**A Novel Method to Facilitate Biodethatching Using Fungal Laccases**

Progress Report

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**INTRODUCTION**

The proposed research was designed to explore the feasibility of utilizing extracellular laccases produced by white rot fungi to enhance the biodegradability of thatch. Through the study, we intend to develop an enzymatic pretreatment method that will significantly enhance the effectiveness of biodethatching processes.

Thatch is a layer of organic matter consisting of tightly intermingled dead and living leaves stem and roots that develop between the soil surface and the green vegetation. Thatch layer intermixed with sand or soil is known as Mat layer (Beard 1973). High organic matter accumulation in the form of thatch or mat causes depletion of oxygen and decreased saturated hydraulic conductivity and increased water content (Hartwiger 2004). This further leads to problems like welt wilt, soft surface, black layer, limited rooting etc. (Carrow 2004; O’Brien and Hartwiger 2003).

Lignin, a 3- dimensional amorphous polymer consisting of methoxylated phenyl propane structure limits the degradation of organic matter (Beard 1973). It resists most microbial degradation mechanisms and serves as a barrier in the cell walls to limit the accessibility to the more biodegradable plant materials, such as cellulose and hemicelluloses, by microbial degraders. Oxidative enzymes such as laccases, lignin peroxidases and manganese peroxidases produced by white rot fungi attack the aromatic components of lignin and leads to its effective degradation.

White rot fungi are recognized as the most active lignin degrading microorganisms among few in the nature (Boyle, et al 1992; Gold and Alic 1993). Oxidative enzymes produced by fungi are able to attack the aromatic contents in lignin and produce free radicals, leading to effective degradation of lignin (Nakayamaa and Kamachi 1999). We hypothesize that thatch that has been directly treated with lignin-degrading enzymes will be more amenable for microbial degradation because the lignin barrier that restricts the microbial accessibility have been effectively removed.

Laccases, lignin peroxidases, and manganese peroxidases are enzymes that have been known to be involved in lignin degradation (Nakayamaa and Kamachi 1999). They have been widely studied and used in pulp and paper industry to remove lignin, which serves as strong basis supporting the hypothesis mentioned above. Laccases, the multi copper oxidases are known to act on a wide variety of aromatic compounds by reducing oxygen to water (Baldrian 2006). The capability of degrading lignin utilizing oxygen as well as their strong extracellular activity makes laccases potentially suitable material for bio dethatching.

The proposed research is aimed at verifying the following hypothesis: 1) degradation of organic matter can be enhanced by applying laccase to the thatch layer; and 2) laccase has no appreciable adverse effects on turf quality.

**OBJECTIVES**

To test the hypothesis listed above represents the overarching goal of this study, and this will be achieved in a three phase studies. Phase 1 was a laboratory study aimed to verify the ability of laccase to facilitate the degradation of the organic matter in thatch layer; Phase 2 is a green house study with bentgrass pots to determine the effects of laccase application on thatch layer and on turf quality; Phase 3 will be a field study to evaluate the overall dethatching effect under field conditions.

**PROGRESS**

Phase 1 and 2 of the study has been completed and the results have been discussed in the reports submitted in November 2009 and February 2010. For phase 3, the field study, we have finalized a treatment plan, based on the results of the greenhouse study, and the experiment will start later in May 2010.

We also started another greenhouse study in December 2009 on dead Bentgrass pots to provide more knowledge about the effects of laccase on organic matter degradation. Laccase is sprayed every two weeks at activity 0, 0.4 and 4 units / cm of soil, respectively. The pots irrigated with distilled water containing 0 units/ ml served as controls. The pots receiving, 0.4 and 4 units / cm were further divided into two groups, one which received guaiacol along with laccase. Guaiacol is a mediator of laccase which is believed to enhance enzyme performance. For all the treatments, twelve replicates were prepared, five of which were sampled during February 2010, two months of treatment. Five replicates will be sampled during June 2010. Two replicates were used to determine the turfgrass firmness.

Thatch layer thickness, organic matter content, and saturated hydraulic conductivity were measured during February 2010 for five replicates, and the results are presented in following three figures. The results again confirmed our earlier finding on live grass that the enzyme treatment effectively reduces thatch. The remaining replicates will be measured at the completion of treatments during June 2010.

RESULTS



