Enzyme Technology to Alleviate Soil Water Repellency in Turfgrass Situations

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Objective:

1. *Based on laboratory results, selected treatments will be evaluated in small field plots on a short–term and long–term basis to remediate soil water repellency (SWR) on normal localized dry spot (LDS) areas and the SWR area within fairy ring. Enzyme–based treatment options include: types of enzymes; enzyme combinations; enzyme activity (rates); and wetting agent + enzyme combinations.*
2. *The most effective and economic treatments from evaluations conducted under objective 1 will be evaluated for short–term and long–term in small scale plot situations on the Griffin Campus and/or golf courses that represent different soil and grass conditions.*

Figure 1. Fairy ring on bermudagrass green.

Soil water repellency (SWR) occurs on sandy turfgrass soils that exhibit localized dry spot (LDS) and within the dry area of fairy ring disease areas; and causes serious soil water infiltration/runoff problems and reduced turf quality. Over 2000 research papers have been published on SWR and 12 remediation strategies have been reviewed by Müller and Deurer (2011), with repeated wetting agent (WA) application being the most effective. But, this strategy is not always effective and often requires repeated treatment (Moore et al. 2010). No reports of direct enzyme application were found; but wax–degrading bacteria have been reported to provide some remediation effects, believed to be due to excreted enzymes or surfactants (Roper, 2006).

In proof–of–concept laboratory studies, we demonstrated potential of four enzymes to alleviate SWR (Liu et al., 2013). Also, we showed in another project the effectiveness and persistence of the direct application of the enzyme laccase for organic matter decomposition in turf thatch/mat situations (Sidhu et al., 2012). Based on these studies, we proposed a new and novel approach to alleviate SWR by using direct application of enzymes or combinations of enzymes that are specific for degradation of hydrophobic organic fractions believed to contribute to SWR – i.e., the organic compounds that are adhered to the surface of

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sand particles and in the particulate organic matter. Since enzymes directly degrade/alter the organic coatings, they should provide for longer term and more effective alleviation of SWR than the most current management approach. The enzymes used are found in natural systems and enzyme activity is much less affected by changes in field environmental conditions, such as temperature, moisture, and aeration, than are specific microbial populations (e.g. wax −degrading bacteria).

Figure 2. Soil water repellency experiment.

Research Methods

Plot size in all studies was 1 x 2 ft with four replications per treatment. Treatments used in all studies were:

1. Control – no enzyme or wetting agent
2. Laccase (L) —– Rate used was 8 units per g soil using the *Pycnoporus* source of 5.3 units/ml of enzyme (Liu et al., 2013).
3. Pectinase (P) —– Rate used was 15.6 unit per g soil using the *Aspergillus niger* source of 1.04 units/mg enzyme or 1040 units per g (Sigma Aldrich 17389 source)
4. Wetting agent (WA) – Aquatrols Aqueduct, Active Ingredients 50% Nonionic polyols; 5% 1, 2−Propanediol applied at 8 oz per 1000 sq. ft.
5. L + WA – rates as above
6. P + WA – rates as above
7. L + P + WA – used only in second run of both studies

Application protocol was designed to maximize effectiveness of WA and enzymes and were:

- Pre−wet the surface with light application of water. This will prevent the enzymes from sticking to dry leave.
- Apply 103 ml total solution (product + water) to each 1.0 sq ft. of plot area for the small plot studies = 0.043 inch water.
- After one hour of treatment but before 2 hours, apply a syringe cycle of about 0.050 inch water (which is about 121 ml per 1.0 sq. ft. plot).
- Thereafter, run similar syringe cycle (i.e. 0.05 inch water) every 3 hour for a total of 3 times.
- Thus, the total solution over about 12 hours, including the pre−wetting, would be about 0.24 inch of water.

Data collection from field studies at 0, 2, 4, 8, and 12 wks after treatment application included: a) volumetric soil water content (%VWC) will be obtained by TDR (Spectrum Field Scout TDR 300) with 3.8 and 7.5 cm probes on a grid pattern with measurements taken within 2−4 hours after irrigation and 24 hours later; b) WDPT (water droplet penetration time) and MED (molarity of ethanol droplet test) tests for SWR after drying at 40 oC for 36 hr (Barton and Colmer, 2011; Hamlett et al., 2011); and c) digital imaging, turf quality and spectral reflectance to quantify turf canopy by NDVI (normalized difference vegetation index).

Normal Localized Dry Spot Studies. Studies (summer/fall 2013, continue thru 2014 UGA Griffin Campus) for normal SWR or LDS were conducted on a SeaIsle I seashore paspalum sand−based simulated sport field (12 yr old) with a USGA green media mowed at 0.62 inch. Application was applied on August 29, 2013 and then repeated on an adjacent area on September 24, 2013. Results analyzed to−date are presented in Table 1.

Fairy−Ring Studies. Experiments (summer/fall 2013, continue thru 2014, local golf course) for SWR associated with fairy−ring were conducted on a Tifeagle bermudagrass USGA sand−based green (4−yr old) and mowed at 0.125 inch. The first study was initiated on August 29, 2013 and the second on September 24, 2013 in an adjacent site. Both were laid out in the SWR area of a large fairy ring.

Results

Since direct application of selected enzymes to soil exhibiting SWR has not been reported, these studies were exploratory to determine the feasibility and to refine field testing protocols. The seashore paspalum sand−based sport field exhibited normal localized dry spot and results to−date are in Tables 1−3. Soil water repellency was found only in the first run where treatments showing the highest statistical ratings (i.e., ´a´) for soil VWC across all 8 rating periods and depths were P + WA (5), L + WA (4), and P (1) (Table 1). The most effective treatments for reducing MED were WA, L + WA (Table 3).

In the fairy ring study, SWR was evident in both runs (Table 4–6). Treatments demonstrating the most frequent improvements in soil VWC across all depths and dates (i.e. 12 ratings) based on highest ´a´ statistical ranking were: WA (8), L + WA (7), P + WA (5) and L (3) (Tables 4, 5). Reduced MED was most evident for L + WA and P + WA (Table 6).

These results do demonstrate that direct enzyme application can aid in alleviation of SWR. Best results

Summary Points

- This is the first report of use of direct application of enzymes for alleviation of soil water repellency in field situations.
- Of the two enzymes, laccase was most effective in the fairy–ring study; and pectinase in the normal localized dry spot study.
- Both enzymes were most effective when in conjunction with a wetting agent.
- These initial studies will aid in refining future field studies in terms of enzymes, rates, and combinations.

Table 1. Percent Volumetric water content (% VWC) at depths of 3.8 and 7.6 cm obtained at different days (d) after treatment application on August 29, 2013 applied to SeaIsle 1 seashore paspalum sand–based sport field with normal localized dry spot (LDS).

Treatment	% VWC, 3.8 cm				% VWC, 7.6 cm			
	14 d	15 d	27 d	28 d	14 d	15 d	27 d	28 d
Control	18.3bc	13.0b	21.4c	19.9bc	13.0c	11.0	18.1b	16.3bc
Laccase	16.9c	12.7b	21.8bc	19.2c	13.6bc	10.2	18.5ab	16.1c
Pectinase	21.2abc	17.5a	24.6abc	23.1abc	14.7abc	12.7	21.7ab	18.5abc
Wet. Agent	21.3ab	14.2ab	25.8ab	23.1abc	14.7abc	12.3	21.2ab	19.7ab
L + WA	22.6a	18.3a	26.2ab	23.2ab	18.5a	13.6	22.4a	19.7ab
P + WA	22.4a	16.9ab	26.8a	24.3a	17.1ab	13.7	22.7a	19.9a
ANOVA								
Replications	<0.0001	<0.0001	<0.0001	0.0004	<0.0001	<0.0001	<0.001	<0.001
Treatment	0.031	0.024	0.028	0.031	0.081	0.435	0.105	0.074

Table 2. Percent Volumetric water content (% VWC) at depths of 3.8 and 7.6 cm obtained at different days (d) after treatment application on September 24, 2013 applied to SeaIsle 1 seashore paspalum sand–based sport field with normal localized dry spot (LDS).

Treatment	% VWC, 3.8 cm			% VWC, 7.6 cm		
	0 d	14d	15d	0 d	14d	15d
Control	26.5ab	25.2	24.7	23.0	24.9	21.1
Laccase	28.0a	27.0	24.8	24.0	25.9	21.7
Pectinase	26.4ab	26.8	25.4	24.0	23.5	21.0
Wet. Agent	23.9bc	24.1	21.9	21.1	22.0	19.1
L + WA	23.0c	25.1	25.1	21.3	23.2	19.1
P + WA	24.2bc	25.6	22.5	20.4	22.2	18.5
L + P + WA	25.1abc	26.7	24.0	22.0	24.5	20.7
ANOVA						
Replications	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Treatment	0.054	0.621	0.175	0.270	0.138	0.379

Table 3. Difference in MED (molarity of ethanol droplet test) from initial MED values (ΔMED) at different days (d) after treatment application on August 29, 2013 applied to SeaIsle 1 seashore paspalum sand-based sport field with normal localized dry spot (LDS). Samples were from the 3.8 cm surface zone. Higher MED values are associated with higher soil water repellency.

Treatment	Initial MED (molarity of ethanol)	ΔMED (decrease)	
		14 d	28 d
Control	3.9	0.2bc	0.4c
Laccase	3.9	0c	0.4c
Pectinase	4.1	0c	0.3c
Wet. Agent	3.8	0.5a	0.7ab
L + WA	4.0	0.4ab	0.8a
P + WA	4.0	0.4ab	0.7ab
ANOVA			
Replications		0.011	0.353
Treatments		0.016	0.010

Table 4. Percent Volumetric water content (% VWC) at depths of 3.8 and 7.6 cm obtained at different days (d) after treatment application on August 29, 2013 to TifEagle bermudagrass sand-based green in the hydrophobic area of a fairy-ring.

Treat.	% VWC, 3.8 cm				% VWC, 7.6 cm			
	14d	15d	27d	28d	14d	15d	27d	28d
Control	13.9c	12.3b	17.6c	16.9c	7.9b	7.4c	11.5b	11.4c
Laccase	18.3ab	15.3a	19.0bc	19.2b	10.3a	9.7ab	13.8a	12.2bc
Pectinase	13.9c	9.5c	16.6c	16.6c	7.8b	6.6c	10.0c	10.2d
Wet. Agent	19.3ab	16.8a	22.3a	21.8a	11.4a	10.2ab	14.8a	13.3ab
L + WA	20.4a	17.0a	22.7a	21.3ab	11.2a	10.9a	15.3a	14.0a
P + WA	17.9b	15.5a	21.2ab	20.3ab	10.5a	9.0b	13.9a	12.7b
ANOVA								
Replications	0.481	0.667	0.505	0.186	0.409	0.535	0.079	0.756
Treatments	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

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Table 5. Percent Volumetric water content (% VWC) at depths of 3.8 and 7.6 cm obtained at different days (d) after treatment application on September 24, 2013 applied to TifEagle bermudagrass sand-based golf green in the hydrophobic area of a fairy-ring.

Treatment	% VWC, 3.8 cm			% VWC, 7.6 cm		
	0 d	14d	15d	0 d	14d	15d
Control	16.1	17.5bc	14.5	12.2a	11.8bc	9.9bc
Laccase	15.1	17.0c	14.8	10.1b	11.9abc	9.7bc
Pectinase	17.3	16.5c	15.7	11.4ab	11.1c	10.3abc
Wet. Agent	17.0	19.8ab	16.9	12.3a	13.7a	11.8a
L + WA	13.8	17.5bc	14.9	11.2ab	11.6bc	9.7bc
P + WA	17.8	19.9a	16.6	12.3a	13.3ab	11.1ab
L + P + WA	15.5	18.0abc	16.3	10.0b	12.0abc	9.0c
ANOVA						
Replications	0.113	0.113	0.033	0.400	0.079	0.131
Treatments	0.199	0.026	0.462	0.058	0.072	0.031

Table 6. Difference in MED (molarity of ethanol droplet test) from initial MED values (ΔMED) at different days (d) after treatment application on August 29, 2013 applied to TifEagle sand-based golf green with hydrophobic area of a fairy-ring. Samples were from the 3.8 cm surface zone. Higher MED values are associated with higher soil water repellency.

Treatment	Initial MED (molarity of ethanol)	ΔMED (decrease) 14 d	ΔMED (decrease) 28 d
Laccase	2.50	0.1abc	0.1bc
Pectinase	2.40	−0.2c	−0.1c
Wet. Agent	2.60	0.3ab	0.3ab
L + WA	2.50	0.4a	0.5a
P + WA	2.50	0.3ab	0.4a
ANOVA			
Replications		0.632	0.088
Treatments		0.032	0.0004