Evaluation of Different Integrate Turf Management Programs to Reduce Microdochium Patch

Results from 2-yr field trials conducted at NIBIO (NO) and STRI (UK)

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TITTEL/TITLE

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SAMMENDRAG/SUMMARY:

For å redusere avhengigheten av fungicider ved behandling av torvgress-sykdommer undersøkte vi bruken av biostimulanter og fargepigmenter og deres evne til å forhindre spredning av mikrodochium og antraknose på ettårig enggress (*Poa annua*). Studien ble utført på to steder (Landvik, Norge og Bingley, Storbritannia) i to år (mai 2020 – mai 2022). Biostimulanten Hicure kan redusere soppdrepende bruk fra tre til to uten tap av effektivitet i behandlingen av soppsykdommene. Biostimulanten bevarte også den visuelle kvaliteten til torvgresset når den reduserte den soppdrepende behandlingen fra tre til to. Fargepigmentet Ryder i alle behandlinger var effektivt til å øke fargeintensiteten til torvgresset sammenlignet med kontrollen. I tillegg kan de biostimulerende behandlingene behandle antraknose bedre enn den eneste soppdrepende behandlingen. Biostimulanten Hicure og fargepigmentet Ryder har potensial for videre forskning og utvikling for å redusere bruken av soppdrepende midler og samtidig bevare den uberørte kvaliteten til torvgress i golfgreens.

To reduce the dependency of fungicides in treating turf grass diseases we investigated the use of biostimulants and colour pigments and their capacity to prevent the proliferation of microdochium and anthracnose on annual meadow grass (*Poa annua*). The study was conducted in two sites (Landvik, Norway and Bingley, United Kingdom) for two years (May 2020 – May 2022). The biostimulant Hicure could reduce the fungicidal use from three to two without loss of efficiency in treating the fungal diseases. The biostimulant also preserved the visual quality of the turf grasses when reducing the fungicidal treatment from three to two. The colour pigment Ryder in all treatments was effective at increasing the colour intensity of the turf grasses compared to the control. Additionally, the biostimulant treatments could treat anthracnose better than the fungicidal only treatment. The biostimulant Hicure and the colour pigment Ryder have potential for further research and development to reduce the use of fungicides while simultaneously preserving the pristine quality of turf grasses in golf greens.

LAND/COUNTRY:	Norway
FYLKE/COUNTY:	Aust-Agder
KOMMUNE/MUNICIPALITY:	Grimstad
STED/LOKALITET:	Landvik

GODKJENT /APPROVED	**PROSJEKTLEDER /PROJECT LEADER**
Håkon Borch	Tatsiana Espevig
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Preface

This report, ‘Evaluation of Different Integrate Turf Management Programs to Reduce Microdochium Patch’, corresponds to WP1.5 in the entire R&D project ‘IPM-Golf: Integrated Management of Important Turfgrass Diseases and Insect Pests on European Golf Courses’, initiated by Norwegian Institute of Bioeconomy Research (NIBIO) and collaborators in 2019. This larger project is a concerted effort by researchers and greenkeepers from the Nordic countries, Germany, Portugal, the UK, Finland and Russia, suppliers from ICL, Syngenta, Suståne and Aqua-Yield. The project received funding from the Scandinavian Turfgrass and Environment Research Foundation (STERF) and the R&A in January 2020 and match funding from Netherlands Golf Federation, German Golf Association, and the Danish Environmental Protection Agency. The companies: Botaniska Analysgruppen (Sweden), Aqua-yield (USA), ICL (The Netherlands), Syngenta (UK), Suståne (UK, USA) and Xema (Finland) also contributed to the match funding.

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Særheim, 11.05.23

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1 Introduction

Microdochium patch (MP) (earlier called fusarium patch or pink snow mould) is a turf grass disease caused by the fungus *Microdochium nivale* (Fr.) Samuels & I.C.Hallett. The fungus is pathogenic to all turf grass species, but incidence and severity varies significantly among turf grass species and varieties (Tronsmo et al. 2001). Annual meadow grass (*Poa annua*), a common turf grass species (Lyman et al. 2007), is one of the most susceptible species to microdochium patch (Mattox 2015). The disease causes significant damage to the turf grass, with a primary result of decreasing surface quality, a main challenge to the performance and aesthetic of golf greens. This in turn is costly to the golf establishments (Kvalbein et al. 2017). To prevent the fungus from proliferating, a variety of fungicidal treatments have been developed over time. However, environmental concerns regarding unnecessary use of fungicides have promoted Integrated Pest Management (IPM) methods, to reduce the use of fungicides and instead utilize other preventative methods of managing pests. In general, these comprise cultural, mechanical, chemical and biological control measures. In the management of MP, a multitude of alternative treatments have been developed and trialled. Examples include compost treatments (Boulter et al. 2002), humic acids (Espevig et al. 2019), microbiological agents (Aamlid et al. 2017), petroleum-derivates (Aamlid et al. 2018), potassium-phosphite (Dempsey et al. 2012) and iron-sulphate heptahydrate (Mattox et al. 2020). Many of these treatments provide low to medium efficacy at preventing disease activity and often are most effective at lower disease pressures. The aim of this study was to establish if the incorporation of a pigment (Ryder) and a biostimulant (Hicure) into an IPM programme would allow less fungicide to be applied when managing MP affected turf, whilst enhancing turf grass health, as expressed through visual quality and colour intensity. We also investigated if these treatments could reduce the proliferation of the turfgrass disease anthracnose (Crouch and Crouch 2009), caused by the pathogen *Colletotrichum graminicola* (Ces.) G.W.Wilson.

2 Material and Methods

2.1 Experimental Setup and Locations

The field trials were conducted over two years (from May 2020 – to May 2022) and in two locations to allow for a generalization of the study findings, both in time and in space. The study was conducted at the NIBIO Turf grass research centre at Landvik, Norway (*58.340 N, 8.526 E*) and at the Sports Turf Research Institute (STRI) at Bingley, United Kingdom (*53.847 N, -1.856 E*). Four treatments were used to assess their respective effectiveness to treating microdochium patch at both sites (and anthracnose at Landvik) (Table 1). The first treatment was a control. The second treatment consisted of timely applications of three fungicides (A19188B, A20323D and Medallion) together with Ryder. The third treatment consisted of timely applications of two fungicides (A20323D and Medallion) and one biostimulant (Hicure) together with Ryder. The fourth treatment consisted of timely applications of one fungicide (Medallion) and one biostimulant (Hicure) together with Ryder. The treatment protocol consisted of a randomized complete block-design with four replicate treatment area (4 treatments x 4 replicates = 16). Total treatment replicate plot size was 2.25 m^2 (1.5 m x 1.5) with the treatment registration area being the central 1 m^2.

Table 1. Experimental treatment plan to reduce microdochium disease development in Landvik and Bingley.

Nr	Treatment	Products and Application Procedures						
		Products	Rate	Ryder[4]	Water	Applications	NIBIO from	STRI from
1	Untreated control							
2	Three applications of fungicides + all tank mixed with Ryder	A19188B[1]	3 L/ha	1 L/ha	250 L/ha	x1	Sept.	Oct.
		A20323D[2]	3 L/ha	1 L/ha	250 L/ha	x1	Oct.	Nov.
		Medallion TL[3]	3 L/ha	1 L/ha	250 L/ha	x1	Nov.	Dec.
3	Biostimulant + Two applications of fungicides + all tank mixed with Ryder	Hicure	20 L/ha	0.5 L/ha	250 L/ha	x5 per 2 wks	End of July	End of July
		A20323D	3 L/ha	1 L/ha	250 L/ha	x1	Oct.	Nov.
		Medallion TL	3 L/ha	1 L/ha	250 L/ha	x1	Nov.	Dec.
4	Biostimulant + One application of fungicide + all tank mixed with Ryder	Hicure	20 L/ha	0.5 L/ha	250 L/ha	x7 per 2 wks	End of July	End of July
		Medallion TL	3 L/ha	1 L/ha	250 L/ha	x1	Before snow cover.	Dec.

[1] *A19188B will be applied just prior first sign of microdochium patch*

[2] *A20323D will be applied 21-28 days after A19188B*

[3] *Medallion TL will be applied 21-28 days after A20323D and before snow cover*

[4] *Total dosage for Ryder is 3, 4.5 and 4.5 L/ha in treatment 2, 3 and 4, respectively. No irrigation after application at least 4 h, ideally 24 h.*

The golf green in the treatment plots were assessed according to visual turfgrass quality, colour intensity and microdochium disease development (and anthracnose disease development at Landvik). The assessments were carried out bi-weekly prior to the first sign of microdochium (always prior to fertilization or treatment) and then weekly until the last application of fungicides, then again bi-weekly if there was no snow present (Supplementary Table 1).

The experimental setup at Landvik was conducted at a USGA annual meadow grass (*Poa annua)* predominant (85%) green and contaminated by creeping bengrass (*Agrostis stolinifera*) or/and Sagina

(*Sagina procumbens*) (15%) with the rootzone 12:88 peat:sand by volume substrate. The green was maintained as a natural golf green except for the treatments themselves. The green was fertilized every week (Tuesday or Thursday). Annual N was 261 kg ha-1 from week 14 to week 44, ratio N:P:K~100:14:72 with 5 mm irrigation after fertilization. The fertilizers used were Walco liquid fertilizer 5-1-4 and Greenmaster cold start 11-5-5 and Greenmaster zero 14-0-10. N rates were deducted by 11.7% in treatments 3 and 4 in weeks when Hicure was applied in order to give the same amount of N as in control treatment 1 (Supplementary Tables 2 - 3). The treatment areas were mowed 3 mm, 3 times per week (one day after fertilization Mon., Wed., Fri) May-September using a John Deere single mower. Initial mowing height was 5 mm, down from 5 to 3 mm during May and up from 3 to 5 mm during October. The green was not mown the same day as treatments (Hicure or fungicides). To recreate natural friction, we used friction wear drums with golf spikes, 3 passes per week in July-September (corresponding to 20000 rounds of golf per season in 20 weeks from May until 1 Oct.). The green was irrigated to field capacity every time soil moisture to 12 cm depth (TDR-measurements) was less than 12%. Topdressing was added weekly (0.25 mm sand) from May to October, totally 5 mm per year. The green was further cut using vertical cutting every 2-4 weeks during peak flowering period for Poa annua, otherwise if needed.

The experimental setup at Bingley was conducted at a natural soil golf green (native sandy soil) with a high proportion of annual meadow grass (*Poa annua*) and the rest being made up browntop bent (*Agrostis capillaris*). The green was maintained as a natural golf green except for the treatments themselves. Annual N was 122 kg ha-1 from week 14 to week 44 with 5 mm irrigation after fertilization, with the rates being deduced slightly to accommodate the Hicure (Supplementary Table 4). Nutrients were supplied using two liquid fertilizers (31-0-26 and 30-7-22). The treatment area was mowed 3 times a week during the growing season and less frequently outside of the growing season. The mower was benchset at 5 mm during the growing season giving an effective height of cut of between 3.75 – 4.25 mm depending on ground conditions. Outside of the growing season, the height of cut was lifted to a benchset of 6 mm. Trial plots were then mown the day after Hicure or fungicide applications. Simulated wear was applied using a golf stud equipped differential slip wear machine. Wear was applied at rate of 2 passes a week between July-September. Fertiliser inputs were made according to the nutrient application plan. On mowing days, fertiliser applications were made after mowing had been completed. Irrigation was applied as required to maintain plant health. After fertiliser application the turf was irrigated to wash excess nutrients off the leaf surface ready for root uptake. Aeration and other cultural operations (such as vertical cutting) were carried out to meet turf requirements, but not in a way that affected treatment effects.

2.2 Meteorological Data

The main meteorological data was acquired from the platform Meteostat (Meteostat 2022). Meteostat provides public open-access weather and climatological data gathered by multiple national and international platforms (e.g., MET Norway, NOAA and the European Data Portal) and hosted by the World Meteorological Organization (WMO). Daily temperature (minimum, mean and maximum) and total daily precipitation were acquired from Landvik, Norway (*58.340 N, 8.526 E*) and Bradford, United Kingdom (*53.794 N, -1.752 E*). The available data coverage for the period 1st May 2020 – 31st May 2022 was 81% for Landvik Temperature and 66% for Landvik Precipitation, while being 79% for Bradford Temperature and 66% for Bradford Precipitation. To supplement the meteorological data, weather data was acquired directly from on-site meteorological weather stations at Landvik and Bingley. This increased the data coverage to 100% for Landvik temperature and precipitation, while increasing the temperature coverage to 93% for min and max temperature and 88% for precipitation at Bingley (mean temperature coverage remained at 79%). The lower weather data coverage at Bingley was primarily during the summer of 2020. Long-term trends in temperature (1961 – 1990) from Landvik were acquired from the Landvik meteorological station. The same trends representing Bingley were acquired from the Centre for Environmental Data Analysis (CEDA) from the long-term meteorological station in Bradford (*53.814 N, -1.774 E*).

2.3 Statistical Analyses

Turf grass visual quality, colour intensity and microdochium disease development were modelled using linear regression models. The models modelled each turf grass response using the categorical variables treatment (four levels), year (two levels) and location (two levels). The models were analysed using Type-II ANOVA to identify significant differences due to the lack of interactions. The models were further analysed using Tukey's Post-hoc test to identify statistically significant differences within each categorical variable (Tukey 1949). Anthracnose disease development was modelled and analysed the same way but containing data from Landvik only. Area under disease progress curve (AUDPC) was further calculated from the disease development data. The AUDPC for microdochium and anthracnose for the separate years were first calculated for each treatment replicate and then averaged within treatments. The AUDPC treatment replicates were further compared using a One-way ANOVA (least-squares) and Sidak's Post-hoc test to identify significant differences. The meteorological trends were analysed using Students T-tests to identify differences between locations.

3 Results and Discussion

3.1 Meteorological Trends

The meteorological trends showed that Landvik had on average lower minimum temperatures than Bingley, but that there were no average differences for the mean and maximum temperatures. There were however larger variations between the daily minimum and daily maximum temperatures in Landvik than Bingley (Figure 1). On average the mean temperature in Landvik was a bit warmer than the long-term norm while in Bingley it was close to average. During the two year period of the experiment Landvik received more precipitation than Bingley (2,715 mm v 1,932 mm), with Landvik having on average more daily precipitation as well. Additionally, precipitation seasonality was stronger in Landvik, with winter being characterized as wetter while summers tended to be drier (Figure 2). The differences in precipitation trends could partly stem from the differences in data availability between the locations. Landvik received snow during January and February of 2021 and then again in the end of November to the middle of January 2022, and then sporadically until the beginning of April.

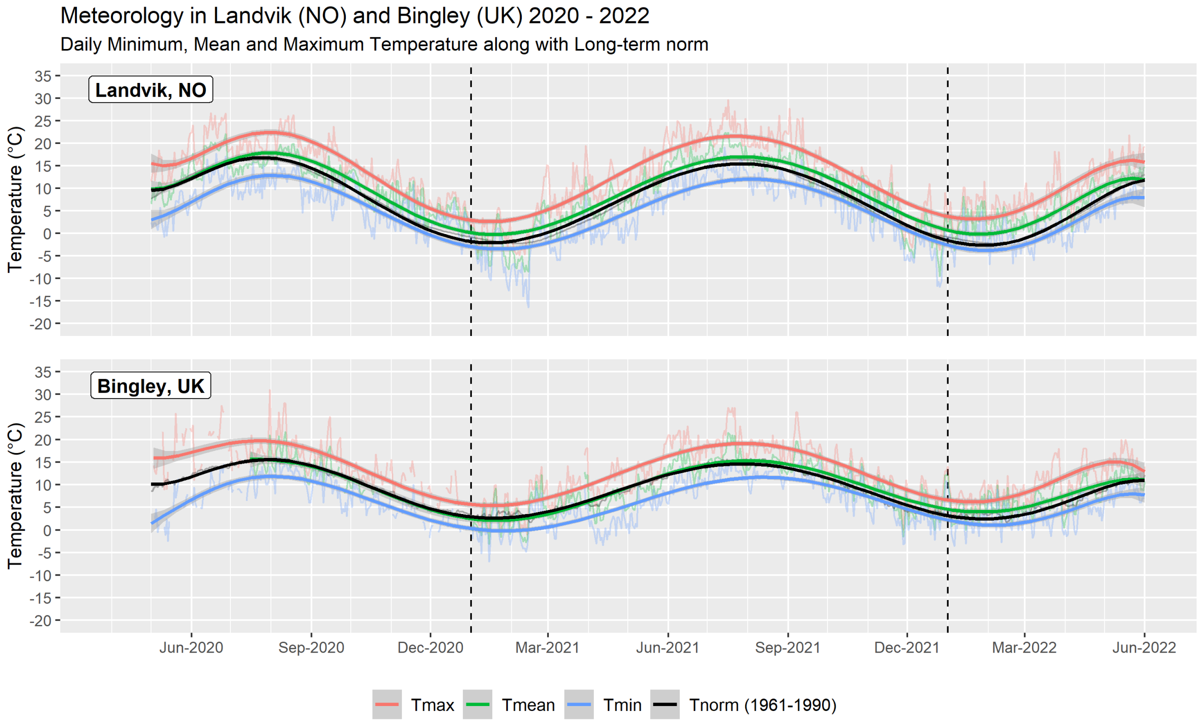

Figure 1: Daily minimum, mean and maximum air temperature for May 2020 – May 2022 and long-term trends between 1961- 1990 for Landvik, NO and Bingley, UK. The trend line is a linear regression smoother that shows the average modelled value with 95% confidence intervals.

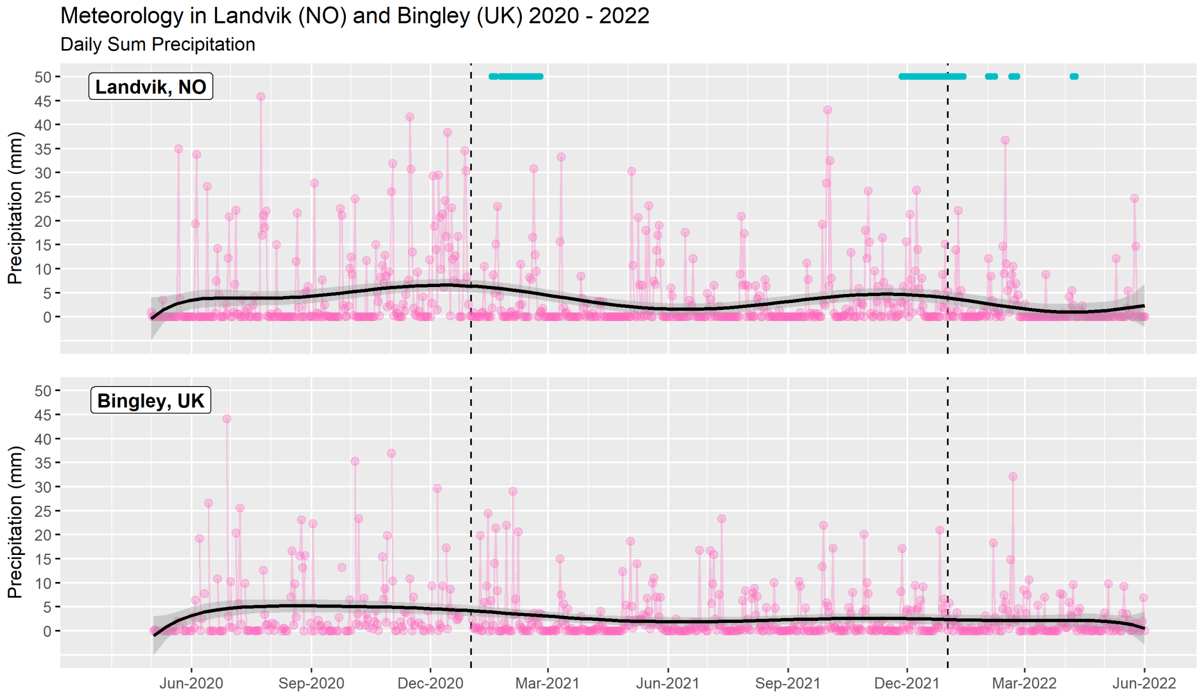

Figure 2: Daily sum precipitation for May 2020 – May 2022 for Landvik, NO and Bingley, UK. The trend line is a linear regression smoother that shows the average modelled value with 95% confidence intervals. The timing and presence of snow for Landvik can be found in the top of the graph.

3.2 Microdochium

Microdochium patch started to affect the turf grass and became visible during early autumn, around September, for both locations and years (Figure 3). Initially, the disease development was the same for all treatments, but around October differences between the treatments could be observed. The differences further continued as the treatments began to take effect to suppress the disease development. The modelling results show that there were significant differences between the treatments and between locations, but that the disease development was the same between years (Table 2a & 2b). Overall, all treatments were significantly better than the control to suppress the disease. Our analysis showed no difference in disease suppression between the treatment of two fungicides and biostimulant and three fungicides, suggesting that the use of a biostimulant can reduce the use of fungicides from three to two. The use of one fungicide and biostimulant was significantly better than the control treatment, but not as good as the two or three fungicide treatments at suppressing the disease. Although the treatment consisting of one fungicide on average is better than the control, it seems to vary between the two locations, with it being much better in Bingley, but barely better in Landvik. This suggests that other factors are involved in modulating the efficiency of this treatment. It is possible that the lower disease pressure in Bingley was involved, or that there were plot specific factors, since there was a large within-treatment variation in Landvik. This was primarily observed during the 2021 – 2022 season. In general, the within-treatment variation for the control suggests that some plots get inherently more infected than others, which could imply that the spread and progression of the disease showed high spatial heterogeneity. The spatial heterogeneity is confirmed by observing the AUDPC numbers, which show large standard error within the less effective treatments (Table 3). This is mostly prominent in Bingley, that showed significant differences in AUDPC between the control and the treatments, while the high standard error within-treatments prevented the same for Landvik. Photographs of the development of the disease in each treatment and replicate for Landvik can be viewed in the supplementary material (Supplementary Figures 1 – 6).

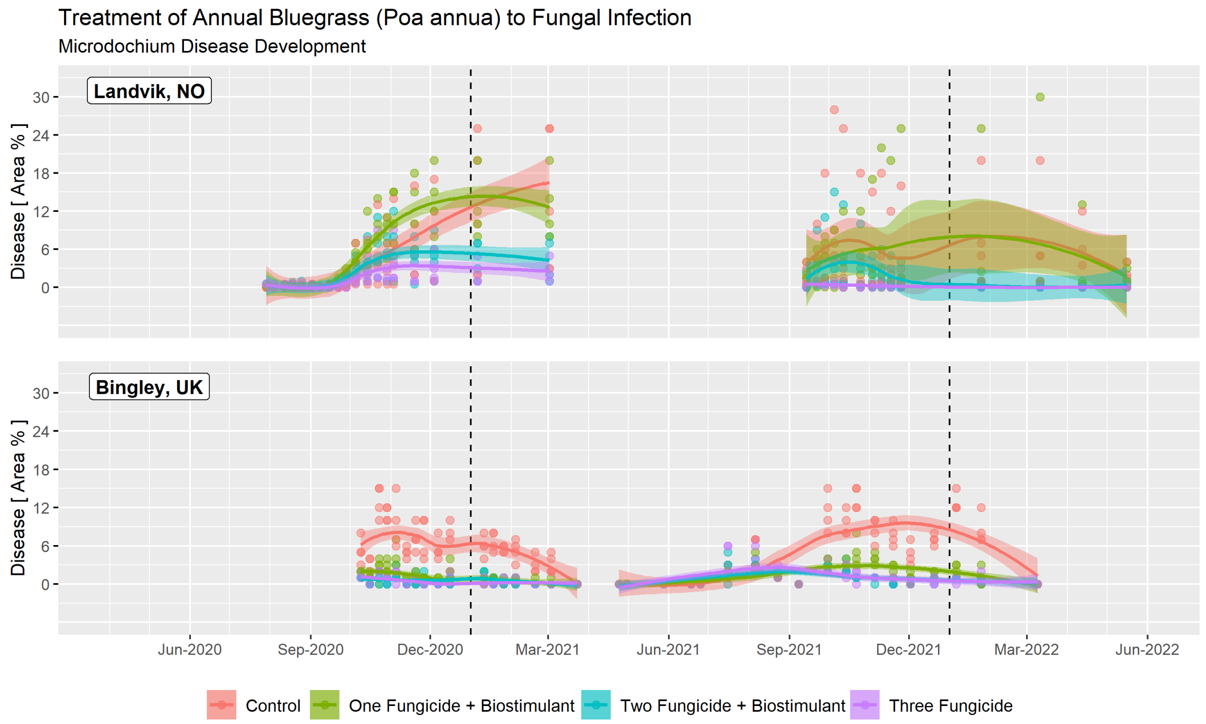

Figure 3: **Turf grass microdochium disease development for each treatment (replicates n = 4). The graph includes the 2020 – 2021 and the 2021 – 2022 season for Landvik, NO and Bingley, UK. The trend line is a linear smoother using a LOESS-function and 95% confidence intervals around the mean value for each treatment, year and location.**

Table 2a. Model statistics and significance levels for the Annual bluegrass (*Poa annua*) microdochium disease development and difference between each treatment, location and year.

Variable	Model Statistics				
	Sum Sq	Df	F - Value	P - Value	Significance
Treatment	2973.70	3	63.09	$< 1 \times 10^{-10}$	***
Location	389.70	1	24.80	7.372×10^{-7}	***
Year	1.00	1	0.06	0.804	ns
Residuals	17250.20	1,098	n/a	n/a	n/a

Table 2b. Continuation of Table 2a with statistical comparisons within each treatment, location and year.

Variable	Model Comparison						
	First	Second	diff	lwr	upr	P - Adj	Significance
Treatment	1 F + B	Control	-1.92	-2.79	-1.05	1×10^{-7}	***
	2 F + B	Control	-3.61	-4.48	-2.74	$< 1 \times 10^{-10}$	***
	3 F	Control	-4.22	-5.09	-3.35	$< 1 \times 10^{-10}$	***
	3 F	1 + F	-2.30	-3.17	-1.44	$< 1 \times 10^{-10}$	***
	2 F + B	1 + F	-1.70	-2.56	-0.83	3.5×10^{-6}	***
	2 F + B	3 F	0.61	-0.26	1.48	0.272	ns
Location	Landvik	Bingley	1.19	0.72	1.66	7×10^{-7}	***
Year	2020 - 2021	2021 - 2022	0.06	-0.41	0.53	0.805	ns

***Significance:** **P < 0.001** - ' *** ', **P < 0.01** - ' ** ', **P < 0.05** - ' * ', **P > 0.05** - ' ns '. **Model Statistics:** Type II ANOVA.*
***Abbreviations: F** - Fungicide, **B** - Biostimulant.*
The model comparison uses 95% confidence intervals (upr and lwr) to compare the First minus the Second treatment (diff).

Table 3. AUDPC average with standard error for each treatment, location and year for Microdochium and Anthracnose.

Variable	Location	Year	AUDPC for Treatments			
			Control	1 F + B	2 F + B	3 F
Microdochium	Landvik, NO	2020 - 2021	1627 ± 587^{a}	1795 ± 333^{a}	724 ± 239^{a}	414 ± 163^{a}
Microdochium	Landvik, NO	2021 - 2022	1524 ± 856^{a}	1449 ± 1090^{a}	284 ± 181^{a}	25 ± 9^{a}
Microdochium	Bingley, UK	2020 - 2021	936 ± 85^{a}	161 ± 28^{b}	91 ± 20^{b}	57 ± 17^{b}
Microdochium	Bingley, UK	2021 - 2022	1516 ± 41^{a}	445 ± 53^{b}	291 ± 33^{b}	361 ± 46^{b}
Anthracnose	Landvik, NO	2020 - 2021	70 ± 10^{a}	32 ± 12^{a}	37 ± 11^{a}	56 ± 24^{a}
Anthracnose	Landvik, NO	2021 - 2022	108 ± 87^{a}	32 ± 21^{a}	87 ± 41^{a}	242 ± 120^{a}

***Abbreviations: AUDPC** - Area under the disease progress curve, **F** - Fungicide**, B** - Biostimulant.*
Treatment post-hoc comparisons (p = 0.05) applies per row.

3.3 Visual Quality

The visual quality grading of the turf grass varied not only within each season (Figure 4) but varied significantly between treatments, locations and years (Table 4a & 4b). The visual quality tended to be the highest during the end of the summer and be the lowest during the end of winter. This corresponds to the optimal growth seasons and temperature conditions. The visual quality grading tended to be the same during the early autumn and then diverge as the weather became more unfavourable for growth and fungal disease development advanced during late autumn and winter. The model analysis revealed that all treatments were significantly better than the control at preserving high visual quality of the turf grass, i.e., suppression of disease improved turf quality. There was no difference in the efficiency of two fungicides and biostimulant and three fungicides treatments in preserving visual quality, suggesting that the use of biostimulants can replace one fungicide in terms of maintaining visual quality. Both these treatments were significantly better than the treatment using one fungicide and biostimulant. The same relationship between the treatment with one fungicide and biostimulant and the control was observed for the visual quality as in was for the microdochium disease development. In other words, there was less difference in this treatment and control at Landvik, but a major difference between these treatments were observed at Bingley. We also observed an average difference between the years, with this mainly being observed for Landvik, as the visual quality was overall higher during the 2021 – 2022 season. The visual quality remained the same between the years for Bingley. Overall, the visual quality of the turf grass was significantly higher in Landvik than in Bingley, although this can be a consequence of the observer conducting the grading.

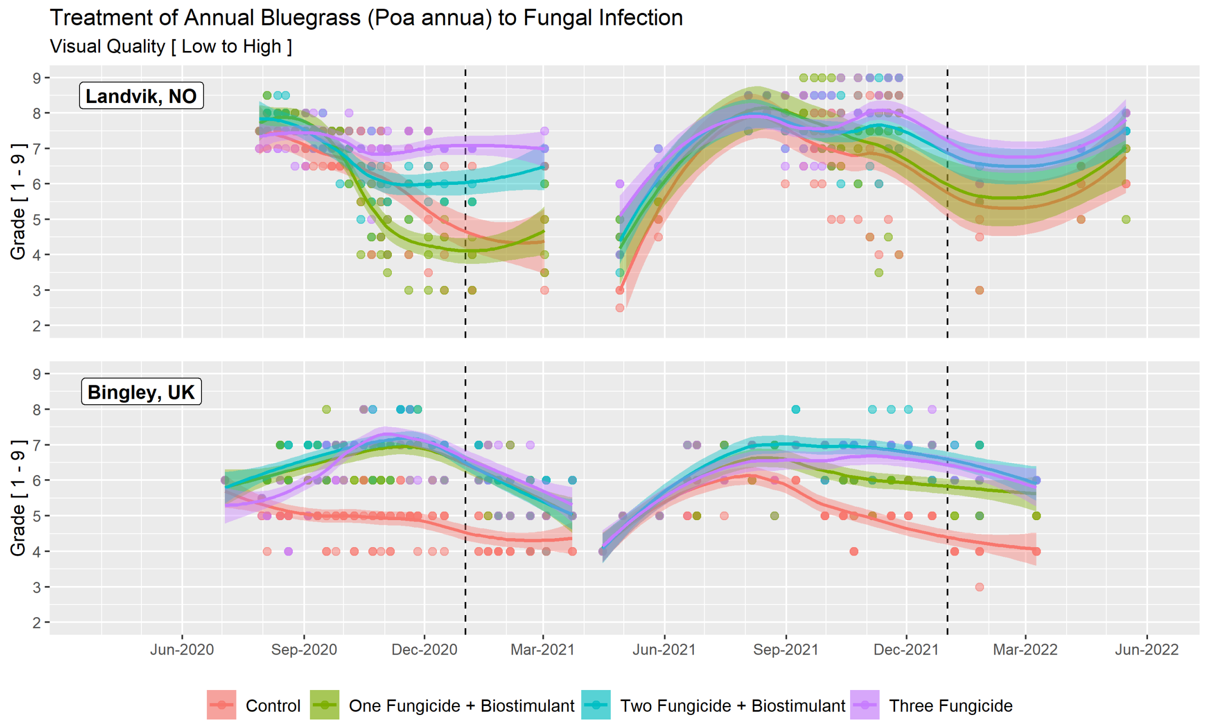

Figure 4: Turf grass visual quality for each treatment (replicates n = 4). The graph includes the 2020 – 2021 and the 2021 – 2022 season for Landvik, NO and Bingley, UK. The trend line is a linear smoother using a LOESS-function and 95% confidence intervals around the mean value for each treatment, year and location.

Table 4a. Model statistics and significance levels for the Annual bluegrass (*Poa annua*) visual quality and difference between each treatment, location and year.

Variable			**Model Statistics**		
	Sum Sq	Df	F - Value	P - Value	Significance
Treatment	266.59	3	73.57	$< 1 \times 10^{-10}$	***
Location	282.23	1	233.66	$< 1 \times 10^{-10}$	***
Year	5.84	1	4.83	0.028	*
Residuals	1616.13	1338.00	n/a	n/a	n/a

Table 4b. Continuation of Table 4a with statistical comparisons within each treatment, location and year.

Variable			**Model Comparison**				
	First	Second	diff	lwr	upr	P - Adj	Significance
Treatment	1 F + B	Control	0.73	0.51	0.95	$< 1 \times 10^{-10}$	***
	2 F + B	Control	1.08	0.86	1.30	$< 1 \times 10^{-10}$	***
	3 F	Control	1.10	0.88	1.32	$< 1 \times 10^{-10}$	***
	3 F	1 + F	0.37	0.15	0.59	7.890×10^{-5}	***
	2 F + B	1 + F	0.35	0.13	0.57	2.308×10^{-4}	***
	2 F + B	3 F	-0.02	-0.24	0.20	0.995	ns
Location	Landvik	Bingley	0.93	0.81	1.05	$< 1 \times 10^{-10}$	***
Year	2020 - 2021	2021 - 2022	0.13	0.01	0.25	0.028	*

Significance: P < 0.001 - ' *** ', P < 0.01 - ' ** ', P < 0.05 - ' * ', P > 0.05 *- ' ns '.* ***Model Statistics:*** *Type II ANOVA.*
Abbreviations: F - *Fungicide,* ***B -*** *Biostimulant.*
The model comparison uses 95% confidence intervals (upr and lwr) to compare the First minus the Second treatment (diff).

3.4 Colour Intensity

Colour intensity of the turf grass tended to vary within each season (Figure 5), likely depending on the optimal growth conditions and local weather conditions, as it tended to become higher during the end of the growth season in late summer. Our model analysis confirmed that the colour intensity varied significantly between treatments, locations and years (Table 5a & 5b). There were significant differences between the three application treatments and the control, suggesting that applying the treatments contributes to better colour intensity of the turf grass. Further on, our analysis revealed that there were no significant differences between the three fungicide treatments. This suggests that applying Ryder improved the colour intensity of the turf grass equally between the three application schedules. The data for Bingley during the 2020 – 2021 season suggests that the delayed Ryder application in September for the three fungicide treatment caused a difference in colour intensity to the other two treatments, which received Ryder already in the end of July. This discrepancy however cannot be observed for any other years or locations. For Landvik, the drop in colour intensity in both years is likely related to the presence of heavy snowfall and snow duration in both years. The lack of difference between the two biostimulant treatments and compared to the three fungicide treatment implies that the biostimulant does not contribute to the variation in colour intensity.

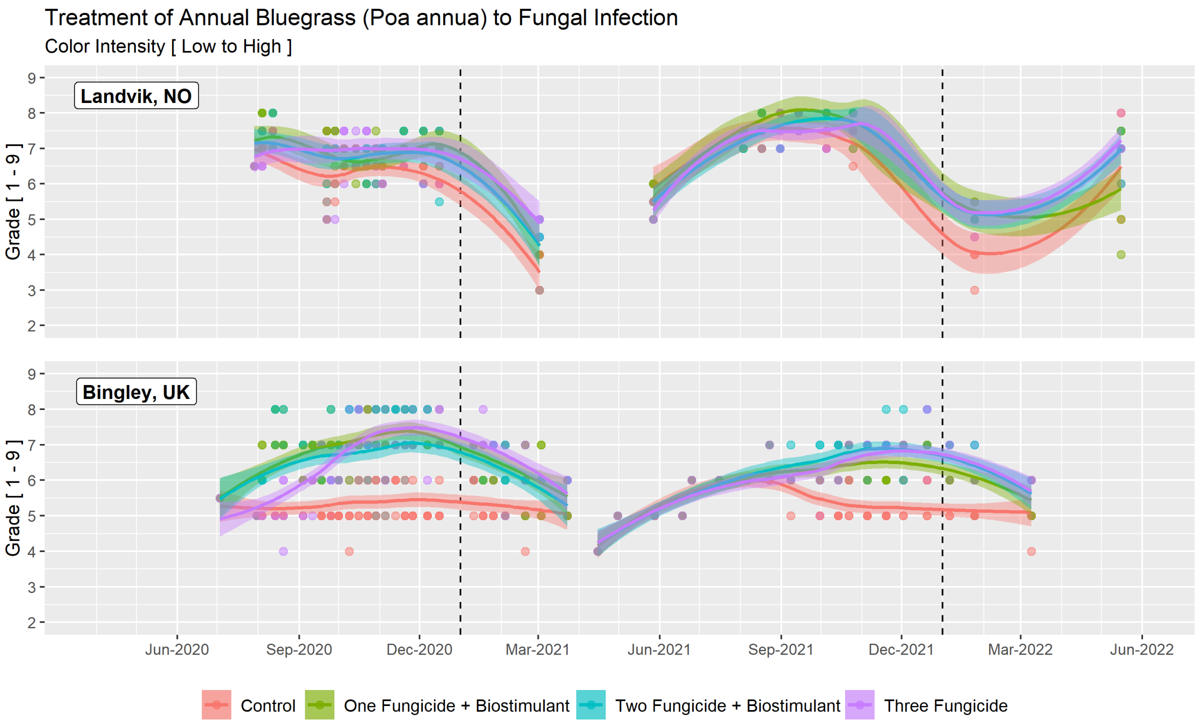

Figure 5: Turf grass color intensity for each treatment (replicates n = 4). The graph includes the 2020 – 2021 and the 2021 – 2022 season for Landvik, NO and Bingley, UK. The trend line is a linear smoother using a LOESS-function and 95% confidence intervals around the mean value for each treatment, year and location.

Table 5a. Model statistics and significance levels for the Annual bluegrass (*Poa annua*) colour intensity and difference between each treatment, location and year.

Variable			**Model Statistics**		
	Sum Sq	Df	F - Value	P - Value	Significance
Treatment	144.81	3	55.76	< 1 x 10^{-10}	***
Location	105.29	1	121.62	< 1 x 10^{-10}	***
Year	7.82	1	9.03	0.003	**
Residuals	950.51	1,098	n/a	n/a	n/a

Table 5b. Continuation of Table 5a with statistical comparisons within each treatment, location and year.

Variable	Model Comparison						
	First	Second	diff	lwr	upr	P - Adj	Significance
Treatment	1 F + B	Control	0.87	0.67	1.08	$< 1 \times 10^{-10}$	***
	2 F + B	Control	0.82	0.62	1.03	$< 1 \times 10^{-10}$	***
	3 F	Control	0.81	0.60	1.01	$< 1 \times 10^{-10}$	***
	3 F	1 + F	-0.07	-0.27	0.14	0.843	ns
	2 F + B	1 + F	-0.05	-0.25	0.15	0.919	ns
	2 F + B	3 F	0.01	-0.19	0.22	0.998	ns
Location	Landvik	Bingley	0.66	0.54	0.78	$< 1 \times 10^{-10}$	***
Year	2020 - 2021	2021 - 2022	-0.17	-0.28	-0.06	0.003	**

Significance: P < 0.001 - *' *** ',* ***P < 0.01 -*** *' ** ',* ***P < 0.05 -*** *' * ',* ***P > 0.05 -*** *' ns '.* ***Model Statistics:*** *Type II ANOVA.*
Abbreviations: F - *Fungicide,* ***B -*** *Biostimulant.*
The model comparison uses 95% confidence intervals (upr and lwr) to compare the First minus the Second treatment (diff).

3.5 Anthracnose

Anthracnose was observed at Landvik in 2020 and 2021 during the late summer and tended to continue until mid-autumn (Figure 6). The disease pressure was characterized as low during the 2020 seasons but reached elevated levels during the 2021 season. Our model analysis showed significant differences in anthracnose disease levels between treatments and years (Table 6a & 6b). Overall, there was no difference between the fungicide treatments and the control. However, the three fungicide treatment was significantly worse at reducing the disease pressure than the two treatments containing one fungicide and biostimulant and two fungicides and biostimulant while there were no differences between the two respective treatments. This suggests that the use of biostimulants can suppress anthracnose disease pressure better than the applied fungicide treatments. One caveat is the large within-treatment variation for the three fungicide treatment, as indicated by the AUDPC numbers (Table X2), suggesting that other factors are involved in the response of the disease development of anthracnose. Further research is needed to determine if biostimulant treatment alone, and in what application rate, could treat anthracnose disease in turf grasses.

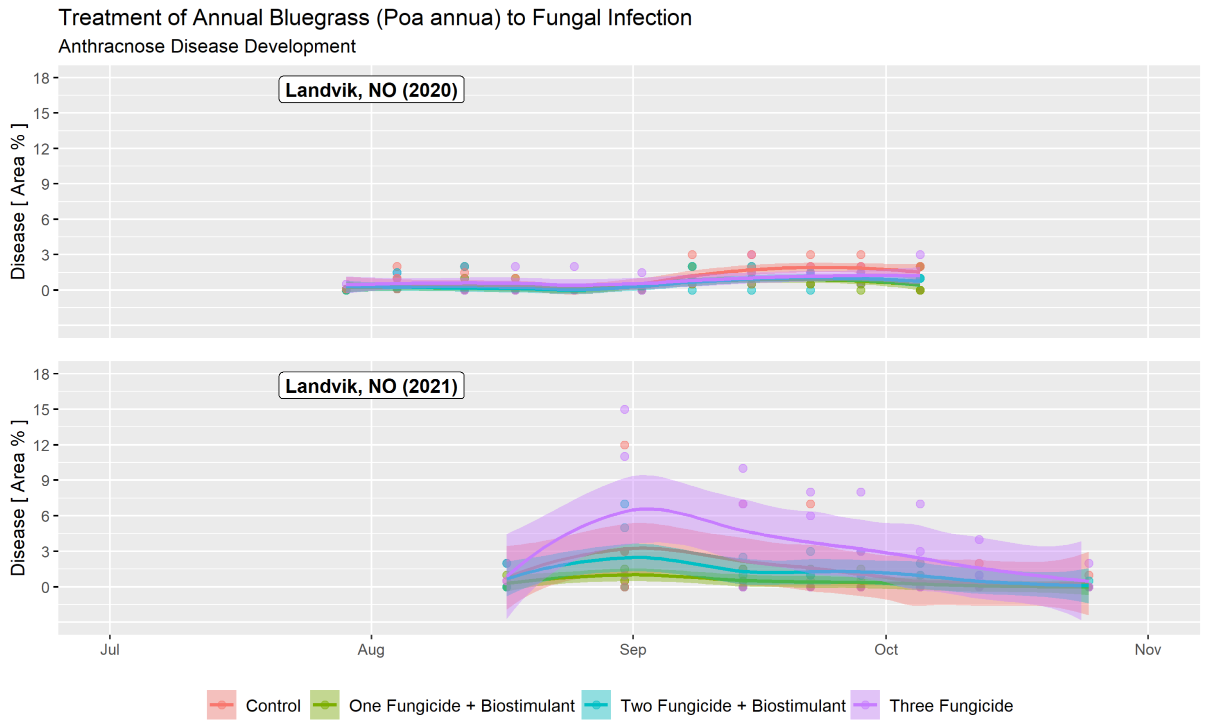

Figure 6: **Turf grass anthracnose disease development for each treatment (replicates n = 4). The graph includes the 2020 and the 2021 season for Landvik, NO. The trend line is a linear smoother using a LOESS-function and 95% confidence intervals around the mean value for each treatment, year and location.**

Table 6a. Model statistics and significance levels for the Annual bluegrass (*Poa annua*) anthracnose disease development for Landvik and difference between each treatment and year.

Variable	Model Statistics				
	Sum Sq	Df	F - Value	P - Value	Significance
Treatment	72.08	3	7.50	7.403 x 10^{-5}	***
Year	40.30	1	12.58	4.518 x 10^{-4}	***
Residuals	957.60	299	n/a	n/a	n/a

Table 6b. Continuation of Table 6a with statistical comparisons within each treatment, location and year.

Variable	Model Comparison						
	First	Second	diff	lwr	upr	P - Adj	Significance
Treatment	1 F + B	Control	-0.71	-1.46	0.04	0.071	ns
	2 F + B	Control	-0.38	-1.13	0.38	0.569	ns
	3 F	Control	0.60	-0.15	1.35	0.165	ns
	3 F	1 + F	1.31	0.56	2.06	5.290 x 10^{-5}	***
	2 F + B	1 + F	0.34	-0.41	1.09	0.655	ns
	2 F + B	3 F	0.98	0.23	1.73	0.005	**
Year	2020 - 2021	2021 - 2022	0.74	0.33	1.15	4.518 x 10^{-4}	***

Significance: ***P < 0.001*** *- ' *** ',* ***P < 0.01*** *- ' ** ',* ***P < 0.05*** *- ' * ',* ***P > 0.05*** *- ' ns '.* ***Model Statistics:*** *Type II ANOVA.*
Abbreviations: F *- Fungicide,* ***B*** *- Biostimulant.*
The model comparison uses 95% confidence intervals (upr and lwr) to compare the First minus the Second treatment (diff).

4 Conclusions

The study found that the addition of a biostimulant (Hicure) allowed a reduction in the number of fungicide treatment three to two fungicide applications with the same prevention and reduction in microdochium patch development. The use of one fungicide with biostimulant was better than the control, but not better than the two and three fungicide treatments. These findings remained similar between years, but the treatment efficiency varied somewhat between locations, most likely to differences in disease pressure and how disease developed at both sites.

Treatment effects on visual turf quality were likely to be the result of disease incidence. The use of a biostimulant (Hicure) can reduce the number of fungicide applications from three to two, whilst still maintaining optimal turf grass visual quality. The treatment with one fungicide and biostimulant was likewise better than the control, but not as good as the three and two fungicidal treatments.

The use of Ryder improves the colour intensity of the turf grass. All treatments were better than the control with no difference in colour intensity between the treatments, one fungicide with biostimulant and Ryder is equally good as three fungicides with Ryder. This suggests that the application of Ryder, irrespective of the number of fungicide treatments, resulted in improved turf colour intensity.

Finally, anthracnose disease development was reduced further with the two biostimulant treatments than with the three fungicidal treatments, suggesting that the biostimulant alone is better than the fungicides at reducing anthracnose.

Our findings support the notion that the biostimulant Hicure can be used to reduce number of fungicide applications when treating microdochium and anthracnose disease development in turf grasses and that the colour pigment Ryder can be used to improve colour intensity and visual quality, and thereby overall impression of the turf grass and the golf green as a whole. This supports the role of integrated approaches at reducing the likelihood of disease development, whilst reducing the environmental loading of fungicide. Future studies should investigate the use of the biostimulant as a stand-alone and in combination with other prominent treatments to further reduce the use of unnecessary fungicides.

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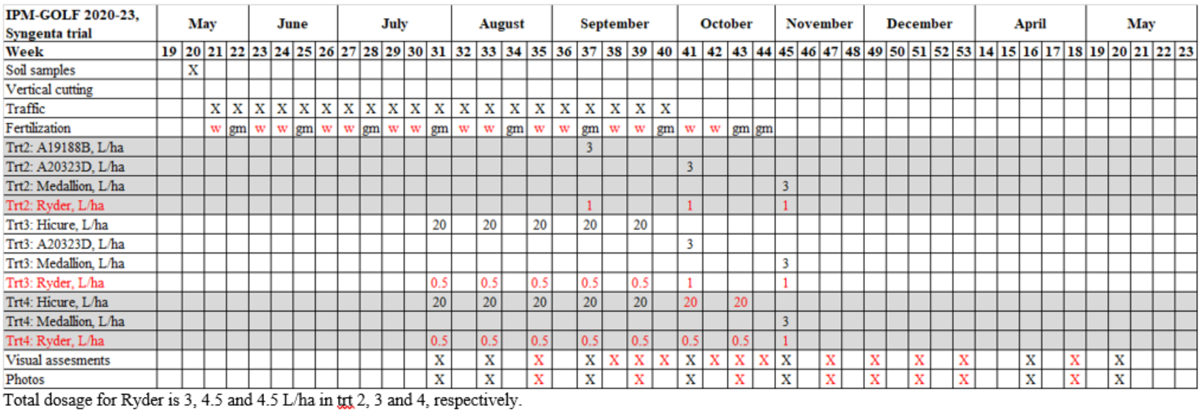
Supplementary Material

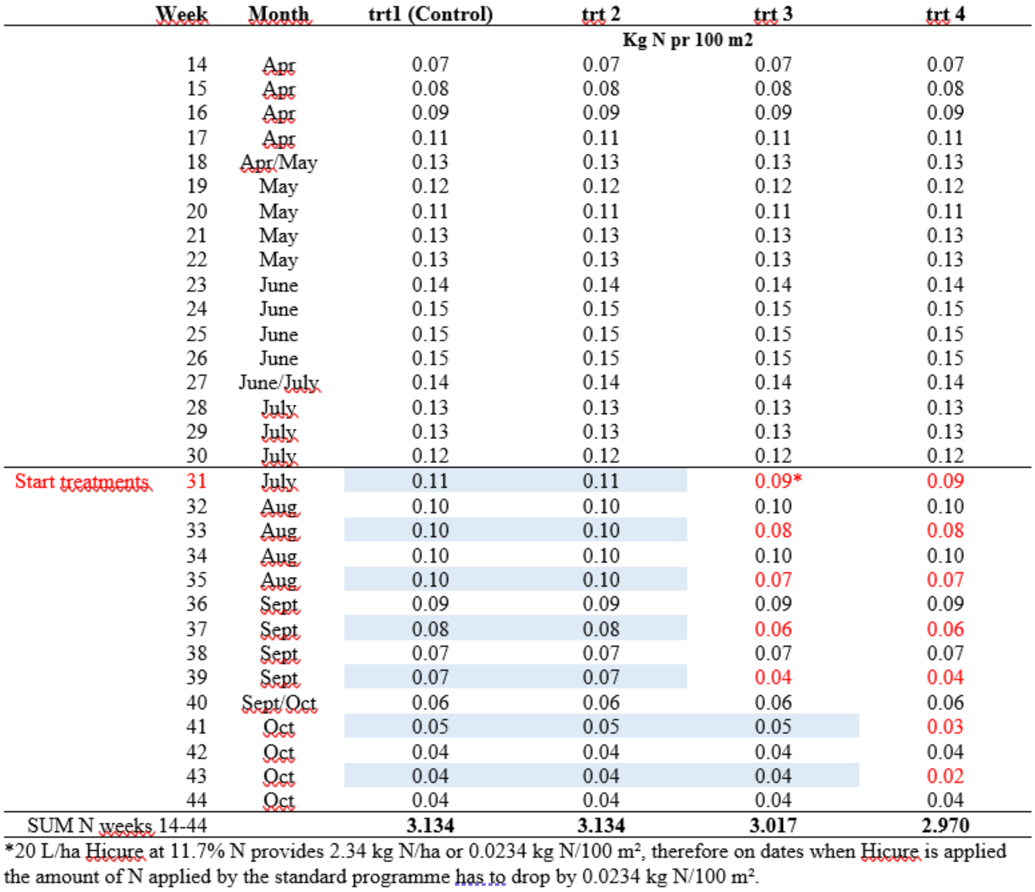
Supplementary Table 1. Treatment application and assessment schedule.



IPM-GOLF 2020-23, Syngenta trial	May				June				July					August				September					October				November				December					April					May				
Week	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	14	15	16	17	18	19	20	21	22	23
Soil samples		X																																											
Vertical cutting																																													
Traffic			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X																							
Fertilization			w	gm	w	w	gm	w	w	gm	w	w	gm	w	w	gm	w	w	gm	w	w	gm	w	w	gm	gm																			
Trt2: A19188B, L/ha																			3																										
Trt2: A20323D, L/ha																							3																						
Trt2: Medallion, L/ha																											3																		
Trt2: Ryder, L/ha																			1				1				1																		
Trt3: Hicure, L/ha													20		20		20		20		20																								
Trt3: A20323D, L/ha																							3																						
Trt3: Medallion, L/ha																											3																		
Trt3: Ryder, L/ha													0.5		0.5		0.5		0.5		0.5		1				1																		
Trt4: Hicure, L/ha													20		20		20		20		20		20		20																				
Trt4: Medallion, L/ha																											3																		
Trt4: Ryder, L/ha													0.5		0.5		0.5		0.5		0.5		0.5		0.5		1																		
Visual assesments													X		X		X		X	X	X	X	X	X	X	X	X		X		X		X		X			X		X		X			
Photos													X		X		X		X		X		X		X		X		X		X		X		X			X		X		X			

Total dosage for Ryder is 3, 4.5 and 4.5 L/ha in trt 2, 3 and 4, respectively.

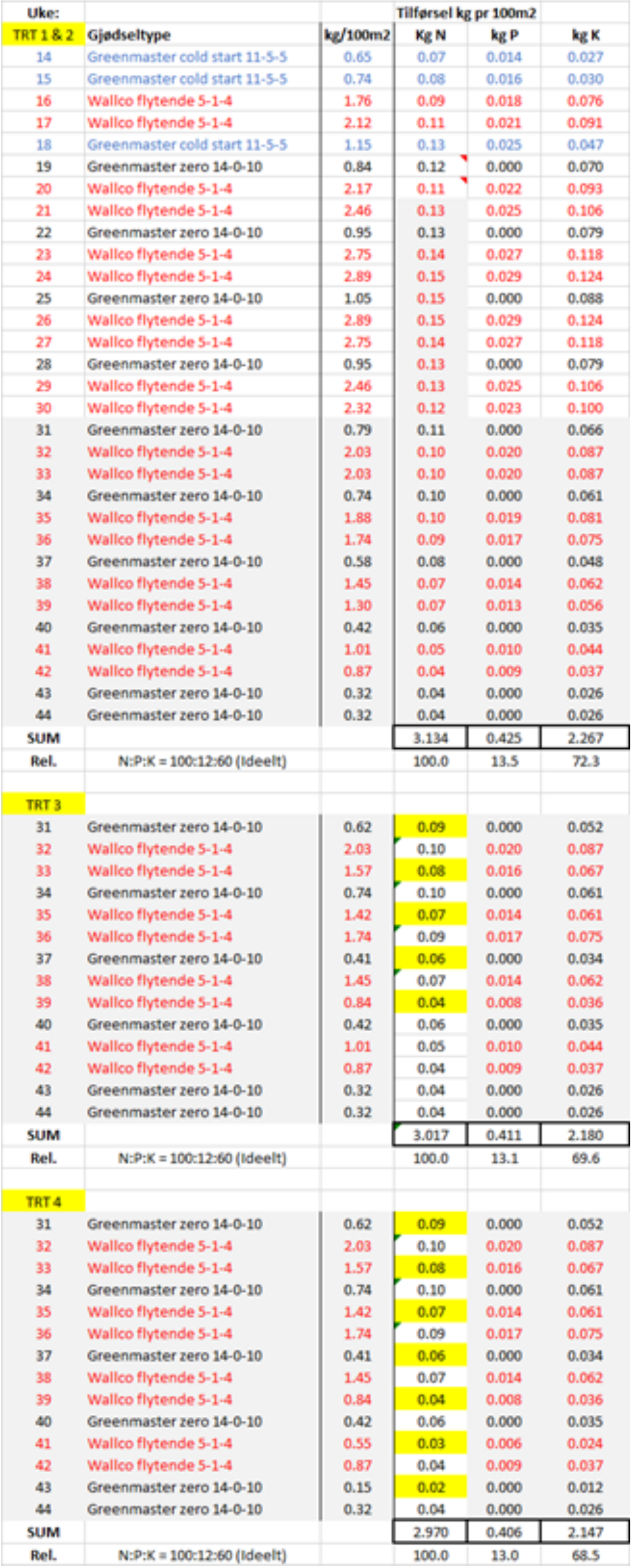
Supplementary Table 2. Nitrogen fertilization plan per Treatment, Landvik.



	Week	Month	trt1 (Control)	trt 2	trt 3	trt 4
				Kg N pr 100 m2		
	14	Apr	0.07	0.07	0.07	0.07
	15	Apr	0.08	0.08	0.08	0.08
	16	Apr	0.09	0.09	0.09	0.09
	17	Apr	0.11	0.11	0.11	0.11
	18	Apr/May	0.13	0.13	0.13	0.13
	19	May	0.12	0.12	0.12	0.12
	20	May	0.11	0.11	0.11	0.11
	21	May	0.13	0.13	0.13	0.13
	22	May	0.13	0.13	0.13	0.13
	23	June	0.14	0.14	0.14	0.14
	24	June	0.15	0.15	0.15	0.15
	25	June	0.15	0.15	0.15	0.15
	26	June	0.15	0.15	0.15	0.15
	27	June/July	0.14	0.14	0.14	0.14
	28	July	0.13	0.13	0.13	0.13
	29	July	0.13	0.13	0.13	0.13
	30	July	0.12	0.12	0.12	0.12
Start treatments	31	July	0.11	0.11	0.09*	0.09
	32	Aug	0.10	0.10	0.10	0.10
	33	Aug	0.10	0.10	0.08	0.08
	34	Aug	0.10	0.10	0.10	0.10
	35	Aug	0.10	0.10	0.07	0.07
	36	Sept	0.09	0.09	0.09	0.09
	37	Sept	0.08	0.08	0.06	0.06
	38	Sept	0.07	0.07	0.07	0.07
	39	Sept	0.07	0.07	0.04	0.04
	40	Sept/Oct	0.06	0.06	0.06	0.06
	41	Oct	0.05	0.05	0.05	0.03
	42	Oct	0.04	0.04	0.04	0.04
	43	Oct	0.04	0.04	0.04	0.02
	44	Oct	0.04	0.04	0.04	0.04
SUM N weeks 14-44			**3.134**	**3.134**	**3.017**	**2.970**

*20 L/ha Hicure at 11.7% N provides 2.34 kg N/ha or 0.0234 kg N/100 m², therefore on dates when Hicure is applied the amount of N applied by the standard programme has to drop by 0.0234 kg N/100 m².

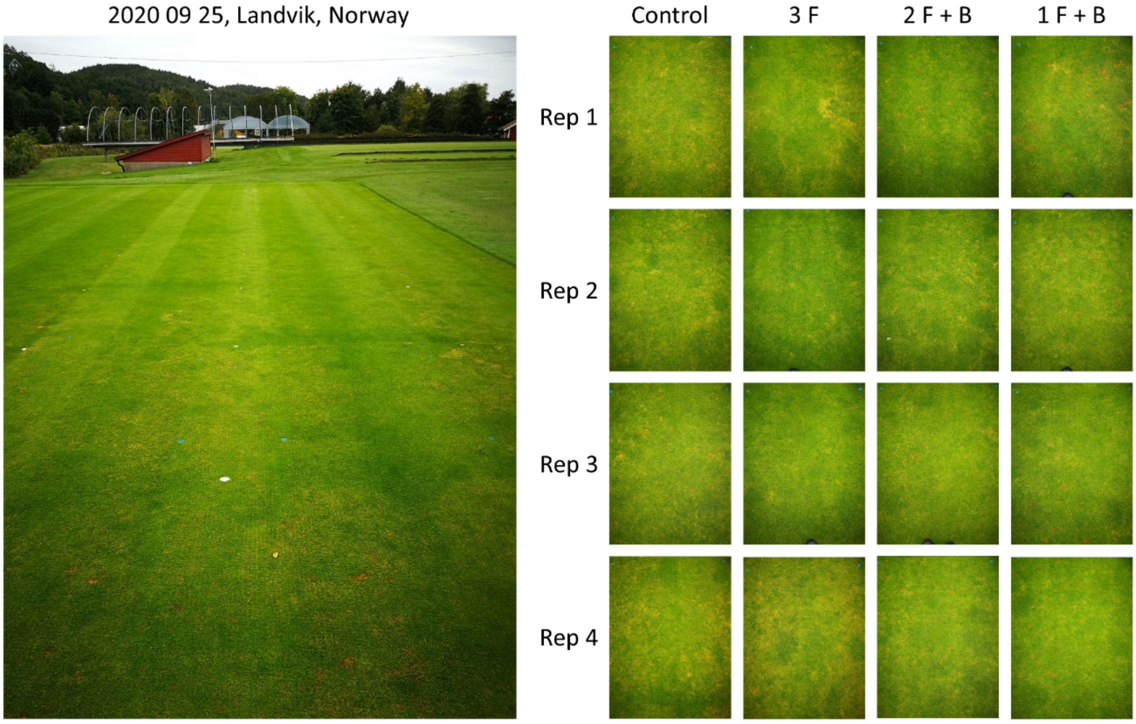
Supplementary Table 3. Total NPK fertilization plan, Landvik.



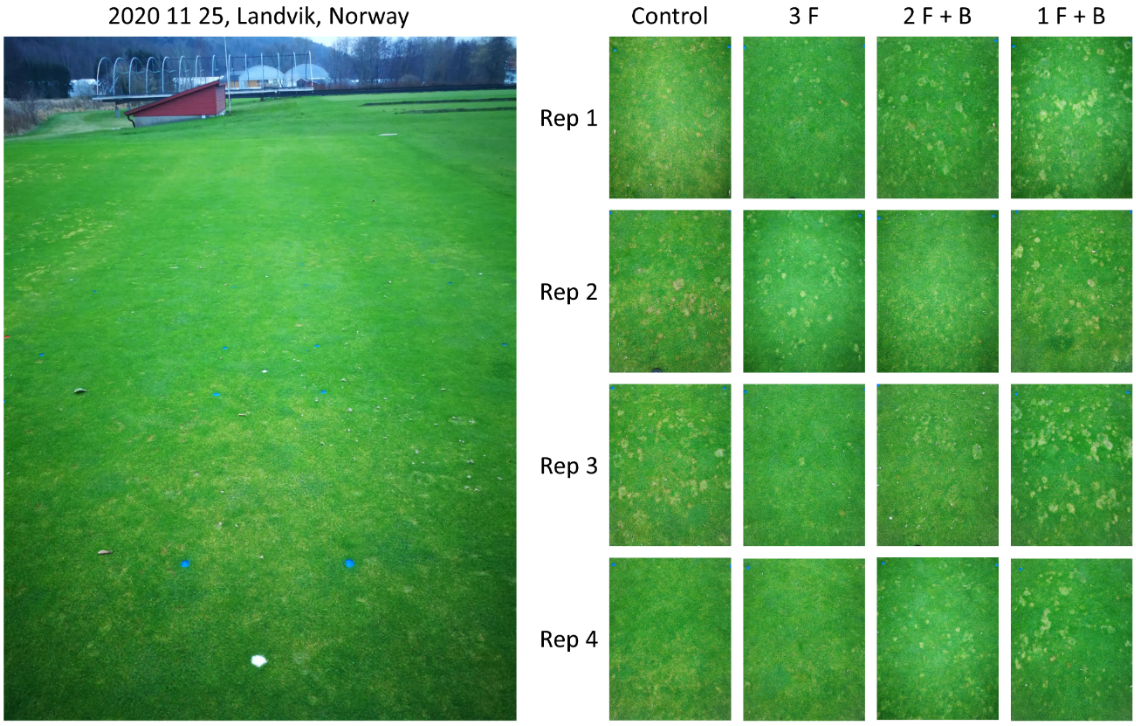
Uke:			Tilførsel kg pr 100m2		
TRT 1 & 2	Gjødseltype	kg/100m2	Kg N	kg P	kg K
14	Greenmaster cold start 11-5-5	0.65	0.07	0.014	0.027
15	Greenmaster cold start 11-5-5	0.74	0.08	0.016	0.030
16	Wallco flytende 5-1-4	1.76	0.09	0.018	0.076
17	Wallco flytende 5-1-4	2.12	0.11	0.021	0.091
18	Greenmaster cold start 11-5-5	1.15	0.13	0.025	0.047
19	Greenmaster zero 14-0-10	0.84	0.12	0.000	0.070
20	Wallco flytende 5-1-4	2.17	0.11	0.022	0.093
21	Wallco flytende 5-1-4	2.46	0.13	0.025	0.106
22	Greenmaster zero 14-0-10	0.95	0.13	0.000	0.079
23	Wallco flytende 5-1-4	2.75	0.14	0.027	0.118
24	Wallco flytende 5-1-4	2.89	0.15	0.029	0.124
25	Greenmaster zero 14-0-10	1.05	0.15	0.000	0.088
26	Wallco flytende 5-1-4	2.89	0.15	0.029	0.124
27	Wallco flytende 5-1-4	2.75	0.14	0.027	0.118
28	Greenmaster zero 14-0-10	0.95	0.13	0.000	0.079
29	Wallco flytende 5-1-4	2.46	0.13	0.025	0.106
30	Wallco flytende 5-1-4	2.32	0.12	0.023	0.100
31	Greenmaster zero 14-0-10	0.79	0.11	0.000	0.066
32	Wallco flytende 5-1-4	2.03	0.10	0.020	0.087
33	Wallco flytende 5-1-4	2.03	0.10	0.020	0.087
34	Greenmaster zero 14-0-10	0.74	0.10	0.000	0.061
35	Wallco flytende 5-1-4	1.88	0.10	0.019	0.081
36	Wallco flytende 5-1-4	1.74	0.09	0.017	0.075
37	Greenmaster zero 14-0-10	0.58	0.08	0.000	0.048
38	Wallco flytende 5-1-4	1.45	0.07	0.014	0.062
39	Wallco flytende 5-1-4	1.30	0.07	0.013	0.056
40	Greenmaster zero 14-0-10	0.42	0.06	0.000	0.035
41	Wallco flytende 5-1-4	1.01	0.05	0.010	0.044
42	Wallco flytende 5-1-4	0.87	0.04	0.009	0.037
43	Greenmaster zero 14-0-10	0.32	0.04	0.000	0.026
44	Greenmaster zero 14-0-10	0.32	0.04	0.000	0.026
SUM			3.134	0.425	2.267
Rel.	N:P:K = 100:12:60 (Ideelt)		100.0	13.5	72.3
TRT 3					
31	Greenmaster zero 14-0-10	0.62	0.09	0.000	0.052
32	Wallco flytende 5-1-4	2.03	0.10	0.020	0.087
33	Wallco flytende 5-1-4	1.57	0.08	0.016	0.067
34	Greenmaster zero 14-0-10	0.74	0.10	0.000	0.061
35	Wallco flytende 5-1-4	1.42	0.07	0.014	0.061
36	Wallco flytende 5-1-4	1.74	0.09	0.017	0.075
37	Greenmaster zero 14-0-10	0.41	0.06	0.000	0.034
38	Wallco flytende 5-1-4	1.45	0.07	0.014	0.062
39	Wallco flytende 5-1-4	0.84	0.04	0.008	0.036
40	Greenmaster zero 14-0-10	0.42	0.06	0.000	0.035
41	Wallco flytende 5-1-4	1.01	0.05	0.010	0.044
42	Wallco flytende 5-1-4	0.87	0.04	0.009	0.037
43	Greenmaster zero 14-0-10	0.32	0.04	0.000	0.026
44	Greenmaster zero 14-0-10	0.32	0.04	0.000	0.026
SUM			3.017	0.411	2.180
Rel.	N:P:K = 100:12:60 (Ideelt)		100.0	13.1	69.6
TRT 4					
31	Greenmaster zero 14-0-10	0.62	0.09	0.000	0.052
32	Wallco flytende 5-1-4	2.03	0.10	0.020	0.087
33	Wallco flytende 5-1-4	1.57	0.08	0.016	0.067
34	Greenmaster zero 14-0-10	0.74	0.10	0.000	0.061
35	Wallco flytende 5-1-4	1.42	0.07	0.014	0.061
36	Wallco flytende 5-1-4	1.74	0.09	0.017	0.075
37	Greenmaster zero 14-0-10	0.41	0.06	0.000	0.034
38	Wallco flytende 5-1-4	1.45	0.07	0.014	0.062
39	Wallco flytende 5-1-4	0.84	0.04	0.008	0.036
40	Greenmaster zero 14-0-10	0.42	0.06	0.000	0.035
41	Wallco flytende 5-1-4	0.55	0.03	0.006	0.024
42	Wallco flytende 5-1-4	0.87	0.04	0.009	0.037
43	Greenmaster zero 14-0-10	0.15	0.02	0.000	0.012
44	Greenmaster zero 14-0-10	0.32	0.04	0.000	0.026
SUM			2.970	0.406	2.147
Rel.	N:P:K = 100:12:60 (Ideelt)		100.0	13.0	68.5

Supplementary Table 4. Nitrogen fertilization plan per Treatment, Bingley.

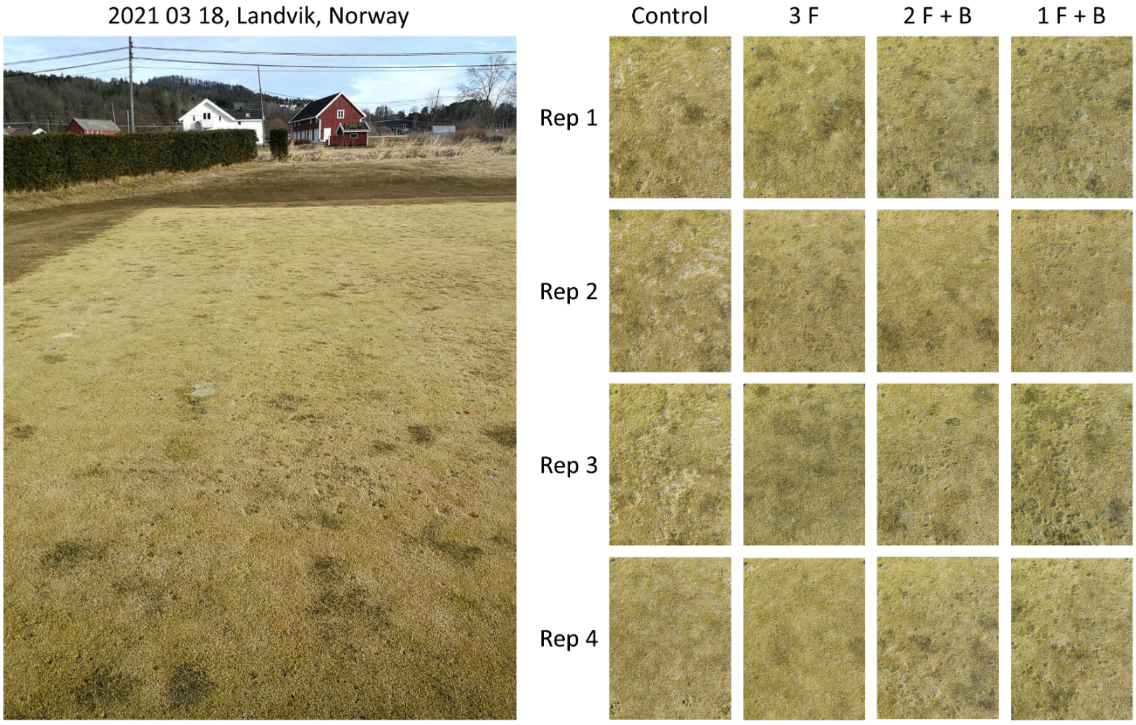
		trt 1	trt2	Hicure trt3	Hicure trt4
Week	**Month**	**Kg N per 100 m²**			
15	Apr				
16	Apr				
17	Apr				
18	Apr/May				
19	May	0.017	0.017	0.017	0.017
20	May				
21	May				
22	May				
23	June	0.050	0.050	0.050	0.050
24	June	0.050	0.050	0.050	0.050
25	June	0.050	0.050	0.050	0.050
26	June	0.050	0.050	0.050	0.050
27	June/July	0.050	0.050	0.050	0.050
28	July	0.050	0.050	0.050	0.050
29	July	0.050	0.050	0.050	0.050
30	July	0.050	0.050	0.050	0.050
31	July	0.050	0.050	0.027	0.027
32	Aug	0.050	0.050	0.050	0.050
33	Aug	0.050	0.050	0.027	0.027
34	Aug	0.050	0.050	0.050	0.050
35	Aug	0.050	0.050	0.027	0.027
36	Sept	0.050	0.050	0.050	0.050
37	Sept	0.050	0.050	0.027	0.027
38	Sept	0.050	0.050	0.050	0.050
39	Sept	0.050	0.050	0.027	0.027
40	Sept/Oct	0.050	0.050	0.050	0.050
41	Oct	0.050	0.050	0.050	0.027
42	Oct	0.050	0.050	0.050	0.050
43	Oct	0.050	0.050	0.050	0.027
44	Oct	0.050	0.050	0.050	0.050
45	Nov	0.050	0.050	0.050	0.050
46	Nov	0.050	0.050	0.050	0.050
	Total N	**1.22**	**1.22**	**1.10**	**1.05**



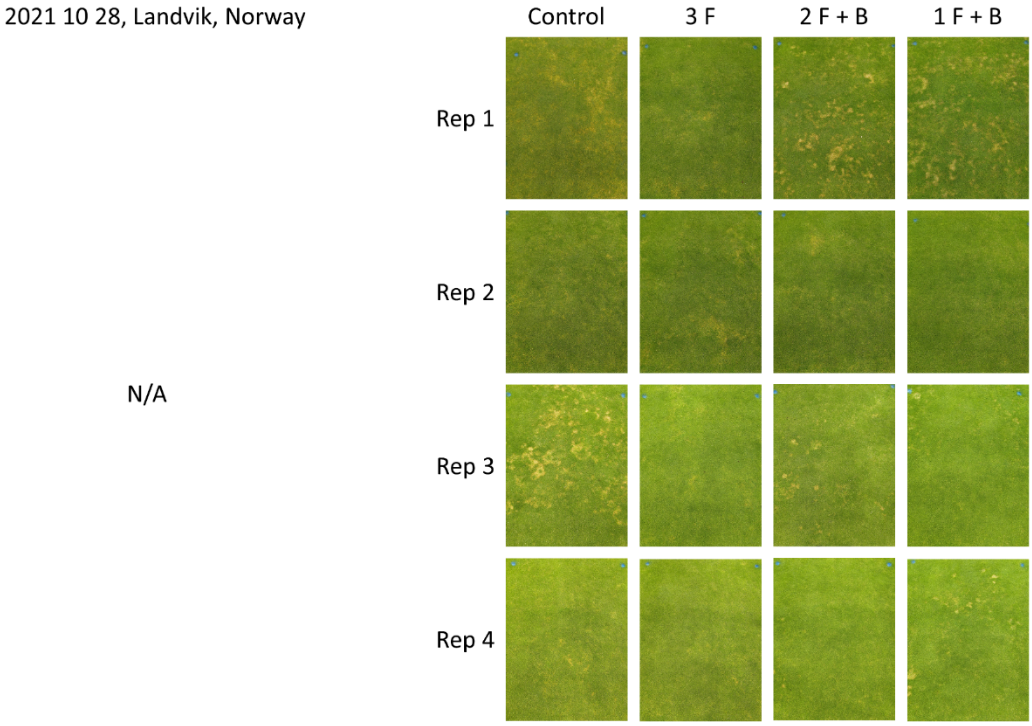
Supplementary Figure 1: Photos of the treatment replicates for each treatment at Landvik, 2020-09-25.



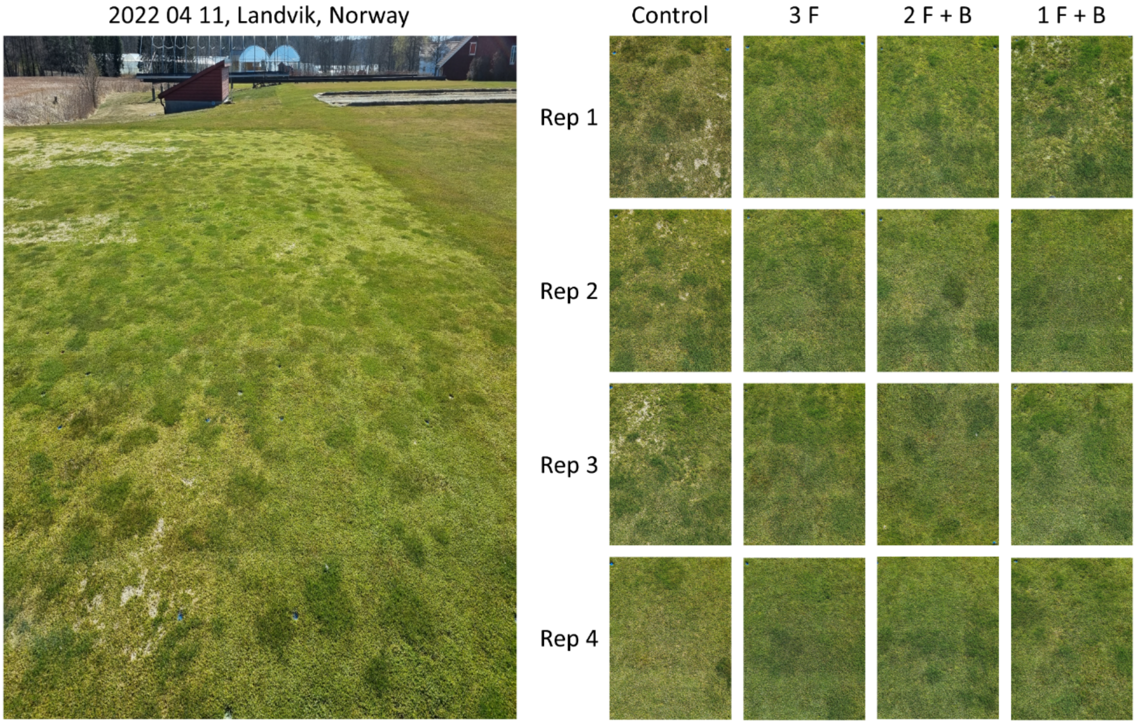
Supplementary Figure 2: Photos of the treatment replicates for each treatment at Landvik, 2020-11-25.



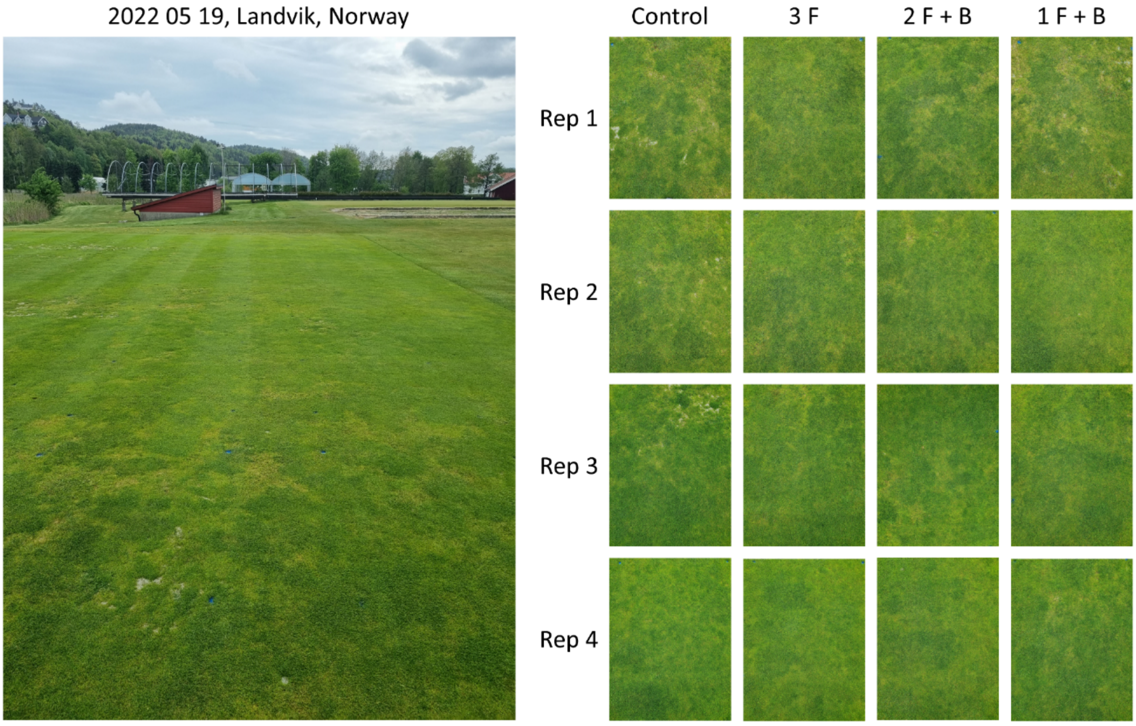
Supplementary Figure 3: Photos of the treatment replicates for each treatment at Landvik, 2021-03-18.



Supplementary Figure 4: Photos of the treatment replicates for each treatment at Landvik, 2021-10-28. Missing overview photograph.



Supplementary Figure 5: Photos of the treatment replicates for each treatment at Landvik, 2022-04-11.



Supplementary Figure 6: Photos of the treatment replicates for each treatment at Landvik, 2022-05-19.

NIBIO
NORSK INSTITUTT FOR
BIOØKONOMI

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.

Bioøkonomi baserer seg på utnyttelse og forvaltning av biologiske ressurser fra jord og hav, fremfor en fossil økonomi som er basert på kull, olje og gass. NIBIO skal være nasjonalt ledende for utvikling av kunnskap om bioøkonomi.

Gjennom forskning og kunnskapsproduksjon skal instituttet bidra til matsikkerhet, bærekraftig ressursforvaltning, innovasjon og verdiskaping innenfor verdikjedene for mat, skog og andre biobaserte næringer. Instituttet skal levere forskning, forvaltningsstøtte og kunnskap til anvendelse i nasjonal beredskap, forvaltning, næringsliv og samfunnet for øvrig.

NIBIO er eid av Landbruks- og matdepartementet som et forvaltningsorgan med særskilte fullmakter og eget styre. Hovedkontoret er på Ås. Instituttet har flere regionale enheter.

Forsidefoto: Tatsiana Espevig

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