Effects of ‘arGrow Turf’ fertilizer on turf quality, freezing tolerance and water soluble carbohydrates in creeping bentgrass

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Agnar Kvalbein, Tatsiana Espevig, Trond Pettersen, Trygve S. Aamlid
Department of Urban Greening and Environmental Technology

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FORFATTER(E)/AUTHOR(S)
Agnar Kvalbein, Tatsiana Espevig, Trond Pettersen and Trygve S. Aamlid

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SAMMENDRAG/SUMMARY:

Denne rapporten er fra et forsøk med sammenlikning av den aminosyre-baserte gjødsla arGrow Turf (SweTree Nutrition AB, Umeå, Sverige) og mineralgjødsla Wallco (kontroll), tilført som ukentlig sprøytegjødsling fra 14.sept til 1.nov 2016 på en krypkveingreen ved NIBIO Landvik, Grimstad.

This report is from a trial comparing the amino-acid based fertilizer arGrow Turf (SweTree Nutrition AB, Umeå, Sweden) and the mineral fertilizer Wallco (control), both given as weekly inputs from 14 Sep. to 1 Nov. 2016 on a creeping bentgrass green at NIBIO Landvik, Grimstad, SE Norway.

GODKJENT /APPROVED	PROSJEKTLEDER /PROJECT LEADER
Håkon Borch	Trygve S. Aamlid
HÅKON BORCH	TRYGVE S. AAMLID

NIBIO
NORSK INSTITUTT FOR BIOØKONOMI

Content

Summary

The amino acid based liquid fertilizer arGrow Turf Turf (70 % of the nitrogen (N) as arginine and 30 % as lysine) was compared with the mineral fertilizer Wallco (also in liquid form, 60 % NO_3-N and 40 % NH_4-N) on a creeping bentgrass (*Agrostis stolonifera* L.) green at NIBIO Landvik Turfgrass Reseach Center, Grimstad, SE Norway. Both fertilizer were applied weekly at the same two decreasing N rates from 14 Sep. to 1 Nov. 2016. In total for eight applications, the low and high N rates were 1.7 and 3.4 g N/m2, respectively. The trial had 16 plots (2 fertilizer type x 2 fertilizer rates x 4 replicates) and was established according to randomized complete block design. Turfgrass overall impression (visual quality) and color were assessed, and the chlorophyll index was measured, at 2-3 week intervals in autumn 2016 and in late March /early April 2017. Plant samples for freezing test and for analyses of nitrogen and water soluble carbohydrates in turfgrass crowns were taken on 5 Dec. 2016.

Plots fertilized with arGrow Turf had better color in both autumn and spring than plots fertilized with Wallco. This may possibly be a nitrogen effect as arGrow had a 14 times higher iron (Fe) content than Wallco. In spring, even the overall impression was significantly better on plots fertilized with arGrow Turf, notably at the lower N rate. There was, however, no difference between the two fertilizer types in freezing tolerance or the content of fructan in the plant samples taken on 5 Dec. 2016. The fructan content decreased with increasing fertilizer rate irrespective of fertilizer type, and the concentration of disaccharides (primarily sucrose) and nitrogen was lower in samples from plots fertilized with arGrow Turf than from plots fertilized with Wallco. Our hypothesis that the turfgrass will save energy and thus survive the winter better if taking up nitrogen in the form of amino acids instead of nitrate and ammonium could therefore not be confirmed.

Sammendrag

Den aminosyre-baserte, flytende gjødsla arGrow Turf (70 % av nitrogenet (N) som arginin, 30 % som lysin) ble sammenlikna med mineralgjødsla Wallco (også flytende; 60 % NO3-N og 40 % NH4-N) på en krypkveinsgreen (*Agrostis stolonifera*) ved NIBIO Landvik forskingssenter, Grimstad. Begge gjødseltyper ble tilført ukentlig ved de samme to avtakende N-mengdene fra 14.sept. til 1.nov. 2016. I sum for åtte gjødslinger utgjorde de to N-mengdene henholdsvis 1.7 og 3.4 g N/m2. Forsøket hadde 16 ruter (2 gjødseltyper x 2 gjødselmengder x 4 gjentak). Rutenes helhetsinntrykk og farge ble bedømt, og klorofyllindeksen i graset ble målt, med 2-3 ukers intervall om høsten og ved vekststart i måneds-skiftet mars/april 2017. Prøver av graset ble tatt ut til frysetest for analyserav nitrogen og vannløselig karbohydrat i plantekronene den 5.des. 2016.

Muligens på grunn av større innhold av jern hadde ruter gjødsla med arGrow Turf bedre farge både om høsten og ved vekststart. Om våren var også helhetsinntrykket bedre på arGrow-ruter enn på Wallco-ruter, særlig ved laveste N-nivå. Det var imidlertid ingen forskjell mellom de to gjødseltypene i frysetoleranse eller fruktaninnhold i prøvene som ble tatt 5.desember. Fruktaninnholdet avtok med økende gjødselmengde uavhengig av gjødseltype, og innholdet av sukrose og nitrogen i plantekronene var lavere på ruter gjødsla med arGrow Turf enn med Wallco. Vår hypotese om at graset sparer energi og dermed overvintrer bedre dersom det tar opp nitrogen som aminiosyrer i stedet for mineral-nitrogen ble dermed ikke bekreftet.

1 Introduction

Nitrogen (N) is the plant nutrient most decisive for growth, and it is widely accepted that it is taken up mainly in the inorganic form of ammonium and/or nitrate. Mineralization denotes the decomposition of plant residues or organic matter to ammonium (ammonification), followed by the oxidiation of ammonium to nitrate via nitrite (nitrification). These processes depend on soil conditions, nitrification usually being more sensitive to temperature, oxygen availability and pH, than ammonification (Haynes & Goh 1978).

After plant uptake, the opposite situation occurs to that in the soil: Nitrate must be reduced to ammonium before it can be incorporated into amino acids. The reduction of nitrate is an energy-demanding reaction catalyzed by the enzyme nitrate reductase in either roots or shoots. Ammonium is, on the other hand, usually assimilated in the roots because high levels of this compound are toxic in plant tissue (e.g. Haynes & Goh 1978, Taiz & Zeiger 1998).

Most textbooks in plant nutrition (e.g. Marschner 1995) mention the possibility that plants can take up N in organic form, e.g. as simple amino acids. That such uptake, thus bypassing the above-mentioned pathways in soils and plants, is not only a curiosity, was proven by Näsholm et al. 1998, 2000). Such direct uptake of amino acids may potentially save the plant for energy that can be used for other purposes, e.g. defence against plant diseases or abiotic stress.

Tolerance to winter stresses such as low freezing temperatures, ice encasement, melting water, desiccation, photoinhibition and winter-active pathogens is extremely important to turfgrasses growing under Nordic climate conditions (Kvalbein et al. 2017). Statistically, about 70 % of the Nordic golf courses suffer some kind of winter injury every year, and these injuries cause heavy economic burdens in the form of extra costs to repair of playing surfaces and reduced revenues due to delayed opening of the golf course. As turfgrass tolerance to freezing temperature is usually correlated with tolerance to other types of winter stress (Ergon et al. 1998, Gaudet et al. 1999, Huang et al. 2014), the LT_{50} values, i.e. the lethal temperature killing 50 % of the plants, is usually considered a useful indictor for overall winter stress tolerance (Huang et al. 2014). The maximum achievable freezing tolerance varies among turfgrass species with creeping bentgrass (*Agrostis stolonifera*) usually acquiring the lowest LT_{50} (Espevig et al. 2014). The process by which plants become able to cope with the low freezing temperatures is referred to as 'cold acclimation' and is primarily mediated by the natural drop in temperature in autumn (Levitt 1980, Espevig et al. 2011). Too abundant N application in the fall may stimulate growth at the expense of acclimation and make the turf more vulnerable to the upcoming winter stress (Webster & Ebdon 2005). In parallel with this, higher N rates will also results in less accumulation of water soluble carbohydrates (WSC), primarily sucrose and fructans of different degrees of polymerization, in turfgrass crowns (Beard 1969, Zanoni et al. 1969, Watschke and Waddington 1974). To what extent freezing tolerance can be causally related to the amount and type of WSC in turfgrass crowns, is however, controversial as there are many inconsistencies reported on the effect of N assimilation on WSC concentration and freezing tolerance (Huang et al. 2014).

Inspired by a study showing nitrate to have a direct negative impact on the enzymes involved in fructan synthesis (Morcuende et al. 2004), the objective of this research was to investigate how the substitution of a conventional mineral fertilizer containing nitrate and ammonium with an amino-acid based fertilizer would influence turfgrass quality, type and amount of WSC in turfgrass crowns, and the freezing tolerance of creeping bentgrass maintained at green mowing height.

2 Materials and Methods

2.1 Experimental site

The experiment was carried out from 14 Sep. 2016 to 3 Apr. 2017 on a 16 plot USGA-spec. golf green lysimeter facility at the NIBIO Landvik Turfgrass Research Center, Grimstad, SE Norway (58º20'N, 8º32' E, 6 m a.s.l). The 10 vol% organic amendment in the rootzone material was peat, the soil pH (H_2O) was 5.9 and the initial ignition loss was 0.8 %.

From seeding creeping bentgrass 'Independence' on 19 May 2016 until 26 July 2016, the green had been used for a grow-in experiment comparing arGrow Turf (70 % of N as arginine and 30 % as lysine) and Wallco 5-1-4 (60 % of N as nitrate, 40 % as ammonium), both given at the same low or high nitrogen rate. The methods and results from the grow-in experiment have been reported by Aamlid et al. (2017). In the experiment reported here, the comparison of arGrow Turf with Wallco 5-1-4 continued on the newly established green from autumn to spring, and with a special focus on overwintering, freezing tolerance and water soluble carbohydrates.

Table 1 shows the concentration of other plant nutrients in the two fertilizer types, an important difference being the 14-fold higher iron-content (Fe) in arGrow Turf than in Wallco.

Table 1. Nutrient content (%) in the two liquid fertilizers 'arGrow Turf' and 'Wallco 5-1-4'

	N	P	K	Mg	Ca	S	Fe	Mn	B	Zn	Cu	Mo
arGrow Turf	5.5	0.9	3.9	0.3	0.0	0.8	0.27	0.05	0.018	0.014	0.002	0.003
Wallco 5-1-4	5.1	1.0	4.3	0.4	0.4	0.4	0.02	0.02	0.010	0.003	0.002	0.00004


Photo 1a,b. Experimental green on 26 July 2016, at the completion of the grow-in experiment. Block 1 and 2 to the left and block 3 and 4 to the right.

Photos: Trygve S. Aamlid.

2.2 Preparations

At the completion of the grow-in experiment on 26 July 2016, the turfgrass coverage was 90-95 % in all treatments (Photo 1). Turfgrass color was, however, better on the plots that had received the higher N rate (Aamlid et al. 2017), and this was probably the case also for turfgrass density and overall impression which were not assessed in the grow-in experiment. To level out these differences, the plots that had been fertilized with the lower rates of arGrow Turf or Wallco from 19 May to 26 July, received 2.0 g N/m² of the respective fertilizer types on each of the dates 8 Aug., 15 Aug. and 22 Aug. 2016. Conversely, plots that had the high rate during grow-in, received 1.0 g N/m² on the same dates. On 30 Aug. and 6 Aug. all plots received the same rates; 0.10 and 0.08 g N/m2, respectively. No changes were made in the assignment of fertilizer type (arGrow Turf vs. Wallco) to different plots from the grow-in experiment to the experiment reported here.

In addition to the differentiation in fertilizer rate, open spots were reseeded with creeping bentgrass 'Independence' on 10 Aug. and again on 31 Aug.to level out differences between the previous fertilizer rates and provide a more uniform coverage at the start of the experiment.

2.3 Experimental treatments

We used the same randomized complete block design with four replicates as in the grow-in experiment (Aamlid et al. 2017).

The experimental factors were

A. Fertilizer type
   a. arGrow Turf
   b. Wallco blomstra 5-1-4

B. Fertilizer rates
   a. Low rate (1x)
   b. High rate (2x)

The experiment had four replicates (blocks). Plot size was 2 m x 3 m of which the central 1 m x 2 m was used for all assessments.

The fertilizers were applied weekly from 14 Sep. to 1 Nov. 2016 at decreasing rates reflecting the seasonal decline in irradiance and temperature (Table 2). We originally planned to continue with the weekly fertilizer inputs for three more weeks, but the applications in November were disrupted due to heavy snowfall on 5 Nov. (see following paragraph on weather conditions).

Table 2. Fertilizer applications from 14 Sep. to 1 Nov. 2016.

Application Date	Low rate			High rate		
	g N/m2	Wallco g/m2	Argrow g/m2	g N/m2	Wallco g/m2	Argrow g/m2
14 Sep.	0.30	5.9	5.5	0.60	11.8	10.9
21 Sep.	0.28	5.5	5.1	0.56	11.0	10.2
29 Sep.	0.25	4.9	4.5	0.50	9.8	9.1
04 Oct.	0.23	4.5	4.2	0.46	9.0	8.4
11 Oct.	0.20	3.9	3.6	0.40	7.8	7.3
19.Oct.	0.18	3.5	3.3	0.36	7.1	6.5
25 Oct.	0.15	2.9	2.7	0.30	5.9	5.5
01 Nov.	0.13	2.5	2.3	0.25	4.9	4.5
SUM	1.72	33.6	31.2	3.43	67.3	62.4

The fertilizers, both in liquid form, were applied in a volume of 800 L/ha using the small walk-behind fertilizer sprayer shown in Photo 2.

In spring 2017, the experiment received no fertilizer applications before the final assessment on 3 April.

Photo 2. Experimental sprayer used for liquid fertilizer applications.

Photo: Trygve S. Aamlid.

2.4 Green maintenance during experimental period

Mowing

The green was mowed at 5 mm with a single (walk behind) green's mower. Mowing frequency was three times per week (Monday, Wednesday, Friday) until the end of September; twice in the first week of October and once per week from 10 Oct. until the last mowing on 31 Oct.

In 2017, the first mowing was to 5 mm on 3 April.

Irrigation

Except on days with natural rainfall, the green was irrigated 5 mm after fertilizer application. No other irrigation was necessary during the experimental period.

Topdressing

Pure silica sand (grain size 0.2-0.6 mm) at a rate corresponding to 0.22 mm sand was applied weekly from 16 Sep. to 7 Oct. (about 0.9 mm of sand altogether).

Fungicide treatment

To control take-all patch (*Gaeumannomyces graminis*) there was a spot treatment with Amistar Duo (azoxystrobin: 200 g/L + propikonazol: 125 g/L) on 21 September. A volume of 20 ml Amistar Duo was dissolved in 40 L water and applied on the take-all patches. These spot-treated areas were late avoided when sampling plants for freezing test and WSC analyses.

2.5 Weather conditions, September 2016 – April 2017

Six out of the eight months from September 2016 to April 2017 had higher than normal temperatures (Table 3). The largest deviation from the 30 year normal values occurred in September (+4.4 °C), December (+3.5 °C) and January (+3.3 °C). These months also had precipitation considerably lower than the 30 year normal values. The highest precipitation compared with the 30 year normal values occurred in November and February.

Table 3. Monthly weather data at NIBIO Landvik weather station (about 200 m from experiment) from September 2016 to April 2017 and 30 year normal values 1961-1990

	Air temperature (°C)				Precipitation (mm)	
Month	**Mean**	**Daily max.**	**Daily min.**	**Normal**	**2016**	**Normal**
Sep.	15.1	19.6	10.7	11.8	37	136
Oct.	7.6	10.1	4.9	7.9	117	162
Nov.	2.7	5.8	-1.0	3.2	258	143
Dec.	3.7	7.4	0.0	0.2	44	102
Jan.	1.7	5.2	-2.2	-1.6	65	113
Feb.	0.4	3.7	-2.5	-1.9	139	73
Mar.	3.4	8.0	-0.9	1.0	118	85
Apr.	6.5	11.1	1.7	5.1	67	58

Figure 1 gives some more details. The high precipitation in November was mainly due to an extreme event of 147 mm on 5 Nov. This is the highest daily precipitation that has been recorded since Landvik weather station was established in 1957. It started as rain but turned into snow as the temperature dropped, and the snow stayed on the unfrozen green for about a week. After that there was no snow until the periods 7-22 Feb., and 5-14 March. The green was, however, frozen during most of December, January and February.

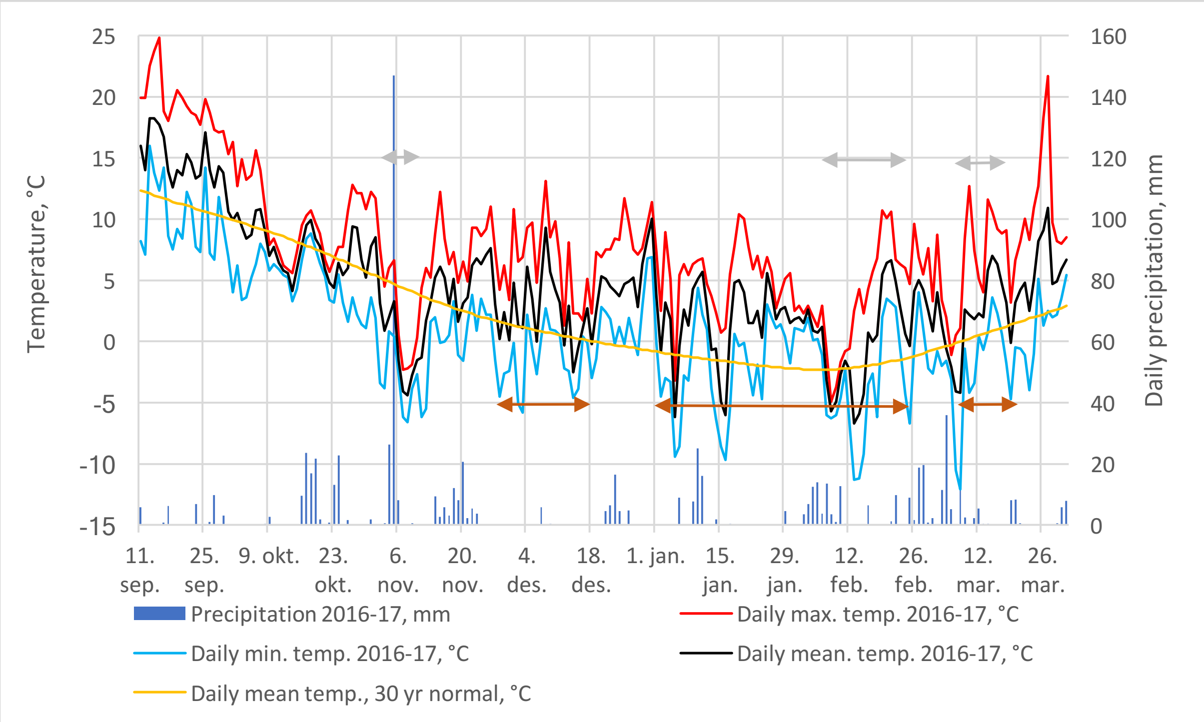

Figure 1. **Daily maximum, minimum and mean air temperatures, as well as daily precipitation during the experimental period. The 30 year normal temperature (1961-90), periods with snow cover (horizontal gray lines) and periods with frozen soil (horizontal brown lines) have been indicated.**

2.6 Field observations

Observations were conducted at the start of the trial on 14 Sep., two to three times during the treatment period in the fall, and two to three times after snow melt in spring. The characters recorded were:

- Turfgrass overall impression (scale 1-9, where 9 is the best turf). These visual assessments focused on turfgrass uniformity and playability, but also on tiller density and color.
- Tiller density (scale 1-9, where 9 reflects the highest tiller number per per unit area). The character was recorded only at the start of the experiment on 14 Sep. 2016)
- Diseases (% plot area affected). The only disease observed in the trial was take-all patch.
- Turfgrass color (1-9, where 9 is the most intensely green turf).
- Chlorophyll index, five readings per plot at 1 m distance with a Field Scout CM 1000 Chlorophyll Meter (model 2950, Spectrum Technologies, Inc.). If too dark (cloudy) on the registration date, the reading was postponed. Mean values and coefficients of variation (CV) among the five observations per plot were calculated, the latter as in indication of plot uniformity.

2.7 Freezing test

The procedures for determining turfgrass freezing tolerance followed Espevig et al. (2014).

Cylinder samples (50 mm deep, 50 mm in diameter) were taken from each plot on 5 Dec. 2016 and stored over night at +4 ˚C. On the following day, once the turf had thawn, the samples were separated into single plants consisting of 2-3 shoots. Roots were washed in cold water and cut to 2 cm length. A total of 110 plants from each plot were prepared for exposure to the temperatures +4 (control), -20, -22, -24, -26, -28, -30, -32, -34, -36, -38 °C). The temperatures were chosen based on experience from earlier freezing tests with creeping bentgrass. Four research technicians worked with samples from one block each (Photo 3).

Bundles of 10 plants were wrapped in moist paper towels and labelled with plot number and freezing temperature. Plant bundles assigned to same freezing temperature were split between two freezers (A: block 1&2, B: block 3&4).

The bundles were placed in plastic boxes with 3 cm moist sand holding 4˚C in the bottom, one box for each freezing temperature in each freezer. When all plants were prepared, more sand was added until the bundles were surrounded by 2 litre moist sand (Photo 4). Surveillance thermometers were installed in two sand filled boxes without plants in each freezer.

The boxes were placed in the freezers and kept at -2 °C until temperature was stable, approximately for 12 hours. (Some minor temperature fluctuations occur when water turn to ice). Then the temperature was lowered by 2° per hour until -12˚C. From this temperature, the temperature reduction was adjusted to 3˚per hour. The boxes were removed from the freezers when the thermometers showed the right temperature. The maximum deviation between the two thermometers was less than 0.8 ˚C. The temperature drop from -2 to – 38 °C took 26 hours.

Photo 3a,b. Research technicians Anne Steensohn (top), Ove Hetland and Elise Pedersen (bottom) working on the washing and separation of single plants for freezing test.

Photos: Agnar Kvalbein.

The sand in the boxes melted after 12-24 hours at 4 ˚C. The bundles were then excavated, unpacked and planted block by block in pot trays (Photo 5) for regrowth in a growth chamber at 23 ˚C (day) /16 ˚C (night), 16-h photoperiod and a PPFD of ca. 150 µmol/m²/s.

The number of surviving plants from the freezing temperatures were recorded after 21 days, on 28 December. The Lethal temperatures killing 50 % of the plants (LT_{50}) values were calculated by PROBIT analysis using the logistic distribution in SAS (version 9.4, SAS Institute Inc., Cary, NC, USA).

2.8 Nitrogen and WSC concentration in turfgrass crowns

On 5 Dec., the same day as taking samples for freezing test, crown samples from each plot were also taken for analyses of total nitrogen (as per cent of dry matter) and WSC with different degrees of polymerization (DP, relative figures). The samples were dried for 48 h at 60˚C before separating crowns totally free of sand. The analyses were conducted at the Swedish Agricultural University's laboratory in Umeå, Sweden. The relative figures for DP did not take into account that longer-chained fructans contained more carbon than shorter-chain fructans (e.g. DP 20 twice as much C per molecule compared with DP 10), and they must therefore not be taken as an expression of proportion of total WSC contained in the different DP fractions.

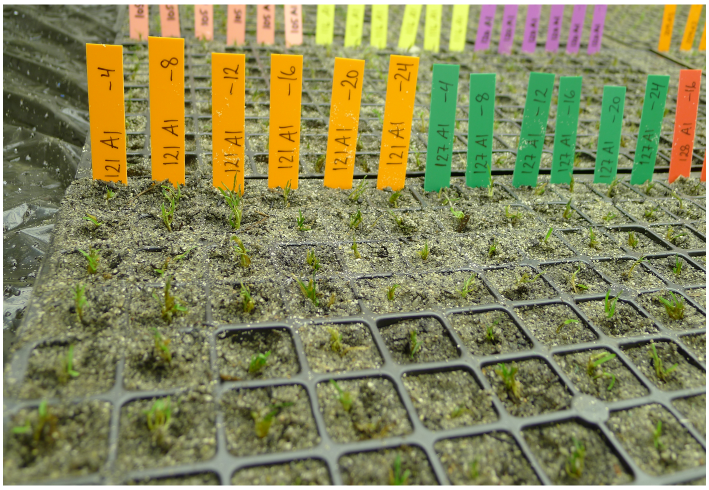

Photo 5. Single plants potted in trays for regrowth in growth chamber. (Illustration photo from a freezing test conducted with Poa annua).

Photo: Agnar Kvalbein

2.9 Statistical analyses

All parameters were analysed using the SAS procedure PROC ANOVA for a RCB design. Significant differences among treatments were identified using the Fisher's protected least significant difference (LSD) at the 5% probability level. Correlation analyses were conducted using the SAS procedure PROC CORR.

3 Results

3.1 Field registrations

3.1.1 Observations at the start of the experiment on 14 Sep. 2016

The overall impression at the start of the experiment was reduced by an attack of take-all patch in August and early September (Photo 6). Numerically, a higher percentage of the surface area was infected on Wallco-treated than on arGrow-treated plots and on plots that had received the higher fertilizer rate during grow-in. However, neither the main effects of fertilizer type or fertilizer rate, nor their interaction, were statistically significant for either disease as a percent of plot area or turfgrass overall impression (Table 4).

The most significant difference at the start of experiment was higher chlorophyll readings on the plots that all the way since seeding on 19 May had received arGrow Turf than on the plots that had received Wallco. The chlorophyll readings on 14 Sep. also showed that the 'opposite' fertilizer applications from 8 Aug. to 22 Aug. had not been sufficient to weigh up for the difference in fertilizer rates during the grow-in period. The coefficient of variation among the five chorophyll readings per plot was not significantly different among treatments and is not shown in Table 4.

An unexpected registration at the start of the trial was a lower tiller density on the plots that during grow-in had received the lower rate of arGrow Turf than in the other treatments (Table 4).

Table 4. Overall impression, tiller density, take-all patch and leaf chlorophyll index at the start of the experiment on 14 Sept. and mean values for overall impression, turfgrass color, take-all patch and leaf chlorophyll index during the experimental period (two observations for overall impression and three observations for the other characters.)

	At start of the experiment, 14 Sep. 2016				Mean of observations from 26 Sep. to 1 Nov.			
	Overall impression (1-9)	Tiller density (1-9)	Take-all patch, % of plot area	Chloro-phyll index	Overall impression (1-9)	Turfgrass color (1-9, 9 is darkest turf)	Take-all patch, % of plot area	Chloro-phyll index
arGrow Turf - Low	6.9	7.4	1.1	307	6.6	6.5	2.4	282
arGrow Turf - High	6.5	7.8	2.6	314	7.0	7.7	2.4	292
Wallco - Low	6.4	8.0	3.9	279	6.2	4.9	2.9	257
Wallco - High	5.9	7.9	6.8	295	6.5	6.1	3.2	279
Significance levels[1]:								
Fertilizer type	NS	*	NS	***	NS	***	NS	***
Fertilizer rate	NS	NS	NS	*	NS	***	NS	**
Interaction	NS	NS	NS	NS	NS	NS	NS	NS

1) ***: P<0.001, **: P<0.01, *: P<0.05, NS: Not significant

Photo 6. Experimental green on 14 Sep. 2016. Notice take-all patches.

Photo: Trond Pettersen.

3.1.2 Observations during the experimental period in autumn 2016

The visual color assessments and the chlorophyll index both showed significant differences among treatments (Table 4): Plots fertilized with arGrow Turf had better color than plots receiving the same amount of N in Wallco, and plots fertilized with the higher N rate had better color than plots fertilized with the lower N rate. The characters for overall impression and take-all patch reflected the same quality trends, but these differences could not be verified statistically. The CV among the five chlorophyll readings per plot was around 5 % in all treatments, thus indicating little difference in color uniformity (data not shown).

3.1.3 Observations in spring 2017

The differences in turfgrass color between arGrow Turf and Wallco in autumn 2016 persisted into the spring 2017 and was also refleced in a better overall impression (Table 5). Doubling the fertilizer rate improved spring color and the average chlorophyll index but led to higher CV among the chlorophyll readings. For the visual color ratings there was a significant interaction as the response to increasing fertilizer rate was stronger if the N was given in the form of Wallco than in the form of arGrow Turf.

The attack of take-all patch in spring 2017 was less conspicuous than in the previous fall and there were not significant differences among treatments (Table 5).

Table 5. Means of spring observations for overall impression, turfgrass color, take-all patch and leaf chlorophyll (two observations for overall impression and chlorophyll index; three observations for disease and color)

	Mean of observations 20 March – 3 April				
	Overall impression (1-9)	Color (1-9)	Take-all patch, % of plot area	Chlorophyll index, mean	Chlorophyll index, CV
arGrow Turf - Low	5.3	4.9	0.3	185	3.6
arGrow Turf - High	5.2	6.3	0.9	224	6.1
Wallco - Low	4.0	4.1	0.5	174	4.2
Wallco - High	4.9	6.1	0.6	224	5.4
Significance levels[1]:					
Fertilizer type	**	**	NS	NS	NS
Fertilizer rate	NS	***	NS	***	**
Interaction	NS	**	NS	NS	NS

1) ***: P<0.001, **: P<0.01, *: P<0.05, (*):P<0.10, NS: Not significant

3.2 Freezing tolerance

Neither fertilizer type nor fertilizer rate had any significant effect on the LT_{50} values for bentgrass plants sampled on 5 Dec. 2016 (Table 6). Absolutely no trends could be detected for fertilizer type, while on average for arGrow Turf and Wallco, there was an insignificant trend for the plants that had received the lower fertilizer rate to have approximately 2 °C less freezing tolerance than the plants that had received the higher rate.

Table 6. Lethal temperatures for 50 % of plants, nitrogen concentration and water soluble carbohydrate (WSC) concentration of different degrees of polymerization (DP) in turfgrass crowns on 5 Dec. 2016 as affected by type and rate of fertilizer.

	LT_{50}, °C	N, % of dry matter	Water soluble carbohydrates x 10^6, relative index				
			Total	Disaccharides, DP 2	Fructans, DP 3-5	Fructans, DP 6-10	Fructans, DP 11-30
arGrow Turf - Low	-31.4	1.27	484	135	206	84	59
arGrow Turf - High	-29.3	1.69	417	138	157	65	57
Wallco 5-1-4 - Low	-31.8	1.30	514	145	226	84	59
Wallco 5-1-4 - High	-29.8	1.94	450	146	174	67	62
Significance levels[1]:							
Fertilizer type	NS	*	NS	**	NS	NS	NS
Fertilizer rate	NS	***	**	NS	***	**	NS
Interaction	NS	NS	NS	NS	NS	NS	NS

1) ***: P<0.001, **: P<0.01, *: P<0.05, (*):P<0.10, NS: Not significant

3.3 Nitrogen and water soluble carbohydrate concentration in turfgrass crowns

On average for fertilizer rates, the N concentration and WSC index in the dry matter of creeping bentgrass crowns were in turn, 9 and 7 % higher for plants fertilized with Wallco than for plants fertilized with arGrow Turf (Table 6). Doubling the N rate increased the N concentration by 41 %, but reduced the total WSC index by 13% on average for the two fertilizer types (Table 6).

Table 6 also shows the WSC index broken down for groups with various degrees of polymerization. The index for disaccharides (DP 2, mainly sucrose) was higher after fertilization with Wallco than with arGrow Turf but not significantly affected by fertilizer rate. The short-chain fructans containing 3-10 units were, in contrast, unaffected by fertilizer type, but significantly reduced by increasing N rate. The longer chain fructans (DP 11 and higher) were not affected by either fertilizer type or fertilizer rate.

Figure 2 gives some more details on the content of the various fractions of WSC in turfgrass crowns. The disaccharides and the short-chained fructans (DP <6) were always in majority.

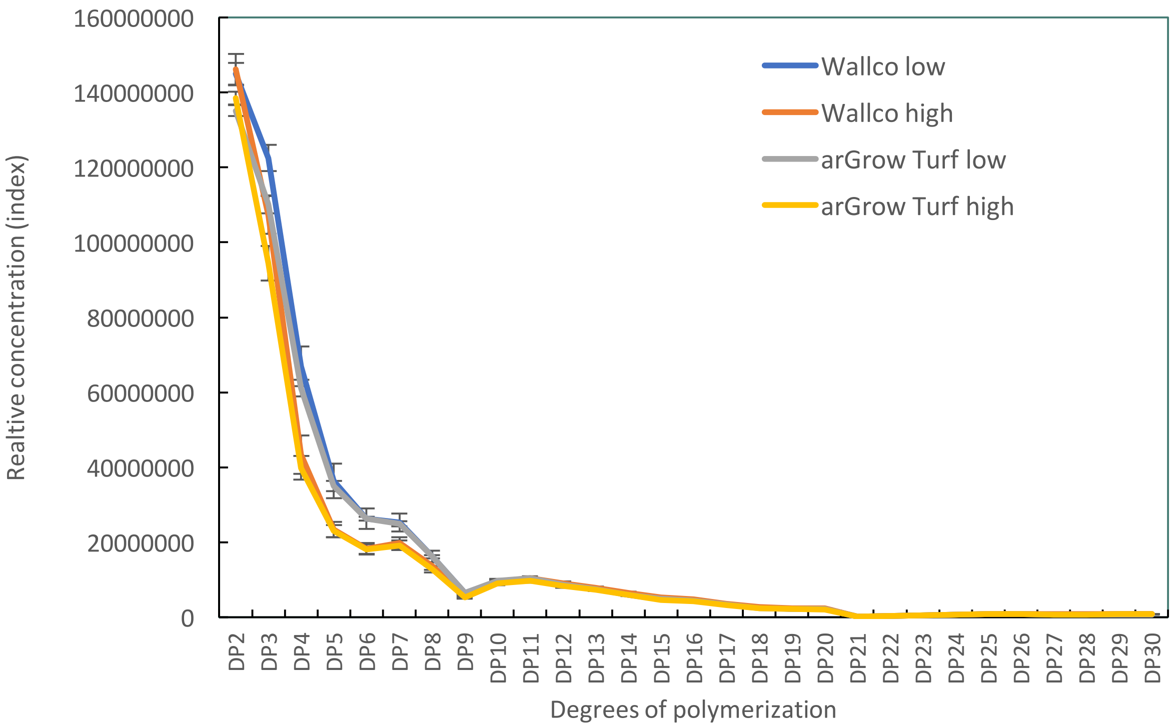

Figure 2. Relative concentration of WSC with increasing degrees of polymerization as affected by various combinations of fertilizer type and fertilizer rate. Bars indicate ± 1 Standard Error (SE).

3.4 Correlations

The simple correlation coefficients showed a trend (P<0.10) for the lethal temperature for 50 % of the plants to be negatively correlated (r=-0.46) with the concentration of short-chained fructans (DP 3-10) in turfgrass crowns (Table 7). However, as shown in Figure 3, this correlation was mainly dictated by two diagonally opposite observations, one of a low freezing tolerance on a plot fertilized with the higher rate of arGrow Turf, and the other of a high freezing tolerance on a plot fertilized with the lower rate of Wallco. Between freezing tolerance and disaccharides (DP = 2) and between freezing tolerance and longer-chained fructans (DP>10), the correlations were even weaker and far from significant.

Table 7. Simple correlation matrix among lethal temperature for 50% of plants (LT_{50}) and characters showing the concentration of nitrogen and water soluble carbohydrates (WSC) with increasing degrees for polymerization (DP) in turfgrass crowns (n=16).

	N, % of dry matter	Disaccharides, DP=2	Fructans, DP<10	Fructans, DP>10	LT_{50}
N, % of dry matter	1.00	0.24^{NS}	-0.51^{*}	0.40^{NS}	0.18^{NS}
Disaccharides, DP=2		1.00	0.08^{NS}	0.43^{NS}	0.34^{NS}
Fructans, DP≤10			1.00	0.39^{NS}	-0.46^{NS}
Fructans, DP>10				1.00	-0.18^{NS}
LT_{50}					1.00

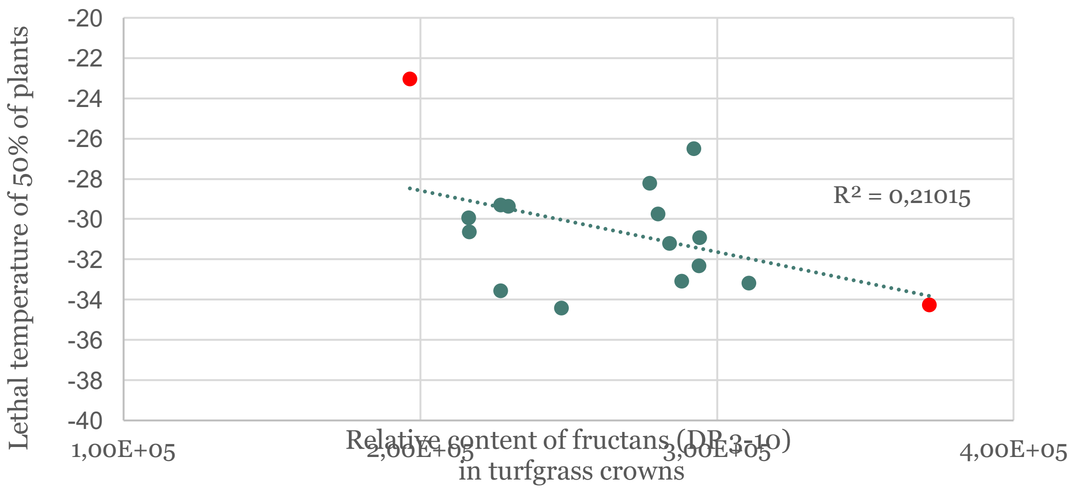

Figure 3. Relationship between relative content of fructans with chain length up to ten units and freezing tolerance. The two observations most influential on the regression have been indicated in red.

As for nitrogen concentration in turfgrass crowns, there was no significant correlation with freezing tolerance (Table 7), but a significant negative correlation with the relative content of short-chained fructans. This is depicted in Figure 4 which also shows that the observations fell into two different groups, mostly reflecting the two fertilizer rates.

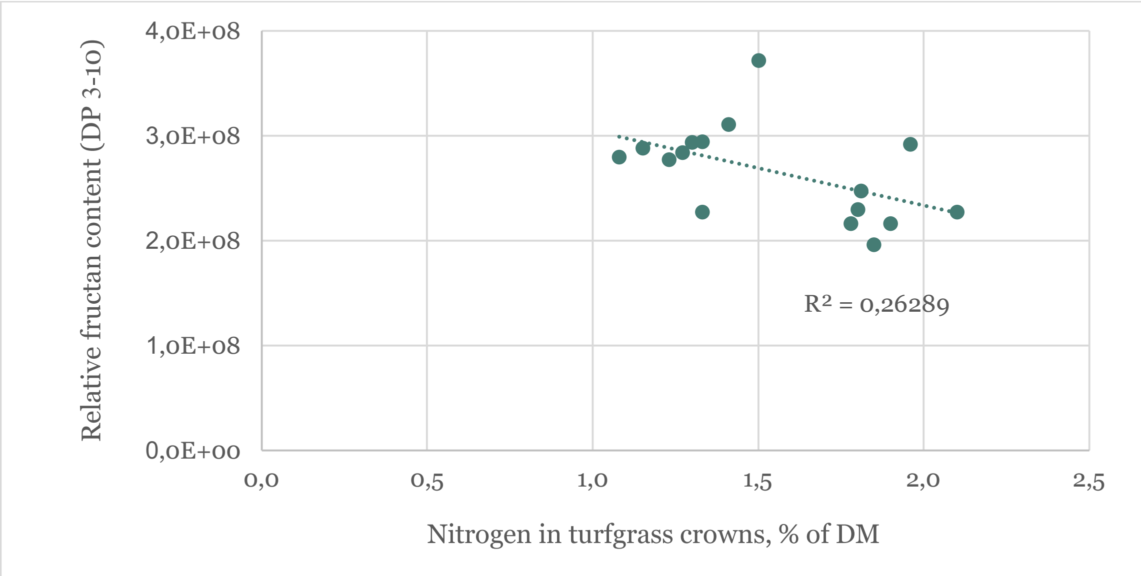

Figure 4. Relationship between nitrogen concentration and relative content of short-chained fructans in turfgrass crowns.

4 Discussion

Replacement of the mineral fertilizer Wallco with arGrow Turf had little impact on turfgrass overall impression but improved turfgrass color and clorophyll index in the fall. The effect on color may be due to easier incorporation of organic nitrogen into chlorophyll, but it is more likely an effect of 13-14 times higher content of iron in arGrow Turf than in Wallco. As far as the spring observations are concerned, a third explanation may be less leaching of nitrogen from November through March since both arginine and lysine will be positively charged at the ambient pH values and therefore less susceptible to leaching than nitrate. Although the take all patch came in as a disturbing factor, our overall results suggest that arGrow Turf had benefits, not only for turfgrass grow-in (Aamlid et al. 2017), but also on established greens and as a fall fertilizer. Furthermore, the overall smaller differences in visual characters between the two fertilizer rates of arGrow Turf than of Wallco also shows a potential for reduced nitrogen rate if mineral fertilizer is replaced with arGrow Turf.

Our results showed no effect of fertilizer type on freezing tolerance, and the effect on water soluble carbofydrates (WSC) were also small except for sucrose (and other disaccharides) being higher after fertilization with Wallco than with arGrow. Otherwise, there was no significant effect of fertilizer type, but a significant reduction in the concentration of short-chain fructans and an accompanying tendency to lower freezing tolerance with increasing N rate. These results are in good agreement with the general pattern of less carbohydrate accumulation with increasing N rate (Lattanzi et al. 2012, Bauer et al. 2012) and regardless of N form (Watschke & Waddinton 1974).

The relative figures for different degrees of polymerization (DP) determined in the laboratory at the Swedish Agricultural University in Umeå does not reflect the proportion of total carbon contained in the different DP fractions and are therefore not in opposition to the early literature showing cool-season grasses within the genera *Agrostis, Dactylis, Phalaris, Phleum* and *Poa* to be 'long-chain accumulators', whereas *Lolium* sp., *Agroyron* sp. and at least some species of *Festuca* are short-chain accumulators (Smith & Grotelueschen 1966). More recently Hoffman et al. (2014) found that 87 % of the WSC in creeping bentgrass crowns after acclimation at 2 °C were contained in long-chained molecules (DP 10-200), the remaining being in the form of sucrose (8%) and short-chained fructans (DP 3-9).

Some studies into turfgrass winter survival suggest that sucrose plays a more important role than fructans in acting as a cryoprotectant that stabilizes cell membranes (Olien & Clark 1993, Dionne et al. 2001, Kim et al. 2001). This was, however, not confirmed by Espevig et al. (2011), and it is also not confirmed by the present results showing no difference in LT_{50} despite a higher concentration of disaccharides after fertilization with Wallco. The higher concentration of disaccharides may at first glance be interpreted as an inhibition of the conversion of sucrose to fructan due to a higher nitrate concentration in plant tissue on plots receiving Wallco (Morcuende et al. 2004), but the fact that there was more than month between the last fertilizer input on 1 Nov. and the sampling for WSC analyses on 5 Dec., makes this rather unlikely. The interconversion between sucrose and (short-chain) fructans in grass leaves usually occur within a few hours (Lattanzi et al. 2012), and even for the less labile pools in turfgrass crowns, a specific effect of N form of the concentration of various WSC and freezing tolerance shall probably be hard to verify unless samples are taken within a day or two after fertilization.

In conclusion therefore, while there may be many other benefits of amino acid-based fertilizers (e.g. (Aamlid et al. 2017), the results of this study showed small prospects for improved turfgrass winter survival by turning from a conventional mineral fertilizer to arGrow Turf.

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Norsk institutt for bioøkonomi (NIBIO) ble opprettet 1. juli 2015 som en fusjon av Bioforsk, Norsk institutt for landbruksøkonomisk forskning (NILF) og Norsk institutt for skog og landskap.

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