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The Yeast *Debaryomyces hansenii* as a Short-Term Biological Control Agent against Fungal Spoilage of Sawn *Pinus sylvestris* Timber

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Previous work has found isolates of the yeast genus *Debaryomyces* to be successful biological control agents against molding of fruits. An isolate of *Debaryomyces hansenii* was tested for its ability to protect sapwood of *Pinus sylvestris* timber against visual degrade by surface growth of molds and staining fungi. Successful protection of presterilized wood blocks inoculated with a mixture of common wood molding fungi was achieved when yeast was applied at a rate of 6×10^8 CFU/cm^2. Yeast cells were sprayed onto wood blocks at the same time as spoilage fungi and blocks were incubated under conditions favorable to fungal development for 15 and 25 days before assessment using a visual scale. Protection of presterilized blocks against blue staining by Ophiostomatoid fungi was achieved when yeast cells were applied at a rate of 2×10^6–6×10^8 CFU/cm^2. Significant reduction in disfigurement of nonsterile wood was demonstrated at a yeast application rate of 9×10^6–9×10^8 CFU/cm^2. Survival and reproduction of *D. hansenii* on sterilized *P. sylvestris* sapwood blocks were also determined across a range of relative humidities and temperatures previously found to support development of wood molding and staining fungi. Following 4 months incubation at 8–25°C and 93–100% relative humidity, between 46 and 473% of the number of colony-forming units applied to the wood were recovered. Maximum increase in viable yeast count on wood blocks occurred at 100% relative humidity and 15°C. © 2001 Academic Press

Key Words: **biological control; *Debaryomyces hansenii; Pinus sylvestris* timber; yeast; mold; blue stain; relative humidity; temperature.**

INTRODUCTION

The use of biological control has been investigated in the forestry and forest products industry as a possible method of limiting the considerable losses to decay of timber in standing trees (Falk, 1987; Davidenko and Tjunova, 1990; Hallaksela, 1993; Werner *et al.*, 1995), fungal disfiguration of freshly sawn timber and wood chips (Croan and Highley, 1992; Highley, 1992; Behrendt *et al.*, 1995; White-McDougall *et al.*, 1998), and decay of timber in service (Oxley, 1977; Highley, 1994; Bruce, 1998; Score *et al.*, 1998). Work has been fueled by the desire to reduce the use of chemical preservatives and fungicides, many of which have detrimental side effects on human health and the environment. Investigations have focused on the use of antagonistic fungi with fewer reports on the use of bacteria (Benko, 1988; Kreber and Morrell, 1993).

Research into biological control of fungal degradation of postharvest fruits and stored grains has included antagonistic yeasts (Droby *et al.*, 1989; Wisniewski *et al.*, 1991; McLaughlin *et al.*, 1992; McGuire, 1994; Mercier and Wilson, 1994; Filonow *et al.*, 1996; Petersson *et al.*, 1998). Tests have indicated that some yeast isolates are effective under conditions in which the fruit and grain are stored commercially. The benefits of the successful yeast antagonists, which can be readily isolated from the natural environment, include nonpathogenicity to plants and animals including humans, ease of maintenance in culture, and rapid reproduction.

Literature on the use of yeasts as biological control agents against wood-inhabiting fungi describes only simple *in vitro* tests and laboratory-based screening tests using wood blocks (Walker and McLeod, 1995; Payne and Bruce, 1999; Payne *et al.*, 2000). Optimal application rates of yeast isolates antagonistic to wood-inhabiting fungi and their tolerance to environmental conditions have not previously been determined *in situ*. The success of yeast in controlling molding of fruit and grain indicates that it is worthwhile to investigate their ability to protect freshly sawn softwood timber from colonization by molds and blue-stain fungi. Timber is vulnerable to disfiguration during air drying in the sawmill yard and during storage and subsequent use if exposed to rewetting.

The objectives of this paper are to investigate the potential of an isolate of the yeast *Debaryomyces han-*

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senii to control fungal degrade, to define the most effective application rate, and to investigate reproduction and survival on wood under conditions representing temperature and relative humidity previously found to allow fungal molding and blue staining of stacked timber in the sawmill yard.

MATERIALS AND METHODS

Effect of Debaryomyces hansenii Abundance on Fungal Disfiguration of Pinus sylvestris Sapwood Blocks

Blocks (100 × 22 × 15 mm) of undried Scots pine (*Pinus sylvestris* L.) sapwood were cut from boards freshly sawn from logs at James Jones & Sons sawmill, Kirriemuir, Scotland. Seventy knot- and bark-free blocks were sterilized by autoclaving twice. A further 35 similar blocks remained unsterilized. Seven replicate blocks were stacked on 90-mm-diameter Petri dish bases placed on the bottom of each of 15 plastic boxes (80 × 140 × 220 mm). High relative humidity was maintained in the boxes by lining the bottom with damp paper towels.

D. hansenii (Zopf) Lodder & Kreger-van Rij (isolate Y7036) was provided by Prof. A. Martini, Sezione Microbiologia Applicata, Universita di Perugia, Borgo XX, Giugno 74, I-06121, Perugia, Italy. Axenic cultures were prepared in 1 L conical flasks containing 600 ml of 2% (w/v) malt extract broth (Oxoid CM57). Cultures were incubated at room temperature on a rotary shaker (160 rpm) for 3 days. Cells were centrifuged out of suspension in 50-ml tubes at 1300*g* for 5 min. Cell pellets were resuspended and washed twice in sterile distilled water.

Concentration of viable cells in the washed suspension was determined using a dilution series-plate-count method before dilution in sterile water and application to the wood blocks using a hand spray bottle at a rate of 2 ml/block. Assuming that cells were spherical, that all cells were deposited on the wood blocks, and that the blocks had smooth surfaces, the yeast was applied to the undried nonsterile wood blocks at 9.85×10^8, 1.97×10^8, 4.93×10^7, and 9.85×10^6 CFU/cm^2. Mean cell diameter of *D. hansenii* was determined to be 3.071 μm using a Coulter Electronics Multisizer 2 (sample size = 2.5×10^5). Using this information, it was possible to calculate the thickness of cell layers deposited on the wood surfaces corresponding to each application rate (Table 1). Autoclaved blocks were inoculated with 6.56×10^8, 1.97×10^8, 1.97×10^7, and 1.97×10^6 CFU/cm^2.

The target blue-staining fungi *Ophiostoma piceae* (Munch) Syd.& P.Syd (IMI 361990) and *Ophiostoma piliferum* (Fr.) Moreau (VTT Z38) were grown in axenic culture on malt extract agar [2% (w/v) malt extract (Oxoid L39) and 1.5% (w/v) agar bacteriological (Oxoid L11)] at 20°C for 7 days. These fungi have previously been shown to be important components of the assemblage of fungi causing blue stain of softwood timber (Seifert, 1993; Uzunovic *et al.*, 1999). Spores and hyphal fragments were dislodged from mycelia by agitating sterile distilled water on the culture surface using a metal bacteriological loop. Suspensions produced in this way from the 2 fungi were combined, diluted to 50 ml, and sprayed onto 35 of the autoclaved wood blocks (7 replicate blocks for each of 4 yeast concentrations and 7 control blocks) at a rate of 0.5 ml/block. The CFU concentration for each fungus was determined using a dilution series-plating-out method. The resulting concentration of CFU/cm^2 wood surface was 1.6×10^5 for *O. piceae* and 2.5×10^5 for *O. piliferum*.

A suspension of the common timber disfiguring mold fungi *Penicillium brevicompactum* Dierckx (IMI 321324), *Aspergillus niger* v. Tieghem (IMI 329399), and *Cladosporium herbarum* (Pers.) Link (IMI 299105) was prepared and applied to the remaining 35 autoclaved blocks using a similar method giving 3014, 3362, and 265 CFU/cm^2, respectively. Unsterilized wood blocks were not inoculated with fungi.

Following inoculation of the wood blocks with fungi and/or yeast, lids were sealed onto the boxes with Parafilm M tape to maintain high relative humidity. Boxes were incubated under darkness at 20°C. After 15 and 25 days each block was examined for disfiguration, which was classified into 1 of 5 categories (Table 2), allowing mean values to be calculated for each treatment.

Control treatments consisted of autoclaved wood blocks incubated following inoculation with either molds or stain fungi but no yeast. Unsterilized wood block controls were incubated without addition of either fungi or yeast.

TABLE 1

Estimated Thickness of the Layer of *Debaryomyces hansenii* Cells Applied to Wood SurfacesExpressed as Number of Cells Deep

CFU/cm^2 block surface	Unsterilized wood	Sterilized wood	Thickness of layer of cells
9.85×10^8	—		72.97
6.56×10^8		—	48.63
1.97×10^8	—	—	14.59
4.93×10^7	—		3.65
1.97×10^7		—	1.46
9.85×10^6	—		0.73
1.97×10^6		—	0.15

Note. Data assume that cells were spherical, that all cells were deposited on the wood blocks, and that the blocks had smooth surfaces. In reality, these assumptions were unlikely to have been satisfied. The values should therefore be taken as maxima.

TABLE 2

Classification Scores for Visual Assessment of Wood Block Disfiguration

Disfiguration score	Description of disfiguration
5	Severe disfiguration over the majority of the wood block surface
4	Substantial disfiguration intensity over a large part of the wood block surface
3	Moderate disfiguration intensity over at least 20% of wood block surface
2	Slight production of pigmented spores or hyphae or coremia/perithecia over more than 10% of the block surface
1	Slight production of pigmented spores or hyphae or coremia/perithecia over less than 10% of the block surface
0	Clean to the naked eye, unaffected by fungal growth

Reproduction and Survival of Debaryomyces hansenii on Pinus sylvestris Sapwood Blocks over a Range of Temperature and Relative Humidity

Wood blocks (60 × 30 × 5 mm) were sawn from the sapwood of a single undried Scots pine board and glued using Ciba Araldite adhesive in pairs onto the inside of screw-cap lids of 250-ml glass jars which were then autoclaved twice. Oven dry moisture content of a sample of wood blocks after autoclaving = 131.64% ± 3.38 (mean ± standard error, $n = 7$). A 25-ml aliquot of saturated salt solution was added to each jar to create known relative humidity (RH) conditions; distilled water = 100%, K_2SO_4 = 98%, KNO_3 = 96%, and $ZnSO_4 \cdot 7H_2O$ = 93% (Viitanen and Ritschkoff, 1991).

Jars containing wood blocks and saturated salt solutions were stored for 5 weeks at room temperature to allow the wood to reach equilibrium moisture content with the RH within each jar. Ten jars at each RH level were then inoculated with 0.2 ml *D. hansenii* suspension (4.5×10^7 CFU/ml) prepared by growth in 0.2% (w/v) malt extract broth for 48 h at room temperature on a rotary shaker at 160 rpm, pelletized by centrifugation, resuspended in autoclaved distilled water, and washed twice.

The wood blocks in 2 jars were inoculated with yeast at each RH level and incubated at 8, 15, and 25°C. Following 4 months incubation, the ability of the yeast to survive and reproduce under the prevailing conditions on each wood block was determined. Individual blocks were removed from the jar lids and placed in 20 ml autoclaved distilled water in clean sterile glass jars before exposure to ultrasound for 30 s (Camlab Transsonic T310). Cells dislodged from the wood surfaces into suspension were counted using a Coulter counter following suitable dilution. A random selection of the washings was also suitably diluted and plated onto 2% malt extract agar for CFU enumeration in order to ensure that the data generated by the Coulter counter were representative of CFU values. Recovery of yeast from a number of blocks was also determined shortly after inoculation to act as controls.

RESULTS

Effect of Debaryomyces hansenii Abundance on Fungal Disfiguration of Pinus sylvestris Sapwood Blocks

The addition of *D. hansenii* to wood blocks reduced disfiguration by mold and blue-stain fungi added to autoclaved wood blocks and by fungi naturally present in undried unsterilized blocks in comparison to control blocks to which no yeast was added (Table 3). However, the number of viable yeast cells necessary to provide significant ($P < 0.05$) protection showed differences

TABLE 3

Disfiguration Scores for Sterilized Wood Blocks Inoculated with Mold or Stain Fungi and for Unsterilized Wood Blocks 15 and 25 Days After Inoculation with *Debaryomyces hansenii* across a Range of Application Rates

D. hansenii CFU/cm^2	Unsterile wood		Sterilized wood			
	15 days	25 days	Molds, 15 days	Molds, 25 days	Stain fungi, 15 days	Stain fungi, 25 days
9.8×10^8	1.00 ± 0.38*	1.14 ± 0.26*	—	—	—	—
6.5×10^8	—	—	0.29 ± 0.18*	0.57 ± 0.29*	0.00 ± 0.00*	0.14 ± 0.14*
1.9×10^8	0.42 ± 0.20*	1.42 ± 0.43*	3.00 ± 0.21	3.85 ± 0.14	0.14 ± 0.14*	1.28 ± 0.47*
4.9×10^7	1.28 ± 0.28*	2.00 ± 0.21*	—	—	—	—
1.9×10^7	—	—	2.86 ± 0.69	3.28 ± 0.75	0.29 ± 0.18*	2.57 ± 0.20
9.8 ± 10^6	1.42 ± 0.20*	1.85 ± 0.34*	—	—	—	—
1.9×10^6	—	—	3.28 ± 0.28	3.57 ± 0.78	0.43 ± 0.20*	2.28 ± 0.18
Control	3.43 ± 0.20	3.85 ± 0.14	3.00 ± 0.0	3.57 ± 0.78	2.71 ± 0.18	3.00 ± 0.37

Note. Data represent mean ± standard error of 7 replicates for each treatment.
* Treatment mean significantly different ($P < 0.05$) from control mean for that column.

TABLE 4

Effect of Temperature (8–25°C) and Relative Humidity (93–100%) on Mean Number of *Debaryomyces hansenii* Cells in Washings from Wood Blocks Following 4 Months Incubation

Temperature (°C)	Relative humidity (%)				Totals
	100	98	96	93	
8	367.4	315.4	307.5	229.4	1219.7
15	473.6	318.8	287.0	46.9	1126.3
25	363.2	143.3	123.4	157.6	787.5
Totals	1204.2	777.5	717.9	433.9	—

Note. Data represent percentages of the number of yeast cells which were dislodged from blocks using the same method immediately after application, when mean = 1.6×10^6 cells/cm^2.

between treatments and in some treatments with increasing incubation time. Nonsterile wood developed significantly lower mean disfiguration scores at all tested application rates of the yeast at both 15 and 25 days incubation than untreated control blocks. For sterile wood inoculated with mold fungi, only the highest tested yeast application rate (6.5×10^8 CFU/cm^2) was effective in significantly reducing mean disfiguration compared to the control. The mean disfiguration score of sterile wood blocks inoculated with stain fungi was significantly reduced at all yeast application rates after 15 days incubation but only the higher 2 application rates (1.9×10^8 and 6.5×10^8 CFU/cm^2) remained successful after 25 days.

Reproduction and Survival of Debaryomyces hansenii on Pinus sylvestris Sapwood Blocks over a Range of Temperature and Relative Humidity

The use of a Coulter counter to determine CFU counts in wood block washings was shown to be acceptable by comparison with the number of colonies produced by a plate-count method when a random selection of washings was tested. Comparison of the data sets indicated a correlation coefficient of 0.929 ($P = 0.007$). Therefore, enumeration of cells dislodged from wood surfaces by ultrasonication provided an acceptable indication of total viable cells present on the surface of the wood blocks. The number of cells available to be dislodged was a factor of the number applied and any reproduction or cell breakdown arising from incubation under the tested conditions.

Washings generated from blocks soon after inoculation with yeast contained $1.6 \times 10^6 \pm 1.88 \times 10^5$ cells/ml (mean ± standard error, $n = 7$). Mean CFU counts for washings from the 4 replicate blocks incubated at each temperature/RH combination indicated that, in most cases, the yeasts had multiplied (Table 4). Greatest growth was found under saturated RH conditions, with 3.6–4.7 times the number of cells in the washings as washed from blocks immediately after inoculation. Of the temperatures tested, the greatest increase in cell number developed at 15°C.

Summing the number of cells washed off wood blocks across all RH levels tested at each temperature indicated an inverse relationship between temperature and cell number over the range 8–25°C. Total number of cells washed off wood blocks at each of the RH levels tested summed across all temperatures indicated a clear positive relationship between RH and cell number. Cell number at each RH level and temperature indicated only a single incidence of decline in comparison to the number recovered soon after inoculation of the blocks, at 15°C and 93% RH. All other incubation conditions resulted in increased cell number.

The number of cells washed off blocks incubated at 15°C approximately trebled after incubation at 96–98% RH and almost 5 times the number of cells were recovered from blocks incubated at 100% RH. At 8°C, RH was of minor importance; only a small change in recovery developed across the range tested. Incubation at 25°C resulted in a large decline in recovery between 100 and 98% RH with little further change at lower RH.

DISCUSSION

Effect of Yeast Abundance on Biological Control Efficacy and Potential Methods of Antagonism

The tested yeast isolate was an effective biological control agent of molding and staining of pine sapwood under the conditions used. While the isolate did not entirely prevent spoilage of timber surfaces, a significant and very marked reduction in visual degrade developed, particularly when the yeast was applied to wood surfaces at the higher application rates tested. The yeast was antagonistic to the development of molds and stain fungi in their natural substrate and under conditions of RH and temperature which support their development in laboratory and field tests (Payne *et al.*, 1999, 2000).

Comparison of the results to work conducted using *Debaryomyces* spp. as biological control agents against molding of fruit is difficult because of the different substrate, target fungi, and incubation conditions. However, comparison of cell density needed to achieve control indicated similarities. Isolates of *Debaryomyces* spp. (sometimes referred to as *Pichia*) inhibited mold fungi when applied to fruit surfaces and wounds at a rate of 10^4–10^7 CFU/cm^2 (McGuire, 1993; Filonow *et al.*, 1996). Such cell densities are comparable with the number reported to reduce wood spoilage in this work. Droby *et al.* (1989) reported a negative correlation between cell abundance and molding over the range 10^5–10^9 CFU/ml when applied to fruit wounds, but did not report the number of cells/cm^2.

A greater yeast abundance was required to reduce disfiguration of autoclaved blocks by molds than stain

fungi. Only the highest density tested significantly reduced degrade by molds, although all the tested concentrations were effective at reducing spoilage by stain fungi (at 15 days incubation). These results suggest that blue-stain fungi are more susceptible to antagonism by the yeast than molds, although disfiguration by blue stain increased more between 15 and 25 days than the increase shown by molds. This may result from a reduction in the rate of growth or melanization of hyphal walls by stain fungi in the presence of the yeast. Efficacy against wood spoilage by mold fungi relies largely on the ability of the biological control agent to prevent or reduce the production of pigmented spores. Mycelial growth by most molds is hyaline and relatively insignificant in causing spoilage to timber.

Disfiguration of wood by stain fungi was significantly reduced by even the lowest yeast application rate tested when compared to control blocks. At the lowest application rate, yeast cells were not sufficiently abundant to cover the wood surface (Table 1). This provides some information about the mode of action of the yeast. Simple physical exclusion of stain fungi inoculum from the wood surface cannot have been solely responsible for reduced disfiguration. However, with molds, a layer calculated to be up to 14 yeast cells thick was insufficient to reduce disfiguration but a thicker layer of up to 48 cells reduced molding considerably. Previous work with *Debaryomyces* sp. by Droby *et al.* (1989) found that killed cells and culture filtrate were ineffective against mold fungi, implying that production of antibiotic substances was not responsible for the observed biological control. The authors concluded that nutrient competition was responsible for mold reduction under conditions of low nutrient availability. Furthermore it has been found that yeasts assimilate nutrients more quickly than germ tubes of fungi (Brodie and Blakeman, 1976; Blakeman and Brodie, 1977; Fokkema, 1981). The observed correlation between number of cells applied to wood surfaces and the resulting reduction in disfiguration might be explained by a rapid uptake of nutrients by yeast from wood surfaces. The greater the abundance of yeast, the more rapidly nutrients may have become depleted. At a certain rate of cell application, total available nutrients or possibly a certain class of nutrients vital to fungal development may have been assimilated by the yeast cells to below a certain threshold level before the fungi had developed sufficiently to cause disfiguration. It is possible that the more abundant yeast cells tolerated by molds reflect quicker germination and a greater ability to compete for nutrients than stain fungi.

Potential of Debaryomyces hansenii as an Effective Biological Control Agent

The results indicate that this isolate of *D. hansenii* can be an effective biological control agent against molding and staining fungi on wood. While laboratory-based tests cannot substitute for field trials (which are currently underway), the experiments were conducted under conditions conducive to fungal degrade of freshly sawn timber in the sawmill yard. Survival, reproduction, and effective antagonism by a potential biological control isolate at saturated RH are particularly important because previous field observations have indicated that fungal disfiguration of softwood timber was particularly severe when this condition was maintained (Payne *et al.*, 1999). However, laboratory-based tests have indicated that the common timber staining fungus *O. piceae* can cause disfiguration of wood surfaces through production of melanized hyphae, perithecia, and coremia at constant RH above 93% (Payne *et al.*, 2000). Common wood molding fungi have been found to grow and sporulate only at RH above 90% (Wang, 1994). The results presented in this paper indicate survival of *D. hansenii* across the range of environmental conditions producing risk of disfiguration. Reduced reproduction of the isolate on wood at lower RH does not necessarily imply poor wood protection because spoilage fungi are also less virulent under such conditions. The yeast generally multiplied to a greater extent on wood at relatively low temperature; cell number following incubation at 8 and 15°C was higher than at 25°C across most of the RH range tested, indicating that the yeast would not produce maximum cell number under temperature conditions particularly favorable to fungal growth. The target fungi generally respond positively to increased temperature within the range used (Payne, 1996), possibly limiting the efficacy of the yeast under extreme conditions.

To be effective at reducing spoilage of timber in sawmill yards, the yeast must maintain effective antagonism for approximately 1 month. Sawn timber rarely remains stored in the sawmill yard for longer periods. Furthermore, timber stacked for air drying would be expected to have developed a sufficiently low moisture content to no longer be at risk of fungal spoilage after this time. The results indicate that the yeast substantially reduced both molding and staining of wood blocks over a 25-day period. Furthermore *D. hansenii* survived on wood for at least 4 months, during which time cells also multiplied 3- to 4-fold at saturated RH across the temperatures tested. An ability to survive across a range of temperature is important because of the diurnal and seasonal variability of outdoor temperature in temperate climates. Other isolates of *Debaryomyces* have previously been found to be tolerant of a wide range of temperature and extremely varied osmotic potentials by Magan and Lacey (1986) which is beneficial if the yeast is to survive on wood under fluctuating moisture conditions. A further promising aspect of the yeast is an undemanding nutritional base, being able to utilize many carbohydrates and organic acids (McLaughlin *et al.*, 1990).

Survival and reproduction of *D. hansenii* on sapwood surfaces indicate that it is not necessary to supplement with nutrients. Addition of nutrients to wood would carry the risk of stimulating development of disfiguring fungi by improving the typically low nutrient status offered by wood, particularly in terms of nitrogen availability, thus potentially reducing the effect of nutrient competition between fungi and yeast. Furthermore, wood surfaces which have become enriched with water-soluble nutrients, as a result of kiln drying, have been found to carry an increased susceptibility to molding (Theander *et al.*, 1993).

The ability of *D. hansenii* to effectively reduce spoilage of sterilized wood inoculated with known fungi indicated an ability to antagonize selected common wood disfiguring fungi in their natural substrate. The use of green wood represented an attempt to obtain information about the ability of the isolate to compete at wood surfaces against the naturally present microorganisms in wood, an aspect not tested by the use of autoclaved wood. The results indicated that the yeast was also successful in this respect. Reduced disfiguration of green wood in the presence of the yeast indicated broad antagonism against many wood disfiguring fungi. The yeast was also presumably able to compete for nutrients against this more complex community of spoilage microorganisms in order to remain active.

A good biological control agent must not only survive on the substrate and remain antagonistic to the targets, but must also remain attached to the substrate. In this respect, the use of yeasts may be at a disadvantage because of the risk of them being washed off the wood. Unlike filamentous fungi, yeasts do not form a mycelium which anchors them to the substrate and are therefore at risk of becoming dislodged and washed away by water. However, *Debaryomyces* (or *Pichia*) isolates were found by Wisniewski *et al.* (1991) to attach strongly to the hyphae of mold fungi. This feature may protect yeast cells in contact with hyphae from becoming easily washed away. Furthermore, at this stage the yeast is primarily being investigated as a potential biological control agent for timber stored in the sawmill yard, where it is exposed to rain mainly on the upper surface of stacks. Such potential loss of yeast through the action of running water is unlikely to be important in practice because previous observations of stain development within stacks of sawn timber indicated that most degrade developed in the center of stacks where yeasts are unlikely to be exposed to running water (Payne *et al.*, 1999).

CONCLUSION

- *D. hansenii* survived and reproduced on *P. sylvestris* sapwood surfaces for at least 4 months under temperature and relative humidity conditions previously found to support growth and sporulation of common wood molds and blue-staining fungi.
- *D. hansenii* significantly and considerably reduced disfiguration of autoclaved sapwood blocks inoculated with mixtures of common wood-inhabiting molds or blue-stain fungi over a 25-day period when applied at a rate of 6.5×10^8 and 1.9×10^8 CFU/cm^2, respectively.
- Disfiguration of unsterilized sapwood blocks was significantly reduced by *D. hansenii* when applied at all tested rates (10^6–10^8 CFU/cm^2).
- Physical exclusion of fungal inoculum from the substrate appeared not to be the primary method of antagonism.

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