Final Report – December 2022

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

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

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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 explored 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 that involves repeated use of wetting agents.

This current research built on our previous research efforts with both laboratory 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 enzyme proposed is 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 were to refine application protocols through laboratory experiments that determine the most 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 laboratory research were evaluated for both short-term (< 2 weeks) and long-term (season-long) effectiveness in small-scale plot trials on the University of Georgia Griffin Campus and at The Old Colliers Golf Course in Naples, Florida.

Objective 1: Research efforts initially focused on goals outlined by Objective 1 of our proposal. 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. Details of the results of this experiment are presented below.

Objective 1: Pot Study 1

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 (Fig. 1). 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 (Table 1) with five replications of each treatment. Treatments are shown in the table below.

Fig. 1. Illustration of design of pots used in Pot Studies 1 and 2. Pot on left shows nylon mesh covering bottom and vertical screen on side of pot. Pots on right show pots filled with hydrophobic soil immediately after application of 40 ml (0.25 inches) of water.

Table 1. List of treatments used in Pot Study 1.

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 / cm^2.
2. Rate of Revolution equivalent to 6.0 oz. / 1000 ft^2 denoted at WA.
3. NH Sand = non-hydrophobic sand. All other treatments are with hydrophobic sand.

Enzyme, wetting agent (WA), 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 (see Fig. 1). 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), many pots reached or neared soil-water field capacity and therefore all pots were re-dried to near 0% moisture in a forced air oven at 120 ^oC 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-week irrigation cycle for later analysis of soil water repellency using the Water Droplet Penetration Time (WDPT).

A summary of soil moisture content of selected treatments for the first 4-week irrigation cycle are presented in Fig. 2 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 illustrated in Fig. 2. 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).

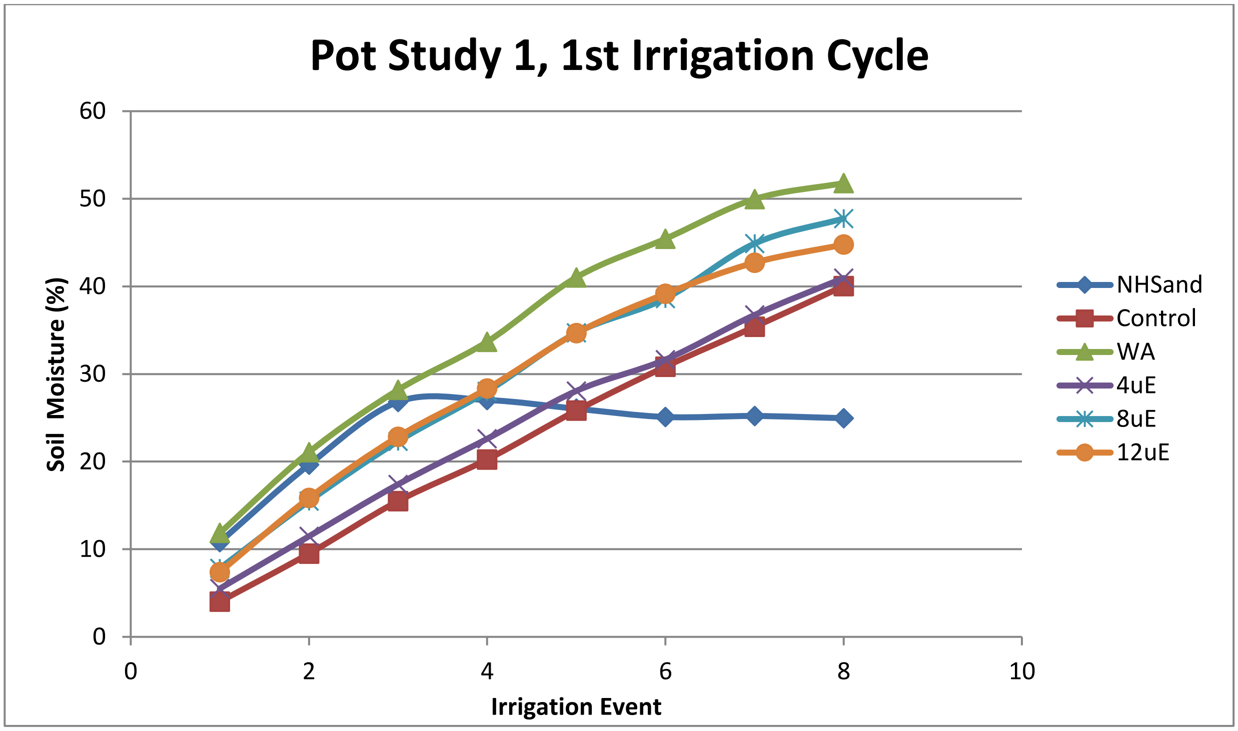

Fig. 2. Treatment responses as observed by differences in soil moisture content over eight irrigation events during a four-week period.

Figure 3 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.

During the second and third irrigation cycles (Fig. 4 and 5, respectively), 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).

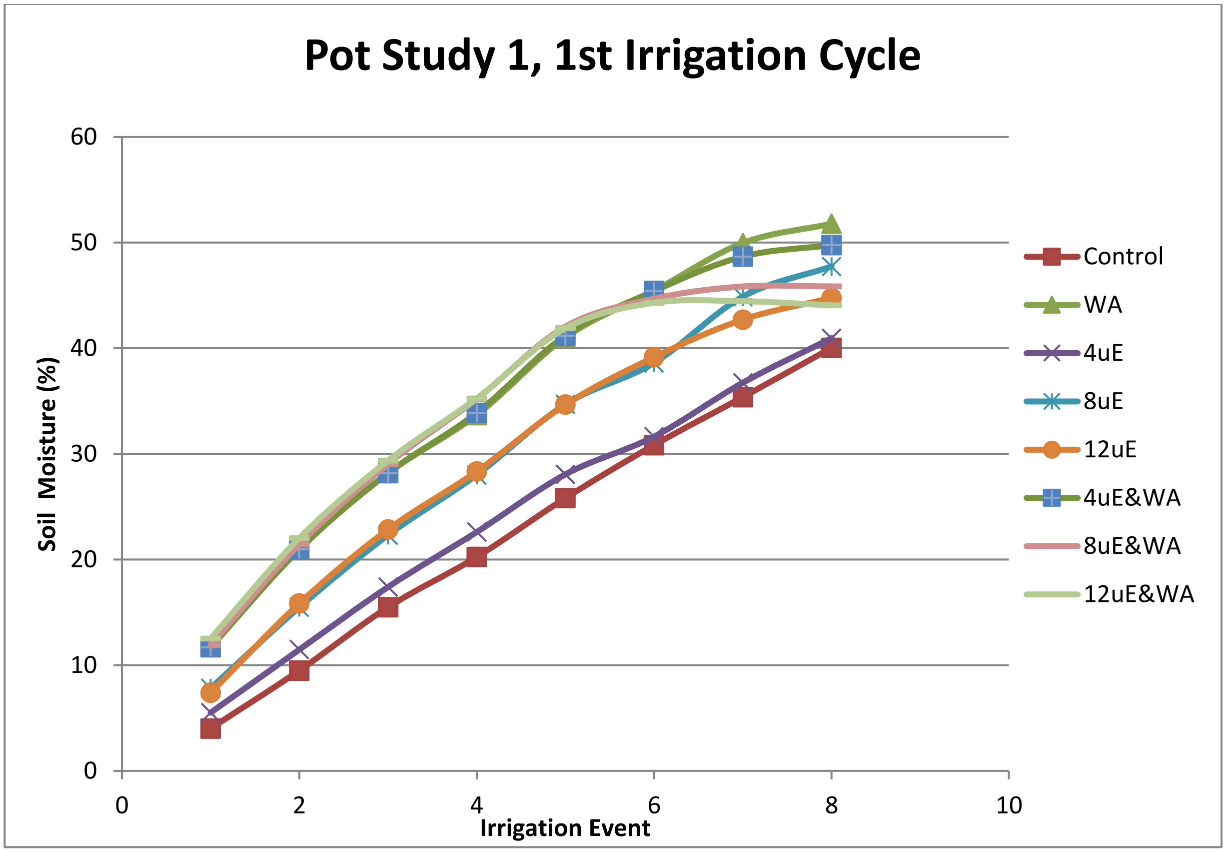

Fig. 3. Comparison of responses to enzyme treatments with and without a wetting agent added as observed by differences in soil moisture content over eight irrigation events during a four-week period.

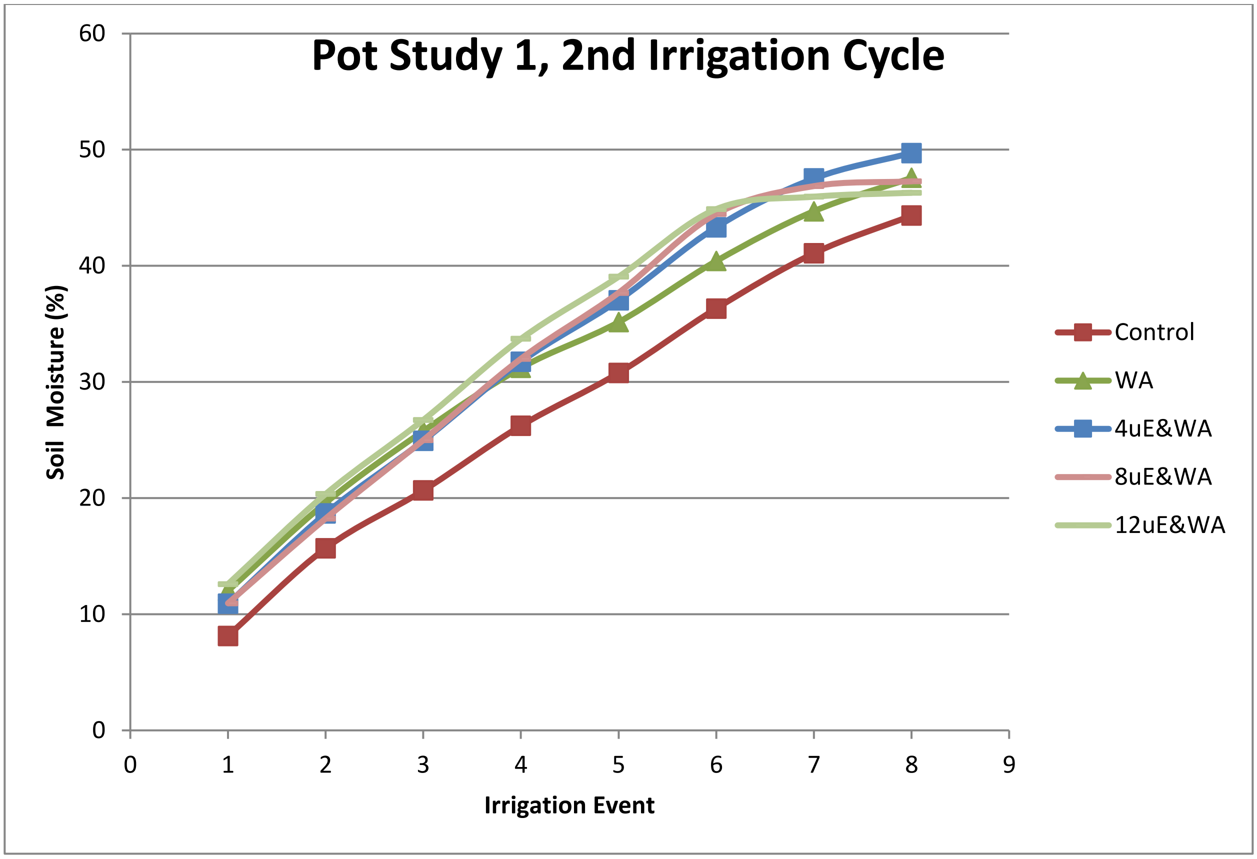

Fig. 4. Comparison of responses to enzyme treatments applied in combination with a wetting agent to control and wetting agent alone treatments during the second irrigation cycle. Treatment responses are indicated as measured differences in soil moisture content over eight irrigation events during a four-week period.

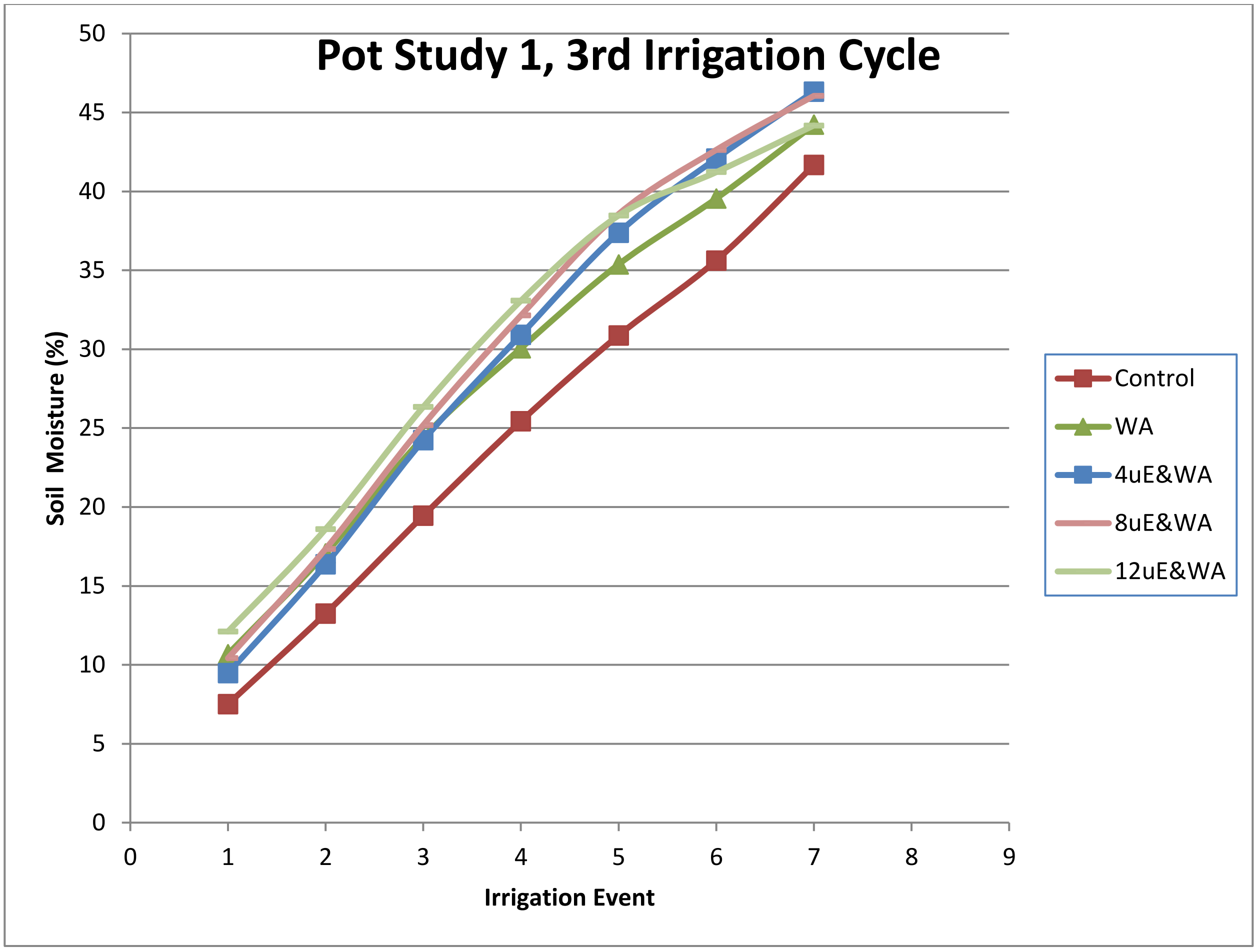

Fig. 5. Comparison of responses to enzyme treatments applied in combination with a wetting agent to control and wetting agent alone treatments during the third irrigation cycle. Treatment responses are indicated as measured differences in soil moisture content over seven irrigation events during a four-week period

In Pot Study 1, we found that 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 a natural product alternative to wetting agents 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 and 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.

Since the overall goal of this research project is to maximize the potential of this new technology to alleviate soil water repellency and we learned much from this first laboratory experiment; we chose to deviate from our original plan by utilizing the same procedures to investigate new treatment combinations in a second set new laboratory trials.

Objective 1 - Pot Study 2

A second laboratory study based on the same protocol used above was initiated. Our findings from the 1st pot study were used to further refine laccase and wetting agent treatments. Specifically, in the second study, we increased the rate of enzyme used and shortened the re-treat interval to 30 days after initial treatment. Treatments for the second laboratory pot study are shown in the table below.

Table 2. List of Treatments used in Pot Study 2.

Treatment #	**Treatment**	**Enzyme Rate[1]**	**App. Freq.**	**Wetting Agent[2]**
1	NH Sand[3]	0	0	0
2	Control	0	0	0
3	8uE	8	1	0
4	8uE & WA	8	1	6 oz.
5	12uE	12	1	0
6	12uE & WA	12	1	6 oz.
7	16uE	16	1	0
8	16uE & WA	16	1	6 oz.
9	WA	0	1	6 oz.
10	8uE + WA fb 8uE	8 + 8	30days	6 oz. + 0
11	12uE + WA fb 12uE	12 + 12	30 days	6 oz. + 0
12	16 uE + WA fb 16uE	16 + 16	30 days	6 oz. + 0

1. Enzyme rates are 8, 12, or 16 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.

The effects of enzyme rate on soil moisture content during the 1st irrigation cycle are also illustrated in Figure 5. The addition of laccase at a rate equivalent to 8 units / cm^2 improved water retention, 8.5 % above that of the untreated control. However, rates of 12 and 16 units / cm^2 greatly improved water retention, increasing soil moisture content by 23 and 25.9 % respectively over that of the non-treated control (Fig, 7).

The addition of a wetting agent to the hydrophobic sand (treatment WA) resulted in a dramatic improvement of the soil's ability to retain water. Pots treated with wetting agent acted similar to pots filled with non-hydrophobic sand and the mean soil moisture content in the WA treatment was 63.2% higher than that of the non-treated control (Fig. 6 and 7).

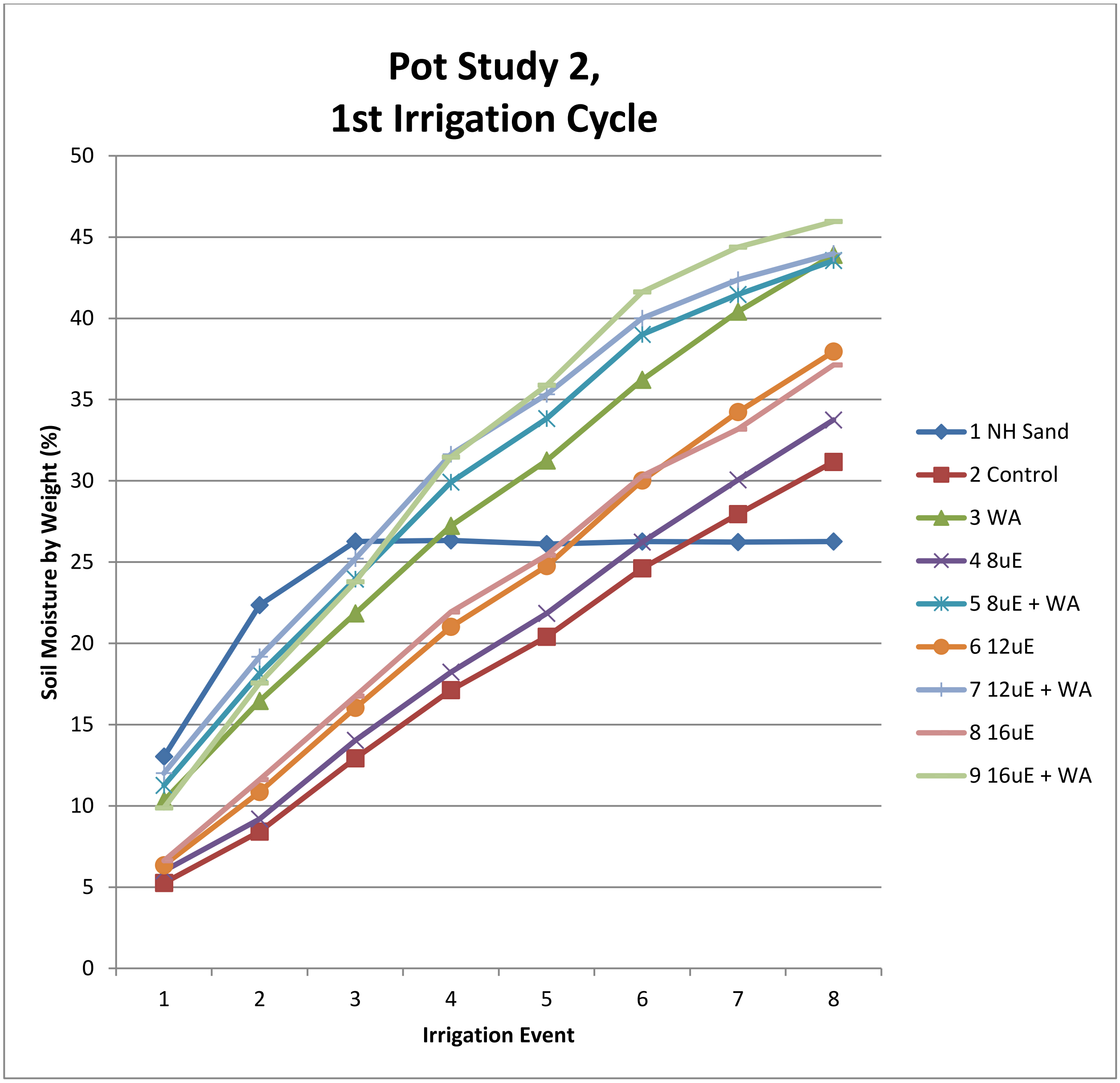

Fig. 6. Treatment responses as observed by differences in soil moisture content over eight irrigation events during a four-week period.

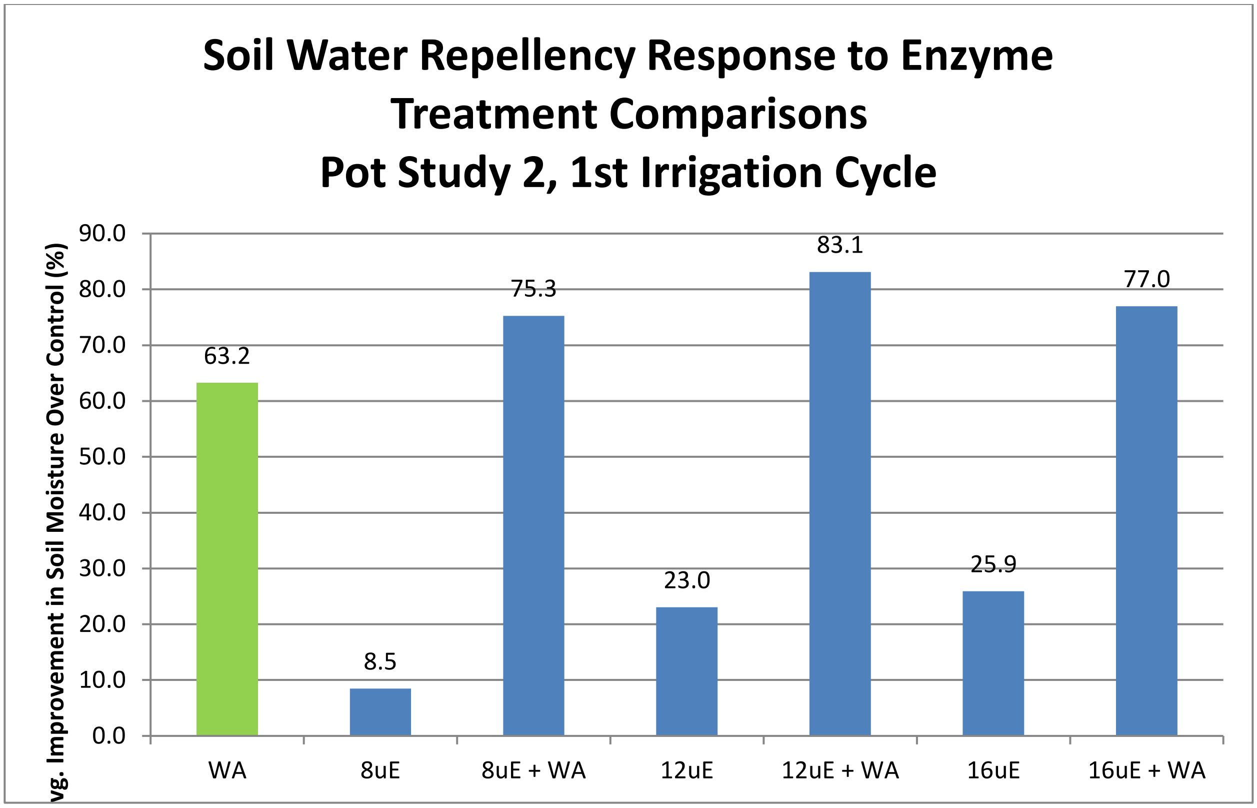

Fig. 7. Comparison of responses to enzyme treatments with and without a wetting agent added as observed by differences in soil moisture content over eight irrigation events during a four week period.

The response of hydrophobic sand to three enzyme rates in combination with a wetting agent can be seen in both Figures 5 and 6. In treatments with wetting agent applied in combination with enzyme rates of 8, 12, and 16 units / cm^2, average soil moisture was improved by 12.1, 19.9, and 13.8%, respectively, over the soil moisture content where wetting agent was applied alone. These results provide further encouragement that laccase enzyme when used in combination with a wetting agent may further improve our ability to effectively treat strongly hydrophobic soils.

In the second round of pot studies, applications 8, 12, and 16 units of laccase were compared with and without a wetting agent and with a second enzyme application after 30 days. Results for each of the three laccase rates are shown in Figures 8, 9, and 10 below.

Comparisons of treatments containing 8 units of enzyme to non-treated control and WA treatments are shown in Fig. 8 during the second cycle of irrigation (weeks 5-8). The second cycle of irrigation began 5 weeks after initial application of enzyme treatments and directly following a second application of 8 units of enzyme in the '8uE + WA fb 8uE' treatment.

During this period, we observed no improvement in water retention as compared to the non-treated control when 8 units of enzyme alone were applied and 8 units of enzyme in combination with WA showed no improvement over WA alone. Additionally, the second application of 8 units of enzyme showed no improvement over a single application.

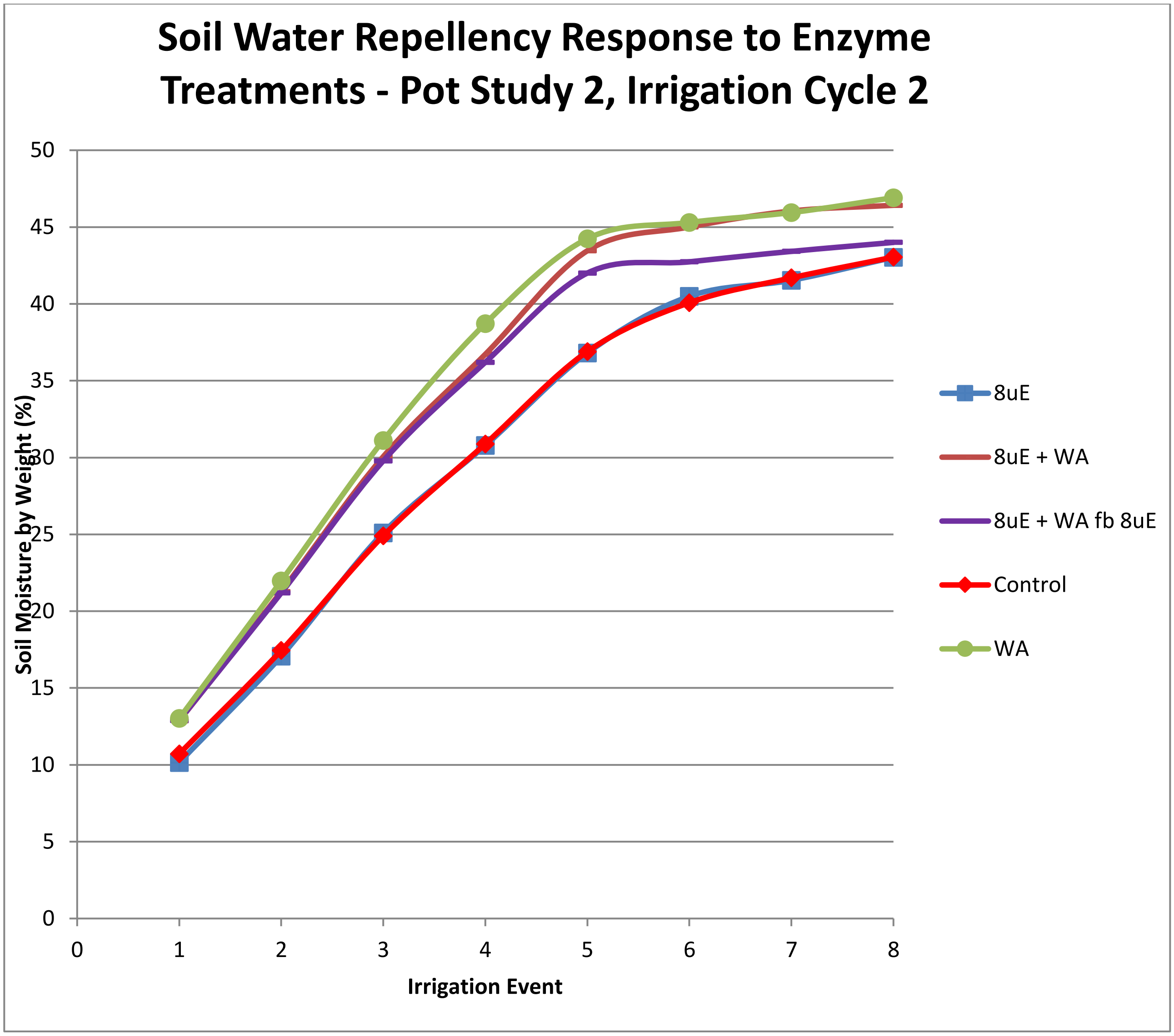

Fig. 8. Treatment responses to 8 units of laccase as observed by differences in soil moisture content during the second cycle over eight irrigation events during a four-week period. Enzyme treatments include a single application of 8 units of laccase, single application of 8 units of laccase plus a wetting agent, and 8 units of laccase plus a wetting agent followed by repeated application of 8 units of laccase 30 days after the first (just prior to cycle 2).

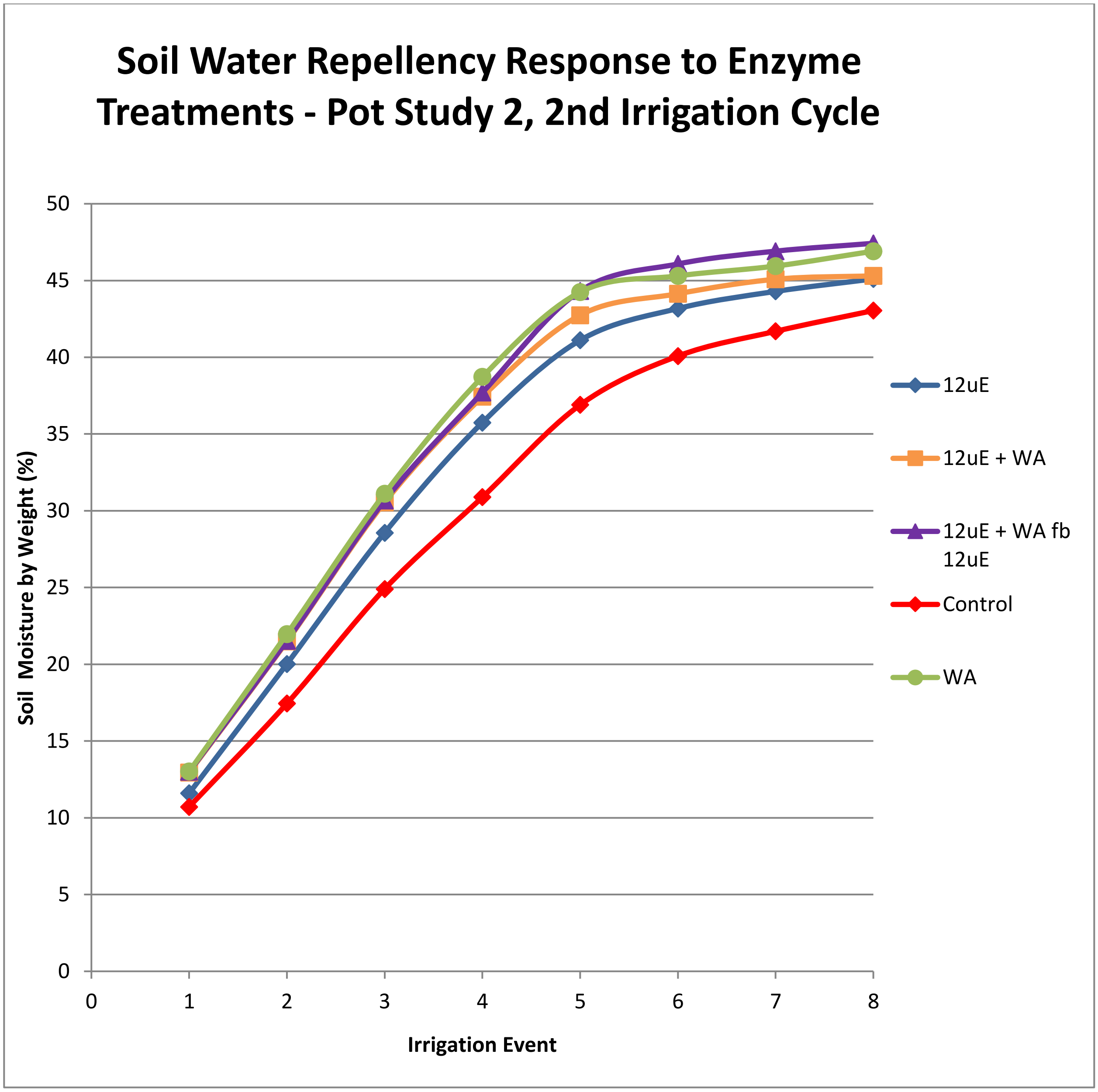

Fig. 9. Treatment responses to 12 units of laccase as observed by differences in soil moisture content during the second cycle over eight irrigation events during a four-week period. Enzyme treatments include a single application of 12 units of laccase, single application of 12 units of laccase plus a wetting agent, and 12 units of laccase plus a wetting agent followed by repeated application of 12 units of laccase 30 days after the first (just prior to cycle 2).

Comparisons of treatments containing 12 units of enzyme to non-treated control and WA treatments are shown in Fig. 9 during the second cycle of irrigation (weeks 5-8). The second cycle of irrigation began 5 weeks after initial application of enzyme treatments and directly following a second application of 12 units of enzyme in the '12uE + WA fb 12uE' treatment.

In contrast to the results from Fig 8, the application of 12 units of enzyme alone did show significant improvement in water retention (approximately 11.5%) over the non-treated control during this period. When 12 units of enzyme were applied in combination with WA, water retention improved by 16% over the non-treated control but was no better than the WA alone treatment. We did observe a modest increase (4%) in water retention when a second application of 12 units of enzyme was applied, but moisture retention was only slightly better than the WA alone treatment.

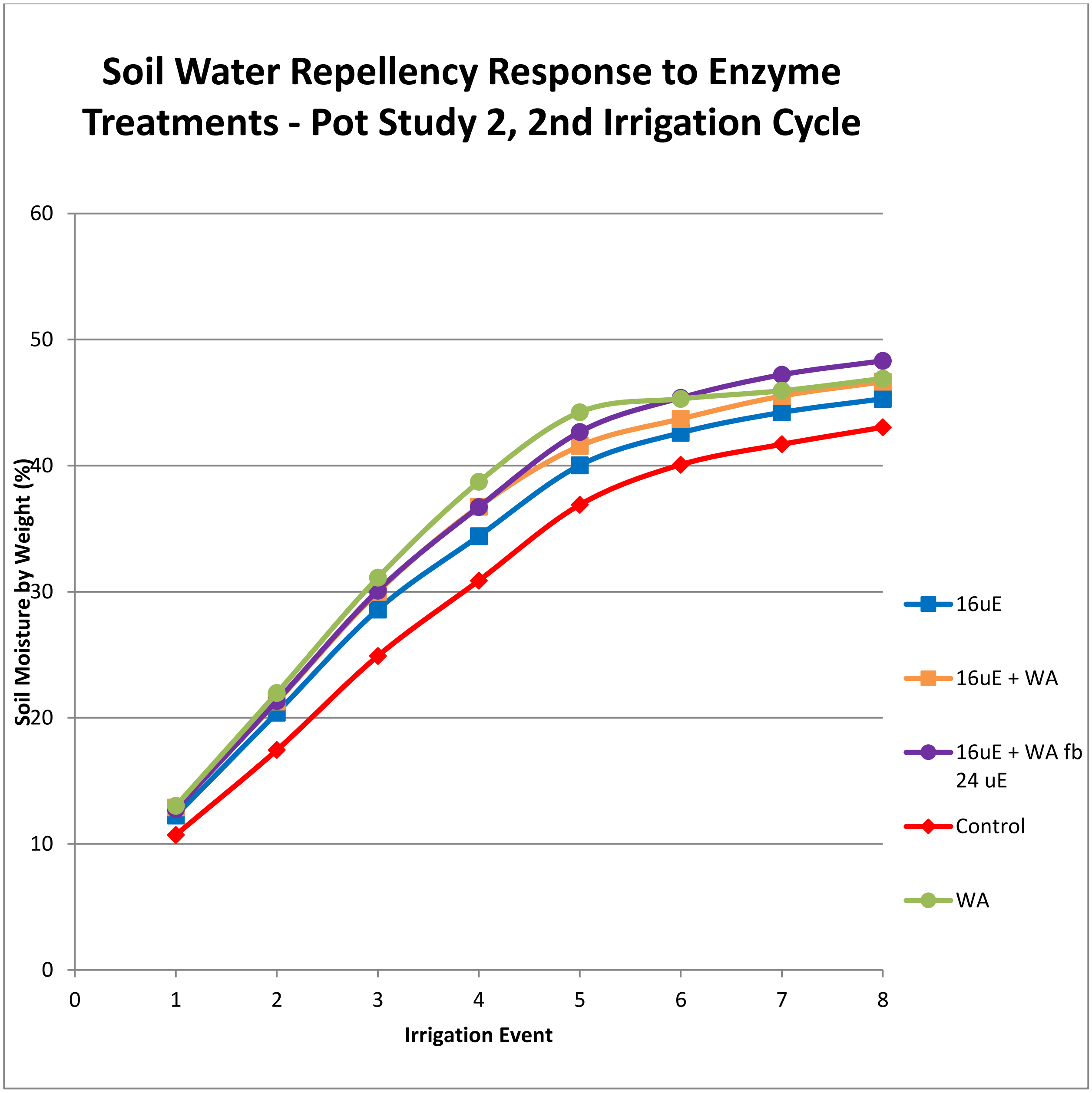

Fig. 10. Treatment responses to 16 units of laccase as observed by differences in soil moisture content during the second cycle over eight irrigation events during a four-week period. Enzyme treatments include a single application of 16 units of laccase, single application of 16 units of laccase plus a wetting agent, and 16 units of laccase plus a wetting agent followed by repeated application of 24 units of laccase 30 days after the first (just prior to cycle 2).

Comparisons of treatments containing 16 units of enzyme to non-treated control and WA treatments are shown in Fig.10 during the second cycle of irrigation (weeks 5-8). The second cycle of irrigation began 5 weeks after initial application of enzyme treatments and directly following a second application of 24 units of enzyme in the '16uE + WA fb 24uE' treatment.

The application of 16 units of enzyme alone showed a similar response to that when 12 units of enzyme alone were applied with a 11% improvement in water retention over the non-treated control during this period. When 16 units of enzyme were applied in combination with WA, water retention improved slightly when compared to the enzyme alone treatment but again was no better than the WA alone treatment. We did observe a modest increase (4%) in water retention when a second application of 24 units of enzyme was applied, but moisture retention was still no better than the WA alone treatment.

Objective 2: Griffin Campus Field Study

Through the two laboratory experiments discussed above, we learned that an enzyme application rate of 12 units /cm^2 or above provides a consistent response and that enzyme should be combined with a wetting agent to provide maximum relief of soil water repellency. Based on these data we selected three treatments for more detailed study in field plots at the Griffin Campus as proposed to fulfill Objective 2 of this research project.

Treatments for Griffin Campus field study included: 1) a single application of 12 units / cm^2 of enzyme in combination with wetting agent (6 oz. Revolution / 1000 ft^2), 2) a single application of Revolution wetting agent alone, and 3) a non-treated control.

Each of these treatments were replicated 10 times in the study area. The study site was a sports field research area planted in seashore paspalum on the UGA Griffin Campus. The field was approximately 20 years old and was constructed using USGA profile specifications. Localized dry spot is frequently observed on this area. Plots were 5 x 5 feet and treatments were randomly assigned based on base-line volumetric soil moisture readings taken using a TDR moisture probes at 3.8 and 7.6 cm. Treatments were applied using a handheld CO_2 spray boom. All plots were irrigated twice weekly with 0.5-inches each application. TDR moisture readings at 3.8 and 7.6 cm were taken weekly for six weeks at 4 hrs. and 24 hrs. after an irrigation event. Ten TDR readings were taken in each plot at each depth providing 100 moisture readings each date for each treatment. Visual ratings of turf quality were taken weekly, and soil samples were taken before and at 4 weeks following treatment to be used for tests of water droplet penetration time (WDPT) and contact angle analysis.

Visual differences in turfgrass quality were apparent among treatments (Fig. 11). However, we, unfortunately, lost all our visual quality data on this experiment when a notebook computer containing our field data unexpectedly crashed. Laboratory analysis of soil samples failed to show significant differences in treatment means at most sampling dates for either WDPT and contact angle analysis.

Soil moisture data taken at the 3.8 cm depth seemed to be the most responsive to our treatments and is presented in Fig. 12 below. Both the wetting agent alone and 12 units / cm^2 of enzyme in combination with wetting agent showed a dramatic improvement in both turf quality (Fig. 11) and soil moisture (Fig. 12). The single application of wetting agent improved soil moisture over the non-treated control by 29% when averaged over all sampling dates while a single application of 12 units / cm^2 of enzyme in combination with wetting agent improved VWC by 36.6% over the non-treated control. Differences between wetting agent alone and wetting agent plus enzyme were not significant at the 5% probability level at any measurement date, but the numerical trend did show an average 6% improvement in soil moisture over wetting agent alone when enzyme was added. These findings largely agree with our laboratory pot experiments showing a slight but not statistically significant improvement in soil water content over wetting agent alone when enzyme was added.

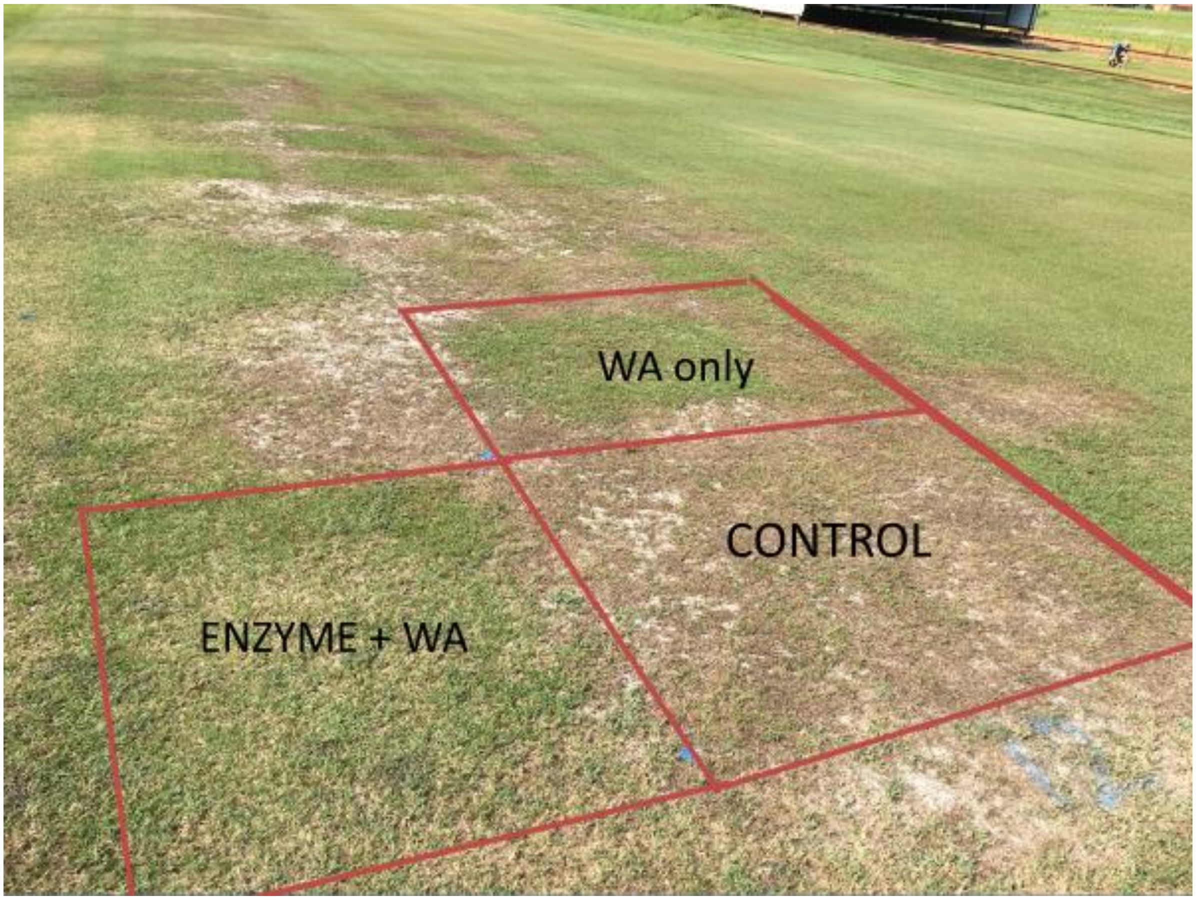

Fig. 11. Visual response to SWR treatments in the Griffin Campus Field Trial 6 weeks after treatment application. Treatments shown are a single application of wetting agent only (WA only), a single application of 12 units of laccase enzyme plus wetting agent (Enzyme + WA), and a non-treated control.

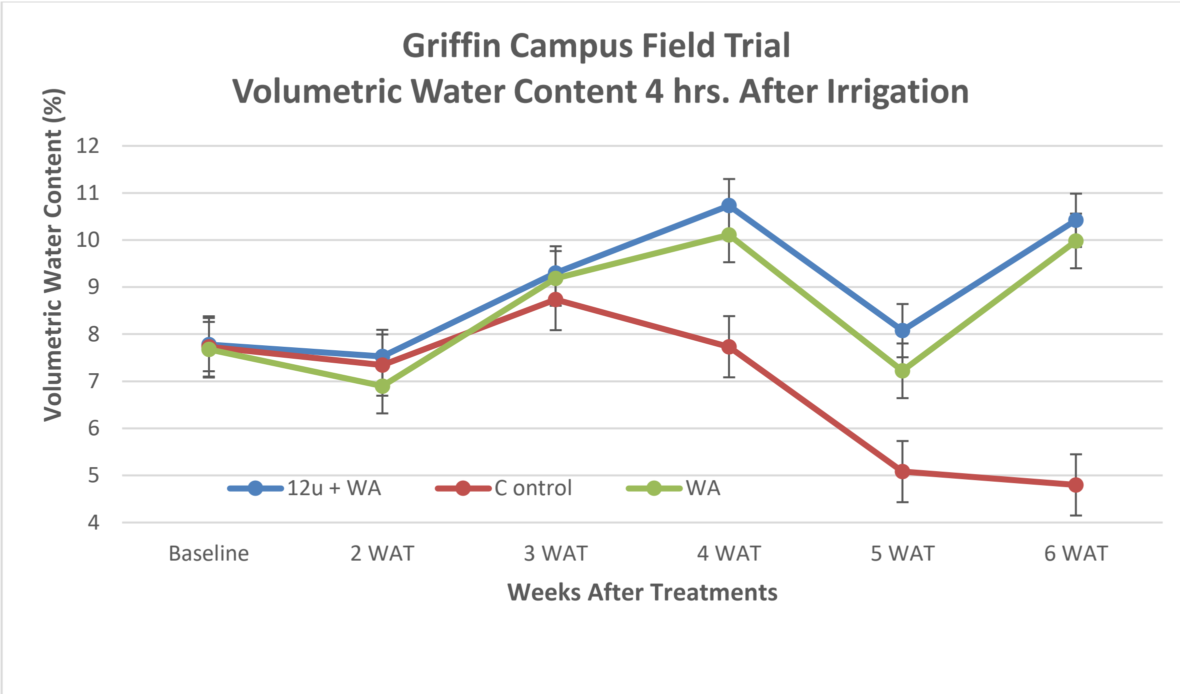

Fig. 12. Comparisons of changes in volumetric soil moisture measured by TDR to a non-treated control, a single application of wetting agent, and a single application of 12 units of laccase enzyme plus wetting agent. Volumetric Water Content (VWC) measurements were taken prior to application of treatments (Baseline) and at 2,3,4,5,6 weeks after treatment (WAT). All VWC measurements were made with 3.8 cm probes.

Objective 2 – The Old Colliers Golf Course Trial

The Old Colliers Golf Course (TOCGC) located in Naples, Florida has chronic problems with LDS due to the sands the course was constructed on, poor quality irrigation water, and the drying conditions that typically occur during the winter months. In spite of routine applications of wetting agents, SWR issues reoccur every year during the winter and spring months. These circumstances make TOCGC an ideal location to test our best treatments under an actual golf course management regime. TOCGC has a long history of collaborative research with The University of Georgia and is supportive of the development of any new technology that can help them to manage SWR on their course. Our approach to this study was to use many replications of only a limited number of our best treatment combinations and placed on tee boxes and fairways with varying degrees of SWR.

Data collection from these field studies was according to the following procedures. Soil samples for SWR evaluation were collected at 0, 4, 8, 12 weeks after treatment application. For each sample date, multiple soil cores were obtained (2 dia. x 9.0 cm deep) air dried and gently sieved to separate the sand from plant residue. These soil samples were used for the WDPT (water droplet penetration time) tests for SWR after drying at 40 °C for 36 hr. At the Griffin Campus, volumetric soil water content (%VWC) was documented weekly using TDR (Spectrum Field Scout TDR 300) with 3.8 and 7.5 cm probes on a grid pattern with measurements taken 24 hours after irrigation. At TOCGC, soil salinity was an issue, and therefore %VWC readings were taken using a POGO (Stevens POGO turf and soil insite tool); otherwise data collection protocols were as described for the Griffin Campus field study. Turf quality was documented based on digital images collected using a lighted camera box and images processed by Assess software (American Phytopathological Society) to obtain estimates of "green cover".

Treatments for this study included a non-treated control, a wetting agent alone (Revolution at 6 oz. / 1000 ft^2, enzyme alone at 12 units / cm^2 of enzyme, and enzyme in combination with the wetting agent. After establishing test plot areas around the golf course, initial treatments were applied on April 13 and second applications of 12 units / cm^2 of enzyme were applied to the laccase alone and laccase plus wetting agent plots on June 8, approximately 8 weeks following the initial treatment. Soil moisture in all plots was monitored weekly using the POGO through September 14 (approximately 5 months).

Analysis of soil samples and digital images and failed to show consistent differences among treatments. Once again, the soil moisture data seemed to be the most response to our treatment applications and are presented in Fig. 13. The wetting agent alone treatment responded much as we expected. Initially, wetting agent treatment increased soil moisture by 3%, for a 13% improvement over the control. However, that effect gradually diminished over a two-month period. The laccase alone treatments showed a slower response with a noticeable improvement over the non-treated control at 5 weeks after treatment, but that positive response also diminished over time even with an additional application of laccase. In this study, we did observe a long-term improvement in soil moisture when laccase was applied in combination with the wetting agent. Plots receiving the laccase plus wetting agent treatment initially showed slightly higher soil moisture than plots treated with the wetting agent alone and this effect continued long after the effects of wetting agent alone had diminished. Over the duration of the experiment, plots treated with the combination of laccase plus wetting agent improved soil moisture on average by 2.3% and had, on average, 10.7% higher soil moisture than the non-treated control. Analysis of variance of treatment means over all the combined dates showed that the laccase plus wetting agent treatment had statistically higher (p=0.0001) soil moisture than any other treatment.

SWR Response to at the Old Colliers Golf Course to Laccase Treatments

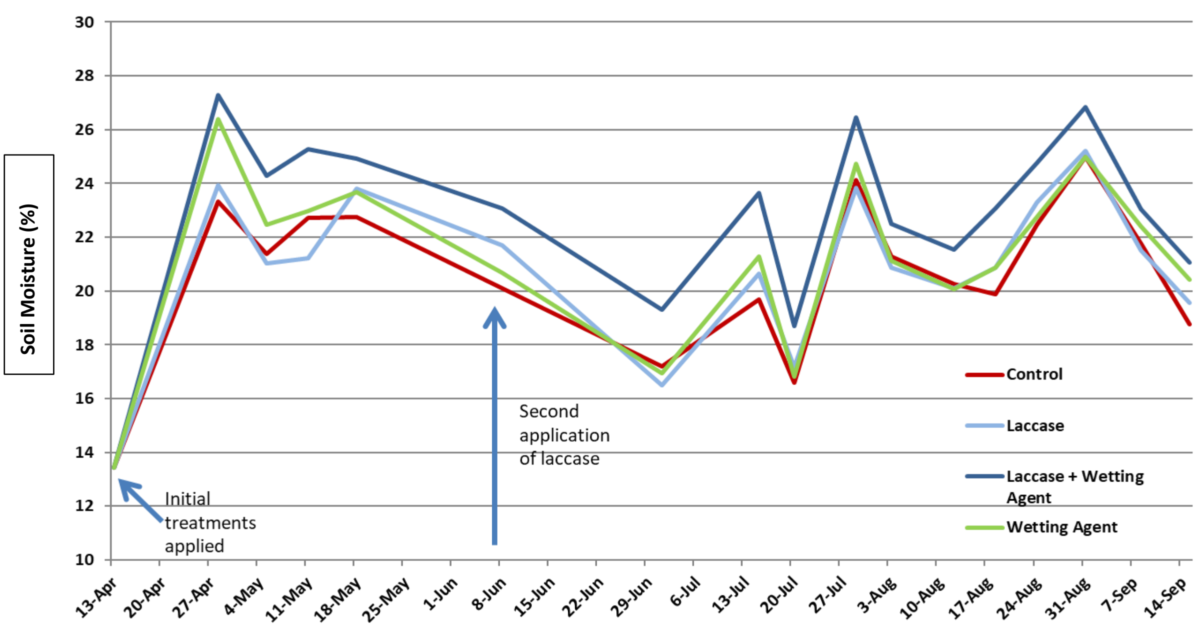

Fig. 13. Effects of four treatment combinations on soil moisture over a five-month period.

Summary:

The specific objectives of the proposed project were to refine application protocols through laboratory experiments that determine the most 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 was evaluated. Field evaluations involving the most effective treatments based on our laboratory research studied both short-term (< 2 weeks) and long-term (season-long) effectiveness in small-scale plot trials on the University of Georgia Griffin campus and at The Old Colliers Golf Course in Naples, Florida.

We found that single applications of laccase enzyme alone did reduce soil water repellency and improve soil moisture content over the non-treated control by as much as 30%. This response was enzyme rate dependent with the higher rates of 12 and 16 units / cm^2 of enzyme showing the most beneficial response. However, 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 our laboratory studies, we did see that soil moisture response to enzyme and wetting agent combination treatments did improve during the second and third month-long irrigation cycles. In some cases, we observed modest increases in soil moisture content of 5 to 10% above those of the wetting agent alone treatment. A field study at the Griffin campus also showed the same trend of modest improvements over wetting agent alone when enzyme was added. In trials at The Old Colliers Golf Course, we did observe a longer-term and statistically significant improvement in soil moisture when laccase was applied in combination with the wetting agent.

This research was an offshoot of prior research exploring the potential use of laccase enzyme to reduce thatch accumulation on highly managed turfgrass sites such as golf course greens. Our findings clearly indicate that laccase enzyme does have the potential to reduce SWR but when used alone, is not as effective at doing so as top performing wetting agents in current use. However, this system does provide an organic/natural product alternative to wetting agents. Our most significant finding is that applications of 12 to 16 units / cm^2 of enzyme in combination with a wetting agent could be effective as a spot treatment and slighly more effective in remediating SWR than wetting agents alone by providing slighly improved and longer-term effects. Furthermore, routine use of this enzyme system for thatch management could have a secondary benefit of reducting SWR issues commonly seen on sand-based soils.

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