THE EFFECT OF CHEMICAL PRESERVATION ON *Pinus patula* WOOD CHIPS DURING OUTSIDE CHIP STORAGE

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Abstract

Accelerated laboratory decay tests using *Pinus patula* chips treated with 7 preservative formulations were conducted at 25°C for 4 months. Biodeterioration was monitored by chemical, microbiological and pulp analyses. Chips treated with 2 % (w/v) propionic acid were not decayed by basidiomycetes and produced pulp of quality indistinguishable from fresh untreated chips. Outside chip storage (OCS) field trials using propionic-acid treated chips were conducted in the subtropical environment of Zululand. Treated chips yielded acceptable pulp after 4 months' OCS. Quantification of microorganisms showed that chip treatment delayed the onset of colonisation for 2 months. Surfaces of untreated chips were extensively colonised after 2 months and after 4 months hyphae penetrated and colonised interior elements. Basidiomycete decay and cellulolytic soft rot by *Chaetomium spp* was observed. Treated chips were relatively uncolonised. No basidiomycete decay or ascomycetous soft rot occurred. Ascomycetous colonisation was restricted to staining fungi such as *Penicillium spp*.

DECLARATION

I declare that this dissertation is my own, unaided work. It is being submitted for the degree of Master of Science in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.

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(Name of candidate)

[illegible] day of [illegible], 19[illegible]

TO MY FATHER (MR I.M. ISMAIL) AND
(LATE) MOTHER (MRS AMINA ISMAIL)

Words cannot express the love, sincerity and happiness
that my parents have bestowed upon me.

My gratitude extends far beyond the measureless miles and enigmatic, endless space of the universe. My love flows freely and faithfully from my heart, my soul, from me to them. Greater still is my gratitude to God Almighty, for the gift of knowing such wonderful two persons who are dearer to me than every precious breath of air I breathe.

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1.0 INTRODUCTION

Wood has been used by man throughout the world, for thousands of years, as a source of cheap and readily available fuel and raw material, with many structural and decorative functions. Its advantages include toughness, strength, and the ease with which it could be worked using the low levels of technology available. Weight for weight, wood is stronger than steel, and from an ecological viewpoint, in contrast with the finite reserves of iron and plastic bases available, wood stocks, with careful management are renewable (KING, 1981).

Wood is however no longer cheap, nor plentiful and forestry management practice in the technically developed world has resulted in considerable control of deforestation and decreased exports to the major wood importing nations. Market forces thus produced have resulted in a steady increase in the value of wood stocks and consequent increased costs to the consumer (KING, 1981). With only 1 % of South Africa's land area under forest, it compares very unfavorably with other countries and regions of the world. For example, approximately 32 % of the world's land area is under forest, whereas the figure for Africa is about 24 % (SPIES, 1988). Delivering the keynote address at the South African Institute of Forestry's Forestry Research 1988 symposium at the CSIR in Pretoria recently, Mr Trahar said projections to the year 2010 show that the supply from existing planted areas will not be able to meet future demand for both hardwood and

softwood. "This shortfall must be addressed as a matter of urgency". (ANON, 1988).

Freshly felled wood is non-durable, and its biodeterioration accounts for losses of up to 10 % total yield (BAECKER, pers. comm., 1987), therefore such losses place greater demands on timber production.

1.1 Timber Storage

Since the period between felling and conversion for end use, during which timber is most susceptible to biodeterioration, storage methods are important determinants of associated economic losses. Timber is stored either as roundwood or as chips.

1.1.1 Roundwood

Timber is felled and may be stored in the forest before extraction and transport to sawmills. The roundwood may be further stored as stocks prior to conversion and planks may in turn be stored prior to end use or during seasoning. Often total storage periods exceed six months.

1.1.2 Outside Chip Storage

Pulp and paper is of course becoming an increasingly valuable commodity as demand continues to grow (HULME, 1979), particularly, in developing South Africa. Rising prices reflect impending shortages on a global basis

(HULME, 1979). Commercial wood chip storage was therefore introduced in the early 1950's in the American West Coast to alleviate some of the problems encountered by the pulp and paper industry in the storage of their vast reserves of raw materials (SCHMIDT, 1969; GREAVES, 1971a; FULLER, 1985). Piles were constructed of chips from sawmill and veneer residues obtained from the wood-using industries (DOYLE, 1963; MICHAUD, 1962 cited by SHIELDS, 1967).

The possibility of storing wood for pulping in the form of chips has received increasing consideration in recent years (LINDGREN and ESLYN, 1961), because chips are more economical to handle than logs (HENSEL, 1958; BAKER, 1960; BURKE, 1962; FORSSBLAD, 1965; HOLEKAMP, 1962a cited by SHIELDS, 1967; HULME, 1979). Handling and economic advantages of outside chip storage (OCS) over log storage (BAKER, 1960 cited by SHIELDS, 1967) have resulted in many mills chipping their logs to provide readily available material for the pulping operations (SHIELDS, 1967). Improved forest management and harvesting policies along with reforestation will obviously help alleviate the problem but it is clear that costly losses attributable to biodeterioration during the outside storage of chips cannot be tolerated (HULME, 1979). The success of chip storage is affected by climate and wood species (HAJNY, 1966), therefore considerable research has been conducted on the biodeterioration of Southern pine, oak and gum chips throughout the world (SHIELDS, 1967), but particularly in U.S.A., Canada, New Zealand, Australia and Sweden. Of all the

pines, none has been grown more extensively for timber in South Africa than *Pinus patula* Schiede et Deppe in Schldl. et Cham (POYNTON, 1979). While it is well suited to very warm and dry regions, and although subject under certain circumstances to damage or attack by such agencies as wind, hail, fungi and insects, it nevertheless thrives on a great variety of soils and yields a general utility timber free from defects and of consistently acceptable quality (POYNTON, 1979). However, the biodeterioration of *Pinus patula* as chips in outside chip storage (OCS) has never been investigated in S.A.

i) Advantages of OCS

Virtually all types of wood ranging from the hard woods and soft-woods of temperate climates to the bamboos (MOKHASI, HANDIGOL, JASPAL and BHARGAVA, 1969 cited by HULME, 1979) and tropical hardwoods (GREAVES, 1971b cited by HULME, 1979) have been stored as chips (HULME, 1979). Operational advantages of OCS over round-wood storage (CLARK, 1963a cited by SHIELDS, 1967) have been reported as follows (SHIELDS, 1967) :

a. *Reduced fibre loss.*
b. Continuous supply (CLARK, 1963b; HOLEKAMP, 1962b; HAJNY, 1960 cited by HAJNY, 1966).
c. Decreased handling costs (CLARK, 1963b cited by HAJNY, 1966).

d. Increased utilisation of residue chips from sawmills to veneer plants (ROTHROCK, SMITH and LINDGREN, 1961).

e. Large manpower saving (CLARK, 1963b; INSERRA, 1961 cited by HAJNY, 1966).

f. 20 to 25 % increase in production from wood-yard wood-room (DIEHL, 1960; LAW, 1963 cited by HAJNY, 1966).

g. Reduced maintenance costs.

h. Elimination of production loss because of wood-room/wood-yard breakdowns.

i. Increased bark yield and consequent reduced fuel costs.

j. Improved uniformity in digester furnish (CLARK, 1963a; HOLEKAMP, 1962c cited by HAJNY, 1966).

k. Greater storage per acre.

l. Less house-keeping (HAJNY, 1961; CLARK, 1963b; CLARK, 1963a cited by HAJNY, 1966).

m. Smooth wood-yard/wood-room operation.

n. Better, more accurate species handling (CLARK, 1963b cited by HAJNY, 1966).

Unfortunately, many costly problems can arise when wood chips are stored outside (BLACKERBY, 1971; CHALK, 1968; GRANT, 1968; HATTON, SMITH and ROGERS, 1968; HOLEKAMP, 1962a cited by HULME, 1979), and unless a chip pile is carefully managed the pulping properties of the chips can be affected in many ways, as mentioned below :

a. The pulp yield may be lowered because the chips have lost weight or require extended pulping; or because more of the chip dissolves when pulped.

b. The pulp strength may be lowered because pulp constituents in the chips have been degraded, or because the chips have to be overcooked to produce the desired grade of pulp.

c. The pulp brightness may be lowered because the chips have darkened.

d. More pulping and bleaching chemicals may be required or chemical recovery (in the kraft process) may be slower.

e. The pulping equipment may corrode more rapidly.

f. Less tall-oil and turpentine by-products may be obtained in kraft pulping.

g. In the extreme case of chip pile fires no pulp or by-products may be obtained.

Thus, deterioration during outside chip storage may be defined as any process which lowers the quantity or quality of pulp, increases the requirement for pulping and bleaching chemicals, or increases the cost of equipment maintenance (HULME, 1979). Such deterioration obviously includes biodeterioration.

In tropical environments and marine environments, insects and molluscs respectively play the major role in wood biodeterioration whereas, in more

temperate climates microorganisms are the main agents of destruction (KING, 1981), particularly during OCS.

An understanding of the economic effects of fungi in wood must involve an appreciation of the end use for that wood. In some uses the presence of fungi in wood may be accepted (e.g sap-stain in construction-grade timber), whereas the same fungi in logs to be used for the production of mechanical pulp may be unacceptable. From the user's viewpoint, wood can be deteriorated without being decayed, this being obvious within the pulp and paper industry. Deterioration of wood by fungi can then be defined as a decrease in value of that material to a specific user because of the presence of these microorganisms (SMITH, 1975).

1.2 Biodeterioration Of Chip Piles

In considering the economic importance of fungi in wood utilised by the pulping industry, we must also consider the time at which fungi may enter the wood. They may enter as listed below :

a. A standing tree before felling.
b. A log during its subsequent period of storage.
c. Wood chips during outside storage following conversion from logs *or sawmill residues (SMITH, 1975).*

When the living tree is attacked the organisms involved are parasites causing disease and this aspect of microbial decomposition is more properly considered as forest pathology or plant pathology. Form the viewpoint of the wood user, the important organisms are generally those which colonise the wood after it is felled (KING, 1981).

1.2.1 Microbiological Succession Patterns

The microorganisms which colonise and degrade wood include bacteria and actinomycetes, but the major group of microorganisms involved in the succession pattern of decaying wood are the fungi.

1.2.1.1 Fungal physiology with respect to wood decomposition

Fungi belong to a primitive branch of the plant super-kingdom PLANTAE and comprise the kingdom MYCETAE. They differ from the photoautotrophic green plants in their lack of chlorophyll and hence their inability to manufacture their own sugars using sunlight, carbon dioxide and water. These primitive chemoheterotrophic microorganisms therefore absorb their energy supplies from external sources. Wood can be such a source and fungi can grow on the surface and within the wood, utilising freely available sugars and breaking down the more solid structure of wood by use of various substrate specific enzyme systems to solubilise and absorb the less freely available food materials. Thousands of different types of fungi exist, showing different forms of growth, types of reproduction and

capacities to attack and colonise wood. Most fungi have at least the following five basic requirements for growth, namely :

a. A *suitable source of food.*
b. Oxygen.
c. *Presence of a minimum degree of moisture.*
d. A pH range between 5 and 8 (von MOLY, 1981).
e. A *suitable temperature range usually* between 25 and 40°C (von MOLY. 1981).

Lack of one of the above requirements will normally prevent the growth of fungi. Therefore, if wood is kept dry as within house timber joists, no growth of fungi will occur. Also, if pulp logs are kept submerged in water, where oxygen will be severely restricted, little fungal growth will occur (SMITH, 1975).

Apart from the carbon sources, wood decomposing fungi require bound nitrogen, for instance, inorganic salts or protein for growth. The carbon : nitrogen ratio of wood is 300;500 : 1, rendering it poor in nitrogen, and this is a limiting factor for the growth and activity of fungi. However, living cell systems (ray parenchyma cells and epithelial cells of the resin canals) have a higher concentration of nitrogen than the rest of the wood material. It has been shown that fungi initially attack these living cells, whereby their content of nitrogen and reserve nutriment like starch and lipophilic extractives is depleted (ASSARSSON, CROON and FRISK, 1970a). The

fungal biomass which developed in this process then dies, releasing nitrogenous materials which provide nutrition for succeeding invaders. In addition, fungi may translocate nitrogen from the exterior, to wood (KING, SMITH, BAECKER and BRUCE, 1981). Simultaneously, carbon is lost from the wood as CO_2 during fungal respiration. These combined processes cause the carbon : nitrogen ratio of wood to narrow from 300/500 : 1 to levels amenable to vigorous microbial growth consistent with rapid decay of the wood. A succession pattern of microorganisms therefore occurs in decaying wood, and different groups invade at different stages of the decay process, depending on their ability to utilise the wood at different stages of its changing nutritional status.

Fungi are classified by microbiologists mainly according to the way they reproduce. In most cases, similar groupings can be used to separate fungi according to the way they damage wood (KAARIK, 1974 cited by HULME, 1979). Three basic groups, which overlap to some extent, can be distinguished as follows :

a. Primary and secondary colonisers;
   1) mould, stain or soft-rot fungus,
   2) yeasts, bacteria and actimomycetes.
b. Tertiary colonisers;
   1) white rot fungus,
   2) brown rot fungus.

These are serious agents of decay and have been reviewed in Chapter 3.

1.2.1.2 Effect of biodeteriogens on the quantity of wood substance in chip piles

Many reports on the losses in wood substance which have occurred during chip storage have come from studies made in the southern U.S.A. and Sweden, and indicate some variability in basic specific gravity losses not only between different piles but also within the pile itself. The type of microorganisms inhabiting chips and the degree of microbiological activity are important factors involved in the amount of wood substance lost during storage (SHIELDS, 1967).

Only minor reductions in specific gravity have been noticed in pine chips stored for up to 2 months (ANON, 1959 cited by SHIELDS, 1967), when compared to non-stored chips. In another study (SAUCIER AND MILLER, 1961 cited by SHIELDS, 1967), losses in specific gravity during the first few weeks of pine chip storage were greater in those chips situated near the centre of the pile. While these losses were unexplained at the time it is possible that very high temperatures resulting from the initial rapid growth of heat tolerant and thermophilic microorganisms may have been involved. With longer storage it was found that losses were not as great in the centre of the pine chip pile (SAUCIER and MILLER, 1961 cited by SHIELDS, 1967). This reduction in specific gravity losses corresponded to

a lowering of the temperatures, an increase in moisture and perhaps depletion of oxygen (SHIELDS, 1967).

The types of microorganisms that will ultimately be responsible for most of the substance losses will depend on the type of material and the prevailing storage conditions, especially the temperature. In the cool outer regions of storage piles, mesophilic fungi prevail; in the warm to hot inner regions thermophilic and thermotolerant fungi are responsible for substance losses (HULME, 1979 cited by SPRINGER, 1983a).

The initial higher losses experienced in the hot areas of some piles within a few weeks after piling has commenced, can perhaps, be attributed to a combination of biological and chemical activity operating at the same time. The metabolic activity of the thermophilic and heat tolerant fungi on the wood probably accounts for some of this loss with other losses occurring from the deacetylation process as a result of high temperatures and moisture, as well as from oxidations of other substances, such as resins, in the wood. With increased storage time however, the losses in the upper interior regions do not surpass the losses experienced in the cooler uncompacted sides of some piles where wood-rotting Basidiomycete fungi and staining fungi are more active (SHIELDS, 1967).

The occurrence of greater numbers of white rot fungi in the lower levels of some soft-wood chip piles is one major cause of wood substance loss in

these areas (SHIELDS, 1967). The observations that chips in the lower part of a pile are frequently dark brown, discoloured and have an acetic acid odour, suggests that there is also a possible hydrolysis of the hemicelluloses by organic acids formed in the pile during biological or chemical processes and that this results in further reductions in wood substance and increased storage (SHIELDS, 1967).

1.2.2 The Detection Of Biodeterioration

The resistance of different species to fungal attack varies, but no wood is immune. A relative degree of resistance is imparted when large amounts of extractives toxic to fungi are present. This is the main reason why some species, such as redwood, oak, chestnut, and black locust, for example, and eucalypt and wattle in S.A. are more durable than others. Similarly heart-wood is generally more durable in comparison to sap-wood of the same species (TSOUMIS, 1968) owing to the higher concentrations of *antimicrobial* extractives such as tannins in heart-wood.

The presence of mould fungi on logs and lumber is often seen as a fluffy surface growth of many colours. Green is a colour frequently seen in *Penicillium* and *Aspergillus* although white *Mucor* and pinkish *Epicoccum* pigmentation are also common.

As stated above (1.2.1.2) there is no indication of weakness in underlying wood and the importance of most staining fungi to pulp mills is probably negligible (SMITH, 1979).

The presence of sap-stain fungi in lumber or chips can easily be ascertained by a visual examination. The wood will appear stained a blue or black colour, frequently in a streaky or radially expanding fashion towards the outside of the wood (SMITH, 1979).

Soft-rot caused by fungi such as Ascomycetes and Fungi Imperfecti cause attacked wood to darken as decay progresses. When dry, affected wood becomes soft and brittle with cubic check marks as with brown rot (below) (CARTWRIGHT and FINDLAY, 1958; LEVI, 1964 cited by TSOUMIS, 1968). However, these symptoms are macroscopic and noted in logs but not in chips. A schematic representation of wood cell wall at micro-level is shown in Figure 1.1 a and b respectively. Microscopically, soft-rotted wood shows characteristic geometrical cavities in cell walls, and these are useful in diagnosis with the microscope (COWLING, 1965; LIESE, 1964 cited by TSOUMIS, 1968; SHIELDS, 1967).

Severe attack of wood by white-rot fungi can result in a white-pocket type or a stringy yellowish type of rot. This would be visually evident, although brashness of the wood when broken would also result. Early stages of

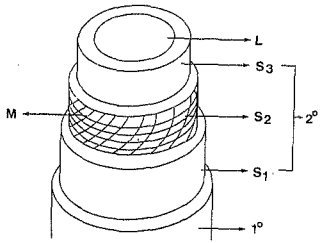
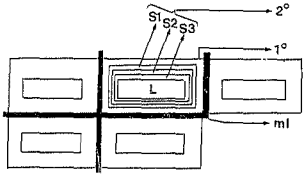
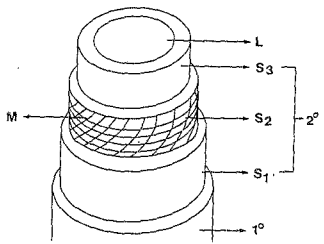


Figure 1.1 Schematic representation of a typical wood cell to show differentiation of cell layers.
a. Longitudinal representation of a transversely sectioned cell (L - lumen; 1° - primary wall; 2° - secondary wall; M - direction of cellulose microfibrils)



b. Transverse section of cells (L - lumen; 1° - primary wall; 2° - secondary wall; ml - lignified middle lamella)



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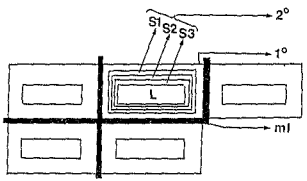


b. Transverse section of cells (L - lumen; 1° - primary wall; 2° - secondary wall; ml - lignified middle lamella)

decay by these fungi can cause brown or reddish discolorations of wood with little physical degrade (SMITH, 1979; LEVY, 1969).

Severe attack of wood by brown-rot fungi generally results in dark brown discolorations of wood which will be crumbly, cracked and broken, particularly as the wood dries (LEVY, 1969). *Considerable shrinkage of the* wood results in drying. Incipient stages of attack by these fungi are difficult to diagnose, but probably at these early levels the reduction effects on pulp yield will be small. However, decay could be diagnosed by a softening of the wood or by a brashness when broken, as well as by a decrease in specific gravity as normally determined (SMITH, 1979). The conclusive method to diagnose white or brown-rot in chips remains the detection of *microscopically visible effects. These include localised* bore holes through cell walls, and disintegration of walls in parts that are not in immediate contact with fungal hyphae - presumably through transfocation of enzymes. The patterns of these effects differ according to the type of fungus, and may be best observed with the electron microscope (TSOUMIS, 1968). In general, white-rotters produce typical lysis zones around hyphae on wood cell walls (Figure 1.2) since the ligninases produced can degrade the S3 *wall. In* contrast, brown rotters produce cellulases which diffuse through the lignin in S3 without causing apparent decay, but these cellulases produce degradation in the *cellulolytic S2 as seen in transverse section* (Figure 1.2).

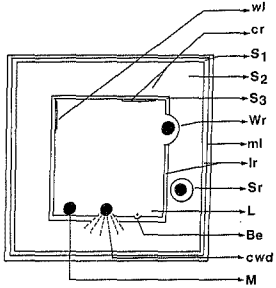


Figure 1.2 Cross section of a wood cell wall showing the composition and *microbial decay patterns.* (wl - warty layer; cr - cellulose rich; S1, S2 and S3 - 2* wall layers; Wr - white rot fungus in erosion trough; ml - middle lamella; lr - lignin rich; Sr - soft rot fungus in soft rot cavity; L - lumen; Be - bacterial erosion; cwd - cell wall dissolution by brown rot fungi; M - mycelium of mould stain fungus).

The visual detection of deterioration by thermophilic fungi is very difficult. In some instances, specific fungi may turn the wood chips a light or dark brown, often in a very spotty pattern. The presence of small brownish-black spherical bodies on the surface of the chips also indicate activity by this group of fungi. However, many of these fungi are capable of growth on wood chips with little external indication of their internal activities. There will be no indication of obvious weakening of the wood chips, at least until long of storage has occurred. It is probable that the limited species of thermophillic fungi involved are common to chip piles of different wood species and to piles stored in different geographic locations. It is unlikely that any large chip pile, untreated in any way, will escape rapid and complete colonisation by these fungi. Their reproductive spores will be present in enormous numbers in the air around a pulp mill and will be quickly drawn into a self-heating chip pile (1.2.3.1) by the developing air convection currents. Obviously, commonly used pneumatic systems for chip transportation to a pile will create ideal conditions for coating the outside of chips with spores of these fungi. As chips pass through the air, they will collect spores which will subsequently grow and colonise the outside and then the inside of chips (SMITH, 1979).

1.2.3 Factors Influencing Deterioration

The durability of the wood species influences the type of fungi and the rate of deterioration in chip piles (SHIELDS, 1967). Temperature and moisture are two of the most important variables in determining the type of

microflora and their succession in chip piles, and the nature and rate of deterioration, while high temperatures over 35°C are important in inhibiting certain wood-staining and decay fungi. The depletion of oxygen and the metabolic evolution of carbon dioxide by these aerobic chemoheterotrophs vary according to the degree of compaction and moisture in a pile, may serve as an indication of the rate of microbial metabolism. While total lack of free oxygen in a water - saturated substrate (55 % wet weight) does inhibit fungal growth; as with submerged roundwood (above) it is expected that oxygen levels are sufficient for growth of fungi in areas of piles where chips are not completely saturated.

1.2.3.1 Temperature and moisture content

Both the moisture content and the temperature existing within many outside chip piles have been found to vary considerably during the storage period. European and American investigators (CRANE and FASSNACHT, 1960; LINDGREN and ESLYN, 1961; ROTHROCK, SMITH and LINDGREN, 1961; BOIS, FLICK and GILMER, 1962; BJORKMAN and HAEGER, 1963a; ANNERGREN, DILLEN and VARDHEIM, 1964; LJUNGQVIST, 1965; BERGMAN and NILSSON, 1966; SAUCIER and MILLER 1961 cited by SHIELDS, 1967), have observed that the drier interior conditions are usually associated with the high temperatures found in the centre of the piles. The presence of excessive moisture (over 20 % dry weight) together with high temperatures has been

suspected both of increasing chip deterioration by chemical means and of decreasing discolorations by the blue-staining fungi (Figure 1.3).

Spontaneous heating in wood chip piles is well known (HAJNY, 1966; ASSARSSON, SPRINGER and HAJNY, 1970; SPRINGER, HAJNY and FEIST, 1971; CROON and FRISK, 1970a cited by FEIST, SPRINGER and HAJNY, 1974). Centre temperatures in commercial chip piles often reach and maintain 55 - 70°C (HAJNY, 1966; SCHMIDT, 1969 cited by FEIST, SPRINGER and HAJNY, 1974). In some chip piles, spontaneous ignition has occurred (COLE, 1972a cited by FEIST, SPRINGER and HAJNY, 1974). Severe degradation of redwood chips recovered from a chip pile in which temperatures exceeded 60°C for long periods have been reported (SCHMIDT, 1969 cited by FEIST, SPRINGER and HAJNY, 1974). The chips darkened, and active alkali requirements for kraft pulping were increased by 1 % to 2 % more than that for normal chips. In addition, screened pulp yields decreased from 38 % to 27 %, burst strength decreased 15 % and tearing resistance as much as 45 % below that normally obtained from redwood. The effects observed were apparently caused by chemical rather than microbiological degradation since temperatures above 50°C have a "biological self-pasteurising effect" (SCHMIDT, 1969 cited by FEIST, SPRINGER and HAJNY, 1974). Within a few weeks of the construction of large chip piles, the phenomenon of "heating" occurs in the upper interior regions of the piles. With longer

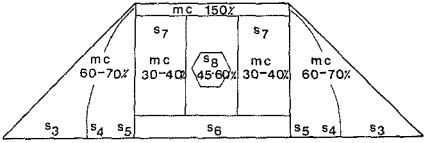


Figure 1.3 Sketch showing areas in cross section of a chip pile *with* different types of microflora and moisture contents (mc) usually observed. (S3 - moulds + basidiomycetes; S4 and S5 - moulds + *Chrysosporium lignorum*; S6 - moulds + *Allescheria terrestris*; S7 - moulds mesophilic; S8 - moulds thermophilic). All mc's are expressed as % dry weight. (ASSARSSON, CROON and FRISK, 1970; GREAVES, 1971)

storage, the temperatures in the interior of the piles were not quite as great as high temperatures (e.g 63 - 65°C) recorded soon after the start of chip storage. The drop in the temperature sometimes occurs rather abruptly (LINDGREN and ESLYN, 1961; ROTHROCK, SMITH and LINDGREN, 1961; SAUCIER and MILLER, 1961 cited by SHIELDS, 1967) but this decline has also been reported to occur more gradually in a larger more compacted chip pile in Sweden (ANNERGREN, DILLEN and VARDHEIM, 1964 cited by SHIELDS, 1967). This difference was attributed to the larger volume of chips in the Swedish pile which gave greater insulatory protection against atmospheric changes.

Temperatures have fluctuated (ANNERGREN, DILLEN and VARDHEIM, 1964; BOIS, FLICK and GILMER, 1962 cited by SHIELDS, 1967), according to the degree of compaction in the piles with greater compaction resulting in higher average temperatures. The cause of the heating which occurs in outside stored chip piles has been attributed in part to the presence of bark, the condensing of water vapour on the chips and the microbiological activity within the pile. It is not expected that solar radiation would influence the conditions deeper within a large pile although heat from the sun will increase the temperature of the surface chips. What is believed to occur in a large chip pile is the transfer of warm moist air by convection from the lower regions of the pile to the upper cooler chips. This creates a chimney effect whereby the evaporation of moisture from the lower chips results in cooler temperatures in these regions while the con-

densing of water vapour on the chips in the upper portion of the piles causes not only an increase in the moisture content but also higher temperatures (ANNERGREN, DILLEN and VARDHEIM, 1964; BOIS, FLICK AND GILMER, 1962; LJUNGQVIST, 1965; ROTHROCK, SMITH and LINDGREN, 1961 cited by SHIELDS, 1967). As a consequence, the wood chips situated in the lower central regions of a pile become cooler and drier. While chemical activity occurring in the part of the pile during early storage is thought to be the cause of higher temperatures which increase the evaporation of some of the moisture (ANNERGREN, DILLEN and VARDHEIM, 1964 cited by SHIELDS 1967), it is also known that heat arising from the increased metabolic activity of microorganisms growing in a favourable environment can occur in a relatively short period of time. It is therefore believed (ROTHROCK, SMITH and LINDGREN, 1961 cited by SHIELDS, 1967) that the excessive heat is generated within the pile as a result of oxidative reactions including the participation of microorganisms (ANNERGREN, DILLEN and VARDHEIM, 1964; SHIELDS, 1967).

Thermophilic or "heat-loving" fungi have been found to contribute considerably to the maintenance of elevated temperatures over long storage periods. The association of thermophilic fungi with hot areas is also found in the storage of grain and other organic materials. Thermophilic fungi grow within the range of 20°C to 60°C while some bacteria will continue their growth at temperatures beyond 60°C. In contrast most wood-stain and

decay fungi are inhibited at temperatures above 35°C except for some heat tolerant wood-decay species which apparently can grow up to 50°C (NILSSON, 1965; SHIELDS, 1967). There is therefore, some probability that chip quality will be adversely affected because of the activity of these heat-tolerant fungi. An additional outcome of increased fungal metabolism in the chip pile is the production of carbon dioxide and water (LINDGREN and ESLYN, 1961 cited by SHIELDS, 1967) as end products of the degradation of carbohydrates in wood.

Moisture content will therefore be unlikely to be a limiting factor in retarding all deterioration in outside chip piles because of the difficulty in maintaining either dry (below 17 % moisture content green weight basis) or the fully water-saturated conditions required to inhibit the growth of fungi. The high temperatures and moisture contents experienced in the upper interior regions of chip piles is thought to be responsible for the reduction in the bluish grey discolorations caused by staining fungi (HOLEKAMP, 1959; HOLEKAMP, 1962a; LJUNGQVIST, 1965 cited by SHIELDS, 1967). There is some indication that excessive moisture content and high temperatures could contribute to other non-microbial, types of chip discolorations. This is due in part to the dark water-soaked appearance of the chips.

The presence of bark has been thought to be responsible for excessive heat within piles and the subsequent rapid development of brown stain due to

fungal attack (WAHLIN, 1965; WRIGHT, 1954 cited by SHIELDS, 1967). According to FEIST, HAJNY and SPRINGER (1973) heat alone can cause serious degradation and solubilization of wood carbohydrates and subsequent loss in pulp yields and pulp properties. High temperatures and moisture content are also factors in contributing to brown chemical staining and are reported (ANNERGREN, DILLEN and VARDHEIM, 1964 cited by SHIELDS, 1967) to result in the deacetylation of hemicellulases in the wood although no change in acetyl content was detected in one hardwood chip pile after 6 months storage (HAJNY, JORGENSEN and FERRIGAN, 1967 cited by SHIELDS, 1967). It has therefore been recommended that every effort should be made to reduce heating during wood chip storage (FEIST, HAJNY and SPRINGER, 1973).

1.2.3.2 Oxygen and carbon dioxide levels

In an early report (BJORKMAN, 1959 cited by SHIELDS, 1967) on storage conditions in a chip pile it was suggested that the oxygen needed for the life process of aerobic organisms might be quickly used up and that the resulting deficiency of oxygen would have and unfavorable effect of the growth of decay fungi in the pile. However, later studies in Louisiana, U.S.A. (HOLEKAMP, 1959 cited by SHIELDS, 1967) and in Sweden (BJORKMAN and HAEGER, 1963a cited by SHIELDS, 1967) indicated that there were no great variations in carbon dioxide and oxygen levels in loosely compacted chip piles during storage. In laboratory experiments

(BJORKMAN and HAEGER , 1963a cited by SHIELDS, 1967) it was observed that certain wood-decay fungi and the mould, *Trichoderma lignorum*, grew well in an atmosphere containing as much as 40 to 50 % CO_2, and that in other tests these same fungi were not inhibited until the concentration of oxygen was *practically zero*. It was concluded that very large changes in oxygen or carbon dioxide concentration would be necessary to inhibit the growth of the fungi which could cause deterioration of chips during storage (SHIELDS, 1967), and this conclusion is supported by on-site observations of the present author.

1.3 Control Of Biodeterioration In Chip Piles

Recognition of the problems caused by the adverse influence of microorganisms in chip storage (ASSARSON, CROON and FRISK, 1970b), and the economic benefits of overcoming them, have led a number of companies and research groups into investigations of chip pile behaviour (ANON, 1969). Control methods are based on manipulation of factors which affect microorganism growth. Control can therefore be achieved by, changing the environment of microorganisms in the pile, treating the chips with fungicides, or suppressing harmful microorganisms by adding dominant but harmless microorganisms (ASSARSSON, CROON AND FRISK, 1970b) as biological control agents. The methods of reducing deterioration during outside chip storage may therefore be classified into mechanical and physical, chemical and biological ones (BERGMAN and NILSSON, 1979).

HATTON (1969) described the benefits derived from the use of smaller piles combined with sensible wood-yard practices (*HATTON, 1970a cited by SMITH, 1975*).

1.3.1 Mechanical And Physical Methods

Chip deterioration can be minimised by mechanical means (HATTON, 1969) such as good woodyard management, systematic storage of chips in the *doughnut pattern* coupled with despatch on a first-in, first-off basis. The ring-shaped pile allows storage of chips on a first-in, first-out principle (BERGMAN and NILSSON, 1979). A piling and reclaim methodology is based upon the following :

a. Nature of wood species stored.
b. Climatological conditions.
c. *Flexibility of wood movement, based on mill* experience.
d. Rate of wood substance loss for a specific location, calculated from literature data or, preferably, test pile results (SMITH and HATTON, 1971).

The physical methods comprise control of storage time, good housekeeping values such as cleanliness, destruction of decayed chips and chip storage on concrete, control of pile size and fines, aeasing of chips, water spraying and irradiation (BERGMAN and NILSSON, 1979).

Preservation of stored chips by a cost-effective system could be used in conjunction with standby storage. Preserved chips might be stored for a longer period than non-preserved, thus the fraction of chips going to storage could be reduced and further increases in overall by-product yields would result (SPRINGER, BENJAMIN, FEIST, ZOCH and HAJNY, 1977; SPRINGER, FEIST, ZOCH and HAJNY, 1977 cited by SPRINGER, 1978).

1.3.1.1 Temperature control by compaction, pile size and climate

The compaction of chips in piles increases the amount of wood stored in a given area and has been accomplished by blow-packing chips (ANNERGREN, DILLEN and VARDHEIM, 1964; BURKE, 1962; HOLEKAMP, 1958; HOLEKAMP, 1962a cited by SHIELDS, 1967). Blow packing has been reported (ANNERGREN, DILLEN and VARDHEIM, 1964; RITCEY, 1962 cited by SHIELDS, 1967) to give superior compaction to other methods. Depending upon the degree of compaction the temperatures have been found to increase in the pile and to remain high throughout winter (ANNERGREN, DILLEN and VARDHEIM, 1964 cited by SHIELDS, 1967). Higher temperatures caused by compaction profoundly affect the types of microorganisms which are able to colonise the chip pile (ANNERGREN, DILLEN and VARDHEIM, 1964; NILSSON, 1965 cited by SHIELDS, 1967), since usual wood-rotting fungi cannot grow at the elevated temperatures experi-

enced in some parts of the chip pile (SHIELDS, 1967). However, these high temperatures are believed to adversely influence the resin content of stored chips and to produce lower by-product yields (LINDGREN and ESLYN, 1961 cited by SHIELDS, 1967). In areas where compaction was the least it was found that more fungal deterioration and greater wood substance losses occurred during storage (BOIS, FLICK and GILMER, 1962; ROTHROCK, SMITH and LINDGREN, 1961 cited by SHIELDS, 1967). Little or no differences were noted (BOIS, FLICK and GILMER, 1962) in chip deterioration between the compacted and uncompacted areas during the first month of storage. However, after 6 months storage the uncompacted chips had lost three to four times more wood substance than the compacted chips. Larger piles have greater compaction of chips together with less exposed surface area, and are reported to be subject to less biodeterioration (ROTHROCK, SMITH and LINDGREN, 1961 cited by SHIELDS, 1967).

A chip pile temperature is affected by the climate and the size and shape of the pile (BERGMAN and NILSSON, 1979). The construction of chip piles during freezing temperatures would appear to be promising in providing protection against subsequent fungal deterioration because the majority of microorganisms are dormant at or near 0°C (SHIELDS, 1967). Wood substance losses due to microbiological activity increase with increasing temperature up to approximately 50°C, after which they decrease. However, with increasing temperature above 50°C increasing thermal de-

gradation occurs (FEIST, HAJNY and SPRINGER, 1973; BERGMAN, 1974). Thus the temperature in a chip pile should be as low as possible or just above 50°C. In cold climates, e.g in Canada, northern U.S.A. and Scandinavia, chips stored during the winter tend to decay most in the centre of the pile. The temperature in the centre is usually between 20 and 50°C, which highly favours the wood-rotting Basidiomycete fungi (BERGMAN and NILSSON, 1966; BERGMAN and NILSSON, 1967; SHIELDS and UNLIGIL, 1968; BERGMAN and NILSSON, 1971; HATTON, 1970b cited by BERGMAN and NILSSON, 1979). During the summer in cold climates, and in warm climates year-round, the temperature in the centre of the pile is usually 50 - 70°C, which is too high to favour wood-rotting Basidiomycete fungi. In the sides, on the other hand, the temperature is between 20°C and 50°C (BERGMAN and NILSSON, 1971; BOIS, FLICK and GILMER, 1962; SAUCIER and MILLER, 1961 cited by BERGMAN and NILSSON, 1979). This suggests that these piles should have large top areas. The volume of the centre would then be much greater than that of the sides, and the total wood substance loss would be reduced (BERGMAN and NILSSON, 1979).

1.3.1.2 Foundation

Many chips are stock-piled on the ground from trucks or pneumatic conveyors (BURKE, 1962 cited by SHIELDS, 1967) without too much preparation for the base. In practice it often happens that fresh chips are blown on the chips from previous piles as a base for fresh chips, while others

stored their chips directly on the ground (ANON, 1961; BLACKERBY, 1958; CRANE and FASSNACHT, 1960; HENSEL, 1958; RITCEY, 1962 cited by SHIELDS, 1967). This leads to faster infection of fresh chips (BERGMAN and NILSSON, 1979). From one to three feet of chips (ANON, 1961 cited by SHIELDS, 1967) have been reported as lost during reclamation where the piles have been constructed on old chips. The larger numbers of wood-decay fungi isolated from the bottom of a soft-wood chip pile in New Brunswick (SHIELDS, 1967), would indicate that the risk of introducing rot into the lower regions of piles is great when piles are constructed on old chips or directly on the ground (SHIELDS, 1967). Considerable amounts of dirt and rot are therefore introduced when unsurfaced bases are used. Furthermore, chips piled on soil are especially susceptible to rapid decay since the soil fungi may form a biotic connection which translocate nitrogen from soil to chips thus accelerating decay as discussed above. The use of surfaced areas is thus useful in decreasing biodeterioration of chips at the bottom of piles.

1.3.1.3 Control of storage time and time management

At a sulphite pulp mill, the pulp wood or chips must be stored in order to season the resins and thereby reduce pitch problems. This is achieved with chip storage times of one or two months provided the chips self-heat. At a kraft pulp mill nothing is gained in pulp processing and pulp quality by storage of the wood. However, the review of microbial succession above (1.2.1.1) shows that wood biodeterioration increases with time. Thus stor-

age time should be as short as possible. Besides increased losses in wood substance and tall oil yield, a storage period of more than six months has been reported to give rise to formation of pitch deposits (AFFLECK and RYAN, 1969 cited by BERGMAN and NILSSON, 1979). Managers must therefore select appropriate storage times to maximise yields with minimal biodeterioration.

1.3.1.4 Control of fines

The ideal chip size for pulping varies according to timber, but is about 19mm x 11mm x 5mm. However, wafer-like chips from chippers are susceptible to breakage and fines formation during pneumatic conveying (GALLOWAY and THOMAS, 1972 cited by BERGMAN and NILSSON, 1979). If fines are left undisturbed, blankets or even pockets (ALLEN, 1968 cited by BERGMAN and NILSSON, 1979) of fines are formed. Blankets of fines reduced heat dissipation, which results in further temperature increase, followed by thermal degradation of the chips. Fines themselves may generate more heat than chips and may also ignite at a lower temperature. Cases of spontaneous combustion in chip piles due to fines have been reported by BLACKERBY (1963), CHALK (1968a), ALLEN (1968), COLE (1972b), and BERGMAN (1974). Control of fines in chip piles is therefore an important measure of reducing chip deterioration and may assume the form of pre-screening an orientation of discharge direction, and disruption of blankets and pockets of fines in the pile. Even if the chips are screened before conveying to the pile, some fines and

dust *always* adhere to the chips, therefore when planning a wood-yard, it should be important to determine the prevailing wind direction and orient the discharge pipe in the same direction to disperse fines. However, present belt conveying systems do not have the flexibility and capacity of pneumatic systems. Also, they are probably more expensive *to install and* maintain (HATTON, *1969 cited by* BERGMAN and NILSSON, 1979).

1.3.1.5 Chip encasement

Since the undesirable microorganisms in chip piles are aerobic, good protection against decay should be obtained if chips are stored under anaerobic conditions. Experiments conducted *by FEIST, SPRINGER and HAJNY (1971) showed that chips* covered by polyethylene film (CO_2 content 1 %) increased the total pulp yield for chips. In addition, experiments by McKEE and DANIEL (1966) and FEIST, SPRINGER and HAJNY (1971) showed that oxygen must be completely excluded (below 1 %) in order to reduce wood deterioration efficiently. For large chip piles encasing between long periods of *building up and reclaim will* give little reduction of *deterioration* (BERGMAN and NILSSON, 1979). Unfortunately airtight encasement of chip piles would probably be fairly expensive.

1.3.1.6 Air exclusion by water

Of all methods for rough pulpwood, storage in water ensures the greatest control of deterioration over extended *periods* (CHESLEY, HAIR and SWARTZ, *1956 cited by* LINDGREN and ESLYN, 1961). To maintain

anaerobic conditions the wood preferably should be freshly cut at the time of storage and kept completely submerged (LINDGREN and ESLYN, 1961). Wetting the chips with water prior to or during and/or after piling might be a cost-effective means for retarding losses in pulp strength during storage (SPRINGER, 1976). SOMSEN (1962) has found that losses in tear strength did not become significant until 28 weeks in a wetted pile of wood compared with 17 weeks in an unwetted pile (SPRINGER, 1976). ESLYN and LAUNDRIE (1973; 1975), reported on two laboratory experiments on the storage of Douglas-fir and aspen chips underwater. After two years the specific gravity losses and chip quality changes were extremely small. However, storage of chips under water will probably be rather expensive to arrange in practice (BERGMAN and NILSSON, 1979).

1.3.1.7 Irradiation

A pilot system for bombardment of chips with electrons during the pneumatic conveying was installed in 1972 at a kraft pulp mill in Oregon as reported by CUMMING (1972). The process is, however, not compatible with chlorine compounds and can therefore not be used on chips intended for bleached pulps. The irradiation kills wood-attacking microorganisms present in the chips but reinfections from air-borne spores are likely to occur. Preliminary results from a large-scale field test indicated that the kraft pulp yield increased 4-6 % due to improved carbohydrate retention (SARKANEN, MITCHELL, PAZNER and HOFFMAN, 1974 cited by

BERGMAN and NILSSON, 1979), but several recent studies refute this claim (HATTULA and PEKKALA, 1977 cited by BERGMAN and NILSSON, 1979). The capital cost of the irradiation installation has been estimated at 2 million dollars for a 500 ton per day pulp mill and the cost of irradiation at about half dollar per ton of oven dry wood (NICHOLLS, LINDEVALL and MITCHELL, 1974 cited by BERGMAN and NILSSON, 1979).

1.3.2 Chemical Methods

The various physical methods tested for the protection of chip piles for example, irradiation, plastic encapsulation (FEIST, SPRINGER and HAJNY, 1971 cited by SMITH, 1975), CO_2 cocooning (ESLYN and LAUNDRIE, 1975) and water spraying (DJERF and VOLKMAN, 1969 cited by SMITH, 1975), have not yet proven to be economically feasible for most of the pulping industry (SMITH, 1975). Chemicals reduce deterioration by inhibiting growth of microorganisms (BERGMAN and NILSSON, 1979). Control of deterioration in chip piles by chemical means has been suggested (LINDGREN and ESLYN, 1961 cited by SHIELDS, 1967) as one means of improving pulp yields and quality-provided that cost of treatment is not too high (SHIELDS, 1967) and the chemicals used are environmentally acceptable and approved by end-users.

Chemical control methods include treatment with fungicides and with chemicals which change the environment for the microorganisms by, for

instance, increasing or decreasing the pH-value of the chips (BERGMAN and NILSSON, 1979). Treatment with chemicals may aim at inhibition of the total microflora or at inhibition of the most harmful wood-destroying fungi (BERGMAN and NILSSON, 1979). It has been proposed that only certain areas of the piles such as the outer layers, need to be treated since greatest biological deterioration seems to occur here (*SHIELDS, 1967*). If these new treatment chemicals live up to the claims of their proponents, then the pulp companies stand to make substantial savings in wood cost, and a large market will develop for chemicals in wood chip treatment (ANON, 1969).

Numerous chemical and mechanical methods for treating chip piles to prevent deterioration have been proposed in the last 20 years, but none have been used in long-term commercial practice (*SMITH and* HATTON, 1971; *BERGMAN and* NILSSON, 1979 cited by FULLER, 1985), however, this search is still going forward in many laboratories (SMITH, 1975) where potential chemical preservative systems are being evaluated (SPRINGER, BENJAMIN, FEIST, ZOCH and HAJNY, 1977; SPRINGER, FEIST, ZOCH and HAJNY, 1977 cited by SPRINGER, 1978). The foregoing indicated that there is a large and growing market for chemicals in chip treatment. The growth of the market will result from the growth of pulp production itself, the increasing share of pulpwood being utilised in the form of chips, and increasing international trade in wood chips (ANON,

1969). The chemical used to prevent chip deterioration must meet the following criteria :

a. Be effective for considerable time.
b. Be reasonable in cost in relation to the losses incurred from chip deterioration.
c. Be compatible with the pulping process.
d. Not cause pollution.
e. Not to be toxic to personnel (SPRINGER, ESLYN, ZOCH and HAJNY, 1970).

Chemical protection of chips in outside storage is not intended for periods longer than 12 - 18 months. It should not be confused with wood preservation in which the desired duration of the chemical treatment is several years. There is a trend to searching for chemicals which cause less environmental pollution. Of all the control methods reviewed above it was felt that chemical methods offered the most appropriate control in the environment studied in this work. Chemical preservatives (reviewed in Chapter 2) include the following :

a. Fungicides.
b. Alkalis.
c. Sulphurous acid.
d. Formaldehyde and formic acid.
e. Propionic acid.

f. Complex organic compounds.

1.4 Scope Of The Present Study

On the basis of the foregoing literature review it was concluded that, in terms of cost and effectiveness, chemical protection of *Pinus patula* chips during OCS would be the most appropriate method to investigate as a means to prevent biodeterioration. It was therefore decided to conduct investigations to establish whether such treatment would be effective.

1.4.1 Hypothesis To Be Tested

It was hypothesised "that *Pinus patula* chips may be chemically protected from biodeterioration during OCS in the sub-tropical climate of Richards' Bay, South Africa".

1.4.2 Objectives

To test this hypothesis rigorously, field trials were essential to provide hard information. The following objectives were therefore set: To monitor during OCS at Richards' Bay, the biodeterioration of *Pinus patula* chips treated with an appropriate chemical with potential in preventing biodeterioration of wood. However, tests of this nature should comprise two major components :

a. Laboratory trials - in which a wide range of chemicals can be evaluated and accelerated tests can be carried out at low cost.
b. Field trials - where the appropriate preservative is selected from (a) and a commercial scale experiment is conducted.

1.4.3 Aims

Overall, the aims of the study were therefore two fold:-

1.4.3.1 Laboratory trials

To apply selected chemical preservatives to *Pinus patula* wood chips and to monitor biodeterioration over a period of 4 months by :

a. Laboratory analyses to determine the extent of biodeterioration of the wood.
b. Pulp test to provide hard information on the paper quality of the preserved wood chips.

1.4.3.2 Field trials

To select one of the above preservatives on the basis of preservative efficiency, cost effectiveness and environmental acceptability for use in field trials at Richards' Bay, and to further monitor biodeterioration using laboratory analyses and pulp tests over a 4 month period, and also the following :

a. To observe degradation patterns of preservative treated and untreated wood chips and classify the fungi invading wood during the 4 month OCS period.

b. To quantify the numbers of microorganisms present in chips sampled from piles during the 4 month OCS period.

2.0 LABORATORY TRIALS

2.1 Introduction

Although no cost effective chemical treatment for long-term chip preservation has been found to date (SMITH, 1975), a wide range of antimicrobial compounds have been used in wood preservation and the major groups of such compounds are reviewed below.

2.1.1 Fungicides

In searching for fungicides cost-benefit analyses some years ago suggested that the value of wood substance and tall-oil saved will roughly equal the cost of the treatment chemicals (SPRINGER, ZOCH, FEIST and HAJNY, 1973b). HUECK (1971) suggests that one should not look for the most stable compounds as fungicides. Ecologically preferable are compounds which are slowly broken down into harmless metabolites. The efficacy of a fungicide should not outlast the useful life of the material it is supposed to protect.

Fungicides for chip protection should therefore be sensitive to slow oxidation. A number of chemicals have been proposed and tested for their ability to reduce deterioration in outside chip storage. Some of these chemicals are simple organic compounds (e.g. chlorinated phenols, alkalis, propionic acid and pulp mill effluents) and some complex organic com-

pounds (e.g. dimethylamides of long-chain, unsaturated fatty acids such as Busperse 47). Pulp mill chemicals have also been proposed (BERGMAN and NILSSON, 1979).

2.1.1.1 Chlorinated phenolic fungicides

Compounds based on chlorinated phenols alone or mixed with borax or Polybor (ROBINSON, 1968; SHIELDS, 1969; GIFFIN, 1970; SPRINGER, HASLERUD, FRIES, CLARK, HAJNY and ZOCH, 1971 cited by SMITH and HATTON, 1971) as active fungicides have been tested by several workers (SELLEBY, 1965; HULME and SHIELDS, 1973), and are widely used for protection of timber (lumber) against blue stain during the yard seasoning period. They are also used in the wood preservation industry. Chlorinated phenols are very effective against microorganisms but they are relatively expensive. Chlorinated phenols inhibit respiration of living sap-wood cells and of microorganisms. The resin content of treated chips after storage remains almost as high as in fresh chips. This is an advantage for a kraft pulp mill with tall-oil recovery, but a disadvantage for a sulphite pulp mill (ASSARSON, CROON and FRISK, 1970b cited by BERGMAN and NILSSON, 1979).

SELLEBY (1965) reported that spruce chips treated with sodium pentachlorophenate (Na-PCP) showed no reduction of brightness after 4 months storage in bags in a pile of untreated pine chips (SPRINGER, HASLERUD, FRIES, CLARK, HAJNY and ZOCH, 1971). GREAVES

(1971b) examined chlorophenol - treated chip samples stored for 3 months in the centre of an uncompacted pile of tropical hardwood chips and found that Na-PCP was the most effective treatment in reducing the size of the invading population of microorganisms and in preserving the brightness of chips. Experiments conducted by SHIELDS (1969) and later repeated by HULME and SHIELDS (1973) found that Na-PCP effectively reduced the number of fungi colonising the chip samples.

However, chlorinated phenols have great environmental disadvantages. They are very toxic to human beings and very irritating to the skin. They must be handled with great care and only by highly trained personnel (SMITH, 1970 cited by BERGMAN and NILSSON, 1979). Dust and sprays of these chemicals must be avoided, which can be a problem when chips are treated during the pneumatic conveying process. Run-off from a pile and wash water from the pulp mill may cause pollution. Fish are extremely sensitive to chlorinated phenols. Chlorinated phenols can also give rise to odour which is stable enough to be retained in pulp after cooking (BERGMAN and NILSSON, 1979). Hence, these disadvantages have caused concern on a global basis and have stimulated the search for alternative preservatives of lower mammalian toxicity.

2.1.1.2 Borax and Na-PCP

Borax has been used to inhibit fungal attack of straw stacks and to prevent blue stain on lumber. It is non-poisonous, odourless and non-corrosive

(FINDLAY, 1953 cited by BERGMAN and NILSSON, 1979), and was reported to have been tested on spruce sawmill chips in eastern Canada (ROBINSON, 1968; GIFFIN, 1970 cited by BERGMAN and NILSSON, 1979). Experiments conducted by GIFFIN (1970) using borax alone were stated to be ineffective apparently because of its only moderately fungicidal properties (SMITH and HATTON, 1971). Contrary to this experiments conducted by HULME and SHIELDS (1973), concluded that borax appeared to be the most effective and economical treatment tested to reduce pulp losses. In addition savings approached approximately 10 % of the wood cost (HULME and SHIELDS, 1973). Although borax retarded growth and spread of blue stain fungi, the fungi were however not killed and it was apparent that chemicals with a more complete inhibiting effect on microorganisms were required. According to HULME and SHIELDS (1973) cited by SMITH (1975) this chemical showed promise but further field trials were required because of irregular results obtained in practice with increasing concentration.

Chlorinated phenols with borax have also been tested by several workers (SPRINGER, HASLERUD, FRIES, CLARK, HAJNY and ZOCH, 1971; GIFFIN, 1970 cited by SMITH, 1975) and were found to be effective in *reducing biodeterioration in chip piles (ROBINSON, 1968; SCHEFFER* and CHAPMAN, 1934 cited by ESLYN, 1973a). Although treatment with borax and Na-PCP resulted in a greater reduction in microbiological activity and hence a positive control of chip degrade (SMITH, 1975;

ASSARSON, CROON and FRISK, 1970b) and lower costs (BERGMAN and NILSSON, 1979), the future availability and usability of NaPCP remains uncertain (SMITH, 1975) because of the drawbacks associated with it

2.1.1.3 Mercuric biocides

The use of mercuric compounds as slimicides led to experiments to test their effectiveness in reducing chip deterioration (SELLEBY, 1965; ANON, 1969 cited by BERGMAN and NILSSON, 1979). Mercuric biocides function in the same manner as chlorinated phenols, but much lower concentrations suffice. They are, however, extremely toxic towards mammals and the pollution hazard therefore precludes their use (BERGMAN and NILSSON, 1979) and has resulted in mercurials being discontinued in many countries (von MOLY, 1981).

2.1.1.4 Nickel sulphate

ASSARSON and NILSSON (1971), and ASSARSSON, CROON and FRISK (1968) cited by SMITH and HATTON (1971), suggested that selective inhibition of the most harmful decaying and staining fungi in a chip pile is achieved by treatment with nickel sulphate. In Sweden, treatment with nickel sulphate reduced the temperature and wood substance losses (ASSARSSON and NILSSON, 1971; ESLYN, 1973a; ASSARSSON, CROON and FRISK, 1968 cited by BERGMAN and NILSSON, 1979) however, in Canada results were not as promising (WORSTER, GUEST

and WIESNER, 1972; SPRINGER, HASLERUD, FRIES, CLARK, HAJNY and ZOCH, 1971 cited by BERGMAN and NILSSON, 1979).

According to SPRINGER, HASLERUD, FRIES, CLARK, HAJNY and ZOCH (1971) cited by ESLYN (1973a) when tested in simulated chip piles, nickel sulfate was found ineffective in controlling wood deterioration. In addition, the chlorophenates, mercurials and nickel compounds that have been used to prevent chip degrade were considered either contributory to water pollution or incompatible with the pulping process (ESLYN, 1973a). According to BERGMAN and NILSSON (1979) since there was the prospect of the chemical cost of nickel increasing in the future due to expected shortages, it was a matter of urgency that a screening program be commenced to investigate additional biocides that would not have these shortcomings (ESLYN, 1973a).

2.1.2 Treatment with alkalis

It has been found that most of the wood-destroying fungi grow between pH 2 and 8. Their optimum is at pH 5-6 (HENNINGSSON, 1967 cited by BERGMAN and NILSSON, 1979). Bacteria are generally considered to grow at higher pH levels. Two ways of reducing the microbial deterioration are possible, either increasing the pH with alkalis or decreasing the pH with acids. According to SPRINGER, ESLYN, ZOCH and HAJNY (1979) kraft green liquor which consisted of 0.76 % sodium sulphide and 3.0 % sodium carbonate was highly effective in reducing wood substance losses,

was non-polluting (SPRINGER, ESLYN, ZOCH and HAJNY, 1969 cited by ESLYN, 1973a) and had no detrimental effects on the kraft pulp quality (CHALK, 1968b cited by BERGMAN, NILSSON and JERKEMAN, 1970; SPRINGER, FEIST, ZOCH, HAJNY and McALILEY, 1974; SPRINGER, HASLERUD, FRIES, CLARK, HAJNY and ZOCH, 1971 cited by BERGMAN and NILSSON, 1979). The green liquor-treated chips also appeared to be free of fungal growth SMITH (1979). A laboratory prepared kraft green liquor (7.7 % sodium carbonate and 1.9 % sodium sulphide) protected wood from two organisms that can cause substantial weight losses to unprotected wood. The green liquor also prevented heat release from chips in laboratory simulators of chip piles (SMITH and HATTON, 1971). Because it is believed that most of the initial heat build-up in chip piles is caused by respiration of living wood cells, the green liquor must act as an enzyme inhibitor as well as a fungicide (SPRINGER, ESLYN, ZOCH and HAJNY, 1969).

Sodium hydroxide and kraft white liquor were tested in outside chip storage in Sweden (SMITH and HATTON, 1971; BERGMAN, NILSSON and JERKEMAN, 1970 cited by BERGMAN and NILSSON, 1979). Two thirds Scots pine *Pinus sylvestris* L and one third Norway spruce *Picea abies* L. Karst were treated with 40 g of NaOH per litre of water. It was found that the microflora in the treated chips were less harmful, lower weight losses resulted, and pulp yield also increased (ASSARSSON, CROON and FRISK, 1970a), however, tall-oil yield decreased more rap-

idly in the treated chips compared to the untreated. In addition pretreating the green wood with alkali (synthetic green liquor and NaOH) resulted in less wood degradation and less loss in pulp yields; however, pulp strength properties were still adversely affected by the action of heat on the green wood. Apparently the alkali neutralises acids in the wood and retards or prevents hydrolysis. However, it does not affect those processes, probably oxidative, that lead to molecular degradation without solubilization of wood components (FEIST, HAJNY and SPRINGER, 1973 cited by FEIST, SPRINGER and HAJNY, 1974). Additional drawbacks when using green liquor or white liquor is that, apart from possible metal corrosion problems (HATTON, 1970b cited by SMITH and HATTON, 1971), there is the risk of hydrogen sulphide formation, which may cause air pollution (SPRINGER, HASLERUD, FRIES, CLARK, HAJNY and ZOCH, 1971; BERGMAN and NILSSON, 1979).

2.1.3 Treatment with sulphur dioxide gas or sulphurous acid

Gaseous sulphur dioxide treatment has been used to prevent the darkening of wood chips during storage (MARSHALL, 1968 cited by SPRINGER, FEIST, ZOCH and HAJNY, 1975a) and has been suggested as treatment to prevent microbial deterioration of chips (KING, STANLEY, JURD and BOYLE, 1971 cited by SPRINGER, FEIST, ZOCH and HAJNY, 1975a). For example treatment of hardwood chips with sulphurous acid or sulphur dioxide gas to improve the brightness of high yield bisulphite and chemimechanical cold soda pulps was performed commercially at some mills in

northeastern U.S.A. (SILBERMAN, 1969; SILBERMAN, 1970 cited by BERGMAN and NILSSON, 1979). Such treatment has also been suggested for the prevention of microbial deterioration of chips (SPRINGER, HASLERUD, FRIES, CLARK, HAJNY and ZOCH, 1971 cited by SPRINGER, FEIST, ZOCH and HAJNY, 1975). Several highly effective mixtures were found, the best of which were mixtures of simple phenolic compounds and sodium bisulphite. Aspen chips immersed in the following mixtures and stored in insulated boxes (ventilated with moist air) showed no heating or microbial growth after 3 months storage:

a. 2 % $NaHSO_3$ + 0.7 % o-cresol,
b. 2 % $NaHSO_3$ + 0.7 % p-cresol,
c. 2 % $NaHSO_3$ + 0.6 % sodium 0-phenylphenol,
d. 2 % $NaHSO_3$ + 0.35 % hydroquinone,
e. 2 % $NaHSO_3$ + 0.35 % resorcinol,
f. 2 % $NaHSO_3$ + 0.3 % 2,4 dinitrophenol.

However, these have not been evaluated in pile simulators (SPRINGER, FEIST, ZOCH and HAJNY, 1975a). In addition the brightness of Western hemlock and Douglas fir chips was enhanced by immersing in 2 % sodium bisulphite solution (SPRINGER, 1983a). HULME and SHIELDS (1973) tested ammonium bisulphite on balsam fir and spruce chips and found that although the weight and pulp yield losses of the treated samples were small, the chip appearance and pulp quality was poor. In addition, problems may also arise during the treatment such as corrosion of chip re-

moval devices (SILBERMAN, 1970 cited by BERGMAN and NILSSON, 1979).

2.1.4 Formaldehyde and formic acid

Formaldehyde has been tested in field experiments in Sweden, however no significant difference between the temperature, wood substance losses and pulp yield between treated and untreated piles was observed (BERGMAN and NILSSON, 1979). On the other hand SPRINGER (1983a) found that 2 % formaldehyde was reasonably effective in maintaining the brightness. Formic acid has been used in laboratory tests but not suggested for field tests owing to its high cost (BERGMAN and NILSSON, 1979).

2.1.5 Propionic acid

Certain organic acids, such as acetic, propionic and sorbic acids, inhibit mould and bacterial growth and have been used in this capacity to protect foodstuffs. The calcium and the sodium salts of propionic acid, for example, have for years been incorporated in bakery goods and used on fruits and vegetables to inhibit growth of moulds.

In laboratory tests using moist wheat (MILNER, CHRISTENSEN and GEDDES, 1947 cited by ESLYN, 1973a) and cornmeal (HALICK and RICHARDSON, 1953 cited by ESLYN, 1973a), propionic acid was effective in the cornmeal tests and at a lower concentration than that of the calcium salt, whereas sodium propionate did not inhibit molding at a con-

centration double that of the calcium salt (RICHARDSON and HALICK, 1957 cited by ESLYN, 1973a). Propionic acid applied at a concentration of 0.8 % permitted safe storage of grain at a moisture content of 20 % whereas grain at a moisture content of 25 % required a concentration of 1 %. The acid prevents microbial deterioration in the grain for at least 12 months if the pile is protected from rain (HELBERG, 1971; RICHARDSON and WEBB, 1961 cited by ESLYN, 1973a). Propionic acid has also been effective on wheat, oats, barley, corn, field beans, soybeans, peas, ground coconut shells and bagasse at concentrations of 0.5 % for plant materials at 15 % moisture content to 2 % for those at 35 % moisture content (HELBERG, 1971 cited by ESLYN, 1973a).

Propionic acid and mixtures of propionic acid and acetic acid have been used on a commercial scale in Canada for preserving moist corn since 1970 (STEVENSON, 1972 cited by ESLYN, 1973a); the mixtures of the acids have been test-marketed in the U.S on a large scale since 1971 (ANON, 1971 cited by ESLYN, 1973a). In addition to being antimicrobial, propionic acid and its salts stop respiration in stored grain, thereby halting heat build-up in the pile. RICHARDSON and HALICK (1957) showed that propionic acid and propionic anhydride at a concentration of 0.1 % effectively inhibited heating in cornmeal. Calcium propionate was effective at a concentration of 0.3 % whereas sodium propionate, at a concentration of 0.6 %, was only capable of delaying heating for 1 week more than did the controls. Later studies (RICHARDSON AND WEBB, 1961 cited by

ESLYN, 1973a) involving storage of mixed feeds indicated that adjusting the high-moisture ingredient (cane molasses) to a pH of 3.0 before adding sodium propionate appreciably improved the ability of this chemical to prevent heating a ground corn-cane molasses mixtures.

Emphasis on investigating and using biocides that are least harmful to the environment is increasing. If a chemical shows promise of controlling degradation and is also apparently harmless environmentally, every effort should be made for thorough testing for potential commercial application. On this basis propionic acid apparently can be considered safe enough for use in foodstuffs and animal feeds. Both the food and drug administration and environmental protection agency have approved acetic acid and propionic acids for use as preservatives in grain for livestock feeds (WILCOX, 1972 cited by ESLYN, 1973a). As part of an extensive biocide screening program, propionic acid was therefore evaluated for its potential *in preventing biodeterioration of stored wood chips. Propionic acid was* first reported to have been evaluated on wood chips by ESLYN (1973b), using blackgum chips.

Propionic acid was found to be effective in controlling fungi capable of de*caying stored wood chips for 12 weeks. Based on its performance in stored* grains, it would most likely be effective, too, in suppressing heating in wood chip storage piles (ESLYN, 1973a). According to SPRINGER, FEIST, ZOCH and HAJNY (1977) at a sufficiently high level, the propionic acid

treatment could be as effective as the sodium bisulfite + 2.4 dinitrophenol treatment. When an 18 % solution of the acid was used to treat pine wood chips it was found to be effective for a 14 week interval. The propionic acid chip pile was also 5°C cooler and in fact suppressed heating for about 70 days and also retained moisture and colour compared to control chips. An added advantage is that propionic acid decomposes quickly to neutral substances after fulfilling its function (KOZLOWSKI and RATAJCZAK, 1978). When red pine *Pinus resinosa* Ait. was treated with concentrations higher than 2.0 % all test fungi were effectively suppressed and weight loss was below 0.5 %. The treatment also reduced heat generation, and the wood substance loss after 6 months storage was about half of that for the untreated chips (SPRINGER, PEIST, ZOCH and HAJNY, 1975b cited by BERGMAN and NILSSON, 1979). In addition, preservation of birch wood chips with 1.5 % propionic acid by weight of oven dry chips limited pathogenic activity, manifested by a reduction in weight loss (from 1 to 4.5 %) (RATAJCZAK, 1983).

2.1.6 Complex organic compounds.

A field experiment with 3 fungicides known by proprietory names was reported by THORNTON, WHITE and STONE (1969) cited by *BERGMAN and NILSSON (1979). The fungicides were Busan 11-M1* (barium metaborate), Busperse 47 (dimethylamides of long chain unsaturated fatty acids) and Busan 72 (60 % 2-thiocyanomethylthiobenzothiazole). Southern pine chips were treated

with various concentrations of the chemicals and stored outdoors in small piles. Microbial growth occurred on all surfaces of the chips, however, treated chips showed reduced populations when compared with the untreated chips (BERGMAN and NILSSON, 1979).

Of the treatments studied, those employing sodium N-methyldithiocarbamate, alone or in combination with other biocides (SPRINGER, ESLYN, ZOCH and HAJNY, 1969; SPRINGER, FEIST, ZOCH, HAJNY and McALILEY, 1974; SPRINGER, FEIST, ZOCH and HAJNY, 1977, SPRINGER, 1983b), show most promise as a basis for a cost effective treatment of chips to preserve by-products during several weeks of outside chip storage (SMITH, 1975). In addition, no fungi were found on a random sample of the treated, stored chips; however many were found on the untreated chips (SPRINGER, HASLERUD, FRIES, CLARK, HAJNY and ZOCH, 1971 cited by SPRINGER, FEIST, ZOCH and HAJNY, 1973a). The sodium N-methyldithiocarbamate treatment was highly effective in maintaining brightness of the chips and preventing losses of wood substance of aspen chips stored for six months in chip pile simulators. KINN and SPRINGER (1985) also used sodium N-methyldithiocarbamate to exterminate the pine wood nematode in wood chips. The treatment had no deleterious effects on the quality of kraft pulp produced from the stored chips. Based on these results, the treatment appears promising for preventing chip deterioration during outside storage. SPRINGER, BENJAMIN, FEIST, ZOCH and HAJNY (1977) recom-

mended the use of sodium N-methyldithiocarbamate for preservation of *Pinus patula* and *Pinus echinata*. Apparently, the treatment lost its effectiveness after about 100 days and permitted the growth of certain types of microorganisms (SPRINGER, FEIST, ZOCH and HAJNY, 1973a). However, according to SPRINGER, HASLERUD, FRIES, CLARK, HAJNY and ZOCH (1971) and SPRINGER, FEIST, ZOCH and HAJNY (1973b) the cost for effective treatment remains high and its economic use seems uncertain. In addition, although sodium N-methyldithiocarbamate at 0.11 % is effective in turpentine preservation, it is only partially effective in tall-oil preservation at the treatment level employed (SPRINGER and ZINKEL, 1986). Contrary to these findings, SPRINGER, FEIST, ZOCH, HAJNY and McALILEY (1974) found that sodium N-methyldithiocarbamate treatment significantly reduced losses of tall-oil and turpentine during the initial 2 months of storage. This undoubtedly resulted because the treatment stopped pile heating for about 1 month. If heating could be suppressed for longer periods, greater savings of by-products and wood fiber would no doubt be found. This might possibly be achieved by using mixtures of sodium N-methyldithiocarbamate and other biocides that are effective for longer periods.

According to SPRINGER, FEIST, ZOCH and HAJNY (1973b); SPRINGER, FEIST, ZOCH and HAJNY (1975b) and SPRINGER, BENJAMIN, FEIST, ZOCH and HAJNY (1977), sodium N-methyldithiocarbamate + Na 2,4 dinitrophenol was by far one of the most

effective chemical treatment tested for preserving wood chips at concentrations of 0.1 % and 0.25 % respectively. The treatment had no deleterious effects on pulp quality, it completely suppressed heating (SPRINGER, HASLERUD, FRIES, CLARK, HAJNY and ZOCH, 1971; SPRINGER, FEIST, ZOCH and HAJNY, 1973a), maintained original chip brightness throughout storage (SPRINGER, SCHMIDT, FEIST, ZOCH and HAJNY, 1975), was the most effective treatment in preventing wood substance loss, pulp strength loss and losses in tall-oil for the entire storage period (SPRINGER, FIEST, ZOCH and HAJNY, 1975b). In addition because of this high degree of effectiveness, no fungi were found on randomly selected treated chips.

Treatment of wood chips with an aqueous solution of 0.2 % potassium N-methyl, N-hydroxymethyldithiocarbamate and 0.05 % sodium 2-mercaptobenzothiazole is highly effective in the prevention of chip heating, brightness loss, and growth of fungi and bacteria, however as to whether the treatment is cost effective or not warrants further extensive evaluations (SPRINGER, ZOCH, FEIST and HAJNY, 1973b).

Because of the effectiveness of the sodium bisulphite treatment in initially inhibiting wood cell respiration and growth of microorganisms, mixtures of this material and other biocides were tested (SPRINGER, FEIST, ZOCH and HAJNY, 1975b). The 2 % sodium bisulphite + 0.3 % 2.4 dinitrophenol treatment is the most effective of those evaluated and re-

ported to date for preserving stored wood chips (SPRINGER, 1976; SPRINGER, FEIST, ZOCH and HAJNY, 1977). No other treatment has been found so effective in preserving tall-oil in Southern pine chips. In addition treatment was highly successful in suppressing heating during the entire storage period and also in preventing weight loss (SPRINGER, FEIST, ZOCH and HAJNY, 1975a). Because of its high level of effectiveness, this treatment may be both technically and economically feasible and was thought to warrant further evaluation in an experimental outside chip pile (SPRINGER, FEIST, ZOCH and HAJNY, 1977). However, a major problem with this treatment is that 2,4 dinitrophenol is a toxic substance and environmental problems may arise through its use (SPRINGER, FEIST, ZOCH and HAJNY, 1977). For this reason, researchers are trying to replace it with less toxic materials, such as certain other phenols, and are continuing research for other treatments (SPRINGER, 1976). In addition, according to SPRINGER (1976) if treated chips were stored on a solid impermeable base such as a asphalt or concrete pad, and the run-off water directed to a treatment plant, the sodium bisulphite + 2,4 dinitrophenol treatment is the cheapest and most effective treatment in terms of chemical costs and would be practical and present no hazard to the environment.

Owing to the lack of information about chemical preservation for the softwood species *Pinus patula* the above literature survey was conducted. On completion of this comprehensive literature search, five compounds were

selected as appropriate test preservatives for trials on the bases of anti-microbial efficacy and cost. The compounds are listed as follows :

a. Sodium N-Methyldithiocarbamate (N-meth).
b. 2.4 Dinitrophenol (DNP).
c. Sodium bisulphite (NaB).
d. Propionic acid (P).
e. Disodium tetraborate (B).

With respect to the laboratory test methods, two major parameters had to be established , namely :

a. Incubation conditions - based on an experiment conducted by ZOCH, SPRINGER and HAJNY (1976) who stored whole - tree chips in insulated boxes supplied with humidified air, it was decided that for this experiment *Pinus patula* chips would be stored in plastic bins and kept in a constant environment room at a temperature of 25°C.
b. Analyses - it was felt that chips should be analysed in three ways;
   1) chemical analyses, which are relatively simple and quick to conduct, and after much research the following five analyses were decided upon: sodium hydroxide solubility, benzene/alcohol extraction, hot and cold water extraction; in addition to this,

2) microbiological analyses, as it is important to adopt a quantitative approach as this would give one an indication of the numbers and types of microorganisms and in addition the succession patterns taking place, and

3) pulp tests, which would give one the hard information on the extent to which wood chips have undergone biodeterioration.

2.2 *Materials And Methods*

2.2.1 Preparation Of Chips

One ton of fresh *Pinus patula* chips were produced from freshly felled timber at Barberton Sawmills (S.A.), transported to the laboratory and immediately treated with preservatives as described below.

2.2.2 Preparation Of Preservative

Five compounds were dissolved separately in water to give stock solutions at the following concentrations (w/v):

a. N-Methyidithiocarbamate (Appendix 1) : 0.4 % ..... (N-meth)

b. 2.4 Dinitrophenol $(NO_2)_2 C_6 H_3 OH$: 0.4 % ..... (DNP)

c. Sodium bisulphite $Na_2 S_2 O_5$: 2 %..... (NaB)

d. Propionic acid $C_3 H_6 O_2$: 1 % and 2 % ..... (P)

e. Disodium tetraborate $Na_2 B_4 O_7$: 1 % and 2 % ..... (B)

These solutions were then used to produce seven preservative formulations (a-g) as follows:

a. N-meth 0.4 % ..... stock solution of N-meth 0.4 %

b. N-meth 0.2 % + DNP 0.2 % ..... equal volumes of stock solutions of N-meth 0.4 % and DNP 0.4 %

c. NaB 1 % + DNP 0.2 % ..... equal volumes of stock solutions of NaB 2 % and DNP 0.4 %

d. P 1 % ..... stock solution of P 1 %

e. P 2 % ..... stock solution of P 2 %

f. B 1 % ..... stock solution of B 1 %

g. B 2 % ..... stock solution of B 2 %

2.2.3 Treatment And Incubation

Nine 60 kg samples were taken from the stock of *Pinus patula* chips. Seven of these samples were then separately treated by dipping in the seven preservative formulations (a-g) respectively. They were drained under gravity for 5 minutes. The eighth sample was dipped in water and the last sample was untreated. These nine samples were then placed in separate 100 litre plastic bins with lids and incubated at 25°C in a constant temperature room.

2.2.4 Sampling And Analyses

A 1 kg sample was then taken at random from each bin at weekly intervals for 4 weeks, then at monthly intervals over a total incubation period of 16 weeks. Each sample was first dried over 5 days at 23°C and thereafter ground for 3 minutes using a rock crusher (SiebTechnik; Molheim, Ruhr). Each crushed 1 kg sample was sieved (Figure 2.1) through a 40 (0.4 mm) and a 60 (0.25 mm) mesh sieve by rotating the sample on the sieve for 5 minutes, and the wood meal remaining on the 60 mesh sieve was subdivided and used to determine the extent of biodeterioration by chemical extractions (TAPPI Standard, T11 m-59) described below (2.2.4.1).

Prior to milling, whole chips were sampled for electron microscopic studies (2.2.4.2).

In addition, 5 kg chip samples were taken for pulp tests and by-product analyses described in (2.2.4.3).

2.2.4.1 Chemical extractions

As wood decays, the quantities of original substrates decrease while quantities of breakdown products increase. For example, Figure 2.2 shows how the proportions of the substrates cellulose and hemicellulose degrade to soluble carbohydrates, and how proportions of waxes degrade to phenols etc. One may therefore monitor decay by one of two ways, namely :

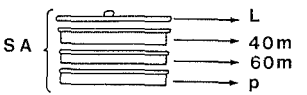


Figure 2.1 Diagram of sieveing apparatus (SA) (L - lid; 40m - 40 mesh sieve; 60 - 60 mesh sieve containing wood meal; p - collecting pan).

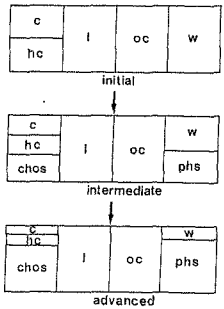


Figure 2.2 Model illustrating the decrease in substrates and increase in breakdown products with biodeterioration of wood. (c - cellulose; hc - hemicellulose; l - lignin; oc - other components; w - waxes; chos - carbohydrates; phs - phenols).

a. Determining quantities of original substrate remaining in decayed wood (these quantities will decrease as decay proceeds).

b. Determining quantities of wood breakdown products in decayed wood (these quantities will increase as decay proceeds).

Loss of material from chips was monitored by five methods listed below :

a. Sodium hydroxide (NaOH) extraction (TAPPI Standard T212 os-76, and T4 os-59) - Hot alkali solution extracts low molecular weight carbohydrates consisting mainly of hemicellulose and degraded cellulose in wood. As the wood decays or degrades the percentage of these wood breakdown products (alkali soluble material) increases (MORGAN, 1931; PROCTER and CHOW, 1973 cited from T212 os-76).

Procedure :

1) the samples were allowed to come to moisture equilibrium in the atmosphere near the balance, then 3 test specimens of 2.0 + 0.01g each were weighed out and the test specimens were each placed in 250 ml conical flasks,

2) to the wood meal, 100 ml of 1 % NaOH solution was added,

3) the flask was covered with a watch glass and placed in a water bath for a period of 60 minutes. The water in the bath was kept boiling and its level above that of the alkali solution in the flask,

4) the flask containing the wood meal and the NaOH was agitated for about 5 seconds at 10, 20 and 30 minutes after placing in the bath,

5) at the end of 60 minutes, the wood meal was transferred to a filtering crucible by suction and washed with 100 ml hot water. Then 50 ml of acetic acid was added and filtered through and finally, 100 ml of hot water was filtered until the material was acid free,

6) the crucible and contents were dried overnight to a constant weight, in an oven at 105°C, cooled in a desiccator and weighed. The percentage solubility was then calculated.

b. Benzene-alcohol extraction (TAPPI standard T6 os-59, T12 os-75) - To determine the percentage of waxes, fats, some resins and possibly some portions of wood gums extracted. As wood decays, the percentage of these substrates originally present in wood, decreases.

Procedure:

The solvent was prepared by mixing 1 volume of 95 % ethanol and 2 volumes of benzene,.

1) the soxhlet extraction flask was cleaned, then dried and weighed to the nearest milligram,

2) then 3 test specimens of 3,0 + 0,01 g were weighed out, and this material which was to be extracted in the extraction

thimble was placed in the soxhlet apparatus. A piece of cotton wool was placed over the thimble to prevent any loss of sample,

3) this was then extracted with 200 ml of solvent for 8 hours in a fume cupboard (Figure 2.3), keeping the liquid boiling briskly so that siphoning from the extractor was no less than 4 times per hour,

4) after extraction with benzene-alcohol, the solvent was allowed to evaporate and the soxhlet flask was placed in the oven at 105°C for at least 5 hours, cooled in a desiccator, and weighed. The percentage benzene-alcohol extraction was then calculated.

e. Ethyl ether extraction (TAPPI Standard T12 os-75, T6 os-59) - To determine the percentage fatty acids and colourants extracted. The procedure was as above but instead of benzene-alcohol, ether solvent was used. Since the fatty acids extracted by ether are original components of decayed wood, the ether extractives decrease as decay proceeds.

d. Hot water solubility (TAPPI Standard T12 os-75, T6 os-59) - To determine the percentage inorganic water soluble compounds such as tannins, gums, sugars, starches and colouring matter. The tannins and gums are not degraded (are anti-microbial) therefore on a proportional basis of final dry weight, these components will

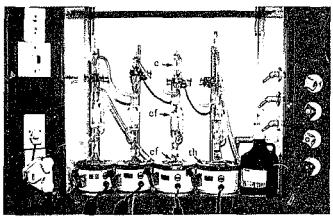


Figure 2.3 Illustrates the apparatus used for benzene/alcohol and ethyl ether extractions. (th - thermal heater; ef - collecting flask; ef - extraction flask; c - condenser).

appear to increase as degradable components are consumed as decay proceeds.

Procedure:

The already extracted wood meal from the benzene-alcohol extraction was used in this analysis;

1) a wood meal sample of 1g was transferred to a round bottom flask,
2) to this, 100 mls of water was added,
3) the flask was then placed in a thermal heater,
4) a reflex condenser was attached to the flask and allowed to digest for 3 hours,
5) the contents of the flask were transferred to a filtering crucible, washed with hot water, dried in an oven at 105°C, cooled in a desiccator and then weighed, and the weight of the moisture free material dissolved was then calculated.

c. Cold water solubility (*TAPPI Standard T207 os-75, T1 os-59*) - To determine the percentage water soluble components and polysaccharides. The principles and procedure are similar to those of hot water solubility, however, instead of digesting the sample for 3 hours in hot water, the wood sample was left to digest for 48 hours at room temperature. The weight of the moisture free material dissolved was then calculated.

2.2.4.2 Microbiological analyses

All bins were monitored visually throughout the incubation period and relative degrees of chip colonisation by microorganisms was recorded. Furthermore, chips were sampled for electron microscope examination to determine the extent and type of colonisation. Small particles of wood (approximately 5mm x 5mm) were removed from the surfaces of these chips. All particles were fixed in 4 % glutaraldehyde in 0,05 % cacodylate buffer (pH 7.2) at 4°C overnight. Samples were washed twice for 15 minutes in the buffer and dehydrated for 10 minutes in each stage of an ethanol series increasing stepwise in concentration from 10 %-100 %. Samples were further immersed for 15 minutes in 100 % ethanol and critically point dried in CO_2. They were then mounted on stubs and coated with 20nm gold-palladium and examined using a Jeol JSM 840 scanning electron microscope. Fungi were identified using standard morphological criteria (with limitations).

2.2.4.3 Pulp tests and by-product analyses

Based on the results of the chemical analyses, samples selected for pulp and by-product analyses are listed in Table 2.1. The major parameters determined from pulp tests were burst and tear indices while turpentine yields were determined from by-product analyses. These analyses were conducted by SAPPI, Springs.

Table 2.1 Samples selected for *pulp tests and by-product analyses*

Incubation period	*Treatment**	*Test*
	fresh	
0 weeks	untreated	pulp test and
	chips : F	by-product analysis
17 weeks	untreated	pulp tests and
	chips : Unt	by-product analysis
	formulation c:	pulp test
	NaB 1%	
	+ DNP 0.2%	
	formulation c:	pulp test and
	P 2%	by-product analysis

* see Section 2.2.2

2.3 *Results*

2.3.1 Chemical Extractions

The results of the caustic solubility or sodium hydroxide solubility; benzene alcohol extraction; ethyl ether extraction; cold water and hot water extractions are presented in Figures 2.4-2.8 respectively, the statistics of which are included as Appendix 2. Refering to these Figures it is seen that after a period of four weeks three chemical analyses were selected and three chemicals that best illustrated control of chip deterioration were analysed further at 8, 12 and 16 weeks after incubation. Of the 5 chemical analyses on each of the 9 treatments conducted, propionic acid 2 % was the only chemical which consistently gave the most protection. Figure 2.4 shows that chips treated with propionic acid 2 % suffered less biodeterioration than those treated with H_2O, B 2 %, DNP + NaB, N-meth + DNP, and P 1 %. Figures 2.5 and 2.6 show that benzene/alcohol and ethyl ether extractions produced results similar to those of caustic solubility, and similarly Figures 2.7 and 2.8 showed that cold and hot water solubilities respectively also reflected these trends.

2.3.2 Microbiological Studies

Visually obvious surface colonisation of chips occurred sequentially as shown in Table 2.2 and Figures 2.9 - 2.15.

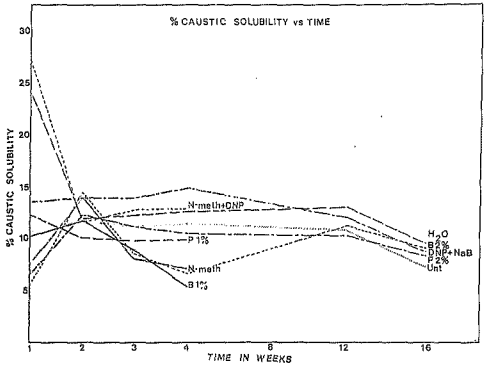


Figure 2.4 The effect of different preservatives (*B 1 %* - Borax 1 %, B 2 % - Borax 2 %, DNP + NaB - 2,4 Dinitrophenol + Sodium bisulphite, H_2O - *Water*, *N-meth* - N-methyldithiocarbamate, N-meth + DNP - N-methyldithiocarbamate + 2,4 *Dinitrophenol*, P 1 % - Propionic acid 1 %, P 2 % - Propionic acid 2 %, Unt - Untreated control) on the NaOH (caustic) solubility of *Pinus patula* chips in laboratory trials.

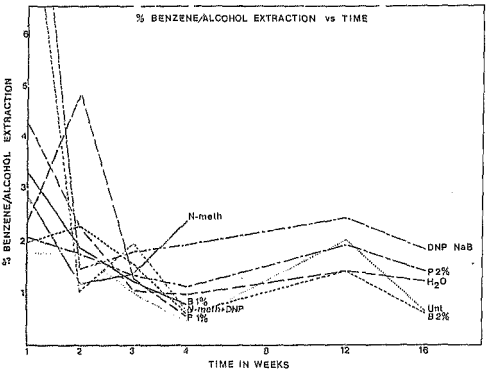


Figure 2.5 The effect of different *preservatives* (B 1 % - Borax 1 %, B 2 % - Borax 2 %, DNP + NaB - 2,4 Dinitrophenol + Sodium bisulphite, H_2O - Water, N-meth - *N-methyldithiocarbamate*, *N-meth* + DNP - N-methyldithiocarbamate + 2,4 Dinitrophenol, P 1 % - Propionic acid 1 %, P 2 % - Propionic acid 2 %, *Unt* - Untreated control) on the benzene/alcohol extraction of *Pinus patula* chips in laboratory trials.

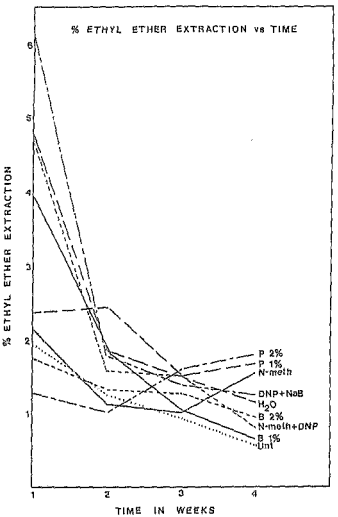


Figure 2.6 *The effect of different preservatives (B 1 % - Borax 1 %, B 2 % - Borax 2 %, DNP + NaB - 2,4 Dinitrophenol + Sodium bisulphite, H_2O - Water, N-meth - N-methyldithiocarbamate, N-meth + DNP - N-methyldithiocarbamate + 2,4 Dinitrophenol, P 1 % - Propionic acid 1 %, P 2 % - Propionic acid 2 %, Unt - Untreated control) on the ethyl ether extraction of Pinus patula chips in laboratory trials.*

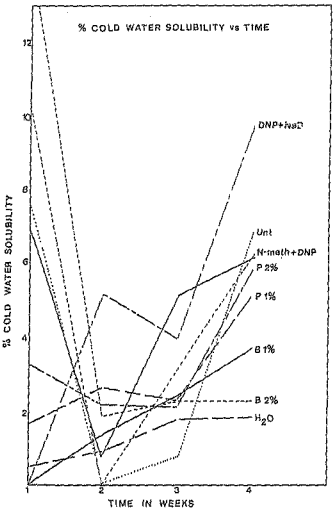


Figure 2.7 The effect of different preservatives (B 1 % - *Borax* 1 %, B 2 % - *Borax* 2 %, DNP + NaB - 2,4 Dinitrophenol + Sodium bisulphite, H_2O - Water, N-meth - N-methyldithiocarbamate, N-meth + DNP - N-methyldithiocarbamate + 2,4 Dinitrophenol, P 1 % - Propionic acid 1 %, P 2 % - Propionic acid 2 %, Unt - Untreated control) on the cold water solubility of *Pinus patula* chips in laboratory trials.

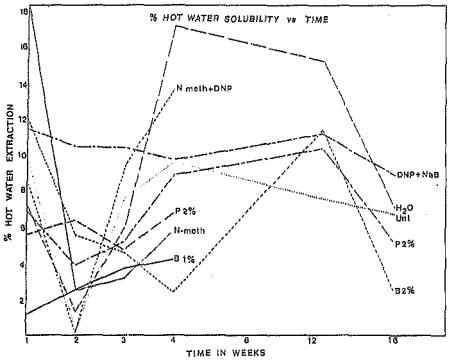


Figure 2.8 The effect of different preservatives (B 1 % - Borax 1 %, B 2 % - Borax 2 %, DNP + NaB - 2,4 Dinitrophenol + Sodium bisulphite, H_2O - Water, N-meth - N-methyldithiocarbonmate, N-meth + DNP - N-methyldithiocarbamate + 2,4 Dinitrophenol, P 1 % - Propionic acid 1 %, P 2 % - Propionic acid 2 %, Unt - Untreated control) on the hot water solubility of *Pinus patula* chips in laboratory trials.

Table 2.2 Incubation periods at which extensive surface colonisation of treated chips was observed visually (- no visible growth; + visible growth).

Incubation	T R E A T M E N T							
Period	F O R M U L A T I O N*							
(wks)	Unt	H_2O	a	b	c	d	e	f	g
1	-	-	-	-	-	-	-	-	-
2	+	+	-	-	-	-	-	-	-
3	+	+	-	-	-	-	-	-	-
4	+	+	-	-	-	-	-	-	-
5	+	+	-	-	-	-	-	+	-
6	+	+	-	-	-	+	-	+	+
7	+	+	-	-	-	+	-	+	+
8	+	+	+	-	-	+	-	+	+
9	+	+	+	-	-	+	-	+	+
10	+	+	+	-	-	+	-	+	+
11	+	+	+	-	-	+	-	+	+
12	+	+	+	-	-	+	-	+	+
13	+	+	+	-	-	+	-	+	+
14	+	+	+	-	-	+	-	+	+
15	+	+	+	-	-	+	-	+	+
16	+	+	+	+	-	+	-	+	+
17	+	+	+	+	-	+	+	+	+

* formulations

a:- N-meth 4 %

b:- N-meth 0.2 % + DNP 0.2 %

c:- NaB 1% + DNP 0.2 %

d:- P 1 %

e:- P 2 %

f:- B 1 %

g:- B 2 %

(see Section 2.2.2)

Figure 2.9 Untreated control chips after 4 weeks' incubation.

Pinus patula (4 weeks)

UNTREATED CONTROL

Figure 2.10 1 % and 2 % borax after 4 weeks' incubation.

Pinus patula (4 weeks)

1% BORAX

2% BORAX

Figure 2.11 1 % and 2 % propionic acid after 4 weeks' incubation.

Pinus patula (4 weeks)

1% PROPIONATE

2% PROPIONATE

Figure 2.12 1 % borax, control and 1 % propionic acid after 4 weeks' incubation.

Pinus patula (4 weeks)

1% BORAX

CONTROL

1% PROPIONATE

Figure 2.13 N-methyldithiocarbamate; 2,4 dinitrophenol + N-methyldithiocarbamate and 2,4 dinitrophenol + sodium bisulphide after 4 weeks' incubation.

Figure 2.13 N-methyldithiocarbamate; 2,4 dinitrophenol + N-methyldithiocarbamate and 2,4 dinitrophenol + sodium bisulphide after 4 weeks' incubation.

Pinus patula (4 weeks)

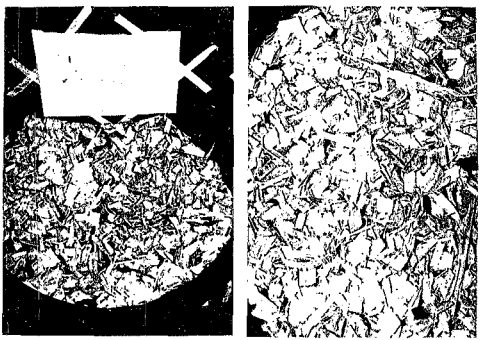
N-METHYLDITHIOCARBAMATE

2,4 DINITROPHENOL +
N-METHYLDITHIOCARBAMATE

2,4 DINITROPHENOL +
SODIUM BISULPHITE

Figure 2.14 2 % propionic acid after 4 weeks' incubation.

Pinus patula (4 weeks)



2% PROPIONATE

Figure 2.15 2 % propionic acid and 2,4 dinitrophenol + sodium bisulphide after 4 weeks' incubation.

Pinus patula (4 weeks)

2% PROPIONATE

2,4 DINITROPHENOL + SODIUM BISULPHITE

On the basis of the information presented in Table 2.2 it was concluded that formulation c (NaB 1 % + DNP 0.2 %) performed as the best preservative treatment tested since no surface colonisation of the relevant chips could be detected visually even after 17 weeks' incubation. Treatment by preservative formulations b (N-meth 0.2 % + DNP 0.2 %) and e (P 2 %) also performed relatively well as anti-microbial agents, whereas untreated (Unt) and water-treated (H_2O) chips became visually colonised after only 1 week's incubation. Formulations b, c and e were therefore considered as suitable preservative treatments for chips, however the cost factors and environmental concerns associated with dinitrophenol and sodium bisulphite precluded the selectability of formulations b and c as appropriate preservative treatments.

Figures 2.9 - 2.15 illustrate the very distinct differences which were manifested between treatments in terms of chip appearance after 4 weeks' incubation in the constant environment room, which is important to purchasers and their agents. The surfaces of untreated control chips (Figure 2.9) were extensively colonised by moulds. Similarly 1 % and 2 % borax (Figure 2.10) and 1 % propionic acid (Figure 2.11) were extensively colonised, apparently to the same extent as untreated control chips (Figure 2.12). The N-methyldithiocarbamate - treated chips were colonised, but in contrast, the 2.4 dinitrophenol + N-methlydithiocarbamate - treated, and 2.4 dinitiophenol + sodium bisulphite - treated chips were uncolonised (Figure

2.13). Similarly, 2% propionic acid - *treated chips were uncolonised* (Figures 2.11 and 2.14), and this preservative appeared to prevent colonisation just as effectively as 2,4 dinitrophenol + sodium bisulphite (Figure 2.15). In summary therefore, two treatments were effective in preventing colonisation of microorganisms, namely :

a. Propionic acid 2 %.
b. 2,4 Dinitrophenol + sodium bisulphite.

Electron microscopic studies of treated chips confirmed the visual observations recorded above. Figure 2.16 shows the extent and nature of chip colonisation by microorganisms at the *end of the incubation period*. Basidiomycete decay fungi had colonised H_2O - treated (Figure 2.16b) and untreated chips (Figure 2.16c and 2.16d). These chips were decayed.

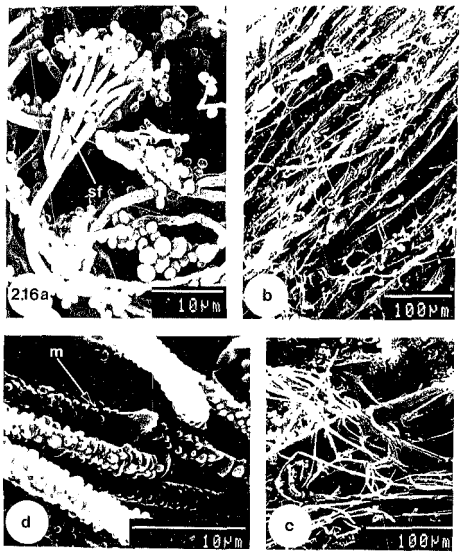
2.3.3 *Pulp Tests And By-product Analyses*

The burst and *tear indices of hand-sheets produced from* pulp tests at various beating revolutions are presented in Figure 2.17. Turpentine yields are presented in Table 2.3.

2.4 *Discussion*

Chips treated with different preservative formulations became visually colonised by microorganisms *at different rates (Table* 2.2 *and* Figures 2.9-2.15) and differences in extent of colonisation were strikingly obvious.

Figure 2.16 Colonised surfaces of chips after 16 weeks' incubation. (a) P 2 %-treated chips supported only non-decay staining fungi such as *Penicillium* spp. H_2O treated chips (b) and Uni chips (c) were more extensively colonised and supported extensive rugose mycelium (m) of basidiomycete decay fungi (d).


sf
2.16a
10µm
b
100µm
m
d
10µm
c
100µm

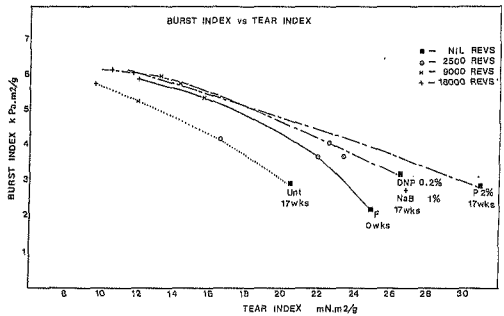


Figure 2.17 Pulp test (REVS : beating revolutions) after 17 weeks' incubation of *Pinus patula* chips. Differences between strengths of paper from untreated and treated (see 2.3.3.) chips are significant.

Table 2.3 Turpentine yields of untreated and treated samples after 17 weeks incubation

SAMPLE	*TURPENTINE YIELD (litre/ton dry timber)*
(0 wk) fresh untreated chips (F)	1.80
(17 wks) untreated chips(Unt)	0.62
(17 wks) treated chips (P 2%)	1.15

It was very clear that chips treated with P 2 %, NaB 1 % + DNP 0.2 %, and N-meth 0.2 % + DNP 0.2 % (formulations e, c and b respectively) showed little or no surface colonisation after 16 weeks' incubation whereas B 1 % and 2 %, P 1 %, N-meth 0.4 % (formulations f, g, d and a respectively), and H_2O - treated and untreated chips became extensively colonised (ISMAIL, SMITH and bAECKER, 1988).

The results of the chemical extractions (Figures 2.4-2.8) however, fluctuated. The variations of these data were within the acceptable limits of reproducibility defined in the TAPPI Standard T212 os-76 used, but it was concluded that the results of the chemical extractions did not suitably identify differences between preservative performances as indicated by chip appearance. For example, percentage caustic solubility and quantities of water extractives present in wood should all increase with degree of biodeterioration of cellulose; percentage benzene/alcohol extractives, i.e waxes, fats and resins, should decrease as utilised by microorganisms during biodeterioration, and percentage ethyl ether extractives, i.e fatty acids, should similarly decrease as biodeterioration proceeds. The explanation for this could be as follows : chemical composition of wood from a tree may vary with respect to nitrogen content (B. KING, pers. comm., 1988) and importantly cellulose and lignin content (van ZYL, 1988). In any given tree there are considerable variations of this nature up and down a tree, within samples from a tree and between samples from different trees. Now

from a 60 kg batch treatment only 2 g samples were taken for each analysis, and depending on which part of the tree this 2 g sample came from, variations could have arisen in the results obtained, owing to variations between samples. Therefore using only part of a tree (2 g) is not a representative sample for a 60 kg batch sample as this sample is minute compared with the whole batch being sampled and may easily over- or underestimate the true abundance. Therefore, to achieve more comparable results, it is felt that 4 sections should be cut throughout the length of a given tree and all chemical analyses should be carried out using chips from these respective sections. In this way, although there would be variation in the analyses between the sections, all chemical analyses from any one particular section of the tree would be comparable. However, it is rarely feasible to subject a whole ecosystem to enumeration or measurement procedures. Instead, these procedures are performed on representative sub-samples, and from these measurements projections are extrapolated to the whole ecosystem (BOARD AND LOVELOCK, 1973 cited by ATLAS AND BARTHA, 1987), and this principle was adhered to in the present case. In addition, chemical analyses are usually carried out because they are relatively simple, require small sample sizes, are less time - consuming and can rapidly provide information about the chemical composition and age of a tree (for example, if the hot water extraction, extracts a high concentration of sugars then the tree is young, but if low concentration of sugars are extracted, then the tree is old). Therefore when chemical extractions are performed this should be in conjunction with pulp tests (M.

GAMBINO, pers. comm., 1988). The present work also considered microbiological criteria.

Since the trends in extractive levels did not conform to observed degrees of chip colonisation, it was decided to establish the nature of the microflora colonising the chips. Electron microscopic examination of these chips confirmed that only sparse colonisation of P 2%, NaB 1% + DNP 0.2 %, and N-meth 0.2 % + DNP 0.2 % - treated (formulations e, c and b respectively) chips had occurred and, importantly, that such colonisation was attributable only to sap-stain fungi such as *Aspergillus* and *Penicillium* spp (Figure 2.16a) which do not significantly affect the strength properties of wood. Conversely, control chips and those treated with the remaining preservative formulations supported extensive fungal growth (Figure 2.16b) and much basidiomycete mycelium was observed (Figure 2.16c and d). It is important to note, however, that although major differences in the degree of colonisation of differently treated chips were recorded after 4 weeks' incubation (Figures 2.9 - 2.15) and that differences persisted throughout the 16 week incubation period (Table 2.1), these differences steadily became less apparent. It was felt that the use of propionic acid 2 % had not simply prevented the microbial succession pattern from occurring, but had only delayed this event. This phenomenon is discussed in more detail in Chapter 5.

Basidiomycetes include the ligninolytic white rot fungi and the cellulolytic brown rot fungi which are serious decay agents of wood material and which produce high losses in the strength properties of affected wood. It was therefore decided to conduct pulp tests to establish whether the wood colonised in these tests yielded paper of differing strength properties. The results of these tests showed clearly (Figure 2.17)that the burst and tear indices of 17 - week old P 2 % treated - and NaB 1 % + DNP 0.2 % (formulations e and e respectively) treated - chips did not differ significantly from those of fresh untreated chips, whereas the strength properties of untreated chips had fallen to commercially unacceptable levels after 17 weeks' incubation had elapsed (ISMAIL, SMITH and BAECKER, 1988). It was therefore concluded that the inhibition of fungal colonisation by certain preservative formulations in chips had maintained wood quality at levels permitting the production of acceptable pulp. In addition it was also found that propionic acid - treated chips produced 1.15 litres of turpentine ton dry timber even after 17 weeks incubation whereas untreated chips produced only 0.62 litres/ton dry timber after 17 weeks incubation. These results therefore support those of pulp tests suggesting that propionic acid 2 % was indeed an effective preservative under the present conditions. On the basis of the consistency of findings of the microscopic investigations, the pulp tests and the by product analyses, it was concluded that the chemical extraction tests had provided rather unreliable results. Possible reasons for the variability in chemical extraction data were discussed above with reference to timber variability in samples in and between

trees. However, it was also felt that specifications of the TAPPI sample preparation technique (TAPPI Standard T212 os-76) used may have affected the data obtained in the present work. Figure 2.1 shows that the crushed wood samples were sieved and that the sieveings used for chemical extraction analyses were those which did not pass a 60 mesh sieve. Sieveings which did pass this mesh were discarded. However it was felt that these sieveings were likely to constitute the most degraded fraction of the crushed sample since decayed wood would probably disintegrate into comparatively small particles. Following this reasoning it was also felt that chemical extraction of these millings, or "fines" (i.e those which passed the 60 mesh sieve) may be more representative of microbiologically - affected wood and therefore provide data more comparable with data obtained *from microbiological observations, pulp tests and by-product analyses.*

Two important considerations of wood preservation are the quantification of costs and the qualification of effectiveness (S. VENTER, pers. comm., 1987) (discussed fully in Chapter 5). Furthermore, only field trials provide hard information on preservative performance in wood. The successful preservatives in the present work obviously prevented chip degradation from reaching unacceptable levels, under the laboratory conditions used, but other aspects of the effectiveness of chemicals in service, such as acceptability to end-users and environmental toxicity, must also be qualified. On the above bases, and the results presented here, it was concluded that propionic acid 2 % constituted the most appropriate preservative formu-

lation to use in field trials to investigate the prevention of losses of pulp quality arising from outside chip storage for extended periods. It was therefore decided to use propionic acid 2 % in field trials and to analyse the samples as before. It was also decided to investigate the unreliability of the chemical extraction tests by performing these on sample sievings as specified (i.e greater than 60 mesh) and to compare these data with identical analyses of "fines" from each sample which passed the 60 mesh sieve.

3.0 FIELD TRIALS

3.1 Introduction

3.1.1 Microorganisms In Wood

Although many microorganisms affect wood, those which do so colonise it in a distinct pattern of microbial succession. The microorganisms can therefore be grouped as primary, secondary and tertiary colonisers, each group of which may be regarded as a distinct "physiological group" (LEVY, 1982) which produces distinct changes in the wood. These groups are as follows :

a. Primary and secondary colonisers - fungi causing mould, stain and/or soft-rot (microbiologists classify most of these fungi with the phycomycetes, ascomycetes and fungi imperfecti). Yeasts, bacteria and actinomycetes (HULME, 1979).
b. Tertiary colonisers - white and brown rot fungi (classified as basidiomycetes).
c. Thermophilic fungi.

The latter includes those fungi which will grow at normal ambient temperatures, but thrive at temperatures between 37-60°C. Their activities are exclusively confined to composts and chip piles, where rapid heating of these large masses of stored wood provides an ideal growth habitat. There

is no evidence to suggest that these fungi utilise lignin, although they can and do utilise cellulose and/or hemicellulose (BERGMAN and NILSSON, 1971 cited by SMITH, 1975). Certainly their presence in a chip pile would cause a progressive loss of wood weight (SMITH and OFOSU-ASIEDU, 1972; OFOSU-ASIEDU and SMITH, 1973 cited by SMITH, 1975) with indications of a subsequent decrease in pulp yields. Although small, these losses when totalised for the entire industry are very important (HATTON, SMITH and ROGERS, 1968 cited by SMITH, 1975). However, it has been shown in previous work at Richards' Bay (BAECKER, pers comm, 1987) that thermophilic fungi were seldom isolated from these chip piles therefore they were not monitored in the present studies.

KRESS, HUMPHREY, RICHARDS, BRAY and STAIDL (1925), BJORKMAN (1946) and HAJNY (1966) conducted extensive studies on the succession of microorganisms during the decay of pulpwood.

3.1.1.1 Effects of primary colonisers

Many fungi in this group are not considered to be serious destroyers of wood (HULME, 1979). This group includes those fungi which normally colonise only the sap-wood of dead or fallen trees, particularly species of pine. Lack of suitable enzyme systems prevents their utilisation of significant amounts of cellulose or lignin components of the wood cell walls. Instead they grow on the freely available soluble carbohydrates in the sap-wood (SMITH, 1975). Because of this method of growth these fungi are

of little consequence to the pulping industry (SMITH, 1975). Moulds which are in the main saprophytes of dead cell contents may sometimes be found to have penetrated into timber along the grain by means of vessel lumina or tangentially into the ray parenchyma by means of pits. Staining fungi with pigmented hyphae and spores cause discolouration of the wood by a build-up of fungal hyphae in the ray parenchyma. The hyphae are of two distinct types and some penetration transversely across tracheids by means of very small bore holes may also be observed. Staining fungi, in addition to the characteristics already mentioned, can form cavities in a helix in the secondary wall of tracheids and fibres or sometimes vertical chains of cavities. These cavities have been observed only in hardwoods (LEVY, 1969). Mould fungi such as *Mucor* appear as furry growths on the wood surface while staining fungi such as *Aspergillis* and *Penicillium* darken the wood usually because they produce pigmented spores or can produce pigments in the wood (HULME, 1979). They therefore cause flecking in the production of high quality papers (SMITH, 1975).

Yeasts and bacteria have also been reported to be present in chip piles as primary colonisers (CRANE and FASSNACHT, 1960; BJORKMAN and HAEGER, 1963a; BJORKMAN and HAEGER, 1963b; LINDGREN and ESLYN, 1961 cited by HAJNY, 1966).

Bacteria are able to attack the organic components of wood separately (TSOUMIS, 1968), however, the precise role these organisms play in the

deterioration of wood is somewhat uncertain. Very finely ground wood has been observed, however, to be readily fermented by certain bacteria, such increased susceptibility may be related to reduction of the degree of crystallinity of wood with grinding (GASCOIGNE and GASCOIGNE, 1960 cited by TSOUMIS, 1968). According to LEVY (1967), bacteria by themselves or in association with other organisms can cause deterioration of wood. Their major role is unlikely to be the attack of structural constituents of wood but these microorganisms may use the wood extractives or the soluble products produced by the decay fungi as substrates (HAJNY, 1966). Resistance of wood is apparently due to toxic extractives or its natural acidity. Recent work by ROGERS and BAECKER (1988) has suggested that strictly anaerobic bacteria may play a role in the biodeterioration of wood.

Bacteria may be involved in discoloration of wood of living trees in association with fungi, as it was reported for American beech, birch and maple (SHIGO, 1965 cited by TSOUMIS, 1968). Presence of saprophytic bacteria may enhance the activity of fungi. Once fungal attack has been initiated, bacteria may play a dominant role in further decomposition of wood (GASCOIGNE and GASCOIGNE, 1960 cited by TSOUMIS, 1968).

GREAVES (1973a) stated that actinomycetes are mycelial bacteria which appear to cause slight damage to the wood however this conclusion was premature because actinomycetes had only received cursory attention at

that time (GREAVES, 1969; GREAVES, 1973b cited by HULME, 1979). Since then extensive studies have been conducted by BAECKER (1981) on the role of actinomycetes in the biodeterioration of wood, who found that only certain actinomycetes are cellulolytic and hence produce decay. Their presence in wood may significantly modify the behaviour of fungal colonisers and may have significant implications in microbial succession patterns in wood.

Bacteria in general grow prolifically during early stages of storage when low molecular weight nutrients are available (SPRINGER, ZOCH, FEIST and HAJNY, 1973; JACQUIOTE and LAPETITE, 1967 cited by HULME, 1979). They are also important because they may retard the development of other microorganisms (JACQUIOTE, 1968; GREAVES, 1970; ROSSELL, ABBOT and LEVY, 1973; GRANT, 1968 cited by HULME, 1979) as may fungi from the first group (SPRINGER, ZOCH, FEIST and HAJNY, 1973).

3.1.1.2 Effects of secondary colonisers

Many of the fungi classified with the ascomycetes can cause extensive damage as soft-rot. Some of these fungi such as *Chaetomium* spp. can form cylindrical cavities inside cell walls in the superficial layers of the wood (NILSSON, 1973a cited by HULME, 1979) causing a type of wood destruction known as soft rot (SAVORY, 1954). This results in loss of cellulose and hence a loss of pulp. Soft-rot fungi give rise to cavities in the

secondary walls of fibres and tracheids in both hardwoods and soft woods and in some woods also appear, under the light microscope, to be capable of direct lysis of the cell walls from hyphae lying in the cell lumen. The formation of cavities has also been observed under the light microscope to occur from a short side branch and it has been postulated that these side branches are 'foraging' hyphae and are acting as a type of haustorium (LEVY, 1969). A few basidiomycetes also cause soft-rot although this is usually slight (NILSSON, 1973b cited by HULME, 1979).

3.1.1.3 Effects of tertiary colonisers

The true wood-destroying fungi (white and brown rot fungi) are all cellulolytic and/or ligninolytic Basidiomycetes. Fungi which break down lignin are called white-rot fungi (*LINDGREN and ESLYN, 1961*). White rot fungi possess an enzyme system capable of degrading both lignin and cellulose and can also produce bore holes both transversely and vertically in the cell wall. White rotters include the more highly developed forms of fungi such as *Phellinus* and *Asterostroma spp.* which generally have the ability to attack rapidly and consume lignin and/or cellulose and hence cause wood damage (CARTWRIGHT and FINDLAY, 1958 cited by HULME, 1979). Holocellulose constitutes 70-75 % of the woody tissue and is the basic material required by the pulping industry for the manufacture of paper, whereas lignin constitutes 25-30 % of similar woody tissues (SMITH, 1975).

If these white-rotters solely degrade lignin, they could be effectively used biologically to pulp wood. However, they degrade cellulose as well as lignin, approximately in proportion to the occurrence of these materials in normal wood. Therefore, these fungi can cause degrade and loss of fibre potentially usable by the pulping industry. Advanced white-pocket rot attack of wood results in only small changes in pulp yield. White-rot fungi cause small increases in alkali solubility during decay, as well as a drop in pH, but not to such a low value as is the cause with brown-rot fungi. Therefore, during the kraft pulping process, the use of wood attacked by white-rot fungi does not result in important economic losses due to increased alkali consumption (SMITH, 1975).

Basidiomycetes such as *Coniophora* which breakdown cellulose and not lignin are called brown-rot fungi (LINDGREN and ESLYN, 1961). Brown rot fungi, where the hyphae are relatively small in cross section and give rise to transverse bore holes through the cell walls, reduce the strength and weight of the wood to a much greater extent than can be accounted for by the volume of wall substances removed in the formation of bore holes. It is known that the enzyme system of these fungi is largely concerned with degrade of only the cellulose fraction of the cell wall. There is some evidence to suggest that the rapidity of this decay must be correlated with a movement of the active enzyme system through the layers of the cell wall (LEVY, 1969). Obviously, these microorganisms can be seriously harmful to the pulping industry, since they not only degrade usable fibre material,

but also they will increase the lignin : cellulose ratio in a given inventory of wood or chips, thereby increasing the consumption of pulping chemicals. When considering mechanical pulp, the presence of wood decayed by brown-rot fungi also give problems of low brightness. As well as decreasing the strength of the wood cells and hence the strength of the manufactured paper, brown-rot fungi cause an increase in the alkali solubility of the wood and a decrease in its pH. Both factors add substantial costs to the kraft - pulping process by increasing the total consumption of alkali, as well as by making less alkali in a given cook available for delignification (PROCTER, 1973 cited by HULME, 1979; SMITH, 1975).

3.1.2 Rationale Of Investigative Test Methods

The experiment was carried out at CTC's Richards' Bay plant (Figure 3.1a) over the Summer and Autumn seasons. It was decided to select only the preservative thought to be most cost-effective for use in field trials, namely 2 % propionic acid (Chapter 2).

With respect to the field test methods, four major parameters had to be established viz :

a. Pile dimensions - it was decided that freshly chipped *Pinus patula* would be erected into pyramid - shaped chip piles. An experiment at the Tugella for pine chip pile heights and corresponding temperatures in winter (August 1987) (E.J. SMITH, pers. comm., 1987)

established that a chip pile constructed to approximately five metres provided a maximum internal temperature of 36°C. Previous workd with *Pinus patula* at Richards' Bay recorded internal pile temperatures of 45°C in four metre high piles (BAECKER, pers. *comm.*, 1987). Therefore in order to approximate to the commercial situation as closely as possible, it was felt that the pile height would have to be at least four meters high in summer (January 1988) in order that temperatures within the chip pile would reach about 45 - 50°C.

b. Sampling stages - it was decided that chips would be sampled at monthly intervals over a four month period. This time interval (four months) was chosen because it was double the time required to chip 60,000 tons of *Pinus patula*, ship it overseas, and deliver to pulp mills at end-user destination. It was felt that the extra eight weeks would allow for possible delays during delivery.

c. Analyses - in the laboratory five biotic analyses were conducted on each treatment per week, however, it was found (SMITH, E.J, pers. *comm.*, 1987) that only four of the above chemical analyses significantly determined the extent of biodeterioration caused by microorganisms, viz: NaOH extraction, benzene/alcohol extraction, hot water extraction, and ethyl ether extraction. Other tests such as the cold water extraction and tall-oil and turpentine yield extractions were therefore not included in the present work. It was felt that confirmatory analyses of biodeterioration would be best

obtained from pulp tests, therefore these pulp tests were conducted monthly in all samples by the CSIR. In addition, microbiological analyses were also pursued, however in contrast to the laboratory trials (Chapter 2), both the outer and inner surfaces of the samples from field trials were examined.

3.2 *Materials and Methods*

3.2.1 Preparation Of Chips

100 tons of freshly felled *Pinus patula* logs were chipped at Richards Bay as detailed below (3.2.3) (Figure 3.1a).

3.2.2 Preparation Of Preservatives

2 % Propionic acid (Figure 3.1b) was made up by adding 28 litres (Figure 3.1c) of Propionic acid to 1400 litres of water (Figure 3.1d) in the cleaned holding tank of a fire tender (Figure 3.1e).

3.2.3 Treatment And Incubation

The hose pipe leading from the fire tender was led to the conveyer belt of the chipper (Figure 3.2a). A specially designed sprayer (Figure 3.2b) was attached to the hose pipe. The sprayer was designed (Appendix 3) to spray

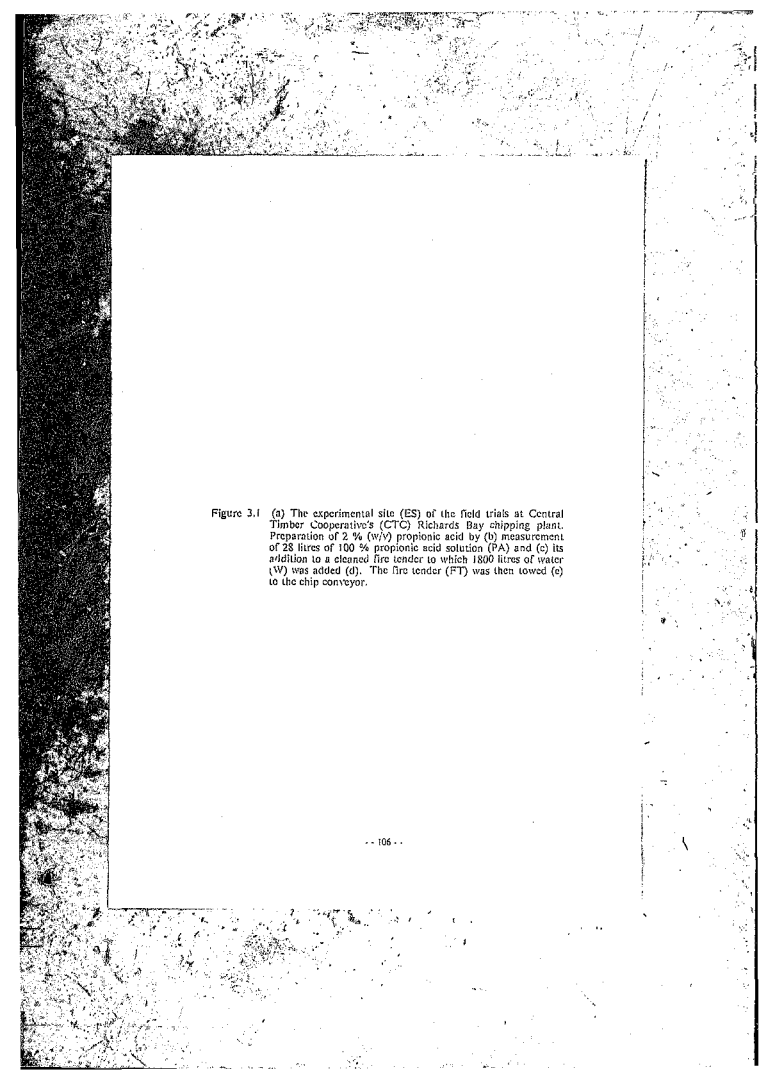


Figure 3.1 (a) The experimental site (ES) of the field trials at Central Timber Cooperative's (CTC) Richards Bay chipping plant. Preparation of 2 % (w/v) propionic acid by (b) measurement of 28 litres of 100 % propionic acid solution (PA) and (c) its addition to a cleaned fire tender to which 1800 litres of water (W) was added (d). The fire tender (FT) was then towed (e) to the chip conveyor.

CTC
31a
PA
b
PA
c
FT
e
d

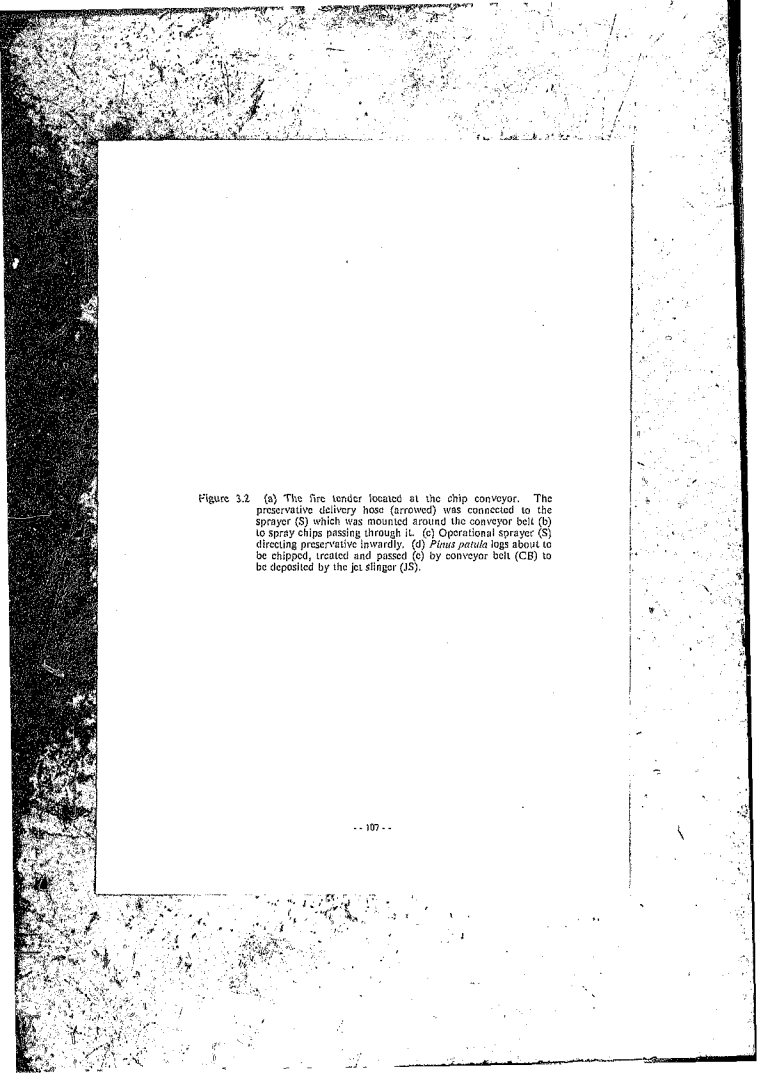


Figure 3.2 (a) The fire tender located at the chip conveyor. The preservative delivery hose (arrowed) was connected to the sprayer (S) which was mounted around the conveyor belt (b) to spray chips passing through it. (c) Operational sprayer (S) directing preservative inwardly. (d) *Pinus patula* logs about to be chipped, treated and passed (e) by conveyor belt (CB) to be deposited by the jet slinger (JS).

S
A
JS
CB

liquid inwardly (Figure 3.2c) under pressure. The sprayer was arranged to surround the chips as they passed through it when leaving the conveyer to be transported in the jet slinger.

The *Pinus patula* logs were separated into 3 batches of 32.5, 32.5 and 39 tons (Figure 3.2d). The fire tender was applied and 39 tons of logs were chipped, passed through the propionic acid spray and deposited via the jet-slinger (Figure 3.2e).

The treated chips were collected in a front-end loader (Figure 3.3a) and transported to the test site nearby. Wire cages (SMITH 1988) were on site (Figure 3.3b). Four cages were each filled with 15 kg samples of treated chips contained in mesh bags (Figures 3.3c and 3.3d) and placed on the 1m high foundation of a treated chip pile (Figure 3.3e). The remainder of the treated chips were added (Figure 3.4a) to cover the cages (Figure 3.4b). The chip pile was built up to 4m height (Figure 3.4c) until the complete batch of 39 tons of treated chips were used.

The remaining 2 batches of 32.5 tons each of logs were chipped and piled with sample cages inside as above, but all were untreated. The 3 experimental chip piles are shown in Figure 3.4d. One of these untreated piles was sprayed with water for 3-4 hours each day during the OCS period of 4 months.

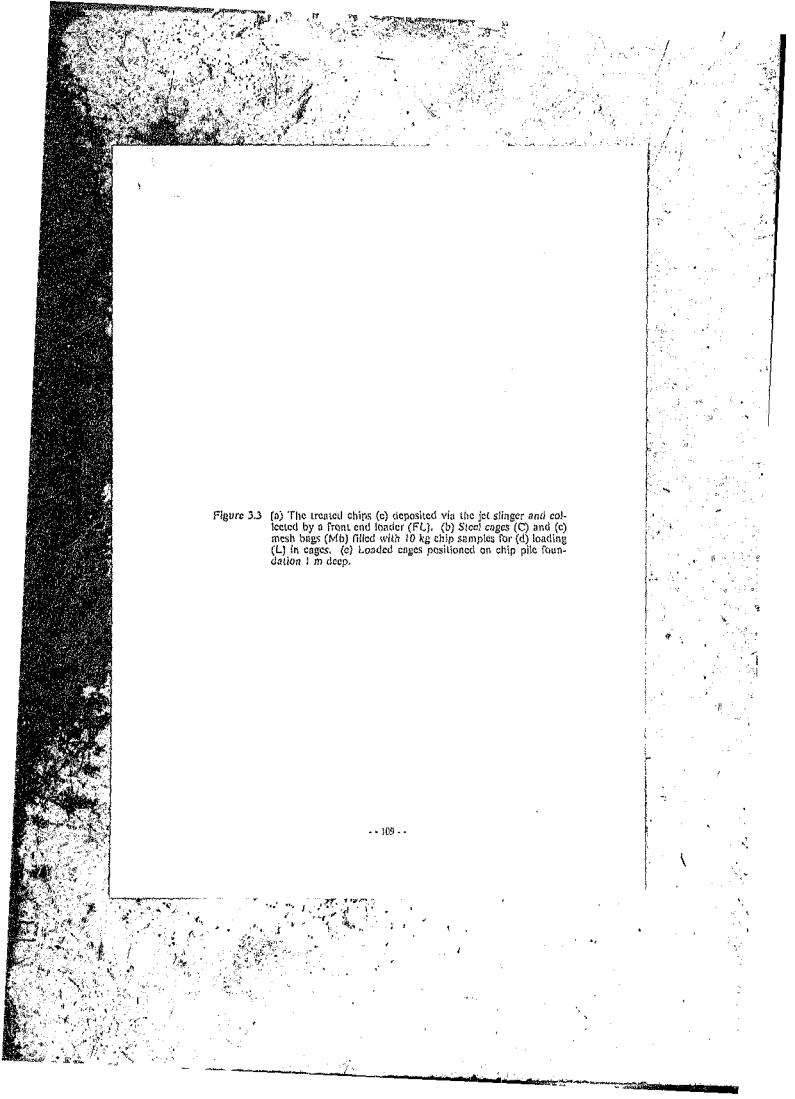
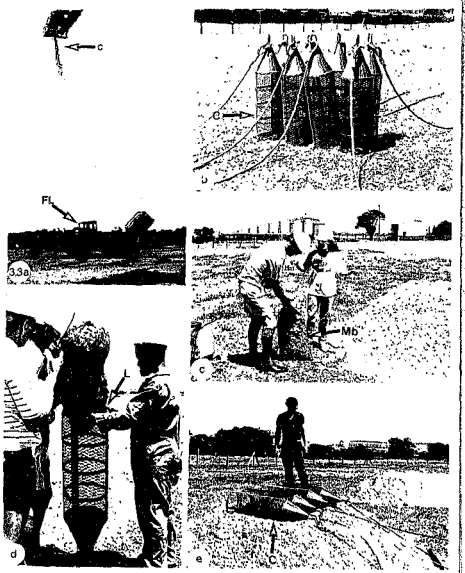


Figure 3.3 (a) The treated chips (c) deposited via the jet slinger and collected by a front end loader (FL). (b) Steel cages (C) and (c) mesh bags (Mb) filled with 10 kg chip samples for (d) loading (L) in cages. (e) Loaded cages positioned on chip pile foundation 1 m deep.


c
b
Fl
33a
Mb
d
e

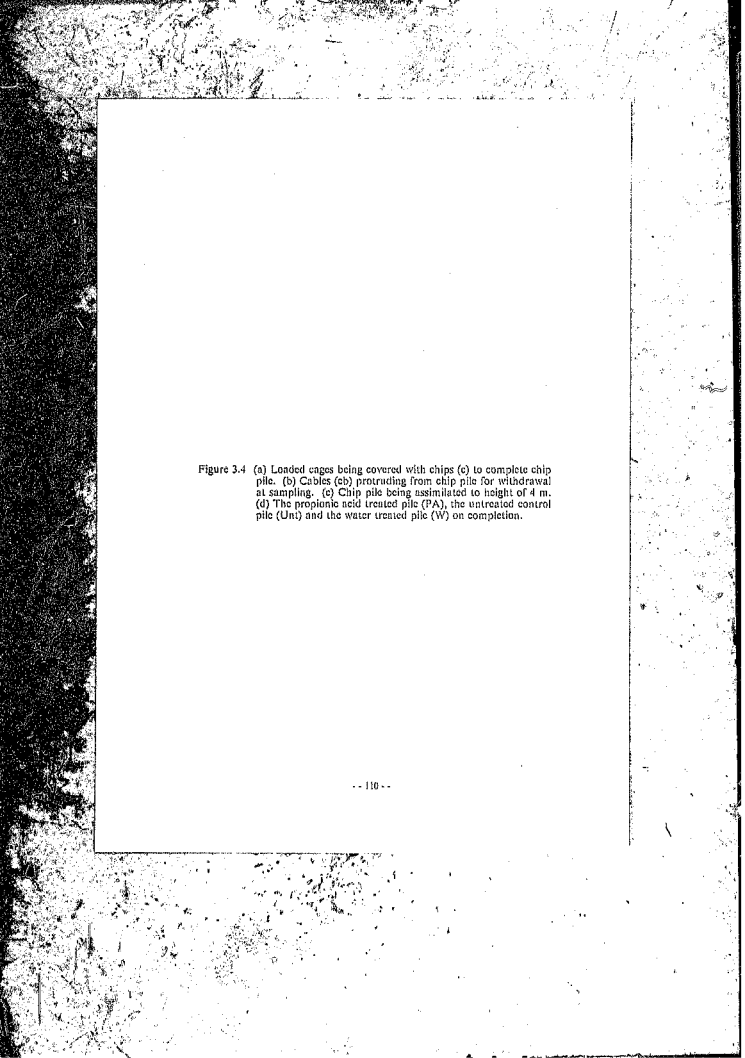
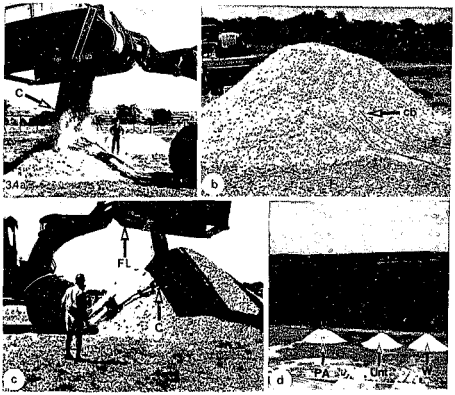


Figure 3.4 (a) Loaded cages being covered with chips (c) to complete chip pile. (b) Cables (cb) protruding from chip pile for withdrawal at sampling. (c) Chip pile being assimilated to height of 4 m. (d) The propionic acid treated pile (PA), the untreated control pile (Unt) and the water treated pile (W) on completion.


C
a
34
b
cb
FL
C
c
d

3.2.4 Sampling And Analyses

At monthly intervals, during the 4 months OCS period (23 March, 25 April, 1 June and 16 June), one cage was withdrawn by cable (Figure 3.5a-d) from each pile. The chips from each cage were taken to the laboratory and divided into 5 kg and 10 kg parts for chemical and microbiological analyses, and pulp tests, respectively. It was ensured that the sample reflected the diversity and density of microorganisms in the entirety of the sampled environment.

3.2.4.1 Chemical extractions

The laboratory trials showed (Chapter 2) that the 4 chemical analyses which best indicated the extent of biodeterioration were benzene-alcohol extraction, ethyl-ether extraction, sodium hydroxide solubility and hot water solubility. These analyses were therefore performed on each sample. The methods have already been described in 2.2.4.1, and were also conducted on fines.

3.2.4.2 Microbiological analyses

The chips contained in the cages that were removed at monthly intervals were monitored visually at sampling and relative degrees of chip colonisation by microorganisms were photographed. In addition one metre long pipes were pushed in the propionic acid and untreated piles and the temperatures in the centre of these two piles were recorded on a daily basis for 4 months.

Figure 3.5 Chip sampling after OCS period. (a) Cage cable (cb) attached to tractor. (b and c) Successive stages of cage (C) withdrawal showing (d) chips (c) in withdrawn cage.

3.5a
b
c
d

Chips were also used for electron microscope examination to determine the extent and type of colonisation. Small particles of wood (approximately 10mm x 10mm) were removed from the surfaces of selected chips that appeared to be most heavily colonised from both the propionic acid - treated and untreated samples. The outer and inner surfaces of these particles were prepared as described in (2.3.4.2) and viewed using the Jeol JSM 840 scanning electron microscope.

3.2.4.3 Pulp tests

Pulp tests were carried out on 9 samples as showed in Table 3.1 over a period of 4 months. The Pulp, Paper and Cellulose Programme of the National Timber Research Institute, C.S.I.R., carried out the pulp tests on the acid treated and untreated control samples while SAPPI (Springs) carried out the pulp tests on the water-treated samples.

3.3 Results

3.3.1 Chemical Extractions

The results of the sodium hydroxide, ethyl ether, benzene/alcohol and hot water extractions are presented in Figures 3.6-3.9 respectively, the statistics of which are indicated in Appendix 4. Figure 3.6 shows that propionic acid 2 % chips suffered less biodeterioration than untreated. Improtantly, this Figure (3.6) also shows that "fines" samples were more degraded than 60-mesh samples of sievings in the cases of both treated and untreated

Table 3.1 Pulp tests carried out on 9 samples over 4 months

Field Tests			
Formulation	e	H_2O	Unt
Weeks			
0			PT
4	PT		PT
8	PT		PT
12	PT		PT
16	PT		PT

Formulation :

e - 2 % propionic acid-treated chips

Unt - Untreated chips

PT - Pulp Tests

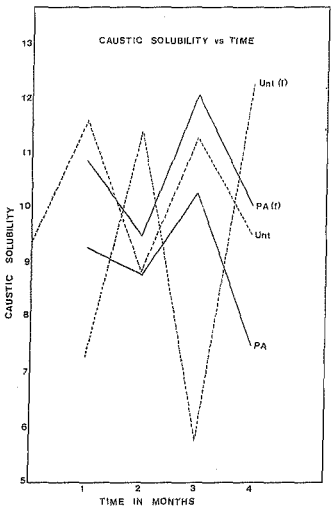


Figure 3.6 The NaOH (caustic) solubility of propionic acid-treated (PA) and untreated (Unt) *Pinus patula* chipsafter OCS (Unt, PA - 60-mesh sample; Unt (f), PA (f) - fines).

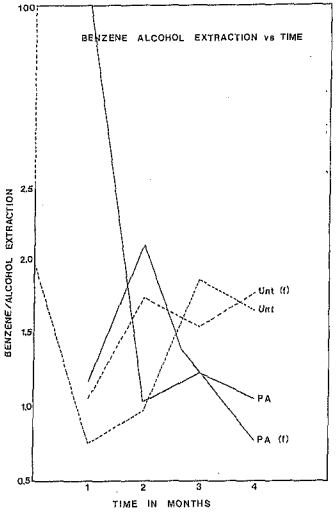


Figure 3.7 *The* benzene/alcohol extraction of propionic acid (PA) and untreated (Unt) *Pinus patula* chips after OCS (Unt, PA - 60-mesh sample; Unt (f), PA (f) - fines).

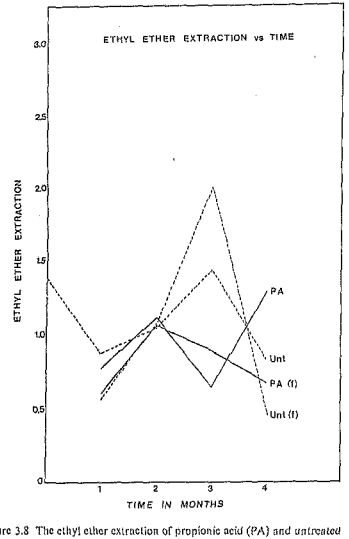


Figure 3.8 The ethyl ether extraction of propionic acid (PA) and *untreated* (Unt) *Pinus patula* chips after OCS (Unt, PA - 60-mesh sample; Unt (f), PA (f) - fines).

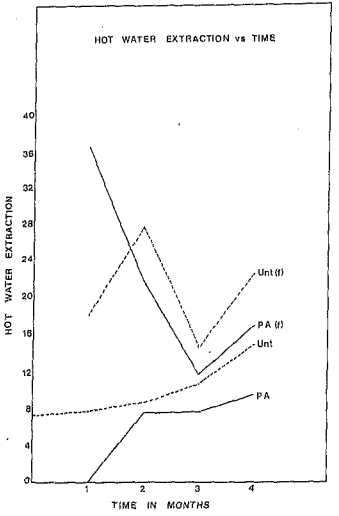


Figure 3.9 The hot water solubility of propionic acid (PA) and untreated (Unt) *Pinus patula* chips after OCS (Unt, PA - 60-mesh sample; *Unt (f), PA (f)* - fines).

chips. Figure 3.7 shows that ethyl ether extractions produced the same results as NaOH extractions, and similarly Figure 3.9 showed that hot water extractions also reflected these trends. The data from benzene/alcohol extractions (Figure 3.8) also showed that "fines" were more degraded than 60-mesh samples of untreated chips but also showed that untreated chips produced more extractives than propionic acid 2 %. Since the latter finding conflicted with those of the other three extraction tests, it was considered unrepresentative, but the reason for the reversed trend is unclear.

3.3.2 Microbiological Analyses

From a distance, differences between the 3 piles were not very apparent (Figure 3.10a) even after 3 months' OCS. However, on closer inspection differences were apparent as indicated on Figures 3.10b and 3.10c which shows discoloration of the water-treated pile compared to the untreated. Towards the end of the field trial it was clear that chips on the surface of the water-treated pile (Figure 3.10d) were more discoloured than those on the untreated control (Figure 3.10e) which were in turn more discoloured than chips of the propionic acid pile (Figure 3.10f). A similar trend was observed when samples withdrawn at monthly intervals from pile interiors were examined. Figure 3.11a shows little differences between untreated and propionic acid-treated at 1 month, however, Figures 3.11b and 3.11c show increasing darkening of untreated chips at 2 and 3 months respectively, whereas propionic acid-treated did not visably discolour. When each

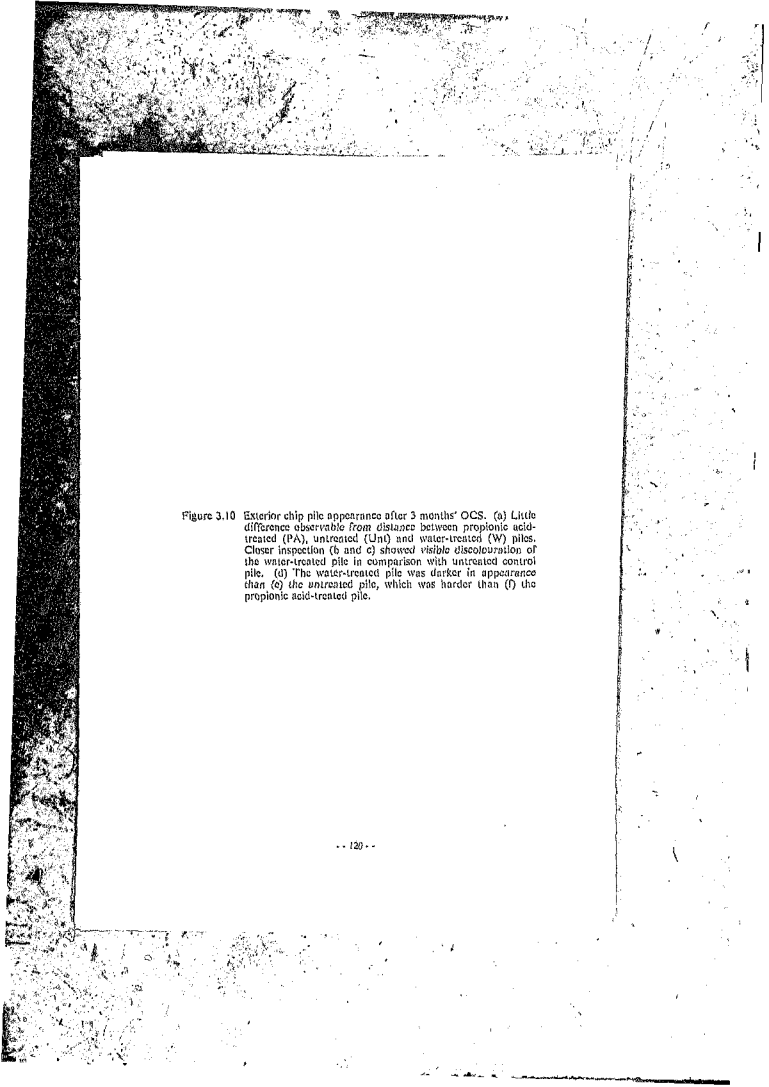
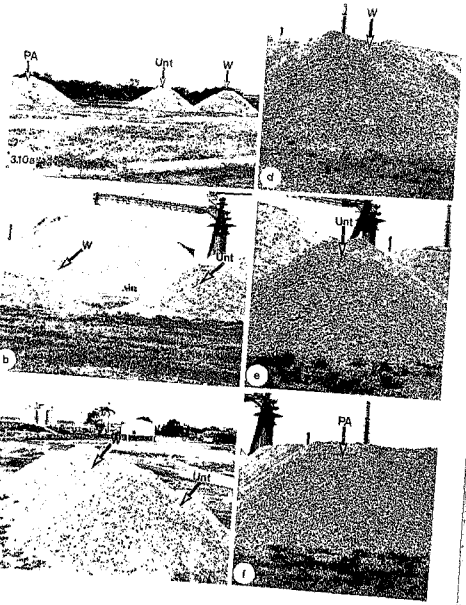


Figure 3.10 Exterior chip pile appearance after 3 months' OCS. (a) Little difference observable from distance between propionic acid-treated (PA), untreated (Unt) and water-treated (W) piles. Closer inspection (b and c) showed visible discolouration of the water-treated pile in comparison with untreated control pile. (d) The water-treated pile was darker in appearance than (e) the untreated pile, which was harder than (f) the propionic acid-treated pile.


PA
Unt
W
a
d
W
Unt
b
e
Unt
PA
c
f

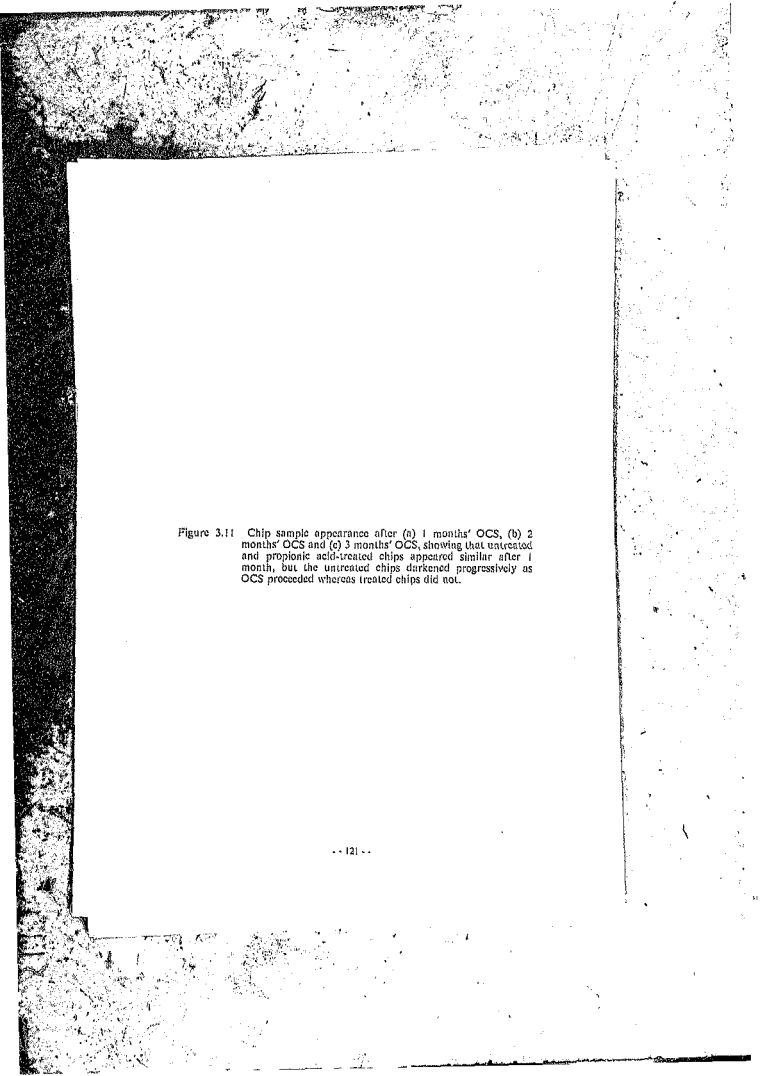
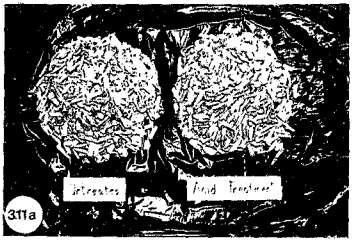
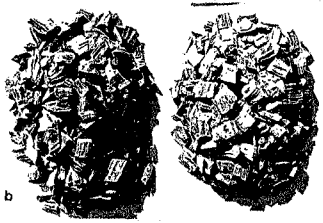
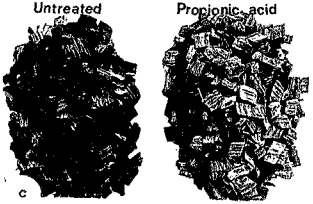


Figure 3.11 Chip sample appearance after (a) 1 months' OCS, (b) 2 months' OCS and (c) 3 months' OCS, showing that untreated and propionic acid-treated chips appeared similar after 1 month, but the untreated chips darkened progressively as OCS proceeded whereas treated chips did not.


Untreated
Acid Treatment
311a


Untreated
Heptoic Acid
b


Untreated
Propionic acid
c

pile was opened at the end of the 4 month trial period, similar differences to those of samples described (Figures 3.10 and 3.11) were observed (Figure 3.12). Figures 3.12a and 3.12b show that chips from the propionic acid-treated pile were not discoloured whereas Figures 3.12c and 3.12d show untreated chips as discoloured and water-treated chips were even more discoloured (Figures 3.12e and 3.12f). The route of infection noted in Figures 3.12d and 3.12e originated at the ground line. Infected chips at the ground line of the propionic acid-treated, untreated and water-treated piles are shown in Figures 3.13a, 3.13b and 3.13c respectively. Most infection was under the water and untreated piles (Figure 3.13d), although mycelium was also evident under the propionic acid pile (Figure 3.13e).

Results of the microbiological investigations are summarised in Table 3.2, and temperature profiles of chip piles are presented in Appendix 5. The surfaces of treated and untreated chips were colonised rapidly. The colonisation patterns observed followed the classical sequence of events reported by Levy (1967) when fungal colonisation of wood was described, Initial infection sites were medullary rays (Fig.3.14a) and mycelium of staining fungi developing in these areas then penetrated adjacent tracheids via pits (Fig.3.14b). Tracheids were progressively colonised (Fig.3.14c, d) until the surfaces of untreated and water treated chips were extensively colonised by staining and soft rot fungi (Fig.3.14e) after 2 months' OCS. After 4 months' OCS, hyphae had penetrated these chips to colonise interior elements. Figure 3.15a shows cellulose fibrils in the S2 layer of the

Figure 3.12 Interior chip pile appearance after 4 months' OCS. (a) Interior of propionic acid-treated (PA) pile contained (b) undiscoloured chips, whereas (c) interior of untreated (Unt) pile contained discoloured chips (d) and (e) the interior of the water-treated (W) pile yielded comparatively darkened chips (f).

312a
b
c
d
e
f

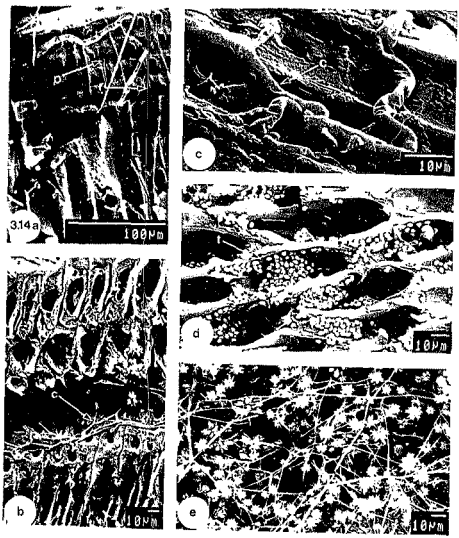
Figure 3.13 Mycelial infection of (a) propionic acid-treated (PA), (b) untreated (Unt), and (c) water-treated (W) chip piles at ground line. (d) Heaviest mycelial infection (M) occurred at ground line of water-treated and untreated piles, but (e) some infection also occurred at ground line of propionic acid-treated pile.

313a
b
c
d
e

Table 3.2 Results of microscopical investigations during field trials

	O C S PERIOD	
	2 MONTHS	*4 MONTHS*
Control Chips	extensive surface colonisation by staining, soft-rot and decay fungi	extensive internal colonisation by 1. cellulolytic soft rot fungi, and 2. *basidiomycete* decay fungi
Untreated Chips	no colonisation	1. localised surface colonisation 2. slight internal colonisation by staining fungi

Figure 3.14 Typical colonisation pattern of treated and untreated chips. (a) Hyphal colonisation of medullary rays (r) occurred initially, associated with pit penetration (p). (b, c and d) Colonisation (c) of tracheids (t) followed by (e) extensive sporulation of surface layers.


3.14a
100µm
b
10µm
c
10µm
d
10µm
e
10µm

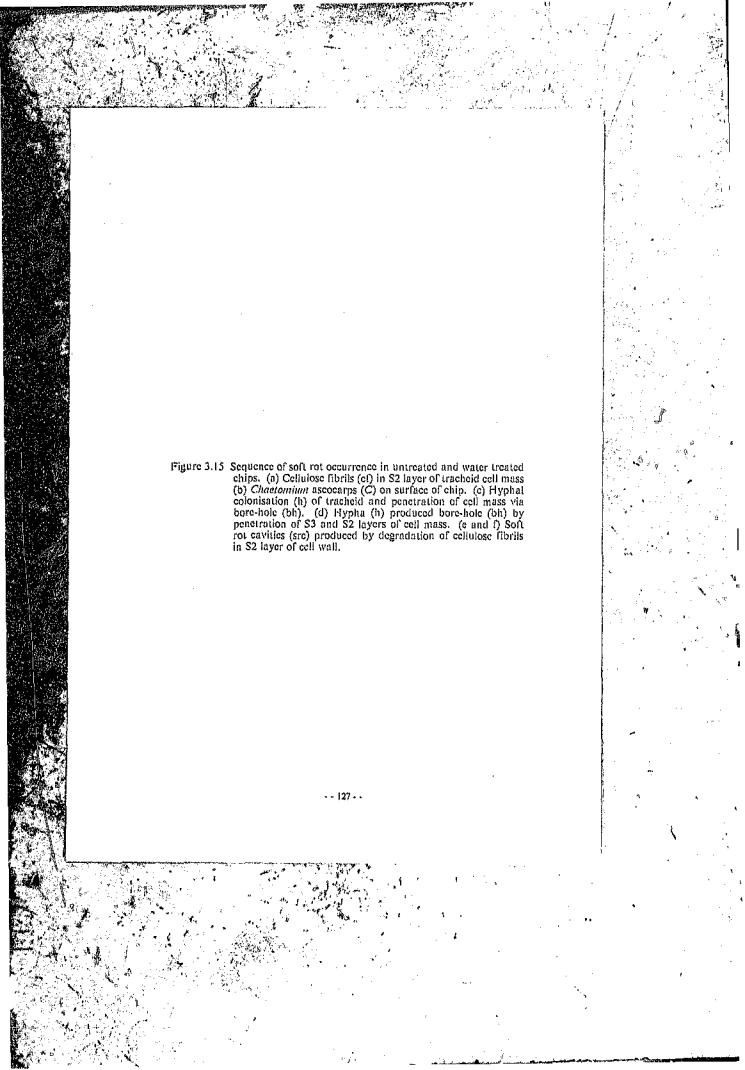
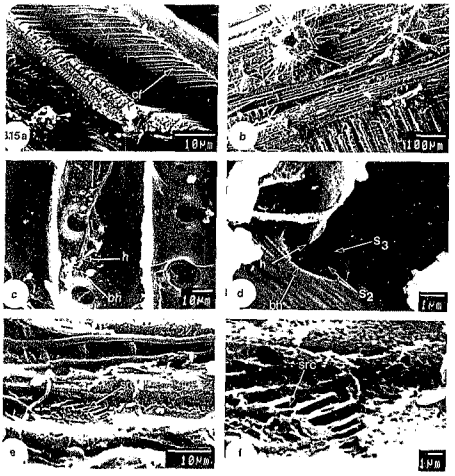


Figure 3.15 Sequence of soft rot occurrence in untreated and water treated chips. (a) Cellulose fibrils (cf) in S2 layer of tracheid cell mass (b) *Chaetomium* ascocarps (*C*) on surface of chip. (c) Hyphal colonisation (h) of tracheid and penetration of cell mass via bore-hole (bh). (d) Hypha (h) produced bore-hole (bh) by penetration of S3 and S2 layers of cell mass. (e and f) Soft rot cavities (src) produced by degradation of cellulose fibrils in S2 layer of cell wall.


315a
cf
10µm
b
100µm
c
h
bh
10µm
d
S3
S2
1µm
g
10µm
f
scc
1µm

tracheid. From 2 months' onwards, ascocarps of soft rot ascomycetes such as *Chaetomium spp.* (Fig.3.15b) were observed on these chips. Members of this genus are cellulolytic and hyphae entered tracheids and penetrated the S3 layers of wood cell walls (Fig.3.15c, f). Towards the end of the OCS period, extensive basidiomycete mycelium showing clamp connections was observed(Fig.3.16a). The mycelium of these decay fungi produced hyphal sheaths connecting it to the wood cell walls (Fig.3.16b, c). Within these sheaths the ligninases and cellulases produced decay (Fig.3.16d, e) causing serious loss of wood material. In contrast, propionic acid-treated chips were relatively uncolonised after 2 months' OCS (Fig.3.17a), even at rays (Fig.3.17b). After 4 months' OCS localised surface colonisation by staining fungi had occurred (Fig.3.17c), but this was never extensive and interior elements remained uncolonised. No soft rotters or decay fungi were observed on propionic acid-treated chips and ascomycetous colonisation was restricted to staining fungi such as *Penicillium spp.* (Fig.3.17d), which do not affect strength properties of wood. Some unidentified fungi were also recorded in these tests (Fig.3.18). Several expert mycologists in S.A. and overseas have been unable to recognise these fungi, but extensive examination by SEM did not reveal any decay associated with these strains.

3.3.3 Pulp Tests

The burst and tear indices of hand-sheets produced from pulp tests at various beating revolutions are presented in Figure 3.19. From the graphs of Tear Index vs Burst Index (Figure 3.19) it is clear that points nearer the

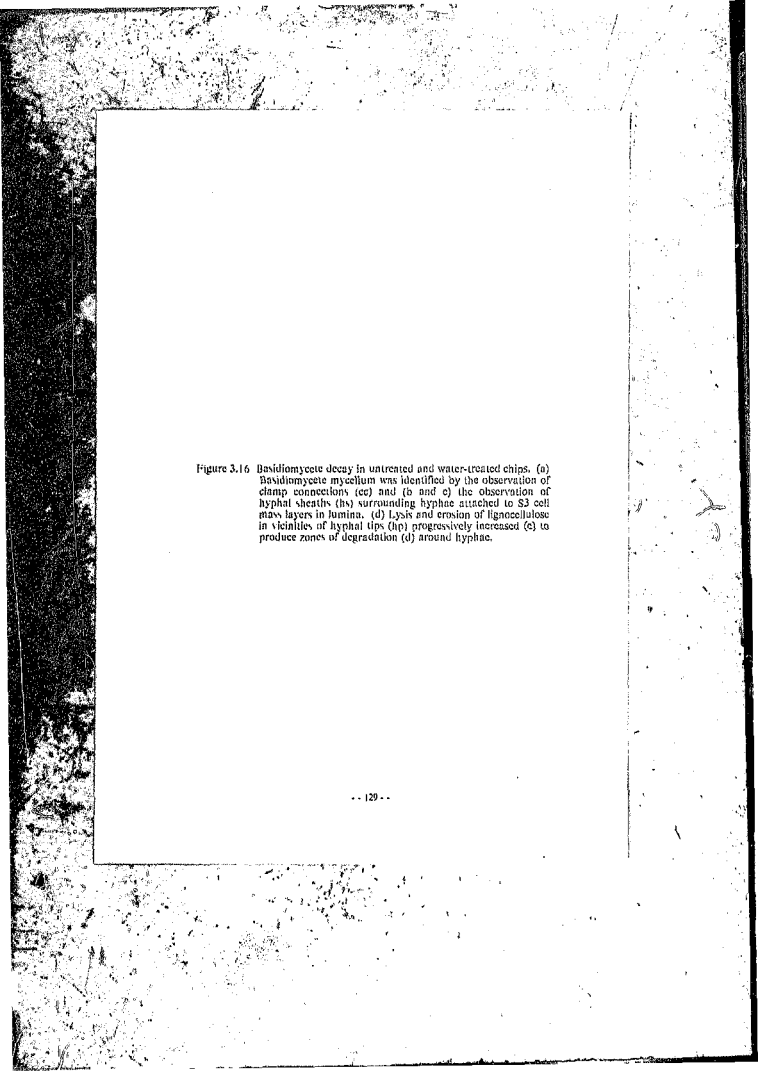
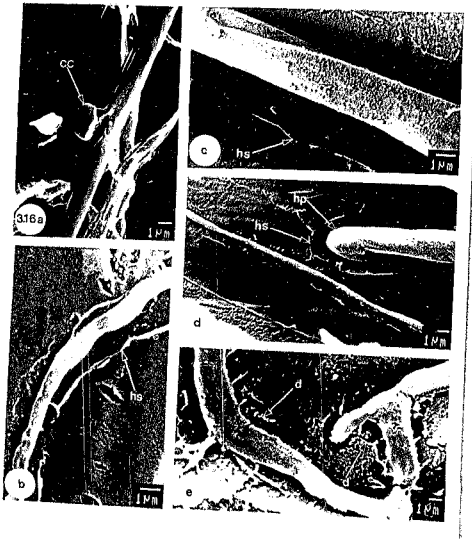


Figure 3.16 Basidiomycete decay in untreated and water-treated chips. (a) Basidiomycete mycelium was identified by the observation of clamp connections (cc) and (b and c) the observation of hyphal sheaths (hs) surrounding hyphae attached to S3 cell wall layers in lumina. (d) Lysis and erosion of lignocellulose in vicinities of hyphal tips (hp) progressively increased (e) to produce zones of degradation (d) around hyphae.


cc
316a
1 µm
c
hs
1 µm
hp
hs
d
1 µm
hs
b
1 µm
d
e
1 µm

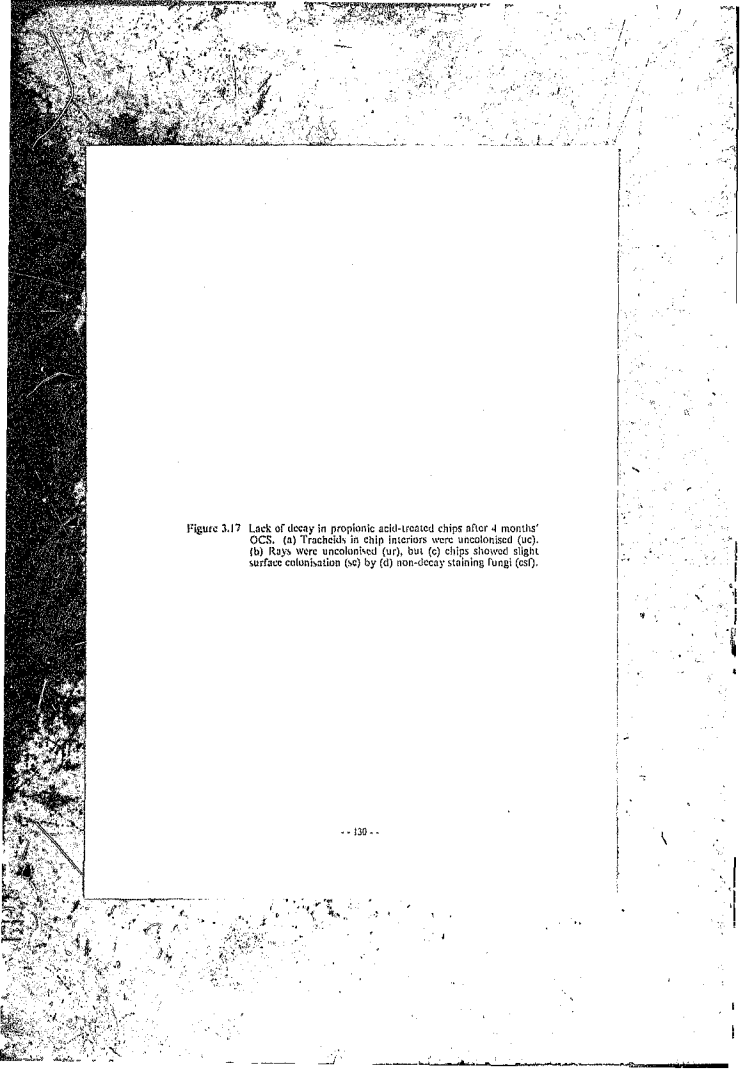


Figure 3.17 Lack of decay in propionic acid-treated chips after 4 months' OCS. (a) Tracheids in chip interiors were uncolonised (uc). (b) Rays were uncolonised (ur), but (c) chips showed slight surface colonisation (sc) by (d) non-decay staining fungi (esf).

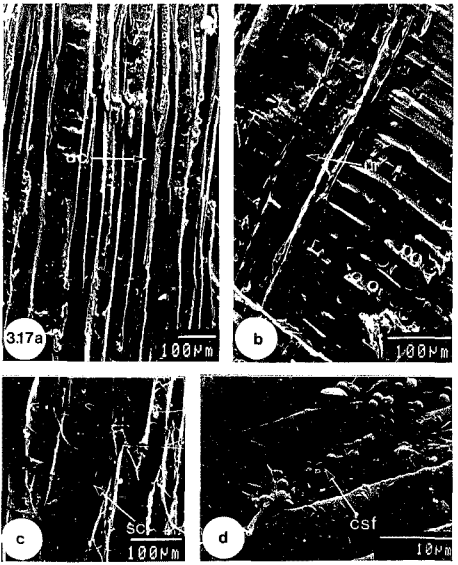

3.17a
100µm
b
100µm
c
sch
100µm
d
csf
10µm

Figure 3.18 Some unidentified fungi observed on chips during OCS at Richard's Bay.

318a
100µm
b
10µm
c
10µm
d
10µm
e
1µm

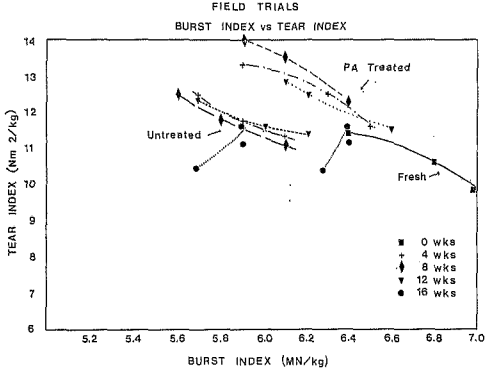


Figure 3.19 Pulp test (REVS : beating revolutions) after 1, 2, 3 and 4 months incubation of *Pinus patula* chips. Differences between strengths of paper treated and untreated chips are significant.

origin represent paper produced from pulp of lower quality. Therefore, graphs furthest from the origin represent paper produced from high quality pulp. Figure 3.19 thus shows that propionic acid 2 % - treated pulp produced paper of quality similar to paper produced from fresh untreated pulp. Although the quality of propionic acid 2 % pulp decreased slightly as the OCS period progressed, at all times this pulp was of superior quality to that of pulp produced from untreated chips (Figure 3.19).

Pulp tests of water treated chips conducted by SAPPI (Appendix 5) showed that water - treated chips produced pulp of quality lower than that of untreated chips (E.J. SMITH, pers. comm., 1988). The results therefore showed that in decreasing order of quality, propionic acid 2 % - treated chips performed better than untreated which in turn perfromed better than water - treated chips.

3.4 Discussion

The general trend for chemical analyses showed that propionic acid prevented deterioration of *Pinus patula* chips. However the benzene/alcohol extraction graph showed contrasting results. These results again suggest that chemical tests should not be relied on exclusively. However, according to TAPPI Standard T4 m-59, to provide an indication of chip quality which detects several properties of deteriorated chips as soon as possible, 1 % caustic solubility is used as the provisional indicator (because caustic solubility has a very close correlation with the pulp yield). Evidently,

caustic solubility (Figure 3.6) indeed showed that propionic acid inhibited deterioration significantly in the present work.

Although chemical extraction analyses are less time consuming to complete and provide information of the chemical composition of wood, only pulp tests will provide hard and reliable information about the biodeterioration of wood chips. However, the results of chemical extractions obtained (Figures 3.6-3.9) did confirm earlier reasoning (2.4) that "fines" samples were more degraded than those which did not pass the 60 - mesh sieve. It was therefore felt that further investigations of this modification of the TAPPI Standard may help confirm the reliability of such analyses.

From the visual observations made, it was apparent that the water-treated pile was most affected by microorganisms, the untreated pile was slightly affected and the propionic acid-treated pile least affected. It was concluded that this was so because of the antimicrobial protection given by the propionic acid. Although the propionic acid chip-treated pile was slightly discoloured on the outside, even after 4 months OCS, when this chip pile was opened, the chips in the interior of the pile were not discoloured. The discoloration of the exterior chips was probably attributable to the volatilisation of the propionic acid coat after about 3 months' OCS, and subsequent weathering of these chips.

According to LINDGREN and ESLYN (1961), of all the methods for rough pulpwood, storage in water ensured the greatest control of deterioration over extended periods, however, the water-treated chip pile was that most extensively affected in the present work. This could have been caused by the fact that water was only sprinkled on the piles for 3-4 hours each day, which was not sufficient to cause anaerobic conditions. Therefore application of intermittent moisture probably enhanced oxygenation and decay. According to FEIST, SPRINGER and HAJNY (1974), the presence of excessive moisture together with high temperatures has been suspected of both increasing chip deterioration by chemical means and of decreasing discolorations by the blue-staining fungi. The chips were successfully thoroughly wetted in the present work but moisture was not in excess. Together with the high humidity in the atmosphere at Richards' Bay and the high ambient temperatures it was felt that a good environment for microorganisms to grow had been created. They therefore proliferated on these chips. Untreated chips were also quite extensively colonised but not as extensively as the water-treated chips. Since specific moisture levels constitute an essential parameter for infection to successfully take place, microorganisms probably did not spread as rapidly in the untreated pile as this was subject to more dehydration than the water - treated pile.

Temperature profiles for propionic acid-treated and untreated chip piles showed similar trends however, in all determinations of temperature in the propionic acid-treated pile the temperature of the chip pile was always

lower than the temperature of the *untreated chip pile*. According to TAPPI Standard T4 m-59 the origin of heat or the "hike" in temperature of chip piles is caused by the following "five factors":

a. Respiration of the living cells of wood.
b. Biological action of bacteria.
c. Biological action of fungi.
d. Chemical oxidation reaction.
e. Acid hydrolysis reaction.

Now since the temperatures of the piles never exceeded 39°C (Appendix 6) the main origin of heat in the untreated and propionic acid - treated piles were probably caused by the respiration by living wood cells. It therefore seemed that propionic acid suppressed heat generation in the chip pile for *up to 4 months which is a very important quality for any preservative* intended to be used during OCS, as chip pile heating has been known to cause detrimental losses during OCS. Such lowering in chip pile temperature in turn causes a significant variation in microorganism population and development (ESLYN, 1967).

Scanning electron microscopic studies showed that both the untreated and water-treated wood chips were very extensively colonised by many types of microorganisms. Although the water-treated piles seemed visually to have deteriorated more than *the untreated piles, at the SEM level, the chips* from both these piles were indistinguishable as both were equally heavily

colonised by bacteria and filamentous fungi. In addition to sapstain fungi. A considerable number of decay fungi were also present and extensive biodeterioration of chips had taken place in the untreated and water-treated chips alike. Fungi entered the ray parenchyma cells where proliferation of hyphae occurred, and subsequently passed through pits into the lumina of the vertical tracheids. Penetration of the wall of the tracheid was effected from a short side branch which grew horizontally into the wall and, after passing through the middle lamella and into the S2 layer of the wall of the adjacent tracheid, branched to form a characteristic T-shape with the 2 branches developing in the S2 layer parallel to the orientation of the helix of the microfibrils of cellulose in that layer. The original horizontal branch did not itself appear to cause enzymic degradation of the wall, but this phenomenon did occur around the vertical branches of the hypha and gave rise to the cavities which are diagnostic feature of soft rot decay (SAVORY, 1954). It is clear that so far as sap wood is concerned all wood inhabiting fungi have a similar initial behaviour. The hyphae will grow passively into any available space, i.e in cell lumina or through pits and may well ramify through a piece of wood with no effect on the cell walls at all. All fungi capable of entering parenchyma cells containing food reserves will utilise these reserves to biosynthesise their mass of hyphal material before ranging through the other cells and destroying all or part of the cell wall (LEVY, 1967). As for the propionic acid - treated chips, no soft rotters or decay fungi were observed even after 4 months' OCS, only sparse colonisation had been observed which was exclusively by staining fungi and such

colonisation was very similar to that observed during early OCS period of water-treated and untreated chip piles (ISMAIL and BAECKER, 1988).

The scope of the present work was to identify soft rot and basidiomycete decay which could cause losses of pulp quality. This was carried out successfully however, work with the SEM has revealed a range of unidentified fungal spores on many samples. It has been stated (3.3.2) that several expert mycologists could not identify these fungi therefore they may be indigenous to S.A and unfamiliar to authors of fungal keys (AINSWORTH, 1966). Perhaps a more comprehensive examination of these spores would lead to the identification of the different microorganisms and hence give further information on the habit of the fungus and interrelationship between fungus and wood species. It was also recorded that most infection set in at the ground line from soil or old wood chips. This was the case even with propionic acid 2 % - treated chips, and it was felt that such colonisation would, once initiated, continue through the chip pile. It was therefore concluded that a concrete foundation is essential for OCS to be carried out successfully.

Pulp tests showed very clearly that propionic acid prevented biodeterioration of wood chips during the 4 month OCS period. The graph of propionic acid (Figure 3.19) was further from the origin and nearest the graph of the fresh untreated chips when compared with the graph of the untreated chips even after 4 months' OCS. However, according to MALE

(1988) of the CSIR, the decrease in the pulp strength during OCS between propionic acid and untreated chips was calculated as only 8 %. Decreases of 10 % are normally considered important, however those shown here were statistically significant differences and are also considered to be microbiologically significant. Since differences between quality of pulp from untreated and propionic acid 2 % chips increased over the 4 month OCS period, it is felt that such differences would be even greater if OCS periods of greater than 4 months were studied. In addition, according to MALE'S (1988) report propionic acid did significantly increase the tensile strength by 12 % (Appendix 7). These data all indicate that propionic acid did prevent biodeterioration. However, even propionic acid 2 % chips were colonised at the ground level therefore, it was felt that such protection would be enhanced, especially at the ground level, if chips were piled on concrete foundations.

The results of the field trials therefore led to the conclusions that propionic acid 2 % - treated chips had been protected from biodeterioration during the 4 month OCS period. It was felt that the protective effect of propionic acid was brought about probably by delaying the onset of the succession pattern which was observed very early during OCS (after 4 weeks) in the untreated and water-treated chip piles. It was therefore decided to quantify microbial presence during the OCS period.

4.0 MICROBIOLOGICAL QUANTIFICATION

4.1 Introduction

In the case of a pure bacterial culture, in a defined medium, the question "How many microorganisms are there?" is relatively simple to answer. When dealing with the mixed communities in complex substrates from environmental samples, however, the problem becomes exceedingly complex and, in most cases, defies a simple and absolute answer. Quantification is possible, but the numbers obtained need to be qualified by the technique used. Indirect quantitative approaches to assess the microbial components of ecosystems include :

a. Determination of microbial biomass - of which the most practical approach is to use;
   1) biochemical assays which assay for specific biochemicals that indicate the presence of microorganisms such as,
      ATP and total adenylate,
      cell wall components, and
      chlorophyll and other photosynthetic pigments;
   2) physiological approaches to biomass determination - which have been developed based upon respiratory activities.
b. Measurement of microbial metabolism by;

1) heterotrophic potential,
2) growth rate based upon nucleotide incorporation,
3) productivity and decomposition, and
4) specific enzyme assays.

However, none of these approaches were considered applicable to the system under study, and it was decided to use two direct methods of total counts in conjunction with a direct viable counting method. The enumeration process can be broken down into three distinct phases: sampling, sample processing and the actual counting procedure (ATLAS and BARTHA, 1987). Sample processing usually includes the dilution of the sample in order to liquefy it and to reduce microbial numbers to levels which may be counted conveniently. Dilution of aqueous samples is straightforward, but dilution of solid substrates requires initial treatment by comminution and possibly by homogenisation to provide representative dilution series (BAECKER, CASIM and RYAN, 1988).

4.1.1 Direct Total Count Procedures Using Cell Counters

Coulter Counters count microorganisms by direct microscopic observations (JONES AND MOLLISON, 1948; FREDERICK, 1965; GRAY, 1967; GRAY, BAXBY, HALL and GOOD FELLOW, 1968; HARRIS, McKEE, WILSON and WHITEHOUSE, 1972; DALEY and HOBBIE, 1975 cited by ATLAS and BARTHA, 1987). Direct count procedures yield the highest estimates of numbers of microorganisms. Direct counts

are often directly proportional to biomass, and thus can be used to estimate microbial biomass. There are, however, several major drawbacks to direct observational methods, including the inability to distinguish living from dead microorganisms, the underestimation of microorganisms in samples containing high amounts of background debris, and the inability to perform further studies on the observed microorganisms. A variety of counting chambers such as a haemocytometer or Petroff-Hauser chamber that hold specified volumes of diluted samples, can be used for counting cells (PARKINSON, GRAY and WILLIAMS, 1971 cited by ATLAS and BARTHA, 1987). Counts ml^{-1} diluted sample may then be calculated, and extrapolated taking dilution factors into account to determine numbers of cell g^{-1} original sample.

A particle counter, such as a Coulter counter, can also be used to estimate directly numbers of microorganisms (KUBITSCHEK, 1958; 1969). The Coulter counter electronically measures the number of particles within a fixed size range in diluted samples. Separate estimates of bacteria and protozoa can be obtained by setting appropriate size ranges. The problem with Coulter counter analysis is that small non-living particles are counted together with microorganisms and this difficulty has limited the usefullness of this method. However, recent advances in image analysis permit the specific recognition of microorganisms in complex samples and it can be expected that cell sorters will see increased usage in the enumeration microorganisms. In comparison with the foregoing procedures, the Coulter

counter for enumeration of cell populations has the obvious advantages of simplicity, accuracy and rapid operation (HARRIS, 1959). According to HARRIS (1959) cell number is perhaps the most useful single parameter in population growth and, when combined with information on cell size, provides a frame of reference for variation in protein, RNA and other changing constituents.

4.1.2 Direct Viable Count Procedures

There are two basic approaches to viable count procedures, namely :

a. Plate count technique.
b. Most probable number (MPN) technique.

The MPN technique is usually confined to studies of liquid substrates such as milk or oils and is therefore not considered here, however all viable count procedures require separation of microorganisms into individual reproductive units. This is achieved by preparing dilution series of the samples. The diluted sample is then spread on plates of culture media which support growth of microorganisms therein, and each microbial colony which develops is counted as having arisen from a single cell in the original. Sample population levels can therefore be calculated. All viable count procedures are selective for certain microorganisms, the degree of selectivity varies with the particular viable count procedure. This selectivity is a disadvantage when trying to estimate the total viable microbial biomass within an

ecosystem, but permits the estimation of numbers of particular types of microorganisms.

4.1.3.1 Plate count methods

The dilution plate count method is convenient and has been widely used for the enumeration of viable microorganisms, especially bacteria (BAECKER and RYAN, 1987), but its indiscriminate use has been severely criticised (POSTGATE, 1969, BUCK, 1979 cited by ATLAS and BARTHA, 1987) and its use in certain cases was discredited 20 years ago (BROCK, 1987). There are many reasons for this problem: Firstly, the most serious difficulty is that viable counts are :

a. Selective for unicellular organisms or organisms which produce many propagules.
b. They are selective for organisms which spread rapidly on the surface of the medium either because of motility or because of growth by extension of filaments.

Alternatively, viable counts are selective against organisms which cannot grow well against the resistance which is offered by the solid surface; finally, a dormant cell, doing nothing in the environment, will show up in a viable count (BROCK, 1987). An additional problem lies in the misuse of the method and/or the misinterpretation of the results. All too often users of this technique fail to recognise that "total counts" can never be achieved using this method. One must consider whether the

microorganisms can survive the plating procedure. Some microbes are killed upon exposure to air in the spread plate method; others cannot tolerate the temperature needed to maintain melted agar in the pour plate method. The real advantage of viable enumeration procedures is that conditions can be adjusted so that only members of a defined group are enumerated. Plate count media may be designed to be selective or differential. Selective plate count procedures are designed to favour the growth of the desired group of microorganisms. Differential media do not preclude the growth of other microorganisms, but permit detection of the desired group by some distinguishing characteristic (ATLAS and BARTHA, 1987).

In spite of the above disadvantages, plate counting remains the most widely used technique in routine analyses to quantify microorganisms in substrates worldwide (BAECKER and RYAN, 1987; BAECKER, CASIM and RYAN, 1988), therefore analyses by this method are directly comparable with those of other workers throughout the world.

4.2 Materials and Methods

The drawbacks associated with each method of microbial quantification if used exclusively led to the decision to use several quantification techniques in the present work. It was hoped that the results of each method could be compared to provide a full appreciation of the numbers of microorganisms in the chip piles as the OCS period progressed.

4.2.1 Preparation Of Wood For Analyses

For the quantification, the fines collected during sieving (Chapter 2) were used (refer to Figure 2.1). It was ensured that the procedures did not alter the numbers or activity of microorganisms, either positively or negatively, in a non-quantifiable manner during collection of the sample. Furthermore, sampling procedures also ensured that samples were representative and were not contaminated with foreign microorganisms by sampling procedures ensuring that the microorganisms came only from the ecosystem being examined. In addition, to make counts reflect accurately the numbers of viable microorganisms present in the sample at the time of collection, processing was accomplished quickly (on arrival from Richards' Bay) because microorganisms reproduce rapidly which can yield artificially elevated counts. In addition, each determination consisted of three phases: sample collection, sample processing, and the actual measurement. Since the manner of sample collection and sample processing can profoundly influence the outcome of the measurements, all three procedures were therefore taken into consideration when the results were interpreted.

4.2.2 Preparation Of Quarter Strength Ringers Solution

The following chemicals were added to make up quarter strength Ringers solution :

CHEMICAL	VOLUME
sodium chloride (NaCl)	2.25 $g.l^{-1}$
potassium chloride (KCl)	0.105$g.l^{-1}$
calcium chloride dihydrate ($CaCl_2.2H_2O$)	0.08 $g.l^{-1}$
sodium hydrogen carbonate (Na_2HCO_3)	0.05 $g.l^{-1}$
ethylenediaminetetra acetic acid ($(CH_2N(CH_2COOH)CH_2COONa)_2.2H_2O$	1 $g.l^{-1}$
triton 100	5 $ml.l^{-1}$

4.2.3 Preparation Of Nutrient Agar

To 1 litre of distilled water 28 g of Nutrient Agar was added, thoroughly mixed and sterilised by autoclaving. The Nutrient Agar was cooled slightly. To prevent over-growth of fungal colonies, fungal inhibitors were added to the media (ATLAS and BARTHA, 1987), so, 50 mg of Nystatin and 50 mg of Cycloheximide were sterilised by filtration and added to the Nutrient Agar (BAECKER and KING, 1980). The Nutrient Agar was thoroughly mixed and ready for pouring.

4.2.4 Preparation Of Malt Extract

50 g of Malt Extract was added to 1 litre of distilled water, thoroughly mixed and sterilised by autoclaving. A technique for suppression of bacteria involves the lowering of the pH of the medium (ATLAS and BARTHA, 1987), therefore, 100 ml of 10 % lactic acid was sterilised and after autoclaving and cooling, 14 ml of sterilised lactic acid was added to the malt extract to maintain a pH 3.5 as most fungi are unaffected by this pH, while most bacteria are suppressed.

4.2.5 Sampling Processing (to be used in 4.2.6, 4.2.7 and 4.2.8.)

In a 250 ml conical flask, 1 g of fines (Chapter 3) and 20 ml quarter strength Ringers solution was added. The flask was then closed with foil and placed on an orbital shaker at medium speed for 3 hours (Figure 4.1). Thereafter, the contents were filtered in a bottle once through Miracloth (manufactured by Calbiochem, La Jolla, U.S.A), which is of finer weave than cheese cloth, covered with foil again and left to stand at room temperature overnight. The supernatant (sample dilution) was used for quantification of bacterial and fungal spores (Figure 4.1).

4.2.6 Method For Total Plate Counts

Using aseptic techniques, to 5 plates of each sample dilution (10^{-3} - 10^{-4}) (4.2.5), 7 ml of Nutrient Agar was added using an automatic dispensing machine. Similarly, 7 ml Malt Extract was added to another 5 plates of

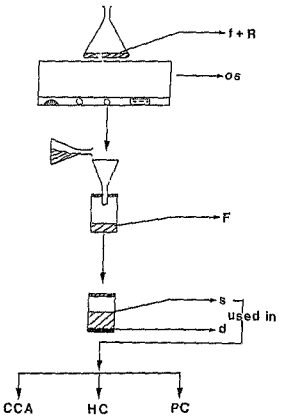


Figure 4.1 The preparation of wound sample before quantification using the Coulter counter, haemocytometer and plate counts. (f + R - "fines" and ringers solution; os - orbital shaker; F - filtrate; s - supernatant containing separated microorganisms; d - debris; CCA - Coulter Counter Analysis; HC - Haemocytometer Counts; PC - Plate Counts)

each dilution (Figure 4.2). The plates were thoroughly mixed and incubated in a 37°C incubator and the fungal and bacterial colonies were counted after 7 days. This procedure was repeated for all samples, i.e untreated and acid-treated samples, on a monthly basis until 4 months' OCS has elapsed. These data were used to calculate the numbers of viable cells g^{-1} wood from the chip piles.

4.2.7 Method For Direct Counts Using The Haemocytometer

A 0.02 ml drop of sample dilution (4.2.5) was placed in the centre of the chamber platform of the haemocytometer. To this 0.02 ml of 5 % methylene blue was added. The cover-slip was placed on the slide and pressed down lightly. The number of bacterial and fungal cells were counted. After taking 20 such readings average counts were determined directly and the number of cells ml^{-1} of diluted sample was calculated. These data were used to calculate total numbers of cells g^{-1} wood from the chip piles.

4.2.8 Method For Coulter Counter Evaluations

To 1 ml of sample dilution (4.2.5), 9 ml of Isoton as surfactant was added in a scintillation bottle. This was thoroughly mixed and placed in the coulter counter, which counted the number of cells in 50 µl aliquots. After taking 5 such readings the average number of cells ml^{-1} of sample dilution was calculated. These data were used to calculate total numbers of cells g^{-1} wood from the chip piles.

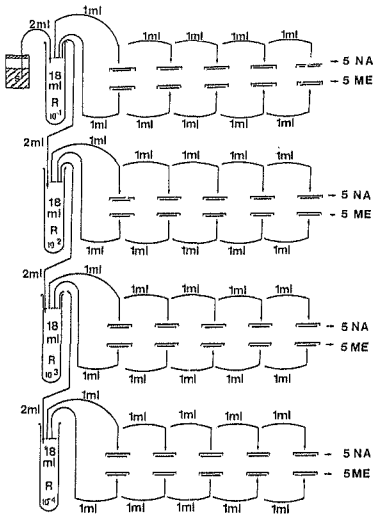


Figure 4.2 The treatment of the supernatant from Figure 4.1 for plate counting at supernatant dilutions of 10^{-1} - 10^{-4}. (S - supernatant containing microorganisms; NA - nutrient agar plates; ME - malt extract plates; R - quarter strength ringers solution).

4.3 *Results*

The results of the methods for indirect viable plate counts, direct total counts and indirect coulter counter evaluations of untreated and treated chips are illustrated in Figures 4.3 and 4.4 respectively, the statistics of which are indicated in Appendices 8 and 9 respectively.

The major difference between untreated and treated chips was that viable plate counts of the latter were two orders of magnitude less than those of the former after 2 and 3 months' OCS and it was only after 4 months' OCS that plate counts of treated chips reached values comparable to those of untreated chips; the statistics (Appendix 9) showed conclusively that this increase was significant. Similarly, Coulter counts of treated chips were slightly lower than those of untreated chips throughout the OCS period. However, haemocytometer counts of treated material were slightly higher than those obtained from untreated material, and this trend persisted throughout the OCS period.

4.4 *Discussion*

Numbers, biomass and metabolic activity are the fundamental biotic parameters of microbial ecosystems (ATLAS and BARTHA, 1987). Though normally correlated with each other, it is wrong to use them in an interchangeable manner. The nature of the ecological problem dictates which

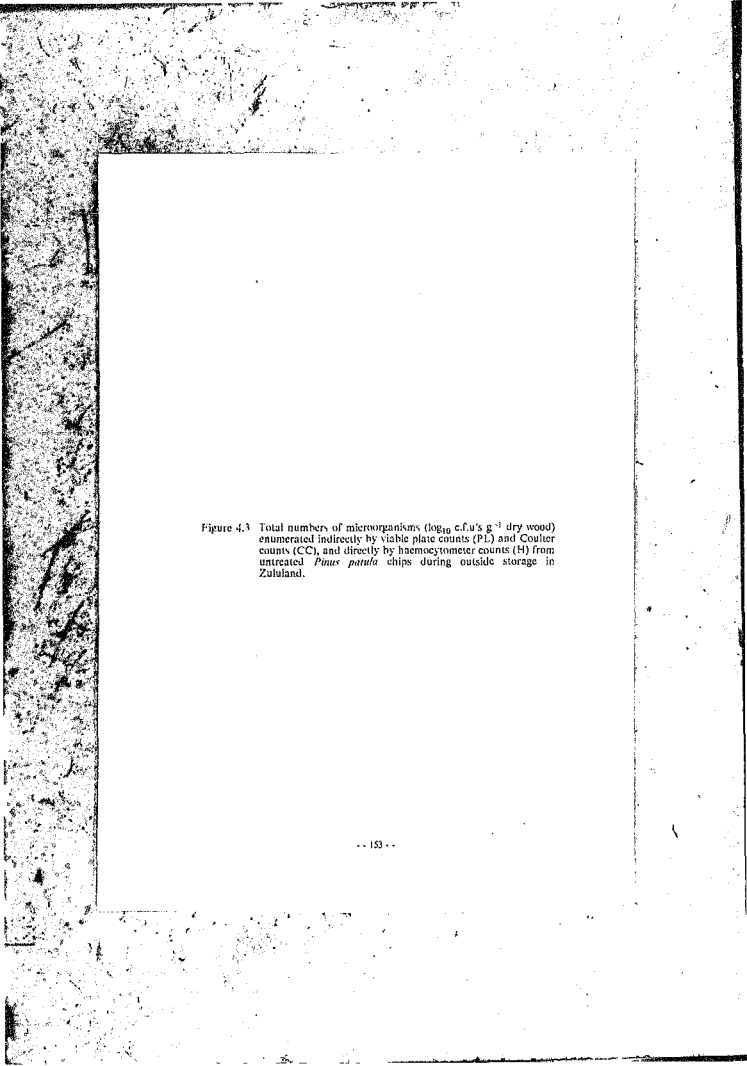


Figure 4.3 Total numbers of microorganisms (log_{10} c.f.u's g^{-1} dry wood) enumerated indirectly by viable plate counts (PL) and Coulter counts (CC), and directly by haemocytometer counts (H) from untreated *Pinus patula* chips during outside storage in Zululand.

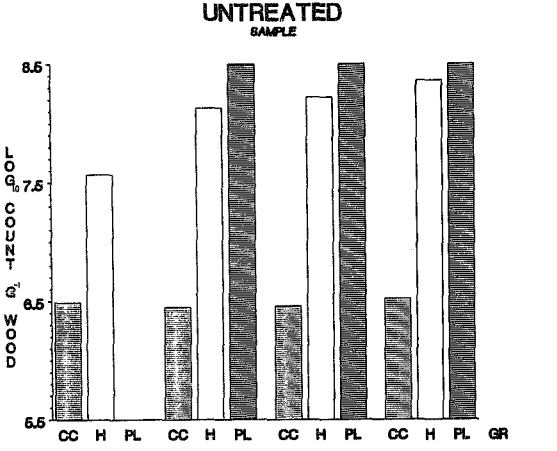
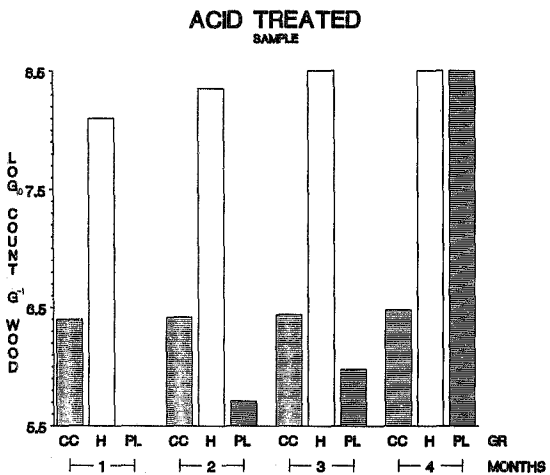

UNTREATED
SAMPLE
8.5
7.5
6.5
5.5
LOG10 COUNT @ WOOD
CC H PL CC H PL CC H PL CC H PL GR
1 2 3 4 MONTHS

Figure 4.4 Total numbers of microorganisms (log_{10} c.f.u's g^{-1} dry wood) *enumerated indirectly by visible plate counts* (PL) and Coulter counts (CC), and directly by haemocytometer counts (H) from 2 % (w v) propionic acid-treated *Pinus patula* chips during outside storage in Zululand.


ACID TREATED
SAMPLE
8.5
7.5
6.5
5.5
LOG10 COUNT G-1 WOOD
CC H PL CC H PL CC H PL CC H PL GR
1
2
3
4
MONTHS

parameter is the most appropriate one to measure. On occasion, technical problems force us to measure a less relevant parameter, such as numbers and to calculate from these a more relevant parameter such as biomass (ATLAS and BARTHA, 1987). Under the prevailing circumstances such extrapolations were avoided. Instead, indirect viable counts obtained from total plate counts were compared with direct total counts from haemocytometer observations and these were also compared with indirect counts obtained using a Coulter counter.

Examination of Figures 4.3 and 4.4 showed that total counts measured by haemocytometer increased as OCS period of each pile progressed, and that at any given time, total counts of treated chips were similar to those of untreated chips. The slight differences observed were statistically microbiologically insignificant. Coulter counts of treated and untreated chips were also broadly similar throughout, but these counts were 2 orders of magnitude less than haemocytometer counts at given times. The reason for this discrepancy lies in the fact that haemocytometer counting, although less convenient, produces results actually observed directly by the investigator. The indirect electronic systems of Coulter counters however may fail to record some particles which the human eye would observe. For example two adjacent cells in the Coulter counter would be recorded as one whereas the human eye would distinguish 2 cells (ISMAIL, SMITH and BAECKER, in press).

However, it was apparent that the absolute counts obtained from the plate counting technique were much higher than those from the Coulter counter technique. One would have expected the reverse situation to have occurred where Coulter counts were higher than plate counts owing to the indirect nature of Coulter counting whereby not only viable cells but also particulate matter in dilutions of wood were also counted. Two factors could have caused this unexpected discrepancy. Firstly, the Coulter counter would not distinguish between single cells and clumped aggregates of cells which would be counted as single particles. The coulter counter is therefore preferable for use in work with isolated cell systems (HARRIS, 1959). It is suggested that two ways of preventing cells from clumping aggregating would be one of the following :

a. To use an ultra turrax.

b. To use a higher concentration of the wetting agent.

Now the first method of alleviating clumping had been investigated however it was found that one disadvantage of using an ultra turrax was that in this process of clump disruption some cells were killed by mechanical damage. Therefore the surfactant method of clump disruption described above (4.2.5) was used instead. However, with respect to the second method of alleviating clumping it is known that high concentrations of the wetting agent used could be toxic to the cells. It was therefore decided to use low concentrations of the wetting agent in order to avoid these further complications of lysis and, toxicity. These lower concentrations of

surfactant did not totally prevent clumping because, haemocytometer observations of the identical samples showed that some cells were indeed clumped. The Coulter counter therefore counted these aggregates as single cells. However, this does not explain why Coulter counter readings were lower than plate counts since similar clumps on spread plates would give rise to only one visible colony (to be recorded as having arisen from a single cell). The explanation for the discrepancy is probably attributable to another consequence of Coulter counts of clumped cells. The Coulter counter can provide information on the distribution of particle sizes in a sample population (MATTERN, BRACKETT, and OLSON, 1957). The change in resistance at the instrument aperture is proportional to the volume of a particle passing through it (KUBITSCHEK and HALDANE, 1950), and thus, as the threshold is progressively increased, weaker impulses fail to register and the cell count declines according to the proportion of the population in successive size ranges (HARRIS, 1959). Now in the experiment, since the size range of the microorganisms in the suspension was not known, a broad threshold was selected. The Coulter was set to record particles of dimensions within the broad threshold of 1 µm-36 µm in order to detect all microorganisms ranging from small bacterial cells to large fungal spores. This lowered sensitivity probably accounted for the lower readings obtained with the Coulter counter when compared with plate counts. Therefore, it was concluded that the Coulter counter is only a reliable technique if one is examining a culture containing non-aggregated cells. Figure 4.3 shows that viable plate counts throughout OCS of un-

treated chips were similar to haemocytometer counts, except at one month, when insignificant numbers of viable cells were detected. However, Figure 4.4 shows that haemocytometer total counts of treated chips were very similar to untreated, whereas plate counts were much lower in treated. These findings confirm that although the numbers of cells/microorganisms observed and counted from untreated and treated chip samples were very similar, most of those from treated samples were actually non-viable or dead.

Although quantification of microorganisms by dilution plating is convenient, all techniques for quantifying mycelial spore formers have specific disadvantages (JONES and MOLLISON, 1948) and may merely estimate numbers present (BAECKER and RYAN,1987). The haemocytometer counts gave conflicting results, in that the numbers of microorganisms from the untreated chips were slightly lower than the numbers from the propionic acid - treated cells at each time interval. However, it must be borne in mind that these slight differences were not significant from a microbiological viewpoint. The results for both the Coulter counts and especially the plate counts showed that propionic acid - treated chips contained significantly fewer microorganisms when compared with the untreated chips throughout the OCS period. In fact total plate counts confirmed the reasoning (Chapter 3) that propionic acid actually delayed the succession pattern considerably, since after one month no viable microorganisms were present in treated chips (ISMAIL, SMITH and

BAECKER, in press). It was therefore concluded that the propionic acid had killed the cells observed during haemocytometer counts investigated of these samples. However, there were large numbers of microorganisms present in the untreated chips.

These findings all showed that it is important to be aware of the true meaning and limitations of each measurement procedure. This dictates caution and critical thinking when one parameter is calculated or projected from another directly measured parameter (ATLAS and BARTHA, 1987).

5.0 GENERAL DISCUSSION AND CONCLUSIONS

According to SPRINGER (1976), probably the best means to conserve by-products prior to the chips reaching the digester is to minimise the time between harvesting the wood and digestion, that is to keep the storage time very short or to eliminate storage completely. Of course, we know it is not practicable to eliminate all wood storage, and it is difficult to operate with very short storage times. Therefore some wood must be kept on hand as insurance against manpower strikes, bad weather, etc. One alternative to no storage or very short storage might be to treat the chips chemically prior to storage and thus preserve the by-products. Hence, very many chemical and mechanical methods for treating chip piles to prevent deterioration have been proposed in the last twenty years, but none have been used in long term commercial practice (SMITH and HATTON, 1971; BERGMAN and NILSSON, 1979, cited by FULLER, 1985). ESLYN (1973) pointed out that emphasis on investigating and using biocides that are least harmful to the environment was increasing. At one time several large scale mill trials were in progress in both Canada and the United States, under a variety of conditions to prove that the use of this process can be effective under North American conditions. If a chemical shows promise of controlling degradation and is also apparently harmless environmentally, every effort should be made for thorough testing for potential commercial application (ESLYN, 1973). On this basis, propionic acid was

considered for use in the present work in a sub-tropical environment. The use of propionic acid treatment on a commercial basis and in large scale mill trials overseas, and in the present field trials at Richards Bay (Natal) has shown that wood losses can be sharply reduced.

From the present study it was clear that, in order to evaluate the performance of preservatives in preserving chips during OCS the results obtained from chemical extractions should not be relied on exclusively as these chemical extractings have certain limitations which have already been discussed in Chapter 2. These tests were conducted by HAJNY, JORGENSEN, and FERRIGEN (1967) and FEIST, HAJNY and SPRINGER (1974) and are still performed on regular bases by SAPPI (Springs); it was agreed with SMITH (pers. comm., 1987) that some of them be conducted in the present study to establish whether there was any correlation between the results obtained here and pulp tests.

In contrast pulp tests (the results of which are presented in Chapters 2 and 3) presented more reliable results in that they correlated with microbiological analyses, and were used by many workers to evaluate preservative performance on wood chips. The tear and burst indices are the conventional indicators of chip deterioration and pulpwood acceptability, however unlike many analyses they cannot be conducted in the routine microbiology laboratory. Pulp tests require specialised heavy duty equipment for chip sample preparation and in addition, to do a pulp test requires large chip

samples of at least 5 kg and not 2 g samples as required for laboratory tests. Nevertheless since it was absolutely necessary that these pulp tests be conducted, they were conducted for laboratory and field trial experiments respectively by prior arrangements with SAPPI and CSIR.

A range of microorganisms (bacteria and fungal spores) were observed as shown in Chapters 2 and 3. A large number of unidentified microorganisms were also viewed which are illustrated in Chapter 3. They did not produce decay. Since it was not the scope of this study to identify these fungi to genus level and since some expert taxonomists could not identify them, future studies could entail further investigations of these fungi. The fact that these were observed only in the water-treated and untreated chips could perhaps help in the classification of these in the various taxa.

The major observation of the work described in this dissertation was that propionic acid delayed the onset of the succession pattern in *Pinus patula* wood chips during OCS. On the propionic acid - treated chips only superficial growth was observed on viewing with the SEM. It was evident that the wood was infected mainly with primary colonizers such as the staining fungi, *Penicilium* and *Aspergillus* (ISMAIL and BAECKER, 1988). This was clearly evident and hence illustrated and discussed with the viable plate counting technique in Chapter 4.

A further very useful investigation would be to do monthly tests to determine the actual composition of the total quantified bacterial and fungal populations in the wood chips i.e proportions of bacteria : stainers : soft rot : basidiomycetes present. This would provide an indication of when in the succession pattern the harmful degradative basidiomycetes actually colonised and subsequently became established. In addition it would be of interest to establish how they affect the populations of the other primary and secondary colonisers. In the case of the propionic acid, the chip pile seemed colonised but when piles were opened colonisation was virtually negligible. SHIELDS (1967) proposed that only certain areas of the piles, such as the outer layers, need to be treated since greatest biological deterioration seems to occur here. This could be important since, if costs permit, the chips on the outside of the pile could perhaps be re-sprayed just before the onset of basidiomycete colonisation and thus give added and longer protection to the inner chips.

With respect to the biocidal effect of propionic acid; it seems to be antimicrobial, and delayed the succession pattern from proceeding; brightness was also kept higher. RATAJCZAK (1983) found that when chips were treated with 1.5 % propionic acid by weight of oven dry chips pathogenic activity which was manifested by a reduction in weight loss was limited. It also seemed to prevent heating by stopping respiration thereby halting heat build-up in the pile (SPRINGER, FEIST, ZOCH and HAJNY, 1975b). This is a very important factor with respect to thermal

degradation because temperature increases have been known to cause chip pile fires and hence caused serious losses to the pulp and paper industry.

Propionic acid at the concentrations used in the present study (2 %) is environmentally acceptable, it is not harmful to man, animals or plants and can be handled directly with no fear for toxicity or contamination. However, as with any other acid it can be harmful if handled directly at very high concentrations. With respect to the industry, it is totally acceptable because it is non-corrosive to machinery at the low concentrations for which it is intended to be used. According to KOZLOWSKI and RATAJCZAK (1978) propionic acid at 18 % has average corrosivity to steel however considering its harmlessness to higher organisms, it could be used, even at that concentration, to protect wood chips.

Propionic acid is also volatile, which means that although it is active initially, after long periods of time the active ingredient seems to become subsequently volatilised. According to the results in Chapter 4 microorganisms were detected by quantitative analyses after 2 months' OCS. Now this is very important since it is preferable that a suitable chemical be eventually volatilised to keep pulping costs low. The present study suggested that after a while the active propionic acid "disappeared" from the environment; this was indirectly shown when the numbers of microorganisms in plate counts started to increase. Such losses would suit the pulping industry because higher yields and decreased bleach demands

are of utmost importance (HAJNY, JORGENSEN and FERRIGAN, 1967). Since chips are usually pulped within 2 months of arrival at mills the volatilisation of propionic acid would alleviate costs incurred by the need to increase the utilisation of pulping chemicals or to increase the usage of alkalis to decrease propionic acid content during pulping. Furthermore, future experiments to actually quantify retention levels of propionic acid would be useful to confirm the above suggestions. For example measurement of propionic acid uptake after treatment i.e retention levels of propionic acid; and after a certain incubation period, measurement of the propionic acid retained in the chips. Such tests could determine exactly how long it would take for the propionic acid to become volatilised. This time interval would be important to the pulping industry in management to keep such pulping costs to a minimum.

The above illustrates that there are many advantages associated with the use of propionic acid, and that the only disadvantage could possibly be costs incurred. All of the foregoing indicates that there is a large and growing market for chemicals in chip treatment. Since propionic acid is considered safe enough for use in food - stuffs and animal feeds, both the Food and Drug Administration and the environmental Protection Agency have approved propionic acid for use as preservatives in grain for livestock feeds (WILCOX, 1972, cited by ESLYN, 1973b). The growth of the market will result from the growth of pulp production itself, the increasing share of pulpwood being utilised in the form of chips, and increasing

international trade in wood chips. In 1973 the use of propionic acid to control deterioration in wood chips stored was considered economically impracticable. However, broader usage of propionic acid, e.g large scale use as in agricultural commodity preservative (ANON, 1971 cited by ESLYN, 1973b), and development of new technology for synthesising the acid (CRADDOCK, HERSHMANN, PAULIK and ROTH, 1971, cited by ESLYN, 1973b) might mean a significant increase in its production which could result in lower costs. Importantly, the value of timber has increased significantly since 1973. The chips in the field trials were treated at a cost of approximately R1 per ton, which represents less than 2 % of their value. With automatic treatment, such costs would be even less. In addition, to facilitate treatment with preservatives, a treatment plant could be installed similar to the apparatus used by SPRINGER, FEIST, ZOCH, HAJNY and McALILEY (1974) which consisted of a hopper containing the treating solution.

An added advantage was that propionic acid prevented biodeterioration in spite of the fact that experiments were conducted in a hostile season (Summer). One can therefore expect greater protection of chips by propionic acid in the Winter months. This statement is agreed with by SHIELDS (1967) who found that less deterioration had been experienced when chips had been piled during the Winter months than during the Summer, and little or no loss in wood substance occurred in chips which remained frozen during storage.

The time period for which chips can be stored without serious deterioration therefore appear to be related to a number of variables including the wood species piled, the size of the pile and the degree of compaction, and the season when storage commenced (SHIELDS, 1967). Greater chip compaction and higher temperatures have been noted in larger piles compared with smaller piles therefore the size of the piles appears to be a determining factor which influences the rate of deterioration and the length of time that chips can be stored outside to obtain good yields of high quality pulp. Taking into consideration all the above factors, it is very clear that good woodyard practices and chip management practices are the most important factors when considering preservation. This together with a concrete foundation, would help maximise the protection of *Pinus patula* wood chips during OCS.

Preservation of pine chips has a favourable effect on wood quality and the pulping process, limits the number of spores of harmful fungi in the chip pile and in the air at places of work, and does not negatively affect the biological purification of pulp mill effluents (LEWANDOWSKI and RATAJCZAK, 1981). While much useful information about the probable value in practice for any new wood preservative can be gathered from the results of laboratory and field tests, it must be realised that even the latter have limitations. Final approval of a wood preservative before its use on a large scale can be recommended but must be finally evaluated on service

trials carried out in the various situations where the treated timber is required to be used. To practical evaluators there is nothing so convincing as satisfactory service data which proves that timber treated with preservative does actually withstand all the destructive agencies it has to contend with in practice. Fortunately with respect to propionic acid data of this type is already available and it has been proved in the present study that propionic acid is effective for use in *Pinus patula* preservation during OCS in the sub-tropical environment of Richards' Bay.

In summary several conclusions were drawn from the previous Chapters :

a. Pulp tests and microbiological analyses showed;
   1) chemical extractions were less reliable than expected, and
   2) propionic acid at 2 % (w/v) was chosen from laboratory trials as the most appropriate for field trials . . . . . Chapter 2.

b. Pulp tests and microbiological analyses confirmed that;
   1) chemical extractions are rather unreliable on a quantitative basis but provided qualitative information of trends in biodeterioration of wood components,
   2) propionic acid at 2 % (w/v) prevented loss of quality of pulp during 4 months OCS. Basidiomycete decay was not observed in treated chips during this period . . . . . Chapter 3,
   3) colonisation of even treated chip piles was extensive at the ground line . . . . . Chapter 3,

4) propionic acid at 2 % (w/v) prevented loss of quality of pulp from biodeterioration by delaying the onset of the succession pattern of microorganisms in treated chip piles , Chapter 4, and,

5) the use of propionic acid at 2 % (w/v) to treat chips as described cost approximately R1 per ton. With management practice described above it is concluded that this treatment would successfully minimise biodeterioration of *Pinus patula* chips during OCS and transit to end-users.

6.0 REFERENCES

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TAPPI Standard. T11 m-59. Sampling and preparation of wood for analysis.

TAPPI Standard. T12 os-75. Preparation of wood for chemical analysis, (including procedures for removal of extractives and determination of moisture content).

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7.0 APPENDICES

Appendix I

Method for N-methyldithiocarbamate

To the round bottomed flask below 137 g of carbon disulphide and cold solution of 72 g of sodium hydroxide in 160ml of water was added. This mixture was cooled to 10-15° and while, stirring, 180 ml of 35°C aqueous methylamine solution was added over a period of 30 minutes. While stirring was still continued, the mixture was warmed gently on a steam bath for 1-2 hours to ensure a complete reaction. The contents were then freeze dried overnight and the precipitate left was N-methyldithiocarbamate (MOORE AND CROSSLEY, cited by HORING, 1955; MICHAEL, personal communication).

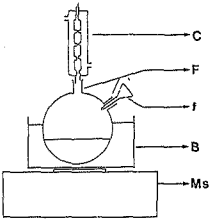


Diagram of apparatus used for preparation of N-methyldithiocarbamate (*C - condensor, F - 2-necked flask, f* - funnel, B - ice-bath, Ms - magnetic stirrer).

ANCOVA ON TREATMENT OVER TIME

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS	LEVELS	VALUES
TREAT	9	BORAX_1 BORAX_2 D+N H2O NNETH NNETH+D PA_1 PA_2 UNT_1
TREAT2	5	BEN ALC CAU.SOL COLD WATER ETYL ETHER HOT WATER

NUMBER OF OBSERVATIONS IN DATA SET = 230

NOTE: ALL DEPENDENT VARIABLES ARE CONSISTENT WITH RESPECT TO THE PRESENCE OR ABSENCE OF MISSING VALUES. HOWEVER, ONLY 210 OBSERVATIONS CAN BE USED IN THIS ANALYSIS.

ANCOVA ON TREATMENT OVER TIME

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: SCORE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	13	3139.64653512	241.51127193
ERROR	196	1849.45499738	9.43599488
CORRECTED TOTAL	209	4989.10153250	

MODEL F =	25.59		PR > F = 0.0001
R-SQUARE	C.V.	ROOT MSE	SCORE MEAN
0.629301	56.5170	3.07180645	5.43449524

SOURCE	DF	TYPE I SS	F VALUE	PR > F
TREAT	8	262.56341288	3.48	0.0009
TREAT2	4	2844.32312644	75.34	0.0001
WEEK	1	32.77999580	3.47	0.0638

SOURCE	DF	TYPE III SS	F VALUE	PR > F
TREAT	8	251.69588815	3.33	0.0013
TREAT2	4	2874.11961525	76.15	0.0001
WEEK	1	32.77999580	3.47	0.0638

PARAMETER		ESTIMATE		T FOR H0: PARAMETER=0	PR > |T|	STD ERROR OF ESTIMATE
INTERCEPT		7.43432310 B		9.43	0.0001	0.78831715
TREAT	BORAX_1	-1.45007607 B		-1.57	0.1180	0.92358006
	BORAX_2	-0.09576923 B		-0.11	0.9106	0.85196582
	O+H	2.25153864 B		2.64	0.0089	0.85196582
	H2O	1.23423077 B		1.45	0.1496	0.85196582
	NHETH	0.00492393 B		0.01	0.9958	0.92358006
	NHETH+O	1.93292393 B		2.09	0.0376	0.92358006
	PA_1	-0.27657607 B		-0.30	0.7646	0.92358006
	PA_2	-0.15823077 B		-0.18	0.8602	0.85196582
	UNT_1	0.00000000 B		.	.	.
TREAT2	BEN ALC.	ANCOVA ON TREATMENT OVER TIME				
		-5.36108696 B		-8.34	0.0001	0.64051592
	CAU.SOL	3.90313043 B		6.09	0.0001	0.64051592
	COLD WATER	1.89434684 B		5.60	0.0001	0.69515177
	ETYL ETHER	5.76823543 B		8.30	0.0001	0.69515177
	HOT WATER	0.00000000 B		.	.	.
WEEK		-0.10745573		-1.86	0.0638	0.05765261

NOTE: THE X'X MATRIX HAS BEEN DEEMED SINGULAR AND A GENERALIZED INVERSE HAS BEEN EMPLOYED TO SOLVE THE NORMAL EQUATIONS. THE ABOVE ESTIMATES REPRESENT ONLY ONE OF MANY POSSIBLE SOLUTIONS TO THE NORMAL EQUATIONS. ESTIMATES FOLLOWED BY THE LETTER B ARE BIASED AND DO NOT ESTIMATE THE PARAMETER BUT ARE BLUE FOR SOME LINEAR COMBINATION OF PARAMETERS (OR ARE ZERO). THE EXPECTED VALUE OF THE BIASED ESTIMATORS MAY BE OBTAINED FROM THE GENERAL FORM OF ESTIMABLE FUNCTIONS. FOR THE BIASED ESTIMATORS, THE STD ERR IS THAT OF THE BIASED ESTIMATOR AND THE T VALUE TESTS H0: (BIASED ESTIMATOR) = 0. ESTIMATES NOT FOLLOWED BY THE LETTER B ARE BLUE FOR THE PARAMETER.

ANCOVA ON TREATMENT OVER TIME

GENERAL LINEAR MODELS PROCEDURE

LEAST SQUARES MEANS

TREAT	SCORE LSMEAN	STD ERR LSMEAN	PROB > |T| HO:LSMEAN=0	LSMEAN NUMBER
BORAX_1	3.33431640	0.69229932	0.0001	1
BORAX_2	4.68862325	0.60532623	0.0001	2
U+H	7.03593096	0.60532623	0.0001	3
H20	6.01862325	0.60532623	0.0001	4
NNETH	4.78931640	0.69229932	0.0001	5
NNETH+D	4.71731640	0.69229932	0.0001	6
PA_1	4.50981640	0.69229932	0.0001	7
PA_2	4.63416171	0.60532623	0.0001	8
UNT_1	4.78639248	0.60532623	0.0001	9

PROB > |T| HO: LSMEAN(I)=LSMEAN(J)

I/J	1	2	3	4	5	6	7	8
1	.	0.1442	0.0001	0.0051	0.1388	0.0006	0.2277	0.1689
2	0.1442	.	0.0044	0.1201	0.9133	0.0292	0.8467	0.9491
3	0.0001	0.0044	.	0.2339	0.0139	0.7305	0.0066	0.0053
4	0.0051	0.1201	0.2339	.	0.1867	0.4503	0.1039	0.1058
5	0.1388	0.9133	0.0139	0.1867	.	0.0486	0.7739	0.8668
6	0.0006	0.0292	0.7305	0.4503	0.0486	.	0.0241	0.0252
7	0.2277	0.8467	0.0066	0.1039	0.7739	0.0241	.	0.8930
8	0.1689	0.9491	0.0053	0.1058	0.8668	0.0252	0.8930	.
9	0.1188	0.9186	0.0089	0.1490	0.9958	0.0376	0.7666	0.8602

PROB > |T| HO: LSMEAN(I)=LSMEAN(J)

I/J	9
1	0.1188
2	0.9186
3	0.0089
4	0.1490
5	0.9958
6	0.0376
7	0.7666
8	0.8602
9	.

NOTE: TO ENSURE OVERALL PROTECTION LEVEL, ONLY PROBABILITIES ASSOCIATED WITH PRE-PLANNED COMPARISONS SHOULD BE USED.

TREAT2	SCORE LSMEAN	STD ERR LSMEAN	PROB > |T| HO:LSMEAN=0	LSMEAN NUMBER
BEN ALC	2.0670760	0.4560628	0.0001	1
CAU.SOL	11.2912934	0.4560628	0.0001	2
COLD WATER	3.4938164	0.5192202	0.0001	3
ETYL ETHER	1.4197275	0.5192202	0.0021	4
HOT WATER	7.3881629	0.4560628	0.0001	5

ANCOVA ON TREATMENT OVER TIME

GENERAL LINEAR MODELS PROCEDURE

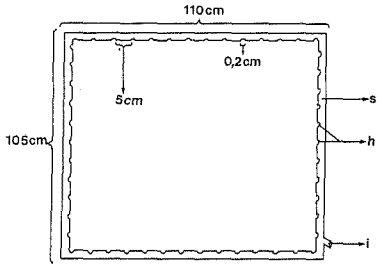
LEAST SQUARES MEANS FOR EFFECT TREAT2
PROB > |T| HO: LSMEAN(I)=LSMEAN(J)

DEPENDENT VARIABLE: SCORE

I/J	1	2	3	4	5
1	.	0.0091	0.0367	0.5396	0.0001
2	0.0091	.	0.0001	0.0091	0.0001
3	0.0367	0.0001	.	0.0106	0.0001
4	0.5396	0.0001	0.0106	.	0.0001
5	0.0001	0.0001	0.0001	0.0001	.

NOTE: TO ENSURE OVERALL PROTECTION LEVEL, ONLY PROBABILITIES ASSOCIATED WITH PRE-PLANNED COMPARISONS SHOULD BE USED.

Appendix 3



Dimensions of sprayer used in field trials for treatment of wood chips (s - sprayer, h - holes, i - inlet).

ANCOVA ON TREATMENT OVER TIME

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS	LEVELS	VALUES
UT	2	A U
RF	2	F R

NUMBER OF OBSERVATIONS IN DATA SET = 64
ANCOVA ON TREATMENT OVER TIME

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: SCORE

SOURCE	DF	SUM OF SQUARES	*MEAN SQUARE*
MODEL	3	512.99918662	170.99972887
ERROR	60	13666.07195431	227.76786594
CORRECTED TOTAL	63	14179.07114094	

MODEL F =	0.75		PR > F = 0.5262
R-SQUARE	C.V.	ROOT MSE	SCORE MEAN
0.036180	180.1859	15.09198019	8.37578125

SOURCE	DF	*TYPE I SS*	*F VALUE*	*PR > F*
UT	1	140.10273225	0.62	0.4360
RF	1	0.44722656	0.00	0.9648
WEEK	1	372.44922781	1.64	0.2059

SOURCE	DF	TYPE III SS	F VALUE	*PR > F*
UT	1	140.10273225	0.62	0.4360
RF	1	0.44722656	0.00	0.9648
WEEK	1	372.44922781	1.64	0.2059

PARAMETER	ESTIMATE	T FOR H0: PARAMETER=0	PR > |T|	STD ERROR OF ESTIMATE
INTERCEPT	12.37403125 B	2.32	0.0238	5.33582077

UT	A	2.95912500 B	0.78	0.4360	3.77299505
	U	0.00000000 B	.	.	.
RF	F	-0.81718750 B	-0.04	0.9648	3.77299505
	R	0.00000000 B	.	.	.
WEEK		-2.15746750	-1.28	0.2059	1.68733468

NOTE: THE X'X MATRIX HAS BEEN DEEMED SINGULAR AND A GENERALIZED INVERSE HAS BEEN EMPLOYED TO SOLVE THE NORMAL EQUATIONS. THE ABOVE ESTIMATES REPRESENT ONLY ONE OF MANY POSSIBLE SOLUTIONS TO THE NORMAL EQUATIONS. ESTIMATES FOLLOWED BY THE LETTER B ARE BIASED AND DO NOT ESTIMATE THE PARAMETER BUT ARE BLUE FOR SOME LINEAR COMBINATION OF PARAMETERS (OR ARE ZERO). THE EXPECTED VALUE OF THE BIASED ESTIMATORS MAY BE OBTAINED FROM THE GENERAL FORM OF ESTIMABLE FUNCTIONS. FOR THE BIASED ESTIMATORS, THE STD ERR IS THAT OF THE BIASED ESTIMATOR AND THE T VALUE TESTS HO: E(BIASED ESTIMATOR) = 0. ESTIMATES NOT FOLLOWED BY THE LETTER B ARE BLUE FOR THE PARAMETER.

ANCOVA ON TREATMENT OVER TIME

GENERAL LINEAR MODELS PROCEDURE

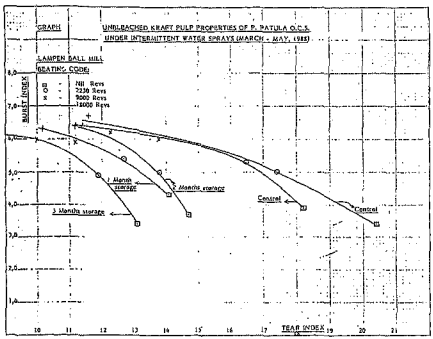
LEAST SQUARES MEANS

UT	SCORE LSMEAN	STD ERR LSMEAN	PROB > |T| HO:LSMEAN=0	PROB > |T| HO: LSMEAN1=LSMEAN2
A	9.85534375	2.66791038	0.0085	0.4360
U	6.89621875	2.66791038	0.0122	

RF	SCORE LSMEAN	STD ERR LSMEAN	PROB > |T| HO:LSMEAN=0	PROB > |T| HO: LSMEAN1=LSMEAN2
F	8.29218750	2.66791038	0.0029	0.9648
R	8.45937500	2.66791038	0.0024	

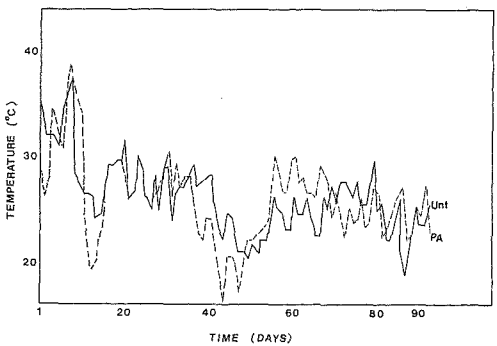
Appendix 5

Graph of burst index versus tear index.



Temperature profiles during OCS.

Appendix 6



Appendix 7

Result from Male's Report (SAPPI).

PULP STRENGTH PROPERTIES OF TREATED AND UNTREATED SAMPLES AT 400 C.S.FREENESS

SAMPLE IDENTIFICATION	BURST	TEAR	TENSILE	GEOMETRIC MEAN	RATING (%)
CONTROL 0 WEEKS	4.6	11.0	92	16.6	100.0
CONTROL 4 WEEKS	5.0	11.9	85	16.0	95.7
ACID TR 4 WEEKS	4.2	12.5	85	16.7	99.5
CONTROL 8 WEEKS	5.8	12.4	83	16.1	96.3
ACID TR 8 WEEKS	6.2	13.2	88	19.3	102.7
CONTROL 12 WEEKS	5.8	11.6	84	17.9	93.2
ACID TR 12 WEEKS	6.3	12.2	89	19.0	101.0
CONTROL 16 WEEKS	5.8	10.8	83	17.3	92.0
ACID TR 16 WEEKS	6.4	10.5	95	18.6	98.9

Statistics for quantification of untreated chips.

ANCOVA ON TREATMENT OVER TIME
USING DIFFERENT METHODS OF ANALYSIS
Untreated cases

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS	LEVELS	VALUES
GR	3	CC H PL

NUMBER OF OBSERVATIONS IN DATA SET = 12
ANCOVA ON TREATMENT OVER TIME
USING DIFFERENT METHODS OF ANALYSIS
Untreated cases

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: LOGCOUNT LOG COUNT G MOOD

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	5	42.54426000	8.50885600
ERROR	6	24.36912000	4.06002000
CORRECTED TOTAL	11	66.90440000	

MODEL F = 2.10 PR > F = 0.1970

R-SQUARE	C.V.	ROOT MSE	LOGCOUNT MEAN
0.635897	28.3796	2.01494913	7.10000000

SOURCE	DF	TYPE I SS	F VALUE	PR > F
GR	2	5.79120000	0.71	0.5274
MONTHS	1	14.56322667	3.59	0.1071
MONTHS*GR	2	22.18985333	2.73	0.1433

SOURCE	DF	TYPE III SS	F VALUE	PR > F
GR	2	21.83201111	2.69	0.1467
MONTHS	1	14.56322667	3.59	0.1071
MONTHS*GR	2	22.18985333	2.73	0.1433

PARAMETER	ESTIMATE	T FOR H0: PARAMETER=0	PR > |T|	STD ERROR OF ESTIMATE

INTERCEPT		-3.641535E-14 B	-0.00	1.0000	2.44779861
GR	CC	6.45500000 B	1.85	0.1139	3.48999427
	H	7.45500000 B	2.14	0.0766	3.48999427
	PL	0.00000000 B	.	.	.
MONTHS		2.70000000 B	3.00	0.0241	0.90111265
*MONTHS*GR*	CC	-2.69000000 B	-2.11	0.0793	1.27436572
	H	-2.45400000 B	-1.93	0.1025	1.27436572
	PL	0.00000000 B	.	.	.

ANCOVA ON TREATMENT OVER TIME
USING DIFFERENT METHODS OF ANALYSIS
Untreated cases

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: LOGCOUNT LOG COUNT G WOOD

NOTE: THE X'X MATRIX HAS BEEN DEEMED SINGULAR AND A GENERALIZED INVERSE HAS BEEN EMPLOYED TO SOLVE THE NORMAL EQUATIONS. THE ABOVE ESTIMATES REPRESENT ONLY ONE OF MANY POSSIBLE *SOLUTIONS TO THE* NORMAL EQUATIONS. ESTIMATES FOLLOWED BY THE LETTER B ARE BIASED AND DO NOT ESTIMATE THE PARAMETER BUT ARE BLUE FOR SOME LINEAR COMBINATION OF PARAMETERS *(OR ARE ZERO). THE* EXPECTED VALUE OF THE BIASED ESTIMATORS MAY BE OBTAINED FROM THE GENERAL FORM OF ESTIMABLE FUNCTIONS. FOR THE BIASED ESTIMATORS, THE STD ERR IS THAT *OF THE BIASED ESTIMATOR AND* THE T VALUE TESTS H0: E(BIASED ESTIMATOR) = 0. ESTIMATES NOT FOLLOWED BY THE LETTER B ARE BLUE FOR THE PARAMETER.

ANCOVA ON TREATMENT OVER TIME
USING DIFFERENT METHODS OF ANALYSIS
Untreated cases

GENERAL LINEAR MODELS PROCEDURE

LEAST SQUARES MEANS

GR	LOGCOUNT LSMEAN	STD ERR LSMEAN	PROB > |T| H0:LSMEAN=0	LSMEAN NUMBER
CC	6.48000000	1.00747457	0.0007	1
H	8.07000000	1.00747457	0.0002	2
PL	6.75000000	1.00747457	0.0005	3

PROB > |T| HO: LSMEAN(I)=LSMEAN(J)

I/J	1	2	3
1	.	0.3071	0.8559
2	0.3071	.	0.3900
3	0.8559	0.3900	.

NOTE: TO ENSURE OVERALL PROTECTION LEVEL, ONLY PROBABILITIES *ASSOCIATED WITH PRE-PLANNED* COMPARISONS SHOULD BE USED.

Statistics for quantification of acid-treated chips.

ANCOVA OF TREATMENT OVER TIME
USING DIFFERENT METHODS OF ANALYSIS
Treated cases

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS	LEVELS	VALUES
GR	3	CC H PL

NUMBER OF OBSERVATIONS IN DATA SET = 12
ANCOVA OF TREATMENT OVER TIME
USING DIFFERENT METHODS OF ANALYSIS
Treated cases

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: LOGCOUNT LOG COUNT G WOOD

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	5	60.33529500	12.06705900
ERROR	6	5.17753000	0.86292167
CORRECTED TOTAL	11	65.51282500	

MODEL F =	13.98	PR > F =	0.0030

R-SQUARE	C.V.	ROOT MSE	LOGCOUNT MEAN
0.920969	13.8492	0.92893577	6.70750000

SOURCE	DF	TYPE I SS	F VALUE	PR > F
GR	2	22.79815000	13.21	0.0063
MONTHS	1	15.21073500	17.63	0.0057
MONTHS*GR	2	22.33461000	12.94	0.0067

SOURCE	DF	TYPE III SS	F VALUE	PR > F
GR	2	34.77243333	20.15	0.0022
MONTHS	1	15.21073500	17.63	0.0057
MONTHS*GR	2	22.33461000	12.94	0.0067

PARAMETER	ESTIMATE	T FOR H0: PARAMETER=0	PR > |T|	STD ERROR OF ESTIMATE

INTERCEPT		-1.64500000 B	-1.65	0.1983	1.13770932
GR	CC	0.01500000 B	4.98	0.0025	1.60896395
	H	9.49000000 B	5.90	0.0011	1.60896395
	PL	0.00000000 B	.	.	.
MONTHS		2.72700000 B	6.56	0.0006	0.41563271
MONTHS*GR	CC	-2.70100000 B	-4.60	0.0037	0.58751057
	H	-2.45900000 B	-4.19	0.0058	0.58751057
	PL	0.00000000 B	.	.	.

ANCOVA OF TREATMENT OVER TIME
USING DIFFERENT METHODS OF ANALYSIS
Treated cases

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: LOGCOUNT LOG COUNT G WOOD

NOTE: THE X'X MATRIX HAS BEEN DEEMED SINGULAR AND A GENERALIZED INVERSE HAS BEEN EMPLOYED TO SOLVE THE NORMAL EQUATIONS. THE ABOVE ESTIMATES REPRESENT ONLY ONE OF MANY POSSIBLE SOLUTIONS TO THE NORMAL EQUATIONS. ESTIMATES FOLLOWED BY THE LETTER B ARE BIASED AND DO NOT ESTIMATE THE PARAMETER BUT ARE BLUE FOR SOME LINEAR COMBINATION OF PARAMETERS (OR ARE ZERO). THE EXPECTED VALUE OF THE BIASED ESTIMATORS MAY BE OBTAINED FROM THE GENERAL FORM OF ESTIMABLE FUNCTIONS. FOR THE BIASED ESTIMATORS, THE STD ERR IS THAT OF THE BIASED ESTIMATOR AND THE T VALUE TESTS HO: E(BIASED ESTIMATOR) = 0. ESTIMATES NOT FOLLOWED BY THE LETTER B ARE BLUE FOR THE PARAMETER.

ANCOVA OF TREATMENT OVER TIME
USING DIFFERENT METHODS OF ANALYSIS
Treated cases

GENERAL LINEAR MODELS PROCEDURE

LEAST SQUARES MEANS

GR	LOGCOUNT LSMEAN	STD ERR LSMEAN	PROB > |T| HO:LSMEAN=0	LSMEAN NUMBER
CC	6.63500000	0.44444789	0.0001	1
H	8.51300000	0.44444789	0.0001	2
PL	5.17250000	0.44444789	0.0001	3

PROB > |T| HO: LSMEAN(I)=LSMEAN(J)

I/J	1	2	3
1	.	0.0194	0.1030
2	0.0194	.	0.0022
3	0.1030	0.0022	.

NOTE: TO ENSURE OVERALL PROTECTION LEVEL, ONLY PROBABILITIES ASSOCIATED WITH PRE-PLANNED COMPARISONS SHOULD BE USED.