An Investigation into the Principle Modes of Action of Surfactants and how a Novel Formulation may Improve Turfgrass Quality by Increasing the Dominance of *Agrostis spp.* in Golf Greens

Thomas Martin, Dr Shane Rothwell, Dr Carly Stevens
Agronomy Research Group, Lancaster University, Lancaster University
t.martin@hitas.co.uk

Abstract

The aim of this study was to establish the mechanisms underpinning observations made in a field trial that suggested a novel surfactant treatment could promote bentgrasses *Agrostis spp.* in golf greens that contain annual meadow grass (*P. annua*). Assessments of the effects of three surfactant treatments (named treatments 1, 2 and 3) on shoot height, biomass accumulation, rhizosheath properties, soil water distribution and rooting characteristics were made over the course of two experiments carried out in controlled conditions. We found that leaf extension rate was significantly affected in the two grass species to different extents depending on the surfactant used. This finding could have positive implications for turf quality in the field for a newly developed formulation (treatment 3), which was observed to be the case in the field trial that pre-dated this study. The same treatment also resulted in significant differences in the grasses in terms of rhizosheath size compared to untreated soil. We found that surfactants affected the distribution of water in the soil by increasing the rhizosheath water content to bulk soil water content ratio, potentially maximising water and nutrient uptake by the roots. The combination of effects observed with use of the novel surfactant treatment may lead to improved water use efficiency and a more desirable sward composition for golf greens.

Background

A two-year field study investigating the effects of different surfactant products on golf green sward composition revealed that a newly developed formulation could influence the prevalence of finer grasses such as bentgrasses and fescues (Baldwin, 2019). This was an exciting finding and highlighted a novel method of annual meadow grass (*Poa annua*) management. However, the field trial was unable to explain why the prevalence of the finer grasses had been affected. As a result, a research project was undertaken to establish the mechanisms underpinning the field observations.

Study Outline

Figure 1. *Two experiments were carried out - a 'mini golf green' trial which mimicked real-world golf green management conditions (left) and an isolated plant trial to investigate direct treatment effects on individual plants and associated soil (right).*

The project incorporated two main experiments, the first being a 'mini golf green' trial. By using a 1:1 v:v mix of washed dune sand and soil collected from a links golf course in South-West Scotland, an attempt was made to mimic real-world golf green management conditions in 2 litre, 20 cm deep pots. The second experiment was an isolated plant trial to investigate direct treatment effects on individual plants and associated soil (Figure 1). Three different surfactant treatments were incorporated into the trials. Treatment 1 was a block co-polymer turfgrass surfactant with a monthly 20 l/ha recommended application rate. Treatment 2 was a 'super spreader' type surfactant which has been shown to reduce water surface tension significantly and to have good soil penetrant properties at a recommended application rate of 1 l/ha. Treatment 3 was a novel formulation (block co-polymer based) that has been shown in preliminary tests to penetrate deep into the soil profile at an application rate of 20 l/ha.

The aim of the 'golf green' trial was to determine if any of the observed effects in the field could be supported by data gathered in controlled conditions. Primarily, sward height change over time of two grass species, highland bentgrass (*Agrostis castellana*) and annual meadow grass, was measured to determine a mechanism for altered competition dynamics in the field. Grasses were raised from seed and cut every 5-6 days to a mean (± SE) height of 6.66 ± 0.075 mm. The change in sward height that had taken place during the period following each cut was recorded. The second experiment using plants grown in isolation was used to determine what was influencing growth rate without cutting, whether that be changing below-ground conditions or direct effects on plant physiology.

Both trials incorporated four treatment groups with 6 replicates – a control (C - water only), treatment 1, treatment 2 and treatment 3. Solutions for each surfactant treatment were made at concentrations based on their recommended per-hectare field application rates (Table 1).

Table 1: The percentages of surfactant present in the surfactant-water mix used to treat each pot/tube. The control treatment is composed of only tap water.

Treatment	Percentage of Surfactant in Water-Surfactant Mix (% v/v)
C	0
1	2.86
2	0.143
3	2.86

For the golf green trial, plants were kept well-watered gravimetrically during the treatment application phase which consisted of four 1 ml doses of each treatment applied over a period of six weeks. For the isolated plant trial, a single application equivalent to six doses of surfactant was used, which amounted to approximately 240 µl of solution per tube (in a real-world golf green, up to six doses of surfactant may be applied throughout the year). Again, plants were watered gravimetrically and kept well-watered throughout the experiment.

Statistical Analysis

Data analysis primarily involved using two-way analyses of variance (ANOVA's) to compare differences between means and identify any interaction effects between treatment and species. Where direct comparisons were required between species for a given treatment, Tukey post-hoc tests were used. In all graphs presented, within species differences are denoted by letters, with 'a.a' corresponding to control values for highland bentgrass (A) and 'p.a' corresponding to control values for annual meadow grass (P). A significant difference from the control is denoted by a 'b' instead of an 'a' following the decimal point above the corresponding bar. Between species significant differences when comparing within a treatment are denoted by an Asterix between the values that are being compared.

Results

'Golf Green' Assessments

A decision was made to split the turf assessments into two phases for data analysis. This is due to the rapid decrease in growth rate after the initial stimulation of growth that was observed after the first time the grasses were cut. The first cut was less vigorous than subsequent cuts, so it is important to make this distinction for analysis of the data. The growth rate remained approximately constant throughout the rest of the trial. As such, a mean growth rate for each replicate within each treatment was calculated using the measurements taken across four dates, i.e., each date was treated as a technical replicate. This provided one value for the six functional replicates in each treatment group, which were then used for subsequent analysis.

Growth Rate

During phase 1 no significant difference ($p = 0.197$) in growth rate was present between treatments for annual meadow grass but for highland bentgrass the growth rate was significantly higher (p = 0.0283) for turfs growing under treatment 3 compared to control turfs (Figure 2-A).

For both species, no significant differences in growth rate (p = 0.312 and p = 0.09043 for annual meadow grass and highland bentgrass, respectively) were present between treatments during phase 2 (Figure 2-B). However, there was a significant difference in the growth rate across treatments (p = 0.00268) between the two species (Figure 2-B). On average, growth rate was 13.5% higher for highland bentgrass than annual meadow grass across treatments. Growth rate was significantly higher (p = 0.0199) for highland bentgrass compared to annual meadow grass under treatment 3 (Figure 2-B), with an average growth rate difference of 32.1%.

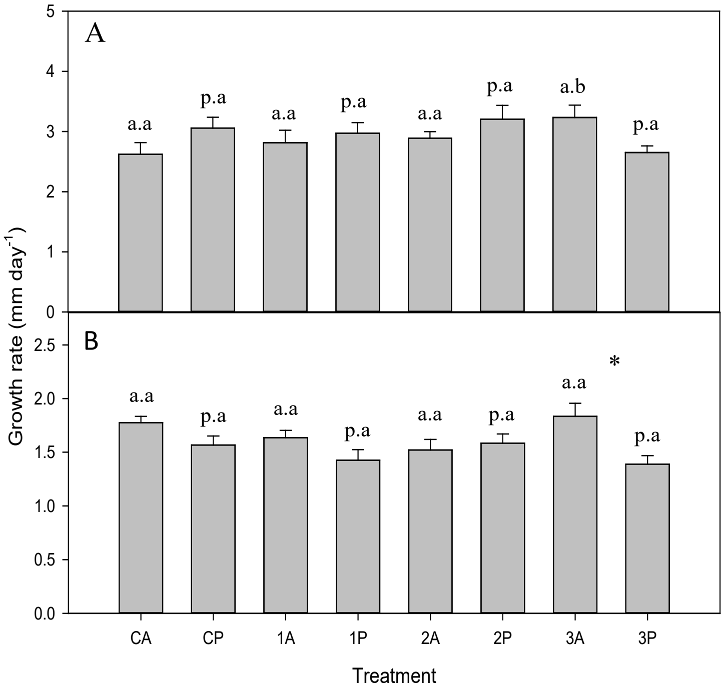

Figure 2. *Sward growth rate expressed in millimetres of growth per day for turf assessments phase 1 (A) and 2 (B). Phase 1 is the initial stimulation of growth that took place after the first time the turfs were cut. Phase 2 includes the growth data that was gathered after all subsequent cuts.*

Single-Plant Assessments

Growth Rate

For within species comparisons, there was no significant difference in plant growth rate between treatments (p = 0.712 and p = 0.425, for annual meadow grass and highland bentgrass respectively). However, there was a significant difference in plant growth rate between the two species ($p < 0.05$), with growth rate being significantly higher ($p = 0.00995$) for highland bentgrass compared to annual meadow grass under treatment 3 (Figure 3-A). Taking the control group as a baseline growth rate difference between the two species, on average the difference was increased by 8.3% under treatment 1, 15.5% under treatment 2 and 52.1% under treatment 3.

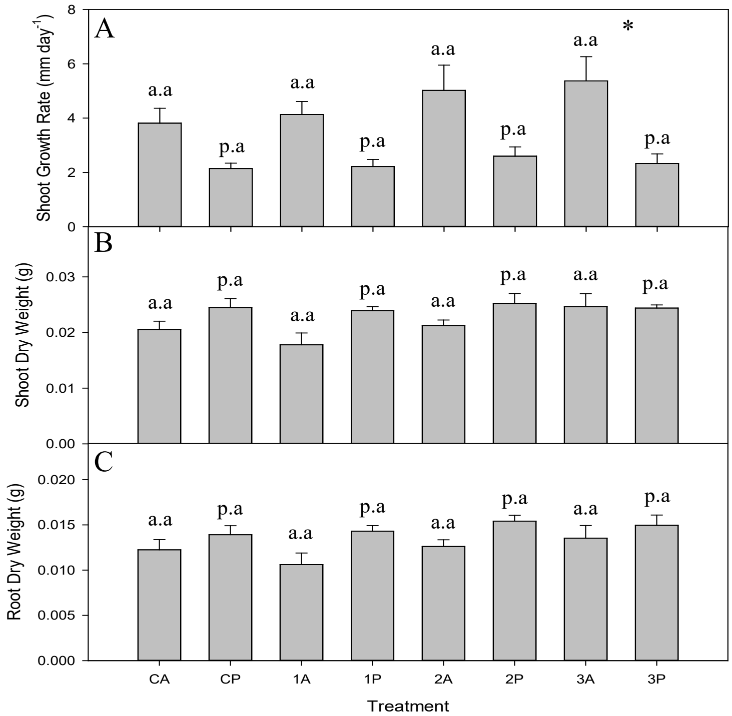

Figure 3. *Shoot growth rate expressed in millimetres of growth per day (A), shoot dry weight (B) and root dry weight (C). Shoot and root dry weight are expressed as grams of dry matter. Graph (A) includes all growth that took place between 26/06/19 and 02/07/19 for both species and each treatment.*

Biomass Accumulation

No significant differences in above-ground biomass between any of the treatments and the control were present for either annual meadow grass or highland bentgrass ($p = 0.911$ and $p = 0.0956$, respectively). Above-ground biomass was statistically identical for both species under treatment 3 ($p = 1.00$) but under all other treatments annual meadow grass had a higher mean (Figure 3-B). Across treatments, above-ground biomass was 16.4% higher for annual meadow grass compared to highland bentgrass on average. This difference dropped to only

1.093% under treatment 3. No significant differences in below-ground biomass (Figure 3-C) between any of the treatments and the control were present for either annual meadow grass or highland bentgrass (p = 0.636 and p = 0.373, respectively).

Below-ground Assessments

Rhizosheath weight was normalised by root length to express it as the weight of rhizosheath per centimetre of root length (Basirat, 2019). Normalised rhizosheath weight (NRW) was significantly higher than the control group for treatment 3 for both annual meadow grass (p = 0.0313) and highland bentgrass (p = 0.00596) (Figure 4-A). On average, NRW was 41.6% higher for annual meadow grass and 88.5% higher for highland bentgrass compared to the control groups for both species.

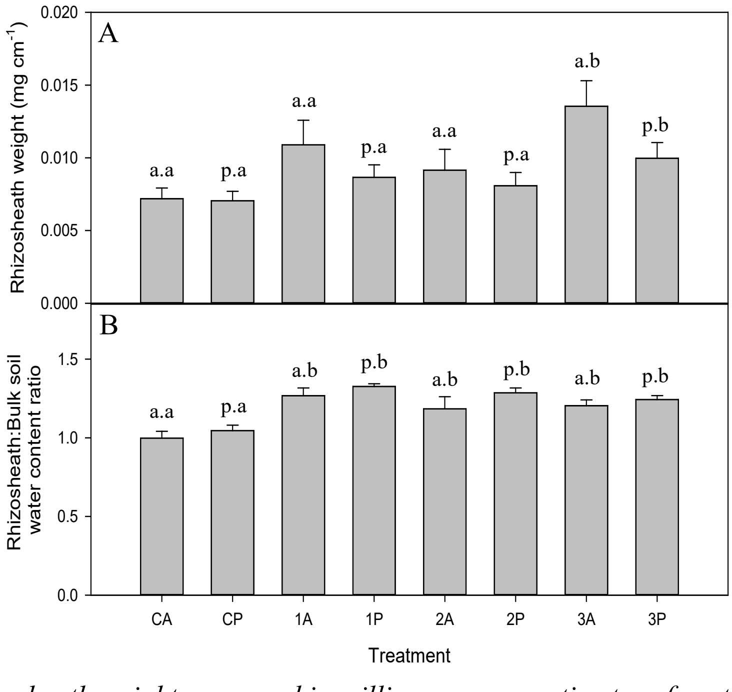

***Figure 4**. Dry rhizosheath weight expressed in milligrams per centimetre of root length (A). The ratio between the gravimetric water content of the rhizosheath and the gravimetric water content of the surrounding bulk soil is also shown (B). Each quantity was expressed on a grams of water per gram of dry soil basis before the ratio was calculated.*

NRW was significantly higher for highland bentgrass than annual meadow grass across all treatments (p = 0.0463), with the difference between the two species being 20.9% on average. On average, the difference between species was 25.9%, 13.4% and 35.9% for treatments 1, 2 and 3, respectively. For both species, the ratio between rhizosheath water content and bulk soil water content was significantly higher for all surfactant treatments ($p < 0.05$ in all cases) compared to the control treatment (Figure 4-B). Compared to the control group, on average this difference was 26.9%, 20.8% and 19.7% for treatments 1, 2 and 3, respectively.

Summary of Key Findings

Plant Growth Rate

Plant growth rate was affected by surfactant use in both undisturbed plants and turfs that were cut regularly. Treatment 3 may alter the competitive dynamic between highland bentgrass and annual meadow grass given the relative difference in growth rate between the two species under this treatment – highland bentgrass had a significantly higher growth rate than annual meadow grass (Figures 2-B and 3-A). This may translate in the field to highland bentgrass becoming more dominant in the sward than it would under natural conditions, as has been shown in the field trials that this study was based on (Baldwin, 2019). The fact that growth rate was higher in highland bentgrass than annual meadow grass under treatment 3 in both the turf (Figure 2-B) and single-plant assessments (Figure 3-A) means we can be confident that the treatment is affecting the two species differently, irrespective of factors such as intraspecific competition.

Shoot Biomass

There is evidence provided by the data for above-ground biomass accumulation to support the suggestion that the competitive dynamic between the two species may be altered by surfactant treatment 3. We would expect under controlled conditions annual meadow grass to have higher shoot biomass than highland bentgrass due to generally being a heavier plant - this was indeed the case for control plants and treatments 1 and 2 (Figure 3-B). However, although there were no significant differences, the above-ground biomass accumulation was close to identical in the two species when under treatment 3. This is potentially very promising for use of the product in the amenity turf industry, as a sward containing individual plants with comparable above-ground biomass will provide a more uniform surface, improving playability. It is likely that the larger difference in growth rate between the two species under treatment 3 (Figures 2-B and 3-A) explains the greater similarity in above-ground biomass accumulation.

Below-ground Interactions

Perhaps the most informative results of the study were provided by the data for the roots and rhizosheath. The rhizosheath can be defined as the portion of soil that is in direct contact with plant roots, and as such is strongly influenced by root exudates. Rhizosheath weight normalised by root length was significantly increased by treatment 3 for both annual meadow grass and highland bentgrass (Figure 4-A). Although not statistically significant, the fact that there was a greater increase on average for treatment 3 in highland bentgrass could help explain some of the observations made in terms of growth rate and biomass accumulation. Rhizosheath's have been shown to assist plant survival in harsh environments and may also help to maximise plant health in favourable conditions (Pang et al., 2017).

Studies have suggested that many root-derived compounds can increase water retention in the rhizosphere (Naveed et al., 2019), the effect of which could be mimicked by the surfactant treatments in the rhizosheath. All surfactants increased the water content of the rhizosheath relative to that of the bulk soil in both species (Figure 4-B). In drying soil, it has often been observed that the moisture content of the rhizosheath is higher than that of the bulk soil (Young, 1995; North and Nobel, 1997; Pang et al., 2017). There is potential that, as the surfactants appeared to be having this effect in well-watered conditions, they were concentrating the soil moisture into the rhizosheath/rhizosphere and the surrounding bulk soil was drier than it naturally would be under well-watered scenarios. Although it focused on the rhizosphere rather than the rhizosheath, the findings from our study are consistent with another study that found the wettability of the rhizosphere could be increased by surfactant application (Ahmadi et al., 2017). This could have positive implications in terms of water and/or nutrient uptake and be partly responsible for the observed growth rate differences (Figures 2 and 3-A).

Conclusions

Although we did not achieve a complete explanation for observed leaf extension rate differences resulting from surfactant application, we generated data that can be used to formulate hypotheses for further research into the possible modes of action. The observations made in the field suggested that surfactant treatment 3 has the potential to alter the competitive dynamic between highland bentgrass and annual meadow grass, increasing the proportion of highland bentgrass in the sward compared to that which would be expected under natural conditions (Baldwin, 2019). We have found evidence to suggest that this could be due to an increase in the growth rate difference between highland bentgrass and annual meadow grass under the treatment compared to controlled conditions. A possible mode of action for this increase based on our data is due to changing below-ground conditions in a way that is more favourable for highland bentgrass, namely the properties of the rhizosheath. Growth rate differences could then potentially be explained by increased water and/or nutrient uptake, but further research is needed in this area to prove this. The observations made in this study have positive environmental implications, as appropriate use of treatment 3 in the field may reduce the need to use herbicides to control the growth of annual meadow grass on golf greens. This potential, coupled with the known positive effects surfactants can have on water infiltration and soil water distribution, provides ground for optimism for improving water conservation and plant health.

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