Use of Fungal Laccases to Facilitate Biodethatching: A New Approach

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Abstract. Accumulation of excessive organic matter as thatch restricts permeability of putting greens and is one of the most difficult problems in turfgrass management. A greenhouse experiment using potted bentgrass (*Agrostis stolonifera* L.) determined the efficacy of a ligninolytic enzyme, laccase, in reducing organic matter accumulation in the thatch-mat layer. Laccase was added biweekly at 0, 0.206, 2.06, and 20.6 units of activity/cm² with and without guaiaicol (2-methoxyphenol), a mediator of laccase, and sampling was performed after two and nine months. Parameters investigated included thickness of the organic layer, thatch layer and mat layer, organic matter content, saturated hydraulic conductivity, and lignin content. Organic matter and thatch layer increased between the two sampling dates in all treatments. Laccase was shown to be effective in slowing the rate of accumulation of organic matter and thatch layer. After two months, application of 20.6 units/cm² of laccase reduced organic layer thickness by 8.7% and extractive-free total lignin content by 8.4% when compared with non-treated control. After nine months, laccase application rates of 2.06 units/cm² reduced organic matter and thatch layer thickness by 15.6% and 45.0%, respectively, below levels observed in the non-treated control. Applications using 0.206 units/cm² of laccase were ineffective. Laccase applications had no influence on turf quality. These positive responses suggest laccase treatments could be a non-disruptive option for thatch and/or mat control in bentgrass.

Formation of thatch and mat layers is one of the major problems in management of modern turfgrass golf greens. Thatch is a layer of organic matter that accumulates between the soil and green turfgrass and contains both living and dead plant tissues intermingled tightly with each other. Thatch consists of stolons, rhizomes, roots, crown tissue, leaf sheaths, and blades (Engel, 1954; Roberts and Bredakis, 1960). The mat layer is generally below the thatch layer and is distinguished from thatch by the presence of sand or soil intermingled with thatch as a result of cultural practices like core aeration and topdressing (McCarty, 2005). A small amount of organic matter reduces surface hardness, moderates soil temperature extremes, increases the resilience, and improves wear tolerance of the turfgrass surface (Beard, 1973); however, excessive thatch and mat layers are undesirable in turfgrass.

High organic matter accumulation in the form of thatch-mat causes problems such as

decreased movement of oxygen through the thatch or mat zone, decreased saturated hydraulic conductivity, and excessive water retention (Carrow, 2003; Hartwiger, 2004; McCarty et al., 2007). These primary problems may further lead to secondary problems like wet wilt, soft surface, black layer, limited rooting, and extra- and intracellular freezing damage (Beard, 1973; Carrow, 2004; O'Brien and Hartwiger, 2003). Although structured organic matter, present in live underground plant tissues, is thought to have no adverse effect on the soil physical properties, rapid root death that results in dead gelatinous organic matter swells in the presence of water during decomposition and plugs the soil macropores (air-filled pores), causing low oxygen levels in the root zones (Carrow, 2004; O'Brien and Hartwiger, 2003). Excessive accumulation of organic matter causes anaerobic conditions, further reducing the rate of organic matter decomposition (McCoy, 1992). Grasses also generally produce more adventitious roots (surface roots) during anaerobic conditions, again further increasing organic matter content (Carrow, 2004).

Thatch management techniques such as core aeration, vertical mowing, grooming, and topdressing are currently the most effective strategies to manage thatch-mat buildup but have shown contrasting results (Barton et al., 2009; Carrow et al., 1987; Dunn et al., 1981; McCarty et al., 2005; McWhirter and Ward, 1976; Weston and Dunn, 1985; White and Dickens, 1984). These cultural practices are intensive in terms of cost, energy, and

labor and have adverse effects on turfgrass quality (Barton et al., 2009; Landreth et al., 2008; McCarty et al., 2007). Several non-destructive thatch control studies using glucose, cellulase solutions (Ledeboer and Skogley, 1967), and commercial inocula containing various microorganisms were ineffective in reducing the amount of thatch (McCarty et al., 2005; Murdoch and Barr, 1976). Reduction in cellulose content and total oxidizable organic matter of bermudagrass (*Cynodon dactylon* L.) and centipedegrass (*Eremochloa ophiuroides*) (Sartain and Volk, 1984) and weight loss of bermudagrass pellets, St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze], and zoysiagrass (*Zoysia japonica* Stued., 'Meyer') stolons (Martin and Dale, 1980) was observed when inoculated with different wood-decaying fungi under controlled greenhouse and laboratory conditions. However, field inoculation experiments on bermudagrass showed no thatch degradation (Martin and Dale, 1980).

The formation of the thatch-mat layer is the result of a greater rate of organic matter accumulation than degradation (Beard, 1973). Most microbial degradation mechanisms are restricted by the presence of lignin, a plant cell wall constituent. The slow decomposition of soil lignin has long been recognized (Kirk and Farrell, 1987). Lignin limits the accessibility of microbial degraders to more biodegradable plant materials such as cellulose and hemicelluloses (Ledeboer and Skogley, 1967). Lignin is formed in plants by oxidative coupling of monolignols of three primary hydroxycinnamyl alcohols: *p*-coumaryl, coniferyl, and sinapyl alcohols. The corresponding lignin monomers are known as *p*-hydroxy phenyl, guaiaicyl, and syringyl units, respectively (Wong, 2009). Lignification is achieved by crosslinking of monomers with a growing polymer through polymer-polymer coupling. Based on the random coupling theory, several models of lignin molecular structure have been proposed but these models do not imply any particular sequence of monomeric units in the lignin macromolecule (Chen and Sarkanen, 2003; Davin and Lewis, 2003).

Natural degradation of lignin occurs in the environment by certain white-rot fungi, which solubilize and mineralize lignin with the help of lignolytic enzymes (Kirk et al., 1975, 1976). White-rot fungi preferentially attack lignin more than cellulose or hemicellulose in the wood tissue (Blanchette, 1984; Mester et al., 2004). This process of selective delignification exposes cellulosic materials for further bacterial degradation in the environment (Otjen and Blanchette, 1987). The presence of naturally occurring (guaiaicol) and synthetic (1-hydroxybenzotriazole) chemicals, known as mediators, have been shown to enhance the activity of the lignolytic enzyme laccase (Kang et al., 2002; Roper et al., 1995). Because lignin content in thatch layer is higher than that of live grass tissues, the thatch layer in turfgrass species with high lignin content is more resistant to microbial decomposition (Beard, 1973; Ledeboer and Skogley, 1967).

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We hypothesize that the use of lignin-degrading enzymes such as fungal laccases can effectively reduce the rate of thatch layer accumulation in golf greens. The objectives of our study were: 1) to determine if degradation of soil organic matter can be enhanced by laccase application; 2) to determine if addition of guaiacol enhances laccase efficacy in organic matter decomposition; and 3) to determine if application of laccase enzyme and guaiacol adversely affect turf quality.

Materials and Methods

A greenhouse experiment was conducted using 'Crenshaw' creeping bentgrass *Agrostis stolonifera* L. (Engelke et al., 1995), established in pots (top diameter 15 cm, height 11.5 cm) at The University of Georgia, Griffin Campus, from Oct. 2008 to July 2009. The bentgrass was acquired from East Lake Country Club, Atlanta, GA. Pots were partially filled with 85:15 sand and organic matter mix and sod ≈ 3 cm in thickness was cut to fit the pots and placed on top of the mix. All pots were established in June 2008 and grown under management conducive to thatch development in a controlled environment greenhouse for about four months before initiation of treatments. Pots were irrigated daily, fertilized monthly with a 50 mL solution of 0.4% (w/v) Macron water soluble 28N-7P-14K fertilizer (Lesco, Strongsville, OH), and maintained by hand clipping weekly at a height of 0.6 cm with clippings removed. The refrigerated air-conditioned greenhouse was maintained at $25 \pm 2/18 \pm 2$ °C day/night temperature by a Wadsworth Step 50 controller (Wadsworth Control System, Arvada, CO) under natural lighting ($\approx 85\%$ ambient light).

The treatment design was as a four-by-two factorial with all combinations of four levels of laccase and two levels of guaiacol (2-methoxyphenol). The four laccase activity levels were 0 (control), 0.206, 2.06, and 20.6 units/cm² and guaiacol levels were 0 and 0.1 M solution. The experimental design was a randomized complete block with five replications and sampling times of two and nine months. Forty-milliliter solutions of the different laccase activity levels and 10 mL of guaiacol solutions were applied uniformly every 2 weeks to each pot using a handheld sprayer. The control pots were treated with equivalent amounts of distilled water. After the two months of treatment applications, the 20.6 units/cm² treatments with and without guaiacol were discontinued as a result of limited availability of laccase enzyme. As a result of unexpected problems in developing protocols for measurement of saturated hydraulic conductivity, one replication was rendered unusable for measurement of other variables. Therefore, only four replications were used for analysis after nine months' treatment duration.

Laccase activity assay. The laccase enzyme from *Trametes versicolor*, a white-rot fungus, was purchased from Sigma-Aldrich (Product 53739; Sigma Aldrich Inc., St. Louis,

MO). Laccase solutions were standardized based on active units, which were quantified using a ultraviolet/VIS-spectrophotometer by a colorimetric assay. One activity unit of laccase corresponds to the amount of enzyme that causes an absorbance change at 468 nm at a rate of 1.0 unit/min in 3.4 mL of 1 mM 2,6-dimethoxyphenol, a specific substrate for laccase, in citrate-phosphate buffer at pH 3.8 (Park et al., 1999). Laccase activity treatments of 0 (control), 0.206, 2.06, and 20.6 units/cm² actually correspond to laccase solutions with activity levels of 0, 0.912, 9.12, and 91.2 units/mL, respectively. The activity level of laccase applied per unit area was calculated by dividing the total number of units of laccase in 40 mL laccase solution by the top surface area of the pot.

Measurements. Effectiveness of treatments was determined by measuring organic matter content (OM) for a depth of 0 to 5.0 cm, organic layer thickness (OL), extractive-free acid-soluble lignin (L_S), and acid-insoluble lignin (L_I) content after two and nine months of treatment application. Total lignin was obtained by addition of acid-soluble and -insoluble lignin contents.

After nine months of treatment application, some additional variables were measured. OL was subdivided into thatch layer thickness (OL_T) and mat layer thickness (OL_M), whereas OM was subdivided into 0 to 2.5 and 2.5 to 5.0 cm depths to more accurately reflect the effectiveness of laccase on the thatch and mat layers. Saturated hydraulic conductivity (SHC) was also measured after nine months.

Organic matter content. The measurement of OM was performed as described by Carrow et al. (1987). Soil cores (2.0 cm diameter) were dried in an oven at 100 ± 5 °C for 48 h and weighed. Soil cores were ashed in a muffle furnace at 600 ± 10 °C for 24 h and weighed again. Organic matter content was determined as the difference in the two readings and percent OM was calculated.

Saturated hydraulic conductivity. Intact cores (diameter 4.7 cm and length 7.7 cm) were obtained from the center of each pot using a soil corer. The cores were collected in brass cylinders. The bottom of the core was covered with a double layer of cheesecloth held in place with a rubber band. The core was saturated overnight in a 0.05 N CaCl₂ solution to minimize dispersion. A clear plastic cylinder of the same diameter as of the brass cylinder was fastened above the brass cylinder with paraffin wax tape. The SHC of the cores was measured by a constant hydraulic head method using a Mariott tube apparatus. A time of 10 min was allowed for the establishment of steady-state flow through the samples. The volume of water that passed through the core was measured for 1 min and repeated three times. Saturated hydraulic conductivity was calculated using Darcy's equation.

Organic layer thickness and thatch-mat layer thickness. After cores were removed for measurement of OM content and SHC, plants were removed from pots and distinct

separations among the thatch, mat, and soil interface were clearly visible. The OL, OL_T, and OL_M were measured from seven different locations around the edges of the plant/root mass and averaged.

Extractive-free lignin content. Thatch was collected from each pot from the top 2.5 cm after sampling for OM and SHC. Extractive-free acid-soluble and -insoluble lignin content in the thatch layer was determined in a two-step hydrolysis procedure according to the laboratory analytical procedure developed by The National Renewable Energy Laboratory (NREL, 2008). In the first step, extractive-free thatch samples were hydrolyzed for 60 min with 72% H₂SO₄ at 30 °C. In the second step, H₂SO₄ was diluted to 4% and the samples were autoclaved at 121 °C for 1 h. Acid-soluble lignin was determined by measuring the absorbance of this hydrolysis liquid at 240 nm in a ultraviolet/VIS spectrophotometer. The solids remaining after acid hydrolysis were dried in an oven at 100 ± 5 °C for 24 h, weighed, ashed in a muffle furnace at 600 ± 10 °C for 24 h, and weighed again. Weight difference was used to calculate the acid-insoluble lignin content.

Turf quality. Turf quality was determined biweekly for the first three months and again for the last two months of the experiment to document the potential for initial and long-term phytotoxicity associated with laccase application. The turf quality of each treatment was recorded every 2 weeks by rating both visual turf quality and canopy spectral reflectance. Visual turf quality ratings were rated on the basis of color, shoot density, and uniformity on a numerical scale where 1 equals no live turf and 9 equals ideal dark green, uniform turf (Johnson et al., 1987). Grass index was determined using a TCM 500 turf color meter (Spectrum Technologies, Plainfield, IL). Grass index is a numerical score of the color and density of grass based on the spectral reflectance at 660 and 850 nm. Three grass index readings were recorded from each pot and averaged for statistical analysis.

Statistical analysis. Analysis of variance (ANOVA) was performed to evaluate the main effects of treatment duration, laccase, and guaiacol and interaction effects of these three factors using the general linear model (SAS Institute Inc., 1994). Strong treatment duration effects ($P \leq 0.001$) were observed in the initial analysis and therefore each treatment duration was analyzed separately using ANOVA as a two-factor study consisting of four levels of laccase enzyme and two levels of guaiacol for the two months' treatment duration and three levels of laccase enzyme and two levels of guaiacol for the nine months' treatment duration. Fisher's protected least significant difference test with $\alpha = 0.05$ was used for determining statistical differences among treatment means after each ANOVA.

Results

The full statistical model was used to compare common parameters at the two- and

Table 1. Analysis of variance table showing the effects of treatment duration, laccase application, guaiacol application, and their interactions on organic layer thickness (OL), organic matter (OM), acid-soluble lignin (L_S), acid-insoluble lignin (L_I), total lignin (L_T), thatch layer thickness (OL_T), mat layer thickness (OL_M), and saturated hydraulic conductivity (SHC) on creeping bentgrass maintained in a greenhouse.

Source of variation	df	OM (0 to 5.0 cm)			L _S (mg·g ⁻¹)			L _I (mg·g ⁻¹)			L _T (mg·g ⁻¹)			Mean square values			OM (0 to 2.5 cm) (mg·g ⁻¹)			OM (2.5 to 5.0 cm) (mg·g ⁻¹)			SHC (cm·h ⁻¹)		
		OL (mm)	OL (mm)	OL (mm)	L _S (mg·g ⁻¹)	L _S (mg·g ⁻¹)	L _S (mg·g ⁻¹)	L _I (mg·g ⁻¹)	L _I (mg·g ⁻¹)	L _I (mg·g ⁻¹)	L _T (mg·g ⁻¹)	L _T (mg·g ⁻¹)	L _T (mg·g ⁻¹)	OL _T (cm)	OL _T (cm)	OL _T (cm)	OL _M (cm)	OL _M (cm)	OL _M (cm)	OM (0 to 2.5 cm) (mg·g ⁻¹)	OM (2.5 to 5.0 cm) (mg·g ⁻¹)	OM (2.5 to 5.0 cm) (mg·g ⁻¹)	SHC (cm·h ⁻¹)	SHC (cm·h ⁻¹)	SHC (cm·h ⁻¹)
Full model																									
Treatment duration	1	3649***	208***	55.8***	102	309**	852***	102	309**	852***	102	309**	852***	102	309**	852***	102	309**	852***	102	309**	852***	102	309**	852***
Laccase	2	99.2***	105***	55.5***	476***	105	50.7	476***	105	50.7	476***	105	50.7	476***	105	50.7	476***	105	50.7	476***	105	50.7	476***	105	50.7
Guaiacol	1	9.69	1.03	9.86	50.7	105	50.7	50.7	105	50.7	105	50.7	105	50.7	105	50.7	50.7	105	50.7	50.7	105	50.7	50.7	105	50.7
Duration*laccase	2	109***	60.8***	22.9**	84.4	193*	172	84.4	193*	172	84.4	193*	172	84.4	193*	172	84.4	193*	172	84.4	193*	172	84.4	193*	172
Duration*guaiacol	1	13.6	4.46	19.6	76.1	172	36.1	76.1	172	36.1	76.1	172	36.1	76.1	172	36.1	76.1	172	36.1	76.1	172	36.1	76.1	172	36.1
Error	42	7.71	5.83	2.82	30.2	36.1	36.1	30.2	36.1	36.1	30.2	36.1	36.1	30.2	36.1	36.1	30.2	36.1	36.1	30.2	36.1	36.1	30.2	36.1	36.1
two months																									
Repetitions	4	1.84	16.9	3.83*	10.6	22.3	22.3	10.6	22.3	22.3	10.6	22.3	22.3	10.6	22.3	22.3	10.6	22.3	22.3	10.6	22.3	22.3	10.6	22.3	22.3
Laccase	3	20.8*	16.7	50.1***	760***	1195***	1195***	760***	1195***	1195***	760***	1195***	1195***	760***	1195***	1195***	760***	1195***	1195***	760***	1195***	1195***	760***	1195***	1195***
Guaiacol	1	0.70	1.84	0.00028	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Laccase*guaiacol	3	3.85	37.5	1.53	3.02	8.58	33.2	3.02	8.58	33.2	3.02	8.58	33.2	3.02	8.58	33.2	3.02	8.58	33.2	3.02	8.58	33.2	3.02	8.58	33.2
Error	28	8.08	7.61	0.96	32.3	33.2	33.2	32.3	33.2	33.2	32.3	33.2	33.2	32.3	33.2	33.2	32.3	33.2	33.2	32.3	33.2	33.2	32.3	33.2	33.2
nine months																									
Repetitions	3	10.1	4.63	7.85	120***	173***	173***	120***	173***	173***	120***	173***	173***	120***	173***	173***	120***	173***	173***	120***	173***	173***	120***	173***	173***
Laccase	2	192***	143***	66.6***	430***	835***	835***	430***	835***	835***	430***	835***	835***	430***	835***	835***	430***	835***	835***	430***	835***	835***	430***	835***	835***
Guaiacol	1	21.9	4.40	25.7*	113	246*	246*	113	246*	246*	113	246*	246*	113	246*	246*	113	246*	246*	113	246*	246*	113	246*	246*
Laccase*guaiacol	2	3.55	2.99	5.80	35.5	71.1	13.0	35.5	71.1	13.0	35.5	71.1	13.0	35.5	71.1	13.0	35.5	71.1	13.0	35.5	71.1	13.0	35.5	71.1	13.0
Error	15	8.72	8.13	4.07	13.1	13.0	13.0	13.1	13.0	13.0	13.1	13.0	13.0	13.1	13.0	13.0	13.1	13.0	13.0	13.1	13.0	13.0	13.1	13.0	13.0

*Significant at the 0.05 *P* level.

**Significant at the 0.01 *P* level.

***Significant at the 0.001 *P* level.

nine-month sampling dates (Table 1). The model included the main and interaction effects of treatment duration, three levels of laccase, and two levels of guaiacol for OM (0 to 5.0 cm), OL, L_S, and L_I. Only three levels of laccase were used because the 20.6 units/cm² laccase treatment was discontinued after two months of application.

Treatment duration strongly affected OM (0 to 0.5 cm; $P \leq 0.001$), OL ($P \leq 0.001$), and L_S ($P \leq 0.001$) (Table 1). Laccase application significantly affected OM (0 to 5.0 cm), OL, L_S, and L_I. The very strong interactions for treatment duration and laccase treatment observed in OM (0 to 0.5 cm; $P \leq 0.001$), OL ($P \leq 0.001$), and L_S ($P \leq 0.01$) were largely the result of the lack of response to laccase treatments after two months as opposed to a strong response seen after nine months. Guaiacol treatment as well as the interaction of guaiacol and treatment duration had no effect on any of the parameters (Table 1).

We observed an overall increase in OM (0 to 5.0 cm) and OL in all treatments between the two sampling dates. However, accumulation of OM (0 to 5.0 cm) and OL was significantly lower in treatments containing laccase when compared with the control. When compared with the control, the rate of accumulation of OM (0 to 5.0 cm) was reduced from 15.8 mg·g⁻¹ in control pots to 9.0 mg·g⁻¹ in pots treated with 2.06 units/cm² laccase (43%) (Table 2). Similarly, application of 2.06 units/cm² laccase reduced OL accumulation from 15.5 mm to 11.7 mm (24%) when compared with the control (Table 2). A reduction in lignin content between the two sampling dates was observed at laccase activity levels of 2.06 units/cm² with and without guaiacol (Table 3).

Analysis after two months' treatment.

After two months of treatment application, laccase treatments had no effect on OM (0 to 5.0 cm) but had significant effects on OL, L_S and L_I (Table 1). Neither guaiacol nor the laccase by guaiacol interaction had significant effects on any of the parameters (Table 1).

Compared with the control, treatment with 20.6 units/cm² of laccase without guaiacol for two months significantly lowered L_S by 5.2, L_I by 20.5, and L_T content by 25.6 mg·g⁻¹ (Table 3). Similarly, treatment at the same laccase activity with guaiacol reduced L_S by 4.2, L_I by 18.9, and L_T by 23.4 mg·g⁻¹ when compared with control. Treatment with 2.06 units/cm² of laccase with guaiacol also significantly reduced L_S by 1.4 mg·g⁻¹ (Table 3).

Analysis after nine months' treatment.

After nine months of treatment application, laccase application impacted OM (0 to 5.0 and 0 to 2.5 cm), SHC, OL, OL_T, L_S, and L_I, and SHC ($P \leq 0.001$) (Table 1). A significant effect of guaiacol was also observed for L_S ($P \leq 0.05$) and SHC ($P \leq 0.01$) (Table 1). The interaction of laccase by guaiacol was significant ($P \leq 0.01$) for SHC. However, none of the treatments affected OM (2.5 to 5.0 cm) or mat layer thickness after nine months of treatment application.

Table 2. Organic layer thickness (OL) and organic matter (OM) content (0 to 5.0 cm depth) after two and nine months of different treatments applied to creeping bentgrass.^z

Treatment ^y	Organic layer thickness		Organic matter (0 to 5.0)	
	two months	nine months	two months	nine months
	------(mm)-----		------(mg·g ⁻¹)-----	
0L (control)	48.4 a ^x B ^w	69.3 a A	33.7 a B	49.5 a A
0L + G	47.4 a B	68.2 ab A	34.4 a B	47.3 a A
0.206L	45.7 a B	67.3 ab A	36.4 a B	50.0 a A
0.206L + G	47.6 a B	63.8 bc A	34.3 a B	49.4 a A
2.06L	48.1 a B	59.8 cd A	32.7 a B	41.7 b A
2.06L + G	47.7 a B	58.5 d A	35.0 a B	41.9 b A
20.6L	44.2 b	—	36.3 a	—
20.6L + G	45.1 a	—	33.9 a	—

^zThe OM values are based on dry weight.

^yL denotes laccase level and G the addition of guaiacol, a mediator.

^xMeans within a column followed by the same lowercase letter are not significantly different according to Fisher's protected least significant difference at $\alpha = 0.05$.

^wMeans in a row within a parameter followed by the same uppercase letter are not significantly different according to Fisher's protected least significant difference at $\alpha = 0.05$.

Table 3. Extractive-free acid-soluble (L_S), acid-insoluble (L_I), and total lignin (L_T) content after two and nine months of different treatments applied to creeping bentgrass.^z

Treatment ^y	Acid-soluble lignin		Acid-insoluble lignin		Total lignin	
	two months	nine months	two months	nine months	two months	nine months
	------(mg·g ⁻¹)-----		------(mg·g ⁻¹)-----		------(mg·g ⁻¹)-----	
0L (control)	43.7 ab ^x A ^w	42.2 b A	259.9 a A	257.4 b A	303.5 ab A	299.7b A
0L + G	43.8 ab A	45.8 a A	260.1 a A	264.8 a A	303.9 a A	310.6 a A
0.206L	44.1 a A	41.4 b A	256.9 a A	254.5 bc A	301.0 ab A	295.9 b A
0.206L + G	43.3 abc A	41.4 b A	255.4 a A	253.9 bc A	298.8 ab A	295.5 b A
2.06L	42.7 bc A	37.1 c B	254.0 a A	243.4 d B	296.7 ab A	280.4 d B
2.06L + G	42.3 c A	39.5 bc B	253.9 a A	249.6 c A	296.2 b A	289.0 c B
20.6L	38.5 d	—	239.4 b	—	277.9 c	—
20.6L + G	39.5 d	—	241.0 b	—	280.1 c	—

^zThe extractive-free lignin values are based on dry weight.

^yL denotes laccase level and G the addition of guaiacol, a mediator.

^xMeans within a column followed by the same lowercase letter are not significantly different according to Fisher's protected least significant difference at $\alpha = 0.05$.

^wMeans in a row within a parameter followed by the same uppercase letter are not significantly different according to Fisher's protected least significant difference at $\alpha = 0.05$.

Treatment with 2.06 units/cm² of laccase with and without guaiacol decreased OL by 10.8 and 9.5 mm, respectively, when compared with the control (Table 2). These same treatments reduced OL_T by 8.3 (45%) and 6.5 mm (35%) when compared with the control (Fig. 1). Laccase applied at the same activity was effective in reducing OL and OM, whereas laccase application at 0.206 units/cm² was not different from the control (Table 2; Fig. 2).

Treatment with 2.06 units/cm² laccase with and without guaiacol reduced OM (0 to 5.0 cm) by 7.6 and 7.8 mg·g⁻¹ and OM (0 to 2.5) by 25.9 and 30.3 mg·g⁻¹, respectively, as compared with the control (Table 2; Fig. 2). Similarly, treatment with 2.06 units/cm² laccase with and without guaiacol increased SHC by 21.6 (322%) and 6.3 cm·h⁻¹ (94%), respectively, over the control (Fig. 3). When compared with control, treatment with 2.06 units/cm² laccase without guaiacol reduced L_S by 5.1, L_I by 14.0, and L_T by 19.0 mg·g⁻¹, respectively (Table 3).

Turf quality. No significant differences in visual quality ratings were observed among the treatments except for the data collected after 38 weeks when 2.06 units/cm² of laccase exhibited a slight but significant reduction in turf quality when compared with the control treatment (Table 4). No significant differences from the control were observed for any treatment when means of the visual ratings were compared for the early (2 to 12

Fig. 1. Thatch (OL_T) and mat layer thickness (OL_M) after nine months of treatment on creeping bentgrass with three different levels of laccase [0 (control), 0.206 and 2.06 units/cm²] with and without the mediator, guaiacol (G). Values are means of four replicates and error bars are standard errors. Bars with the same letter (OL_M = bolded and OL_T = standard) are not considered to be statistically different according to Fisher's protected least significant difference at $\alpha = 0.05$.

Fig. 2. Organic matter (OM) for 0 to 2.5 cm depth and 2.5 to 5.0 cm depth after nine months of treatment on creeping bentgrass with three different levels of laccase [0 (control), 0.206 and 2.06 units/cm²] with and without the mediator, guaiacol (G). Values are means of four replicates and error bars are standard errors. Bars with the same letter [OM (0 to 2.5 cm) = bolded and OM (2.5 to 5.0 cm) = standard] are not considered to be statistically different according to Fisher's protected least significant difference at $\alpha = 0.05$.

Discussion

Laccase application. This is the first study to report the direct application of laccase enzyme to manage thatch-mat accumulation on creeping bentgrass. Application of laccase, especially at the 2.06 units/cm² activity level, proved to be effective in reducing thatch-mat depth, OM, and significantly

weeks), late (32 to 38 weeks), and all periods (Table 4). When compared with the control, pots receiving 20.6 units/cm² laccase treatment had a small but significant decrease in grass index at 4 and 6 weeks after treatment initiation (Table 5). However, no significant differences in grass index values were observed after 6 weeks of treatment application. No visual differences among treatments for turf color or growth rate were observed over the duration of the experiment.

increasing SHC. Carley et al. (2011) noted that OM accumulates rapidly near the soil surface by small annual accumulations that result in long-term effects. Our results indicate that application of 2.06 units/cm² laccase alters OM dynamics in a positive manner by effectively reducing OM (0 to 2.5 cm) and OL_T in comparison with the control. However, an increase in OM (0 to 5.0

cm) and OL was observed for all the treatments over the experiment duration. Application of laccase enzyme was effective in reducing the rate of accumulation of thatch layer thickness and OM.

The 2.06 units/cm² laccase treatment, after nine months of application, also resulted in increased SHC in which a three- and two-fold increase in SHC was observed with applications of 2.06 units/cm² laccase with and without guaiacol, respectively. This increase can be explained on the basis of thatch layer thickness of the corresponding treatment. Thatch layer thickness more than 1.3 cm was reported to adversely affect water infiltration (McCarty et al., 2005). Thatch layer thickness for the treatment of 2.06 units/cm² with and without guaiacol after nine months of treatment was 11.2 and 13.0 mm, respectively.

For both the two and nine months' sampling, no effect of guaiacol was observed and no interaction effect with laccase was observed except for SHC after nine months of application. The 2.06 units/cm² laccase treatment was ineffective when applied with guaiacol after two months of application except for a reduction in extractive-free L_S. The significant replication effect observed for L_S and L_I for two and nine months, respectively, may be associated with incomplete acid hydrolysis for some replications during autoclaving and unavailability of additional sample materials for reanalysis.

The lowest level of laccase application (0.206 units/cm²) proved to be an ineffective treatment even after nine months of application for all the parameters measured. The 20.6 units/cm² treatment was applied for two months and resulted in no reduction of OM (0 to 5.0 cm). However, this treatment did result in a significant reduction in OL and extractive-free lignin content (L_S, L_I, and L_T) of the thatch layer.

Laccase application had only minor influences on turfgrass quality. A slight reduction in turf quality was indicated by lower grass index values during the first 4 to 6 weeks in response to the 20.6 units/cm² laccase treatment. However, visual quality ratings were not significantly different from controls except for one treatment combination at 38 weeks.

Treatment duration. If laccase was effective in enhancing OM degradation, it would seem reasonable to expect that effects would become more apparent over time. Samples were analyzed after two and nine months of treatment application. Laccase activity levels of 0.206 and 2.06 units/cm² area were continued for nine months. It was observed that treatment duration and the interaction of treatment duration with laccase treatments had a significant effect on OM (0 to 5.0 cm), OL, and L_S content.

Why laccase application? Studies in the past using various cultural management practices with different cultivation frequencies

Fig. 3. Saturated hydraulic conductivity (SHC) after nine months of treatment on creeping bentgrass with three different levels of laccase [0 (control), 0.206 and 2.06 units/cm²] with and without the mediator, guaiacol (G). Values are means of four replicates and error bars are SES. Bars with the same letter are not considered to be statistically different according to Fisher's protected least significant difference at $\alpha = 0.05$.

Table 4. Mean visual turf quality ratings of creeping bentgrass made over time following continued treatment with different laccase and guaiacol solutions to greenhouse-grown plants.

Treatments ^z	Weeks												Early	Late	All
	Early						Late								
	2	4	6	8	10	12	32	34	36	38					
	-----Visual turfgrass quality ^y -----														
0L (control)	7.1 ab ^x	6.7 a	8.3 ab	7.8 a	7.2 a	8.0 ab	7.9 a	7.9 a	8.3 a	8.5 a	7.5 a	8.0 a	7.8 a		
0L + G	7.0 ab	6.9 a	8.1 ab	7.7 a	7.1 a	8.1 a	7.8 a	7.7 a	8.1 a	8.4 a	7.4 a	7.9 a	7.8 a		
0.206L	7.2 a	6.8 a	8.2 ab	7.6 a	7.1 a	7.9 ab	8.0 a	8.0 a	8.1 a	8.4 a	7.4 a	7.8 a	7.8 a		
0.206L + G	7.0 a	6.7 a	8.0 b	7.8 a	7.3 a	7.8 b	7.7 a	7.6 a	8.3 a	8.3 ab	7.4 a	7.8 a	7.5 a		
2.06L	7.2 a	7.0 a	8.3 ab	7.8 a	7.4 a	7.9 ab	7.9 a	7.9 a	8.1 a	8.1 b	7.5 a	7.9 a	7.6 a		
2.06L + G	6.9 ab	6.7 a	8.4 ab	7.8 a	7.3 a	7.7 b	7.9 a	8.0 a	8.2 a	8.4 a	7.4 a	7.9 a	7.6 a		
20.6L	6.7 b	6.9 a	8.4 a	7.8 a	—	—	—	—	—	—	7.5 a	—	—		
20.6L + G	6.9 ab	6.7 a	8.2 ab	7.9 a	—	—	—	—	—	—	7.5 a	—	—		

²L denotes laccase level and G the addition of guaiacol, a mediator.

³Turf quality was visually rated on a 1 to 9 scale with 9 = outstanding, 6 = acceptable, and 1 = dead.

⁴Means within a column followed by the same letter are not significantly different according to Fisher's protected least significant difference at $\alpha = 0.05$.

Table 5. Mean grass index values of creeping bentgrass made over time following continued treatment with different laccase and guaiacol solutions to greenhouse-grown plants.

Treatments ^z	Weeks												
	Early						Late				Early	Late	All
	2	4	6	8	10	12	32	34	36	38			
	Grass index ^y												
0L (control)	7.03 ab ^x	6.47 a	7.47 a	6.71 a	7.70 a	8.17 ab	8.15 a	8.23 a	9.14 a	9.34 a	6.92 a	8.46 a	7.84 a
0L + G	7.11 ab	6.45 a	6.96 ab	6.53 a	7.94 a	8.31 a	8.20 a	8.47 a	9.04 a	9.06 a	6.76 a	8.50 a	7.81 a
0.206L	7.21 a	6.40 ab	7.11 ab	6.55 a	7.70 a	7.96 ab	8.22 a	7.93 a	9.07 a	9.10 a	6.82 a	8.33 a	7.73 a
0.206L + G	7.15 ab	6.33 ab	6.90 ab	6.53 a	7.61 a	7.84 b	8.36 a	8.10 a	9.06 a	9.34 a	6.73 a	8.39 a	7.72 a
2.06L	7.10 ab	6.44 a	7.27 ab	6.53 a	7.66 a	8.14 ab	8.10 a	8.29 a	9.07 a	9.33 a	6.84 a	8.43 a	7.79 a
2.06L + G	6.94 ab	6.19 ab	7.50 a	6.49 a	7.84 a	8.20 ab	8.16 a	8.10 a	9.20 a	9.12 a	6.78 a	8.43 a	7.77 a
20.6L	6.68 b	6.02 b	6.70 b	6.72 a	—	—	—	—	—	—	6.53 b	—	—
20.6L + G	6.92 ab	6.16 ab	6.96 ab	6.74 a	—	—	—	—	—	—	6.73 a	—	—

²L denotes laccase level and G the addition of guaiacol, a mediator.

³Grass index was recorded using TCM 500 based on the spectral reflectance with higher value representing higher quality.

⁴Means within a column followed by the same letter are not significantly different according to least significant difference at $\alpha = 0.05$.

have reported contrasting results for reduction in thatch-mat accumulation (Callahan et al., 1998; Carrow et al., 1987; Engel and Alderfer, 1967; McCarty et al., 2005; Rieke, 1994). Degradation of thatch-mat is reported either in terms of thatch-mat depth (Smiley et al., 1985; Soper et al., 1988) or in terms of thatch-mat depth and OM content by weight (Barton et al., 2009; McCarty et al., 2007). The OM content by weight in different studies is observed for different depths further making it difficult to compare the results (Barton et al., 2009; McCarty et al., 2005; Murray and Juska, 1977). In our study, however, we observed both OL thickness (thatch layer and mat layer) and OM content to provide a better comparison of the effectiveness of laccase on thatch-mat degradation.

Cultural practices like core aeration and vertical mowing are disruptive in nature and have shown to reduce the turf quality both aesthetically and physically, further reducing the playability of the turf (Barton et al., 2009; Landreth et al., 2008; McCarty et al., 2007). However, application of laccase is not disruptive and the effective treatment of 2.06 units/cm² laccase for nine months showed no overall reduction in turf quality of bentgrass.

Several non-destructive studies in the past using different treatments like sugars, mixtures of sugars and microbial inocula, and some enzymes like cellulase proved ineffective (Ledeboer and Skogley, 1967; Martin and Dale, 1980; McCarty et al., 2005; Murdoch and Barr, 1976). Most of these studies intended to increase microbial population to degrade OM. However, it is difficult to maintain higher microbial populations over sustained periods of time under field turfgrass management systems as a result of the inability to maintain proper microenvironment conditions required by particular microbial populations. Another reason that such studies were ineffective may be that they were focused on degradation of cellulose and hemicellulose by using cellulase enzyme and by increasing bacterial populations, whereas our hypothesis is that lignin degradation will open the cell wall structure of thatch biomass, hence making cellulose and hemicellulose more available for further microbial degradation. In our study, we used the end product from the white-rot fungi *Trametes versicolor*, the laccase enzyme, which is stable over a wide pH and temperature (Baldrian, 2006; Munoz et al., 1997; Stoilova et al., 2010; Thurston, 1994) to degrade lignin and to facilitate dethatching.

This greenhouse research demonstrated that biweekly application of laccase enzyme at 2.06 units/cm² can be effective in reducing the rate of accumulation of OM in highly maintained turf. However, low activity levels of laccase (0.206 units/cm²) were ineffective in reducing the rate of thatch accumulation. Laccase application had little effect after two months but significantly reduced OM after nine months. Implications of these findings point to a novel approach to reduce OM in thatch or mat and its associated problems on

golf greens. This approach can lead to the development of a new non-disruptive method for thatch management. Future research is needed to observe the effectiveness of laccase under field conditions as well as to optimize the activity level of laccase and the frequency of its application.

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