Presented at CHINA PAPER / CHINA FOREST 2005 –
The International Exhibition and Conference for the Pulp/Paper and Forestry Industries.

Albino Fungal Effects on TMP and Dissolving Pulp Mill Operations

Roberta L Farrell[1, 2], Romuald Krzywanski[3], Stewart Pikula[4]

[1] Department of Biological Sciences, The University of Waikato, Hamilton, New Zealand
[2] Parrac Limited, 74 Nevada Road, Hamilton, New Zealand
[3] Rayonier Marketing and Research Center, Jesup, Georgia, USA
[4] Rayonier Fernandina Beach Pulp Mill, Fernandina Beach, Florida, USA

INTRODUCTION

Biotechnology, combining wood chemistry, mycology, biochemistry and genetics, developed for the pulp and paper industry an albino fungal product that when applied to wood reduced pitch and improved overall brightness levels prior to pulping, with resultant decreased pitch (resin) problems, decreased pulp bleaching requirements, and some suggested modifications to fibre (Blanchette et al., 1992; Farrell et al., 1993; Bush et al., 1994; Farrell et al 1997; Forde Kohler et al., 1997). The original fungal product described for resin degradation, Cartapip®97, was made by classical mating of isolated ascospores from various *Ophiostoma piliferum* isolates of the United States. *Ophiostoma piliferum* is a saprophytic Ascomycete found throughout the world in the forest, in chip piles and at mills, and is commonly referred to as one of the sapstain fungi. Marketed as Cartapip™97, the colorless strain is an absolute albino and has no stain effect. This fungal product has been demonstrated to have no harmful effects to wood or pulp.

Development of *Ophiostoma piliferum* as a fungal treatment prior to pulping began in 1987 and within 5 years, a fungal product was being commercially used to treat chips producing a brighter pulp with resin extractives significantly decreased (Farrell et al 1992). The product was first developed as part of a research programme with the thermomechanical pulp mill Bear Island Paper Co. of Ashland, Virginia, USA in order to reduce resin extractives in southern yellow pine wood chips. The original product, Cartapip 97, consists of a specific colorless (albino) strain #97 of *Ophiostoma piliferum*. Generally, this technology can refer to any albino *Ophiostoma* sp. that can be used for resin decrease and also resulting in maintenance of brightness levels in transportation and storage of wood chips prior to pulping. Efficacy of the albino *Ophiostoma* technology has been demonstrated on loblolly pine (*Pinus taeda*), as well as other wood species including red pine (*Pinus resinosa*), radiata pine (*Pinus radiata* D. Don.), aspen (*Populus tremuloides*) and *Eucalyptus* sp. (Rocheleau et al 1998; Farrell et al 2000; Farrell et al., 2004).

BACKGROUND – FUNGI, ENZYMES and EXTRACTIVES

Sapstain, also called blue stain, is caused by pioneer (early) colonizing fungi, such as *Ophiostoma*, *Ceratocystis* or *Leptographium* species that utilize simple carbohydrates, fatty acids, triglycerides and other components of the sapwood. The dark stain produced by these fungi is due to melanin and melanin-like compounds that are localized within the fungal hyphae (Zimmerman et al., 1993). Colorless, albino fungal products were made by natural mating techniques used between various fungal strains isolated from wood chip piles and logs – neither mutation nor genetic engineering was used. In this development, single ascospore isolations yielded many fungal strains that had varying degrees of desirable traits, such as reduced hyphal pigments and/or little to no synnemata production. These isolates were then incorporated into mating studies with other isolates of the same species to obtain additional colorless strains with desired characteristics, especially the ability to reduce resin extractives and grow quickly to dominate colonization of wood chips in a chip pile. Thousands of single ascospore isolations were screened to obtain the colorless isolates of each species. The colorless (albino) isolate designated #97 of *O. piliferum*, marketed as Cartapip 97, was shown to be blocked in the 1,8-dihydroxynaphthalene (DHN) melanin synthetic pathway by the inability to produce the intermediate scytalone (Zimmerman et al 1995). Later work showed that *O. piceae* albino strains could also be generated by the same methodology (White-McDougall et al, 1998). Melanin production in fungi is thought to be important for resistance to microbial lysis, protection from ultraviolet light and desiccation. It also plays a role in perithecial development (Zimmerman *et al.*, 1995). The lack of melanin in the albino strains obtained does not appear to inhibit the aggressiveness or growth characteristics of the fungus. Fungal isolates are fast growing and capable of out competing different genera of wild type fungi that cause sapstain.

Albino *Ophiostoma* fungal species, therefore, developed as products for the pulp and paper industry, have the advantage of being able to readily grow on wood chips as long as they are relatively fresh. The *Ophiostoma* fungal product dominates and is present in significantly higher concentrations than background blue stain fungus so that it achieves the desired objectives faster and more efficiently. Ability of any fungus to grow on chips is dependent on the type of fungus and the condition and species of the wood to which it is applied. Competition, possibly involving hyphal interactions, occurs to some extent in connection with primary resource capture and is almost always involved in secondary resource capture, such as with extractives decrease (Holmer and Stenlid, 1993). It is essential to establish the correct loading, or amount of fungal propagules on the wood surface, to allow for proliferation and dominance of the applied *Ophiostoma* fungal product to wood chips entering the chip pile. Inoculation of the *Ophiostoma* fungal product onto the wood chips is achieved either by a closed box type of inoculation with chips being conveyed on a moving belt and fungal suspension sprayed by nozzles onto passing chips, or an open chip conveyor system with the stream of fungal inoculum being focused directly on the chips. The key point for application is to maximize the fungal product being dosed onto the wood chips.

Inherent abilities to capture nutrients & compete with other micro-organisms, distinguishes early from late fungal colonizers of any given substrate and the advantage of the *Ophiostoma* fungal products is that they are early colonizers of wood (Farrell & Thwaites, 2001). Biopulping is typically defined as treatment of wood with fungi prior to pulping - typically lignin degrading white-rot fungi, which are late fungal colonizers of wood. One of the best studied biopulping fungi, *Ceraporiopsis subvermispora*, does not compete well with early colonizers of wood chips so a decontamination step is required for chip treatments with this fungus, and basidiomycetes in general. Kirk and Farrell (1987) called the process of delignification "enzymatic combustion" referring to the non-specific nature of the oxidative pathway as initiated by the ligninase (also known as lignin peroxidase) enzymes. With the biopulping results published so far there has been little lignin loss but good extractives decrease measured and substantial modification of cell walls has been observed (Blanchette et al 1997). The small amount of lignin loss has not been well considered, though the literature still suggests that biopulping can only be done by lignin degrading fungi. For many reasons the lignin biodegradation pathway has not been completely elucidated, although roles have been proposed for more than a dozen enzymes that have been identified and characterized. For an excellent review of biopulping fungi, see Akhtar et al., 1998.

In the last 12 years, albino *Ophiostoma* species have also been studied for their potential when used on solid wood to exert a biocontrol, anti-sapstain effect to maintain a clean, unstained, white appearance (Held et al 2003). In addition to *Ophiostoma piliferum*, these new albino fungal species were developed as product prototypes in New Zealand in a programme commenced in 1996 with the same group of academics that earlier developed Cartapip – Professor Robert Blanchette of University of Minnesota, Professor Thomas Harrington of Iowa State University, Professor Yitzhak Hadar of Hebrew Univ of Jerusalem and the team leader of the project, the co-author (Farrell), currently at University of Waikato (Farrell et al., 1998). The New Zealand albino fungal strains are *O. piliferum*, as well as other natural species of *Ophiostoma,* specifically *O. floccosum, O. piceae, O. pluruanulatum and O. coronatum.* Additionally, albino *Ophiostoma* species have been developed in Chile from fungi isolated in Chile with the same group led by Dr Jose Navarrete of University of Bio-Bio (Navarrete et al., 2005). A collaboration between the Taiwan Forest Research Institute in Taipei and University of Waikato in New Zealand has also developed albino *Ophiostoma* strains from fungi isolated in Taiwan from Eucalyptus sp and from tropical hardwoods from Indonesia (Farrell et al., 2004). For the original Cartapip product, as well as the newly developed albino *Ophiostoma* species from around the world, the fungi are absolutely natural and no mutations, nor genetic engineering, were done to create the fungal products which are colorless.

None of the *Ophiostoma* fungi degrade lignin or structural carbohydrates (arabinose, galactose, glucose, xylose and mannose), as demonstrated conclusively by a study in radiata pine sapwood after 16 weeks' fungal incubation (Schirp et al 2003a). All New Zealand sapstaining isolates tested secreted low amounts of xylanase and pectinase as extracellular enzymes. Under the conditions tested, mannanase was secreted only by *Ophiostoma piceae*. Cellulase was not detected from any of the *Ophiostoma* fungi, supporting the mill results and use of the Cartapip product that no effect on cellulose is observed by the use of the product. *Ophiostoma* species seem to be exceptional since fifteen years ago it was considered that practically all cellulase-producing microorganisms also produce xylanases and vice versa (Eriksson et al., 1990). Amylase activity of the *Ophiostoma* fungal species tested was more significant than xylanase and mannanase and pectinase activities, which confirms that these fungi preferably metabolize easily accessible, non-structural wood components like starch (Schirp et al 2003b). A possible function of these enzymes may be the facilitation of the colonization of wood cells, specifically, pectinase may support the fungal penetration of pit membranes. Scanning electron microscopy of *O. piliferum* 97 colonised ray cells of loblolly pine show that the pit membranes are for the most part removed.

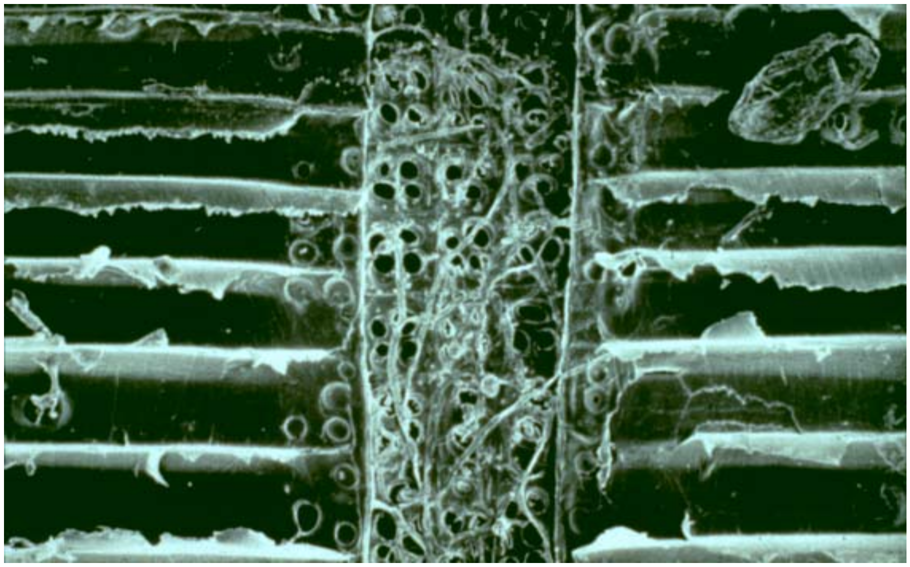

Figure 1. Scanning electron microscopy of *O. piliferum* 97 colonised loblolly pine, (from Blanchette, Farrell et al., 1992)

The confirmation of production of pectinases by these fungi may be important to pulping operations, as the action of pectinases on vessel pits allows the flow of protoplasm from adjoining parenchyma cells into the vessels and contributes to plugging of the vessels (Gagnon, 1967); the pectinase activity of the albino fungal products if optimised may make liquor and steam diffusion in wood cells considerably more efficient.

Resin, also known as pitch, or collectively extractives, consists of low molecular weight oleophilic materials extracted from wood chips by neutral, nonpolar, organic solvents. Resin contains triglycerides, fatty acids, di-terpenoid resin acids, sterols, waxes and other compounds. Though the total percentage on wood of extractives is less than 3.5% for most wood species, this represents a major problem for most pulp mills, particularly TMP, CTMP, and sulfite pulp mills. The total percentage and composition of extractives is dependent upon the wood species, within the tree itself, geographical location and seasons of the year, as well as the period of time between harvesting and pulping of chips. Pitch problems are dependent upon the type of pulp processing, chemical versus mechanical. Pitch is liberated from the pulp fibers at various processing time points, and particularly where there is a change of pH and/or temperature. Pitch can be deposited alone or with fibers, filler, defoamers, coating binders and insoluble inorganic salts (Farrell et al., 1993).

Besides the original published results on pine species, *Eucalyptus* species have recently been the focus of extended studies in a collaborative project between New Zealand and Taiwan Forest Research Institute, in order to develop more efficient pitch reducing *Ophiostoma* fungal products that can also improve the brightness of unbleached *Eucalyptus* pulp. New Zealand albino *Ophiostoma pluriannulatum* isolates, and the original marketed Cartapip 97, were recently screened for their ability to produce the desired effect of rapid growth on fresh *E. globulus* chips, with concomitant increased brightness. Taiwanese *Ophiostoma* strains, New Zealand *O. floccosum* and Cartapip 97 were screened on *Eucalyptus camaldulensis* for their ability to reduce pitch, as measured by the amounts of acetone extractive degraded. Organic solvent extractable substances from wood are complex fractions consisting of fats, waxes, resin acids, free and esterified sterols, alcohols, terpenoids, and phenolics These substances often interact during the pulping and papermaking processes to cause serious pitch troubles, thus decreasing the quality of paper products and hamper productions. After 2 weeks of fungal treatment, the changes in acetone extractive contents were recorded and 70% of the lipophiles were destroyed. Nearly all component parts showed high amplitudes of reduction. Despite that sterols had the highest residual contents; their removal rates still reached 63%, indicating a high efficacy of pitch degradation by the fungi (Farrell et al 2004).

Classification of Fungal Product

Ophiostoma piliferum has official United States Department of Agriculture classification as a non-pathogen and all components of Cartapip 97 (Code #299972) are registered under the regulations of the Toxic Substances Control Act. Additionally, studies were conducted in conformation with EPA Pesticide Assessment guidelines, and the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) and all the studies conducted showed the fungal product was safe for use. The Food Drug Administration (FDA) confirmed that Cartapip has an acceptable regulatory status for use as a pitch control agent and as a pulping aid for wood chips that are processed into pulp and then into paper used in contact with food. For this status, FDA accepted that presence of Cartapip in the final pulp product is below the Threshold of Regulation level of 0.5 parts per billion (ppb), rendering it de facto non-existent in the final pulp. Thus, Cartapip use is in compliance with the Federal Food, Drug, and Cosmetic Act and all applicable Food Additive Regulations.

Enhanced Softwood Mechanical Pulp Properties

It was shown that pulp produced by Cartapip 97 had improved handsheet strength properties as studied by producing refiner mechanical pulps (Forde Kohler et al 1997) analogous to biopulps. In this doctoral research study, Cartapip 97 treatment of loblolly pine wood chips decreased extractive levels and increased fiber length, tear, tensile, and zero-span tensile strengths over and above the control, non-treated wood chips, which were aged for the same time period. Cartapip 97 treated and control chips were refined in a Sprout Waldron atmospheric refiner, executed in 5-7 refining passes and handsheet production ranged in freeness from 250 to 30 mL Canadian Standard Freeness (CSF). Statistical analysis of the data was performed as multiple regression analysis. Results showed that the Cartapip 97 fungal treatment chips refined with greater fiber lengths, which may partly account for increased tear strength (Forde Kohler et al., 1995). Cartapip treatment yielded concurrent significant decreases in fines content. The resultant Cartapip treated pulps resembled chemimechanical pulps where strength properties increase while freeness changes minimally. Fiber and/or extractive chemical compositional changes were suggested to explain strength differences (Forde Kohler et al., 1995).

Use of the Albino Fungal Product in the Dissolving Pulp Mill

Rayonier Fernandina Beach pulp mill located in Northern Florida where temperatures generally stay above 5° C produces premium dissolving grade, high alpha cellulose pulps. The Fernandina Beach pulp mill uses southern pine chips that arrive from satellite facilities. The pulping process is an ammonium sulphite process and bleaching uses proprietary sequences that remove most of the lignin and hemicellulose while preserving cellulose structure to a degree higher than conventional bleaching sequences.

The end use applications require not only a very high alpha cellulose content, but also very low content of extractives. The required low level of extractives can normally be achieved by controlling the duration of chip pile storage. This can result in high inventory costs for the mill. Cartapip 97 offers a way of meeting extractives target in the final product with lower inventories.

Cartapip 97 fungal product is received as a freeze dried powder. The Powder is dissolved in water in a make-down tank and applied to the chips at specified rates. Adequate dispersion across the chip supply is essential as migration of product within the storage pile is limited. Cartapip97 activity rates are a function of chip temperature at the time of application.

Cartapip 97 supplements the naturally occurring blue stain fungi, which produce enzymes that degrade extractives in wood. Hence, use of Cartapip 97 can be viewed as an augmentation or acceleration of the natural aging process that occurs in the chips due to the blue stain fungus, but in a more complete and effective manner. This is very different from conventional use of pulping or bleaching chemicals. The thoroughness of the chip treatment is an important factor in overall Cartapip effectiveness. Some mills that tried Cartapip in the past were unsuccessful due to inability to treat all their incoming chips. When the treatment is applied to a small percentage of chips only, the overall results will be disappointing since Cartapip will not reduce the extractives to very low levels in treated chips and not much of an averaging impact will be achieved.

The Florida climate is ideal for Cartapip treatment. However, Cartapip will do well under more adverse conditions and the fungus will not die if temperature drops too low – the range of temperature for effective treatment with Cartapip and other albino *Ophiostoma* fungal products is from freezing, 2° C to 32° C, and at higher temperatures, though the fungal product is not actively growing, the extracellular enzymes produced by Cartapip, and any of the albino *Ophiostoma* sp, are still active at temperatures in excess of 65°C (McNaughton 1996).

Cartapip is used during winter months and it accelerates aging on the mill's chip pile by approximately two weeks.

While Cartapip does an excellent job of reducing extractives in chips, the extractives content does not drop below a certain plateau at the pulp mill. Hence, Cartapip 97 use complements chip aging when too short in duration, but it does not reduce extractives to a level lower than can be achieved with sufficiently long chip pile storage, and aging due to background fungi. This makes it the ideal additive from the quality control point of view. Its use ensures that the level of extractives through pulping remains the same throughout the year. The organism is destroyed during the pulping process and any residual cells are removed from pulp during washing stages before the bleach plant and throughout subsequent bleaching.

Cartapip treatment was shown to have the following impact on pulp properties during some trials:

1. Kappa or K number – slightly lower digester K# was recorded with Cartapip use in certain grades.
2. Viscosity – digester pulp viscosity tends to be slightly higher with Cartapip use. The viscosity of the final pulp is unchanged.

3. Pitch dispersant use and dirt content – Cartapip was shown in early trials to reduce pitch dispersant use and hence lower the pulp dirt content.
4. Brightness – brightness increase of 0.5-1.0% ISO was observed with Cartapip use. However, brightness is not the most important property of Fernandina Beach mill pulps and the mill's process allows meeting brightness targets with little difficulty.
5. Less cellulose degradation is present when Cartapip is applied as evidenced by generally lower S10 values.

The differences between properties with Cartapip treatment on and off stayed mostly within process variability limits, especially when the final product is concerned. The most clear and documented benefit has been a significant reduction in extractives in chips aged three weeks or less. The difference in extractives gradually decreases and becomes not existent with chips aged six weeks or longer.

In general, while brightness is slightly higher for Cartapip treated chips due to the difference in chip coloring introduced by colorless fungus versus the untreated chips having colonization by native sapstain fungi, the impact on bleaching has been negligible. This could well be different in a mill in which brightness is a primary control parameter and where brightness increase should result in lower use of bleaching chemicals.

Conclusions

Chip pile storage has long been known to be effective at decreasing problematic pitch levels. However, there are disadvantages associated with seasoning chips, such as non-uniformity, as well as brightness and yield losses. Albino *Ophiostoma* fungal (Cartapip) application with an automated spray system is easily instituted and changes required in wood yard management are usually minimal. Some of the research aspects to further optimize albino *Ophiostoma* fungal (Cartapip) products' effects in the mill include the following:

a. Improve colonization and growth in the woodpile.
b. Investigate whether one fungal isolate is sufficient for wood extractive decrease as well as brightness improvement or if multiple isolates achieve a greater effect not equaled simply by increased dosage.
c. Determine the components of wood extractives in various wood species that are most important to decrease in pulp.

Acknowledgements

Many people have contributed to the study and use of the albino *Ophiostoma* fungal products. We particularly want to acknowledge the following for their many contributions:
Robert Blanchette, Hou-min Chang, Shona Duncan, Esther Hadar, Yitzhak Hadar, Thomas Harrington, Kunio Hata, Benjamin Held, Jose Navarrete, Arvina Ram, Yu-chang Su, Joanne Thwaites, Eugene Wang and Shoichi Yamanami. For information about availability of Cartapip/ *Ophiostoma* fungal products, please contact Roberta Farrell at r.farrell@waikato.ac.nz.

References

Akhtar, M., Blanchette, R.A., Myers, G. & Kirk, T.K. (1998) An overview of biomechanical pulping research. *Environmentally Friendly Technologies for the Pulp and Paper Industry* (ed. Young, R.A. & Akhtar, M.), John Wiley & Sons, Inc., New York, USA, pp. 309-340.

Blanchette, RA, Farrell, RL, Burnes, TA, Wendler, PA, Zimmerman, W, Brush, TS, Snyder, RA. Biological Control of Pitch in Pulp and Paper Production by *Ophiostoma piliferum*. *Tappi Journal* 75 (12), 102-106. (1992).

Blanchette, R.A., E.W. Krueger, J.E. Haight, M. Akhtar & D.E. Akin. (1997) Cell wall alterations in loblolly pine wood decayed by the white rot fungus *Ceraporiopsis subvermispora*. *J. Biotechnol.* **53**:203-213.

Brush, TS, Farrell, RL, Ho, C. Biodegradation of Wood Extractives from Southern Yellow Pine by *Ophiostoma piliferum*. *Tappi Journal* 77, 155-159 (1994).

Eriksson, K.-E., Blanchette, R. A., Ander, P. (1990): Microbial and Enzymatic Degradation of Wood and Wood Components. Springer-Verlag Berlin Heidelberg. 407 pp.

Farrell, R.L., Blanchette, R.A., Brush, T.S., Gysin, B., Hadar, Y., Perollaz, J.-J., Wendler, P.A. & Zimmerman, W. (1992) Cartapip™: A biopulping product for control of pitch and resin acid problems in pulp mills. *Biotechnology in Pulp and Paper Industry* (ed. Kuwahara, M. & Shimada, M.), Uni Publishers Co. Ltd., Tokyo, Japan, 27-32.

Farrell, R.L., Blanchette, R.A., Brush, T.S., Hadar, Y., Iverson, S., Krisa, K., Wendler, P.A., Zimmerman, W. (1993). Cartapip TM: a biopulping product for control of pitch and resin acid problems in pulp mills. *J. Biotechnol*. 30: 115-122.

Farrell, R.L., Hata, K. and Wall, M.B. (1997). Solving Pitch Problems in Pulp and Paper Processes by the Use of Enzymes or Fungi. *Advances in Biochemical Engineering/Biotechnology*, Vol. 57: p 198-210.

Farrell, R.L., Kay, S.J., Hadar, E., Hadar, Y., Blanchette, R.A., Harrington, T.C. (1998). Sapstain in New Zealand - the causes and a potential anti-sapstain solution, In “Biology and prevention of Sapstain”, Forest Products Society Publication No. 7273.

Farrell, R.L., J. Mulcahy, R. Nobbs, T. Rose, D. Richardson, A. Ram, J. Thwaites, T. Haryati, B.W. Held, D. McNew, R.A. Blanchette, and T.C. Harrington. (2000) Research in progress: resin degradation and brightness increase of radiata pine with fungal treatment in lab and mill trials. p.279-284. Su, Yu-Chang and E. I. C. Wang (Eds), *In* Proceedings of the international symposium on environmentally friendly and emerging technologies for a sustainable pulp and paper industry. Taiwan Forestry Institute, Taipei, Taiwan.

Farrell, R.L., Thwaites, J.M. (2001). Future Directions for Biological Control & BioActivity®. International Research Group for Wood Preservation Secretariat, KTH, Sweden, 2001IRG/WP 00-10329.

Farrell, R.L., Thwaites, J. M., Su, Y-c., Wang, E.I., Ho, C-l., Chang, H-m. 2004. Screening of fungi for removal of wood extractives. 58th Annual Appita Proceedings, Canberra, Australia.

Forde Kohler, L.J., Dinus, R., Malcolm, E., Rudie, A., Farrell, R. & Brush, T. (1995) Enhancing softwood mechanical pulp properties through chip treatment with *Ophiostoma piliferum*. Proceedings of the 6th Conference on Biotechnology in the Pulp and Paper Industry: Advances in Applied and Fundamental Research, Ed. E. Swebotnik and K. Messner, p.225-228.

Forde Kohler, L.J., Dinus, R.J., Malcolm, E.W., Rudie, A.W., Farrell, R.L. & Brush, T.S. (1997) Improving softwood mechanical pulp properties with *Ophiostoma piliferum*. *Tappi Journal* **80**(3):135-140.

Gagnon, C. (1967): Histochemical studies on the alteration of lignin and pectic substances in white elm infected by *Ceratocystis ulmi*. Canadian Journal of Botany 45: 1619-1623.

Held, B.W., Thwaites, J.M., Farrell, R.L., Blanchette, R.A. (2003). Albino Strains of *Ophiostoma* Species for Biological Control of Sapstaining Fungi. *Holzforschung,* **57**, 237-242.

Holmer, L. and Stenlid, J. (1993) The importance of inoculum size for the competitive ability of wood decomposing fungi. FEMS Microbiol. 12:169-176.

Kirk, T.K. & Farrell, R.L. (1987) Enzymatic "combustion": the microbial degradation of lignin. *Annu. Rev. Microbiol.* **41**:465-505.

McNaughton, C. The Purification and Characterisation of an Extracellular Lipase from *Ophiostoma piliferum* 97. MSc. Thesis, The University of Waikato, 1997.

Navarrete, J., Moreira, A., Santanda, A., Vera, R., Herrara, P., Reyes, L., McNew, D., Harrington, T.C., Farrell, R.L., Thwaites, J.M., Held, B., Blanchette, R.A. *Ophiostoma* species that cause blue stain in *Pinus radiata* in Chile. Manuscript submitted, 2005.

Rayner, A.D.M. and Boddy, L. (1988) Fungal Decomposition of Wood. Its Biology and Ecology. John Wiley & Sons, New York.

Rocheleau, M.J., Sithole, B.B., Allen, L.H., Iverson, S., Farrell, R., Noel, Y. (1998). Fungal Treatment of Aspen Chips for Wood Resin Reduction: A Laboratory Evaluation. *Journal of Pulp and Paper Science,* Vol 24. Number 2, p 37-42.

Schirp, A., Farrell, R.L., Kreber, B. Effects of New Zealand Sapstaining Fungi on Structural Integrity of Unseasoned Radiata Pine. (2003). Holz als Rohund Werkstoff, 61, 369-376.

Schirp, A., Farrell, R.L., Kreber, B., Singh, A.P. (2003). Advances in understanding the ability of sapstaining fungi to produce cell-wall degrading enzymes. Wood and Fiber Science, 35, 434–444.

White-McDougall, W.J., Blanchette, R.A., Farrell, R.L. (1998). Biological control of the stain fungi on *Populus tremuloides* using selected Ophiostoma isolates. *Holzforschung,* 52 (3), p 234-240.

Zimmerman, W.C., Blanchette, R.A., Burnes, T.A., Farrell, R.L. (1993). Melanin and perithecial development in Ophiostoma piliferum. *Mycologia* 87: 857-863.