5 (0.7) [50%]	2.9 (0.3) [78%]
3% Solution	10.3 (1.4)	4.9 (0.7) [52%]	2.8 (0.4) [73%]

* Values in parenthesis are standard deviations.

Isolations from mini stakes after a three month period.

The mini stakes taken from the soil bed showed three distinct regions (Figure 3.3.3.1.) which were previously described. The differences in these regions may indicate that several

mechanisms could cause the loss of DDAC from the wood. Isolations were taken from the mini stakes from the various regions, the results of the isolations are shown in Table 3.3.3.4. Isolation frequency refers to the number of plates containing the organism present from the total number of plates used in each set of isolations. The isolations from the untreated wood did not produce a large frequency of any isolate. As expected, bacterial isolations were most frequent from the mini stake region that had been submerged in the soil in the untreated and treated mini stakes. However, the isolation taken from the DDAC treated mini stakes in the A and C regions showed an increase in the frequency of fungi isolated. Several fungal genera isolated were common to the isolation taken from DDAC treated wood in chapter 4.3.3. Examples were *Verticillium* spp. and *Gliocladium* spp. The *Gliocladium* sp. isolate was subcultured, sent for identification and was later confirmed as *Gliocladium roseum* SB. This isolate was later tested against DDAC and found to be able to degrade DDAC in the agar media. The presence of both bacteria and fungi indicated that there may be a link between the loss of DDAC and their presence in this particular soil bed. Bacteria have been known to degrade AACs in activated sludge systems and waste water treatment systems (Micales, Richter & Highley, 1985) and may contribute to the loss of DDAC from the mini stakes in the soil bed.

The above results indicate that organisms can affect the loss of DDAC from the wood and may be able to achieve a similar influence in field exposure. The loss of DDAC especially in the above ground line regions may reduce the retention to below toxic threshold values as seen in Table 3.3.3.3. allowing decay organisms to invade the wood.

Table 3.3.3.4.: Isolations from mini stakes exposed to a flooded soil bed for three months.

Organisms isolated	Frequency of Isolation (%)								
	Untreated			1% DDAC			3% DDAC		
	Isolate Location								
	A	B	C	A	B	C	A	B	C
Penicillium spp.	-	-	25	-	-	-	-	-	-
Gliocladium spp.	-	-	-	-	-	16	-	-	-
Verticillium spp.	-	-	-	-	-	-	25	-	25
Fusarium spp.	-	-	-	-	-	-	25	-	-
Fungi*	25	-	-	42	-	25	-		-
Slime Moulds	50	-	-	8	-	8	25	-	25
Mucor	25	-	50	16	-	-	25	-	25
Bacteria	-	25	-	42	33	42	50	25	50

A: Above soil surface

B: Below soil surface

C: Soil surface contact

*Unidentified Fungal genera

4. AACs Interaction with Fungi.

4.1. Introduction.

Alkylammonium compounds (AACs) have been used as biocides since the early 1900's. They have found application as bactericides, algaecides and fungicides (Hendrick, Adema & Wiegmann, 1966). Their applications include paints, nappy sanitizers, household disinfectants, and eye drops. An early review by Hugo (1964) proposed five possible modes of action for the biocidal activity of AACs. These included interactions with proteins, metabolic reactions, enzyme systems, the glycolysis reaction and the cytoplasmic membrane. Research has shown that AACs can denature proteins, but at concentrations in excess of biocidal activity (Putnam, 1948). The action of AACs on certain metabolic pathways and enzyme systems has been studied by numerous researchers. The results do not indicate that AACs act on specific metabolic pathways or enzyme systems (Hugo, 1964). The glycolysis reaction can be stimulated by AACs at concentrations, too low to be considered biocidal (Bihler, Rothstein & Bihler, 1961). Therefore, interaction with the cytoplasmic membrane appears to be the most likely mode of action to explain the biocidal activity of AACs. Electron microscope studies of the yeast *Candida albicans* (Robin) Berkhout, revealed that the cell membrane lost it integrity after exposure to benzalkonium chloride solutions (Gale, 1963). Research on the effect of an alkylammonium bromide on the mitochondria of *Agaricus bisporus* LGE. found no interference with any enzyme system. It was concluded that the chemical affected the mitochondria membrane (Steffan, *et al.*, 1988). Recent studies on *Aspergillus ustus* (Bainier) Thom *et* Church reported that exposure to AACs resulted in

the release of amino acids and carbohydrates from the cells, suggesting loss of membrane integrity (Zlocherskaya, *et al.*, 1981). These results are in agreement with the physiochemical knowledge of AACs and their interaction with surfaces (Rosen, 1975).

AACs were first seriously proposed as wood preservatives in 1977 by Butcher, Hedley and Drysdale. Since then considerable research into their application as wood preserving agents has been under taken. Initial laboratory trials indicated that AACs were almost as effective as CCA in preventing decay (Butcher & Drysdale, 1977; Hedley, Tsunoda & Nishimoto, 1982; and Preston & Chittenden, 1982). However, the laboratory success was not replicated in field trials, where initial studies found that AAC-treated wood decayed at the same rate as untreated wood and in some instances more rapidly (Ruddick, 1983; and Butcher, 1985). Consequently many researchers have investigated the use of AACs in combination with other biocides (Drysdale, 1983[a]; Sundmann, 1984; Wallace, 1986; and Ruddick, 1987).

Several hypotheses have been proposed to explain the poor field performance of AAC treated wood. One theory suggested that non-decay fungi may interact with AACs reducing their bioefficacy. This was supported by an investigation of AAC-treated blocks exposed to standard decay fungi. After a pre-exposure to a 'soup' of non-decay fungi, the toxic thresholds and limits increased for the decay organisms in the standard soil jar test (Ruddick, 1986). Several possible explanations may be offered for these observations. Pre-exposure may have reduced the effectiveness by accumulation of protein from the initial invading organisms deactivating some of the AAC (Washam, Sandine & Elliker, 1976[ab]). A serial exposure can cause a similar effect as observed in the above study by Ruddick (Butcher, 1979[b]). Pre-exposure could increase the leachability and reduce the loading of the AAC in

the wood. However, this last possibility was addressed in the same study and was found not to occur (Ruddick, 1986). Finally it is possible that the AAC was degraded by one or more of the non-decay fungi during the pre-exposure. Reduction in the AAC concentration by degradation would increase the toxic threshold and limits for standard decay organisms in a soil jar test.

The potential of fungi to detoxify or degrade preservatives, designed to prevent wood decay, has been previously observed. Many researchers have proven that soft-rot fungi, decay fungi and non-decay fungi can degrade certain preservatives (Francis & Leightley, 1983; Micales, Richter & Highley, 1989; and Lee, Takahashi & Tsunoda, 1992).

As a result of their wide industrial use and their potential to be released into the environment, the biodegradation of AACs has been investigated by many researchers. Initial studies concentrated on chemical reduction in activated sludge and waste water treatment systems. The majority of these studies found that monoalkyl, dialkyl and benzalkonium derivatives of AACs were all readily biodegraded (Boethling, 1984; and Pauli & Franke, 1970). However, numerous reviews of these investigations have concluded that AACs adsorption was not taken into account and that primary biodegradation had occurred (Mackell & Walker, 1978; & van Ginkel, van Dijk & Kroon, 1992). Primary biodegradation can be defined as the reduction in the AAC content without accounting for adsorption and no actual breakdown of the AAC. Ultimate biodegradation is the conversion of AACs into carbon dioxide, water and mineral salts. Later studies using radioactive carbon found that 80% of the C^{14} was evolved as carbon dioxide after the AAC had been exposed in an activated sludge system for 20 days. Addition of clay to the system reduced the extent of biodegradation to 10% after 20 days

(Krzeminski, *et al.*, 1973). These studies indicate the importance of adsorption of AACs on to the surfaces of solids within systems. Further studies have isolated and identified bacteria capable of degrading AACs in activated sludge (Dean-Raymond & Alexander, 1977; Mackell & Walker, 1978; and van Ginkel, van Dijk & Kroon, 1992). Experiments with these bacteria carried out in liquid culture against a range of AACs demonstrated that the simple short chain AACs were readily utilized as a sole carbon source by many bacteria (Hampton & Zatman, 1973; Mackell & Walker, 1978; and Ghisalba & Küenzi, 1983). However, research on longer alkyl chains as sole carbon sources has proven more difficult. The addition of a secondary carbon source did allow the degradation of a monoalkyl AACs to occur (Dean-Raymond & Alexander, 1977; and Mackell & Walker, 1978). One study found the combination of two bacteria in the liquid culture did enable them to grow on an AAC as the sole carbon source (Dean-Raymond & Alexander, 1977). Recently, it was reported that an isolate of *Pseudomonas* had the ability to utilize an monoalkyl AAC as the sole carbon source (van Ginkel, van Dijk & Kroon, 1992). It must be noted that all of the isolated bacteria are limited in that they can only degrade monoalkyl AACs. No bacteria to date have been identified that can degrade dialkyl AACs in liquid culture. One possible explanation for this, is the steric hindrance afforded by the presence of two alkyl chains on the nitrogen or the hydrophobic nature of the dialkyl AACs.

Two possible mechanisms have been proposed for the degradation of AACs. Dean-Raymond and Alexander (1977) found that *Xanthomonas* caused the ω-oxidation of the terminal carbon on the decyl chain of the AAC. The carboxylic acid that was formed then under went further β-oxidation removing acetyl groups from the alkyl chain. van Ginkel, van Dijk and

Kroon (1992) isolated a *Pseudomonas* strain that caused the fission of the carbon-nitrogen bond to form trimethylamine and hexadecanal. Hexadecanal was then degraded through β-oxidation removing acetyl groups on the alkyl chain.

Bacteria have proven to be very important with respect to AACs degradation in waste water treatment systems. However, the role of bacteria is minor in the decomposition of wood in service and only contributes under adverse conditions were fungal decay does not occur (Rayner & Boddy, 1988). Examples of such conditions are in the living tree, where the moisture content is generally too high for most fungal growth, due to limited oxygen levels. Fungi on the other hand, are extremely important in the decomposition of wood in service. The failure of AAC-treated wood during field exposure was linked to rapid discolouration of the stakes by staining fungi (Ruddick, 1986). Studies using a *Scopulariopsis* sp. did not show any reduction in the AAC content in liquid culture after incubation (Briscoe, *et al.*, 1990). However, no comprehensive study of the possible biodegradation of AACs by fungi has been undertaken to date.

The main objectives of this phase of the research was to: isolate and identify fungi which appear to be tolerant to DDAC (representing dialkyl AACs); determine their tolerance to other AACs (as exemplified by monoalkyl AACs and benzylalkyl AACs); demonstrate that the tolerance of these fungi is related to their ability to at least partially degrade the DDAC; and establish if these organisms could degrade DDAC in a wood matrix.

4.2. Methodology.

4.2.1. Bioassay of Common Wood Deteriorating Fungi.

Chemicals.

The alkylammonium compounds used in this investigation were of commercial grade, Bardac® 2280 (Lonza Inc.) contained 80% didecyl(C_{10},C_{10})dimethylammonium chloride (DDAC) active ingredient plus 10% ethyl alcohol and 10% water and Arquad® C33 from Akzo Chemicals Inc., contained 33% trimethylcoco(C_{12},C_{16},C_{18})ammonium chloride (CAC) active ingredient in water. Stock solutions were made up with distilled water to give 0.4%, 4% and 40% active ingredient of DDAC and 0.165%, 1.65% and 16.5% active ingredient of CAC.

Fungal Species.

Each "wood deteriorating fungal group" was represented by one species, obtained from Forintek Canada Corp. and included: a mould: *Aspergillus niger* van Tiegham (207A:v Tiegh. H:814); a staining fungus: *Aureobasidium pullulans* (de Barry) Arnaud (132B: host tissue isolate, *Picea* sp. lumber, Williams Lake, B.C., B:, R S Smith); a soft-rot fungus: *Chaetomium globosum* Kunze : Fries (127B: stored cotton, I:ATCC 6205); a brown-rot fungus: *Postia placenta* (Fries) M. Larsen *et* Lombard (120F:Cke. (L:MAD 698); and a white-rot fungus: *Trametes versicolor* (Linnaeus : Fries) Pilát (105E: host tissue isolate, *Quercus* sp. flooring, Vancouver, B.C., B:, R S Smith).

Bioassay.

Solutions of 4% malt agar were made up and sterilized at 121°C for 20 minutes (at 103 kPa). Once the malt agar had cooled to "hand hot", the previously prepared DDAC and CAC solutions were added to give concentrations of 0 ppm (control), 50 ppm, 100 ppm, 250 ppm, 500 ppm, 1,000 ppm, 5,000 ppm and 10,000 ppm of each preservative in the malt media. For example: 7.5 ml of the 40% stock solution of DDAC was added to 292.5 ml of 4% malt agar to give a concentration of 10,000 ppm DDAC in the media. From each solution, 10 ml petri plates were poured and allowed to solidify. Using a core borer, individual 6 mm cores of the A. *niger* inoculum, from the outer growing edges, were transferred from culture plates to the centre of each test plate. Three replicates of each concentration of DDAC and CAC were prepared plus three control plates. This was repeated with A. *pullulans*, C. *globosum*, P. *placenta* and T. *versicolor* resulting in a total of 45 plates for each test fungus. The labeled plates were incubated at 25°C. The diameter of fungal growth present on each plate was measured daily using a ruler, until the fungi on the control plates had completely colonized the plate (~6 days). The diameter values used were the averages of the cross diameters on three replicated plates. The treated plates were further monitored after 9 and 22 days to determine the long term effect of each chemical against the individual organisms. Regression analysis on the slopes on the various graphs were carried out to determine the growth rates of each fungi and the effect of the two AACs tested.

4.2.2. Isolation and Identification of Tolerant Organisms.

Isolation Material.

Isolations were carried out on AAC-treated wood, made available by the western laboratory of Forintek Canada Corp. An anti-sapstain investigation was conducted by Forintek using 480 pieces of hem-fir lumber, sprayed with three anti-sapstain formulations containing DDAC, which were Timbercote™, NP-1™ and F2™. Two of these formulations contained a secondary biocide as shown in Table 4.2.2.1. Timbercote™ contains a mixture of DDAC as the sole biocide with latex. The other two formulations have a secondary biocide, F2™ contains sodium octaborate and NP-1™ has 2-iodo-2-proponyl butyl carbamate (IPBC) combined with the DDAC (Table 4.2.2.1.).The lumber was sprayed with each formulation to provide the surface of each board with 90 $\mu g/cm^2$ of DDAC. After treatment the boards were cut in half into 1.3 m long sections giving two matched samples. One set was pasteurized by steam sterilization for 48 hours and the other was placed directly in the field. This was done to determine the effect of post-treatment pasteurization on colonization by mould and sapstain fungi. The isolations in this study were carried out after the six packages of lumber had been exposed in the open for seven months.

Isolations.

Isolations were carried out using four media: 4% malt, 4% malt with 100 ppm of tetracycline, 4% malt with 100 ppm DDAC; and 4% malt with 2,500 ppm DDAC. The concentrations of DDAC present in the media were selected based on the results found in the

bioassay of deteriorating fungi (chapter 4.3.1). Ten wood sections were removed from areas where visible fungal colonization had occurred in each of the six test packages (20 wood sections were removed from the non-pasteurized Timbercote™ treated lumber). Each section was split and a small sliver of freshly exposed wood was placed on the culture media. The plates were incubated for four weeks at 25°C, and any fungal growth was tentatively identified. Selected organisms were cultured and sent to the National Identification Service in Ottawa for formal identification.

Table 4.2.2.1.: Formulations used in the isolation of DDAC tolerant fungi.

Sapstain Formulation	Chemical Formulation	Concentration of DDAC $\mu g/cm^2$
Timbercote™	DDAC plus Latex	100
F2™	DDAC plus Sodium Octaborate	100
NP-1™	DDAC plus 2-Iodo-2-Proponyl Butyl Carbamate	90

4.2.3. Bioassay of DDAC Tolerant Isolates.

Chemicals.

The alkylammonium compounds used in this experiment were of commercial grade, including both didecyldimethylammonium chloride (DDAC) and trimethylcocoammonium chloride (CAC) as mentioned in section 4.2.1. A third AAC was included which was Barquat® OJ-50 (Lonza Inc.) containing 50% n-alkyldimethylbenzylammonium chloride (DMBC) active ingredient in water. The structures and chain length distributions are shown in Table 4.2.3.1. Stock solutions of 1% (10,000 ppm) active ingredient were made up with distilled/deionized water for each preservative.

Fungal Species.

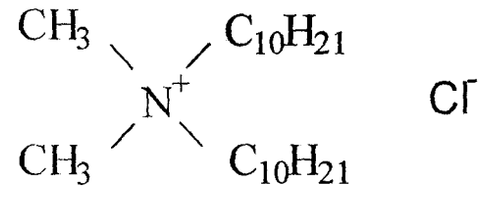
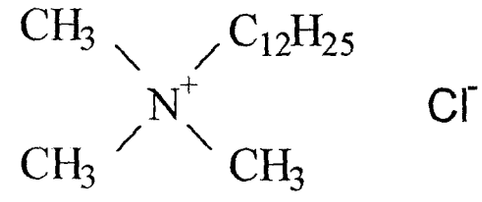
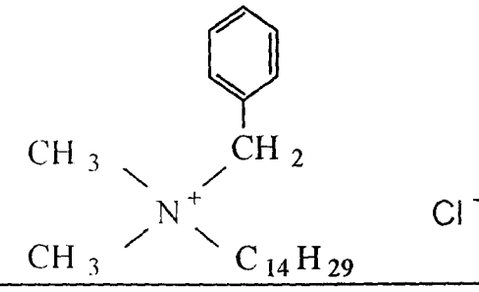
Four previously isolated organisms, which had shown tolerance to DDAC were tested. They were: *Verticillium bulbillosum* W. Grams & Mulla, an *Acremonium* sp. and two *Gliocladium roseum* Bainer Complex isolates- one from the anti-sapstain field trial referred to as G. *roseum* FT, while the second was isolated from mini stakes removed from the soil bed exposure referred to as G. *roseum* SB (see chapter 3.2.3. and 4.2.2.).

Exposure.

Solutions of 4% malt agar were made up and sterilized at 121°C for 20 minutes (at 103 kPa). The previously prepared preservative stock solutions were filtered through a 0.45 μm PTFE (Teflon) sterile filter before the addition to the media. Once the malt agar had cooled to "hand hot", the filtered DDAC, CAC and DMBC solutions were added to the media to

produce concentrations of 0 ppm (control), 100 ppm, 200 ppm, 400 ppm and 800 ppm of each preservative. From each solution, 10 ml petri plates were poured and allowed to solidify. Using a core borer, individual 6 mm cores of each fungal inoculum were transferred from the growing edges of the culture plates to the centre of each test plate. Three replicates for all four concentrations of DDAC, CAC and DMBC were prepared with each test fungus, plus three control plates. The plates were labeled and placed in an incubator at 25°C. The diameter of fungal growth present on each plate was measured every three to five days with a ruler over a 24 day period. The diameter values used were averages of the cross diameter on three replicate plates. Regression analysis was carried out on the various slopes to determine the growth rates of each fungi and the effect of the three AACs on their growth rates.

Table 4.2.3.1.: Chemical structure of the three AACs tested

Chemical	Structure	Chain Distribution
DDAC Didecyldimethylammonium Chloride		C_{10} 100%
CAC Trimethylcocoammonium Chloride		C_8 6%, C_{10} 7% ,C_{12} 51%, C_{14} 19% , C_{16} 9% & C_{18} 2%
DMBC n-alkyl dimethylbenzyl ammonium Chloride		C_{12} 25%, C_{14} 60% & C_{16} 15%

4.2.4. Fungal Degradation of AACs.

Test Chemicals.

The alkylammonium compounds (AACs) used in this investigation were of commercial grade, which included didecyldimethylammonium chloride (DDAC) and trimethylcocoammonium chloride (CAC) as mentioned in section 4.2.1.

Fungal Species.

The four fungi included in this study were isolated from DDAC-treated wood (see section 4.2.2.). They have been identified as *Verticillium bulbillosum* W. Grams & Mulla, an *Acremonium* sp and two *Gliocladium roseum* Bainer Complex strains previously utilized. Two brown rot fungi which were *Coniophora puteana* (Schum. : Fr.) P. Karsten (9G: *Schum.*:Fr, sporocarp tissue isolate, F:FPRL 11E) and *Postia placenta* (Fr.) M. Lars. *et* Lomb. (120F: *Poria monticola* Murr., L:MAD 698) were also tested.

Wood Species.

Two different softwood species were employed. They were western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) in the form of cylinders 80 mm long and 15 mm in diameter and ponderosa pine (*Pinus ponderosa* Laws.) sapwood in the form of mini stakes with dimensions of 3 x 28 x 44 mm.

DDAC Treatment of the Wood Samples.

Oven dried and weighed T. *heterophylla* and P. *ponderosa* samples were impregnated with a 1% solution of DDAC. The labeled samples were placed in a beaker inside a desiccator that was evacuated for 30 minutes. The 1% DDAC solution was added and the wood samples allowed to soak for one hour at atmospheric pressure. Upon removal from the treating solution the wood samples were carefully wiped to remove excess solution, weighed, wrapped in polyethylene and stored in the laboratory for seven days to ensure complete fixation. At the end of this storage the polyethylene was removed and the samples air dried for one week.

The T. *heterophylla* cylinders were split into 3 mm thick disks with their order being noted. The estimated retention based on weight uptake was 4 kg/m^3. Sawdust was obtained from the ponderosa pine mini stakes by grinding them through a Wiley mill with a 20 mesh (850 μm) filter. The estimated DDAC retention of the mini stakes was 8 kg/m^3.

Solid Media Exposure.

Solutions of 4% malt agar were made up and sterilized at 121°C for 20 minutes (at 103 kPa). The previously prepared preservative stock solutions were filtered through a 0.45 μm PTFE (Teflon) sterile filter before adding to the media. Agar concentrations of 500 and 800 ppm CAC and DDAC were prepared with "hand hot" malt agar, which were poured into 10 ml petri plates and allowed to solidify.

Using a core borer, individual 6 mm cores of each fungal inoculum were transferred from the edge of the growing colony on the culture plates to the centre of each test plate. The plates were labeled and placed in an incubator at 25°C for 4 weeks.

After incubation, 6 mm cores were removed from plates treated with 500 ppm DDAC, 800 ppm DDAC and 800 ppm CAC. The cores were removed from specially selected locations on each plate. These regions included the outer edges, uncolonized by fungal mycelium and the central region, that had been colonized by fungal mycelium. In the initial study a known volume of agar was removed, but the weight was not noted. In later studies the number of agar cores and their weights were recorded, in order to calculate the amount of AACs present in the samples. Two replicate cores were removed from various locations within plates treated with AACs. The locations were located in 0 to 10 mm, 10-20 mm, 20-30 mm and 30-40 mm regions from the colony centre.

The addition of DDAC to solid agar raised concern of uneven distribution due to the nature of the chemical involved. Therefore, plates containing 800 ppm DDAC were prepared as previously described. Two of the plates had cores removed from various locations within the plates. The DDAC was extracted with acidified ethanol solution using either formic acid or acetic acid. The acetic acid was used to determine if it could replace the formic acid previously used in the extracting solution. The recovery results are shown in Table 4.2.4.1., which indicate that formic acid containing extracting solution removes the DDAC more efficiently. The results across individual plates and from plate to plate indicate that there was no significant difference in distribution. These results suggest that uneven distribution is not

a major concern. The concentration is slightly higher than the amount estimated and maybe due variations in weighing. accuracy

Table 4.2.4.1. Distribution of DDAC in control plates.

Control Plate Sample	**Acid Used**	**DDAC Content**
Plate I		**(ppm)**
A	Formic Acid	**856**
B	Formic Acid	**875**
C	Formic Acid	**854**
D	Acetic Acid	**822**
Plate II		
A	Acetic Acid	**746**
B	Acetic Acid	**761**
C	Acetic Acid	**852**
D	Formic Acid	**850**
		827 (±48)

Liquid Media Exposure.

Fungal cultures of G. *roseum* and *Acremonium* sp. were initially grown on solid Czapek media. Once the culture had obtained a reasonable growth on the agar plate, 5 ml of "sterile" liquid media was added to each plate. Using an inoculum needle to release the mycelium from the agar into the liquid, the fragmented mycelia solutions were decanted into a sterilized glass homogenizer. The mycelium fragments were homogenized to produce a mycelium suspension to be used in the inoculation of the test flasks.

The Czapek liquid media was prepared by dissolving 30 g sucrose, 2 g sodium nitrate, 1 g potassium phosphate, 0.5 g magnesium sulphate, 0.5 g potassium chloride, 0.01 g iron sulphate, 0.01 g zinc sulphate and 0.005 g copper sulphate, in one litre of distilled/deionised water. Aliquots of the liquid media (50 ml) were measured into 250 ml flasks and autoclaved at 121°C for 20 minutes (103 kPa). Once cooled to ambient temperature, 1 ml of media was removed and 1 ml of the homogenized mycelia suspension added. Eight flasks were inoculated with the mycelium suspensions of G. *roseum* and eight flasks with the *Acremonium* sp. Five flasks were retained as media controls, giving a total of twenty-one flasks used. Glass beads were also added to the flasks to prevent the inoculum from forming a single colony. The flasks were incubated at 25°C for two days with a shaking speed of 125 rpm.

After two days, DDAC was added to the flasks in various forms as shown in Table 4.2.4.2. The quantity of material added to each flask was calculated to introduce approximately 10 mg (200 ppm) of DDAC into each of the test flasks, with the exception of sawdust samples

Table 4.2.4.2. : DDAC addition to liquid media.

Sample	DDAC Form	Amount Added
Acremonium sp.		
I	*(2 day growth control)*	*None*
II	Disk 4, Sections 1, 3 & 5	1.1657 g
III	Liquid	0.25 µl of 4% DDAC
IV	*(14 day growth control)*	*None*
V	Pine Sawdust	0.5339 g
VI	Disk 5, Sections 1, 3 & 5	1.0291 g
VII	Liquid	0.25 µl of 4% DDAC
VIII	Pine Sawdust	0.5122 g
Gliocladium roseum		
I	*(2 day growth control)*	*None*
II	Pine Sawdust	0.1088 g
III	Pine Sawdust	0.1104 g
IV	Disk 2, Sections 1, 3 & 5	0.9946 g
V	Disk 4, Sections 4, 8 & 9	0.9253 g
VI	Liquid	0.25 µl of 4% DDAC
VII	Liquid	0.25 µl of 4% DDAC
VIII	*(14 day growth control)*	*None*
Controls		
A	Disk 4, Sections 11 & 13	0.5291 g
B	Disk 5, Sections 11 & 13	0.7219 g
C	Disk 2, Sections 10 & 12	0.6250 g
D	Pine Sawdust	0.4682 g
E	Liquid	0.25 µl of 4% DDAC

in flasks containing G. *roseum*. At the same time one flask of each fungal inoculum was removed with the biomass being determined. The remaining flasks were incubated at 25°C for an additional 12 days with a shaking speed of 125 rpm.

After twelve days of incubation in the presence of DDAC the wood disks were removed from the flasks. The fungal biomass values were determined by centrifuging the culture at 1,500 rpm for 30 minutes at 5°C. The supernatant was decanted and the remaining mycelia filtered through a pre-weighed Whatman glassfibre filter paper. The filter paper plus mycelia were dried by microwaving for 2 minutes and weighed to give the biomass. The volumes of liquid remaining in each flask were recorded and retained for analysis.

Soil Jars Exposure.

The use of a soil jar exposure was selected to provide a suitable environment for fungal decay, in a shorter time frame than is necessary in field trials. The organization of the soil jar prepared was very important and the following method was adapted from the North American standard preservative screening test (AWPA Handbook. Standard E10-91). The soil was sifted through a US No. 5 sieve (4 mm) which met the ASTM standard for wire-cloth sieves for testing purposes. The moisture content was determine for the soil after sieving and adjusted by the addition of water to produce a moisture content between 45 - 50 %. The moisture content of the soil is very important as this will strongly influence fungal growth and the amount of decay. The moisture content of the amended soil was determined before being added to the soil jars.

Feeder strips were prepared from P. *ponderosa* sapwood. The feeder strips were cut to give dimensions of 3 x 28 x 44 mm with the grain of the wood parallel to either of the long dimensions and with the edge grain exposed to the flat face. The role of the feeder strip was to provide nutrients to the fungus to sustain initial growth.

The design of the incubation lid is important as it must allow respiration, to ensure normal fungal growth and the decay of the wood to proceed. However, the lid should prevent mite infestation and contamination by other micro-organisms (Smith, 1978). The incubation lids were metal lids in which a 5 mm hole had been drilled in the centre. A two part epoxy glue was then applied to the inside of the lid around the hole. A 25 mm Gelman membrane with a pore size of 2 μm (GA-85) was placed onto the glue, making sure the membrane area over the hole did not become blocked with glue. The metal lids were sealed in autoclavable plastic bags and steam sterilized for an hour at 103.4 kPa before being used.

A total of twenty-four jars were prepared. The jars were filled to a third of their capacity with the previously prepared soil. The soil was lightly compacted by tapping the jar on the bench. A feederstrip was placed on the soil surface in each culture jar, then loosely fitted plastic lids were placed onto the culture jars before sterilization. The culture jars were placed inside a steam autoclave and sterilized for an hour at 103.4 kPa and 121°C. After one hour, the autoclave exhaust opened automatically and the pressure slowly decreased. When atmospheric pressure was reached, the autoclave door was opened and the jars removed. Sterilization of the jars was required to kill any micro-organisms present in the soil or feeder strip. Steam sterilization has been found to be one of the most convenient and effective methods for sterilization of soil jars.

Once the sterilized soil jars and cooled, they were transferred to a sterile bench and inoculated with previously prepared fungal cultures. Using a sterile spatula, two 6 mm cores of the fungal inoculum were removed from culture plates close to the growing colony edge and placed on either side of the feeder strip in each of the jars. The plastic lids were replaced with previously prepared sterile incubation lids. The inoculated soil jars were incubated at 22 - 25°C for three weeks, or until the feeder strips were covered with mycelium, before placement of the treated mini stakes occurred. Twelve DDAC-treated mini stakes and untreated mini stakes halves were added to the various soil jars. Three replicates of C. *puteana* and P. *placenta* were used, plus six replicates of V. ***bulbillosum*** for DDAC treated and untreated mini stakes.

The soil jars were incubated for 24 weeks at 22 - 25°C, after which the mini stakes were removed and the weight loss was determined. The exposed halves of the mini stakes and the reference halves were ground up. The DDAC content determined by the method describe below.

AAC Analysis.

DDAC and CAC analysis were carried out with an Indirect Photometric using High Performance Liquid Chromatography (HPLC) Technique (Larson & Pfeiffer, 1983). The system consisted of a Spectra Physics 1200 series HPLC pump equipped with a 8 mm x 10 cm Partisil SCX Column (Water 85753) and 10 μm particles and a Spectra Physics variable wavelength UV detector set at 262 nanometers. The mobile phase was a mixture of 0.025 M of benzyltrimethyl-ammonium chloride, 1% acetic acid and water:methanol mixture with a

ratio of 5:1 which was filtered through a 0.45 μm Telfon filter. The flow rate used was 2.2 ml/min.

Approximately 0.1 to 0.25 gram agar samples were extracted with 1 ml of acidified ethanol (formic acid) solution by ultrasonication at 40 Hz for 2 hours. The extracting solutions were allowed to settle overnight, after which the solutions were filtered through a PTFE filter (0.45 μm) and analyzed with the HPLC to determine their AAC content. (Initial studies used 0.3 to 0.6 grams of agar and 5 ml of extracting solution.)

The T. *heterophylla* disks were ground in a Wiley mill through a 20 mesh filter (850 μm). Approximately 0.2 grams of the sawdust was extracted with 2 ml of extracting solution (acidified ethanol -formic acid) solution by ultrasonication at 40 Hz for 2 hours. The solutions were allowed to settle for 12 hours, after which the solutions were filtered through a PTFE filter (0.45 μm). The solutions were analyzed by the above HPLC technique to determine the DDAC content. The same method was used for the P. *ponderosa* sawdust, the fungal mycelium and the pine sawdust/mycelium mixture, to determine the amount of DDAC present.

Analysis of the liquid remaining involved addition of 10 ml of dichloromethane to 10 ml of media into a separatory funnel. After shaking the dichloromethane phase was separated and a further 10 ml of dichloromethane added to the media. This was repeated until 30 ml of dichloromethane was obtained, which was rotary-evaporated, then 5 ml of acidified ethanol were added to each flask. The acidified ethanol samples were filtered through a Teflon filter (0.45 μm) and DDAC analysis by the above HPLC technique.

Flasks used for the control samples were washed with 4 ml of acidified ethanol to determine the amount of DDAC absorbed on to the glass. The samples were filtered through a Teflon filter (0.45 μm) and analyzed with the HPLC.

4.3. Results and Discussion.

4.3.1. Bioassay of Common Deterioration Fungi.

The bioassay was carried out to estimate the relative effectiveness of two AACs against a range of wood deteriorating fungi and to indicate the possible resistance to such chemicals by these organisms. The simplest method of assessing fungal growth is by linear measurement. This was carried out by monitoring the radius of a developing colony on agar over a period of time. This method has the virtues of being extremely simple, valuable as a rough estimate of growth, while being non-destructive; and allowing repeated observations of the same mycelium (Moore-Landecker, 1990). However, it fails to account for difference in aerial mycelium or mycelium submerged in the agar, does not distinguish between mycelium growth rate and the total biomass production. A bioassay compares the linear growth of each fungus on agar plates treated with selected amounts of the two preservatives with control plates (no chemical present). Growth rates for each fungus was compared with growth rate on the media treated with the two AACs. To correct for the weakness of the linear measurements, visual observations of colony morphology were taken into account (represented by pictures). These observations plus the linear growth values were compared to indicate the relative effectiveness of DDAC and CAC against the five deteriorating fungi tested.

Growth curves of fungal cultures normally show three stages of growth. Stage I is called the lag phase, where the organism adjusts to the growing medium. However, in this study the transfer of a highly active growing mycelium should eliminate the lag phase. Stage II, the

linear phase, is where the hyphal tip extension doubles at a constant rate and can be seen on control plates immediately. Stage III or IV, the declining phase or stationary phase, is where no growth occurs and biomass declines (Moore-Landecker, 1990). A decline phase was not observed in this study due to the type of measurements used - but a stationary phase was observed on the control plates.

Mould and Staining Fungi

Mould and stain are caused by the Ascomycetes and Fungi Imperfecti classes. These fungi use the wood substance primarily as a habitat and obtain the majority of their nutrients from the simple sugars stored in the wood. The major portion of the degrade attributable to moulds and staining fungi is in fact not wood decomposition, but discolouration caused by pigments within the penetrating hyphae in the case of stains, or by pigments in the surface-formed conidia in the case of mould (Wilcox, 1973). Moulds and staining fungi cause little damage to the structure of wood they inhabit, provided their action does not reach a more aggressive stage where they would be considered soft-rots or cellulolytic "moulds" (Nilsson, 1985).

The staining fungus used in this investigation was *Aureobasidium pullulans*. No growth was recorded on any of the DDAC or CAC treated plates over a two week period. However, the control plates showed normal growth and were totally covered within eight days. This indicated that the inoculum used was viable and the lack of growth in the treated plates can only be attributed to the presence of DDAC and CAC in the media. The growth of A. *pullulans* was completely controlled by 50 ppm or less DDAC and CAC. Therefore, both

preservatives appeared to be very effective against this staining fungus and may find useful application in anti-sapstain formulations. However, the sensitivity of A. *pullulans* to the test chemicals may not be common to all staining fungi. Previous research suggests that A. *pullulans* and other common staining fungi may not be as sensitive to either chemical as suggested by this study. A mixed fungal inoculum containing A. *pullulans* with two other staining fungi, was not controlled by treatment of approximately 25,000 ppm CAC (Linderborg, 1984). Other reports of experiments where DDAC was examined as an anti-sapstain suggest that 1,000 to 1,500 ppm DDAC did not control the growth of staining fungi used (Rustenburg & Klaver, 1990; and Wakeling, Maynard & Narayan, 1993).

Aspergillus niger was the mould utilized in this study. The growth of A. *niger* was difficult to measure due to the spore dispersal mechanism of the organism. This dispersal resulted in numerous colonies over the plates containing low levels of both preservatives. The effect of the chemicals could only be estimated by the number of spores in combination with the colony diameters. Reproduction is associated with the decline or cessation of vegetative growth and a number of factors interacting to induce the shift from vegetative growth to sporulation. Even the presence of a simple barrier can induce sporulation in some fungi (Moore-Landecker, 1990). In this study, the only factor to account for the sporulation was the presence of the test chemical. Despite the above problem, A. *niger* is commonly used in bioassays (Scheffer & Morrell, 1986), but the spore dispersal mechanism is usually inhibited by the presence of volatiles from the preservative, which is not a distinctive characteristic of AAC preservatives.

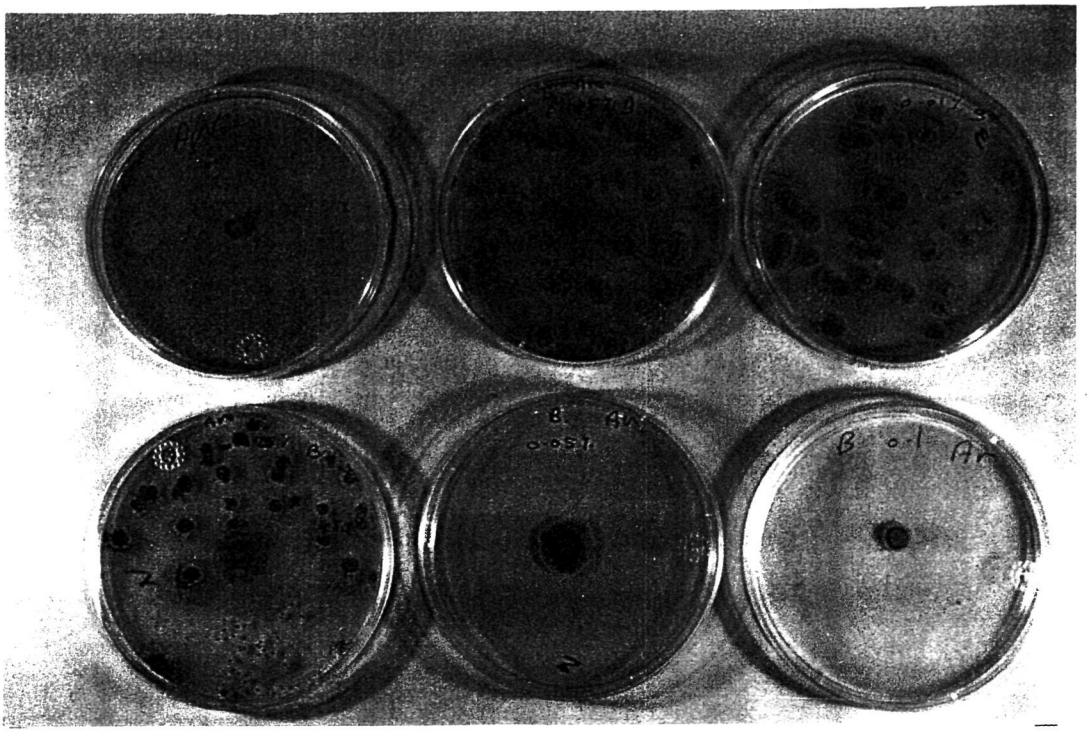


Figure 4.3.1.1.: The growth of A. *niger* on media containing 0 ppm, 50 ppm, 100 ppm, 250 ppm, 500 ppm and 1,000 ppm DDAC after a ten day incubation period (from top to bottom, left to right).

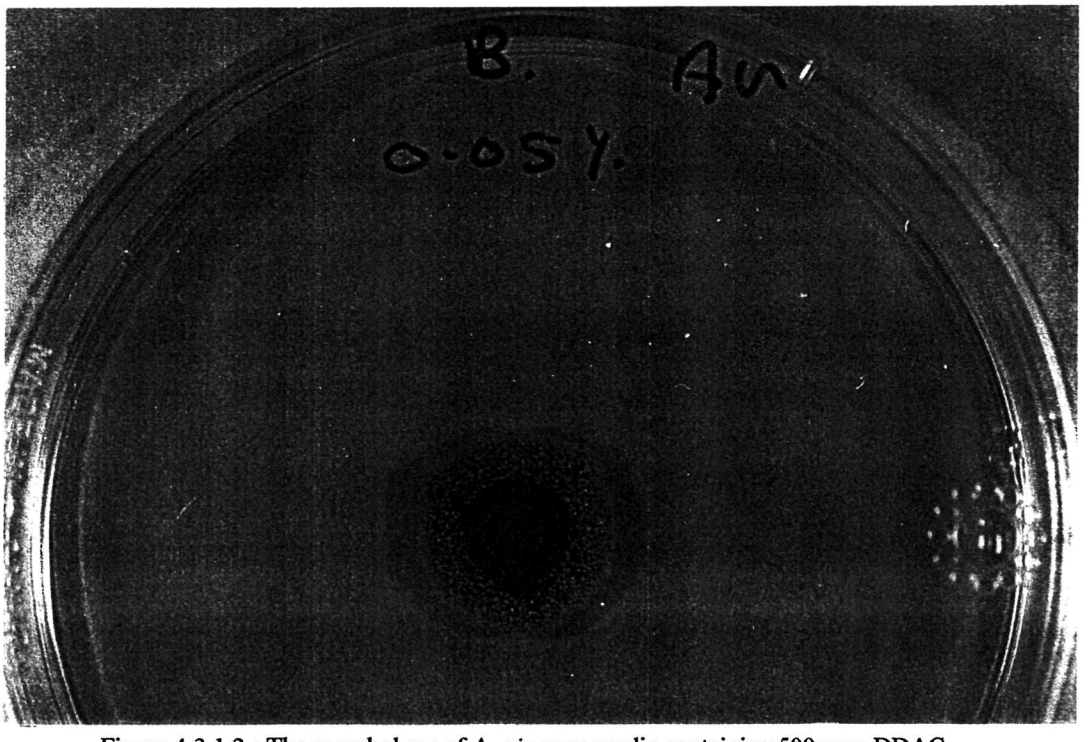


Figure 4.3.1.2.: The morphology of A. *niger* on media containing 500 ppm DDAC.

Regardless of this difficulty it can be clearly seen from Figure 4.3.1.1. that as the DDAC concentration increased, both the colony quantity and size were effectively reduced. The excessive sporulation at the lower concentrations could be an indication of the fungus being under stress due to the presence of DDAC. As the DDAC concentration increased, either the spore dispersal became inhibited or the germination of the spores was inhibited, resulting in fewer colonies. On the plates treated with 500 ppm DDAC only a single circular colony was formed. As no black spores were observed on these plates, it can be concluded that spore dispersal was inhibited. The morphology of the fungal colony on plates treated with 500 ppm DDAC completely changed. The morphology of the colony is yeast-like in structure with an intense yellow colour (Figure 4.3.1.2.). This type of morphology change is often associated with the interaction of the fungus and its' surrounding medium. These sites indicate areas of high enzyme activity and mycotoxin production. This interaction zone is thought to be important in the micro-habitat and may increase the organism's Saprobic competitive ability (Rayner & Webber, 1983). From these results A. *niger* was completely controlled by 1,000 ppm DDAC. The growth was approximately reduced by 50% at 250 ppm DDAC and very little growth observed on 500 ppm DDAC.

The effect of CAC on A. *niger* can be seen in Figure 4.3.1.3. As with DDAC, the growth of A. *niger* was reduced as the concentration of CAC increased. The number of fungal colonies declined in medium containing 250 ppm CAC (Figure 4.3.1.3.). On the plates modified with 1,000 ppm CAC, A. *niger* developed a single circular colony indicating that spore dispersal was inhibited at higher concentration of CAC. The yellow colouration observed with DDAC was not exhibited with CAC containing media, suggesting that the two preservatives do not

have the same toxic effect on A. *niger*. A. *niger* was not completely controlled by CAC, the growth observed on plates containing 1,000 ppm CAC was less than 10% of the control plates. The growth dropped by ~50% with the addition of 250 ppm CAC. The minimum inhibitory concentration that had been previously reported was 500 ppm CAC against A. *niger* in liquid culture, which is half of the value observed here (Hueck, Adema & Wiegmann, 1966). The most likely explanation for this difference, is the lack of any partitioning of the chemical in the liquid culture compared to the solid agar medium. This phenomenon may be a result of the reduction of the mobility of AACs by the solid agar, limiting the interaction with the fungal mycelium. In the liquid culture system, the surfactant properties of AACs will be at their optimum.

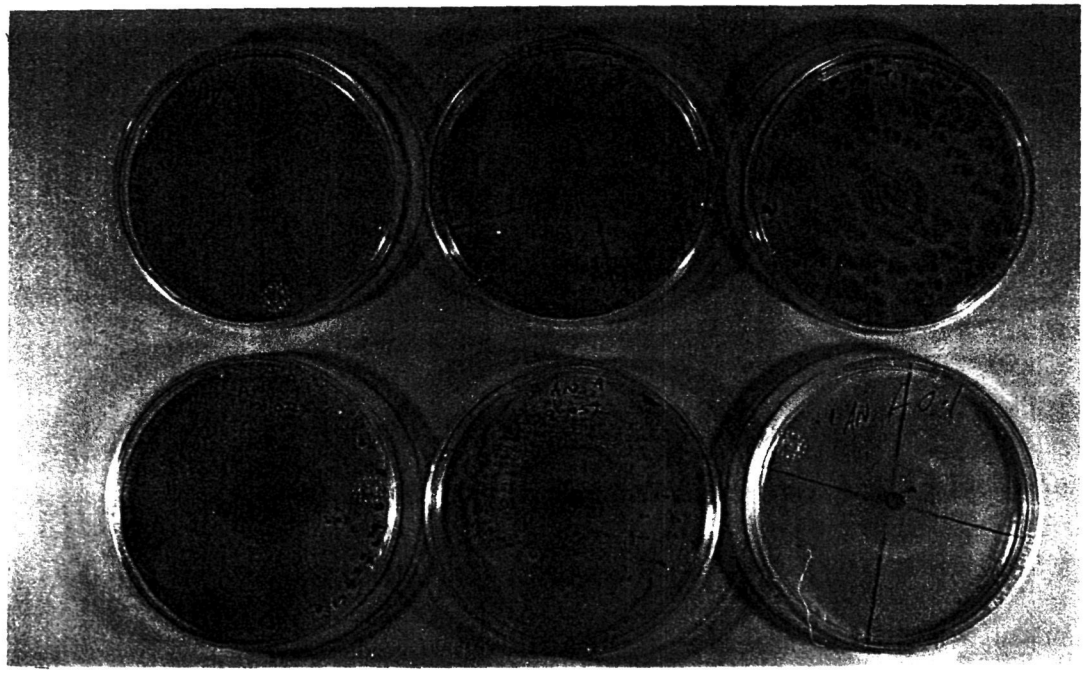


Figure 4.3.1.3.: The growth of A. *niger* on media containing 0 ppm, 50 ppm, 100 ppm, 250 ppm 500 ppm and 1,000 ppm CAC after a ten day incubation period (from top to bottom, left to right).

These results may indicate that DDAC could be more effective against moulds than CAC. However, other researchers have found that CAC was more effective than DDAC against moulds (Hulme & Thomas, 1979; & Linderborg, 1984). These studies used wood as the substrate, and it is well known that changing the substrate can induce different fungal growth responses even without the presence of biocides (Bravery & Carey, 1977). For that reason, general laboratory based conclusions cannot be directly related to in vivo situations.

Soft-Rot Fungi.

Although soft-rot fungi have been clearly differentiated from basidiomycetes fungi, the distinction between soft-rot fungi and other non-basidiomycete wood-inhabiting fungi remains unclear. Soft-rot fungi generally belong to either Ascomycetes or Fungi Imperfecti. However, these same classes of fungi also contain stain and mould causing fungi. In the past differentiation has been based upon the anatomical aspects of the deterioration: the distinctive, often diamond-shaped, spiraling cavities in the S_2 wall layer, and the stage of wood decay that usually separates out soft rot fungi (Wilcox, 1973). The modern definition of soft rot is a form of decay caused by Ascomycetes and Fungi Imperfecti where discrete cavities are formed around hyphae growing within the wood cell walls. There is little difference from the original definition, except the fungi in group D causing moderate decay (Nilsson, 1985).

Chaetomium globosum was the representative soft-rot fungus used in this experiment. The graph in Figure 4.3.1.4. shows the effect of adding DDAC to the growth medium. The

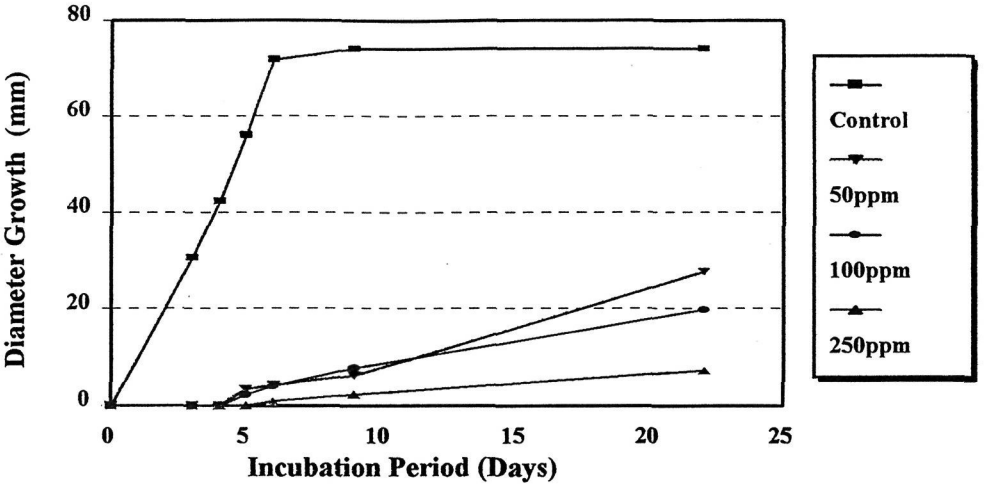


Figure 4.3.1.4.: The growth of C. *globosum* on media containing DDAC.

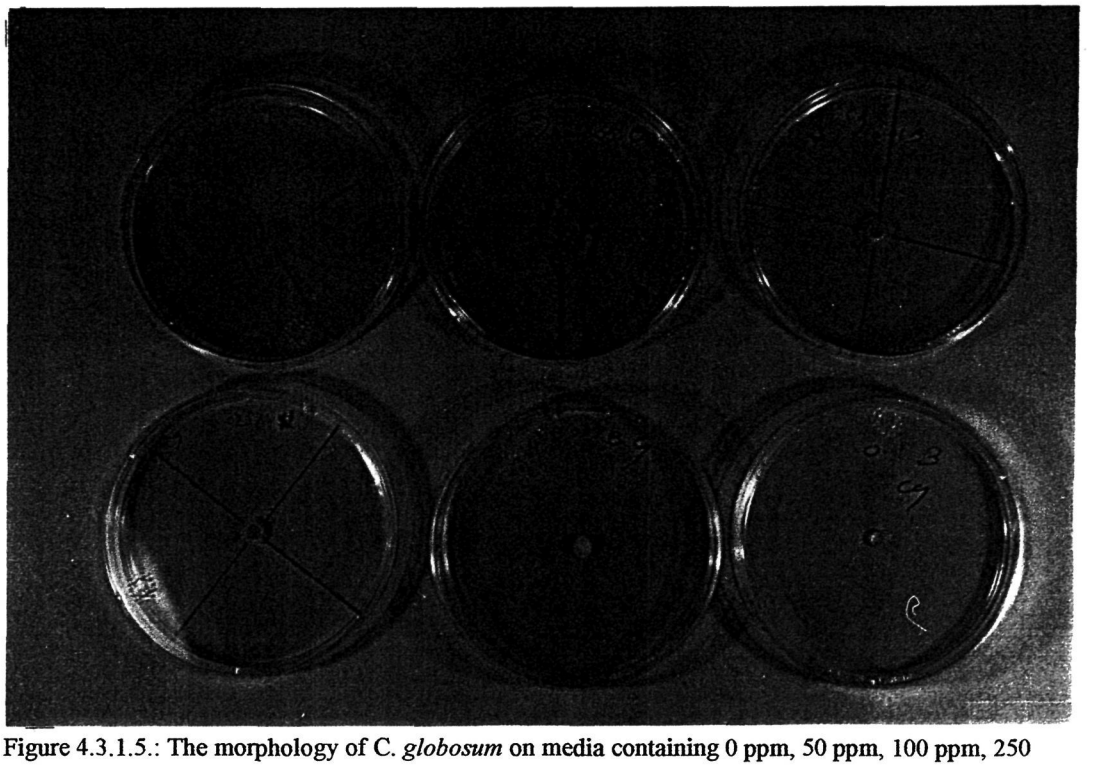


Figure 4.3.1.5.: The morphology of C. *globosum* on media containing 0 ppm, 50 ppm, 100 ppm, 250 ppm, 500 ppm and 1,000 ppm DDAC after a ten day incubation period (from top to bottom, left to right).

growth of C. *globosum* on the control medium was linear, 11.5∓1.1 mm/day, until it had totally covered the plate (six days). The C. *globosum* growth rate on plates treated with DDAC declined. The DDAC treated plates did show a lag phase of four to five days before any growth was observed. Growth after a lag phase may indicate that the fungus had developed a tolerance to DDAC, possibility as a result of enzyme modification. After this initial delay in growth, the growth rates on plates treated with 50 ppm and 100 ppm DDAC were statistically the same, with a reduced rate compared with the control plates of 1.2∓0.1 and 1.4∓0.1 mm/day, respectively. A further decline in the growth rate of C. *globosum* was observed on plates treated with 250 ppm DDAC(0.4∓0.04 mm/day). The growth of C. *globosum* was completely control by 500 ppm DDAC in the media. Figure 4.3.1.5. shows that after a ten day incubation period there was very little growth on any of the DDAC treated plates. Any growth present was very thick and limited to the area around the inoculating core, indicating stress. From these results it can be concluded that DDAC was very effective against C. *globosum* even at low concentrations.

The influence of CAC on the growth of C. *globosum* can be seen in Figure 4.3.1.6. On the plates amended with 50 ppm and 100 ppm CAC, the growth rate declined slightly compared with the control plates growth rate. The growth rate went from 11.5∓1.1 mm/day to 7.8∓0.3 and 6.8∓0.4 mm/day after the addition of 50 ppm and 100 ppm CAC. These results suggest that C. *globosum* has a slight tolerance to low levels of CAC. However, the growth rate dropped significantly to 2.4∓0.1 mm/day after the addition of 250 ppm CAC. The

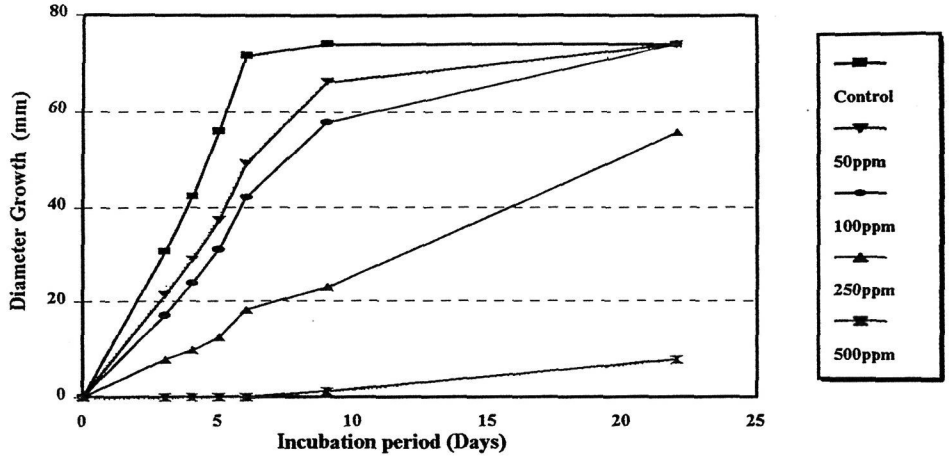


Figure 4.3.1.6.: The growth of C. *globosum* on media containing CAC.

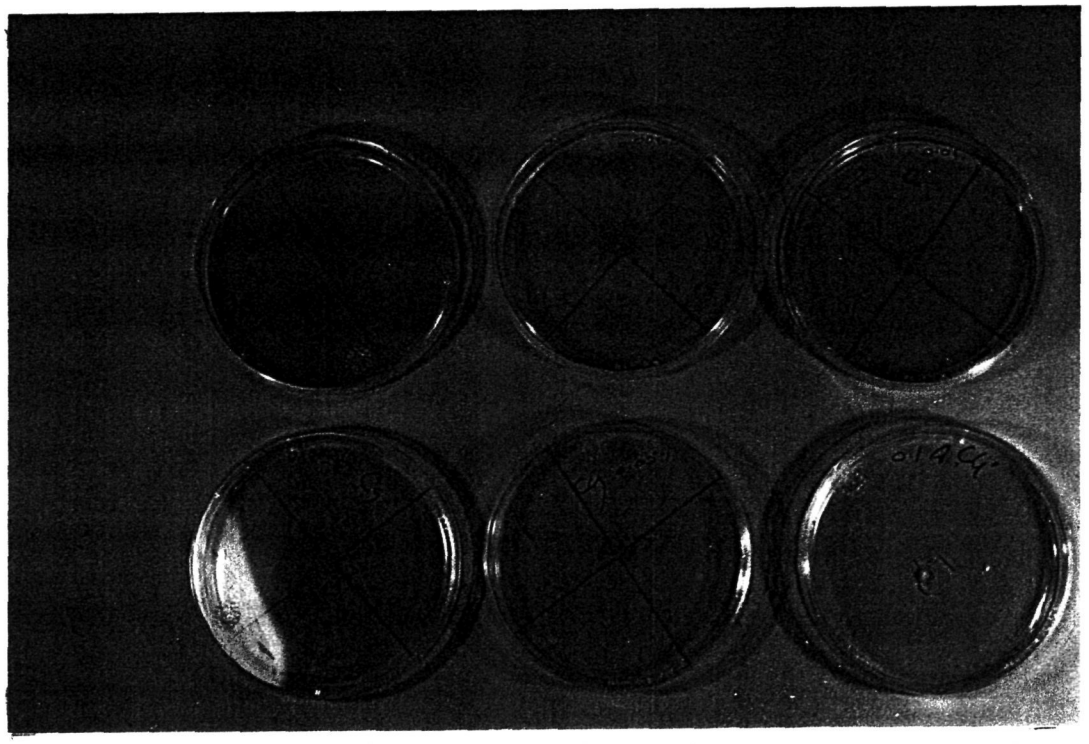


Figure 4.3.1.7.: The morphology of C. *globosum* on media containing 0 ppm, 50 ppm, 100 ppm, 250 ppm, 500 ppm and 1,000 ppm CAC after a ten day incubation period (from top to bottom, left to right).

addition of 500 ppm CAC the growth rate was 0.4∓0.04 mm/day. Figure 4.3.1.7. shows C. *globosum* after a ten day incubation period. It can be seen that the plate treated with 1,000 ppm CAC had no fungal growth, while at lower concentrations normal fungal growth occurred. The lowest concentrations had little effect on the fungal morphology, with respect to visual observations. However, on the plate treated with 250 ppm CAC the morphology was distinctly different. The colony structure shows branching occurring less frequently, distance between hyphal branches being greater and mycelium pigment changing. A more ordered mycelium structure occurs to increase the coverage of an unfavorable substrate and in order to optimize the chances of reaching a more suitable substrate (Watkinson, 1983). These results indicate that DDAC was more effective against C. *globosum* than CAC. However, findings by early workers that had suggested that n-alkyldimethylbenzyl ammonium chlorides were effective against C. *globosum* (Butcher, Hedley & Drysdale, 1977).

Decay Fungi.

The term "decay" will be applied strictly to effects produced in the wood by the action of fungi belonging to the class basidiomycetes. Two major types of basidiomycete decay are recognized, and these are based on action within the wood and on reaction of the causal fungus to tests such as extracellular oxidase (white-rot fungi). When only the carbohydrate fraction of the wood is removed to a significant degree the decay is referred to as 'brown-rot'. When both the carbohydrate and lignin fractions of wood are removed, the decay is referred to as 'white-rot' (Wilcox, 1973).

Brown-Rot Fungi.

The modern definition of this decay type has changed little and is quite distinct from all other wood deterioration types. It is a form of fungal decay caused by basidiomycetes that mainly degrade the polysaccharides through extensive depolymerization, with little effect on the lignin which Nilsson (1985) classified as group E.

Postia placenta was the brown-rot fungus used in this investigation. Figure 4.3.1.8. shows the effect of DDAC on the growth of P. *placenta*. The plot of the growth rate against time shows a growth rate of 10.6∓0.25 mm/day for P. *placenta* on the control plates. The growth was linear with no lag phase, until the plate was totally covered after nine days. The addition of 50 ppm DDAC to the media reduced the fungal growth rate to 3.2∓0.2 mm/day, with further increases in DDAC concentration producing additional reduction in the growth rate. Plates treated with 100 ppm to 500 ppm DDAC exhibited a delay of three days in the development of the inoculum. The three lower concentrations of DDAC showed growth rates of 2∓0.2, 1.2∓0.1 and 0.6∓0.1 mm/day for plates treated with 100, 250 and 500 ppm DDAC. Growth of P. *placenta* after ten days of incubation can be seen in Figure 4.3.1.9. The change in morphology can be clearly seen when DDAC was added to the agar plates. The addition of 50 ppm DDAC resulted in a colony that had increased its aerial mycelium at the edge of the colony yielding a thicker hyphal mat. This increase in aerial mycelium is certainly a response to an unfavorable environment. This feature was more prominent in the plates treated with 100 ppm, 250 ppm and 500 ppm DDAC.

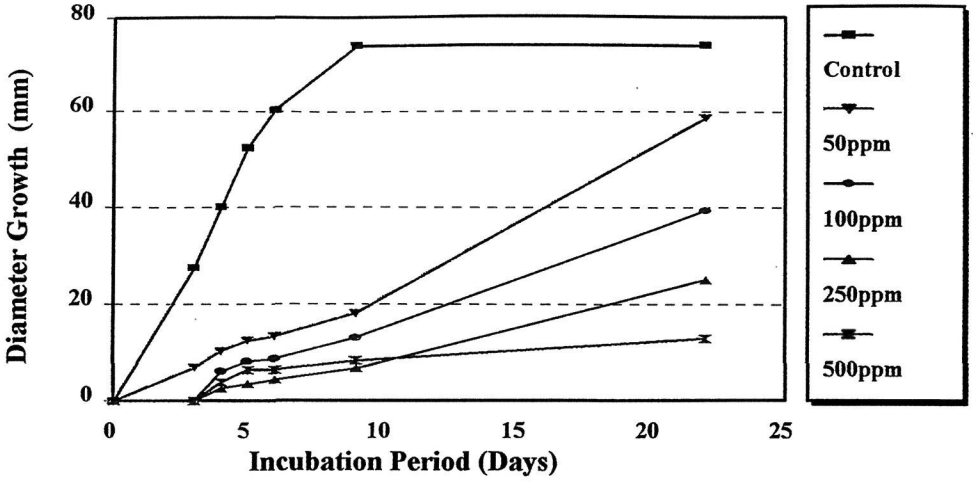


Figure 4.3.1.8.: The growth of P. *placenta* on media containing DDAC.

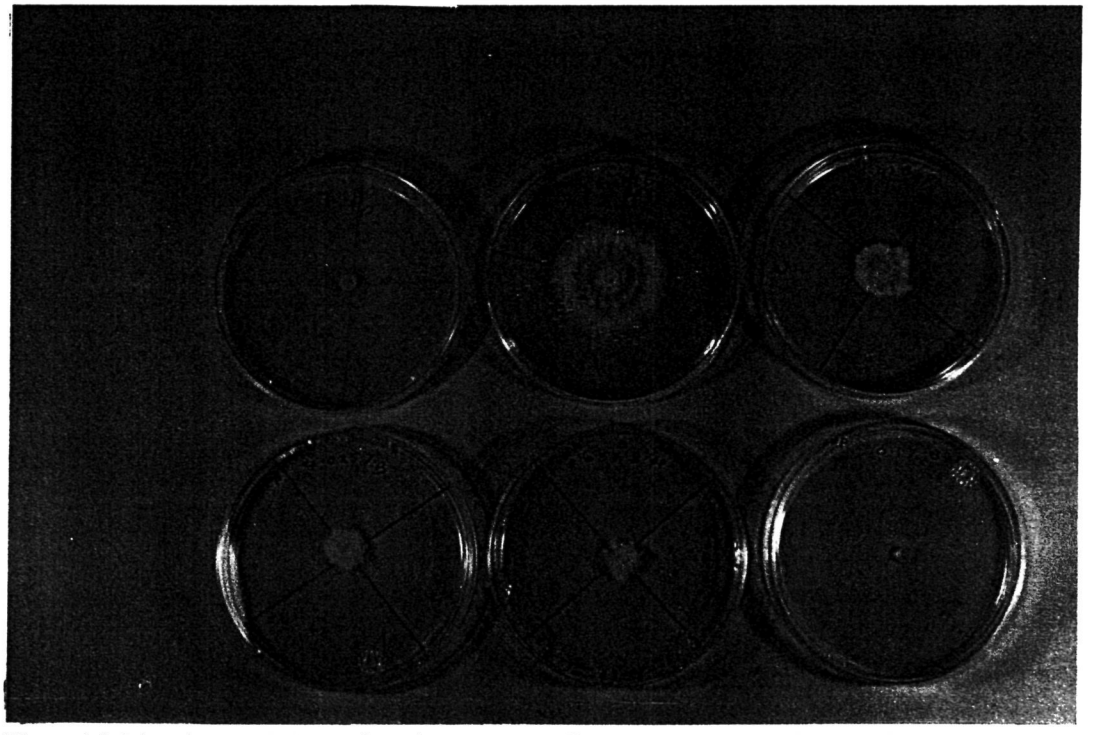


Figure 4.3.1.9.: The morphology of P. *placenta* on media containing 0 ppm, 50 ppm, 100 ppm, 250 ppm, 500 ppm and 1,000 ppm DDAC after a ten day incubation period (from top to bottom, left to right).

The influence of CAC on the growth of P. *placenta* can be seen in Figure 4.3.1.10. The graph shows that the addition of 50 ppm CAC reduced fungal growth rate from 10.6∓0.25 mm/day to 2.6∓0.2 mm/day. With an additional increase in the concentration of CAC, fungal growth was totally inhibited. As with DDAC, there was an initial three day delay after which fungal growth increased dramatically to almost cover the plate in 22 days. The effect of CAC after a ten day incubation can be seen in Figure 4.3.1.11. The fungus growing on the plate with 50 ppm CAC has a slightly thicker hyphal mat and more aerial mycelium present, than observed on the control medium. The change in morphology was not as distinct as in the DDAC plates.

From these results, it may be concluded that CAC was more effective than DDAC against P. *placenta*. However, previous research using a wood substrate had suggested that DDAC was more effective than CAC against P. *placenta* (Butcher, Preston & Drysdale, 1977). Thus the above results illustrate the effect of substrates on fungal growth.

White-Rot Fungi.

The modern definition of white-rot is a form of fungal decay caused by basidiomycetes in which extensive breakdown of cellulose, hemicellulose and lignin occurs. These fungi are members of group C in the classification by Nilsson (1985).

Trametes versicolor was the white-rot fungus used in this experiment. Figure 4.3.1.12. shows the effect of adding DDAC to the medium on growth of T. *versicolor*. Normal linear growth was observed on the control plates until they were totally covered (six days) with a growth rate of 13.6∓0.4 mm/day. The presence of 50 ppm DDAC reduced growth rate to 3.0∓0.1 mm/day. Any further increase in the DDAC concentration resulted in additional inhibition.

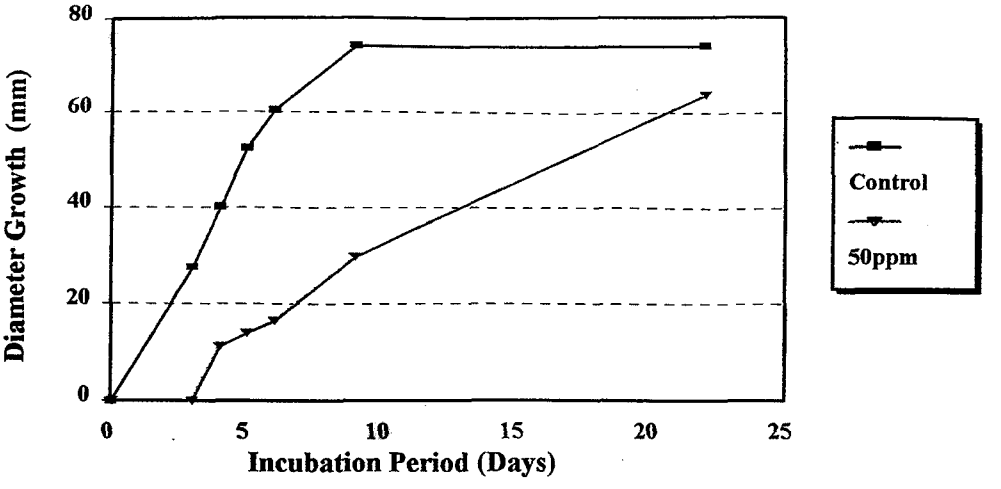


Figure 4.3.1.10.: The growth of P. *placenta* on media containing CAC.

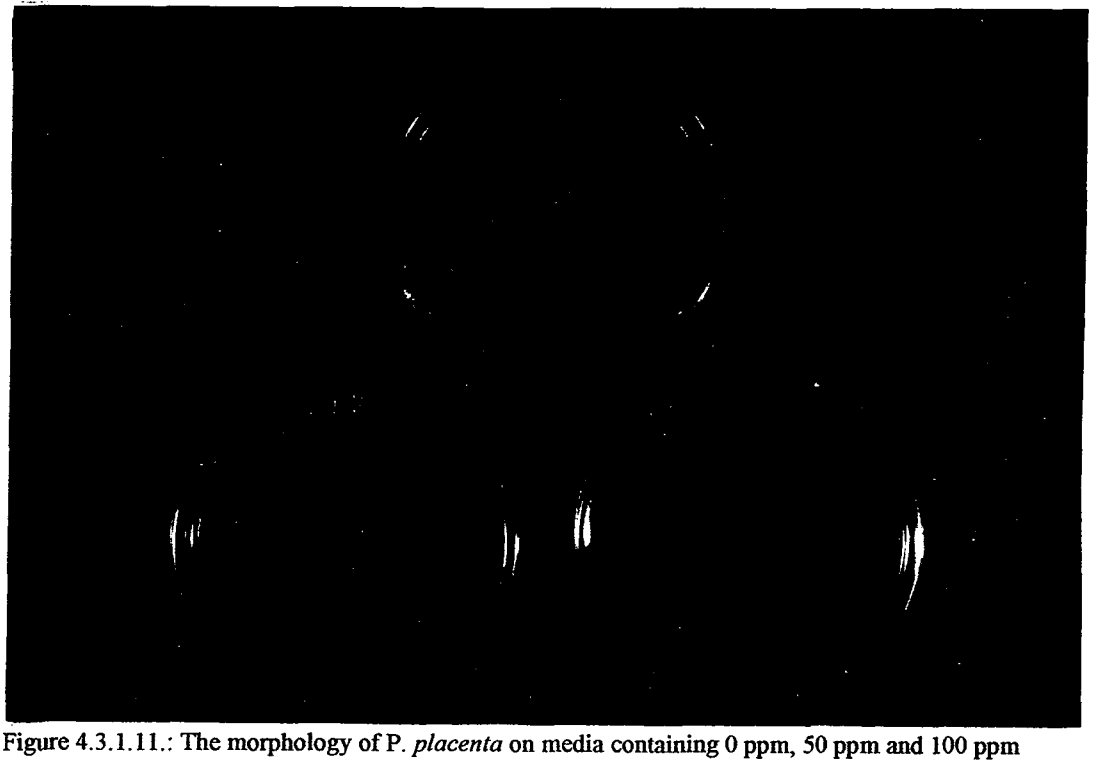


Figure 4.3.1.11.: The morphology of P. *placenta* on media containing 0 ppm, 50 ppm and 100 ppm CAC after a ten day incubation period (from top and bottom, left to right).

Growth rates on plates treated with 100 and 250 ppm DDAC had statistically the same growth rates of 2.6∓0.2 and 2.4∓0.1 mm/day. Plates treated with 500 ppm DDAC exhibited an initial delay of three days before growth occurred, then the growth rate obtained was 1.4∓0.1 mm/day. After ten days of incubation the reaction of T. *versicolor* to different levels of DDAC can be seen in Figure 4.3.1.13. As the concentration of DDAC in the plates increased the morphology of the colony changed. The hyphal mat of the colony became much thicker as the level of DDAC increased, until the fungus could no longer grow on the medium. On the plates treated with 100 ppm DDAC the outer edges of the colony were much thinner. This could be a result of the fungus becoming adapted to the preservative, so that the presence of the preservative did not have the same effect. This observation corresponded with changes in the linear growth, which also suggested an increase in tolerance (Figure 4.3.1.12.).

The effect of adding CAC to the medium on growth of T. *versicolor* can be seen in Figure 4.3.1.14. Normal linear growth of T. *versicolor* on the control plates was observed, with a rate of 13.6∓0.4 mm/day. The addition of 50 ppm, 100 ppm and 250 ppm CAC resulted in a decline in the growth rates to 3.5∓0.1, 3.0∓0.1 and 2.4∓0.2 mm/day respectively. The growth of T. *versicolor* was totally inhibited by the addition of 500 ppm CAC to the medium. Plates treated with 250 ppm CAC had a three day lag phase after which growth increased. This change was thought to be caused by the fungus overcoming the initial suppression of the growth by CAC. Figure 4.3.1.15. shows the growth of T. *versicolor* after a ten day incubation period. The morphology of the cultures was affected by the presence of CAC, but to a lesser extent than with DDAC. On plates treated with 50 ppm CAC, the

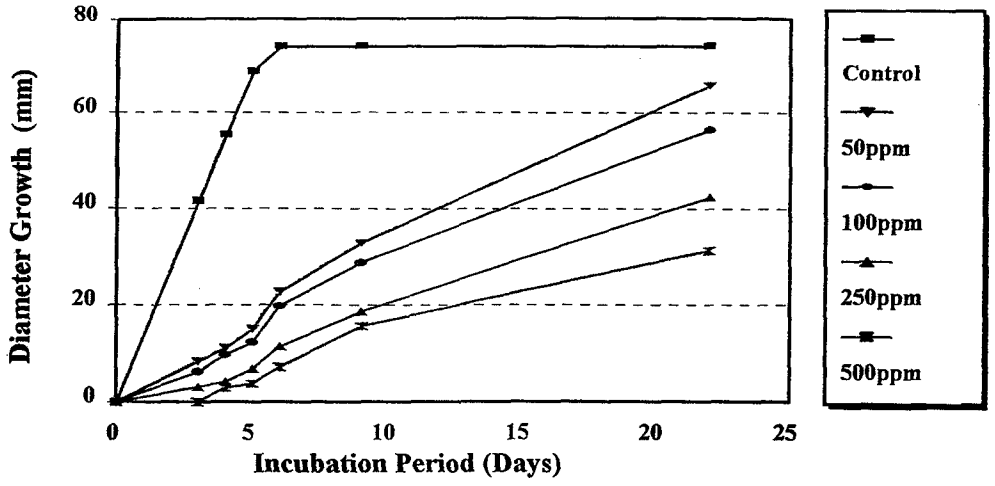


Figure 4.3.1.12.: The growth of T. *versicolor* on media containing DDAC.

Figure 4.3.1.13.: The morphology of T. *versicolor* on media containing 0 ppm, 50 ppm, 100 ppm, 250 ppm, 500 ppm and 1,000 ppm DDAC after a ten day incubation period (from top to bottom, left to right).

hyphal mat was much thinner at the edge of the growing colony, corresponding to the sudden change in growth rate as noted in Figure 4.3.1.14. The thickening may be a result of stress and the growth change indicative of adaptation to the substrate. A similar effect was observed in the plate containing 100 ppm CAC.

CAC appears to be more effective than DDAC against T. *versicolor*. Previous research with DDAC against T. *versicolor* found good control, but variable in its effectiveness against other white rot fungi (Tsunoda & Nishimoto, 1987[b]). CAC has not previously been tested against T. *versicolor*, CAC maybe effective against white-rot decay.

DDAC Versus CAC.

The bioassays showed that DDAC was more effective against A. *niger* and C. *globosum*, while CAC provided greater control against P. *placenta* and T. *versicolor* when added to the media. The effective levels of preservative were different for each individual fungi (Table 4.3.1.1). However, both preservatives were found to be effective against all the test fungi at concentrations of approximately 1,000 ppm or less, as seen in Table 4.3.1.1. One possible exception was A. *niger* where growth occurred on plates treated with 1,000 ppm CAC. However, even in this investigation the growth pattern showed that the complete control would be probably achieved just above 1,000 ppm CAC. These threshold values are much lower than those found for similar fungi on a wood substrate (Butcher, Preston & Drysdale, 1977). Results in Table 4.3.1.1. showed the growth achieved on the treated plates compared with the control plates at six day incubation period being assumed to be 100%. The results indicate that the decay fungi growth was reduced by at least 50% with the addition of either

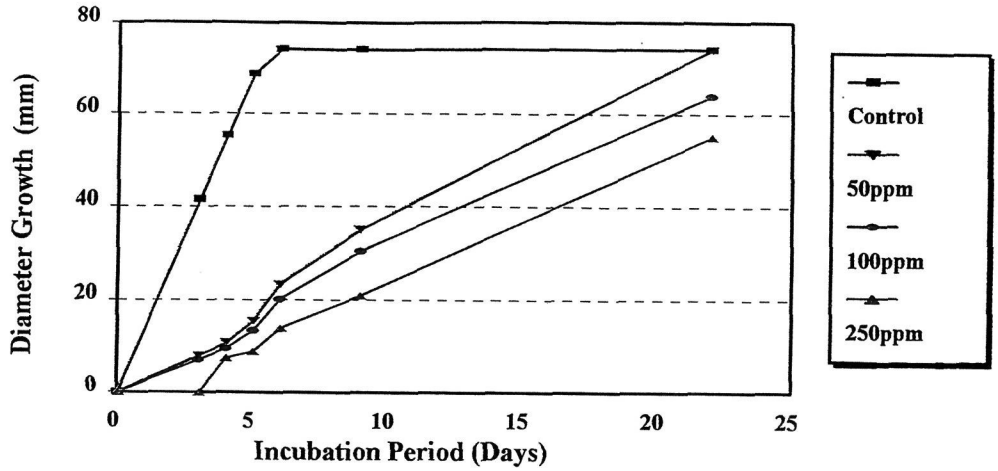


Figure 4.3.1.14.: The growth of T. *versicolor* on media containing CAC.

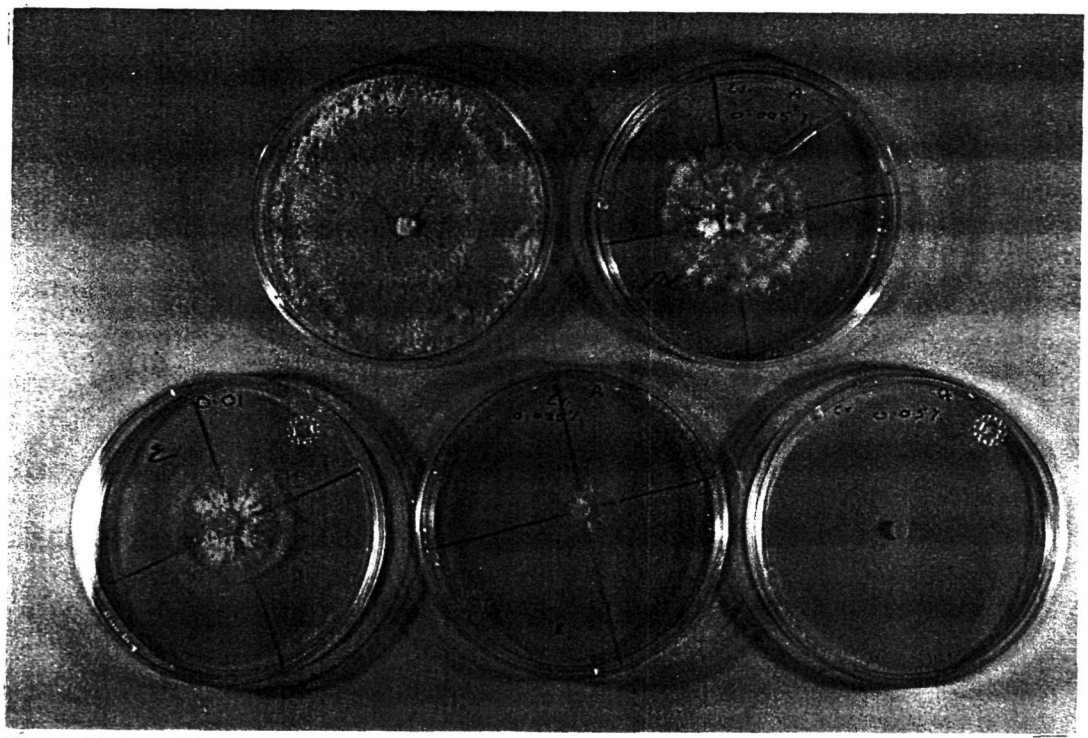


Figure 4.3.1.15.: The morphology of T. *versicolor* on media containing 0 ppm, 50 ppm, 100 ppm, 250 ppm and 500 ppm CAC after a ten day incubation period (from top to bottom, left to right).

CAC or DDAC. Only the C. *globosum* showed greater than 50% growth on CAC at 50 ppm. C. *globosum* appears more sensitive to DDAC than CAC and P. *placenta* was more sensitive to CAC than DDAC, while, T. *versicolor* showed similar results on both chemicals. The results suggest that each organism responses different to the chemicals tested.

The morphology of the culture was changed by the presence of both preservatives. In nature, morphology responses of a fungus to changing conditions might be seen as an essential part of its life cycle. These include, the exploitation of a substratum by production of enzymes and mycelium growth; extension of the colony from the food base over non-nutrient areas; colonization of a second food base; production of different enzymes (depending on the medium) and mycelium growth...(Watkinson, 1983). Changes in the morphology were more distinct when the various fungi were exposed to DDAC. These changes are a response of the fungus to an unsuitable substrate, in this case due to the presence of the preservative. The rate of reproduction may increase to raise the probability of spores landing on a more suitable substrate or the formation of resting spores until the medium becomes more suitable. These changes could result in the production of new enzymes or tolerant strains.

Table 4.3.1.1: Effect of two alkylammonium compounds on the growth of five fungi after a six day incubation period.

Organism	**Fungal Growth (%)**					
Conc (ppm)	0	50	100	250	500	1,000
DDAC						
A. *pullulans*	100	0	0	0	0	0
A. *niger*	100	*90	*70	50*	10*	0
C. *globosum*	100	6	6	1	0	0
P. *placenta*	100	22	15	8	11	0
T. *versicolor*	100	31	27	16	10	0
CAC						
A. *pullulans*	100	0	0	0	0	0
A. *niger*	100	*90	*80	50*	25*	10*
C. *globosum*	100	69	59	25	0	0
P. *placenta*	100	27	0	0	0	0
T. *versicolor*	100	32	27	19	0	0

*Values estimated due to difficulties in measurements.

In the normal bioassay procedure, measurements are only recorded until the control plates are totally covered appears to bias the results. From all of the graphed results it can be seen that some of the fungi in the preservative treated plates had a lag growth phase and an increased growth rate that occurred in most cases after the control plates were totally covered. The various chemical concentrations showed distinct differences after the 22 days. This can be seen in the growth rates. The lag phase was probably due to adaptation by the

fungi, which resulted in the removal of the preservative suppression. Therefore, stopping the measurements when the control plates were totally covered does not account for the ability of the fungus to adapt to a new substrate, after an initial delay. This type of delay is common when yeast is transferred from a glucose based medium to a lactose based medium. The delay is a result of switching on of lactase production that is normally suppressed at the level of the gene by the presence of glucose. It may be suggested that a similar activity was occurring in the treated plates during the lag phase (Moore-Landecker, 1990).

4.3.2. Isolation and Identification of Tolerant Organisms.

Many researchers have shown that AACs are very effective against wood deteriorating fungi in laboratory trials (Butcher, Preston & Drysdale, 1977). However, field trials have not confirmed the laboratory results (Ruddick, 1983). One possible explanation for the poor field performance is the influence of staining fungi on AACs. Initial studies found that toxic threshold values for decay organisms were increased by a pre-exposure to staining fungi (Ruddick, 1986).

DDAC treated lumber isolations were carried out on four types of media in order to obtain and identify fungal species, which may have tolerance to DDAC. When isolating from material such as wood it must be kept in mind that the isolates may not be actively growing in the wood, but in a resting state. Therefore, if they come into contact with a suitable substrate they will proliferate and may out-compete organisms actively growing in the wood. Malt media will allow all fungal types present in the inoculum (wood) to grow. However, the rapidly growing moulds tend to out compete other slower growing fungi, such as basidiomycetes. The addition of tetracycline to the media was used to suppress the faster growing organisms and increase the chances of the less competitive organisms. DDAC was added to the media to identify fungi, which may have a competitive edge in the presence of the DDAC. The levels of DDAC were selected based on the bioassay carried out with five wood deteriorating fungi in chapter 4.3.1. The isolates used in the bioassay were controlled by 1,000 ppm DDAC or less. Therefore, by using 2,500 ppm DDAC, the majority of the organisms should be controlled, except for extremely tolerant organisms. The second

treatment level chosen (100 ppm DDAC) reduced the growth of these wood deteriorating organisms by at least 50% initially. The lower levels of DDAC would prove useful if no fungi showed tolerance at 2,500 ppm DDAC.

Isolations were taken from hem-fir lumber treated with three anti-sapstain formulations containing DDAC, which had been exposed for seven months in the field. Isolation results from pasteurized and non-pasteurized lumber are shown in Tables 4.3.2.1. and 4.3.2.2. The frequency of the organism was based on the number of plates in which it was isolated compared with the total number of plates used for isolation. The frequencies of fungal isolations from both pasteurized and non-pasteurized treated lumber have similar trends. The least number of isolates came from the lumber treated with NP-1™ sapstain formulation. These results may indicate that NP-1™ was providing better protection than F2™ or Timbercote™ against sapstain and moulds. NP-1™ contains IPBC as a secondary biocide. Previous work has shown that both DDAC and IPBC alone are equally effective against stain, mould and decay fungi (Wakeling, Maynard & Narayan; 1993; and Hansen, 1984). However, when they are combined in one formulation they appear to have a synergetic effect (Ward, 1990) giving superior protection. The F2™ antisapstain formulation gave slightly better protection than Timbercote™. The F2™ formulation also contains a secondary biocide, in the form of sodium octaborate that is less effective than IPBC as a biocide. An improvement in AACs against sapstain has been noted by the addition of alkaline solutions (Hulme & Thomas, 1979). This may explain the fewer isolations obtained from the F2™ treated wood. The Timbercote™ formulation contains only DDAC as a biocide, therefore isolations from this lumber may give organisms more likely to be tolerant to DDAC.

Pasteurization of the lumber reduced the frequency of *Aureobasidium* spp., *Ceratocystis* spp., *Gliocladium* spp. and *Verticillium/Acremonium* spp., while *Trichoderma* spp. increased compared with non-pasteurized lumber. All other fungal genera remained unchanged (see Tables 4.3.2.1. & 4.3.2.2.). Similar isolation results were noted between branches, which had been exposed to the forest floor and virgin branches (Rayner & Boddy, 1988). These results suggest that airborne inoculum contains organisms such as *Trichoderma* spp. which can out-compete other organisms and reduce their frequency. Secondly the natural inoculum within the wood prior to pasteurization is different than the air inoculum at the time of exposure.

Non-Pasteurized Wood Isolations.

A wide spectrum of micro-organisms isolated on malt and malt plus tetracycline media changed depending on the antisapstain treatment used (Table 4.3.2.1.). The number of isolates from NP-1™ treated wood were considerably lower than those from either F2™ and Timbercote™ treated wood. The difference can be attributed to the presence of a secondary biocide. For each wood treatment the media type used altered the number and genera distribution of the isolates. The addition of tetracycline to the media caused little change in genera and numbers for fungi obtained. *Trichoderma* spp. and *Penicillium* spp. were isolated with a high level of frequency on the malt and tetracycline amended media. However, when the media contained DDAC these organisms were reduced. On media containing 2,500 ppm DDAC very few different fungal isolates were obtained, with the majority coming from one genus. This was most noticeable from isolations taken from wood treated with Timbercote™. The frequency of *Acremonium/Verticillium* spp. increased from 20% to 80% as the DDAC

Table 4.3.2.1. Isolations from non-pasteurized wood treated with three DDAC formulations.

Organisms isolated	Frequency of Isolation (%)										
	Timbercote				F2				NP-1		
	DDAC (ppm) in Medium										
	c	T	100	2500	c	T	100	2500	c	100	2500
Trichoderma spp.	30	45	20	-	70	30	20	-	20	-	-
Penicillium spp.	40	50	35	-	40	30	10	10	50	-	-
Aureobasidium spp.	5	-	5	-	40	-	10	10	-	-	-
Ceratocystis spp.	20	15	5	5	-	-	-	-	-	10	-
Gliocladium spp.	-	20	-	5	50	50	30	20	-	30	-
Verticillium/Acremonium spp.	5	5	20	80	-	-	-	10	-	-	-
Basidiomycetes	-	-	-	-	10	-	10	-	-	-	-
Mucor	-	5	10	-	-	-	-	-	-	10	-
Bacteria	-	-	-	-	10	-	-	-	-	-	-

T: Tetracycline

c : 0 DDAC

concentration went from 100 ppm to 2,500 ppm. The high level of these organisms isolated from wood treated with Timbercote™ could indicate tolerance (Table 4.3.2.1.). Isolates taken from the wood treated with F2™ and NP-1™ did not show the same frequency of *Acremonium/Verticillium* spp. The failure to recover these isolates is attributed to the presence of the secondary biocides. *Gliocladium* spp. showed a consistent frequency of isolation particularly from the F2™ treated wood. The addition of DDAC to the media produced only a slight reduction in the frequency of this isolate.

Pasteurized Wood Isolations.

When the wood was pasteurized prior to field exposure the distribution of organisms isolated changed, particularly when using the DDAC containing media. Pasteurization will destroy all organisms present in the freshly cut green lumber. Any isolation from pasteurized lumber will be attributed to airborne inoculum. As with non-pasteurized lumber, the antisapstain treatment altered the number and genera of fungi isolated. The number of isolates from NP-1 ™ was considerably lower than F2™ and Timbercote™ treated wood as seen with non-pasteurized lumber. These differences can be attributed to the addition of the secondary biocides. The addition of tetracycline to the media did not affect the genera or numbers of fungi isolated compared with untreated media. *Trichoderma* spp. and *Penicillium* spp. were isolated with a high level of frequency, using both malt media and tetracycline modified media (Table 4.3.2.2.). When media was treated with DDAC the numbers of these organisms were considerably reduced. However, isolation from the Timbercote™ treated wood on media containing 2,500 ppm DDAC, had a 90% frequency of *Penicillium* spp. The

Table 4.3.2.2. Isolations from pasteurized wood treated with three DDAC formulations.

Organisms Isolated	Frequency of Isolation (%)										
	Timbercote				F2				NP-1		
	DDAC (ppm) in Medium										
	c	T	100	2500	c	T	100	2500	c	100	2500
Trichoderma spp.	80	90	70	10	90	60	20	-	60	-	-
Penicillium spp.	30	30	40	90	20	40	70	-	-	30	-
Aureobasidium spp.	20	10	10	-	30	-	20	-	10	-	-
Ceratocystis spp.	-	-	-	-	-	-	-	-	-	-	-
Gliocladium spp.	-	10	-	-	10	-	-	-	-	-	-
Verticillium/Acremonium spp.	-	-	-	-	-	-	-	-	-	-	-
Basidiomycetes	10	10	-	-	-	10	10	-	-	-	-
Mucor	-	-	-	-	-	10	20	-	-	-	-
Bacteria	20	-	-	-	-	-	-	-	-	-	-

T: Tetracycline

c : 0 DDAC

Penicillium spp. obtained were very restricted in their growth, to no more than 3 mm in diameter with a tight conidia formation. Unlike the non-pasteurized lumber no *Acremonium/Verticillium* spp. were isolated and the frequency of *Gliocladium* spp. was also greatly reduced. Pasteurization of the lumber eradicated these fungi and indicates that they inhabit wood and are not associated with the airborne inoculum available.

As can be seen from the results in Table 4.3.2.1., *Verticillium* spp. have a particular tolerance to DDAC. These organisms have not been associated with either wood deteriorating fungi or superficial discolouration. Both *Verticillium* spp. and *Acremonium* spp. isolates were initially classified together due to the difficulty in separating these genera. However, subsequent information has confirmed that 90% of the *Verticillium/Acremonium* spp. were *Verticillium* spp. The *Acremonium* spp. have been associated with soft-rot in decaying wood and standing trees (Rayner & Boddy, 1988). This species was used in a mixed inoculum of soft-rot fungi employed to test DDAC, IPBC and NP-1™(Ward, 1990). Other isolates, which showed promise as a DDAC tolerant organisms were *Gliocladium* spp. that are classified as moulds. They were also used in the fungal mixture to test DDAC, IPBC and NP-1™ (Ward, 1990). The results from that study showed that DDAC did not prevent mould or staining fungi at 5,000 ppm, while NP-1™ did. The same effect was observed with the soft-rot fungi in the above study by Ward (1990). *Verticillium* spp. has not been associated with any deteriorating effects in wood, while it has been quite frequently associated with plant diseases.
A selection of the fungi isolated were chosen for further research (see Table 4.3.2.3.). These fungi were cultured and sent to the National Identification Bureau, Ottawa for identification.

All fungal genera were correctly identified and the species for some of the organisms were identified as shown in Table 4.3.2.3. The isolates were maintained on malt media containing DDAC and utilized in further experiments.

Table 4.3.2.3.: Confirmation of the fungal identification of isolated fungi.

Isolation Code*	Initial Identification	Confirmation
TC-NP-16	*Gliocladium* sp.	*Gliocladium roseum* Bainer Complex
TC-NP-13 (a)	*Verticillium* sp.	*Verticillium bulbillosum* W. Gams & Mulla
TC-NP-13 (b)	*Verticillium* sp.	*Verticillium bulbillosum* W. Gams & Mulla
SB-03 (a)	*Gliocladium* sp.	*Gliocladium roseum* Bainer Complex
TC-NP-18	*Acremonium* sp.	*Acremonium* sp.

*TC: Timbercote™ NP: Non-pasteurized SB: Soil Bed (from laboratory isolations)

4.3.3. Bioassay of DDAC Tolerant Isolates.

The same type of bioassay was used with the DDAC tolerant fungi as previously used for the standard deteriorating fungi. The organisms used in this bioassay were obtained from isolations of DDAC treated wood and appeared to have extreme tolerance to DDAC. These included *Verticillium bulbillosum*, an *Acremonium* sp. and two *Gliocladium roseum* isolates. The two G. *roseum* isolates included one that was isolated from the antisapstain field trials referred to as G. *roseum* FT and one that was isolated from DDAC treated mini stake exposed in a soil bed trial referred to as G. *roseum* SB.

Verticillium spp. are members of the dematiaciaceous hyphomycete genus that produces slimy masses from phialides. These are borne in verticils directly or on branches. They are wide-spread in soil, as pathogens of other fungi, insects and plants. However, the importance of this genus in wood deterioration has not been determined. While this fungus has been isolated from wood it has not been associated with either soft rot or staining problems in wood. However, certain species can produce coloured hyphae that could produce stain within the wood. This fungal genus was included in the staining fungal soup used by Ruddick (1986) which reduced the toxicity of AACs in a standard soil jar exposure.

Gliocladium spp., are members of the hyphomycete genus that produces one-celled hyaline or coloured conidia which form on penicillate condiophores in slimy heads. Verticillate condiophores may also be present with the phialides arranged in whorls of three or four; the conidia are borne at the tips in discrete clusters. The conidia are elongated and often slightly curved. *Gliocladium roseum* is a significant colonizer on the surface of green timber producing white to pink aggregations of spores.

Acremonium spp. are the members of the hyphomycete genus that produce single verticillates, which are not branched similar to *Verticillium* spp. This organism has been associated with soft rot decay in the living tree and has been used as a soft rot testing organism by several workers.

The isolation of specific fungal genera in extreme conditions, such as high DDAC concentrations, can be associated with a lack of competitiveness against more common micro-organisms. However, given the opportunity, the isolate would preferentially grow on a media without AACs, but if it had to compete with other organisms such as *Trichoderma* spp. it may not be able to compete. However, the addition of AACs to the media can reduce these highly competitive organisms allowing less competitive organisms, such as *Verticillium* spp. to proliferate.

Tolerance to DDAC exhibited by the above fungi does not necessary indicate that these organisms will be tolerant to a range of AACs. Previous work with bacteria studying AACs biodegradation showed that bacteria could grow on monoalkyl AACs, but could not utilize the dialkyl AACs (Dean-Raymond & Alexander, 1977). Three structurally distinct AACs were included in this bioassay, which were DDAC, CAC and DMBC (see Table 4.2.3.1.). As with the previous bioassay linear growth measurements were taken over the incubation period. Visual observations were also recorded to account for any morphological changes due to the presence of the AAC in the media.

Didecydimethylammonium chloride.

Didecyldimethylammonium chloride (DDAC) was the dialkyl AACs used in this investigation. The growth of V. ***bulbillosum*** on media that supplemented with DDAC is shown in Figure 4.3.3.1. The growth of V. ***bulbillosum*** on the control plate was quite different from the growth seen with the wood deteriorating fungi in chapter 4.3.1. The growth rates for all three decay fungi was greater than 10 mm/day, while V. bulbillosum had a growth rate of 2.8∓0.1 mm/day on the untreated malt plates. The growth does not show a lag in growth, but does not cover the plate even after 24 days. A longer incubation period would be difficult due the possibility of the agar plates drying out. Therefore, the growth of this particular organism is much slower on malt media than previously tested organisms. The removal of DDAC from the media did not appear to increase the growth rate significantly of the V. ***bulbillosum*** as would be expected if the growth occurred due to the suppression of other more competitive organisms. The addition of DDAC to the media resulted in a small reduction of V. ***bulbillosum*** growth during incubation. All four DDAC concentrations showed similar growth over the first 11 days. After which the growth rates began to diverge. The growth on media containing 100 ppm DDAC had a slightly lower growth rate than the control wt 2.1∓0.1 mm/day. Plates treated with 200 and 800 ppm DDAC had statistically the same growth rate of 1.7∓0.1 mm/day. Surprisingly the growth rate on the plates treated with 800 ppm DDAC was greater than that obtained on the plates treated with 400 ppm DDAC (1.4∓0.1 mm/day) These results were also obvious in Figure 4.3.3.2., where the fungus on plates containing 400 ppm DDAC had the smallest growth at the end of the incubation. The visual observation indicated that the growth on 800 ppm DDAC could not be attributed to

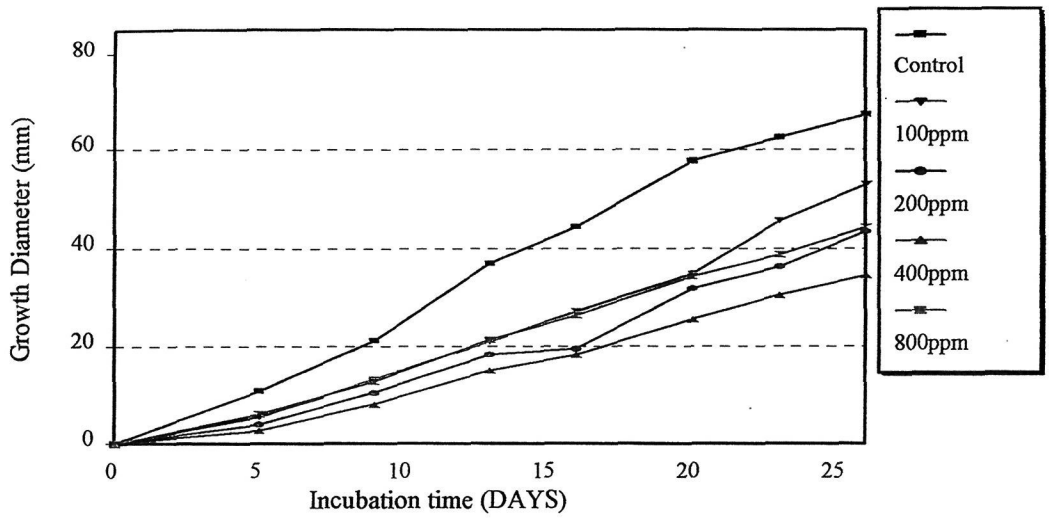


Figure 4.3.3.1.: The growth of V. *bulbillosum* on media containing DDAC.

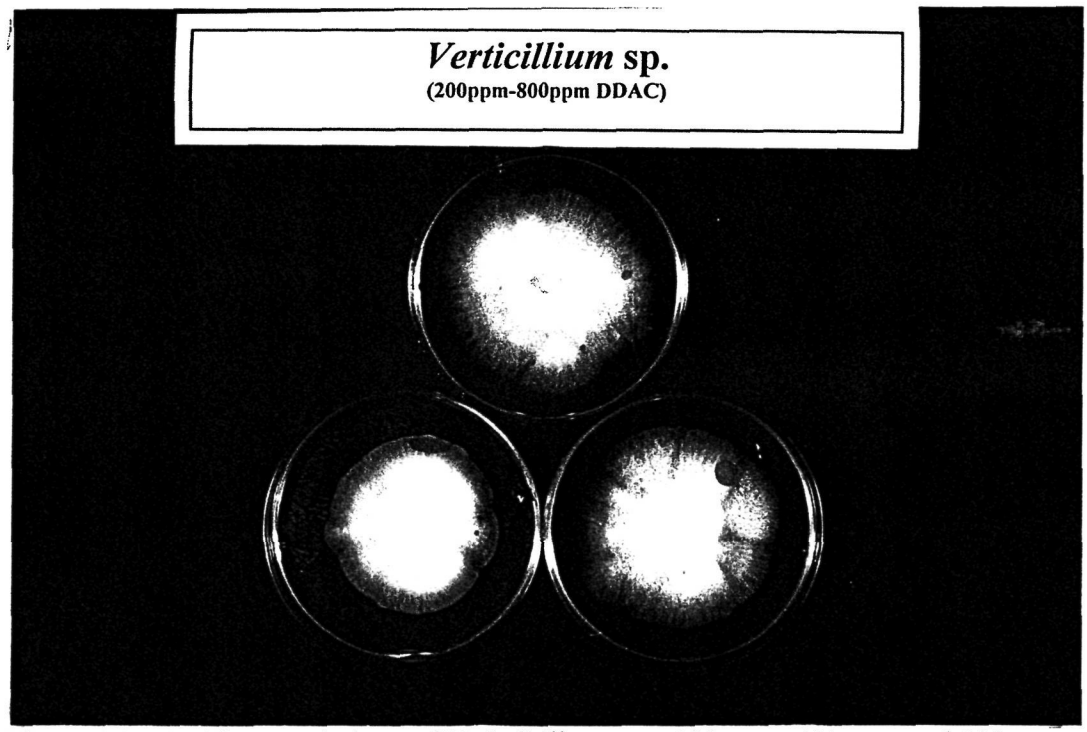


Figure 4.3.3.2.: The morphology of V. *bulbillosum* on 200 ppm, 400 ppm and 800 ppm DDAC after a 35 day incubation period (from top to bottom left to right).

morphological changes (Figure 4.3.3.2.). Morphological changes, which could have accounted for this observation were a thinning and highly structured culture that can occur when the fungus is in an unfavorable medium. This type of structure occurs in order to optimize the chances of reaching a suitable substrate. However, the cultures on 200 ppm, 400 ppm and 800 ppm DDAC were very similar with the outer edges thinning in both. An initial explanation was that the plates were incorrectly labeled, however chemical analysis confirmed that the plates did contain 800 ppm DDAC.

The growth of the *Acremonium* sp. on plates treated with DDAC are shown in Figure 4.3.3.3. The growth rates were very similar to that seen with V. *bulbillosum.* The growth rate on the control plates was the same at 2.8∓0.1 mm/day, which was much slower than that seen for the wood deteriorating fungi in chapter 4.3.1. Again the addition of DDAC resulted in a small drop off in the growth rate with little dose response observed. *Acremonium* sp. growth rate was greatest on plates treated with 100 ppm DDAC at 2.2∓01 mm/day. Like V. *bulbillosum,* the growth rate on 800 ppm was 1.6∓0.04 mm/day, which was similar to that obtained on 200 ppm DDAC (1.8∓0.1 mm/day). The growth rates on these plates were both greater than 400 ppm DDAC for *Acremonium* sp at 1.3∓0.1 mm/day. Visual observation of the cultures again indicated that the growth on 800 ppm DDAC was not due to a thin well structured mycelium mat (Figure 4.3.3.4.).

The G. *roseum* isolates growth on DDAC treated media are shown in Figures 4.3.3.5 and 4.3.3.7. The growth rates of the two isolates are quite different and their morphologies of the colony reflect these differences. G. *roseum* FT growth on the control plates shows an initial lag phase that turns to a linear growth after 4 days at a rate of 3.2∓0.4 mm/day. One possible explanation for this was that subculture media contained DDAC and this change of substrate

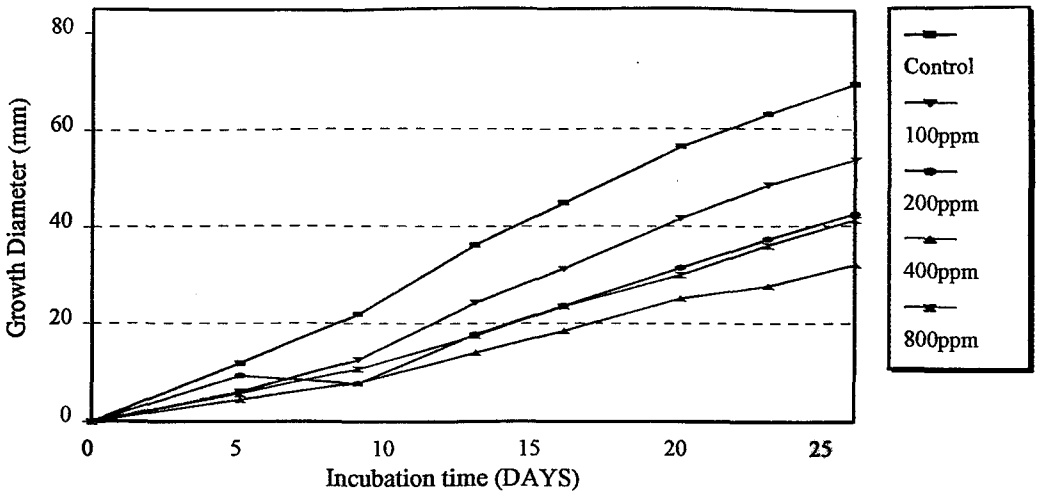


Figure 4.3.3.3.: The growth of *Acremonium* sp. on media containing DDAC.

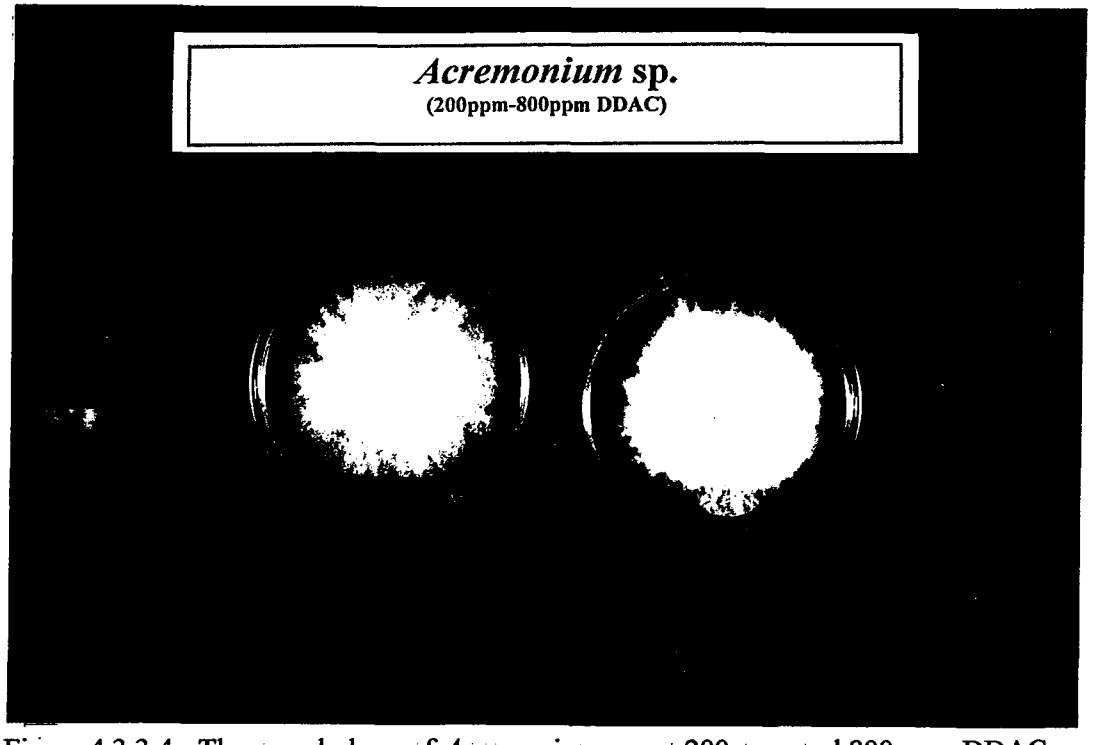


Figure 4.3.3.4.: The morphology of *Acremonium* sp. on 200 ppm and 800 ppm DDAC after a 35 day incubation period (from left to right).

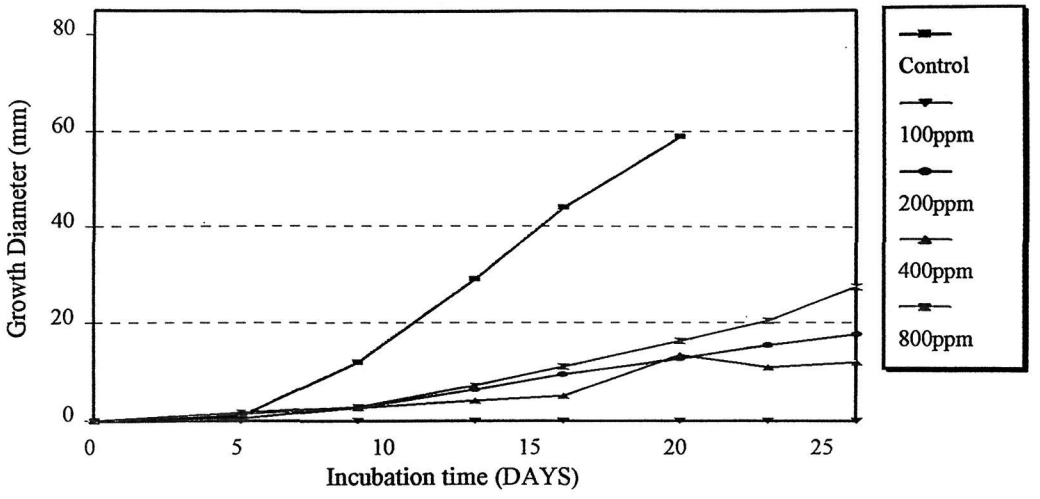


Figure 4.3.3.5.: The growth of G. *roseum* FT on media containing DDAC.

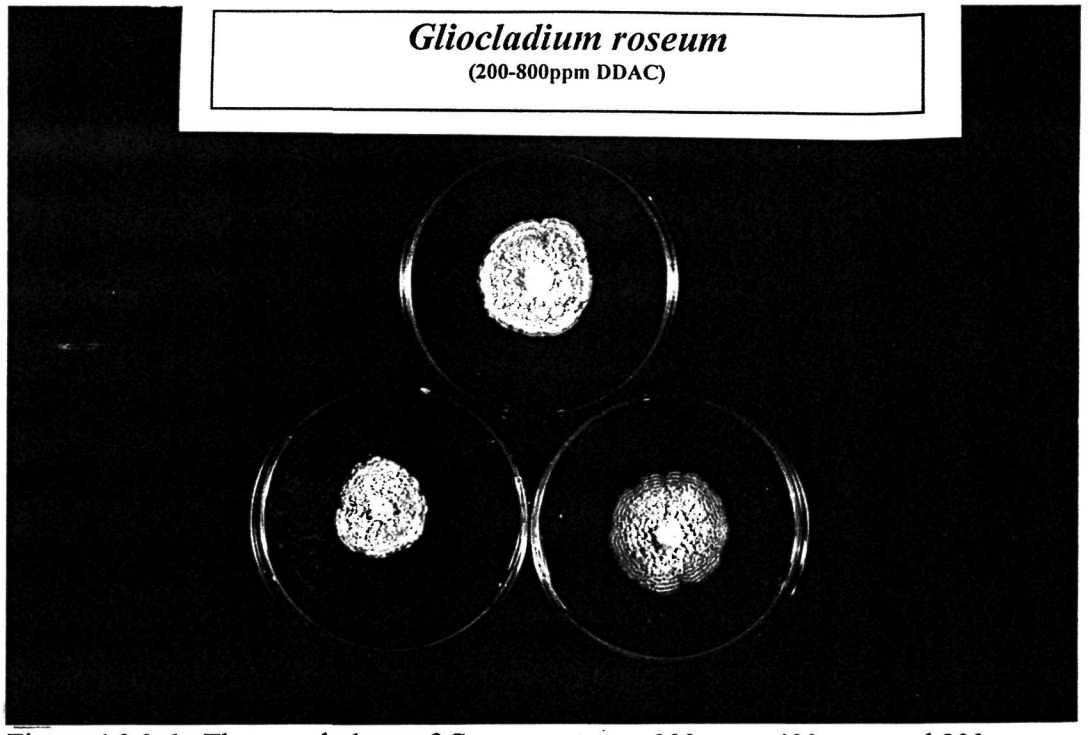
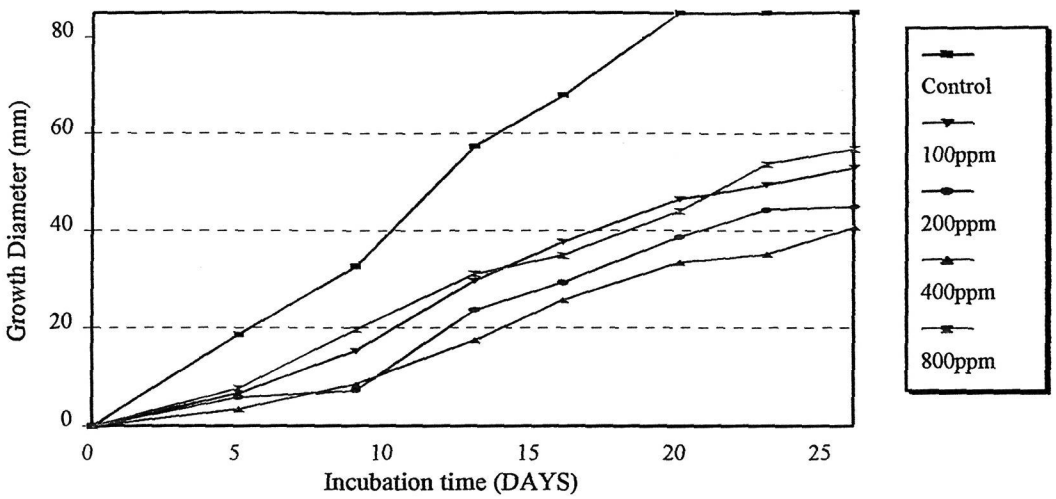


Figure 4.3.3.6.: The morphology of G. *roseum* FT on 200 ppm, 400 ppm and 800 ppm DDAC after a 35 day incubation period (from top and bottom left to right).

required adjustment. The readings were stopped after 16 days due to the plates drying out. The addition of DDAC to the media shows a decrease in the growth rate similar to that observed with wood deteriorating fungi. Results indicate that the growth rates on the plates treated with 200 and 400 ppm DDAC were statistically the same. These rates were 0.7∓0.1 and 0.5∓0.1 mm/day. Growth rate on the plates containing 800 ppm DDAC was 1.1∓0.1 mm/day, which was greater than the lower concentrations. At 100 ppm DDAC all three replicates did not show any growth, which could be a result of either the plates being too dry or the inoculum being non-viable. As with V. *bulbillosum* and the *Acremonium* sp. the diameter of G. *roseum* FT on the plates treated with 800 ppm DDAC was greater than 200 ppm or 400 ppm. The visual observation shown in Figure 4.3.3.6. indicated that the DDAC media resulted in a highly concentrated conidia formation in a small area with highly distinct yellow colouration. Growth of G. *roseum* SB on DDAC treated media are shown in Figure 4.3.3.7. Unlike the G. *roseum* FT the growth rate obtained with G. *roseum* SB was much greater. The growth on the control plates looks similar to that obtained with the wood deteriorating fungi in chapter 4.3.1. Linear growth occurs until the plate has been covered in 20 days. The growth rate of 4.4∓0.2 mm/day was slower than that obtained with the wood deteriorating fungi. The addition of DDAC reduces the growth of G. *roseum* SB, but not to the same extent as seen with G. *roseum* FT, V. *bulbillosum* and the *Acremonium* sp. Results indicate that the growth rates on the plates treated with 100, 200 and 800 ppm DDAC were statistically the same. These rates were 2.3∓0.2, 1.9∓0.2 and 2.3∓0.1 mm/day. Growth rate on the plates containing 400 ppm DDAC was 1.7∓0.1 mm/day, which was less than 800 ppm treated plates and statistically the same as the plates containing 200 ppm DDAC. The morphological difference seen in Figure 4.3.3.8. shows why the growth of G. *roseum* SB was



.Figure 4.3.3.7.: The growth of G. *roseum* SB on media containing DDAC.

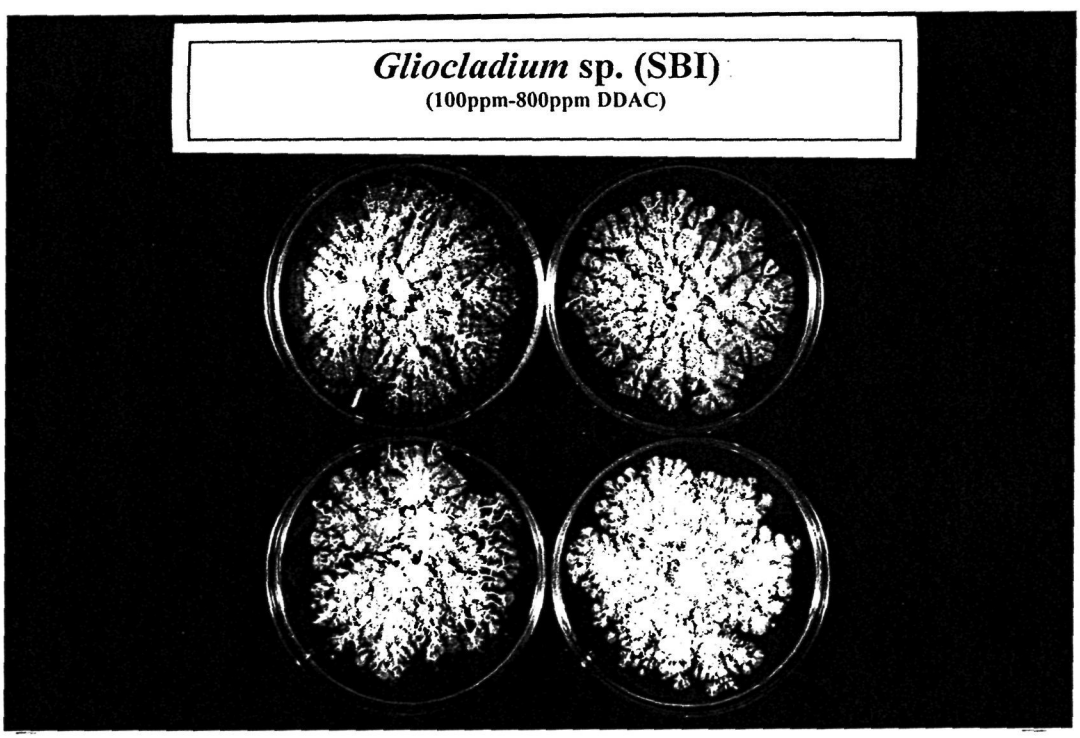


Figure 4.3.3.8.: The morphology of G. *roseum* SB on 100 ppm, 200 ppm, 400 ppm and 800 ppm DDAC after a 35 day incubation period (from top left to bottom right).

greater than G. *roseum* FT as the colony is much thinner and the ordered structure provides a wider coverage, but not necessarily more biomass production. As the DDAC concentration increases the structure becomes less ordered and the yellow colouration decreases.

Trimethylcocoammonium chloride.

Trimethylcocoammoniun chloride (CAC) was the monoalkyl AAC used in this investigation. The growth of V. *bulbillosum* on CAC treated media is shown in Figure 4.3.3.9. The growth of V. *bulbillosum* on the malt media was linear at a rate of 2.8∓0.1 mm/day, but did not totally cover the plate in the 24 day incubation period. The addition of CAC to the malt media gave a slight reduction in growth. The plates containing 100 ppm, 200 ppm and 400 ppm CAC showed statistically the same rate of growth of 2.4∓0.1, 2.6∓0.1 and 2.4∓0.1 mm/day. The addition of 800 ppm CAC to the media resulted in a growth rate of approximately half at 1.4∓0.1 mm/day, this change is also obvious in the visual observations seen in Figure 4.3.3.9. The growth obtained with V. *bulbillosum* on CAC treated media was greater for all concentrations except 800 ppm than seen with DDAC treated plates.
Figure 4.3.3.11. shows the growth of the *Acremonium* sp. on CAC. Again growth on the control was linear, at a rate of 2.8∓0.08 mm/day. The addition of CAC caused a small reduction in growth. The plates containing 100 ppm, 200 ppm and 400 ppm CAC had statistically the same growth rate, of 2.1∓0.1, 2.2∓0.2 and 2.7∓0.5 mm/day over the incubation period. The addition of 800 ppm CAC to the malt media resulted in a slower growth rate (1.4∓0.1 mm/day), which became more obvious over time. This was similar to that observed with V. *bulbillosum* on media containing 800 ppm CAC. This was very

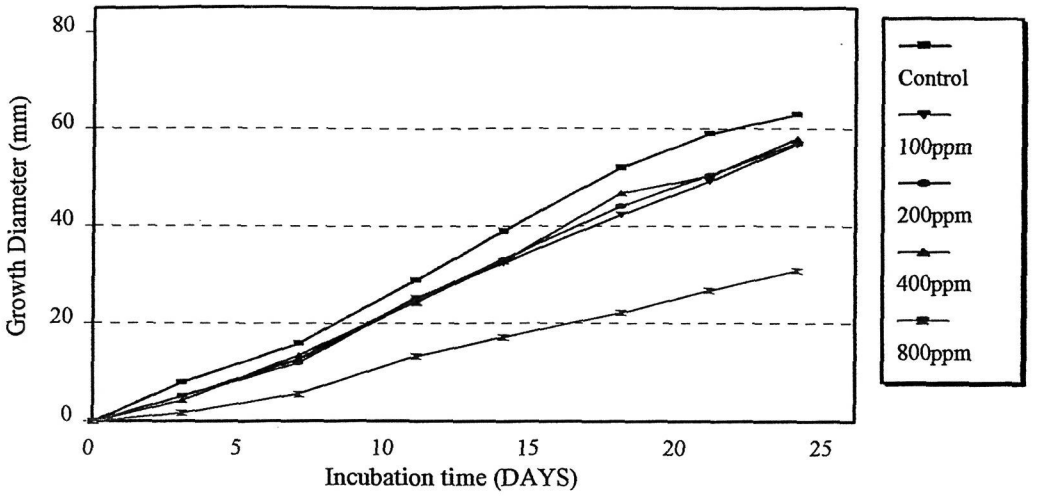


Figure 4.3.3.9.: The growth of V. *bulbillosum* on media containing CAC.

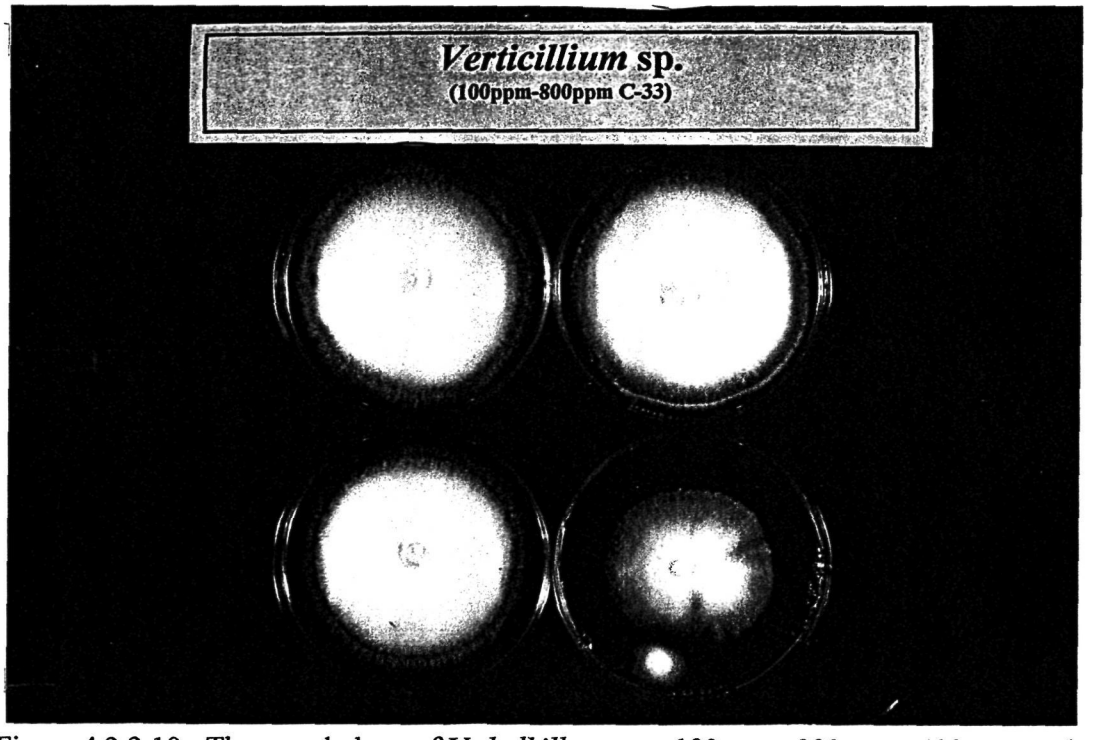


Figure 4.3.3.10.: The morphology of V. *bulbillosum* on 100 ppm, 200 ppm, 400 ppm and 800 ppm CAC after a 35 day incubation period (from top left to right bottom).

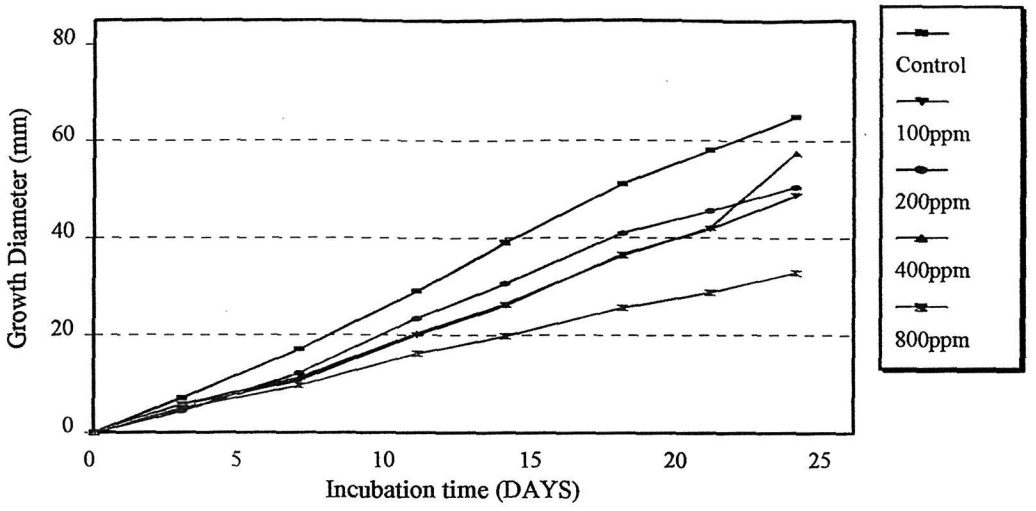


Figure 4.3.3.11.: The growth of *Acremonium* sp. on media containing CAC.

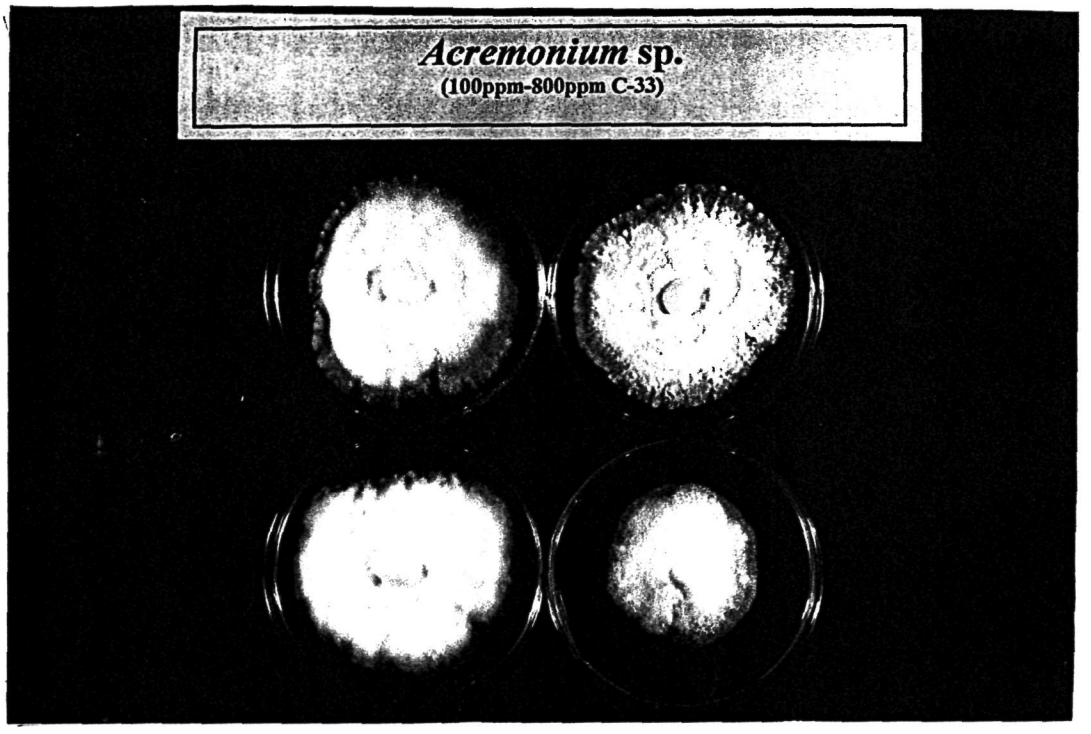


Figure 4.3.3.12.: The morphology of *Acremonium* sp. on 100 ppm, 200 ppm, 400 ppm and 800 ppm CAC after a 35 day incubation period (from top left to bottom right).

obvious in the visual observation seen in Figure 4.3.3.12. The growth rates observed in the 100 ppm, 200 ppm and 400 ppm CAC were similar to that obtained with DDAC. *Acremonium* sp. growth did not appear to be effected differently with either dialkyl AACs or monoalkyl AACs.

The G. *roseum* growth on CAC treated plates are shown in Figures 4.3.3.13 and 4.3.3.15. The growth of G. *roseum* FT on the malt media had an initial lag phase followed by a linear phase of 3.0∓0.4 mm/day that had to be stopped early due to sporulation that covered the edge of the plates preventing accurate measurements. The addition of CAC to the malt media showed a reduction in growth rate to 1.6∓0.1, 1.8∓0.2 and 1.5∓0.2 mm/day on plates treated with 100 ppm, 200 ppm and 400 ppm CAC, which were statistically the same. All three concentrations show a similar lag phase before linear growth occurred, which was not as rapid as on the control plates. With an addition of 800 ppm CAC to the malt media, the growth of G. *roseum* FT was significantly reduced to 0.4∓0.04 mm/day. This observation was confirmed visually as seen in Figure 4.3.3.14. The morphology of the G. *roseum* FT was quite different from the plates treated with DDAC that had a concentrated conidia mat. These observations suggest that the CAC was not as effective against G. *roseum* FT compared with DDAC. Growth measurements also agree with visual observation were the growth on CAC was greater than the media containing DDAC. The growth of G. *roseum* SB on the CAC containing media are shown in Figure 4.3.3.15. The growth of G. *roseum* SB on the control media showed linear growth rate of 4.2∓0.1 mm/day until it covered the plate after 20 days. Media containing with 100 ppm, 200 ppm and 400 ppm CAC showed a slight reduction in the growth rates of 3.1∓0.1, 3.1∓0.2 and 3.1∓0.2 mm/day, which was linear until it reached

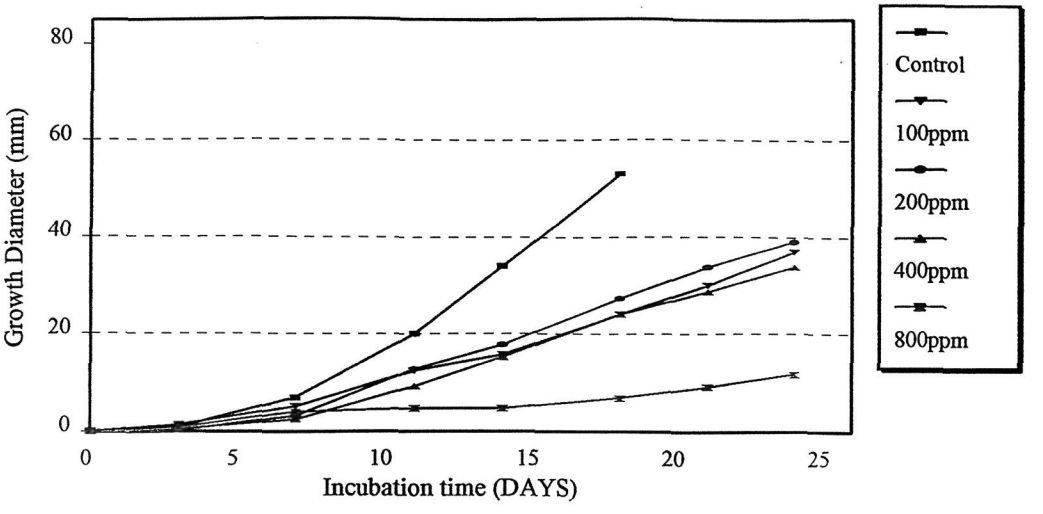


Figure 4.3.3.13.: The growth of G. *roseum* FT on media containing CAC.

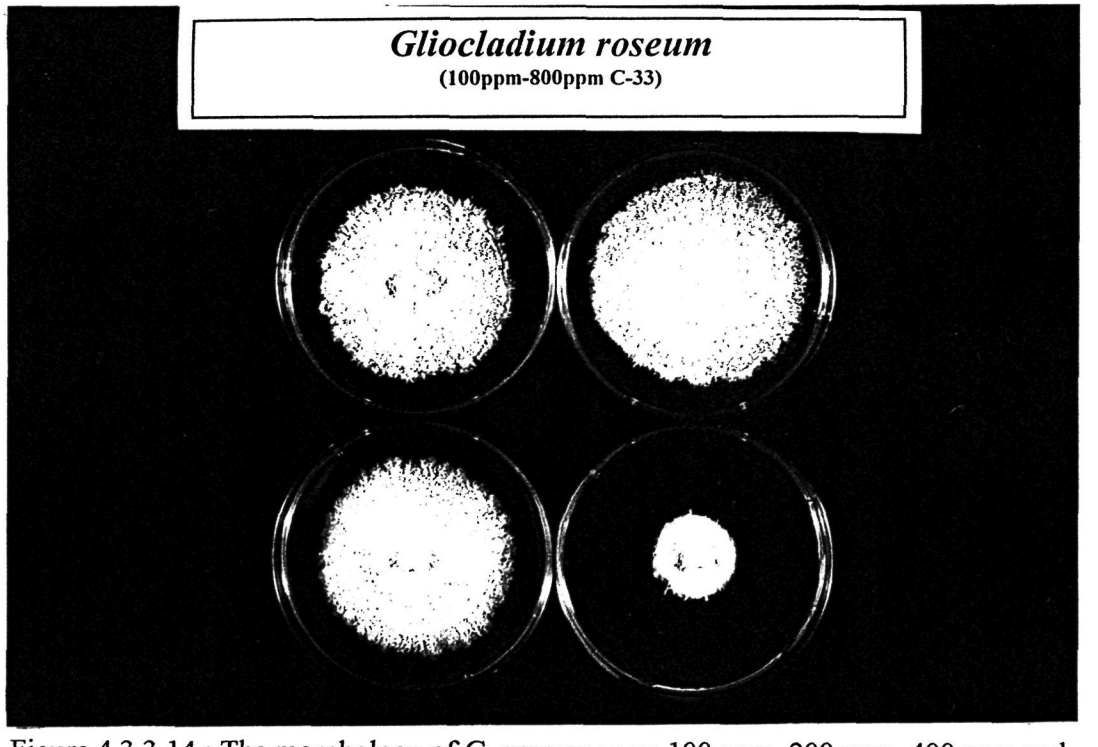


Figure 4.3.3.14.: The morphology of G. *roseum* FT on 100 ppm, 200 ppm, 400 ppm and 800 ppm CAC after a 35 day incubation period (from top left to bottom right).

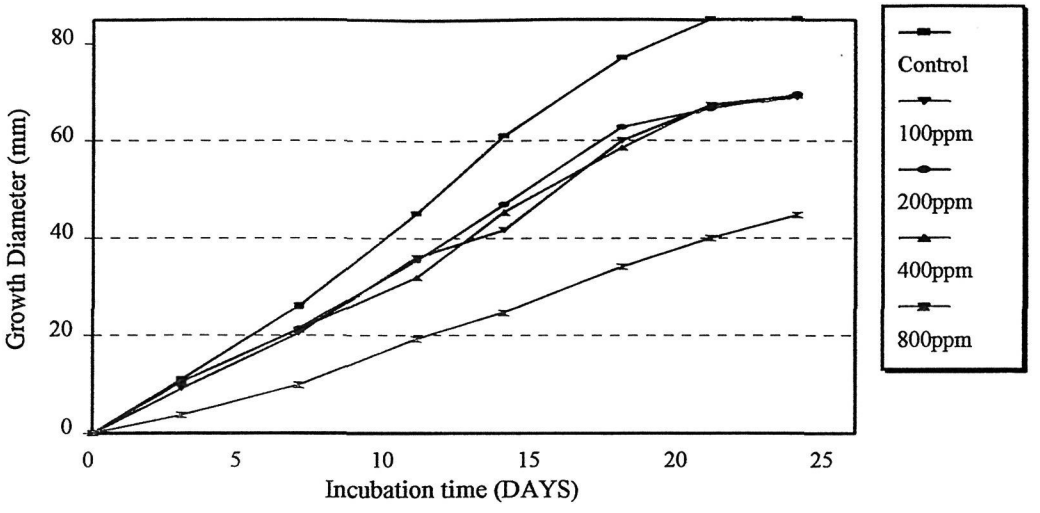


Figure 4.3.3.15.: The growth of G. *roseum* SB on media containing CAC.

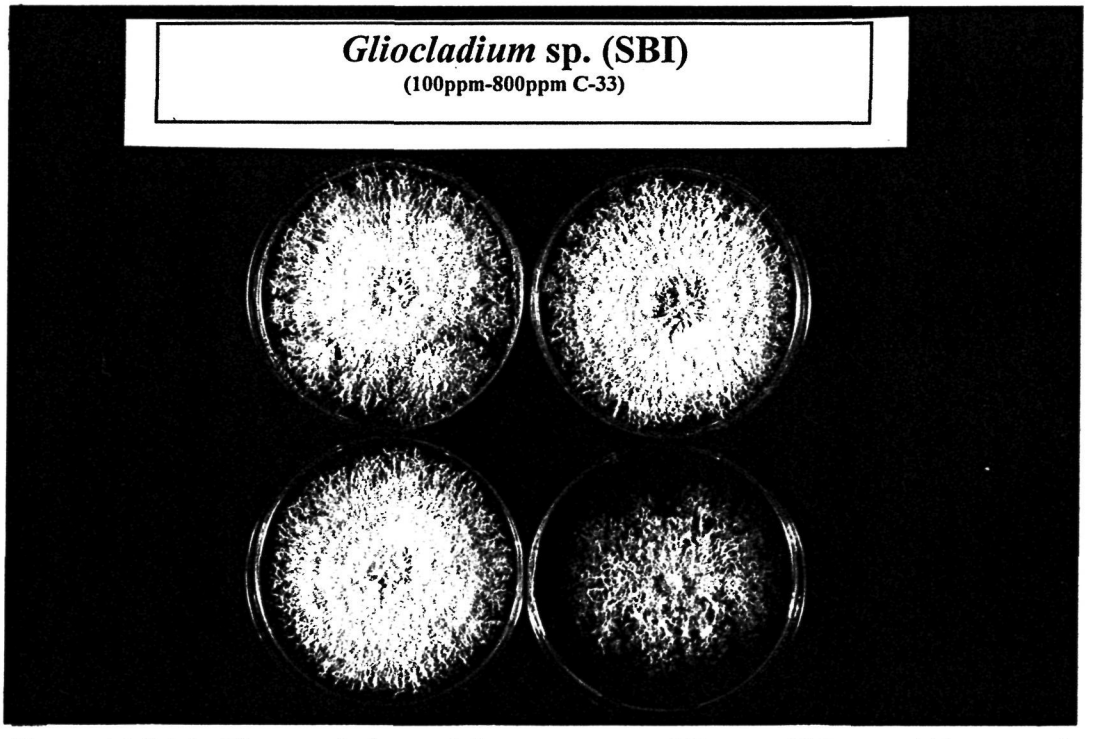


Figure 4.3.3.16.: The morphology of G. *roseum* SB on 100 ppm, 200 ppm, 400 ppm and 800 ppm CAC after a 35 day incubation period (from top left to bottom right).

a stationary phase after 20 days. The addition of 800 ppm CAC lead to a significant decline in the growth rate to 2.0∓0.1 mm/day. This reduction was observed visually (Figure 4.3.3.16.) and the morphology was noted as being quite different from that in the plates treated with 100 ppm, 200 ppm and 400 ppm CAC. The morphology of G. *roseum* SB was not as thin or structured as that seen with DDAC. This indicates that CAC was not as effective at controlling the growth of G. *roseum* SB as was the DDAC. Growth rate measurements also show that G. *roseum* SB growth was greater on CAC treated media than on media treated with DDAC except for 800 ppm DDAC that were the same.

Alkylbenzyldimethylammonium chloride.

Alkylbenzyldimethylammonium chloride (DMBC) was the benzyl AAC used in this investigation. The growth of V. *bulbillosum* on DMBC treated media is shown in Figure 4.3.3.17. The growth rate of V. *bulbillosum* on malted media was linear at 2.8∓0.1 mm/day, but not as rapid as with wood deteriorating fungi. The addition of 100 ppm, 200 ppm and 400 ppm DMBC did not affect the growth rate of V. *bulbillosum* with rates of 2.7∓0.1, 2.7∓0.2 and 2.6∓0.1 mm/day. However, the addition of 800 ppm DMBC shows a significant reduction in the growth rate to 1.4∓0.1 mm/day. This reduction was also obvious under visual observation as seen in Figure 4.3.3.18. The morphology of the V. *bulbillosum* on plates treated with 800 ppm DMBC was quite different with the edges thinning. Sporulation can be seen on the plate treated with 800 ppm DMBC that is a second indication of stressed growth of an organism. Growth on DMBC was very similar to growth obtained

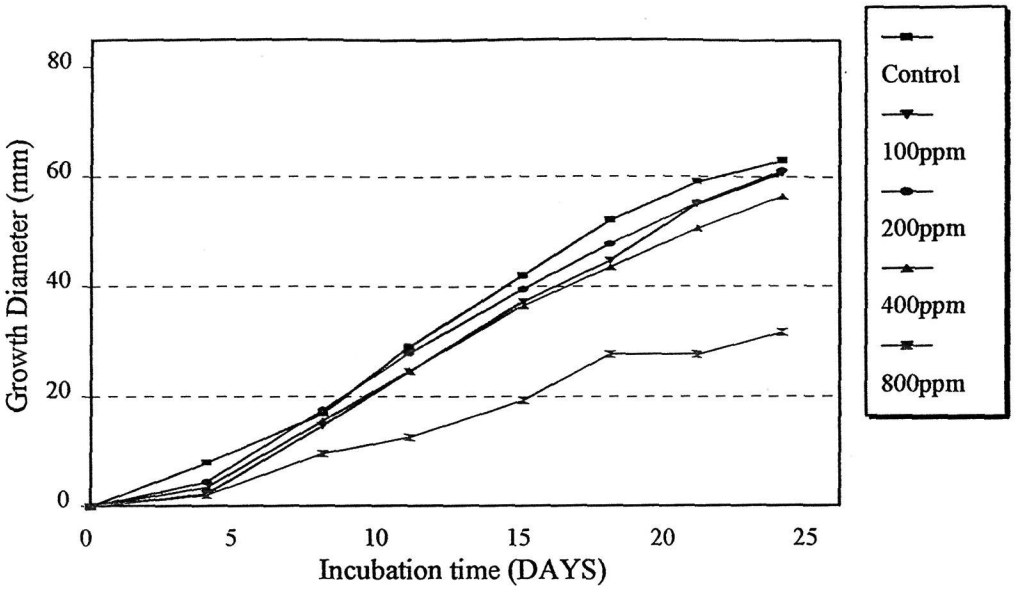


Figure 4.3.3.17.: The growth of V. *bulbillosum* on media containing DMBC.

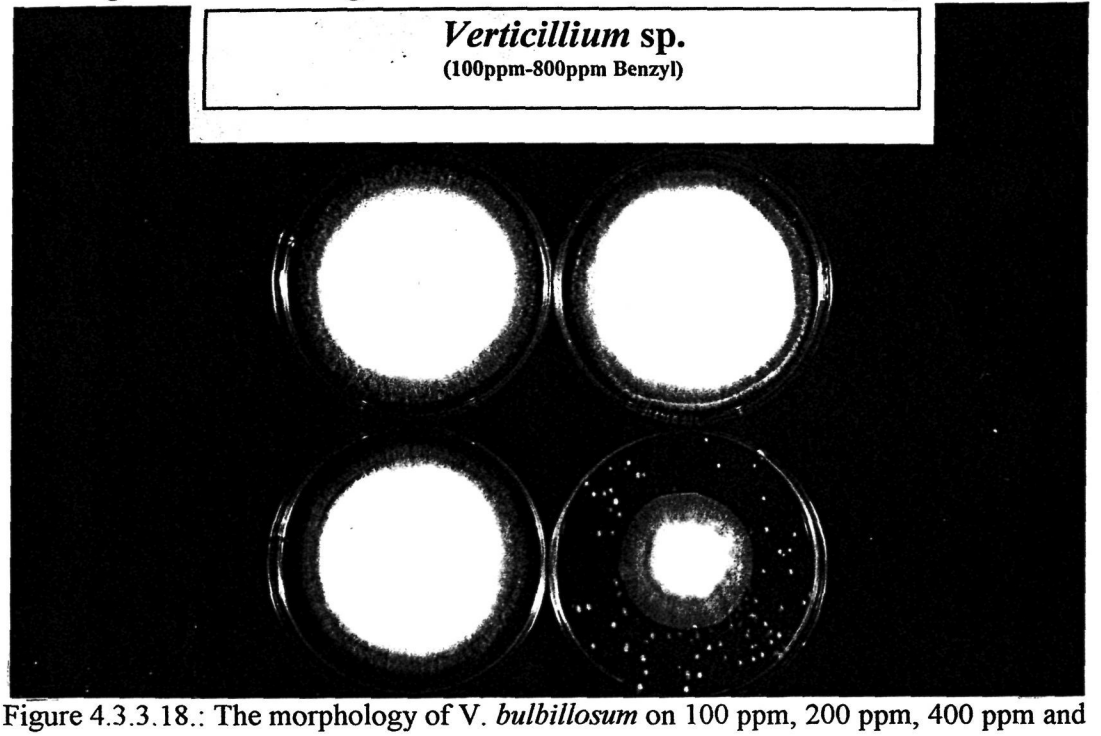


Figure 4.3.3.18.: The morphology of V. *bulbillosum* on 100 ppm, 200 ppm, 400 ppm and 800 ppm DMBC after a 35 day incubation period (from top left to bottom right).

on the CAC treated plates, which is greater than DDAC treated plates (for the 100 ppm, 200 ppm & 400 ppm).

Figure 4.3.3.19. shows the growth of *Acremonium* sp. on media containing DMBC. The growth of the *Acremonium* sp. on malt media was linear at a rate of 2.8∓0.07 mm/day, but not as rapid as normal decay test fungi. The addition of 100 ppm, 200 ppm and 400 ppm DMBC to the media resulted in little change in growth rates to 2.2∓0.1, 2.6∓0.1 and 2.3∓0.2 mm/day. However, the addition of 800 ppm DMBC resulted in a significant reduction in the rate to 1.4∓0.1 mm/day. This reduction is clearly shown in Figure 4.3.3.20. where the culture on 800 ppm DMBC was half the that of the other culture. The morphology was different for the *Acremonium* sp. grown on 800 ppm DMBC where the aerial mycelium was prominent. *Acremonium* sp on plates with less than 800 ppm DMBC had variable morphology. The growth on DMBC was greater than growth on DDAC and CAC treated plates. The growth rates of the G. *roseum* FT on media containing DMBC are shown in Figures 4.3.3.21 and 4.3.3.22. The growth of G. *roseum* FT on the malt media shows an initial delay with a linear growth at a slower rate of 3.0∓0.5 mm/day than normally observed for wood deteriorating fungi. The addition of 100 ppm (2.7∓0.3 mm/day) and 200 ppm (2.5∓0.1 mm/day) DMBC into the malt media did not affect the growth rate of G. *roseum* FT. The addition of 400 ppm DMBC reduced the growth rate to 2.2∓0.1 mm/day. Further addition of 800 ppm DMBC to the media resulted in a significant reduction in the growth rate to 0.7∓0.1 mm/day. This was also observed visually in Figure 4.3.3.21. where the colony diameter change was very dramatic. The morphology was quite different in these cultures than others previously observed. The colony appearance was closer to that observed

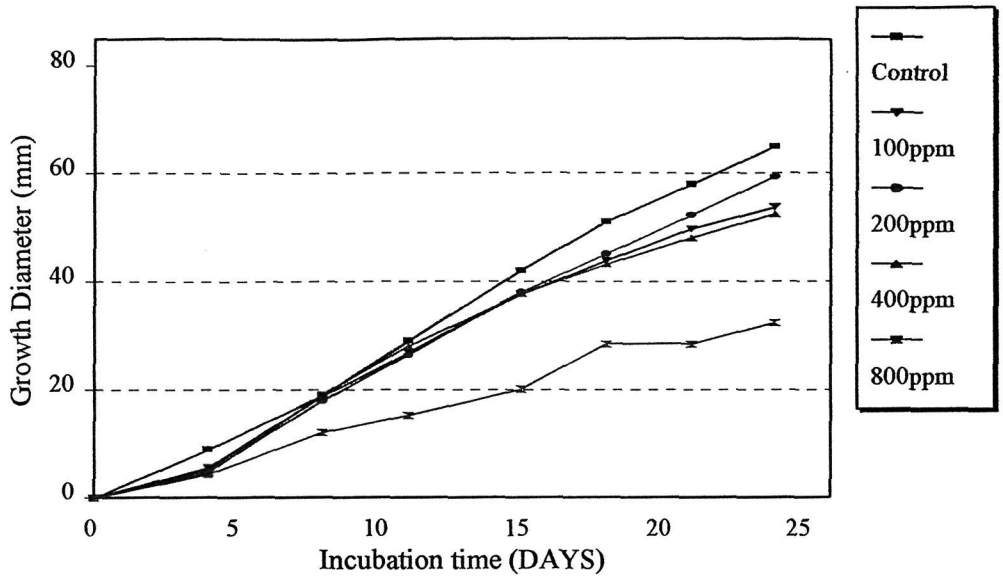


Figure 4.3.3.19.: The growth of *Acremonium* sp. on media containing DMBC.

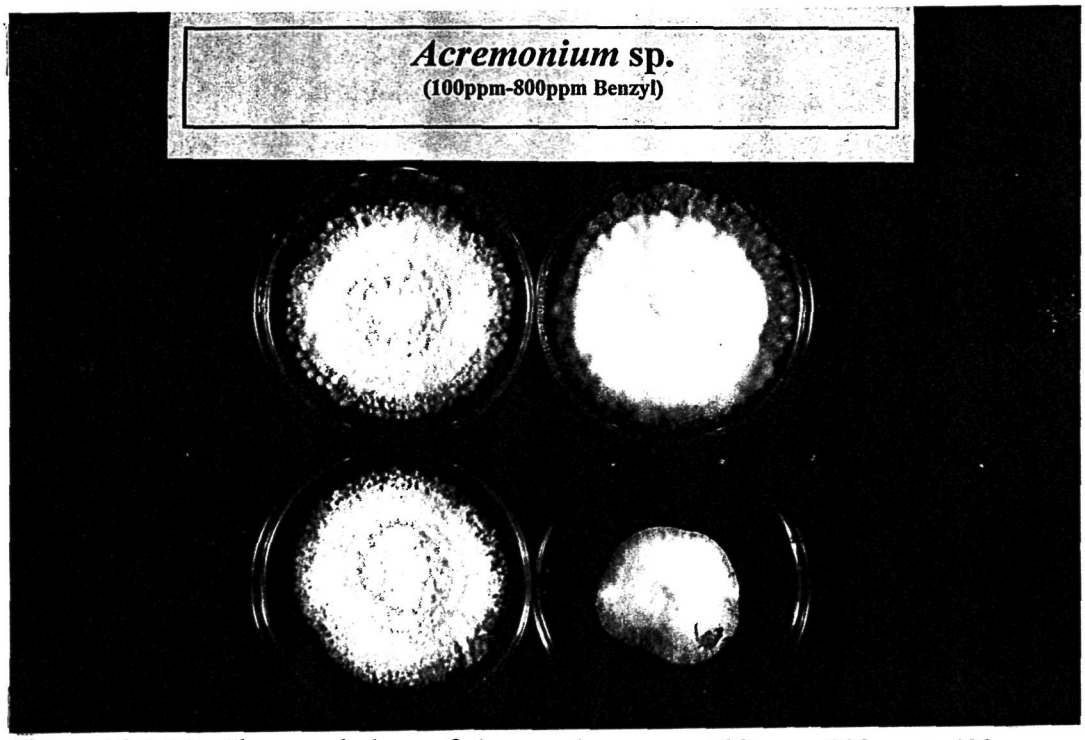


Figure 4.3.3.20.: The morphology of *Acremonium* sp. on 100 ppm, 200 ppm, 400 ppm and 800 ppm DMBC after a 35 day incubation period (from top left to bottom right).

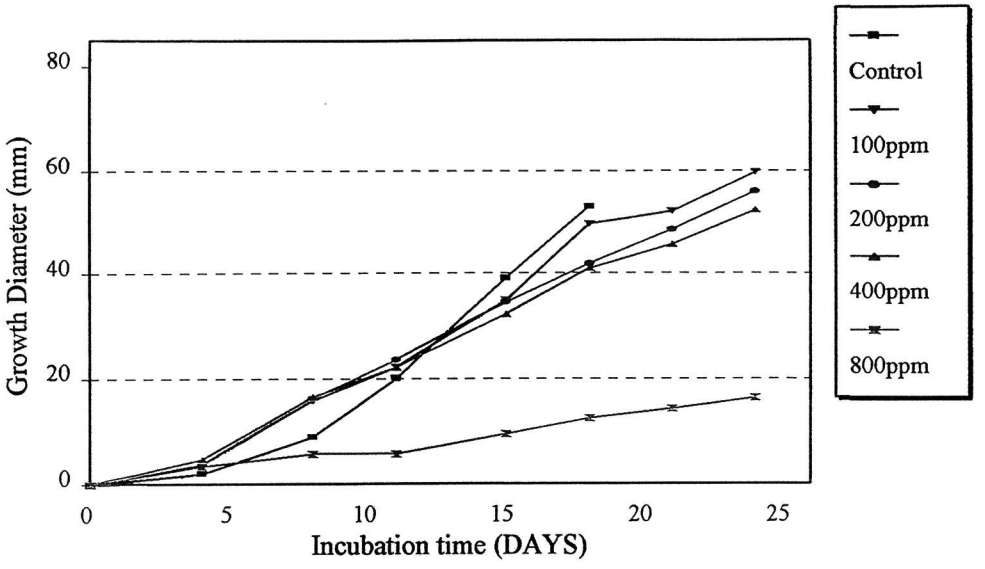


Figure 4.3.3.21.: The growth of G. *roseum* FT on media containing DMBC.

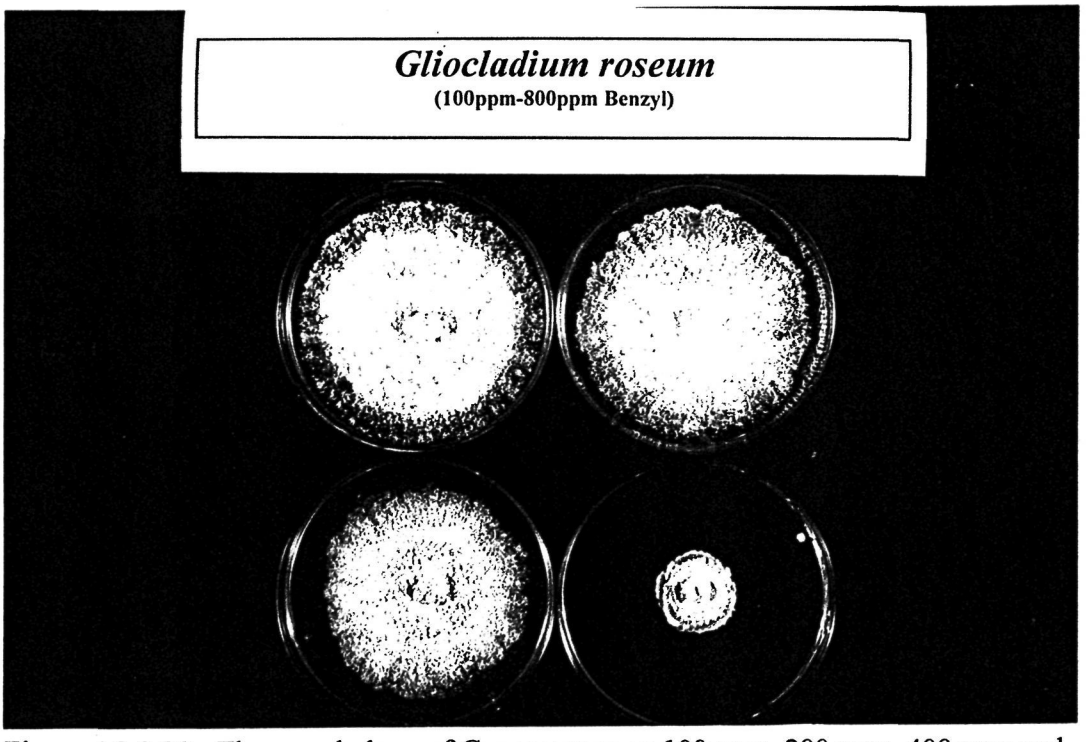


Figure 4.3.3.22.: The morphology of G. *roseum* FT on 100 ppm, 200 ppm, 400 ppm and 800 ppm DMBC after a 35 day incubation period (from top left to bottom right).

with the untreated media. However, the addition of 400 ppm DMBC shows an increase in density. The density appears even greater in the plates treated with 800 ppm DMBC. The growth obtained with the DMBC treated plates were much larger than either DDAC and CAC with exception of the media containing 800 ppm DMBC. The growth of G. *roseum* SB on DMBC treated media is shown in Figure 4.3.3.22. A growth rate of 4.2∓0.1 mm/day for G. *roseum* SB on malt media was the half to the growth rate observed with wood deteriorating fungi in chapter 4.3.1. Plates treated with 100 ppm (3.2∓0.5 mm/day), 200 ppm (3.4∓0.2 mm/day) and 400 ppm (3.8∓0.3 mm/day) DMBC had statistically the same growth rate as the G. *roseum* SB on the control plates. Figure 4.3.3.22. showed that the measurement in plates treated with 100 and 400 ppm DMBC were discontinued after 10 and 15 days respectively. This was due to sporulation that covered the plate edges and did not produce reliable measurements. The addition of 800 ppm DMBC to the malt media induced a significant decline in the rate to 1.8∓0.2 mm/day. The growth on these plates showed a stationary phase occurring at 16 days. A photograph of these cultures was not taken due to the sporulation completely covering the plates treated with 100 ppm and 400 ppm DDAC. The growth pattern on DMBC treated media was very similar to growth on the CAC treated media, which were both greater than the DDAC treated media (except 800 ppm).

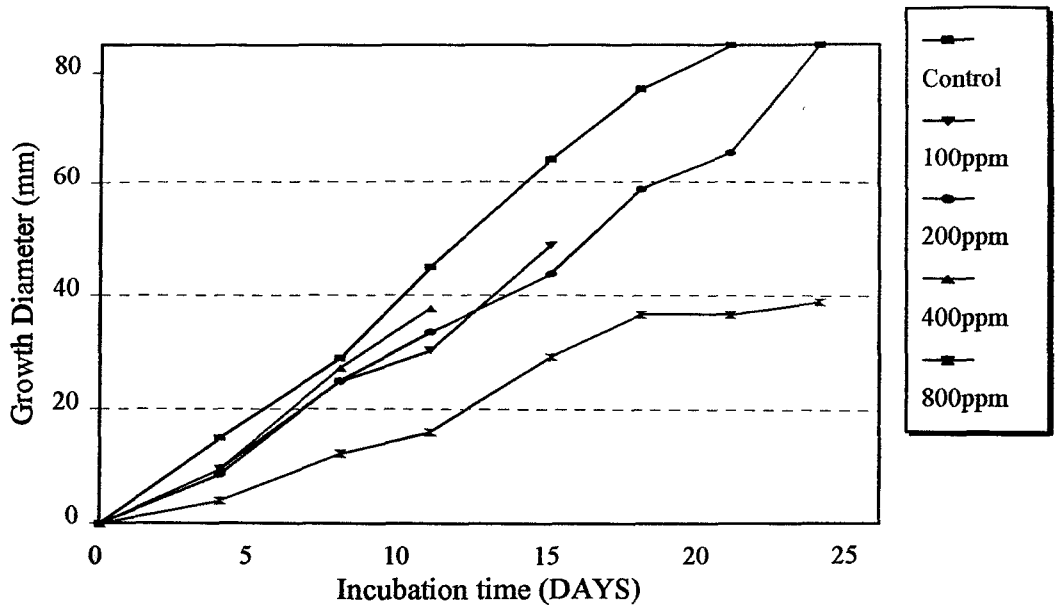


Figure 4.3.3.23.: The Growth of G. *roseum* SB on Media Containing DMBC.

DDAC Versus CAC Versus DMBC.

Comparison of the AACs type and organisms indicated two different trends with concentrations occurred one at 100-400 ppm level and 800 ppm level. V. ***bulbillosum*** has equal growth on both DMBC and CAC at the 100-400 ppm level and both are greater than the organism on DDAC. This agrees with previous research where dialkyl AACs were more fungicidal than the monoalkyl AACs (Preston, 1983). Growth on DDAC at the 800 ppm level is greater than either DMBC or CAC for V. ***bulbillosum***. The *Acremonium* sp. used has the maximum growth on DMBC followed by CAC and finally DDAC at the 100-400 ppm levels. However, at the 800 ppm level of DDAC, the greatest growth is obtained with *Acremonium* sp. then CAC and DMBC being smaller, but statistically the same. G. *roseum* FT

shows the best growth on DMBC then CAC and finally DDAC at the 100-400 ppm level. At the 800 ppm level the highest growth obtained with G. *roseum* FT was on DDAC then DMBC and finally CAC. The G. *roseum* SB shows the largest growth on both DMBC and CAC, which is larger than DDAC on the 100-400 ppm levels. At the 800 ppm level the highest The G. *roseum* SB growth is obtained on DDAC. Both DMBC and CAC had the same growth rate at the 800 ppm level, which was less than the growth shown on 800 ppm DDAC.

All organisms grown on plates treated with DMBC and CAC show a dramatic reduction in growth at the 800 ppm level. One possible explanation for this phenomenon could be related with adsorption of the chemical by the agar. Groves and Turner (1959) found a maximum adsorption of 0.001 moles/gram that would be equivalent to 556, 678 and 738 ppm for CAC, DMBC and DDAC respectively. Therefore, at the 800 ppm level there would be an excess of the AAC in the media. The excess may over load the fungal mycelium and attack their plasma membranes reducing the ability of the fungi to tolerant the AAC. At the 100 ppm to 400 ppm level all the AAC would be bound and not available to interfere with the growing mycelium. However, this phenomenon was not observed with any of the organisms grown on the DDAC treated plates, which could be a result of the smaller amount of free DDAC compared with CAC and DMBC. A compounding factor could be that the different structures of AACs affect their mobility in the media with the DDAC being the least mobile. Research has shown that DMBC related AACs are less mobile than CAC related AACs. The shorter the alkyl group, the more mobile the molecule (Groves & Turner, 1959). Comparing AACs mobility, CAC would be the most mobile then DMBC with DDAC being the least mobile. At the 800 ppm level there would be a smaller amount of free DDAC that could be

more available for utilization and result in the increased growth seen at the 800 ppm level compared with 400 ppm treatment seen in all four organisms.

The adsorption of the AAC by the agar does not deactivate its biocidal activity as the standard deteriorating fungi tested were affected by AACs at concentration which were lower than the maximum adsorption of the agar. A second explanation for the dramatic change in the growth with the 800 ppm CAC and DMBC present in the media could be the concentration being too high for these fungi to tolerant. The lack of the same response in the media containing DDAC (800 ppm) could be due to higher tolerance to this AAC.

4.3.4. Fungal Degradation of AACs.

Previous research with DDAC, treated wood that was pre-exposed to a soup of microflora, which included some moulds, soft rot and staining fungi, found an increase in the toxic thresholds and limits against common decay fungi in subsequent soil jar tests (Ruddick, 1986). There are several possible explanations for this observation. One possibility was that the microflora action had increased the leachability of the DDAC. However, post leaching of the samples that had been pre-exposed to the mould, staining fungi and soft rot fungi showed similar toxic threshold values as unleached samples, eliminating this possibility (Ruddick, 1986). A second explanation could be a simulation of a serial exposure, where the toxic threshold increased with the number of exposures to decay fungi in soil jar tests (Butcher, 1979[b]). A third possible explanation could be the removal of unbound AACs by adsorption onto the non-decay fungi (protein), protecting the decay fungi and possibly providing an alternative nutrient source, in essence increasing the threshold value (Washam, Sandine & Elliker, 1976[ab]). Finally it is possible that the degradation of the DDAC by these non-decay organisms resulted in a reduction in the AAC concentration present in the wood. This reduction causes the toxic threshold to appear to increase.

Degradation studies are normally carried out in liquid culture. However, liquid degradation studies to date have found only very limited degradation of AACs even with bacteria. A few organisms have been isolated which can grow on specific AACs such as hexadecyltrimethyl-ammonium chloride (Van Ginkel, van Dijk & Kroon 1992), but can not utilize other related monoalkyl AACs. None of the organisms tested appear to have the ability to degrade dialkyl

AACs in liquid culture. Researchers have found the addition of silica increased the ability of *Pseudomonas* to tolerate and degrade the test AAC (Van Ginkel, van Dijk & Kroon, 1992). The addition of silica gel would adsorb the AAC and release it slowly back into the media as the organism degrades it. Researchers have indicated that the addition of a second carbon source gave better results than using AACs as a sole carbon source (Dean-Raymound & Alexander, 1977; and Mackrell & Walker, 1978).

The purpose of this study was to determine whether AACs had been degraded during exposure to non-decay fungi. The plates used in this study were initially setup for bioassay evaluations in chapter 4.3.3. Bioassay studies indicate that the fungi were tolerant to the AACs tested. However, tolerance does not distinguish between the organisms ability to degrade the AACs, translocate AACs away from the growing tip or simply tolerate the AACs.

The second and third phases of this investigation were to determine if the DDAC tolerant organisms could degrade DDAC in liquid and wood. Previous work using liquid cultures showed variable results in the recovery of DDAC (Brisco, *et al.*, 1990). Therefore, control samples were included to monitor the mass balance and recovery of DDAC from the test system. The addition of DDAC in the form of wood disks and sawdust in the liquid culture may result in a slow release of the DDAC into the liquid, while the solution containing DDAC gave immediate exposure.

The ability of fungi to degrade AACs in liquid or solid media with the presence of simple nutrients does not necessarily indicate that the fungi can degrade AACs in the wood. It was decided to use V. *bulbillosum* in the following soil jar test to determine whether it could

degrade DDAC in an environment as close to natural decay conditions as possible, without the microflora competition. Two brown rot fungi were included in the following test to identify if these decay fungi could detoxify DDAC in the wood, without showing any decay. Previous research found that fungi, including decay fungi have the ability to detoxify preservatives designed to prevent decay (Lee, Takahashi & Tsunoda, 1992).

Initial Solid Agar Studies.

The investigation of the fate of DDAC during colonization by G. *roseum* FT and an *Acremonium* sp. isolates were determined using plates containing 500 ppm DDAC. The results of DDAC analysis by an Indirect Photometric HPLC technique and these tolerant isolates growth on DDAC treated media are shown in Figures 4.3.4.1., 4.3.4.2., 4.3.4.3. and 4.3.4.4. The *Acremonium* sp. isolate showed the greater tolerance obtaining twice the diameter of growth compared with G. *roseum* FT as seen in Figures 4.3.4.2. and 4.3.4.4. Figure 4.3.4.1. shows the chromatograms of samples taken from 0-10, 10-30 and 30-40 mm from the centre of the *Acremonium* sp. growing colony (locations shown on Figure 4.3.4.2.) and samples taken from a control plate. As *Acremonium* sp. colonize the substratum the DDAC peak intensity declines (a retention time 9.8 ±0.4 minutes), the closer to the colony centre the smaller the amount of DDAC recovered. Cores taken from 0-10 mm, closest to the initial inoculum core showed that the DDAC concentration was below the limits of this analysis method. A small positive peak developed as the DDAC concentration declined with a shorter retention time on the column (Figure 4.3.4.1.). This positive peak was not associated with the growth of the *Acremonium* sp. on the malt media (without DDAC), as the

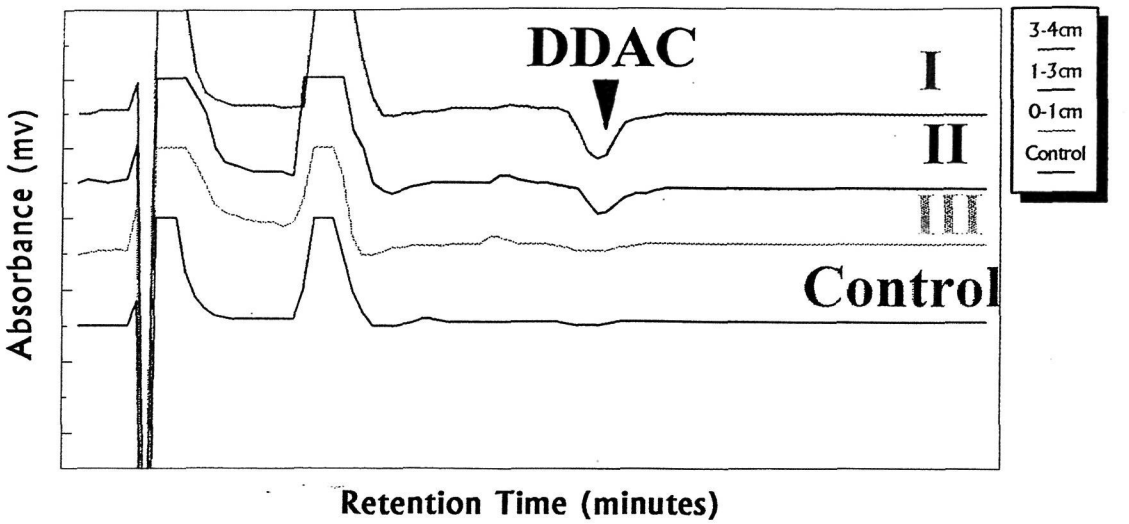


Figure 4.3.4.1.: The influence of an *Acremonium* sp.on DDAC present in the media.

Figure 4.3.4.2.: The growth of an *Acremonium* sp. on 500 ppm DDAC.

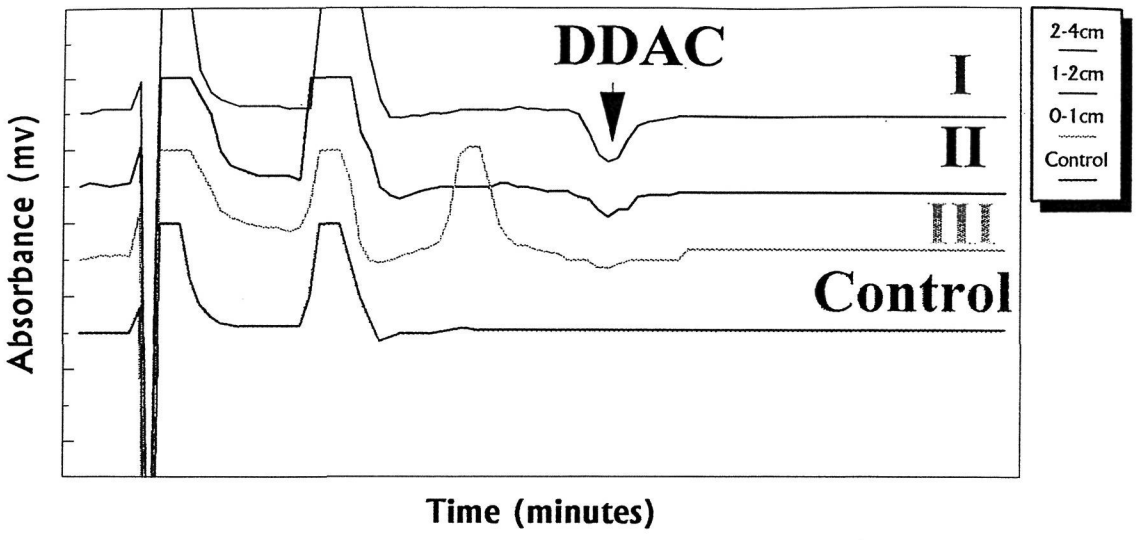


Figure 4.3.4.3.: The influence of G. *roseum* on DDAC present in the media.

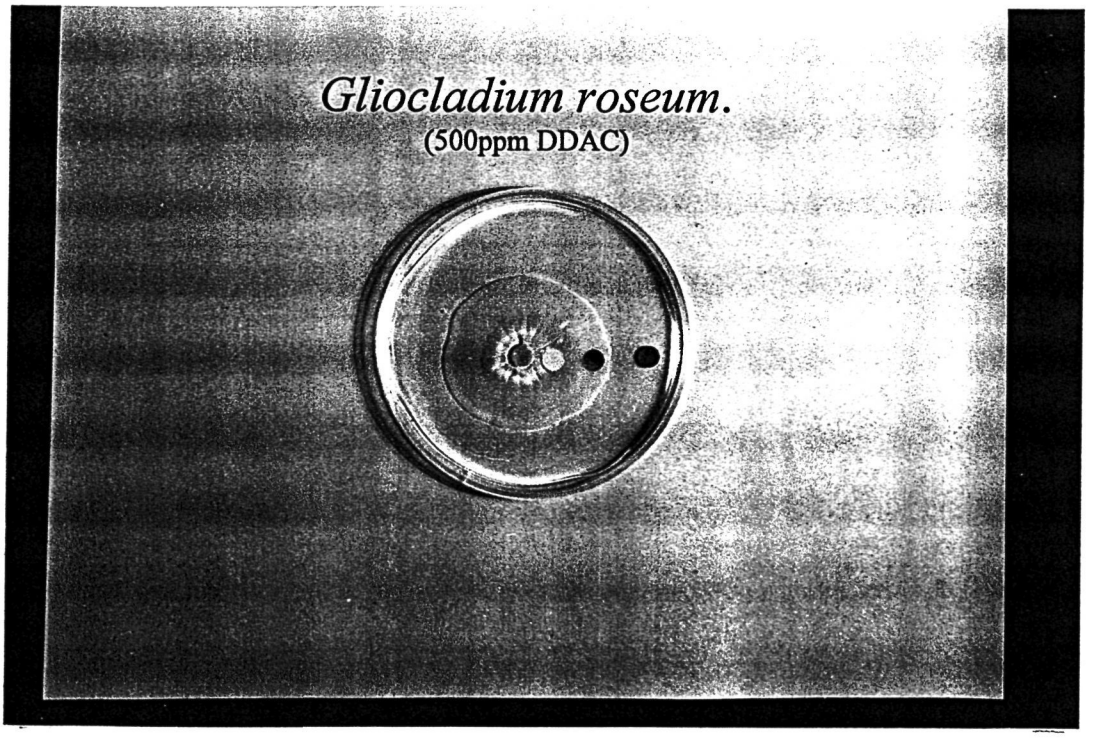


Figure 4.3.4.4.: The growth of G. *roseum* on 500 ppm DDAC.

HPLC chromatogram of a sample taken from a control plate did not show this peak. As the mycelium proliferates the amount of chemical was reduced and the older the colony the less chemical would be expected. These results indicate that the *Acremonium* sp. does not translocate or tolerate (without effect) DDAC in the media, but does degrades the DDAC present in the media.

The results indicate that G. *roseum* FT was less tolerant to DDAC compared with the *Acremonium* sp. As the growth seen in Figure 4.3.4.4. was only half that obtained by the *Acremonium* sp. in Figure 4.3.4.2. Secondly, results of DDAC analysis indicate that the reduction of DDAC was not as complete as with the *Acremonium* sp. The HPLC chromatograms in Figure 4.3.4.3. are taken from 0-10, 10-20 and 20-40 mm from the colony centre of G. *roseum* FT (locations shown in Figure 4.3.4.4). A chromatogram of samples taken from control plate was also included. The amount of DDAC obtained declines as the samples taken approached the centre of the colony centre. DDAC in cores taken from 0 to 10 mm, showed reduction in the DDAC concentration by G. *roseum* FT, but not to the level obtained by the *Acremonium* sp. As the DDAC declines, shoulders on the DDAC peak were observed. A positive peak (retention time 8 minutes) occurs with a greater intensity than that observed with the *Acremonium* sp. The chromatograms taken from control plates do not show this positive peak at 8 minutes. Therefore, this peak could correspond to either a DDAC degradation product, metabolite produced as a stress response, or specifically to DDAC presence in the media. As this chemical accumulates in the media it may have a toxic effect on the growth of G. *roseum* FT and may correspond to the yellow coloration seen in

Figure 4.3.4.4. Like the Acremonium sp. the G. *roseum* FT was able to degrade the DDAC present in the media.

Table 4.3.4.1. The reduction of DDAC by an *Acremonium* sp over 28 day incubation period.

Sample Location	Incubation Period	Amount of DDAC (ppm)	DDAC Reduction (%)
Centre of Colony	2 Weeks	280	44%
(0-10 mm)	4 Weeks	26	96%
Edge of Colony	2 Weeks	-	-
(10-40 mm)	4 Weeks	129	70%
Away from Colony	2 Weeks	501	-
(40-80 mm)	4 Weeks	433	-

To confirm the degree of DDAC degradation occurring, a second analysis was carried out with the *Acremonium* sp. culture plates, using a known weight of agar in the analysis (Table 4.3.4.1.). After a two week incubation the amount of DDAC declined from 501 ppm to 280 ppm, a reduction of 44%. A final DDAC reduction of 96% was obtained in the samples closest to the centre of the colony after a four week incubation period, on plates containing 433 ppm DDAC. At the edge of the of the colony a 70% loss of DDAC was obtained over

the same period. Therefore, an *Acremonium* sp. can convert between 70% and 90% DDAC with ~500 ppm DDAC initially present during a four week incubation period (Doyle & Ruddick, 1993).

DDAC Degradation in Solid Agar.

A second analysis was carried out on plates, which were used in the bioassay study in chapter 4.3.3. The plates were analyzed for DDAC content after 26 days and 41 days of incubation. The plates used for this investigation contained initially 800 ppm of DDAC. Analyses of the uncolonized areas show that the DDAC content had increased up to a maximum of 1,100 ppm. This increase was a result of the plates drying out during incubation and concentrating the concentration of DDAC in the plates.

V. *bulbillosum* appears to be very limited in its ability to degrade DDAC. After 26 days the DDAC concentration went from 847 ppm to 538 ppm a reduction of 37% (Table 4.3.4.2.). However, the number of isolates obtained from the DDAC treated wood in chapter 4.3.2 indicated that under the test conditions this particular organism had a competitive edge. Bioassay results indicated that next to G. *roseum* SB, which had the maximum growth on DDAC treated plates, the V. *bulbillosum* cultures had superior growth than G. *roseum* FT or the *Acremonium* sp. The combination of these results suggests that DDAC degradation was not essential for growth of V. *bulbillosum* on malt media. The chromatograms of these samples showed other peaks with increased retention times.

The *Acremonium* sp. cultures exposed to 800 ppm DDAC did not show the same capacity for DDAC degradation that was shown in Table 4.3.4.1. After 26 days of incubation the DDAC content was reduced by 27% which was less than half of that obtained culture on plates containing 500 ppm DDAC (Table 4.3.4.1.). This DDAC degradation doubles after incubation for 41 days. These results suggest that 800 ppm DDAC required longer for the *Acremonium* sp. to totally degrade it, which could be associated with absorption. Therefore, the tolerance of *Acremonium* sp. to DDAC was close to its limit at 800 ppm, but 500 ppm DDAC present in solid media could be easily degraded. This poor degradation ability could be a result of resistance loss, due the presence of a second carbon source, a similar response was observed with *Serratia marcesens* (Chaplin, 1951). The complete conversion of DDAC does not appear to be absolutely necessary for growth of the *Acremonium* sp. As observed previously the amount of DDAC degradation declines as the core/sample location (age) moves away from the centre of the colony. Chromatograms of these samples also shows tailing or shoulder peaks emerging as the amount of DDAC declines.

The G. *roseum* FT from the anti-sapstain lumber did not show the same capacity to degrade DDAC as the G. *roseum* SB isolate from the soil bed. This would be consistent with growth measured in chapter 4.3.3., G. *roseum* SB had the maximum diameter growth, while G. *roseum* FT had minimum diameter growth on all three AACs tested. The G. *roseum* SB showed the greatest ability to degrade DDAC, with a maximum reduction of 73% after 26 days of incubation. G. *roseum* FT had a DDAC reduction of 25% after 26 days and 50% at day 41 in the outer regions. The inner regions, close to the colony centre

show a 43% and 89% reduction after 26 and 41 days incubation periods. The G. *roseum* FT growth requires degradation of almost all of the DDAC before it can continue to colonize the substrate. Chromatograms of the above samples showed a widening of the DDAC peak and the presence of shoulder peaks.

Table 4.3.4.2. DDAC content in solid agar before and after incubation with various tolerant organisms.

Organism	Sample location (mm)	DDAC Content (ppm) Control	Exposed	DDAC Reduction
26 Days				
V. *bulbillosum*	10-15	847	538	**37%**
V. *bulbillosum*	10-25	889	735	**17%**
Acremonium sp.	10-20	1124	825	**27%**
G. *roseum* FT	5-10	951	535	**43%**
G. *roseum* FT	10-15	866	651	**25%**
G. *roseum* SB	10-20	1114	316	**72%**
G. *roseum* SB	10-20	917	314	**63%**
41 Days				
Acremonium sp.	0-10	1070	470	**56%**
Acremonium sp.	30-40	1070	520	**51%**
Acremonium sp.	50-60	1070	850	**21%**
G. *roseum* FT	0-5	1085	120	**89%**
G. *roseum* FT	10-20	1085	550	**49%**

Based on this study G. *roseum* SB has the greatest ability to degrade DDAC then G. *roseum* FT and V. *bulbillosum,* with *Acremonium* sp. having the smallest degradation at 800 ppm DDAC. These results reflect the results seen in chapter 4.3.3. where the G. *roseum* SB shows the maximum growth on plates treated with 800 ppm compared with the other three organisms.

CAC Degradation in Solid Agar.

The fate of CAC at the end of incubation in chapter 4.3.3. was determined in the same manner as DDAC. The HPLC analysis of this chemical gave a broader peak than the one obtained for DDAC, therefore area analysis was critical in the determination of CAC content. The peak representing the CAC was a wide peak and it would be difficult to identify shoulders.

The CAC degradation results for the four fungi are shown in Table 4.3.4.3. V. *bulbillosum* reduced the concentration of CAC by 55% over 40 days. The plate appeared very dry and this may have limited the amount of CAC degradation obtained. This is consistent with the number of cores used to obtain the required weight, which was 3 to 4 times normally used. *Acremonium* sp. shows the greatest ability to breakdown CAC with reductions of 84% and 96% during a 40 day incubation. The above finding would be consistent with the results observed in chapter 4.3.3. where this particular organism showed the maximum growth on media containing CAC. Both G. *roseum* isolates showed similar reduction in the CAC content of ~50% over the 40 days.

Table 4.3.4.3. CAC content in solid agar before and after 40 days incubation with various tolerant organisms.

Organism	Sample location (mm)	DDAC Content (ppm)		DDAC Reduction
		Control	Exposed	
V. *bulbillosum*	10-20	1692	768	**55%**
Acremonium sp.	0-10	1237	54	**96%**
Acremonium sp.	10-20	1237	196	**84%**
G. *roseum* FT	0-10	1695	961	**43%**
G. *roseum* SB	10-20	1015	451	**55%**

The above results indicate that all four organisms have the ability to degrade not just DDAC, but other AACs such as the CAC. From the literature, the monoalkyl CAC should be degraded more readily than the DDAC. The results agrees with this theory, were CAC content reduction was greater with *Acremonium* sp. The exception to the rule was G. roseum isolates where the amount of degradation declined.

DDAC Degradation in Liquid Culture.

A second investigation was set up to determine the ability of G. *roseum* FT and the *Acremonium* sp. to degrade DDAC in liquid culture. The DDAC was introduced in various forms including solid wood disks, pine sawdust and liquid DDAC.

Biomass

At the end of the incubation period the liquid volumes were determined for each flask. Control samples show a much greater evaporation rate than the samples containing fungal mycelia (Table 4.3.4.4.). Typically control flasks lost between 40% and 60%, while fungal flasks lost between 30% and 50%. One possible explanation for this difference was the presence of a 'gelling' agent produced by both fungi. This substance made it very difficult to filter the mycelium directly without prior centrifugation.

Table 4.3.4.4.: Remaining liquid volume in the control samples after 14 days incubation.

Sample	Form of DDAC	Remaining Volume (%)
Control		
A	Disk 4- 11 & 13	38
B	Disk 5- 11 & 13	50
C	Disk 5- 10 & 12	50
D	Pine Sawdust	40
E	Liquid	60

In the flasks containing mycelia the biomass was determined in g/l. The biomass of the *Acremonium* sp. increased from 0.938 g/l (I) after 2 days to 7.178 g/l (IV) after 14 days of incubation (Table 4.3.4.5.). The biomass results are consistent with other results obtained for decay organisms with 25 g/l glucose over a similar time period compared with 30 g/l of sucrose available from the liquid media over this The addition of liquid DDAC resulted in

its growth (VI & VII).The addition of the DDAC treated disk appeared to increase the growth of one sample while the other remained unchanged. The samples treated with sawdust showed an increase in the fungal growth up to 13 g/l (II). The increase in biomass obtained in the samples exposed to DDAC treated sawdust is surprising and possible reasons are nutrients available in wood may promote the growth or the surfaces of the wood encourage mycelium growth.

Table 4.3.4.6.: Biomass accumulation in G. *roseum* FT. after 14 days incubation with various forms of DDAC.

Sample	**DDAC**		**Days**	**Biomass**	**Remaining Volume**
	Form	**Wt (mg)**		**(g/l)**	**(%)**
G. *roseum* FT					
I	None	0	2	0.712	90
II	Sawdust	2.1	14	8.56	72
III	Sawdust	2.1	14	13.222	74
IV	Disk	11.0	14	4.774	66
V	Disk	9.3	14	7.178	50
VI	Liquid	10	14	0.722	76
VII	Liquid	10	14	0.524	70
VIII	None	0	14	5.27	72

Reduction of DDAC.

The fate of the DDAC was determined by the amount recovered compared against the amount initially introduced on the second day of incubation. Table 4.3.4.7. shows the recovery of DDAC from the five control flasks over the fourteen day incubation period. A recovery of 84% of DDAC from the liquid control (E) after 14 days. Freshly prepared DDAC containing media had a recovery of 93%. Complete recovery of DDAC from the control samples may be hindered by adsorption on glassware during recovery or incomplete extraction with the dichloromethane. Therefore, ~10% of the DDAC in the liquid can be lost during recovery. DDAC recovery from the T. *heterophylla* disks was between 80% and 90%. The lower recovery may be a reflection of DDAC retention variations due to the heterogeneous nature of wood. The pine sawdust had the lowest recovery of DDAC being only 70%. This may reflect a higher variation in the retention in the P. *ponderosa* mini stakes compared with T. *heterophylla* disks. Later studies confirmed that the retention in the P. *ponderosa* mini stakes with DDAC retention of between 4 to 18 kg/m^3, while T. *heterophylla* retention was more homogeneous between 3.9 to 4.6 kg/m^3.

The flasks, which contained no DDAC were also analyzed using the HPLC method for DDAC analysis. Both the mycelia from G. *roseum* ft and the *Acremonium* sp. from the control flasks had a peak occur at a similar retention time as DDAC (10 minutes). Therefore, all samples were corrected for the amount which this interfering peak corresponds too, values shown in Tables 4.3.4.8. and 4.3.4.9. This was not exhibited in samples grown on the solid agar.

Table 4.3.4.7.: DDAC recovery from control samples after incubation.

Sample	Form of DDAC	DDAC Content (mg)					DDAC Recovery (%)
		Initial	Final				
			Wood	Liquid	Glass Ab	Total	
Control							
A	Disk 4- 11 & 13	5.3	3.5	0.6	0.04	4.14	78
B	Disk 2- 11 & 13	6.6	4.7	0.6	0.09	5.4	82
C	Disk 5- 10 & 12	7.2	5.5	0.6	0.25	6.35	88
D	Sawdust Pine	8.9	5.3	0.9	-	6.2	70
E	Liquid	10	-	7.8	0.6	8.4	84

Reduction of DDAC by the *Acremonium* sp. is shown in Table 4.3.4.8. Both samples exposed to the liquid DDAC show almost no reduction in DDAC content in sample (III & VII). This would agree with the lack of biomass accumulation noted in these two flasks. DDAC recovered from the mycelium was 70% to 80% of the DDAC added indicating that the chemical had completely adsorbed onto the mycelium. The assumption can be made that the DDAC concentration around the mycelium increased from 200 ppm to 8,000 ppm in a small volume (1 ml) which may be too high for this fungi to tolerate.

The reduction of DDAC content by G. *roseum* FT results are shown in Table 4.3.4.9. The organisms treated with liquid DDAC showed, what was considered to be negligible degradation. DDAC degradation would not be expected as no growth was monitored after the addition of DDAC liquid on the second day of the incubation. Mycelia content indicated that up to 85% of the DDAC added to the medium adsorb onto the mycelium and may well overwhelm the fungi preventing it from growing. Again the concentration of DDAC can be estimated to increase from 200 ppm to 8,500 ppm in a small volume (1 ml).

These results suggest that the DDAC present in the liquid quickly binds to the cytoplasmic membrane and cause it to lose its' integrity. Krzeminski, *et al.* (1973) found a 91% adsorption of benzalkonium chloride by river silt within 60 seconds. Other researchers showed that *Aspergillus niger* mycelium mat adsorbed 50% of the AAC solution within the first five minutes (Mackell & Walker, 1978). Studies using *Candida albicans* indicated that benzalkonium chloride causes the cytoplasmic membrane to become less dense (Gale, 1963). Work using *Aspergillus ustus* treated with a range of AACs found them to cause amino acid

Table 4.3.4.8.: DDAC remaining in the *Acremonium* sp. culture after incubation with the different forms of DDAC.

Sample	Form of DDAC	DDAC Content (mg)					DDAC
		Initial	Final				Degraded
Acremonium sp.			Wood	Mycelium*	Liquid	Total	(%)
I	None	-	-	-	-	-	-
II	Disk 4- 1, 3 & 5	11.14	4.5	0.4	-	4.9	56
III	Liquid	10	-	7.7	1.9	9.6	4
IV	None	-	-	1.4	-	1.4	-
V	Sawdust Pine	10		2.16**	0.85	3.01	70
VI	Disk 5- 1, 3 & 5	11.64	4.55	0.28	-	4.8	59
VII	Liquid	10	-	8.4	n/a	8.4	16
VIII	Sawdust Pine	9.7		2.2**	-	2.2	77

* Mycelium Values Corrected for Controls.

**Due to the difficulty in separating the sawdust and mycelium the analysis was carried out on the mixture.

and carbohydrate leaks from the mycelium (Zlochevskaya, *et al.*, 1981). These results suggest that the DDAC present in the liquid quickly binds to the cytoplasmic membrane and cause it to loose its' integrity.

G. *roseum* FT was exposed to a fifth of DDAC in the form of treated pine sawdust compared with the *Acremonium* sp. isolate. DDAC analysis of the mycelium and pine sawdust was carried out together due to difficulty in separating them. A 70% and 77% loss of DDAC in the samples were obtained after exposure to the *Acremonium* sp. (Table 4.3.4.8.) Accounting for a 70% recovery from the pine sawdust control D, then *Acremonium* sp. degraded 49% and 54% of the DDAC present in the pine sawdust during a 12 day incubation period. Chromatograms of both samples indicated two separate negative peaks with retention times of 11 and 12.6 minutes, plus a shoulder peak at 9.4 minutes (present in all four chromatograms). G. *roseum* FT produced a DDAC reduction of 91% to 95% (Table 4.3.4.9.). Correcting for the control recovery of 70% the degradation of DDAC by G. *roseum* FT would be 61% and 67% over a 12 day incubation period. The HPLC chromatograms of these samples showed one extra negative peak at 11.8 minutes, one positive peak at 8 minutes and a shoulder peak at 9.6 minutes.

A DDAC concentration reduction of 56% and 59% was obtained in flasks containing DDAC in the form of treated T. *heterophylla* disks which were exposed to the *Acremonium* sp. inoculum (Table 4.3.4.8.). Accounting for 80 to 90% recovery, the DDAC degradation was 45% and 54% of the DDAC added after 12 days. The HPLC chromatogram of sample VI had one extra negative peak with a retention time of 11 minutes. Flasks containing DDAC-treated T. *heterophylla* disks showed a 33% and 59% reduction when exposed to G. *roseum*

Table 4.3.4.9.: DDAC remaining in the G. *roseum* cultures after incubation with the different forms of DDAC.

Sample	Form of DDAC	DDAC Content (mg)					DDAC
		Initial	Final				Degraded
G. roseum			Wood	Mycelium*	Liquid	Total	(%)
I	None	-	-	-	-	-	-
II	Sawdust Pine	2.1		0.1	-	0.1	95
III	Sawdust Pine	2.1		0.19	-	0.19	91
IV	Disk 2- 1, 3 & 5	11	7.4**	-	-	7.4	33
V	Disk 4- 4, 8 & 9	9.3	3.8**	-	-	3.8	59
VI	Liquid	10	-	4.7	2.4	7.1	29
VII	Liquid	10	-	8.5	2.3	10.8	-8
VIII	None	-	-	0.14	-	0.14	-

* Mycelium Values Corrected for Control reading in the HPLC

**Due to the difficulty in separating the sawdust and mycelium the analysis was carried out on the mixture.

FT after 12 days (Table 4.3.4.9.). Adjusting for the control recovery, 18% and 54% DDAC was degraded by G. *roseum* FT. The chromatograms of the samples exposed to DDAC in the form of treated disks showed one extra negative peak with a retention time of 12 minutes. Results indicate that both organisms were capable of degrading DDAC released from the treated pine sawdust and hemlock disks. All wood samples, which were exposed to either fungi, showed a presence of unaccounted negative peaks. The extra negative peaks seen in the pine sawdust samples exposed to *Acremonium* sp. had retention times of 11 and 12.6 minutes, plus a shoulder peak at 9.6 minutes. A negative peak at 11 minutes was also detected in the T. *heterophylla* disks and agar. A positive peak seen in the G. *roseum* FT cultures at 8 minutes were also detected in the control cultures this supports the theory that this product is a metabolite which is produced under stress conditions. The wood samples exposed to G. *roseum* FT have an extra negative peak with a retention of 11 minutes and a shoulder peak at 9.6 minutes. The negative peak at a retention time of 11 minutes was also observed in the chromatogram for the soil bed mini stake which the G. roseum SB was isolated from in chapter 3.3.3.

The literature has described two possible pathways for AAC degradation. The mechanism proposed by Van Ginkel, van Dijk & Kroon (1992) appears to be a highly unlikely pathway in the above study, as the amine standards used in the HPLC do not correspond to any breakdown products. These are not detected in the above samples. However, the mechanism suggested by Dean-Raymond & Alexander (1977) appears to be a more plausible mechanism. The presence of the shoulder peaks with a shorter retention time suggest an increase in the molecular weight. This would be consistent with ω-oxidation of the terminal

carbon resulting an increase in molecular weight. The presence of negative peaks with longer retention times would be consistent with β-oxidation of the long chain alkyl group cleaving acetyl groups reducing the molecular weight.

The enzyme responsible for ω-oxidation was identified mono-oxygenase (Hampton & Zatman, 1973). Studies on this particular enzyme found that Cu^{2+} would inhibit its' activity and would explain the success of AACs formulated with Copper in combination with it being a known biocide. While the addition of Fe^{2+} would stimulate the activity of this particular enzyme. This may answer the reason for the rapid failure of AAC-treated wood in Westham Isle, the Forintek Canada Corp. field test site where the soil has a high iron content (Ruddick, 1986) and the improvement seen with copper addition to AACs (Drysdale, 1983[a]).

DDAC Degradation in Solid Wood.

The ability to degrade DDAC in the presence of simple carbon sources does not indicate that these tolerant fungi can degrade DDAC present in wood under field conditions. Therefore, a soil jar test was set up to determine if these fungi could degrade DDAC in wood without the presence of simple carbon source, except the ones available in the wood.

Weight Loss.

The weight loss results shown in Table 4.3.4.10. indicate as expected that V. *bulbillosum* does not cause any substantial weight loss in either the untreated or DDAC treated mini stakes.

The two brown rot fungi cause approximately 60% weight loss in the untreated mini stakes. These results are consistent with previous studies using P. *ponderosa* with the brown rot

fungi under similar conditions (Ruddick, 1986). However, the DDAC treated mini stakes have less than 2% weight loss with one exception. It can be concluded that DDAC treatment resulted in the control of the two brown rot fungi tested. These results are consistent with observed toxic threshold values of ~4 kg/m^3 for both organisms (Ruddick, 1986). The mini stake which had a retention of DDAC 4 kg/m^3 did show physical signs of decay upon removal. Further observations found the interior of the wood was discolored and showed typical cubic brown rot appearance. This particular mini stake had a 30% weight loss after 24 weeks exposure to P. *placenta*. This result indicates that 4 kg/m^3 maybe at the lower the toxic threshold limit for P. *placenta*. These results are consistent with previous results where the toxic threshold value of 4.5 kg/m^3 for P. *placenta* was found (Ruddick, 1986). A weight loss of 30% is more consistent with a retention of 1.53 kg/m^3 observed in serial exposures study (Butcher, 1979[b]). The decay pattern suggests that the exterior of the wood was adequately protected, while the interior was poorly protected.

DDAC Degradation.

DDAC analysis suggests that the treatment of the mini stakes used in this experiment were unpredictable as the estimated retention, base on treating solution, and measure retention are quite different. The DDAC retention measured varying from 4 to 18 kg/m^3.

The two brown rot fungi clearly show that they do not cause any reduction in the DDAC present in the mini stakes (Table 4.3.4.10.). These results suggest that the variation between the mini stakes halves was ±9%, which is in agreement with the control samples.

Mini stakes exposed to V. *bulbillosum* all show a reduction in DDAC content after a 24 week incubation period. The amount of degradation appears to be influenced by the initial

retention of DDAC in the mini stake. At the lowest retention there appears to be no reduction in the DDAC content and any reduction observed is within the sample deviation. One possible reason for this is the irreversible adsorption of DDAC at 4 kg/m^3 onto the various wood components leaving none available for degradation by V. *bulbillosum*. At a retention of ~9 kg/m^3 the amount of degradation is at a maximum of 39% to 42%. The possible explanation for this observation could be the availability of free or loosely bound DDAC for degradation by this organism. However, a further increase in retention to 14 to 18 kg/m^3 shows a drop in DDAC degradation to 15%. At higher levels of DDAC there may be too much free DDAC for the organism to handle and its' ability to degrade declines. Two chromatograms of DDAC treated samples exposed to V. *bulbillosum* showed shoulder peaks and a separate negative peak. These results suggest similar breakdown products as previously noted in agar and liquid culture. A maximum reduction of 40% for mini stakes with a retention of ~9 kg/m^3 will reduce the retention close to the toxic limit observed for several brown rot fungi to ~4 kg/m^3 (Ruddick, 1986) which could explain the poor field performance.

The recommended retention of 6-8 kg/m^3 for DDAC treated wood is within the range where this organism could cause a 40% reduction over a 24 month period, bring the retention close to the toxic threshold value for certain brown rot fungi (Ruddick, 1986). This reduction in DDAC may be one possible reason for poor field performance found in DDAC treated wood.

Table 4.3.4.10. Reduction of DDAC in wood exposed in the soil jar test.

Organism	DDAC Retention (kg/m3)		DDAC Reduction	Treated mini stakes	Untreated mini stakes
	Exposed	Unexposed		Weight loss (%)	
V. *bulbillosum*	15	17.7	**15.3%**	1.45	1.34
V. *bulbillosum*	11.7	14.4	**18.8%**	1.42	0.96
V. *bulbillosum*	12.1	14.2	**14.8%**	1.79	1.05
V. *bulbillosum*	5.7	9.4	**39.4%**	1.60	1.39
V. *bulbillosum*	5.3	9.2	**42.4%**	1.69	0.72
V. *bulbillosum*	4	4.1	**2.5%**	1.33	1.14
P. *Placenta*	12.9	11.8	(-9%)	0.51	60.99
P. *Placenta*	9.3	8.5	(-9%)	2.07	63.74
P. *Placenta*	4.9	3.8	(-30%)	30.65	58.88
C. *puteana*	14.1	14.8	(5%)	2.12	61.03
C. *puteana*	10.4	10.9	(6%)	1.51	62.36
C. *puteana*	4	4.1	(2%)	1.80	60.92
Control		9.45±0.9	(9%)		

5. Conclusions.

The cellular distribution of AACs suggest that the main preservative penetration pathway was via the ray cells. Earlywood tracheids tend to absorb more AACs than the latewood tracheids in the peripheral regions (outer 5 mm) of the wood. However, at a greater depth of penetration the retention of AACs in the latewood tracheids was higher than the earlywood tracheids. Factors, which influence the cellular distribution of AACs are the wood type, solution concentration, sample size and solution pH, especially in the basic region.

The microdistribution in the tracheid cell wall shows an even distribution, while an accumulation of AACs in the compound middle lamella was noted at high solution concentrations and alkaline treating solution pH.

Ion exchange fixation mechanism plays a minor role in the wood when the treating solution concentration was well above the CMC. The main DDAC interaction site in the wood was the lignin component.

Mobility studies concluded that DDAC depletion from mini stake in a flooded soil bed was uneven. Isolations from the exposed mini stakes indicated that biological factors may contribute to the loss of DDAC.

Isolations, bioassay and degradation studies concluded that non-decay fungi had the ability to degrade AACs in the wood, while standard deterioration fungi did not affect the AACs.

In conclusion the failure of AACs to protect wood against decay appears to be a complex process involving distribution, physical and biological factors. Earlywood regions have a very thin shell of protection that is prone to leaching. As a result of weathering this region may have a failure in its integrity allowing invasion of decay fungi under field conditions. Isolated fungi such as V. ***bulbillosum***, an *Acremonium* sp. and G. *roseum* can degrade AACs in the wood, reducing the AAC retention to levels that cannot protect the wood against decay fungi.

6. Summary.

Wood Interaction.

Ion exchange fixation of DDAC accounts for one tenth of the DDAC adsorbed in wood using a 1% DDAC solution. The other 90% of DDAC will be fixed by ion pairing, dispersion forces and hydrophobic bonds. However, the importance of ion exchange cannot be ignored due to the dilution of the AAC solution as it penetrates the wood block. This may reduce the concentration below the CMC where the primary mechanism of surfactant adsorption is ion exchange.

The interaction of DDAC with cellulose and lignin found that DDAC preferably adsorbed onto the lignin with almost negligible adsorption onto the cellulose. Ion exchange adsorption in TFA lignin accounts for one quarter of the DDAC retained and is greater than in wood samples. The importance of the acidic sites was highlighted as the Klason lignin did not show any ion exchange adsorption of DDAC. Estimated retentions based on preferential adsorption onto lignin were similar to actual DDAC retentions measured in wood.

Cellular distribution of AACs found that ray cells retain the highest levels, indicating that the rays were the main preservative penetration pathway in the softwoods. Earlywood tracheids generally retain more AACs in the outer 5 mm of a block of wood than latewood tracheids. Further analysis found that lumen surface area limited the amount of the AAC absorption in the cell wall. The larger lumen in the earlywood resulted in higher absorption. However, analysis of samples taken from deeper in the blocks found that latewood tracheids had a more even distribution, while earlywood tracheid absorption declined rapidly. Maximum absorptions for the various cells was between 5% and 6%.

Analysis of chlorine in the various wood cell types indicated that ion exchange was not the sole adsorption mechanism. The iodine trends were duplicated by the chlorine, suggesting that the two ions remain together in the wood. Chlorine analysis of two AACs commonly used in wood preservation indicated that the iodo-benzalkonium chloride was a good model for AACs distribution in the wood.

Penetration of AACs into a small block of wood indicated that the retention in the earlywood drops by ~50% further in than the outer 5 mm shell. AAC retention in latewood tends to remain fairly constant throughout the block. Larger blocks of wood showed a greater decline of AACs in the earlywood as the depth increases. However, latewood retention remains fairly constant throughout the wood block (0-30 mm region).

The permanency of AACs in the different tracheids showed that earlywood tracheids retention level is higher. However, it can lose 50% after a 12 hour soak. Latewood tracheids initially have a lower retention level, which loses ~30%, resulting in similar retentions after a 12 hour soak.

The influence of treating solution pH on the cellular retention of the AAC found that the addition of acetic acid did not cause any major changes in the retentions compared to the unbuffered treating solutions. The main reason for this was the ability of the wood to buffer the unbuffered treating solution to acidic condition. However, the addition of sodium carbonate to the treating pH resulted in increased retention in all three cell types at 1% and 3% iodo-benzalkonium chloride. The increases of AAC cation measured in the SEM-EDX agreed with retentions based on HPLC analysis. The anion retention in the 3% AAC solution did not increase correspondingly with the cation indicating increased retention in the

tracheids was due to ion exchange. The addition of sodium carbonate to a 10% treating solution found that earlywood tracheids retention was not increased, but latewood tracheids had an increase in the AAC cation retention.

Early work suggested that the increased retention caused by alkaline treating conditions was on the outer regions at the expense of the inner core. However, SEM-EDX and HPLC analysis suggested that this was not the case. At the 1% treating solution the inner retention remained similar, while the 3% treating solution found a slight increase in retention of the AAC in the inner core. Again the chlorine retentions did not match the increase in cation retentions observed at the 3% treating solution, indicating that the excess AAC retention was by ion exchange.

The introduction of copper into an ammonia solution containing the iodo-benzalkonium chloride, resulted in a distribution of copper that was even in all three cell types. The distribution of the iodo-benzalkonium chloride was similar to that previously monitored. The combination of both did not effect the distribution of either copper or the AAC. However, the chloride P/B ratio was less than expected indicating that ion exchange of the AAC may have increased in this treatment.

The distribution of the iodo-benzalkonium chloride in the two hardwood species was quite different from the softwood. The cellular distribution was even and overall higher than that monitored for the earlywood and latewood tracheids. The amount of iodo-benzalkonium chloride present in the various hardwood cells suggested that the main preservative penetration pathway was different from softwoods.

The microdistribution of the iodo-benzalkonium chloride in the tracheid cell wall indicated that the iodine cation tended to build up in the compound middle lamella in the earlywood tracheid as the concentration increased. This agrees with early work where AACs preferentially adsorbed onto the lignin and the compound middle lamella, which is rich in lignin. The latewood tracheids did not show the same AAC build up in the compound middle lamella. However, the latewood tracheids at a 13% treating solution showed high levels of iodine close to the lumen in the S_3 region. Chlorine analysis found no change in compound middle lamella retention suggesting that the extra cation was adsorbed by ion exchange. Spot analysis found higher iodine retentions in the compound middle lamella compared with the secondary wall regions. This was observed in the unbuffered 6% treating solution concentration. The addition of sodium carbonate resulted in iodine accumulated in the compound middle lamella to occur at a 3% concentration compared with 6% for unbuffered solution. Chlorine retention did not indicate differences in the compound middle lamella and secondary wall region and confirmed that the extra iodine retention was by ion exchange. Loss of DDAC from the mini stakes by soil leaching appeared to be a continuous loss over a 26 month exposure with a maximum loss of 70%. The loss of DDAC from the mini stake was not even as the upper region that was not in contact with the soil. This region lost 78% over the 26 months while the lower region in soil contact lost 50% over the same time period.

Isolations taken from the mini stakes exposed for three months in the flooded soil bed, resulted in a high frequency of bacteria and exclusively bacteria from the regions, which

were in soil contact. The upper region that was not soil contact resulted in a few fungal isolates which previously showed tolerance to DDAC.

Fungal Interactions.

The interaction of two AACs with a range of typical deteriorating fungi found that 1,000 ppm or less of CAC or DDAC completely controlled all five organisms tested. A typical dose response was observed with the growth of these deteriorating fungi. The two AACs tested found that they had different effects on these organisms. The CAC was more effective against P. *placenta* and T. *versicolor*, while DDAC was more effective against A. *niger* and C. *globosum*. Each organism showed different responses to the two AACs and the concentrations tested.

Isolations taken from DDAC treated lumber that had been exposed to a field trial for seven months, resulting in numerous fungal genera and numbers obtained. However, plates containing 2,500 ppm DDAC resulted in a significant number of isolates, which were classified into a few genera. These included *Verticillium* spp., the frequency of this particular genus increased as the DDAC concentration increased in the media. These isolates were obtained from wood which was treated with Timbercote™ which contained DDAC as the sole biocide. However, the other types of treated wood used a formulation containing a secondary biocide that reduced the frequency of *Verticillium* spp. in particular. Pasteurization of the treated lumber also reduced these fungi that appeared to show tolerance to DDAC. The results indicate that these DDAC tolerant fungi genera previously isolated had been present in the wood prior to pasteurization and not from the air inoculum.

A bioassay of four fungi, which showed DDAC tolerance, against three AACs did not respond in the same way as the deteriorating fungi did. The removal of AACs from the media did not show a dramatic increase in the growth of the organisms. This suggested that the presence of DDAC did not limit the growth of the competing organisms, which allowed these organisms to grow and that these genera were tolerant to DDAC. The growth of all four organisms did not show a dose response as was seen earlier. The media containing DDAC found that the growth on the treated media with 800 ppm was not less than on the media treated with 400 ppm. However, the media containing CAC and DMBC showed no dose response, but a dramatic fall off in growth at 800 ppm. This concentration is where there is free AACs in the agar and may be too high for the organism to cope.

The studies found that the organisms did not simply tolerate the AAC or translocate it, but reduced the amount of the AAC present in the media. *Acremonium* sp. reduced the concentration of 400 ppm DDAC by 96% over a 28 day incubation period, while 800 ppm DDAC was reduced by 56% over 41 days. G. *roseum* FT reduced the content of 800 ppm DDAC by 90% over 41 days and G. *roseum* SB by 70% over 26 days. V. *bulbillosum* reduced 800 ppm DDAC by 37% over 26 days. The same effect was observed with CAC were V. *bulbillosum* and G. *roseum* FT reduced the initial 800 ppm CAC by ~50%. The *Acremonium* sp. reduced 800 ppm CAC by 90% over 40 days.

The addition of the same amount of DDAC in the form of liquid to the media containing G. *roseum* FT and *Acremonium* sp. resulted in the termination of fungal growth and no reduction in DDAC. The addition of DDAC in the form of wood did not stop fungal growth of G. *roseum* FT and *Acremonium* sp. DDAC in the wood was reduced by 49-54% and 45-54%

exposed to *Acremonium* sp. in the form of wood disks and sawdust. While G. *roseum* FT exposed wood reduced DDAC content by 18-54% and 61-67% from the wood disks and sawdust.

Mini stakes treated with DDAC were exposed to V. *bulbillosum*, C. *puteana* and P. *placenta* in a soil jar exposure. The results indicated that the two brown rot fungi did not affect the DDAC present in the media, but could degrade the untreated mini stakes. V. *bulbillosum* did not cause weight loss in the untreated mini stakes, while the DDAC treated mini stakes showed concentration reduction of DDAC. The amount of DDAC degraded by V. *bulbillosum* depended on the amount of DDAC initially present. At the highest retention (17 kg/m^3) the DDAC degradation was only 15%, which was less than at the lower retention the reason for this could be the amount of free or available DDAC maybe too high a level for the fungi to cope with. However, at the 10 kg/m^3 the DDAC degradation was in the order of 40% over 24 week exposure. At the lowest concentration 4 kg/m^3 no DDAC degradation occurred.

References.

Anonymous. 1976. 408 Chloride. Argentometric method. Standard methods for estimation of water and waste water. 302-304.

Anonymous. 1984. Alkyl Ammonium Compounds as Wood Preservatives. American Wood-Preservers' Association Proceedings. **80**: 191-204.

AWPA A9-70. 1970. Standard Method for Analysis of Treated Wood and Treating Solutions by X-Ray Emission Spectroscopy. American Wood-Preservers' Association Handbook on Standards.

AWPA E10-91. 1991. Method of Testing Wood Preservative by Laboratory Soil-Block Cultures. American Wood-Preservers' Association Handbook on Standards.

Baechler, R.H. 1949. "Reports of preservatives committees." American Wood-Preservers' Association Proceedings. **45**: 14-16.

Barnes, H. M. 1993. Wood protecting chemicals for 21st century. Internal Research Group on Wood Preservation. IRG/WP/93-30018.

Barnes, H. M., B. Buchanan, and T. L. Amburgry. 1985. Treatment and Durability of Wooden Roofing Materials. American Wood-Preservers' Association Proceedings. **81**: 89-107.

Bethell, J. 1838. British Patent 7731.

Bihler, I., A. Rothstein and L. Bihler. 1961. Mechanism of stimulation of aerobic fermentation in yeast by a quaternary ammonium detergent. Biochemistry Pharmacology. **8**: 289-99.

Blow, D. P. 1986. Alkyl dimethyl benzyl ammonium chloride: Toxicity to *Coniophora puteana* when formulated in water and organic solvent. Internaational Research Group on Wood Preservation. IRG/WP/2250.

Bodner, J. and G. Pekny. 1991. Distribution of wood preservatives within the cell wall. Holzforschung und Holzverwertung. **43**(2): 46-48.

Boethling, R. S. 1984. Environmental fate and toxicity in wastewater treatment of quaternary ammonium surfactants. Water Research. **18**(9): 1,061-1,076.

Bolton, A. J., J. M. Dinwoodie and P. M. Beele. 1985. The microdistribution of UF resins in particleboard. Proceedings Symposium on Forest Products Research International Achievement and the Future. **6**(17-12): 18p.

Bravery, A. F. and J. K. Carey. 1977. Development of an improved technique for rapid screening of woof preservative fungicides. 1. Selection of test substrate. International Biodeterioration Bullitin. **13**(1): 18-24.

Brezny, R., J. Schraml, J. Cermak, M. M. Micko and L. Paszner. 1990. Specific ion-exchangers based on lignins and their characterization by ^{29}Si-NMR Spectroscopy. Tappi Journal. 199-204.

Briscoe, P A, G R Williams, D G Anderson and G M Gadd. (1990) Microbial tolerance and biodetoxification of organic and organometallic biocides. International Research Group on Wood Preservation. IRG/WP/1464.

Browning, B. L. (1967). Methods in Wood Chemistry. Volume 1 Specific Gravity of Wood. pp 336-337. Intersune Publishers New York.

Butcher, J. A. 1979[a] . Testing New Preservatives for Protection of Wood Exposed in Aboveground Situations. Material und Organismen **14**(1): 43-53.

Butcher, J. A. 1979[b]. Serial Exposure Technique For Assessing Performance of Wood Preservatives. Wood Science and Technology. **13**: 127-135.

Butcher, J. A. 1980. Current status of AAC preservatives in New Zealand. International Research Group on Wood Preservation. IRG/WP/3141.

Butcher, J. A. 1985. Benzalkonium Chloride (An AAC Preservative); Criteria for Approval, Performance in Service, and Implications for the Future. International Research Group on Wood Preservation. IRG/WP/3328.

Butcher, J. A., and J. Drysdale. 1977. Relative Tolerance of Seven Wood-destroying Basidiomycetes to Quaternary Ammonium Compounds and Copper-chrome-arsenate Preservative. Material und Organismen. **12**(4): 271-277.

Butcher, J. A., and J. Drysdale. 1978[a]. Laboratory Screening trials with chemicals for protection of sawn timber against mould, sapstain and decay. International Bioteterioration. Bullitin. **14**(1): 11-19.

Butcher, J. A. and J. A. Drysdale. 1978[b]. Efficacy of Acidic and Alkaline Solutions of Alkylammonium Compounds as Wood Preservatives. New Zealand Journal of Forest Science. **8**(3): 403-409.

Butcher, J. A. and H. Greaves 1982. AAC Preservatives : A recent New Zealand and Australian Experience. International Research Group on Wood Preservation. IRG/WP/3188.

Butcher, J. A., A., M. E. Hedley and J. Drysdale. 1977. Comparison of a Quaternary Ammonium Compound And Copper-Chrome-Arsenate as Wood Preservatives. Forest Products Journal. **27**(7): 22-25.

Butcher, J. A., A. F. Preston and J Drysdale. 1977. Initial screening trials of some quaternary ammonium compounds and amine salts as potential wood preservatives. Forest Products Journal. **27**: 19-22.

Butcher, J., A. F. Preston and J. Drysdale. 1979. Potential of unmodified and copper-modified alkylammonium compounds as groundline preservatives. New Zealand Journal of Forestry Sciences. **9**(3): 348-358.

Byrne, T. 1991. Lumber protection in the 90's. Proceedings of a meeting held at: Forintek Canada Corp. Special Publication No. SP33 ISSN 0825-2119.

Byrne, T. and R.S. Smith. 1987. Protection of lumber in Canada: Current pratices, future trends. International Union of Forest Research Organization.

Cassen, D. L. and W. E. Eslyn. 1981. Fungicides to prevent sapstain and mold on hardwood lumber. Forest Products Journal. **31**(9): 39-42.

Chaplin, E. 1951. Observations on quaternary ammonium disinfectants. Canadian Journal of Botany. **29**: 373-382

Chen H. H. 1989. In situ determinations of calcium carboxymethycellulose on a cellulose film via ion exchange. Journal of Applied Polymer Science. **37**: 360-370.

Clark, E. 1868. On engineering philosophy: The duarbility of materials. Minutes of proceedings Institution of Civil Engineers (London). **25**: 554-581.

Connor P. and R. H. Ottewill. 1971. The adsorption of cationic surface active agents on polystyrene surfaces. Journal of Colloid and Interface Science. **37**(3): 642-651

Cross, D. J. 1979. Alkylammonium compounds as insecticidal wood preservatives. Material und Organismen. **14**(2): 105-116.

Cserjesi, A. L., and E. L. Johnson. 1982. Mold and sapstain control: laboratory and field tests of 44 fungicidal formulations. Forest Prod. J. 32(10): 59-68.

Cserjesi, A. L., and J. W. Roff. 1975. Toxicity tests of some chemicals against certain wood-staining fungi. International Biodeterioration Bullitin. **11**(3): 90-96.

Dean-Raymound D. and M. Alexander. 1977. Bacterial metabolism of quaternary ammonium compounds. Applied and Environmental Microbiology. **33**(5): 1,037-1,041.

DeGroot, R. C. and T. A. Kuster. 1986. SEM X-ray microanalysis of tracheid cell walls in southern yellow pine sapwood treated with water-dispersible pentachlorophenol. Wood and Fiber Science. **18**(1): 58-67.

DeGroot, R. C., B. M. Woodward and S. LeVan. 1992. Alternative species and preservatives for wood roofing: Laboratory decay studies. Forest Product Journal. **42**(11/12): 57-60.

Doyle A. K. and J. N. R. Ruddick. 1993. Isolation and identification of non-decay fungi affecting the performance of alkylammonium compounds. International Research Group on Wood Preservation. IRG/WP/93-10020 F1.

Doyle A. K. and J. N. R. Ruddick. 1994. The microdistribution of alkylammonium compounds in ponderosa pine sapwood. Holzforschung. **48**(2): 106-112.

Drysdale, J. A. 1983[a]. Performance of Unmodified and copper-modified alkylammonium treated stakes in ground contact. Material und Organismen. **13**(3): 354-363.

Drysdale, J. A. 1983[b]. A technique for measuring preserative loss or redistribution during leaching. International Group on Wood Preservation. IRG/WP/2199.

Drysdale, J. A. and A. F. Preston. 1982. Laboratory screening trials with chemicals for the protection of green lumber against fungi. New Zealand Journal of Forestry Science. **12**(3): 457-466.

Eaton and Hale. 1993. Wood. Decay, pests and protection. Chapman and Hall, New York.

Fengel, D. and G. Wegener. 1989. Wood chemistry, ultrastructure and reactions. Walter de Gruyter, Berlin.

Francis, D. M. and L. E. Leightley. 1983. Extracellular layers of wood decay fungi and copper tolerance. International Research Group on Wood Preservation. IRG/WP/1180.

Gale, G. R. 1963. Cytology of *Candida albicans* as influenced by drugs acting on the cytoplasmic membrane. Journal Bacteriology. **86**: 151-157.

Ghisalba, O. and M. Kuenzi. 1983. Biodegradation and utilization of quaternary alkylammonium compounds by specialized methlotrophs. Experienta. **39**: 1,264-1,271.

Gjovik, L. R., H. G. Roth and L. F. Lorenz. 1970. Quantitative differences in preservative penetration and retention in summerwood and springwood of longleaf pine. American Wood-Preservers' Association. **66**: 260-263.

Goldstein, J. I, D. E. Newbury, P. Echlin, D. C. Joy, C. Fiori and E. lifshin. 1981. Scanning electron microscopy and X-ray microanalysis. Plenum Press, New York and London.

Goodhew, P. J. and F. J. Humphreys. 1988. Electron microscopy and anlysis. Taylor and Francis, London, New York, Philadelphia.

Gotshal, Y., L. Rebenfeld and H. J. Howard. 1959. The adsorption of cationic surfactants by cellulosic material. II. The effects of esterificaton of the carboxyl groups in the cellulosic substrates. Journal of Colloid Science. **14**: 619-629.

Greaves, H. 1972. Structural distribution of chemical components in preservative-treated wood by energy dispersion X-ray analysis. Material und Organismen. **7**: 277-286.

Greaves, H. 1974. The microdistribution of copper-chrome-arsenic in preservative treated sapwood using X-ray microanalysis in scanning electron microscopy. Holzforschung **28**: 193-200.

Greaves, H., L .J. Cookson., and M.A. Tighe. 1988. Laboratory tests on light ogranic solvent preservative for use in Australia 2. Assessments of forther candidate fungicides. J. Inst. Wood Science. **11**(3): 103-107.

Greaves, H. and J.F. Levy. 1978. Penetration and distribution of copper-chrome-arsenic preservative in selected wood species. Holzforschung **32**: 200-208.

Greaves, H. and T. Nilsson. 1982. Soft rot and the microdistribution of water-borne preservatives in three species of hardwoods following field test exposure. Holzforschung **36**: 207-213.

Groves, M. J. and H. A. Turner. 1959. A study of factors affecting the inactivation of quaternary ammonium compounds on agar, Lancet. **65**: 169-172T

Hampton, D. and L. J. Zatman. 1973. The metabolism of tetramethylammonium chloride by bacterium 5H2. Biochemical Society Transactions. **1**: 667-668.

Hansen, J. 1984. IPBC- a new fungicide for wood protection. International Reseach on Wood Preservation. IRG/WP/3295.

Hartig, R. 1894. Textbook of diseases of trees. M^{ac}Millian and Co (Translation from German by W. Sommerville).

Hayward, P. and J. Duff. 1987. Movement of water through quaternary ammonium treated wood. International Research Group on Wood Preservation. IRG/WP/3440.

Hayward, P., W. Rae, and J. Duff. 1984. Mixtures of fungicides screened for the control of sapstain on *Pinus radiata*. International Research Group on Wood Preservation. IRG/WP/3307.

Hedley, M. E., K. Tsunoda, and K. Nishimoto. 1982. Evaluation of alkylammonium compounds for use as low toxicity wood preservatives in Japan. Wood Science. **68**: 37-46.

Hendrik, J. H., D. M. M. Adema and J. R. Wiegmann. 1966. Bacteriostatic, fungistatic and algistatic activity of fatty nitrogen compounds. Applied Microbiology. **14**(3): 308-319.

Highley, T. L. 1984. In-place treatments with waterborne preservatives for control of decay in hardwoods and softwoods above ground. Material und Organismen. **19**(2): 95-103.

Highley, T. L. 1990. Performance of surface-treated hardwoods and softwoods out of ground contact. International Research Group on Wood Preservation. IRG/WP/3592.

Howick, C. D., J. W. Creffield, J. A. Butcher, and H. Greaves. 1983. The Terminal Efficacy of a quaternary ammonium compound and a tertiary amine salt in wood protection. Material und Organismen. **17**(2): 81-92.

Hueck, H. J., D. M. M. Adema and J. R. Wiegmann. 1966.Bacteriostatic, fungistatic and algistatic activity of fatty nitrogen compounds. Applied Microbiology. **14**(3): 308-319.

Hugo, W. B. 1964. Some aspects of the action of cationic surface-active agents on microbial cells with special reference to their action on enzymes. p67-82.

Hulme, M. A., and J. F. Thomas. 1979 Control of fungal sap stain with alkaline solutions of quaternary ammonium compounds and with tertiary amine salts. Forest Products Journal. **29**(11): 26-29.

Hulme, M. A., and J. F. Thomas. 1983. Control of brown stain in eastern white pine with reducing agents. Forest Products Journal. **33**(9): 17-20.

Jin, L. and K. J. Archer. 1991. Copper based wood preservatives: observations on fixation, distribution and performance. American Wood-Preservers' Association Proceedings. **87**: 16pp.

Jin L. and A. F. Preston. 1991. The Interaction of Wood Preservatives with Lignocellulosic Substrates. I. Quaternary Ammonium Compounds. Holzforschung. **45**(6):455-459.

Jin L. and A. F. Preston. 1992. Commercial development of ACQ in the United States. CWPA.

Jin L., K. J. Archer and A. F. Preston. 1992. Depletion and biodeterioration studies with developmental wood preservative formulations. American Wood Preservers' Assocation Proceedings. **88**

Knezovich, J. P., M. P. Lawton, and L. S. Inouye. 1989. Bioaccumulation and tissue distribution of a quaternary ammonium surfactant in three aquatic species. Bullitin on Environmental Contamination Toxicology. **42**: 87-93.

Krzeminski, S. F. et al. 1973. The enviromental impact of quaternary ammonium bactericide. Household and Personal Products Industry. 22-25.

Larson, J. R. and C. D. Pfeiffer 1983. Determination of alkyl quaternary ammonium compounds by liquid chromatography with indirect photometric detection. Analytical Chemistry. **55**: 393-396.

Lee, D., M. Takahashi and K. Tsunoda. 1992. Fungal detoxification of organoiodine wood preservative. Holzforschung. **46**(6): 467-469.

Levy J. F. and H. Greaves. 1978. Penetration and distribution of copper-chrome-arsenic preservative in selected wood species. Holzforschung **32**: 209-213.

Linderborg, I. 1984. A potential antisapstain chemical for sawmills. IRG/WP/3300.

Loubinoux, B. and H. Malek. 1992. Interactions of quaternary ammonium salts with wood. 1. Fixation of benzalkonium bromide and chloride. Holzforschung. **46**(6): 537-539.

Loubinoux, B., H. Malek, J. P. Joly and G. Kilbertus. 1992. Interactions of quaternary ammonium salts with wood: influence of cation and anion structure on fixation and leaching. Forest Products Journal. **42**(10): 55-58.

Mackrell, J. A. and R. L. Walker. 1978. The biodegradation of quaternary ammonium compounds. International Biodeterioration Bullitin. **14**(3): 77-83.

Micales, J. A., A. L. Richter and T. L. Highley. 1985. Extracellular carbohydrate production by isolates of *Postia placenta*. International Research on Wood Preservation. IRG/WP/1388.

Moore-landecker, E. 1990. Fundamentals of the fungi. Pretice Hall, Englewood Cliffs, New Jersey 0732.

Morris, P.I. 1987[a]. Examination Of L-Joints Treated with Bardac 20. Forintek Canada Corp. Report: 02-17-93-0684, Vancouver, BC.

Morris, P.I. 1987[b]. Mycological Examination Of L-Joints Treated with Bardac 20 Supplementary Report. Forintek Canada Corp. Report: 02-17-93-0684, Vancouver, BC.

Morris, P.I., and J. Ingram. 1988. Stake tests of wood preservatives at Westham island 1987 data. Forintek Canada Corp. Report: 87-02-17-10-E051, Vancouver, BC.

Nicholas, D.D., and J. Drysdale. 1980. The evaluation of alkylammonium compounds as potential wood preservatives. American Wood-Preservers' Association Proceedings. **76**.

Nicholas, D. D. and A. F. Preston. 1980. Evaluation of alkyl ammonium compounds as potential wood preservation. American Wood-Preservers' Association. pp13-21.

Nicholas D.D., A.D. Williams, A.F. Preston and S. Zhang. 1991. Distribution and Permanency of Didecyldimethylammonium Chloride in Southern Yellow Pine Sapwood Treated by the Full Cell Process. Forest Products Journal. **41**(1): 41-45.

Nilsson, T. 1985. Defining fungal decay types-A proposal for discussion. International Research Group on Wood Preservation. IRG/WP/1264.

Oertel, J. 1965. Novel wood preservatives of good leaching resistance based on water soluble organic compounds, and their potential uses. Holztechnologie. **6**(4): 243-247.

Pauli, O. and G. Franke. 1970. Behaviour and degradation of technical preservatives in the biological purification of sewage.

Plackett, D. V. 1982. Field evaluation of alternative antisapstain chemical. International Research Group on Wood Preservation. IRG/WP/3198.

Plackett, D. V., C. M. Chittenden and A. F. Preston. 1984. Exterior weathering trials on *Pinus radiata* roofing shingles. New Zealand Journal of Forestry Science. **14**(3): 368-381.

Preston, A. F. 1983. Dialkyldimethylammonium Halides as Wood Preservatives. Journal of American Oil Chemists' Society. **60**(3): 567-570.

Preston, A. F., and C. M. Chittenden. 1982. Alkylammonium compounds as above-ground wood preservatives. New Zealand Journal of Forest Science. **12**(1): 102-106.

Preston, A.F., and D.D. Nicholas. 1982. Efficacy of a series of alkylammonium compounds against wood decay fungi and termites. Wood and Fiber. **14**(1): 37-42.

Preston, A. F., P. J. Walcheski and P. A. M^{c} Kaig. 1985. Recent studies with ammoniacal copper carboxylate preservatives. American Wood-Preservers' Association Proceedings. **81**: 30-39.

Preston, A. F., P. J. Walcheski, P. A. M^{c}Kaig and D. D. Nicholas. 1987. Recent research on alkylammonium compounds in the US. American Wood-Preservers' Association. **83**: 331-348.

Putam, F. W. 1948. The interaction of proteins and synetic detergents. Advanced Protein Chemistry. **4**: 79

Rayner, A. D. M. and L. Bobby. 1988. Fungal decomposition of wood: its biology and ecology. John Wiley and Sons, New York.

Rayner A. D. M. and M. K. D. Todd. 1979. Population and community structure and dynamics of fungi in decaying wood. Advances in Botanical Research. pp 333-420.

Rayner, A. B. M. and J. F. Webber. 1983. Interspecific mycelium interaction. An over view. The ecology and physiology of the fungal mycelium in nature.

Reid, V.W., G.F. Longman, and E. Heinerth. 1967. Determination of anionic active detergents by two phase titration. Tenside. **4**:292-304.

Resuggan, J. C. L. 1951. Quaternary ammonium compound in chemical sterilization. United trade press ltd, London.

Roomans G.M. 1978. Quantitative X-ray Microanalysis of thin Sections. *In*: M.A. Hayat (eds.): X-ray Microanalysis in Biology. pp. 401-453. University Park Press, Baltimore.

Rose, G. R. F., A. S. Weatherburn and C. H. Bayley. 1951. The sorption of synthetic surface-active compounds by carbon black. Textile Research Journal. 472-432.

Rosen, M. J. 1972. The relationship of structure to properties in surfactants. Journal of the American Oil Chemists' Society. **49**: 291-297.

Rosen, M. J. 1975. Relationship of structure to properties in surfactants. III. Adsorption at the solid-liquid interface from aqueous solution. Journal of American Oil Chemists' Society. **58**: 431-435.

Ruddick, J. N. R. 1983. Field testing of alkylammonium wood preservatives. International Research Group on Wood Preservation. IRG/WP/3248

Ruddick, J. N. R. 1984. Determination of alkyl ammonium compounds by titration. American Wood-Preservers' Association Proceedings. **80**:205-210.

Ruddick, J. N. R. 1986. The influence of staining fungi on the decay resistance of wood treated with alkyldimethylbenzylammonium chloride, Material und Organismen. **21**(2).

Ruddick, J. N. R. 1987. A field evaluation of modified and unmodified alkylammonium compounds. International Group on Wood Preservation. IRG/WP/3436.

Ruddick J N R and A K Doyle.1992. Unpublished results.

Ruddick J. N. R. and A. Lum. 1991. Soil and water leaching of didecyldimethylammonium chloride treated ponderosa pine stakes. Unpublished Report. 80 pages.

Ruddick J. N. R. and A. R. H. Sam. 1982. Didecyldimethylammonium chloride- a quaternary ammonium wood preservative: it's leachability and distribution in four softwoods. Material und Organismen. **17**(4):299-313.

Ruddick, J. N. R., and J. K. Ingram. 1987. An evaluation of selected wood preservatives by the standard stake test. Forintek Canada Corp. Report 02-17-10-051, Vancouver, BC.

Rustenburg, Gb. and C. J. Klaver. 1990. Evaluation of the effectiveness of defence anti-stain in the control of sapstain in laboratory and field tests. International Research Group on Wood Preservation. IRG/WP/3593.

Ryan, K. G. 1986. Preparation techniques for X-ray analysis in the transmission electron microscope of wood treated with copper-chrome-arsenate. Material und Organismen. **21**:223-234.

Saka, S. and D. A. I. Goring. 1988. The distribution of lignin in white birch wood as determined by bromination with TEM-EDXA. Holzforschung **42**: 149-153.

Scheffer, T. C. 1982. Microbiological Degradation and the Causal Organisms. Wood Deterioration and It's Prevention by Preservative Treatments. Vol 1, Chp 2. pp 31-106. Editor Nicholas, D.D. Syracuse University Press. New York.

Scheffer, T. C. and J. J. Morrell. 1986. The influence of method variable on the Aspergillus bioassay of wood preservative. Holzforschung. **40**:131-136.

Sexsmith, F. H. and H. J. White. 1959. The adsorption of cationic surfactants by cellulosic materials. I. The uptake of cation and anion by a variety of substrates. Journal of Colloid Science. **14**: 598-618.

Smith, R. S. 1978. New Lid Closure for Fungal Culture Vessels Giving Complete Protection Against Mite Infestation and Microbiological Contamination. Mycologia. **LXX**(3):499-508.

Stahl, W. F. 1992. Wood preservatives- United States. CEH Data Summary. Chemical Economics Handbook -SRI International. 597.5000A-Z

Steffan S., J. Joly, B. Loubinoux and P. Dizengremel. 1988. Effects of an alkyl ammonium bromide on oxidative and phosphorylative properties of mitochondria isolated from *Agaricus bisporus*. Pesticide Biochemistry and Physiology. **32**: 38-45.

Sterling, T. D., L. D. Stoffman, D. A. Sterling, and G. Mate. 1986. Health effects of chlorophenol wood preservatives on sawmill workers. Int. J. of Health Services. **12**(4): 519.

Sundman, C. 1984. Tests with Ammonical Copper and Alkyl Ammonium Compounds as Wood Preservatives. IRG/WP/3299.

Tillott, R. J., and C. R. Coggins. 1981. Non-arsenical water-borne preservatives- A review of performace and properties. BWPA Annual Convention. pp 32-48.

Tsunoda, K. 1990. Effect of alkyl chain length on the fungicidal efficacy of benzalkonium chlorides. Journal of Antibacterial and Antifungal Agents. **18**(4): 185-189.

Tsunoda, K. and K. Nishimoto. 1983. Fungicidal and termiticidal effectiveness of alkylammonium. International Research Group on Wood Preservation. IRG/WP/3232.

Tsunoda, K. and K. Nishimoto. 1987[a]. Effectiveness of alkylammonium compounds as above-ground wood preservative. Journal of Japan Wood Research Society. **33**(7):589-595.

Tsunoda, K. and K. Nishimoto. 1987[b]. Fungicidal effectiveness of amended alkylammonium compound. International Research Group on Wood Preservation. IRG/WP/3421.

van Ginkel, C. G., J. B. van Dijk and A. G. M. Kroon. 1992. Metabolism of hemadecyltrimethylammonium chloride in *Pseudomonas* stain B1. Applied and Environmental Microbiology. **58**(9): 3,083-3,087

Vinden, P. 1982. Treatability trials with alkylammonium compound preservatives. International Journal of Wood Preservation. **2**(1):5-12.

Vinden, P. 1984. The effect of temperature on the rate of fixation of an alkyl ammonium compound (AAC) wood preservative. International Research Group on Wood Preservation. IRG/WP/3293.

Vinden, P., and A. J. McQuire. 1979. Treatability trials with alkylammonium compound preservatives. International Journal of Wood Preservation. **1**(4): 185-195.

Wakeling, R. N., N. P. Maynard and R. D. Narayan. 1993. A study of efficacy of antisapstain formulations containing Triazole fungicides. International Research Group on Wood Preservation. IRG/WP/93-30021.

Wallace, L. R. 1986. Mathematical Modelling in Fungus Cellar Screening of Ammoniacal Copper/Quaternary Ammonium Preservative Systems Efficacy. American Wood-Preservers' Association Proceedings. **82**: 1-12.

Ward, H. A. 1990. Wood preservatives. US Patent Number 4,950,685.

Washam, C. J., W.E. Sandine and P. R. Elliker. 1976[a]. A strain of *Pseudomas aeruginosa* resistant to quaternary ammonium compound I. Physiologiacal properties. Journal Milk Food Technology. **39**: 101-106.

Washam, C. J., W.E. Sandine and P. R. Elliker. 1976[b]. A strain of *Pseudomas aeruginosa* resistant to quaternary ammonium compound II. Factors influencing degree of resistance. Journal Milk Food Technology. **39**: 273-279.

Watkinson, S. C. 1983. Morphogenesis of Serpula lacrimans colony in relation to it's function in nature. The ecology and physiology of the fungal mycelium in nature.

Weatherburn A. S. and C. H. Bayley. 1952. The sorption of synthetic surface-active compounds by textile fibres. Textile Research Journal. **22**: 797-804.

White H. J. 1970. Adsorption of Cationic Surfactants by Cellulosic Substrates. in J. Jungermann (eds): Cationic Surfactants. Vol.4 Surfactant Sci. Series. pp.311-339. Marcel Dekker Inc, New York.

Wilcox, W. W. 1973. Degradation in relation to wood structure. iin Degradation and Protection of Wood (Edited by D. D. Nicholas). Syracuse University Press. **1**(3): 107-148.

Zhi-ping, L. and M. J. Rosen. 1981. Two phase mixed indicator titration method for the determination of anionic surfactant. Analytical Chemistry. **53**: 1,516-1,519.

Zlochevskaya, I. A., S. Ya. Absalyamov, L. M. Galimova and I. A. Reshetnikova. 1981. Study of the mechanism of fungicidal action of quaternary ammonium compounds. Biologicheskava nauka. **3**: 1-11.