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Biological Processing of Pine Logs for Pulp and Paper Production with *Phlebiopsis gigantea*†

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***Phlebiopsis gigantea* (=*Phanerochaete gigantea*) is a white rot fungus that rapidly colonizes cut stumps, stems, and branches of pine. Two laboratory and several field studies showed that inoculation of red pine logs, *Pinus resinosa*, with *P. gigantea* reduced the pitch content of wood, facilitated bark removal, modified wood cells, and controlled detrimental sapstain. Isolations from inoculated logs revealed up to 100 and 80% colonization of the sapwood by *P. gigantea* after 8 weeks in the field and 32 days in the laboratory, respectively. Logs colonized by *P. gigantea* in both the laboratory and field showed a 9 to 71% reduction in pitch content, as well as a significant enhancement of bark removal. Examination with Simons' stain of refined wood fibers from inoculated logs revealed an increase in cell wall porosity. Blue stain fungi that cause dark discoloration of the sapwood were inhibited by inoculation with *P. gigantea*. These studies demonstrate that biological processing of logs with *P. gigantea* can result in substantial benefits to the pulp and papermaking process.**

Phlebiopsis gigantea (Fr.) Jül. (synonyms, *Phanerochaete gigantea* and *Peniophora gigantea*) is an aggressive saprophytic white rot fungus that colonizes freshly cut wood of conifers. Hyphae of *P. gigantea* invade tracheids and ray parenchyma cells of sapwood utilizing readily available nutrients. Several decades ago this fungus was studied extensively for use as a biocontrol agent of *Heterobasidium annosum* (Fr.) Bref, a root rot fungus (14, 15, 27–29). It was found that *P. gigantea*, when applied to cut stumps, could inhibit subsequent colonization by the pathogen.

Certain white rot fungi are able to selectively degrade lignin from wood, removing a far greater proportion of lignin than cellulose (10). In advanced stages of delignification, the middle lamella is degraded and the cellulose-rich secondary walls separate. Although biological delignification would be a great benefit to the paper-making process through reducing chemical demand and environmental pollution, the time needed for extensive microbial delignification to take place is currently too long for incorporation into existing pulping operations. Recent information suggests that some fast-growing white rot fungi can provide significant benefits to biopulping processes with relatively short incubation times (1, 17). Fungi such as *Phanerochaete chrysosporium* and *Ceriporiopsis subvermispora* can significantly reduce the amount of energy used during refiner mechanical pulping (1, 8, 19, 33) and increase paper strength properties (1, 20, 21, 33). Pretreatment of wood chips with *P. chrysosporium* prior to the Kraft pulping process also appears to yield significant benefits (22, 23).

Additional benefits from pretreating wood with fungi for biological processing include the reduction of wood extractives such as triglycerides, fatty acids, and resin acids (9, 11–13). These components, commonly called pitch, cause major problems during pulping and papermaking processes by increasing the frequency and length of machine shutdowns, increasing breaks in the paper roll, and decreasing paper strength (3, 36). Some fungi such as *Ophiostoma piliferum* are able to colonize wood chips and utilize resin components of the wood (9, 11–13, 39). A colorless strain of *O. piliferum* has recently been developed and is available commercially for the depitching of wood chips (9, 12). Wild-type blue stain fungi commonly colonize recently cut trees causing discoloration of the sapwood that lowers pulp and timber quality (30, 32). This colorless strain of *O. piliferum* has also been shown to inhibit detrimental wild-type blue stain fungi from colonizing freshly cut wood (4).

Prior to pulping, the majority of bark is removed through a variety of methods including mechanical tumbling of logs, shearing of the bark with knives, or using pressurized water to forcibly remove the bark (5). These processes often require large amounts of energy and can cause significant loss of wood fibers. Complete bark removal is essential before papermaking, since bark fragments contribute little useful fiber, darken pulp, consume chemicals, and leave dark flecks in the final paper product (5). A previous laboratory investigation using pectinolytic enzymes to facilitate bark removal has shown that savings in energy consumption of as high as 80% can be obtained during the debarking of spruce (26). The use of fungi to facilitate bark removal has only recently been shown to be effective (7).

This study investigated the use of *P. gigantea* as a fungal pretreatment for logs before they are processed for pulp and reports information on the extent of sapwood colonization, reduction in resin content, biopulping potential, and capacity to facilitate bark removal and to control detrimental blue stain fungi.

MATERIALS AND METHODS

Laboratory studies. Red pine trees (*Pinus resinosa* Aiton), 25 to 40 years old, were felled at the Cloquet Forestry Center, Cloquet, Minn. Logs had an average diameter of 10 cm and were cut into approximately 20-cm lengths. Cut logs were transported to the laboratory and inoculated 2 to 3 days after being cut. Twenty logs inoculated with *P. gigantea* and 20 noninoculated control logs were used for each laboratory study. A fungal culture of *P. gigantea* UM1993-3-3 (NRRL 21054), obtained from a red pine log at the Cloquet Forestry Center, was used as an inoculum source. This inoculum was grown on 2% malt extract broth (20 g of Malt Extract, Difco, Detroit, Mich.) for 2 weeks prior to inoculation. Fungal mats were removed from the petri dishes with a sterile glove, squeezed to remove excess broth, and pressed firmly against both ends of the log. Each inoculated log was placed in a clear plastic bag with one moist paper towel, and the bags were filled with air and closed. Inoculated logs were placed on a shelf in the laboratory at room temperature (20°C) under normal lighting conditions. Sampling of logs occurred at 16, 32, 64, and 100 days after inoculation, and five logs were randomly sampled at each sample date. A second laboratory study (study two) was

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set up in the same manner as described above to replicate study one. Five additional fungal mats of *P. gigantea*, not used in inoculations, were dried and weighed for both studies and found to have an average mycelial dry weight of 0.101 g (± 0.009 g).

Field studies. Red pine trees, 40 to 50 years old, were felled at the Cloquet Forestry Center in May of 1994 and 1995. Three field studies were carried out, one in 1994 and two in 1995 (designated a or b). Logs were cut into lengths of approximately 0.6, 0.8, and 2.5 m for the 1994, 1995a, and 1995b field studies, respectively, with an average diameter of 0.2 m. Inoculation of logs occurred 1 day after cutting. The 0.6- and 0.8-m logs were piled directly on the forest floor in a pyramidal shape, while the 2.5-m logs were stacked in a 4- by 4-log rectangular solid. The 1994, 1995a, and 1995b field studies consisted of 10, 10, and 16 logs per pile with three, four, and one log pile per treatment, respectively. A similar number of noninoculated control log piles were used. For the 1995 studies, 2.5-cm-diameter holes were drilled at four locations around the circumference of each log every 20 cm down its length. Sampling of logs occurred 4, 8, and 12 weeks after inoculation with an additional sampling at 16 weeks for the 1994 field study. In the 1994 study, two log segments (30 cm in length) from the ends of different logs were sampled from each of three piles (for a total of six logs per treatment). In the 1995 field studies two log segments 20 cm in length were sampled, one from the end of the log and one from the middle of the same log (drill hole in center of sample). Two logs were sampled from each of four piles (16 log segments) per treatment in the 1995a study, and three logs were sampled from each pile (six log segments) per treatment in the 1995b study. All field studies included a noninoculated control receiving only a water spray and an inoculated *P. gigantea* treatment. Spores from 2-week-old cultures of *P. gigantea* (same isolate and growing conditions as previously described) were harvested and used for the field studies. Spore concentrations in distilled water were calculated with a hemacytometer, and the concentrations for the 1994, 1995a, and 1995b field studies were 10^5, 3.2×10^5, and 3.1×10^5 spores/ml, respectively. The inoculum was sprayed with a hand sprayer at a pressure of 30 to 40 lb/in^2 onto the log ends and the outer surface of the log piles. The total volume of inoculum sprayed per log pile was 800, 1,000, and 2,000 ml for the 1994, 1995a, and 1995b field studies, respectively.

Analysis. Assessment of treatments both in the laboratory and in field studies included analyses of *P. gigantea* growth in logs, changes in pitch content, removal of bark from logs, and Simons' staining of refined wood fibers to determine fiber modifications. Analysis of *P. gigantea* growth in whole logs was carried out by aseptically splitting logs and removing wood chips (approximately 3 by 3 by 1 mm) from the sapwood and placing them in a semiselective medium for basidiomycetes similar to that used by Worrall (40) (15 g of malt extract, 15 g of agar, 0.06 g of benlate, 2 ml of lactic acid, and 0.1 g of streptomycin sulfate). Fungal colonies were allowed to grow for 1 to 2 weeks on the semiselective medium, and the percent colonization was then determined.

To determine pitch content, sampled logs were debarked and cut to a length of 20 cm. The heartwood was removed (if present), and only the sapwood portion of the log was used. One randomly selected log per treatment in the second laboratory study was examined along with one randomly selected log per treatment in all three field studies. Wood was manually cut into small segments that could be dried and ground with a Wiley mill. Ground wood was sifted to pass a 40-mesh screen and used for pitch analysis. At the time of cutting, extra freshly cut logs were placed in a freezer and were used to determine the amount of extractives present in logs at the time of cutting. Extraction of pitch in wood was carried out according to Tappi procedure no. 204 (37) by using dichloromethane. Percent pitch reduction was determined by comparing values between treated wood and noninoculated control wood.

Analysis of bark removal was conducted using five logs from each sample time in the laboratory study, 16 log segments (8 ends and 8 middles) from each sample time in the 1995a field study, and 6 log segments (3 ends and 3 middles) from each sample time in the 1995b field study. Each log pile in the 1995 field studies was sprayed with Dursban 4E insecticide (active ingredient Chlorpyrifos; Dow Elanco, Indianapolis, Ind.) to repel insects so bark removal would not be influenced by bark beetle colonization. A 0.8-cm-wide strip of bark was removed from the log longitudinally. The remaining bark was then peeled back from this strip, and the number of pieces removed as well as the resistance to peeling was rated. The following rating scale was used: 0, bark does not peel off; 1, bark peels off with great resistance in many pieces; 2, bark peels off with some resistance in a few pieces; 3, bark peels off with little to no resistance in one piece; 4, bark falls off in one piece with no resistance. Intermediate values of 0.5, 1.5, 2.5, and 3.5 were also used.

Fiber modification after mechanical refining was evaluated with the use of Simons' stain (6). One log was randomly selected from each treatment in the first laboratory study and from all three field studies. Wood chips obtained as described above were soaked for 24 h prior to mechanical refining with a Sprout-Waldron refiner. Wood chips were passed through the refiner with a distance of 0.04 in. between plates. Procedures described by Blanchette et al. (6) were used in these studies, with staining patterns based on cell wall modification determined over the entire length of the fiber instead of just at the fiber ends. Color change from blue to orange-yellow or to an intermediate transition color of green was used to rate the extent of modification as described by Yu et al. (41). Individual fibers were counted in three random fields of view at a magnification of ×320.

TABLE 1. Percent colonization of wood chips, removed from the sapwood of nonsterilized pine logs, by *P. gigantea* in two laboratory studies at different times after inoculation[a]

Study	Treatment	% Colonization after inoculation (days)			
		16	32	64	100
One	Control	0	0	20	8
	Treated	76	80	84	40
Two	Control	0	0	0	20
	Treated	80	76	76	84

[a] Each percentage is an average value from five logs.

Temperature study. A third field study was set up in 1995 to evaluate the effect of temperature on growth of *P. gigantea* in logs. Red pine logs were cut, piled, and treated as in the 1994 field study with two log piles per treatment. Four piles were placed in an open field and four piles were placed under the shade of a thick spruce stand (lower temperature). Logs were inoculated with spores as stated above with a concentration of 4.1×10^5 spores/ml. Logs were sampled at 2, 4, 6, and 8 weeks after inoculation. Analysis of four logs per treatment (two logs) was done as described above for the 1994 study. Maximum and minimum temperatures were obtained from the sites on a weekly basis.

Blue stain study. A fourth study was established in 1995 to evaluate the effect of *P. gigantea* as a biological control agent against blue stain fungi. Red pine logs were cut, piled, and treated as in the 1994 field study with holes drilled in the side of each log as stated above. Inoculation of logs was done with a spore concentration of 3.2×10^5 spores/ml. Analysis of six logs per treatment (two logs per pile) occurred at 4, 8, and 12 weeks after inoculation as stated for the 1994 study. Two wood chips were removed from each isolation point. The first chip was placed on a semiselective medium for basidiomycetes, and the second chip was placed on a medium selective for blue stain fungi (SDA; Sabouraud dextrose agar with 0.40 g of cycloheximide/liter, 0.05 g of chloramphenicol/liter, and 0.05 g of streptomycin sulfate/liter) (25). Percent colonization of *P. gigantea* and blue stain fungi was calculated. Percent blue stain for each log was also calculated with the use of an image analyzer and print monitor (Delta-T Devices, Burwell, Cambridge, England, and Ikegami Tsushinki Co., Utsunomiya-City, Japan, respectively). Stained areas on the surface of the split sapwood were traced onto acetate sheets, followed by calculation of the area stained using the image analyzer.

Statistical analyses. Data obtained from the laboratory and field studies were subjected to statistical analyses using the computer program SAS (SAS Institute Inc., Cary, N.C.). Logs in the laboratory study were each considered one replicate. In field trials, individual logs sampled from each pile were considered a replicate. Comparison of treatments was conducted using analysis of variance and least significant difference tests with a probability of 95% (0.05).

RESULTS

In field and laboratory studies, *P. gigantea* rapidly colonized sapwood of nonsterilized, inoculated logs (Tables 1 and 2). After 16 days in the laboratory studies, up to 80% of the wood chips removed from the sapwood were colonized. Maximum colonization (84%) of the log sections occurred 64 days after inoculation in laboratory study one and 100 days after inoculation in study two (Table 1). Logs from the 1994 field study

TABLE 2. Percent colonization of wood chips, removed from the sapwood of nonsterilized pine logs, by *P. gigantea* in three field studies at different times after inoculation[a]

Study	Treatment	% Colonization after inoculation (weeks)		
		4	8	12
1995a	Control	0	21	39
	Treated	82	100	100
1995b	Control	0	11	19
	Treated	67	100	97
1994	Control	0	0	19
	Treated	91	85	100

[a] Percentages for the 1995a, 1995b, and 1994 studies are average values from eight, three, and six logs, respectively.

TABLE 3. Analysis of pitch components in sapwood of pine logs treated with *P. gigantea* in the laboratory

Treatment	Percentage of pitch extracted from wood chips after inoculation (days)				
	0	16	32	64	100
Control	2.45	2.30	2.33	2.50	1.45
Treated	—[a]	2.55 (11)[b]	1.58 (−32)	1.25 (−50)	0.58 (−60)

[a] At 0 days only control logs were analyzed.
[b] Values in parentheses are percent differences in pitch content of treated logs compared to control logs.

TABLE 5. The effect of *P. gigantea* on bark removal of pine logs in two laboratory studies

Study	Treatment	Bark removal value after inoculation (days)[a]			
		16	32	64	100
One	Control	0.1 b	0.0 b	0.2 b	0.0 b
	Treated	1.2 a	3.0 a	3.1 a	2.7 a
Two	Control	0.0 b	0.1 b	0.3 b	0.5 b
	Treated	1.6 a	2.4 a	3.2 a	3.2 a

[a] See Materials and Methods for rating scale. Values in a column followed by the same letter are not significantly different according to the least significant difference test ($P = 0.05$).

were completely colonized after 12 weeks. Logs in both 1995 field studies, all of which had holes at 20-cm intervals, reached 100% colonization after 8 weeks (Table 2). A slight amount of colonization occurred in noninoculated control logs by *P. gigantea* after 8 weeks (Table 2). Percent colonization by *P. gigantea* in the noninoculated treatments varied between studies, with a maximum colonization of 20% obtained in the laboratory and 39% obtained in the field after 64 days and 12 weeks, respectively. Colonization of logs appeared faster and was more complete in the field studies compared to the laboratory study. Observations at 4 weeks showed a slower rate of colonization in the middle log segments compared to isolation attempts made from the log ends. However, after 8 weeks, colonization was similar throughout the log.

Pitch content in inoculated logs after 32 days in the laboratory study and 8 weeks in the field studies was reduced compared to noninoculated logs. As the length of incubation increased, the concentration of wood extractives decreased. After 64 days in the laboratory, 50% of the pitch components in the wood were removed compared to control wood (Table 3). Increases in the pitch content of treated logs compared to control logs were observed in both the field and laboratory studies after the first sampling date (4 weeks or 16 days, respectively), with percentages ranging from 7 to 52%. Reductions in pitch content of treated logs compared to control logs from the field studies ranged from 9% at 8 weeks in 1995b to 71% in 1994 after 12 weeks (Table 4). In general, a 25 to 71% reduction in pitch was observed after 8 weeks in field trials.

Bark removal was enhanced as time of incubation increased from 16 to 64 days in the laboratory, with maximum ratings of 3.1 and 3.2 obtained in study one and two, respectively (Table 5). As values for bark removal increased larger pieces of bark were removed with less resistance. Debarking values associated with enhanced bark removal were obtained in both laboratory studies after 32 days. A significant difference between inoculated and noninoculated treatments was observed at each sample time in both laboratory studies. In the 1995a field study, log segments from the ends of 0.8-m logs showed significant differences in bark removal values between inoculated and noninoculated treatments at both 8 and 12 weeks after cutting (Table 6), while segments from the middles of logs showed significant differences after 12 weeks (Table 6). In the 1995b field study, segments from the ends of 2.5-m logs showed significant differences between inoculated and noninoculated treatments at both 4 and 12 weeks (Table 6). A significant difference between inoculated and noninoculated logs was also observed at 4 weeks when middle log segments were evaluated in the 1995b field study. No significant differences between inoculated and controls were observed in either end or middle segments at 8 weeks.

Refined wood chips from logs inoculated with *P. gigantea* and treated with Simons' stain showed increased color changes suggesting modification of fibers. After 64 days in the laboratory, 12 weeks in the 1995a and -b field studies, and 16 weeks in the 1994 field study, high percentages of fibers stained yellow-orange (Tables 7 and 8). As the incubation time increased in the 1994 and 1995a studies, wood treated with *P. gigantea* yielded higher percentages of orange-yellow to green fibers. Noninoculated control treatments and fresh wood controls (frozen upon initiation of the study) in laboratory and field studies showed very little orange-yellow to green fiber coloration of refined wood. The percentages of orange-yellow to greenish fibers ranged from 13 to 20% for the fresh wood control samples and 12 to 30% for the noninoculated control treatments (Tables 7 and 8). Treatments inoculated with *P. gigantea* yielded maximum orange-yellow coloration percentages of 69 to 76% for refined fibers from the 1995a, 1994, and laboratory one studies (Tables 7 and 8), while the 1995b field study using 2.5-m logs yielded 43% maximum coloration (Table 8). During the mechanical refining process, fibers are shredded and torn, creating many fine fractions of individual

TABLE 4. Analysis of pitch components in sapwood of pine logs treated with *P. gigantea* in three field studies

Study	Treatment	Percentage of pitch extracted from wood chips after inoculation (weeks)			
		0	4	8	12
1995a	Control	5.38	2.45	2.10	1.39
	Treated	—[a]	3.00 (22)[b]	1.57 (−25)	0.98 (−29)
1995b	Control	5.38	2.65	1.73	1.75
	Treated	—	4.03 (52)	1.58 (−9)	1.27 (−27)
1994	Control	3.91	1.95	2.78	3.08
	Treated	—	2.08 (7)	1.08 (−61)	0.88 (−71)

[a] At 0 weeks only control logs were analyzed.
[b] Values in parentheses are percent differences in pitch content of treated logs compared to control logs.

TABLE 6. The effect of *P. gigantea* on bark removal of 0.8-m and 2.5-m pine logs in 1995a and 1995b field studies

Log section	Treatment	Bark removal value after inoculation (weeks)[a]					
		1995a (0.8 m) study			1995b (2.5 m) study		
		4	8	12	4	8	12
End	Control	0.1 a	0.2 b	0.1 b	0.3 c	0.0 a	0.2 b
	Treated	0.2 a	2.5 a	3.3 a	1.0 ab	0.0 a	2.5 a
Middle	Control	0.3 a	0.0 b	0.3 b	0.5 bc	0.2 a	0.2 b
	Treated	0.2 a	0.3 b	3.1 a	1.2 a	0.0 a	0.3 b

[a] See Materials and Methods for rating scale. Values in a column followed by the same letter are not significantly different according to the least significant difference test ($P = 0.05$).

TABLE 7. Percentage of refined wood fibers from logs inoculated with *P. gigantea* in the laboratory staining orange-yellow to green in color after application of Simons' stain

Treatment	% Wood fibers staining orange-yellow to green after inoculation (days)[a]			
	0	16	32	64
Control	16	28	19	17
Treated	—[b]	21	19	69

[a] Values in table are percentages of whole wood fibers, counting each individual fiber in the field of view, stained orange-yellow to green based on three random fields of view.
[b] At 0 weeks only control logs were analyzed.

whole fibers. When exposed to Simons' stain, these fibers stain orange to yellow, while intact fibers stain blue. The percentages of orange-yellow fibers in the fresh wood or noninoculated controls consisted mainly of these fine fiber fractions.

The difference in the extent of *P. gigantea* colonization in logs was not significant in an open field compared to a shaded environment at 4, 6, or 8 weeks after inoculation (Table 9). However, a significant difference was observed between field and shaded sites after 2 weeks (Table 9). *P. gigantea* colonized 100% of the sapwood after 8 weeks in both the field and forest sites. Temperatures in the open field site obtained a maximum of 39°C and a minimum of 1°C, while temperatures in the shaded area reached a maximum of 31°C and minimum of 14°C. The weekly average temperatures at the open field site were 6°C warmer during the day and 5°C cooler during the night than those in the shaded area.

Inoculation of *P. gigantea* to freshly cut logs inhibited subsequent growth and colonization by wild-type blue stain fungi (Table 10). No blue stain fungi were isolated from chips taken from treated logs, while 53% of the wood chips removed from noninoculated logs after 12 weeks had blue stain fungi present. In untreated controls colonization of the sapwood by blue stain fungi increased from 4 to 12 weeks, with the maximum colonization occurring after 12 weeks. Significant differences in the area of stained wood between logs from control and inoculated treatments were also found after 8 weeks based on assessment with an image analyzer (Table 10).

DISCUSSION

Results from both laboratory and field studies demonstrated that *P. gigantea* is able to quickly and successfully colonize freshly cut nonsterile wood. The ability of *P. gigantea* to be a pioneer colonizer of cut stumps in forest environments was shown by Rishbeth and Greig many years ago (14, 15, 27–29). Rapid colonization of stumps by *P. gigantea* inhibited subsequent colonization of the wood by *H. annosum*, proving that *P. gigantea* is an effective biocontrol agent. Primary colonization of cut wood typically begins with pioneer organisms such as blue stain fungi or common molds (e.g., *Trichoderma* sp.) (16, 34). Basidiomycetes are often considered as secondary colonists during microbial successions in wood since they commonly follow organisms that remove wood extractives or other toxic compounds from wood (16, 34). However, there are some wood-colonizing basidiomycetes, such as *P. gigantea*, that preferentially colonize fresh sapwood. Results presented here also show that *P. gigantea* invades the sapwood and degrades resin and other wood extractives, demonstrating that this fungus is an ideal candidate for use in biological processing.

Inoculation of holes on the sides of logs with *P. gigantea*, as well as the log ends, enhanced the amount of growth in logs compared to those inoculated only at the ends by reducing the distance that *P. gigantea* needed to grow in order to completely colonize the entire log. As shown in the 1995a and 1995b field trials, maximum colonization was obtained after 8 weeks, indicating that colonization of any size commercial log would be

TABLE 8. Percentage of refined wood fibers from logs inoculated with *P. gigantea* in 1994 and 1995 field studies staining orange-yellow to green after application of Simons' stain

Study	Treatment	% Wood fibers staining orange-yellow to green after inoculation (weeks)[a]				
		0	4	8	12	16
1995a	Control	20	24	26	25	ND[c]
	Treated	—[b]	7	54	76	ND
1995b	Control	20	30	12	14	ND
	Treated	—	22	19	43	ND
1994	Control	13	ND	23	ND	30
	Treated	—	ND	50	ND	73

[a] Values in table are percentages of whole wood fibers, counting each individual fiber in the field of view, stained orange-yellow to green based on three random fields of view.
[b] At 0 weeks only control logs were analyzed.
[c] ND, not determined.

TABLE 9. Percent colonization of logs by *P. gigantea* in an open field or shaded location[a]

Site	Treatment	% Colonization after inoculation (weeks)[b]			
		2	4	6	8
Open field	Control	0 c	0 b	0 b	0 b
	Treated	25 a	63 a	83 a	100 a
Shaded forest	Control	0 c	0 b	13 b	0 b
	Treated	17 b	63 a	83 a	100 a

[a] Maximum temperatures in the open field and shaded forest were 39 and 31°C, respectively, and respective minimum temperatures were 1 and 14°C. On average the field was 6°C warmer during the day and 5°C cooler during the night than the shaded forest.
[b] Values in table are the mean percentages of wood chips colonized by *P. gigantea* from a total of four logs per treatment. Values in a column followed by the same letter are not significantly different according to the least significant difference test ($P = 0.05$).

TABLE 10. Percentage of pine logs pretreated with *P. gigantea* colonized by blue stain fungi in a field study after 4, 8, and 12 weeks, as determined by isolation attempts and quantification of discolored wood using an image analyzer[a]

Treatment	% Log colonized by blue stain fungi after inoculation (wk)[b]			% Wood chips colonized by blue stain fungi after inoculation (wk)[c]			% Wood chips colonized by *P. gigantea* after inoculation (wk)[d]		
	4	8	12	4	8	12	4	8	12
Control	2 a[e]	22 a	28 a	3 a	22 a	53 a	0 a	8 a	31 a
Treated	0 a	0 b	0 b	0 a	0 a	0 b	67 b	100 b	100 b

[a] Values in table are mean percentages of wood colonized by blue stain fungi or *P. gigantea* from a total of six logs per treatment.
[b] Percentage of split sapwood surface discolored by blue stain fungi, determined by using an image analyzer.
[c] Percentage of wood chips that yielded growth of blue stain fungi on a semiselective medium for isolating blue stain fungi.
[d] Percentage of wood chips that yielded growth of *P. gigantea* on a semiselective medium for isolating basidiomycetes.
[e] The same letters within a column indicate values that are not significantly different according to the least significant difference test ($P = 0.05$).

possible if multiple inoculation points were used along the length of the log. Inoculation of pulpwood at the time of cutting would allow *P. gigantea* to colonize logs while they are in transport or storage. Our results also suggest that either shaded or full sun environments appear favorable for growth of this fungus in logs. *P. gigantea* has been reported from pine forests in many geographic regions and should be well suited for biological processing throughout the temperate regions of the world.

The maximum colonization percentage obtained from logs in laboratory studies (84%) was less than the maximum colonization percentage obtained from field studies (100%) (Tables 1 and 2). This was likely due to the artificial conditions associated with the plastic bag and increased moisture at wood surfaces. Previous studies have suggested that logs in plastic bags may decrease colonization of some fungi, as a result of increased moisture content or possibly reduced air exchange (4).

Degradation of wood resins by *P. gigantea* may serve to alleviate pitch problems at the pulp mill and increase wood porosity. These changes should enhance the diffusion of pulping chemicals into wood. In a previous study, inoculation of wood chips with a colorless strain of *O. piliferum* reduced the pitch content of wood and increased the penetration of Kraft pulp cooking chemicals (9, 12, 38). Improved penetration into wood chips and diffusion of cooking liquors into cell walls greatly influences pulping processes (31). Increased diffusion may decrease cooking time, reduce the amount of chemicals needed, and allow a greater concentration of lignin to be removed, resulting in easier bleaching. Economic benefits such as reduced number of paper breaks, reduced machine shutdown time, and increased paper strength may also occur when pitch is degraded. Results from logs in noninoculated treatments demonstrate that some wood extractives may be removed by naturally occurring fungi as the wood ages. Sapstain fungi such as *Ophiostoma* spp. and *Ceratocystis* spp. are pioneer colonizers capable of quickly colonizing freshly cut wood and utilizing the resinous pitch components (12, 39). Although these fungi degrade pitch, they impart detrimental stain to the wood that lowers wood quality (30, 32).

Successful colonization of logs by *P. gigantea* resulted in enhanced removal of bark. These results support Kubler's suggestion that fungi are important for facilitating bark removal (18). Fungal growth in the cambial layer, through physical and enzymatic activity, degrades the cambium, breaking the bond between sapwood and bark (24, 26). Increased moisture has also been suggested to facilitate bark removal, most likely due to the increased microbial activity with higher moisture contents (18). Excessive water, however, may create anaerobic conditions that inhibit microbial growth, making bark removal more difficult. In the study reported here, lower debarking values associated with the 2.5-m log study indicate that logs piled higher off the ground may have a more unfavorable microclimate due to decreased moisture. Little is known about the fungi that may enhance bark removal, but in our study it is clear that inoculation of logs with *P. gigantea* results in rapid colonization of the cambium by the fungus. Most likely, *P. gigantea* is using both physical and enzymatic processes to degrade the cambium. Introducing this fungus to the cambial region of cut logs enhances the rate of fungal colonization and greatly accelerates bark removal processes.

Examination of fibers made from refined wood colonized by *P. gigantea* revealed differences in staining patterns when Simons' stain was used. Staining of mechanically refined wood is mainly associated with fiber ends (6). Greater fibrillation during refining causes the fiber ends to stain orange-yellow compared to fibers from untreated wood that stain blue (35). Simons' stain has proven to be an excellent method for evaluating the effectiveness of various fungi for biological pulping prior to mechanical pulp production (2, 6). In our studies, it was noticed that the whole fiber was staining orange-yellow after treatment with *P. gigantea* and not just the fibrillated ends. This suggested that an increase in cell wall porosity of fibers from *P. gigantea*-treated material was taking place. Yu et al. (41) demonstrated that the high-molecular-weight orange dye has a greater affinity for large pores than the low-molecular-weight blue dye. Therefore, increased pore size in wood fibers leads to an increase in absorption of the orange dye. Yu et al. also observed fibers that stained green following Kraft pulping and indicated that this was due to a mixture of different pore sizes in cell walls (41). Results from both the laboratory and field studies presented here have shown an increase in whole fiber staining with coloration ranging from orange-yellow to green for logs treated with *P. gigantea*. These results demonstrate that pretreated logs have fibers with enhanced porosity and greater fibrillation after refining. These characteristics strongly suggest that significant improvements will be realized during both mechanical and chemical pulping processes. Additional studies are needed to determine specifically how much energy may be saved during commercial mechanical pulping processes and to elucidate how increased cell porosity may improve pulping efficiency.

P. gigantea appears ideally suited for biological processing of whole logs. Inoculation of logs immediately after cutting insures extensive colonization by this fungus. During the 4 to 12 weeks after inoculation, the period when logs are in transit to the mill or being stored for future use, modifications occur in the wood that improve pulping efficiency (i.e., pitch degradation and increased cell wall porosity). As the fungus grows it also inhibits detrimental sapstain fungi from colonizing the wood and accelerates bark removal.

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REFERENCES

1. **Akhtar, M., M. C. Attridge, G. C. Myers, T. K. Kirk, and R. A. Blanchette.** 1992. Biomechanical pulping of loblolly pine with different strains of the white rot fungus *Ceriporiopsis subvermispora*. TAPPI J. **75:**105–109.
2. **Akhtar, M., R. A. Blanchette, and T. A. Burnes.** 1995. Using Simons stain to predict energy savings during biomechanical pulping. Wood Fiber Sci. **27:** 258–264.
3. **Allen, L. H.** 1980. Mechanisms and control of pitch deposition in newsprint mills. TAPPI J. **63:**81–87.
4. **Behrendt, C. J., R. A. Blanchette, and R. L. Farrell.** 1995. Biological control of blue stain fungi in wood. Phytopathology **85:**92–97.
5. **Biermann, C. J.** 1993. Essentials of pulping and papermaking. Academic Press Inc., San Diego, Calif.
6. **Blanchette, R. A., M. Akhtar, and M. C. Attridge.** 1992. Using Simons' stain to evaluate fiber characteristics of biomechanical pulps. TAPPI J. **75:**121–124.
7. **Blanchette, R. A., R. L. Farrell, and C. J. Behrendt.** May 1996. U.S. patent 5518921.
8. **Blanchette, R. A., T. A. Burnes, M. M. Eerdmans, and M. Akhtar.** 1992. Evaluating isolates of *Phanerochaete chrysosporium* and *Ceriporiopsis subvermispora* for use in biological pulping processes. Holzforschung **46:**109–115.
9. **Blanchette, R. A., R. L. Farrell, T. A. Burnes, P. A. Wendler, W. Zimmerman, T. S. Brush, and R. A. Snyder.** 1992. Biological control of pitch in pulp and paper production by *Ophiostoma piliferum*. TAPPI J. **75:**102–106.
10. **Blanchette, R. A.** 1991. Delignification by wood-decay fungi. Annu. Rev. Phytopathology **29:**381–398.

11. **Brush, T. S., R. L. Farrell, and C. Ho.** 1994. Biodegradation of wood extractives from southern yellow pine by *Ophiostoma piliferum*. TAPPI J. **77:** 155–159.
12. **Farrell, R. L., R. A. Blanchette, T. S. Brush, Y. Hadar, S. Iverson, K. Krisa, P. A. Wendler, and W. Zimmerman.** 1993. Cartapip: a biopulping product for control of pitch and resin acid problems in pulp mills. J. Biotechnol. **30:**115–122.
13. **Fisher, K., M. Akhtar, R. A. Blanchette, T. A. Burnes, K. Messner, and T. K. Kirk.** 1994. Reduction of resin content in wood chips during experimental biological pulping processes. Holzforschung **48:**285–290.
14. **Greig, B. J. W.** 1976. Biological control of *Fomes annosus* by *Peniophora gigantea*. Eur. J. For. Pathol. **6:**65–71.
15. **Greig, B. J. W.** 1976. Inoculation of pine stumps with *Peniophora gigantea* by chainsaw felling. Eur. J. For. Pathol. **6:**286–290.
16. **Käarik, A. A.** 1974. Decomposition of wood, p. 129–174. *In* C. H. Dickinson and G. J. F. Pugh (ed.), Biology of plant litter decomposition. Academic Press, New York, N.Y.
17. **Kirk, T. K., J. W. Koning, Jr., R. R. Burgess, M. Akhtar, R. A. Blanchette, D. C. Cameron, D. Cullen, P. J. Kersten, E. N. Lightfoot, G. C. Myers, I. Sachs, M. Sykes, and M. B. Wall.** 1993. Biopulping: a glimpse of the future? U.S. Department of Agriculture Forest Service Research Paper FPL-RP-523. U.S. Department of Agriculture, Washington, D.C.
18. **Kubler, H.** 1990. Natural loosening of the wood/bark bond: a review and synthesis. Forest Prod. J. **40:**25–31.
19. **Leatham, G. F., G. C. Myers, T. H. Wegner, and R. A. Blanchette.** 1990. Energy savings in biomechanical pulping, p. 17–25. *In* T. K. Kirk and H.-M. Chang (ed.), Biotechnology in pulp and paper manufacture, application and fundamental investigations. Butterworth-Heinemann, Boston, Mass.
20. **Leatham, G. F., G. C. Myers, T. H. Wegner, and R. A. Blanchette.** 1990. Biomechanical pulping of aspen chips: paper strength and optical properties resulting from different fungal treatments. TAPPI J. **73:**249–254.
21. **Myers, G. C., G. F. Leatham, T. H. Wegner, and R. A. Blanchette.** 1988. Fungal pretreatment of aspen chips improves strength of refiner mechanical pulp. TAPPI J. **71:**105–108.
22. **Oriaran, T. P., P. Labosky, Jr., and P. R. Blankenhorn.** 1990. Kraft pulp and paper making properties of *Phanerochaete chrysosporium* degraded aspen. TAPPI J. **73:**147–152.
23. **Oriaran, T. P., P. Labosky, Jr., and P. R. Blankenhorn.** 1991. Kraft pulp and paper making properties of *Phanerochaete chrysosporium* degraded red oak. Wood Fiber Sci. **23:**316–327.
24. **Perem, E.** 1958. Bark adhesion and methods of facilitating bark removal. Pulp Paper Can. **59:**109–114.
25. **Prince, H. N.** 1989. Diagnostic medical microbiology, p. 595. *In* W. O'Leary, (ed.), Practical handbook of microbiology. CRC Press Inc., Boca Raton, Fla.
26. **Rätto, M., A. Kantelinen, M. Bailey, and L. Viikari.** 1993. Potential of enzymes for wood debarking. TAPPI J. **76:**125–128.
27. **Rishbeth, J.** 1963. Stump protection against *Fomes annosus*. III. Inoculation with *Peniophora gigantea*. Ann. Appl. Biol. **52:**63–77.
28. **Rishbeth, J.** 1952. Control of *Fomes annosus* Fr. Forestry **25:**41–50.
29. **Rishbeth, J.** 1950. Observations on the biology of *Fomes annosus*, with particular reference to East Anglian pine plantations. I. The outbreak of disease and ecological status of the fungus. Ann. Bot. **14:**365–383.
30. **Roff, J. W., A. J. Cserjesi, and G. W. Swann.** 1980. Prevention of sapstain and mold in packaged lumber. Forintek Canada technical report no. 14R. Forintek Canada Corp., Vancouver, Canada.
31. **Rydholm, S.** 1965. Pulping processes. Wiley, New York, N.Y.
32. **Seifert, K. A.** 1993. Sapstain of commercial lumber by species of *Ophiostoma* and *Ceratocystis*, p. 141–151. *In* M. J. Wingfield, K. A. Seifert, and J. E. Webber (ed.), *Ceratocystis* and *Ophiostoma*: taxonomy, ecology, and pathogenicity. American Phytopathological Society, St. Paul, Minn.
33. **Setliff, E. C., R. Marton, S. G. Granzow, and K. L. Eriksson.** 1990. Biomechanical pulping with white-rot fungi. TAPPI J. **73:**141–147.
34. **Shigo, A. L.** 1967. Successions of organisms in discoloration and decay of wood. Int. Rev. For. Res. **2:**237–299.
35. **Simons, F. L.** 1950. A stain for use in the microscopy of beaten fibers. TAPPI J. **33:**312–314.
36. **Smook, G. A.** 1992. Handbook for pulp and paper technologists, 2nd ed. Angus Wilde, Vancouver, Canada.
37. **Technical Association of the Pulp and Paper Industry, Chemical Properties Committee of the Process and Product Quality Division.** 1987. Solvent extractives of wood and pulp. T204 om-88. Technical Association of the Pulp and Paper Industry, Atlanta, Ga.
38. **Wall, M. B., J. B. Brecker, A. Fritz, S. Iverson, and N. Yves.** 1994. Cartapip^{R} treatment of wood chips to improve chemical pulping efficiency, p. 67–76. *In* Proceedings of the TAPPI Biological Sciences Symposium, Minneapolis, Minn. TAPPI Press, Atlanta, Ga.
39. **Wang, Z., T. Chen, Y. Gao, C. Breuil, and Y. Hiratsuka.** 1995. Biological degradation of resin acids in wood chips by wood-inhabiting fungi. Appl. Environ. Microbiol. **61:**222–225.
40. **Worrall, J. J.** 1991. Media for selective isolation of Hymenomycetes. Mycologia **83:**296–302.
41. **Yu, X., J. L. Minor, and R. H. Atalla.** 1995. Mechanisms of action of Simons' stain. TAPPI J. **78:**175–180.