Biodegradation of Turf Thatch With Wood-Decay Fungi

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ABSTRACT

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Thirteen wood-decay fungi were tested for ability to degrade turf thatch components. Four of these, *Fomes fomentarius, Coriolus versicolor, Gloeophyllum trabeum,* and *Ganoderma applanatum* grew on and substantially reduced the weight of bermudagrass pellets, and St. Augustine grass and zoysia grass stolons under laboratory conditions. Zoysia grass stolon decay occurred in an atmosphere saturated with water, but not at vapor pressure deficits of 7.59 or 15.17 mbar. Optimum temperatures for fungus growth on agar media were not correlated with thatch weight loss at corresponding temperatures. Weight losses of zoysia grass stolons exposed to the four test fungi at 24 and 32 C were not significantly different, indicating that temperature was not as critical as vapor pressure for stolon decay. Electron microscopy revealed that lignocellulose was degraded in zoysia grass stolons exposed to these fungi. The effectiveness of the fungi in reducing thatch in the field was examined, but under the test conditions employed there was no indication that thatch was degraded.

Additional key words: biological control, lignocellulose breakdown, turf thatch control.

In modern turfgrass culture, an excessive layer of dead rhizomes, stolons, leaves, and roots may accumulate between the living grass and the soil surface. This layer of organic matter, called thatch, when excessive, causes grass to be shallow-rooted, less drought resistant, and more prone to heat and cold injury (3,13,19).

Thatch is often removed mechanically by mowing, coring, slicing, and spiking (3); however, these processes are expensive, time-consuming, and temporarily disrupt the turf.

Attempts were made to control thatch biologically by adding topdressing and various soil amendments (8,13). In these tests topdressing appeared to increase thatch decomposition, water penetration, and soil aeration (8). Murdoch (156) tested two commercial products for biological degradation of thatch and found no effect.

Thatch consists of cellulose, hemicelluloses, and lignin, and contains approximately twice the lignin content of live grass, which accounts for its high degree of decay resistance (13). Several microorganisms are known to be lignolytic (6,7,12,16,17). Lignin- and cellulose-decomposing basidiomycetous wood-decay fungi that cause white or brown rot probably have been studied the most intensively. White- and brown-rot fungi both metabolize cellulose, but only the white-rot fungi completely decompose and assimilate lignin (12).

Since no completely satisfactory method of thatch control by accelerated biological degradation has been developed, tests were conducted under controlled conditions to determine the feasibility of degrading turf thatch with white- and/or brown-rot fungi.

MATERIALS AND METHODS

The following fungi were tested for relative ability to decompose various thatch or grass substrates: *Coriolus versicolor* (L. ex Fr.) Quel. (*Polyporus versicolor* L. ex Fr.); *Schizophyllum commune* Fr.; *Gloeophyllum trabeum* (Pers.) Murr. (*Lenzites trabea* Pers. ex Fr.); *Inonotus tomentosus* (Fr.) Gilbn. (*Polyporus tomentosus* (Fr.); *Phellinus robustus* (Karst.) Bourd. et Glaz. (*Fomes robustus* Karst.); *Phellinus pini* (Tjore per Fr.) Pilát (*Fomes pini* [Brot. ex Fr.] Karst.); *Fomes fomentarius* (L. per Fr.) Kickx.; *Lentinus lepideus* Fr.; two isolates of *Ganoderma applanatum* (Pers. ex S. F. Gray) Pat.; *Piptoporus betulinus* (Bull. per Fr.) Karst. (*Polyporus betulinus* Bull. ex Fr.); *Poria placenta* (Fr.) Cooke (*Poria monticola* Murr.); and *Inonotus dryophilus* (Berk.) Murr. (*Polyporus dryophilus* Berk.). The isolates were obtained from F. H. Tainter (Department of Plant Pathology, University of Arkansas, Fayetteville) and were maintained in culture on Difco potato-dextrose agar (PDA).

Weight-loss measurements were made to estimate tissue degradation of: bermudagrass (*Cynodon dactylon* [L.] Pers., 'Common') pellets containing compressed leaf, stem, and stolon tissue; St. Augustine grass (*Stenotaphrum secundatum* [Walt.] Kuntze) stolons; and zoysia grass (*Zoysia japonica* Steud., 'Meyer') stolons. The weight-loss experiments involving bermudagrass pellets and St. Augustine grass stolons were conducted in 237-ml French-square bottles (Fisher Scientific Co., Dallas, TX 75240), a modified version of the soil-block method of evaluating wood decay, with soil-block chambers prepared according to ASTM standards (2). All bottles containing soil and southern yellow pine feeder strips were autoclaved for 20 min at 1.4 kg/cm^2. Feeder strips were inoculated with 8-mm-diameter plugs of mycelium cut from stock petri dish cultures of the fungi. All weight determinations before and after exposure to the test fungi were made after specimens had been oven-dried at 100 C to a constant weight.

Weight loss of bermudagrass pellets. Containers for the bermudagrass pellets and later stolon tests consisted of 18-mm diameter plastic test tube caps (Bacti Capall, Fisher Scientific Co., Dallas, TX 75240) with a 15-mm hole cut through the bottom. The hole was covered from the inside with a circular piece of 0.75-mm plastic screening (National Greenhouse Co., Pana, IL 62557). Soil-block chambers were prepared as previously described. Treatments consisted of individual inoculation with the 13 fungus isolates and a noninoculated control with four replications per treatment. Pellets in preweighed containers were placed in preweighed paper sacks, sterilized for 30 min at 1.4 kg/cm^2, and then oven-dried to a constant weight. Bermudagrass pellets in the plastic containers were aseptically placed on the inoculated feeder strips and exposed to the fungi for 90 days at 24 C after which the decayed substrate was dried to a constant weight and the percent weight loss was determined. Weight losses were expressed as a percent of the original oven-dry weight of the samples.

Weight loss of St. Augustine grass stolons. Air-dried internodal tissue was cut from field-grown saint augustine grass turf. Approximately 1 g of oven-dried tissue was autoclaved and used as substrate per soil-block chamber. There were 13 fungal inoculation treatments and a noninoculated control with three replications per treatment. Exposure time and weight loss

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determinations were as previously described.

Relative linear growth of fungus isolates in pure culture. Experiments with bermudagrass pellets and saint augustine grass stolons indicated that *C. versicolor, G. trabeum, F. fomentarius,* and *G. applanatum* isolate 2 were most effective in decaying these substrates. Therefore, only these fungi were used and temperature optima for linear growth in culture were determined for use in later controlled temperature weight loss experiments with thatch substrates. Comparative growth rates of these four fungi were determined by measuring radial growth on agar media during incubation at temperatures of 12, 16, 20, 24, 28, 32, and 36 C. The media, 20 ml per plate, were PDA, a mixture of PDA plus sawdust (4.5 g dry weight per plate, PDA-S), and Difco malt agar (MA).

TABLE 1. Bermudagrass pellet weight losses caused by various wood decay fungi after 90 days of incubation at 24 C

Fungus isolate	Average weight loss (%)[y]
Fomes fomentarius	58.7[z]a
Coriolus versicolor	41.6 b
Ganoderma applanatum isolate 2	41.5 b
Phellinus robustus	37.5 bc
Gloeophyllum trabeum	35.5 bcd
Schizophyllum commune	35.2 bcd
Ganoderma applanatum isolate 1	31.3 cd
Inonotus dryophilus	30.4 cd
Phellinus pini	29.1 d
Inonotus tomentosus	20.7 e
Piptoporus betulinus	20.5 e
Lentinus lepideus	20.4 e
Poria placenta	19.9 e
Uninoculated control	1.1 f

[y]Results from four replications.
[z]Means followed by same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).

TABLE 2. Weight losses of St. Augustine grass stolons induced by various wood decay fungi after 90 days of incubation at 24 C

Fungus isolate	Average weight loss (%)[y]
Fomes fomentarius	27.9[z]a
Gloeophyllum trabeum	26.6 ab
Ganoderma applanatum isolate 2	17.7 abc
Coriolus versicolor	17.5 abc
Ganoderma applanatum isolate 1	10.9 abc
Inonotus dryophilus	8.7 bcd
Piptoporus betulinus	8.0 bcd
Phellinus robustus	5.3 bcd
Phellinus pini	5.2 bcd
Poria placenta	5.0 bcd
Inonotus tomentosus	3.7 cd
Schizophyllum commune	3.6 cd
Lentinus lepideus	1.2 de
Uninoculated control	0.1 e

[y]Results from three replications.
[z]Means followed by same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).

TABLE 3. Weight loss of zoysia grass stolons by wood-decaying fungi as influenced by temperature after 90 days of incubation

	Average weight loss (%)[y]	
Fungus isolate	24 C	32 C
Ganoderma applanatum isolate 2	46.2[z]a	50.6a
Coriolus versicolor	48.8 a	40.8a
Fomes fomentarius	35.1 a	39.0a
Gloeophyllum trabeum	36.5 a	38.6a
Uninoculated control	2.1 b	1.8 b

[y]Results from four replications.
[z]Means followed by same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).

There were four replications of each fungus with each medium at each temperature.

Effect of vapor pressure on decay and weight loss of zoysia grass stolons. Decay of zoysia grass stolons was evaluated in 237-ml French-square bottles saturated with water at a vapor pressure of 29.66 mbar, and also in atmospheres with vapor pressure deficits of 7.59 and 15.17 mbar. The vapor pressures were maintained with differing concentrations of glycerol-water solution, 50 ml per bottle (5). Internodal stolon tissue of greenhouse-grown zoysia grass was placed in 1.5 × 6-cm glass vials and autoclaved for 20 min at 1.4 kg/cm^2. The sterile vials containing stolons were placed aseptically into the bottles having differing vapor pressures and inoculated directly with an 8-mm-diameter plug of mycelium of *C. versicolor, G. trabeum, F. fomentarius,* or *G. applanatum* isolate 2. There were four replications of the fungal treatments and an uninoculated control. Incubation was at 24 C for 90 days.

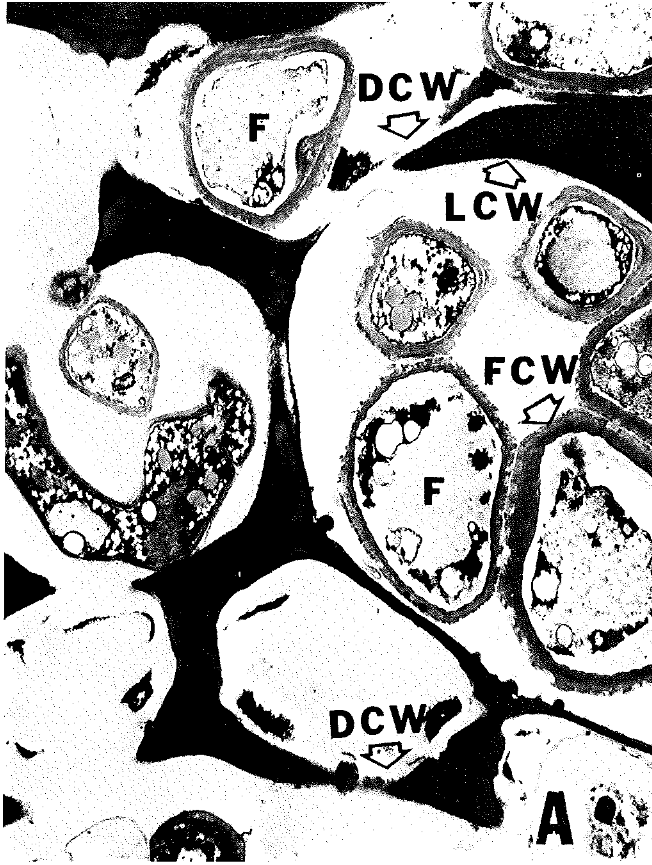
Effect of temperature on decay and weight loss of zoysia grass stolons. Decay and weight loss of zoysia grass stolons was compared at 24 and 32 C to test for correlation between growth on agar and stolon weight loss at these temperatures. French-square bottle decay chambers with feeder strips were prepared and inoculated as before. Four replications with each of the four fungi and an uninoculated control were incubated at each temperature for 90 days.

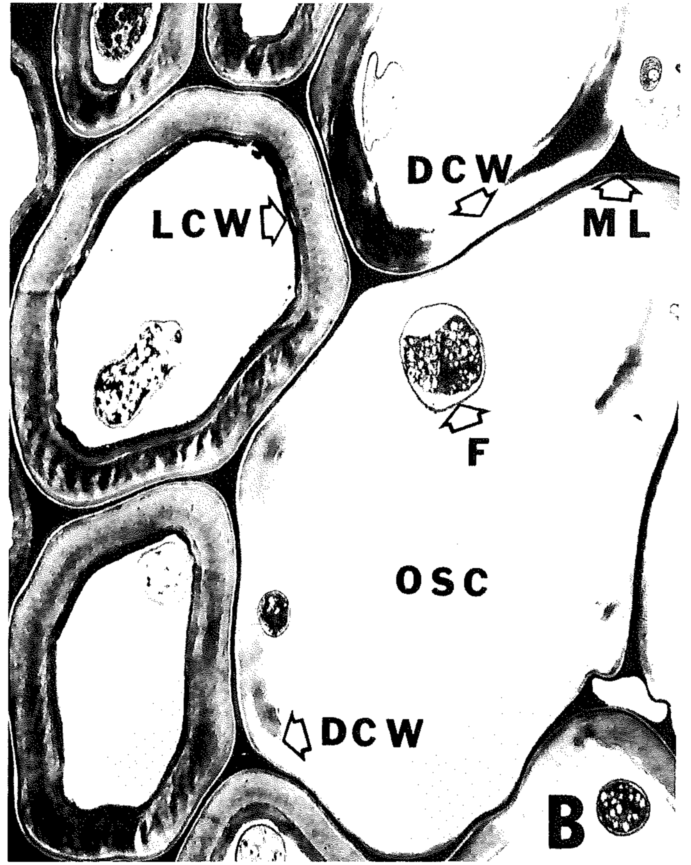
Field test. An exploratory test was conducted to determine whether *C. versicolor, G. trabeum,* and *G. applanatum* isolate 2 could decompose turf thatch under field conditions. A planting of common bermudagrass with only a moderate amount of thatch was divided into 0.11 m^2 plots in a randomized complete block design with four replications.

The test fungi were grown on 45 g of sterile vermiculite saturated with 200 ml of Richard's solution fortified with V-8 juice (Campbell Soup Co., Camden, NJ 08101) contained in 500-ml Erlenmeyer flasks. This inoculum was grown for 3 mo until mycelium had permeated the medium. A sterile control inoculum consisted of uninoculated vermiculite-Richard's medium. On 7 July 1978, 126 g of each inoculum treatment was spread onto the respective plots and thoroughly watered. Plots were watered periodically to maintain a high soil moisture level. Ratings were taken 6 September 1978, and 25 October 1978. In addition to visual observation, 11.2-cm diameter cores were removed from the plots and individually broken up by hand in a large bucket of water. After vigorous agitation, the supernatant, which contained a large amount of the dead plant material, was poured through a 0.87-mm (20-mesh) sieve. All live grass was removed and the remaining thatch was dried to a constant weight and compared with cores taken from untreated plots.

Light and electron microscopy of zoysia grass stolons. Tissue of inoculated stolons was examined microscopically to determine the site of fungal activity. Internodal stolon tissue of greenhouse-grown zoysia grass was sterilized in an autoclave for 20 min at 1.4 kg/cm^2. These stolons were transferred aseptically to petri plates containing sterile PDA from which a 3-cm strip of agar was removed aseptically from the middle of each plate. Individual stolons were laid across the trough to bridge the gap between the portions of agar remaining. One side of the agar was inoculated with a culture plug of mycelium of each of 13 test fungus isolates. The plates were placed in plastic bags to retain moisture and incubated at 24 C in the dark for 67 days. After incubation, 2-mm sections of the fungus-permeated stolons were excised and fixed in 4% glutaraldehyde in 0.05 M cacodylate buffer, pH 7.4, for 2 hr. The specimens were washed in three changes of the same buffer for 1 hr. Tissue was postfixed in 1% osmium tetroxide in 0.05 M buffer for 2 hr, washed in distilled water, and prestained overnight in 0.5% uranyl acetate. The specimens were dehydrated in a graded ethanol series, infiltrated with 50% Spurr's embedding medium for 2 hr, followed by 100% Spurr's medium overnight. Specimens were transferred to fresh 100% Spurr's medium and polymerized at 70 C for 10 hr.

For light microscopy, 1-μm thick sections were cut with a Sorvall "Porter-Blum" MT2-B ultramicrotome (Du Pont Co., Newton, CT 06470) and stained with Schiff's reagent, basic fuchsin, and





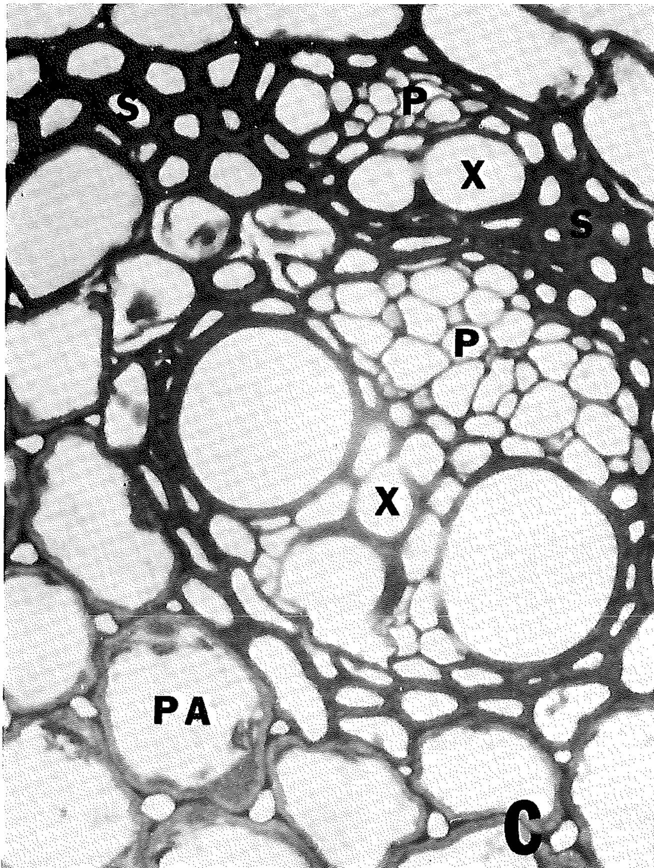


Fig. 1. Ultrastructure of zoysia grass stolon tissue after inoculation with: **A,** *Gloeophyllum trabeum,* or **B,** *Coriolus versicolor* and incubation at 24 C for 67 days, and **C,** light micrograph of similarly incubated uninoculated control tissue. **A,** Electron micrograph showing fungal mycelium and degradation of lignified cell walls of sclerenchyma sheath adjacent to xylem; **B,** Electron micrograph showing extensive degradation of walls of outer sheath cells, but little or no breakdown of middle lamellae; and **C,** light micrograph of vascular tissue of uninoculated stolon tissue showing no evidence of cell wall degradation or breakdown. DCW = degraded cell wall; F = fungus; FCW = fungus cell wall; LCW = lignified cell wall; ML = middle lamella; OSC = outer sheath cell; P = phloem; PA = parenchyma cells; S = sclerenchyma cells; and X = xylem. A, ×4,000; B, ×10,000; C, ×2,000

toluidine blue. The staining method used was a PAS procedure and a quick stain adapted by W. M. Harris, Botany Department, University of Arkansas (*personal communication*) from several different sources (1,12,21). Ultrathin sections were cut from the specimens showing the best evidence of fungal degradation. Grids with thin sections were stained with 2% uranyl acetate and lead citrate immediately after sectioning (14) and examined in a Siemens Elmiskop 1A electron microscope (Siemens Corp., Cherry Hill, NJ 08002) at 75 kV.

RESULTS

Weight loss of bermudagrass pellets. Bermudagrass pellets inoculated with all the test fungi showed weight losses that ranged from 19.9 to 58.7%, which differed significantly from the noninoculated controls (Table 1). *Fomes fomentarius* caused significantly greater pellet weight reduction than those caused by the other fungi. Analysis according to Duncan's multiple range test showed degrees of association between the weight losses induced by the other fungi in this test.

Weight loss of St. Augustine grass stolons. All test fungi, except *L. lepideus*, produced weight losses which were significantly different from the control (Table 2). Weight losses of saint augustine grass stolons varied from 27.9 to 1.2%. The lower weight loss of the St. Augustine grass stolon substrate suggested that it was more decay-resistant than were bermudagrass pellets.

Relative linear growth of fungus isolates in pure culture. The four fungi most active in degrading thatch components were grown on PDA, PDA-S, and MA. Linear growth of all fungi was greatest on MA. The optimum temperature for growth of the fungi on MA was: *C. versicolor*, 28–32 C; *F. fomentarius*, 28 C; and *G. applanatum* isolate 2, 32–36 C. Less growth occurred on PDA and PDA-S. Temperature optima for growth on PDA-S was 32 C for *C. versicolor*, *G. trabeum*, and *F. fomentarius*. *G. applanatum* isolate 2 grew best at 36 C on PDA-S.

Effect of vapor pressure on weight loss of zoysia grass stolons. No fungal growth or weight loss occurred at vapor pressure deficits of 7.59 or 15.17 mbar for any of the four test fungi. Average percent weight loss of the stolons incubated in a saturated atmosphere were: *C. versicolor*, 19.9%; *G. trabeum*, 28.9%; *F. fomentarius*, 20.5%; *G. applanatum* isolate 2, 17.0%; and control, 1.6%. All fungal treatments differed significantly from the control, but not from each other.

Effect of temperature on decay and weight loss of zoysia grass stolons. When *C. versicolor*, *G. trabeum*, *F. fomentarius*, and *G. applanatum* isolate 2 were inoculated on zoysia grass stolons and incubated for 90 days at 24 and 32 C, there were no significant differences between the respective weight losses at 24 and 32 C. All differed significantly from the control at each temperature (Table 3).

Field test. Field observations and measurements showed no evidence of a decrease in thatch. There were no adverse phytotoxic or other effects observed on the turf due to treatments.

Light and electron microscopy of zoysia grass stolons. After examination of 1-μm-thick sections of zoysia grass stolons exposed to the 13 test fungi, and examination of results of weight loss data, four specimens were picked for ultrathin sectioning and electron microscopy. These specimens had been degraded by *C. versicolor*, *G. trabeum*, *F. fomentarius*, and *G. applanatum* isolate 2.

All four fungi degraded the cell walls of the stolons similarly. In each case the cortical cell walls were severely decomposed. Sclerenchyma tissue, bundle sheath fibers, and epidermal cell walls were much more resistant to decay. Figs. 1A and 1B show effects of *C. versicolor* and *G. trabeum* on zoysia grass stolons.

DISCUSSION

Weight-loss data and histological and ultrastructural evidence showed that some wood decay fungi can decompose certain turf thatch components and the highly decay-resistant stolon components (13) of such thatches. Both brown-rot and white-rot fungi (eg, *G. trabeum* and *C. versicolor*, respectively) substantially reduced stolon weight under certain controlled conditions. Bermudagrass pellets were decayed more easily than the other substrates tested.

Whether these fungi effectively destroy turf thatch in the field was not proven in this study. One possible reason for the lack of noticeable decomposition of on-site thatch is that the fungi may not have been able to become established immediately on the grass substrate in competition with successional organisms colonizing it. In our soil-block experiments a source of nutrition other than the material being tested always was available in the form of pine feeder strips. It was apparent from the zoysia grass stolon decomposition (vapor pressure test) that *C. versicolor*, *G. trabeum*, *F. fomentarius*, and *G. applanatum* isolate 2 could become established on these substrates with few supplementary sources of nutrition. In this test, extraneous nutrients were provided only by the small initial disk of inoculm.

A saturated atmosphere was of prime importance for decay of zoysia grass stolons by these fungi. Because topdressing increases the water-holding capacity of thatchy turf (8) and presumably would enhance contact of the thatch with microorganisms, a sawdust-loam fungus-growing medium applied as a topdressing might accelerate thatch decay. This would both retain moisture and also provide a source of supplemental nutrition for the fungi.

Weight loss of zoysia grass stolons incubated at 32 C were not significantly different from those at 24 C; however, linear growth of the four fungi on agar media at 32 C was significantly greater than that at 24 C. Other investigators (9,10) reported a lack of correlation between amount of growth and enzyme production by wood-decay fungi.

Combinations of certain wood-decay fungi might prove more effective in reducing thatch than did single microorganisms. Sundman and Nase (18) reported synergism among certain wood decay fungi decomposing lignins and lignosulfonates. Blanchette and Shaw (4) reported association of certain yeasts and bacteria with a wood-decay fungus in nature. They reported greater increases in decay of slash pine wood chips by bacteria (*Enterobacter* spp.) and yeasts (*Saccharomyces bailii* var. *bailii* and *Pichia pinus*) in combination with *Coriolus* (*Polyporus*) *versicolor*, *Hirschioporus abietinus*, or *Poria placenta* than for those caused by the basidiomycete species alone.

There also have been reports of lignolytic bacteria (16,17) and actinomycetes which decay lignocelluloses to variable extents. Perhaps combinations of different lignolytic microorganisms as inoculum could serve as an effective thatch control treatment as well as be useful in other waste decomposition applications.

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