Annual Progress Report – December 2016

Funding Agencies: The Environmental Institute for Golf and Georgia Golf Environmental Foundation

Project Title: An enzymatic approach to remediate water repellency of turfgrass soils

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Soil water repellency occurs on sandy turfgrass soils as localized dry spots (LDS) and within the dry area of fairy ring disease areas. Soil water repellency causes serious soil water infiltration/runoff problems and reduces turf quality. Our research explores a new and novel approach to alleviate soil water repellency by using direct application of enzymes that are specific for degradation of hydrophobic organic fractions believed to contribute to soil water repellency. Since these enzymes directly degrade or alter the organic coatings, they should provide for longer-term and more effective alleviation of soil water repellency than the current management approach, repeated use of wetting agents.

In proof-of-concept laboratory studies funded by the University of Georgia Technology Commericalization Office, we identified a number of enzymes and multiple extracts from biomass fermentation that have the potential to alleviate soil water repellency. We submitted a patent application (Huang et al., 2015) based on this laboratory data and have published two journal articles (Liu et al., 2013; Meng et al., 2014). The first article represents the first report of the use of enzymes for alleviation of soil water repellency. With USGA-sponsored research funds, we compared the effectiveness of wetting agents and two enzymes and their combinations in relieving soil water repellency associated with both LDS and fairy ring in small field plots on the University of Georgia Griffin Campus. In April 2015, we established research at The Old Colliers Golf Club in Naples, Fla., comparing an enzyme and a wetting agent (Revolution) and their combination for long-term effectiveness in treating LDS. These previous studies clearly establish the potential of this technology to provide more effective and lasting alleviation of soil water repellency issues on golf courses.

This current research builds on these previous efforts and proposes both greenhouse and field studies using direct application of enzymes or combinations of enzymes and wetting agents as a means to degrade certain organic fractions believed to contribute to soil water repellency. The enzymes proposed are found in natural systems, and enzyme activity is much less affected by changes in field environmental conditions than are specific microbial populations. We would anticipate that enzyme treatments could be confined to the localized soil water repellency areas (spot treatment) as a corrective and possibly preventive measure.

The objectives of the proposed project are to refine application protocols through greenhouse experiments that determine the most cost-effective enzyme application rate and application frequency for the treatment of soil water repellency/LDS. Additionally, the effectiveness of adding wetting agents in combination with enzyme to enhance enzyme penetration is being evaluated. Field evaluations involving the most effective and economic treatments based on our greenhouse research will be evaluated for both short-term (< 2 weeks) and long-term (seasonlong) effectiveness in small-scale plot trials on the University of Georgia Griffin Campus and with more limited treatments at The Old Colliers Golf Course in Naples, Fla.

2016 Research

Research efforts during 2016 were focused on research goals outlined by Objective 1 of our proposal. In July of 2016, we initiated laboratory studies designed to refine application protocols with regard to enzyme application rate, application frequency, and the potential to improve results by adding a wetting agent.

Collected soil cores of hydrophobic sand were acquired from Dr. Gerald Henry at UGA-Athens. Core material was hand sieved using a 720 micron sieve to remove large particles of organic material. The sieved sand was oven dried to near 0% moisture content. The bottoms of 4-inch square pots used in this experiment were first covered with fitted nylon mesh to prevent sand from moving through the openings in the bottom of the post. A rectangular piece of nylon door screen was placed vertically on one side of the pot to allow for slow downward movement of water unable to penetrate the surface. Pots were filled to a level approximately 2 cm from the top of the pot with 275 grams of sand. A total of eleven treatments were used in this study with five replications of each treatment. Treatments are shown in the table below.

Treatment #	**Treatment**	**Enzyme Rate[1]**	**App. Freq.**	**Wetting Agent[2]**
1	NH Sand[3]	0	0	0
2	Control	0	0	0
3	4uE	4	1/16 wk	0
4	4uE & WA	4	1/16 wk	6 oz.
5	8uE	8	1/16 wk	0
6	8uE & WA	8	1/16 wk	6 oz.
7	12uE	12	1/16 wk	0
8	12uE & WA	12	1/16 wk	6 oz.
9	WA	0	1/16 wk	6 oz.
10	8uE + 8uE	8 + 8	1/8 wk	0 + 0
11	8uE & WA + 8uE	8 + 8	1/8 wk	6 oz. + 0

1. Enzyme rates are 4, 8, or 12 units of enzyme activity / cm2.
2. Rate of Revolution equivalent to 6.0 oz. / 1000 ft2
3. NH Sand = non-hydrophobic sand. All other treatments are with hydrophobic sand.

Enzyme, wetting agent, and enzyme + wetting agent treatments were applied using an experimental spray chamber at an application rate of 80 gal. / acre. Controls were treated with the equivalent rate of water. Pots were allowed to dry overnight before beginning irrigation treatments. Pots were placed in molded greenhouse trays with drainage and set on top of conetainer racks placed in cafeteria trays on a lab bench. Initial weights of all pots were recorded. Irrigation was applied bi-weekly by slowing pouring 40 ml of water into each pot. This is roughly equivalent to 0.25 " of rainfall. Pots were allowed to drain for 24 hrs. and re-weighted to determine water retention. Pots were weighed again prior to each irrigation event.

After each four week cycle of irrigation (eight irrigation events), all pots were dried to near 0% moisture in a forced air oven at 120 ^{o}C for 48 hrs. before resuming the next irrigation cycle. The experiment continued for 16 weeks with treatments 10 and 11 receiving a re-application of enzyme after eight weeks. Soil samples were taken following each 4 wk irrigation cycle for later analysis of soil water repellency using the Water Droplet Penetration Time (WDP).

A summary of soil moisture content of selected treatments for the first 4-week irrigation cycle are presented in Figure 1 below. Note that water retention of non-hydrophobic sand (NH sand) was significantly higher that the non-treated control (hydrophobic sand) for the first three irrigation events until the NH sand reached field capacity at around 25% soil moisture. In contrast, the hydrophobic sand used for all other treatments showed a field capacity of 50% soil moisture or above.

The effects of enzyme rate on soil moisture content during the 1st irrigation cycle are also illustrated in Figure 1. The addition of laccase at a rate equivalent to 4 units / cm^2 did not improve water retention above that of the untreated control. However, rates of 8 and 12 units / cm^2 did significantly improve water retention increasing soil moisture content by approximately 30% over that of the non-treated control.

The addition of a wetting agent to the hydrophobic sand (treatment WA) resulted in a more dramatic improvement of the soil's ability to retain water. Pots treated with wetting agent acted very similar to pots filled with non-hydrophobic sand and the mean soil moisture content in the WA treatment was 50% higher than that of the non-treated control (Control).

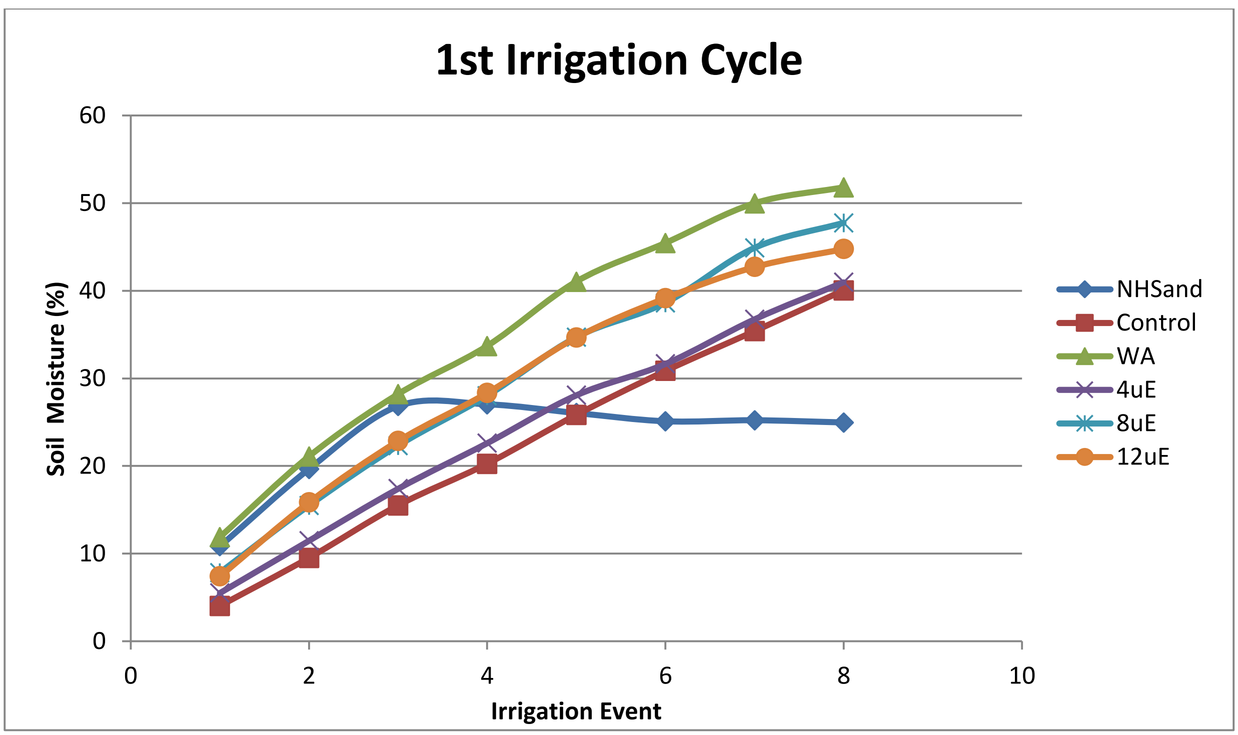

Figure 1. Treatment responses as observed by differences in soil moisture content over eight irrigation events during a four week period.

Figure 2 below compares the soil moisture response curves among three enzyme rates with and without the addition of a wetting agent during the 1st irrigation cycle. Response curves indicate that the addition of wetting agent to enzyme treatments was better than enzyme alone across all enzyme rates. During the first irrigation cycle, enzyme & wetting agent combination treatments were equal to, but not better than the wetting agent alone treatment.

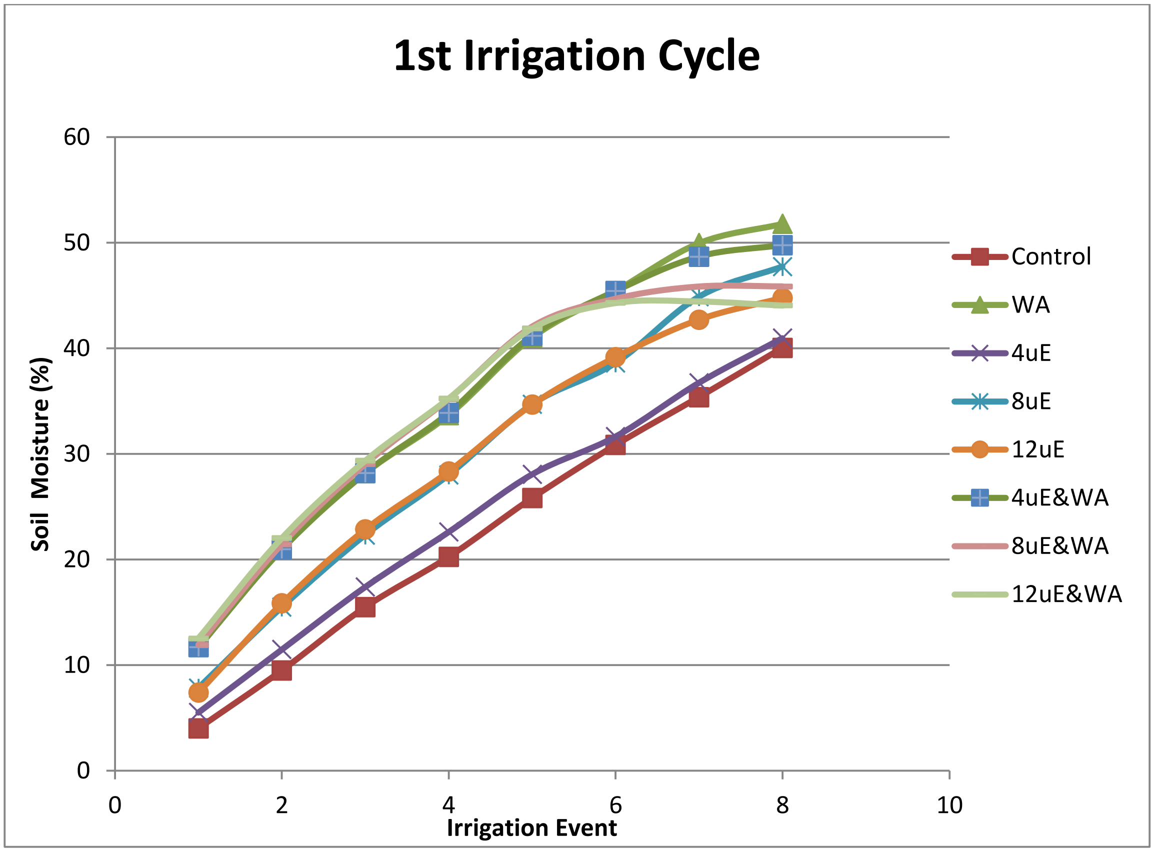

Figure 2. Comparison of responses to enzyme treatments with and without a wetting agent added as observed by differences in soil moisture content over eight irrigation events during a four week period.

During the second and third irrigation cycles (Figures 3 and 4), enzyme and wetting agent combination treatments did show slightly better performance that the wetting agent alone treatments. However, in only a few cases were enzyme & wetting agent treatment means for soil moisture content statistically higher than the means for the wetting agent alone treatment. By the 4th irrigation cycle, soil moisture content means for enzyme & wetting agent treatments had dropped back below the means of the wetting agent alone treatment (data not shown).

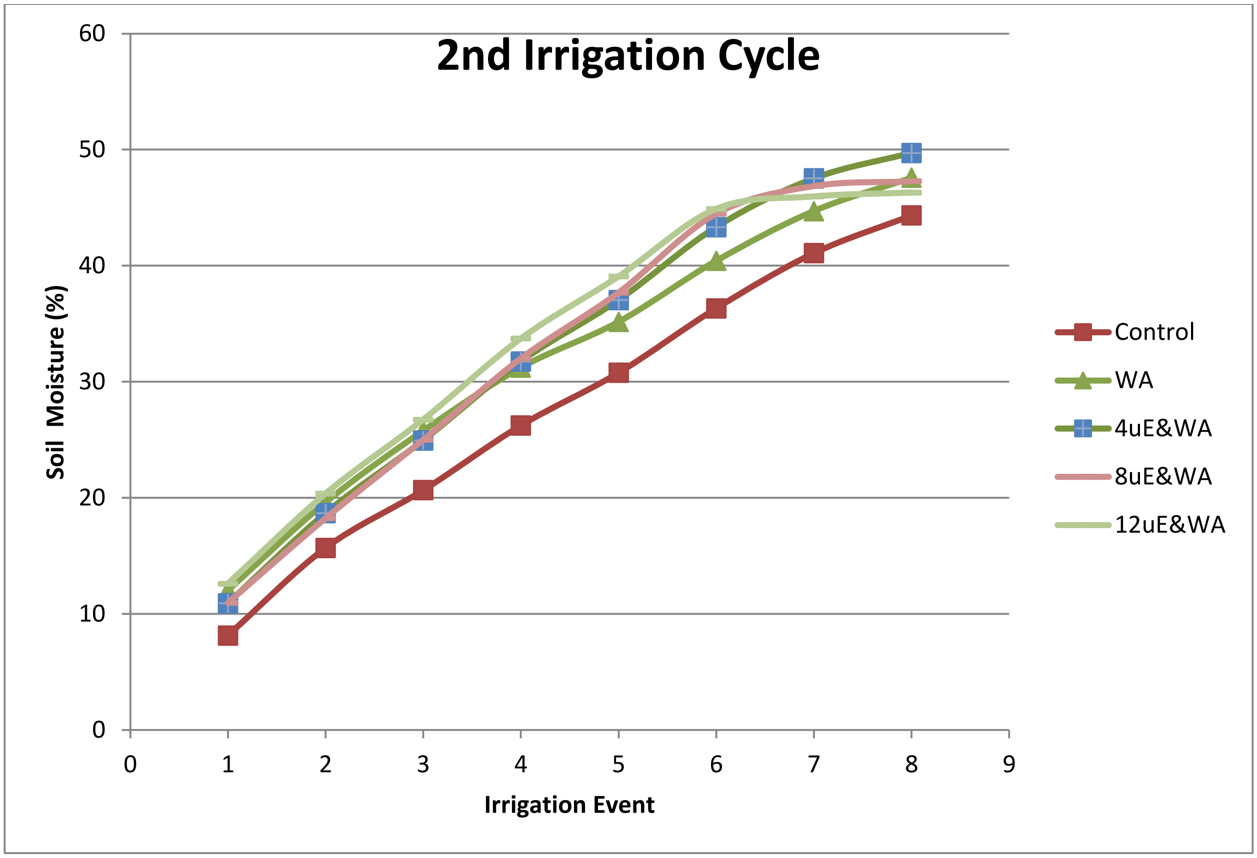

Figure 3. Comparison of responses to enzyme treatments applied in combination with a wetting agent to control and wetting agent alone treatments during the second irrigation cycle. Treatment responses are indicated as measured differences in soil moisture content over eight irrigation events during a four week period.

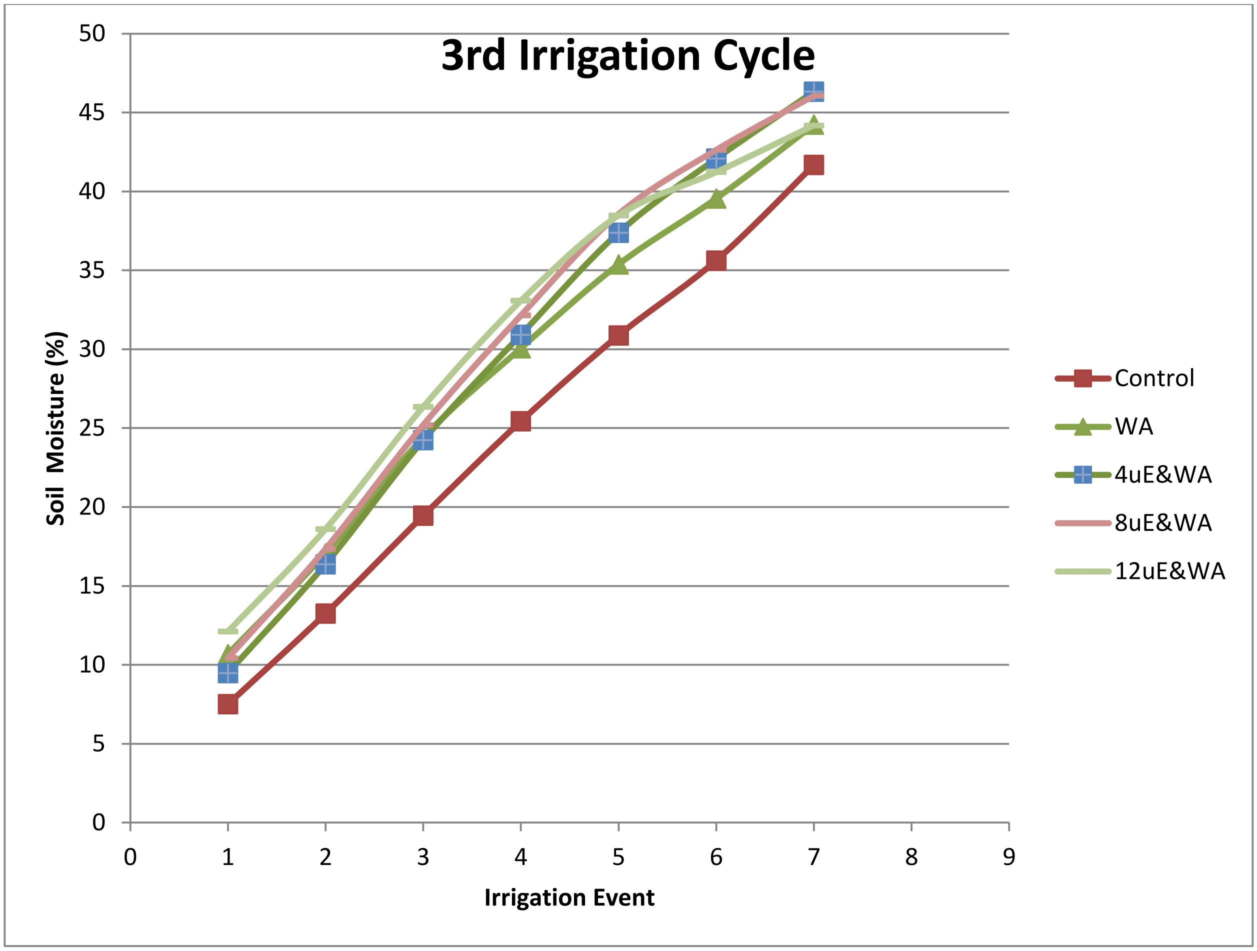

Figure 3. Comparison of responses to enzyme treatments applied in combination with a wetting agent to control and wetting agent alone treatments during the third irrigation cycle. Treatment responses are indicated as measured differences in soil moisture content over seven irrigation events during a four week period

In summary, single applications of 8 and 12 units / cm^2 of laccase enzyme alone did reduce soil water repellency and improve soil moisture content over the non-treated control by approximately 30%. This result indicates that enzyme treatments could be used as natural product alternative to wetting agents as a means to alleviate soil water repellency associated localized dry spot or fairy ring. Our results also clearly show that the wetting agent, ‘Revolution’, was more effective than any of the enzyme alone treatment rates that we used in this experiment.

When we designed this experiment, we expected that the impact of the wetting agent treatment would diminish over time while the impact of the enzyme treatments would continue to gradually increase over time. However, after four irrigation cycles of 4-weeks each we could still clearly see the positive impact of a single application of Revolution.

We did see that soil moisture response to enzyme & wetting agent combination treatments did improve during the second and third irrigation cycles. In some cases, we observed increases in soil moisture contents of 5 to 10% above those of the wetting agent alone treatment which is encouraging.

The overall goal of this research project is to maximize the potential of this new technology to alleviate soil water repellency. We have learned much from this laboratory experiment and propose to deviate from our original plant by utilizing the same procedures to investigate new treatment combinations in new laboratory trials early in 2017. We have postponed field studies planed for the Griffin Campus and at the Old Colliers G.C. outlined as objective 2, until we have completed the second set of laboratory experiments. Currently, we anticipate field trials with best treatment options will be conducted later in the spring of 2017 and fall of 2017.

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Literature Cited

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