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Laccase mediated changes in physical and chemical composition properties of thatch layer in creeping bentgrass (*Agrostis stolonifera* L.)☆

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ABSTRACT

Thatch is a tightly intermingled layer of dead and living organic matter present below the turf aerial shoots and above the soil. Excess thatch can be a major problem on many turfgrass sites, especially golf greens. A greenhouse experiment was conducted on dead potted bentgrass (*Agrostis stolonifera* L.) to eliminate the confounding effects of continual growth as we attempted to determine the efficacy of a ligninolytic enzyme, laccase, along with mediator guaiacol in reducing organic matter content in thatch layer biomass. Laccase was added bi-weekly at 0, 2.06, and 20.6 units of activity cm^{-2} with and without guaiacol (2-methoxyphenol) and sampling was performed after two and six months. Parameters investigated included thickness of thatch layer, organic matter, saturated hydraulic conductivity, lignin content, and structural sugars. After two months of treatment application, thatch layer thickness was reduced by 22.1% and extractive-free acid-soluble lignin by 12.3%. Lignin content decreased by 6.5% and saturated hydraulic conductivity improved to 124.7% higher than the non-treated control. No reduction in organic matter and sugar content was observed after two months of treatment application. After six months, thatch layer thickness was reduced by 62.0%, total organic content (0–2.5 cm) by 24.7%, and total sugar content by 29.3%. Extractive-free acid-insoluble lignin and saturated hydraulic conductivity increased by 17.1 and 70.8%, respectively in comparison to the control. These positive responses suggest laccase treatments could expedite organic matter degradation in the thatch layer of creeping bentgrass.

1. Introduction

A major problem in management of recreational turfgrass sites and especially golf greens is the formation of thatch and/or mat layer that accumulates between the soil and green turfgrass and contains both living and dead plant tissues intermingled tightly with each other. Thatch, a layer of high organic matter content, consists of stolons, rhizomes, roots, crown tissue, leaf sheaths, and blades (Engel, 1954; Roberts and Bredakis, 1960). The occurrence of mat layer is generally due to the presence of sand or soil intermingled with thatch as a result of cultural practices like core aeration and topdressing (McCarty, 2005). A thin layer of thatch aids in reducing surface hardness, moderating soil temperature extremes, and increasing resilience and wear tolerance of the turfgrass surface (Beard, 1973); however, excessive thatch and mat layers are undesirable in turfgrass.

Thatch–mat causes problems such as decreased movement of oxygen through the thatch or mat zone, decreased saturated hydraulic conductivity, low oxygen levels within the thatch/mat layer during wet periods, and increased water retention (Carrow, 2003; Hartwiger, 2004; McCarty et al., 2007). These conditions often lead to secondary problems like wet wilt, soft surface, increased mower scalp, black layer, limited rooting, and extra- and intra-cellular freezing damage (Beard, 1973; Carrow, 2004; O'Brien and Hartwiger, 2003). Adverse effects on the soil physical properties are caused by rapidly decaying dead gelatinous organic matter that swells in the presence of water during decomposition and plugs the soil macro-pores (air-filled pores), causing low oxygen levels in the root zones (Carrow, 2004; O'Brien and Hartwiger, 2003).

Mechanical practices such as core aeration, vertical mowing, grooming, and topdressing are the most effective to manage thatch–mat buildup but have shown contrasting results (Barton et al., 2009;

Abbreviations: L_I, acid-insoluble lignin; S_{ARA}, arabinose; L_S, acid-soluble lignin; S_{GAL}, galactose; S_{GLU}, glucose; SHC, saturated hydraulic conductivity; L_T, total lignin; TLT, thatch layer thickness; OM_L, organic matter content (2.5–5.0 cm depth); OM, organic matter (0–5.0 cm depth); OM_U, organic matter (0–2.5 cm depth); S_T, total sugars; S_{XYL}, xylose.

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Carley et al., 2011; 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 practices often adversely impact turf quality and are intensive in terms of cost, energy, and labor (Barton et al., 2009; Landreth et al., 2008; McCarty et al., 2007).

When accumulation of organic matter exceeds the degradation rate, formation of thatch–mat layer is increased (Beard, 1973). The presence of lignin, a plant cell wall constituent, acts as a protective matrix and 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 mono-lignols of three primary hydroxycinnamyl alcohols: *p*-coumaryl, coniferyl, and sinapyl alcohols (Wong, 2009). Several models of lignin molecular structure have been proposed that do not imply any particular sequence of monomeric units in the lignin macromolecule (Chen and Sarkanen, 2003; Davin and Lewis, 2003). Ledeboer and Skogley (1967) proposed that lignification is achieved by random cross linking of monomers through different bonds resulting in a heterogeneous structure resistant to degradation. Other researchers have indicated that the structure of lignin is not heterogeneous but rather that the lignin polymer is composed of repetitive units suggesting a homogeneous structure (Banoub and Delmas, 2003).

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). The rate of microbial decomposition is more dependent on the lignin content of the organic matter in the degradation progress (Taylor et al., 1989). A plant litter decomposition study reported a close relationship of mass loss with activity of lignocellulose-degrading enzymes (Sinsabaugh et al., 1993). Extra-cellular lignolytic enzymes produced by certain white-rot fungi are responsible for natural degradation of lignin (Kirk et al., 1975, 1976). The preferential degradation of lignin by extra-cellular enzymes produced by white-rot fungi exposes cellulosic materials for further bacterial degradation in the environment (Blanchette, 1984; Mester et al., 2004; Otjen and Blanchette., 1987). Inoculation with different wood-decaying fungi under controlled greenhouse and laboratory conditions reduced cellulose content and total oxidizable organic matter of bermudagrass (*Cynodon dactylon* L.) and centipedegrass (*Eremochloa ophiuroides*) (Sartain and Volk, 1984). Similar inoculation studies reported weight loss of bermudagrass pellets as well as St. Augustinegrass (*Stenotaphrum secundatum* [Walt.] Kuntze) and zoysiagrass (*Zoysia japonica* Stued., 'Meyer') stolons (Martin and Dale, 1980). However, field experiments on bermudagrass inoculated with these same wood-decaying fungi showed no thatch degradation (Martin and Dale, 1980).

The presence of naturally-occurring (guaiacol) and synthetic (1-hydroxybenzotriazole, HBT) chemicals, known as mediators, have been shown to enhance the activity of the lignolytic enzyme laccase (Kang et al., 2002; Roper et al., 1995). Low oxidation potential of laccase restricts its ability to oxidize non-phenolic lignin components (Kersten et al., 1990; Ten Have and Teunissen, 2001). However, addition of low molecular weight substances such as mediators increases the substrate range of laccase enzyme to non-phenolic groups including benzyl and allyl alcohols and ethers (Bourbonnais and Paice, 1992; Bourbonnais et al., 1997; Crestini and Argyropoulos, 1998; Fabbrini et al., 2002, 2001) which comprise the major moieties in lignin macromolecule (Fritz-Langhals and Kunath, 1998; Johannes and Majcherczyk, 2000; Potthast et al., 1995). In a laccase-mediator system, laccase acts to oxidize the mediator increasing its redox potential and then the oxidized mediator oxidizes the substrate (Cantarella et al., 2003).

A novel approach developed to facilitate thatch–mat degradation using direct application of laccase, an extra-cellular lignolytic enzyme produced from white-rot fungi *Trametes versicolor*, on turfgrass was reported to be effective in reducing the rate of accumulation of organic matter content and thatch layer thickness in creeping bentgrass (Sidhu et al., 2012). However, a net accumulation of organic matter in thatch layer treated with laccase was observed over time (Sidhu et al., 2012). This study was designed to observe the potential of laccase enzyme to facilitate organic matter decomposition in a system where accumulation of organic matter is ceased. The major objectives of this study were: 1) to determine the potential of laccase enzyme to facilitate organic matter degradation in thatch layer; and 2) to determine the changes in physical and chemical composition properties of thatch layer due to application of laccase.

2. Materials and methods

2.1. Greenhouse and experimental setup

A greenhouse experiment was conducted using 'Crenshaw' creeping bentgrass (Engelke et al., 1995) *Agrostis stolonifera* L., established in pots (top diam. 15 cm, height 11.5 cm) at The University of Georgia, Griffin Campus from December 2009 to June 2010. The bentgrass was acquired from East Lake Country Club, Atlanta, Georgia. Pots were partially filled with 85:15 sand and organic matter mix and sod approximately 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 in a controlled environment greenhouse for approximately eighteen months prior to initiation of treatments to facilitate development of thatch layer in the pots. The refrigerated air conditioned greenhouse was maintained at $25 \pm 2/18 \pm 2$ °C, day/night temperature maintained by a Wadsworth Step 50 controller (Wadsworth Control System, Arvada, Co) under natural lighting (approximately 85% ambient light). Pots were irrigated daily, fertilized monthly with a 50-mL solution of 0.4% (w/v) Macron water soluble 28-7-14 fertilizer (Lesco. Strongsville, OH), and maintained by hand clipping weekly at a height of 0.6 cm with clippings removed to develop favorable conditions for thatch development in the pots.

Prior to the treatment initiation in December 2009, the growth of creeping bentgrass in the pots was ceased by application of a herbicidal solution containing 1.3% (v/v) of Roundup Pro® (isopropylamine salt of glyphosate, Monsanto, St. Louis, MO) and 1.3% (v/v) Finale® Herbicide (glufosinate ammonium, Bayer Environmental Science, Montvale, NJ). One week after herbicide application, dead creeping bentgrass was clipped down to the thatch layer. To block any natural or artificial light from reaching the pots and thereby avoid stimulation of re-growth, the pots were covered with two 76.2 μm thick sheets of black plastic sheeting cut from Husky Contractor Clean-up bags (item no HK42WC032B, Poly America, Grand Prairie, TX). The treatment design was a three by two factorial with all combinations of three levels of laccase and two levels of guaiacol (2-methoxyphenol). The three laccase activity levels were 0 (control), 2.06, and 20.6 units cm^{-2} 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 six months. Forty milliliter solutions of the different laccase activity levels were applied uniformly every two weeks to each pot using a hand-held sprayer. The control was applied as 40 mL of distilled water. Guaiacol levels were applied as 10 mL of 0.1 M solution. Guaiacol is a natural co-substrate and mediator of laccase believed to enhance enzyme performance (Roper et al., 1995). The pots were irrigated to drainage twice a week and maintained near field capacity to favor microbial activity during the six month study. Laccase treatments were applied at least 24 h after and at least 24 h prior to irrigation.