Biomechanical pulping with *Phlebiopsis gigantea* reduced energy consumption and increased paper strength

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ABSTRACT *Biomechanical pulping of whole logs pretreated with Phlebiopsis gigantea was investigated in several studies using loblolly and red pine. Results from these studies showed P. gigantea was able to colonize 90 to 100% of the freshly cut logs after 8 weeks, with little variation between replicate treatments. Up to a 59% decrease in resinous wood extractives was observed in loblolly and red pine logs inoculated with P. gigantea as compared to non-inoculated logs. Simons' staining, used to evaluate cell wall changes in mechanically refined pulp fibers during biological pulping processes, showed 55 to 77% of the fibers from treated logs stained, while 25 to 58% of the fibers from aged control logs stained. Refined wood from inoculated logs required less energy (9 to 27%) to reach a freeness of 100 Canadian Standard of Freeness than wood from non-inoculated logs. Pretreatment of red pine logs with P. gigantea also resulted in a 17%, 20%, and 13% increase in burst, tear, and tensile strength properties, respectively, as compared to paper derived from non-inoculated logs.*

KEYWORDS *Biopulping, energy, mechanical pulping, white-rot fungi, paper strength, pitch, wood extractives, Simons' stain*

Biomechanical pulping, the pretreatment of wood chips with fungi prior to mechanical refining, is a process with potential for reducing energy consumption, environmental pollution, and improving paper strength properties. Research investigating the pretreatment of wood chips with white rot fungi has demonstrated several benefits of the process to the mechanical pulping industry (1, 2, 3, 4, 5, and 6). Although mechanical pulp produces high yields, it consumes large amounts of energy and produces paper with reduced strength properties as compared to chemical

pulps (7). Biological pre-treatments appear well suited for paper production since they have been shown to significantly reduce energy consumption during refining and increase paper strength properties (1, 2, 3, 4, 5, and 8).

Two white rot fungi, *Ceriporiopsis subvermispora* and *Phanerochaete chrysosporium*, have been extensively studied on both hardwood and softwood chips for biomechanical pulping (9, 10, 11, 12). Results from these earlier studies have shown that energy consumption is reduced and paper strength properties are improved (1, 2, 3, 4, and 8). However, further investigations with *C. subvermispora,* have shown that the biomechanical process requires steam sterilization of the wood chips, addition of nutrients during inoculation, and aeration of the chip pile with temperature and moisture-controlled air to achieve optimum results (13). In contrast, inoculation of whole logs with white rot fungi would eliminate the requirement for steam sterilization and aeration in large chip piles, since non-sterilized logs could be inoculated at the time of cutting. Recent investigations have demonstrated the ability of *Phlebiopsis gigantea* (synonym *Peniophora gigantea)* to colonize freshly cut logs.

To evaluate the biomechanical pulping potential of various fungi a staining procedure, commonly called Simons' stain, can be used to predict energy savings during the refining process (14, 15, 16, 17, and 18). The ends of refined fibers from wood chips treated with *C. subvermispora* are greatly fibrillated and stain orange-yellow, while untreated fibers stain blue (16). The extent of orange-yellow fiber staining has been recently correlated with increased energy savings during mechanical pulping (17). Although, Simons' stain is usually associated with fibrillated fiber ends, recent investigations have shown that staining of the entire fiber can occur in wood treated with *P. gigantea* suggesting an increase in cell wall porosity (14).

Another benefit from the biological pretreatment of wood includes a reduction in wood extractives such as triglycerides, fatty acids, and resin acids that can be problematic to the paper making industry. These compounds cause breaks in the paper rolls, increased machine shut downs, holes in the final paper product, and decreased paper strength (19, 20). Successful removal of such components from wood has previously been demonstrated with a colorless strain of *Ophiostoma piliferum* (Cartapip 97) (21, 22, and 23). Research by Fisher et al. (24) demonstrated that *C. subvermispora* and *P. chrysosporium* reduced the resin content of spruce and loblolly pine. Recently, *P. gigantea* was shown to reduce the pitch content of red pine by as much as 71% (14).

The objective of the study reported here was to evaluate the effectiveness of *P. gigantea* as a biological pretreatment to improve mechanical pulping processes of pine wood. Specific goals were to determine the effect of *P. gigantea* at reducing wood extractives, decreasing energy consumption, increasing fiber porosity, and increasing paper strength properties.

RESULTS AND DISCUSSION

Red and loblolly pine logs inoculated with *P. gigantea* had 90 to 100% of the sapwood colonized after 8 weeks, indicating that *P. gigantea* is a superb pioneer colonist capable of rapidly colonizing non-sterilized logs **(Table I).** The ability of *P. gigantea* to colonize freshly cut stumps of conifers was first shown by Rishbeth (25, 26, and 27), who demonstrated that *P. gigantea* could act as a biocontrol agent against the root rot fungus *Heterobasidion annosum.* Recently Behrendt and Blanchette (14) demonstrated the ability of *P. gigantea* to colonize freshly cut red pine logs, and prevent subsequent colonization by sapstain fungi. The ability of

P. gigantea to tolerate resinous extractives present on freshly cut wood allows the fungus to quickly colonize the sapwood and prevents other fungi from becoming established. Inoculating the fungus into cut, exposed slits on the sides of the log enables *P. gigantea* to completely colonize any length of log in a short period of time (14). Application of fungal inoculants to logs immediately after cutting would allow beneficial biopulping effects to take place while logs *are* in transport and storage.

Table I: Percent of sapwood colonized by *Phlebiopsis gigantea* in pine logs inoculated in the field.

	Loblolly Pine (244 cm)		Red Pine (61 cm)			Red Pine (122 cm)	
Treatment	10 wk	20 wk	8 wk	11 wk	17 wk	9 wk	22 wk
Control	30	65	8	31	56	14	11
Treated	90	90	100	100	100	100	48

Red and loblolly pine logs inoculated with *P. gigantea* yielded 10 to 59% fewer wood extractives than non-inoculated logs 9 to 12 weeks after inoculation. Previous research has shown a 33 to 35% reduction in loblolly pine wood chip extractives when inoculated with *Ceriporiopsis subvermispora* for 4 weeks (24). In our studies, loblolly pine log extractives were reduced by as much as 29% 8 to 12 weeks after inoculation with *P. gigantea* **(Table II).** Variation in the percent of wood extractives removed from loblolly pine logs (10% to 59%) is most likely due to differences in log lengths (61 cm versus 244 cm) and the time needed to colonize entire logs, the rate of sapwood colonization, or variation in resin content of the trees studied.

Table II: Percent of resin extractives present in pine logs inoculated with *Phlebiopsis gigantea* and non-inoculated logs.

	Loblolly Pine (244 cm)			Red Pine (61 cm)				Red Pine (122 cm)		
Treatment	0 wk	10 wk	20 wk	0 wk	8 wk	11 wk	17 wk	0 wk	9 wk	22 wk
Control	3.1	2.0	1.4	3.0	2.4	1.7	1.2	3.0	2.1	1.4
Treated	--[z]	1.8	1.0	--[z]	2.3	0.7	0.7	--[z]	1.2	0.7
% change		(-10%)	(-29%)		(-4%)	(-59%)	(-42%)		(-43%)	(-50%)

[z] At 0 weeks only control logs were analyzed.

Staining of refined wood fibers from logs inoculated with *P. gigantea* revealed a larger percent (55 - 77%) of the fibers staining orange-yellow than fibers from non-inoculated logs (25 - 58%) after 17 weeks in the field **(Table III).** Only 8 to 9% of the fibers from control logs sampled at the time of set up were stained. The staining observed in the aged non-inoculated logs was apparently due to colonization by native *P. gigantea.* Although colonization was delayed compared to inoculated logs, the amount of *P. gigantea* increased with time (Table I).

Table III: Percent of refined wood fibers, from logs inoculated with *Phlebiopsis gigantea,* staining orange-yellow to green in color.

	Loblolly Pine (244 cm)			Red Pine (61 cm)				Red Pine (122 cm)		
Treatment	0 wk	10 wk	20 wk	0 wk	8 wk	11 wk	17 wk	0 wk	9 wk	22 wk
Control	9	14a[Y]	25a	8	18a	23a	58a	8	2a	38a
Treated	--[Z]	36b	55b	--[Z]	21a	54b	77b	--[Z]	50b	69b

[Y] Values within a column with the same letter are not statistically different at $P < .05$ based on Chi-square test statistics.

[Z] At 0 weeks only control logs were analyzed.

Mechanical refining of logs inoculated with *P. gigantea* required less energy to reach a freeness of 100 (Canadian Standard of Freeness, CSF) than non-inoculated logs **(Table IV).** Red pine logs inoculated with *P. gigantea* showed reductions in energy consumption of 9 to 27% after 17 weeks, while non-inoculated logs in the field showed reductions of 1 to 19% (Table IV). Although aged non-inoculated logs showed a reduction in energy consumption when compared to freshly cut wood, logs inoculated with *P. gigantea* at the time of cutting yielded an additional 6 to 8% decrease in energy consumption. Up to 100% of the inoculated red pine logs were colonized by *P. gigantea,* while non-inoculated logs had up to 56% colonization (Table I). Thus, the reduction in energy consumption in non-inoculated logs was most likely the result of natural colonization by *P. gigantea.* The cool, moist conditions at our field site and large amount of native *P. gigantea* resulted in unusually high levels of colonization in the non-inoculated logs over time. These conditions would not be present at most dry, sunny log storage sites and limited natural inoculum would be present.

Table IV. Percent energy consumed during mechanical refining of wood from pine logs inoculated with *Phlebiopsis gigantea.*

		Loblolly Pine (244 cm)		Red Pine (61 cm)			Red Pine (122 cm)				
Data Collected	Treatment	0 wk	20 wk	0 wk	17 wk	% change[Z]	0 wk	9 wk	% change[Z]	22 wk	% change[Z]
Freeness (CSF)[V]	Control	430	400	98	108	---[X]	98	105	---[X]	109	---[X]
	Treated	---[W]	278	---[W]	98	---[X]	---[W]	110	---[X]	80	---[X]
Energy Consumption[Y]	Control	---[X]	---[X]	3118	3085	1	3118	2741	12	2532	19
	Treated	---[W]	---[X]	---[W]	2825	9	---[W]	2558	18	2279	27

[V] Canadian Standard of Freeness (CSF) for loblolly pine after 2 passes through mechanical refiner and CSF for red pine after 5 passes through the refiner, refining red pine logs to a freeness of approximately 100.

[W] At 0 weeks only control logs were analyzed.

[X] ND, not determined.

[Y] Energy consumed in WH/kg (watt hours/kg) after standardizing to a CSF of 100.

[Z] Percent reduction in energy consumption, compared to 0 week control.

Since mechanical refining consumes large quantities of electrical energy during processing (7), savings of up to 27% in energy consumption would be significant to the pulp and paper industry. Previous laboratory investigations have shown a reduction in energy consumption during mechanical refining of wood chips inoculated with either *C. subvermispora* or *P. chrysosporium* (1, 3, 4, and 12). Although energy use was not determined for the loblolly pine used in our refining study (Table IV), a drop in freeness of 100 or more after the first or second pass has been associated with approximately a 20% energy savings (authors unpublished data). Therefore, we predict that the refined loblolly pine logs inoculated with *P. gigantea* exhibiting freeness of 122 (CSF) after 2 passes would also reduce energy consumption by as much as 20%. Energy savings were confirmed in our studies using 8 foot red pine logs inoculated with *P. gigantea* with savings up to 27%. Inoculating whole logs instead of wood chips appears to be an effective way to treat pulpwood for biological processing. The large initial set up costs required for successful biopulping of wood chips, such as steam sterilization and aeration of chip piles, would not be necessary.

Successful colonization of red pine logs by *P. gigantea* also led to an increase in the physical strength properties of handsheets made from treated logs. An increase in burst, tear, and tensile strength properties of 17, 20, and 13%, respectively, was observed 22 weeks after inoculation when compared to paper made from the freshly cut control logs **(Table V).** Previous studies (4, 12) have demonstrated that biomechanically pulped wood pretreated with white-rot fungi can increase paper strength properties. The small increase in burst, tear, and tensile strength properties of handsheets made from aged non-inoculated logs in our studies was likely due to natural colonization of *P. gigantea* after 22 weeks. Results from this study also showed a decrease in pulp brightness after 22 weeks in *P. gigantea* treated logs when compared to pulp made from freshly cut logs. However, previous research has shown that the loss of pulp brightness from fungal treatment can be easily regained by bleaching with either alkaline hydrogen peroxide or sodium hydrosulfite (6).

Table V. Physical properties of handsheets made from pine logs treated with *Phlebiopsis gigantea* for 9 or 22 weeks.

	Burst Index (KN/g)			Tear Index (mN-m^2/g)			Tensile Index (Nm/g)			Brightness (%)			Opacity (%)		
Treatment	0[y]	9	22	0[y]	9	22	0[y]	9	22	0[y]	9	22	0[y]	9	22
Control	.85	.89	.98	2.74	2.86	3.10	22.1	23.5	23.5	56.8	54.3	50.4	96.9	94.6	94.3
% change[z]	--	4	13	--	4	12	--	6	6	--	-4	-11	--	2	3
Treated	--	.97	1.03	--	3.06	3.42	--	24.3	25.5	--	39.9	38.6	--	94.8	94.2
% change[z]	--	12	17	--	10	20	--	9	13	--	-30	-32	--	2	3

[y] At 0 weeks only control logs were analyzed.
[z] Percent reduction in energy consumption, compared to 0 week control.

Biological pulping of logs with *Phlebiopsis gigantea* resulted in the degradation of wood extractives, the reduction of energy consumed during mechanical refining, and the improvement of paper strength properties. The application procedure for this process is relatively easy and allows colonization and bioprocessing to occur during transport and storage of logs. As with any

application of white-rot basidiomycetes, extended storage of 6 months or longer should be avoided so biomass losses to decay do not occur.

EXPERIMENTAL

Field Investigations

Loblolly pine experiment. Loblolly pine trees 30 to 40 years old with an average diameter of 20 cm were felled at the Solon Dixon Forestry Center in Alabama during March of 1996 and cut into 244 cm lengths. Prior to inoculation the logs were slit with a chain saw through the bark to expose the sapwood four times around the outer circumference of the log every 20 cm down the length of the log. Both log ends and slits were sprayed. Two piles per treatment were set up in a pyramid shape on the forest floor with 16 logs per pile. Treatments included inoculation with *P. gigantea* and a non-inoculated water control. A spore suspension prepared as previously described by Behrendt and Blanchette (14), consisted of 9.0×10^5 oidial spores/ml. Three liters were sprayed on each log pile. Logs were examined 10 and 20 weeks after inoculation by sampling 3 logs per pile (6 logs / treatment). Two sub samples, one from the end and one from the middle, were cut from each log.

Red pine experiment. Red pine trees 40 to 50 years old with an average diameter of 20 cm were felled at the Cloquet Forestry Center in Minnesota during May of 1995 and 1996 cutting the logs into 61 and 122 cm lengths, respectively. Logs in the 1995 study received 2.5 cm holes, to expose the sapwood, 4 times around the outer circumference of the log every 20 cm down the length of the log. Logs in the 1996 study were slit with a chainsaw through the bark to expose the sapwood 2 times around the outer circumference of the log every 15 cm down the length of the log. The log ends, holes, and slits were inoculated. Ten logs were piled in a pyramid shape on the forest floor in 1995 with 3 piles per treatment, while 16 logs were piled in a 4 X 4 log pile with 2 piles per treatment in 1996. Treatments included inoculation of logs with *P. gigantea* and a non-inoculated water control. Spore suspensions were produced as stated above. Spore concentrations for the 1995 and 1996 studies consisted of 3.2×10^5 and 6.2×10^5 oidial spores/ml, respectively. A volume of 1 L was sprayed onto each pile in the 1995 studies, while 1.5 L was sprayed onto each pile in the 1996 study. Logs were sampled at 8, 11, and 17 weeks after inoculation in 1995, and 9 and 22 weeks after inoculation in 1996. Two logs per pile were sampled (6 logs per treatment) in 1995, by removing a section from the log end. Three logs per pile were sampled (6 logs per treatment) in 1996, taking segments from the end and middle.

Analysis. Treatment evaluation included the percent of logs colonized by *P. gigantea*, the percent of wood extractive present in logs, and the percent of refined wood fibers stained with Simons' stain. Treatments were also examined for change in energy consumption during mechanical pulping, and changes in the physical properties of handsheets. The percent colonization was determined by aseptically splitting each log and removing approximately 3 X 3 X 1 mm wood chips from the surface of the sapwood at designated intervals. Wood chips were placed in a medium selective for basidiomycete fungi similar to that used by Worrall (28) allowed to grow for 1 to 2 weeks, and examined for colonies of *P. gigantea*.

Analysis of the wood extractives began by debarking logs, removing the heartwood if present, and chipping logs. Sapwood from two representative logs from each field study was screened so no chips greater than 4 cm in width were used for extractive analyses. Wood chips

were ground with a Wiley Mill and sifted to pass a 40-mesh screen. Ground wood was analyzed according to the procedures listed in Tappi standard no. 204 using dichloromethane (29). Extra logs collected at the time of cutting were placed in the freezer in order to determine the resin content at time of cutting (week zero). The percent of wood extractives in logs inoculated with *P. gigantea* was compared to extractives in aged non-inoculated logs.

Wood chips not used for extractive analyses were mechanically refined. Fiber modification was analyzed after mechanical refining with the use of Simons' stain. Pulp sampled from the Sprout Waldron refiner after 1 pass was stained and analyzed according to procedures described by Behrendt and Blanchette (14). Chi-square test statistics ($P < 0.05$) were used to compare control and treated logs.

Energy consumed during the mechanical refining process and changes in the physical properties of handsheets, made from inoculated and non-inoculated red pine logs were conducted at the Forest Products Laboratory, USDA, Forest Service, Madison, WI. Wood chips were passed through the refiner, calculating the percent of energy consumed, until a freeness of 100 CSF was reached. Handsheets were made from refined wood (pulp) with a CSF of approximately 100. One sample from each treatment was analyzed. Procedures similar to those previously published by Akhtar were followed (12). The amount of energy consumed during refining and changes in the physical properties of wood inoculated with *P. gigantea* as well as non-inoculated wood were compared to fresh control wood.

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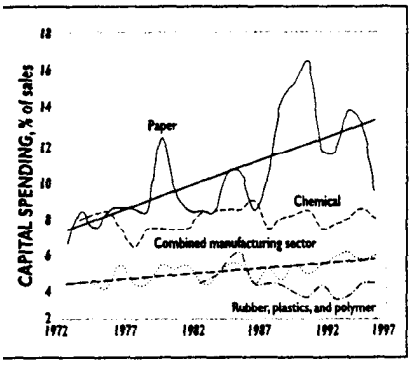

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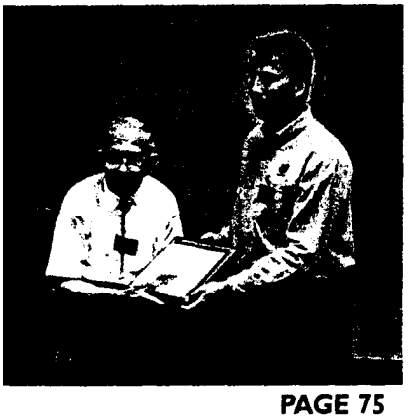

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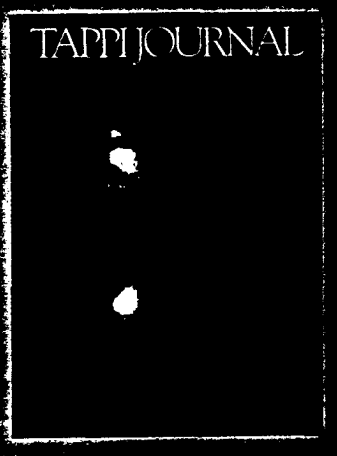

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