Biological Control of Blue-Stain Fungi in Wood

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ABSTRACT

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Biological control of blue-stain fungi, such as *Ophiostoma* spp., that are detrimental to the wood products industry, was demonstrated in laboratory and field trials by a colorless strain of *O. piliferum,* Cartapip-97. This strain lacks melaninlike compounds responsible for the discoloration of sapwood. Inoculation of logs with Cartapip in the laboratory 2 wk before challenging with other fungi resulted in 58–68% colonization for Cartapip in isolated wood chips, while *O. piliferum, O. piceae, O. minus, Phanerochaete gigantea,* or *Trichoderma harzianum* colonized 0, 0, 0, 0, and 61%, respectively. Inoculation of logs with Cartapip 4 wk before other fungi resulted in similar trends with strong inhibition of blue-stain fungi. Simultaneous inoculation of logs with Cartapip and other fungi resulted in decreased colonization by both Cartapip and *Ophiostoma* species. When blue-stain fungi, *P. gigantea* or *T. harzianum* were inoculated 2 wk before Cartapip, colonization for these fungi ranged from 19 to 64% in cultured wood chips, whereas Cartapip ranged from 0 to 45% among the different treatments. Inoculation of *O. piliferum* and *O. piceae* prior to Cartapip resulted in inhibition of Cartapip. Two field trials demonstrated the exclusion of blue-stain fungi with prior colonization of the sapwood by Cartapip. Four weeks after inoculation of logs in the field, 92–100% of cultured wood chips were colonized by Cartapip in both trials, while blue-stain fungi colonized only 0–8%. In contrast, blue-stain fungi colonized 63% of the cultured wood chips in untreated control logs during the first field trial, and 29 and 71% for untreated control and antitranspirant treatments, respectively, during the second field trial. Results from both laboratory and field trials show the effectiveness of Cartapip for protecting freshly cut wood from blue-stain fungi.

Additional keywords: biocontrol, blue stain, biodeterioration, sap stain.

Blue-stain fungi cause a reduction in the value of timber or timber products by discoloring sapwood. The blue to black discoloration greatly reduces the aesthetic quality of timber but has little affect on wood strength (6,28,31,37). However, in mechanical pulping processes, blue-stained wood chips can cause a detrimental reduction in pulp quality, requiring an increase in bleaching chemicals to achieve high paper brightness (3,7). Methods used to control blue-stain fungi include saturating logs with water to inhibit fungal colonization (25) and application of inhibitory fungicides or chemicals (34,35,37). Previous investigations using biological control to prevent blue-stain fungi have had limited success in the laboratory but the ability of these organisms to successfully control blue stain under field conditions has not been demonstrated (2,15,23,30).

Blue-stain fungi, such as *Ophiostoma* and *Ceratocystis* spp., are primary colonizers that rapidly invade freshly cut wood. Hyphae can be found in all cells of the xylem, but ray parenchyma cells and resin canals are colonized preferentially (1,4,13,29). The discoloration imparted to the wood is due to melaninlike pigments in the hyphae (38).

Recent investigations have demonstrated that some stain fungi are able to degrade wood extractives such as triglycerides, fatty acids, and diterpenoid resin acids during wood colonization (3,5, 14). In paper manufacturing processes, prior removal of these compounds can be beneficial. Colorless strains of *Ophiostoma piliferum* (Fr.:Fr.) Syd. & P. Syd. lacking melanin have been identified and are currently being used in biological pulping processes, in which wood chips are inoculated before mechanical pulping, to remove problematic pitch. A colorless strain of *O. piliferum* is commercially available under the trade name Cartapip-97 (Sandoz Chemicals Corp., Charlotte, NC). Previous investigations have shown that unsterilized wood chips inoculated with this strain are rapidly colonized, have reduced amounts of pitch, and are protected from colonization by blue-stain fungi (3,5,14). This research evaluates the effectiveness of a colorless *O. piliferum* strain at protecting cut logs from wild-type blue-stain fungi in the laboratory and field and demonstrates how the colorless strain interacts in wood when inoculated with other fungi and at different time intervals.

MATERIALS AND METHODS

Laboratory study. Red pine (*Pinus resinosa* Aiton) trees approximately 15–20 yr old were felled at the Cloquet Forestry Center, Cloquet, MN. The trees were cut into 30.5-cm sections and transported to the laboratory. Inoculation of randomly chosen, unsterilized logs occurred 1–3 days after cutting.

Fungi used in the laboratory study included a colorless strain of *O. piliferum,* Cartapip-97 (obtained from Sandoz Chemicals Corp., Charlotte, NC), three wild-type blue-stain fungi (*O. piliferum*, *O. piceae* (Münch) Syd. & P. Syd., *O. minus* (Hedg.) Syd. & P. Syd.), *Phanerochaete gigantea* (Fr.:Fr.) Rattan et al in S. S. Rattan, and *Trichoderma harzianum* Rifai. *P. gigantea* and *T. harzianum* were obtained from red pine during a previous field study in Minnesota. Blue-stain fungi were obtained from *Pinus* spp. in the north central United States. To inoculate logs, cultures were grown in 2% malt-extract broth for 14 days prior to inoculation in order to allow fungal mat formation. Cultures were grown at room temperature (20 C) under natural laboratory lighting. To determine the average weight of the mycelial inoculum, five mats not used in inoculations were dried and weighed.

Averaged dry mat weights were as follows: Cartapip 0.105 g ±0.009, *O. piliferum* 0.093 g ±0.008, *O. piceae* 0.086 g ±0.013, *O. minus* 0.043 g ±0.002, *T. harzianum* 0.083 g ±0.009, and *P. gigantea* 0.180 g ±0.015.

Treatments included inoculation with *O. piliferum, O. piceae, O. minus, P. gigantea, T. harzianum,* or Cartapip alone; a water control; Cartapip inoculated simultaneously with each of the above fungi; Cartapip inoculated 2 wk after each of the other fungi; Cartapip inoculated 2 wk before the other fungi; and Cartapip inoculated 4 wk before the other fungi. A total of twenty-seven treatments were used with 8 logs per treatment.

Log ends were inoculated 1–3 days after cutting by placing one fungal mat on each end of a red pine log. Fungal mats were evenly spread over the entire end of the log using a sterile glove pressed firmly enough to ensure adherence. Individual inoculations occurred by placing one inoculum mat on the log end. Simultaneous inoculation of Cartapip with the other fungi involved mixing both mats by hand in a beaker, vortexing for 20 s, and placing them on a log end.

After inoculation, each log was stored at room temperature (20 C) in a clear plastic bag with two moist paper towels for 14 wk. After 3 wk, bags were opened to allow air exchange. Sampling and analysis of logs was carried out 6 and 14 wk after inoculation, with 4 logs randomly chosen at each sampling date. Sampled logs were flamed on both ends and split with a sterile ax using one half of the log for culturing. Isolation of fungi was done aseptically by removing wood chips with a dimension of approximately 3 × 3 × 1 mm from the sapwood of the split surface. Isolated wood chips were removed from the end of the log and at 1.3, 2.5, 5.1, 7.6, 10.2, 12.7, and 15.2 cm from the end of the log. At each interval, chips were taken from the edge of the log at regular spacing toward the heartwood. Isolated wood chips were placed on one of three media; a semiselective medium for basidiomycetes modified slightly from that used by Worrall (36) (1.5% malt-extract agar amended with 0.01 g/L streptomycin sulfate, 2 ml/L lactic acid, and 0.06 g/L 50% WP benlate), Sabouraud Dextrose media with 0.40 g/L cycloheximide, 0.05 g/L chloramphenicol, and 0.05 g/L streptomycin sulfate (27), and 1.5% Difco Malt Extract and Difco Agar. After 1–2 wk, fungal colonies growing on media were identified. The percentage of cultured wood chips that were colonized was determined by dividing the number of fungal colonies growing on media by the total number of wood chips removed from the end of the log to a depth of 10.2 cm into the sapwood (average 22 chips).

Field trial 1. The June field study at the Cloquet Forestry Center, Cloquet MN, had plots located in the southwest corner of the station (T-49, R-18, section 36). The site was located between a 2-yr-old clear-cut area and a mature red pine plantation. Red pine trees, approximately 60–70 yr old with an average diameter of 20.3 cm, were felled and cut into lengths of approximately 61 cm. Logs were inoculated 1–2 days after cutting.

Treatments consisted of a water control, an antitranspirant, Cartapip at a concentration of 5.1×10^7 cfu/ml with an antitranspirant, Cartapip treatment at 5.1×10^7 cfu/ml, and Cartapip treatment at 5.1×10^6 cfu/ml. The last concentration represents the inoculum concentration used in paper mills for spraying fresh wood chips (14). The antitranspirant used to retain moisture on the log surface was ForEverGreen (25% acrylic co-polymer from Mycogen Corporation, San Diego, CA). ForEverGreen (202 ml) was diluted in 1,420 ml of water.

Cartapip-97 was added to 1,420 ml of distilled water, mixed, and sprayed with a hand sprayer with a pressure of 207–276 MPa. Each log was individually sprayed, including bark and sawn ends, until slight runoff. Thirteen logs per treatment were sprayed individually, then piled into a pyramidal shape. The antitranspirant treatments were applied immediately after the Cartapip inoculation or after the water treatment used for controls.

Logs were sampled 4 wk after inoculation by cutting lengths of approximately 20.3 cm from 2 randomly chosen logs per treatment. Isolations were made from the sapwood as previously described for the laboratory study with the exception of the sample intervals. Wood chips were removed from the log end and at 1.3 and 2.5 cm from the end of the log. Four chips evenly spaced across the sapwood were removed at each interval. Wood chips were placed on selective media for isolating *Ophiostoma* spp. modified slightly from that used by Harrington (19) (0.01 g/L cycloheximide and 0.01 g/L streptomycin sulfate). The percentage of cultured wood chips colonized was determined by dividing the number of fungal colonies growing on media by the total number of wood chips removed from the log end to a distance of 2.5 cm into the sapwood (12 chips per log).

Additional Cartapip treatments, including Cartapip at 5.1×10^6 cfu/ml, Cartapip at 5.1×10^7 cfu/ml and Cartapip (5.1×10^7) cfu/ml with an antitranspirant, were added in order to examine the effect that time of inoculation after cutting had on sapwood colonization by Cartapip and blue-stain fungi. The additional treatments were carried out as stated above, except the time of inoculation for logs was 1–2 days, 2 wk, and 4 wk after cutting.

Field trial 2. A second field trial was initiated in late August 1992. Treatments were the same as those described for the previous field trial but with the Cartapip (5.1×10^6 cfu/ml) treatment deleted. Sampling of logs occurred as described above, with 3 logs assayed at each sampling time. Wood chips for culturing were removed from the log end, 0.95, and 1.90 cm in from the end of the log, with 4 chips evenly spaced across the sapwood at each interval. The percentage of cultured wood chips colonized was determined by dividing the number of fungal colonies growing on media by the total number of wood chips removed from the log end to a distance of 1.9 cm into the sapwood (12 chips per log).

Analysis of the field and laboratory data was performed using the statistical program STATISTIX (NH Analytical Software, Roseville, MN). Each log per treatment in the laboratory trial was considered a replicate, therefore the data was analyzed as a completely randomized design. Each log in field treatments, though sampled from the same log pile, was analyzed as a replicate using a standard ANOVA. ANOVA and Fisher's LSD test ($P = 0.05$) were performed, comparing the mean percentage of colonization between treatments.

RESULTS

Laboratory investigations. Visual observations of Cartapip-inoculated logs in the laboratory showed growth on log ends within 7 days, and dense mycelial growth over the entire cut surface 10–12 days after inoculation. The maximum distance colonized from the cut end by Cartapip was 7.6 cm and 15.2 cm at 6 and 14 wk, respectively, with an average growth of 6.8 mm per wk. The percentage of isolated chips colonized by Cartapip ranged from 0 to 65% at 6 wk and 19 to 66% at 14 wk (Table 1). The percentage of chips colonized at 6 and 14 wk showed a reduction in colonization, as the depth of the sampling interval from the end of the log increased (Table 1). Fourteen weeks after inoculation, 30 and 42% of the sapwood isolations yielded various Deuteromycete fungi (*Trichoderma* and *Penicillium* spp. were the most prevalent) at intervals of 1.3–2.5 cm and 1.3–5.1 cm, respectively.

Treatments with Cartapip inoculated 2 or 4 wk before the pigmented wild-type strains of *O. piliferum, O. piceae,* or *O.*

TABLE 1. Mean percentage[z] of isolated wood chips from the sapwood in inoculated treatments colonized by Cartapip at different intervals from the log end, at 6 and 14 wk after inoculation in the laboratory trial

Intervals (cm)	Time after inoculation (wk)	
	6	14
0.0–2.5	65	66
2.6–5.1	59	57
5.2–7.6	13	43
7.7–10.2	0	27
10.3–15.2	0	19

[z] Each value represents the mean from results of four logs.

minus, and before *P. gigantea* or *T. harzianum,* resulted in 48–76% of the isolated chips colonized by Cartapip (Table 2). Results showed a significant difference ($P = 0.05$) between treatments in which Cartapip was inoculated before *O. piliferum, O. piceae, O. minus,* or *P. gigantea,* and those in which Cartapip was inoculated after each of these fungi. Logs treated with Cartapip were free of blue stain (Fig. 1).

Inoculation of Cartapip simultaneously with *O. piliferum, O. piceae, O. minus, P. gigantea,* or *T. harzianum* resulted in Cartapip colonization percentages of 50, 36, 43, 7, and 72%, respectively (Table 2). Inoculation of Cartapip simultaneously with these fungi resulted in lower colonization percentages of Cartapip (Table 2).

Inoculation of *O. piliferum* to log ends 2 wk prior to Cartapip resulted in no Cartapip (0%) isolated from the wood chips (Table 2). Similarly, inoculation of *O. piceae* and *P. gigantea* prior to Cartapip resulted in severe inhibition of Cartapip, yielding colonization percentages for Cartapip of 6 and 5% (Table 2). Cartapip colonized 19% of the sapwood when inoculated 2 wk after *O. minus,* and 45% of isolated chips when inoculated after *T. harzianum* (Table 2). Logs inoculated with pigmented strains of blue-stain fungi were darkly discolored (Fig. 1).

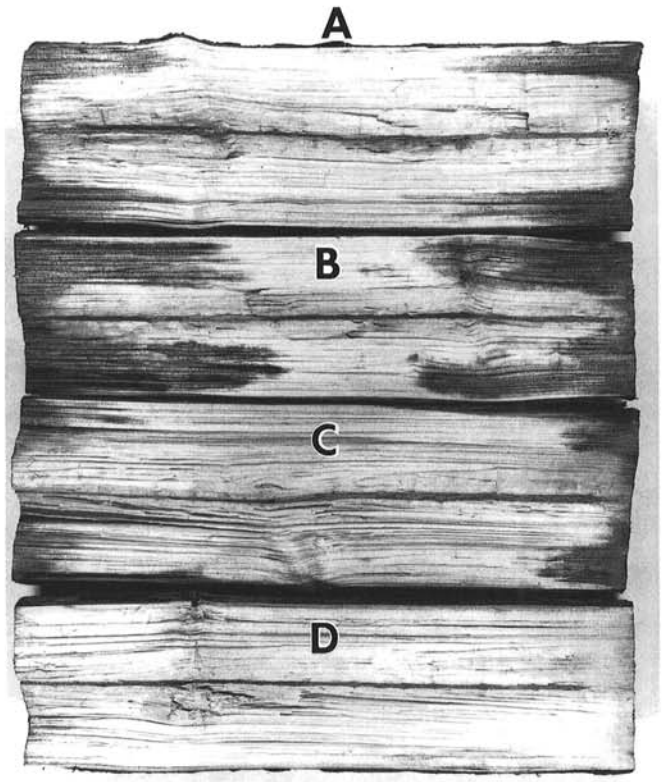

Fig. 1. Split logs of red pine from laboratory trial 6 wk after inoculation with wild-type *Ophiostoma piliferum* and Cartapip. Treatments from top to bottom represent **A,** inoculation of *O. piliferum* alone, **B,** *O. piliferum* inoculated before Cartapip, **C,** *O. piliferum* inoculated simultaneously with Cartapip, and **D,** *O. piliferum* inoculated 2 wk after Cartapip. Blue stain was most prolific in treatments in which wild-type strain of *O. piliferum* was inoculated before or simultaneously with Cartapip (**A** to **C**). No blue stain is evident in log treated with Cartapip 2 wk before treatment with *O. piliferum* (**D**).

TABLE 2. Mean percentage[y] of isolated wood chips from the sapwood colonized by Cartapip after 14 wk, when Cartapip was inoculated after, simultaneously, or before other fungi in the laboratory

Inoculation of logs with Cartapip	*Ophiostoma* spp.			*Phanerochaete gigantea*	*Trichoderma harzianum*
	piliferum	*piceae*	*minus*		
2 wk after	0 a[z]	6 a	19 a	5 a	45 a
Simultaneously	50 b	36 ab	43 ab	7 a	72 c
2 wk before	62 bc	58 b	66 b	59 b	68 bc
4 wk before	76 c	58 b	55 b	48 b	52 ab

[y]Each value represents the pooled mean from results of four logs analyzed to a depth of 10.2 cm from the end of the log.
[z]Letters within a column are significantly different according to Fisher's LSD test, $P = 0.05$.

Individual inoculation of log ends with *O. piliferum, O. piceae, O. minus, P. gigantea,* or *T. harzianum* resulted in wood-chip colonization percentages of 77, 44, 38, 44, and 77%, respectively, for each fungus (Table 3). Average laboratory colonization rates for wild-type blue-stain fungi were 7.3, 5.0, and 5.9 mm/wk for *O. piliferum, O. piceae,* and *O. minus.* Variations in the fungal colonization and growth rate of these fungi were observed at the species levels.

Sapwood was not colonized by blue-stain fungi or *P. gigantea* when inoculated 2 wk after Cartapip (Table 3). Exclusion of *O. minus* and *P. gigantea* was also observed when inoculation of Cartapip occurred 4 wk prior to these fungi (Table 3). Inoculation of *O. piliferum* and *O. piceae* to log ends 4 wk after Cartapip resulted in colonization percentages for *O. piliferum* and *O. piceae* of 1 and 10%, respectively (Table 3). *O. piliferum* and *O. piceae* were isolated from only 1 of 4 logs sampled for each treatment. There was no significant difference ($P = 0.05$) between Cartapip treatments when inoculated 2 wk before *O. piliferum, O. piceae, O. minus, P. gigantea,* or *T. harzianum* and 4 wk before these fungi (Table 3). A significant difference ($P = 0.05$) was observed between Cartapip treatments inoculated prior to *O. piliferum, O. piceae, O. minus,* and *P. gigantea* and individually inoculated control treatments (Table 3). When inoculated 2 and 4 wk after Cartapip, *T. harzianum* colonized 61 and 55% of the isolated chips, respectively.

Simultaneous inoculation of logs with blue-stain fungi and Cartapip resulted in chip colonization of 53, 22, and 0% for *O. piliferum, O. piceae,* and *O. minus,* respectively (Table 3). Colonization of the sapwood by *P. gigantea* and *T. harzianum* when simultaneously inoculated with Cartapip resulted in 62 and 76%, respectively (Table 3). Logs inoculated simultaneously with Cartapip and blue-stain fungi contained some blue stain (Fig. 1).

Isolations of wood resulted in 56, 55, 19, 51, and 64% colonization, by *O. piliferum, O. piceae, O. minus, P. gigantea,* or *T. harzianum,* respectively, when inoculated 2 wk before Cartapip in the laboratory (Table 3). Significant colonization of the isolated wood chips was obtained with all species when inoculated before Cartapip except *O. minus,* which colonized only 19% of the chips.

Field investigations. Visual observations of fungal growth on log ends in the first field trial showed good colonization by Cartapip 2 wk after inoculation. However, after 8 wk, Cartapip growth on the cut log ends was visually less obvious. The percentage of isolated chips colonized by Cartapip in treated logs was 100, 100, and 92% for treatments of Cartapip (5.1×10^6 cfu/ml), Cartapip (5.1×10^7 cfu/ml), and Cartapip with an antitranspirant, respectively (Table 4). No growth (0%) of Cartapip was observed in the chips of control or antitranspirant-

TABLE 3. Mean percentage[x] of isolated wood chips from the sapwood colonized by *Ophiostoma piliferum, O. piceae, O. minus, Phanerochaete gigantea,* or *Trichoderma harzianum* after 14 wk, when these fungi were inoculated after, simultaneously, or before Cartapip in the laboratory

Inoculation of logs with Cartapip	*Ophiostoma* spp.			*Phanerochaete gigantea*	*Trichoderma harzianum*
	piliferum	*piceae*	*minus*		
2 wk after	56 bc[y]	55 d	19 b	51 b	64 ab
Simultaneously	53 b	22 bc	0 a	62 b	76 b
2 wk before	0 a	0 a	0 a	0 a	61 ab
4 wk before	1 a	10 ab	0 a	0 a	55 a
Control[z]	77 c	44 cd	38 c	44 b	77 b

[x]Each value represents the pooled mean from results of four logs analyzed to a depth of 10.2 cm from the end of the log.
[y]Letters within a column are significantly different according to Fisher's LSD test, $P = 0.05$.
[z]Inoculation of logs with *Ophiostoma* spp., *P. gigantea,* or *Trichoderma harzianum* only, and not challenged by Cartapip.

alone-treated logs (Table 4). A significant difference ($P = 0.05$) was observed between Cartapip-treated and untreated logs, but no significant difference was observed among any of the Cartapip-treated logs. Growth of Cartapip on the bark of logs was not observed, and attempts to isolate Cartapip from the bark were unsuccessful. Colonization of blue-stain fungi from isolated chips yielded percentages of 63, 63, 0, 8, and 8% for control, antitranspirant alone, Cartapip (5.1×10^6 cfu/ml), Cartapip (5.1×10^7 cfu/ml), and Cartapip with antitranspirant treatments, respectively (Table 4). A significant difference ($P = 0.05$) in colonization of blue-stain fungi was observed between treated and untreated logs.

Visual observations of logs in the second field trial showed similar results. Cartapip colonized log ends 2 wk after inoculation and log ends appeared slightly white, but evidence of the fungus was not as obvious after 8 wk. Colonization of the chips by Cartapip yielded 4, 0, 96, and 96% for control, antitranspirant, Cartapip (5.1×10^7 cfu/ml), and Cartapip with antitranspirant treatments (Table 4). Statistical analysis of the results showed a significant difference ($P = 0.05$) between treated and untreated logs. The percent wood chips colonized by blue-stain fungi was 29, 71, 0, and 4% for control, antitranspirant, Cartapip (5.1×10^7 cfu/ml), and Cartapip with an antitranspirant (Table 4). No significant difference ($P = 0.05$) was observed between control logs and Cartapip-treated logs, but a significant difference was observed between antitranspirant- and Cartapip-treated logs.

Effect of time of inoculation on colonization of the sapwood. Results from the additional Cartapip treatments inoculated at different time periods showed that colonization percentages of blue-stain fungi increased as the time of Cartapip inoculation after cutting increased from 1–2 days to 4 wk (Table 5). Colonization percentages increased for blue-stain fungi from 0 to 33%, 8 to 50%, and 8 to 29% for treatments of Cartapip (5.1×10^6 cfu/ml), Cartapip (5.1×10^7 cfu/ml) and Cartapip with an antitranspirant, respectively (Table 5). In general, Cartapip colonization percentages decreased as the inoculation time increased from 1–2 days to 4 wk after cutting. Cartapip percentages decreased from 100 to 54%, 100 to 42%, and 92 to 38% for treatments of Cartapip (5.1×10^6 cfu/ml), Cartapip (5.1×10^7 cfu/ml) and Cartapip with an antitranspirant, respectively (Table 5). Greatest inhibition of blue-stain fungi and maximum colonization of Cartapip in sapwood were obtained when inoculation occurred 1–2 days after cutting.

TABLE 4. Mean percentage of isolated wood chips from the sapwood in field trial treatments (logs inoculated 1–2 days after cutting) colonized by Cartapip and blue stain fungi, at 4 wk after inoculation

Treatments	Field trial 1[w]		Field trial 2[x]	
	Cartapip	Blue stain fungi	Cartapip	Blue stain fungi
Control	0 a[y]	63 a	4 a	29 b
Antitranspirant	0 a	63 a	0 a	71 a
Cartapip (5.1×10^6)/ml	100 b	0 b	...[z]	...[z]
Cartapip (5.1×10^7)/ml	100 b	8 b	96 b	0 b
Cartapip and antitranspirant	92 b	8 b	96 b	4 b

[w] Each value represents the pooled mean from results of two logs analyzed to a depth of 2.5 cm from the end of the log.
[x] Each value represents the pooled mean from results of three logs analyzed to a depth of 1.9 cm from the end of the log.
[y] Letters within a column are significantly different according to Fisher's LSD test, $P = 0.05$.
[z] Treatment not carried out in second field study.

DISCUSSION

Results from laboratory and field studies demonstrated that Cartapip colonized freshly cut sapwood and excluded blue-stain fungi from becoming established. To successfully control blue stain it is essential that Cartapip colonize available substrates before other fungi become established. This colorless strain appears to be adapted for rapid resource capture, and, like blue-stain fungi, quickly dominates the sapwood, utilizing readily available compounds. Fatty acids, triglycerides, and resin acids within wood extractives, commonly referred to as pitch, are degraded during the early stages of Cartapip colonization (3,5,14). The ability of Cartapip and blue-stain fungi to colonize freshly cut wood appears to be associated with their capacity to tolerate and metabolize wood extractives (1,33).

Cartapip growth in the laboratory was most prevalent within the first 5 cm of the log end and decreased as distance increased into the interior of the log. The percent colonization of Cartapip in laboratory-inoculated logs was lower than in logs observed in the field studies, probably due to incubation conditions associated with the plastic bag. These conditions tended to cause elevated moisture levels on wood surfaces due to condensation within the bag, promoting growth or dispersal of Deuteromycetes, while inhibiting the growth of blue-stain fungi (25,31). The promotion of mold growth, such as *Trichoderma* and *Penicillium* spp., may have restricted some colonization of Cartapip and may be responsible for lowering the successful isolation of Cartapip from sapwood.

Successful colonization of Cartapip in laboratory logs resulted in nearly complete inhibition of all blue-stain fungi from the sapwood. In treatments in which blue-stain fungi were inoculated before Cartapip, colonization of the sapwood by blue-stain fungi resulted in the inhibition of Cartapip. Colonization of sapwood treated with *O. minus* was less than colonization with treatments using other blue-stain fungi or Cartapip. Other investigations have also found poor growth by *O. minus* compared with other blue-stain fungi (18,26).

Inoculation with Cartapip and *T. harzianum* in laboratory treatments resulted in colonization of sapwood by both fungi with little inhibition of either species. Colonization of the sapwood by *T. harzianum* and Cartapip occurred whether Cartapip or *T. harzianum* was inoculated first. Although the percentage of *T. harzianum* within the sapwood was high, it did not appear to interfere with the growth of Cartapip within the wood.

Colonization of the sapwood by *P. gigantea* occurred in the laboratory when inoculated before or simultaneously with Cartapip, but not when inoculated after Cartapip. This inhibition is most likely due to competition for readily available nutrients in the fresh sapwood. Previous studies have shown that *P. gigantea* is able to colonize logs in forest environments when blue stain is present (17,18,32). Logs in forest situations that have been previously colonized by Cartapip for an extended period of time would likely be colonized by *P. gigantea* or other white rot fungi following a typical succession of microorganisms (17,22,32).

In field trials, successful colonization of the sapwood was

TABLE 5. Mean percentage[z] of isolated wood chips from the sapwood in field treatments colonized by Cartapip and blue stain fungi 4 wk after inoculation of cartapip to the log end

Time of inoculation after cutting	Cartapip			Blue stain fungi		
	(5.1×10^6/ml)	(5.1×10^7/ml)	with antitranspirant	Cartapip (5.1×10^6/ml)	Cartapip (5.1×10^7/ml)	Cartapip (with antitranspirant)
1–2 days	100	100	92	0	8	8
2 weeks	100	92	96	17	21	8
4 weeks	54	42	38	33	50	29

[z] Each value represents the pooled mean from results of two logs analyzed to a depth of 2.5 cm from the end of the log.

obtained in all of the Cartapip treatments. The effectiveness of Cartapip at inhibiting blue-stain fungi appears to be related to colonization of the first 2.5 cm of sapwood, and not the inoculum concentration or the antitranspirant treatment used in the trials. However, materials such as antitranspirants that reduce water loss (8,12) may be desirable in dry environments to ensure germination by Cartapip and promote rapid growth in stacked logs.

Successful colonization by Cartapip and maximum exclusion of blue-stain fungi from the sapwood occurred when Cartapip was inoculated 1–2 days after cutting. Colonization by Cartapip was also substantial when inoculated 2 wk after cutting, but the percentage of blue-stain fungi colonizing the sapwood increased. To obtain the best control of blue-stain fungi, Cartapip should be inoculated as soon as possible after cutting to utilize the available substrate before other blue-stain fungi can become established.

The low percentage of blue-stain fungi colonizing sapwood in untreated control logs for the second field trial (initiated in August) may have resulted from lower amounts of rainfall and cooler temperatures associated with this field trial (climate weather data, Cloquet Forestry Center, Cloquet, MN). Certain environmental factors such as moisture and temperature have previously been shown to reduce blue stain growth (18,31). Reduced populations of bark beetles observed late in the season may also have an effect on the extent of blue-stain fungi in wood (16). The contamination of a few control logs with Cartapip also occurred. Cartapip growth in the control logs most likely resulted from rain and wind dispersing spores from treated to untreated logs, inadvertent contact during inoculation, or possibly animal or insect vectors. The spores of *Ophiostoma* spp. are easily dispersed by rain splash, wind, insects, animal vectors, or during harvest and processing operations (9,10,24).

Bark beetle activity was prevalent during the June field trial, and observations of logs 2 wk after treatment showed bark beetle colonization in the cambial region of many logs. Observations during the second field trial, initiated in August, showed very low levels of beetle activity and colonization. Bark beetles are able to carry spores of blue-stain fungi and introduce the spores beneath the bark (11,20,21). Since Cartapip is apparently unable to colonize the outer bark of logs, bark beetles may transmit propagules of blue-stain fungi already adhering to their bodies into the sapwood. While blue stain is controlled on the log ends by Cartapip, bark beetles, if present, may introduce blue stain into the sides of the logs. To keep logs free of blue stain during times of high populations of bark beetles, additional control procedures would be needed for preventing bark beetle attack. These could include debarking, application of insecticides or other methods (16).

This study demonstrates that a colorless strain of *O. piliferum*, is effective for protecting cut logs from colonization by blue-stain fungi. These results support previous observations of reduced blue stain in wood chips after treatment at pulp mills (3,5,14). This novel approach to controlling blue stain should have wide application in the forest-products industry to reduce detrimental sap stain in conifers.

LITERATURE CITED

1. Ballard, R. G., Walsh, M. A., and Cole, W. E. 1982. Blue-stain fungi in xylem of lodgepole pine: a light-microscope study on extent of hyphal distribution. Can. J. Bot. 60:2334-2341.
2. Benko, R. 1988. Bacteria as possible organisms for biological control of blue stain. International Research Group on Wood Preservation Document No. IRG/WP/1339.
3. Blanchette, R. A., Farrell, R. L., Burnes, T. A., Wendler, P. A., Zimmerman, W., Brush, T. S., and Snyder, R. A. 1992. Biological control of pitch in pulp and paper production by *Ophiostoma piliferum*. Tappi J. 75:102-106.
4. Boyce, J. S. 1961. Forest Pathology, 3rd ed. McGraw-Hill, New York.
5. Brush, T. S., Farrell, R. L., and Ho C. 1994. Biodegradation of wood extractives from southern yellow pine by *Ophiostoma piliferum*. Tappi J. 77:155-159.
6. Chapman, A. D., and Scheffer, T. C. 1940. Effect of blue stain on specific gravity and strength of southern pine. J. Agr. Res. 61:125-134.
7. Chidester, G. H., Bray, M. W., and Curran, C. E. 1938. Characteristics of sulphite and kraft pulps from blue-stained southern pine. Paper Trade J. 106:43-46.
8. Davis, T. S., and Fretz, T. A. 1972. The antitranspirant effect on harvested christmas trees. Am. Christmas Tree J. 16:3-5.
9. Dowding, P. 1970. Colonization of freshly bared pine sapwood surfaces by staining fungi. Trans. Br. Mycol. Soc. 55:399-412.
10. Dowding, P. 1973. Effects of felling time and insecticides treatment on the inter-relationship of fungi and arthropods in pine logs. Oikos 24:422-429.
11. Dowding, P. 1984. The evolution of insect-fungus relationships in the primary invasion of forest timber. Pages 133-135 in: Invertebrate-Microbial Interactions. J. M. Anderson, A. D. M. Rayner, and D. W. H. Walton, eds. Cambridge University Press, Cambridge.
12. El-Tamzini, M. I., and Elyatem, S. M. 1982. Antitranspirants: Vapor Gard and Wilt Pruf maintained the quality of Hamlin orange fruits. The Libyan J. of Agr. 11:103-108.
13. Eriksson, K. E., Blanchette, R. A., and Ander, P. 1990. Microbial and Enzymatic Degradation of Wood and Wood Components. Springer-Verlag, Berlin.
14. Farrell, R. L., Blanchette, R. A., Brush, T. S., Hadar, Y., Iverson, S., Krisa, K., Wendler, P. A., and Zimmerman, W. 1993. Cartapip: a biopulping product for control of pitch and resin acid problems in pulp mills. J. Biotechnol. 30:115-122.
15. Freitag, M., Morrell, J. J., and Bruce A. 1991. Biological protection of wood: Status and prospects. Biodeterioration Abstracts 5:1-13.
16. Furniss, R. L., and Carolin, V. M. 1992. Western Forest Insects; Miscellaneous Publication No. 1339. U.S. Dept. of Agriculture, For. Serv.
17. Gibbs, J. N. 1967. The role of host vigor in the susceptibility of pines to *Fomes annosus*. Ann. Bot., n.s. 31:803-815.
18. Gibbs, J. N. 1993. The biology of Ophiostomatoid fungi causing sapstain in trees. Pages 153-160 in: *Ceratocystis* and *Ophiostoma;* Taxonomy, Ecology, and Pathogenicity. M. J. Wingfield, K. A. Seifert, and J. F. Webber, eds. American Phytopathological Society, St. Paul, MN.
19. Harrington, T. C. 1981. Cycloheximide sensitivity as a taxonomic character in *Ceratocystis*. Mycologia 72:1123-1129.
20. Harrington, T. C. 1988. *Leptographium* species, their distributions, hosts and insect vectors. Pages 1-39 in: Leptographium Root Diseases on Conifers. T. C. Harrington and F. W. Cobb, Jr., eds. American Phytopathological Society, St. Paul, MN.
21. Harrington, T. C. 1993. Diseases of conifers caused by species of Ophiostoma and Leptographium. Pages 161-172 in: *Ceratocystis* and *Ophiostoma;* Taxonomy, Ecology, and Pathogenicity. M. J. Wingfield, K. A. Seifert, and J. F. Webber, eds. American Phytopathological Society, St. Paul, MN.
22. Käärik, A. A. 1974. Decomposition of wood. Pages 129-174 in: Biology of Plant Litter Decomposition, Vol. 1. C. H. Dickinson and G. J. F. Pugh, eds. Academic Press, New York.
23. Kreber, B., and Morrell, J. J. 1993. Ability of selected bacterial and fungal bioprotectants to limit fungal stain in ponderosa pine sapwood. Wood Fiber Sci. 25:23-24.
24. Land, C. J., Banhidi, Z. G., and Albertsson, A.-C. 1985. Surface discoloring and blue staining by cold-tolerant filamentous fungi on outdoor softwood in Sweden. Mater. Org. (Berl.) 20:133-156.
25. Liese, W., and Peek, R. 1984. Experiences with wet storage of conifer logs. Skovtek. Dansk. Skov. Tidsskr. (Sonderheft) 69:73-91.
26. Paine, T. D., Stephen, F. M., and Cates R. G. 1993. Host defense reactions in response to inoculation with *Ophiostoma* species. Pages 219-224 in: *Ceratocystis* and *Ophiostoma;* Taxonomy, Ecology, and Pathogenicity. M. J. Wingfield, K. A. Seifert, and J. F. Webber, eds. American Phytopathological Society, St. Paul, MN.
27. Prince, H. N. 1989. Diagnostic medical microbiology. Page 595 in: Practical Handbook of Microbiology. W. O'Leary, ed. CRC Press, Inc., Boca Raton, FL.
28. Scheffer, T. C. 1973. Microbial Degradation. Pages 31-106 in: Wood Deterioration and Its Prevention by Preservative Treatments. Vol. I. Degradation and Protection of Wood. D. D. Nicholas, ed. Syracuse University Press, Syracuse, NY.
29. Scheffer, T. C., and Lindgren, R. M. 1940. Stains of sapwood and sapwood products and their control. U.S. Dept. Agr. Tech. Bull. No. 714:1-123.
30. Seifert, K. A., Breuil, C., Rossignol, L., Best, M., and Saddler, J. N. 1988. Screening for microorganisms with the potential for biological control of sap stain on unseasoned lumber. Mater. Org. (Berl.) 23:81-95.
31. Seifert, K. A. 1993. Sapstain of commercial lumber by species of *Ophiostoma* and *Ceratocystis*. Pages 141-151 in: *Ceratocystis* and

Ophiostoma; Taxonomy, Ecology, and Pathogenicity. M. J. Wingfield, K. A. Seifert, and J. F. Webber, eds. American Phytopathological Society, St. Paul, MN.
32. Shigo, A. L. 1967. Succession of organisms in discoloration and decay wood. Int. Rev. For. Res. 2:237-299.
33. Shrimpton, D. M., and Whitney H. S. 1968. Inhibition of growth of blue-stain fungi by wood extractives. Can. J. Bot. 46:757-761.
34. Tarocinski, I. E., and Zielinski, M. H. 1982. Protection of pine sawtimber and sawn timber against blue stain in Poland. International Research Group on Wood Preservation Document No. IRG/WP/3193.
35. U.S. EPA, Office of Pesticide Programs, Registration Division. 1984. Wood preservative pesticides: Creosote, pentachlorophenol and the inorganic arsenicals: position document 4. Washington D.C.: Registration Division, Office of Pesticide Programs, Office of Pesticides and Toxic Substances, U.S. Environmental Protection Agency.
36. Worrall, J. J. 1991. Media for selective isolation of Hymenomycetes. Mycologia 83:296-302.
37. Zabel, R. A., and Morrell, J. J. 1992. Wood Microbiology: Decay and Its Prevention. Academic Press, San Diego, CA.
38. Zink, P., and Fengel, D. 1988. Studies on the colouring matter of blue-stain fungi. Holzforschung 42:217-220.