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Golf Course 2030

Integrated Management of Turfgrass Diseases and Pests

Study on European Golf Courses

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The overall aim of the project is to investigate cultural practices and new technologies for prevention and control of the two most important turfgrass diseases on golf course putting greens and insect pests on golf courses with minimum use of pesticides.

This project is a joint effort by researchers and greenkeepers from the Nordic countries, Germany, Portugal, UK, Finland and Russia, suppliers, Golf to investigate cultural practices, alternative products and new technologies for managing important diseases: microdochium patch and dollar spot with no or strongly reduced pesticide inputs.

Detailed project updates, reports and whitepapers can be downloaded from www.sterf.org.

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R&A

Preface

Integrated Pest Management (IPM) refers to the integration of all available techniques for the control of diseases, harmful insects and weeds, and to keeping the use of pesticides to levels that are economically justified and environmentally sustainable (FAO, 2016). In compliance with regulations 2009/128/EU and 2009/1107/EU, the five Nordic countries, the UK, the Netherlands, Germany, Portugal and Italy have all imposed strict regulations on pesticide use (STERF, 2016). In this context, a main challenge for golf courses is to secure high-quality playing conditions for current and future generations, while at the same time reducing the dependency on chemical plant protection products.

IPM has for many years been one of STERF's highest research priorities with a focus on the:

(i) Evaluation and management of turfgrass species, varieties and mixtures to create more disease resistant, stress tolerant and weed-competitive turf

(ii) Identification and understanding the biology and proliferation of harmful organisms in turf

(iii) Safer and more efficient use of pesticides (including reduced risk for surface runoff and leaching into the environment

Due to common EU directives, global warming and other factors, golf courses in other parts of Northern Europe mostly experience the same IPM challenges as in Nordic countries. This project addresses the UN's Sustainable Development Goals 12, 13 and 15 as described by The R&A and STERF in 'Golf Course Scandinavia 2030'.

The overall goal of this project was to investigate cultural practices and new technologies for prevention and control of the two most important and destructive turfgrass diseases on golf course putting greens – microdochium patch and dollar spot – and gain insight into the situation and methods for the prevention and control of insect pests on golf courses, with a minimum use of pesticides in the Nordic countries. Therefore, the objectives of the project were to:

1. **Investigate the effect of cultural approaches such as rolling UV-C radiation and alternative products against microdochium patch and dollar spot. To achieve this objective seven field trials were established in May-June 2020 and were conducted until Autumn 2021 - Spring 2022 in Denmark (1), Norway (2), the UK (2) and Germany (2);**
2. **Identify the fungal species causing dollar spot in Northern and Central Europe and investigate immonoassay for identification of *Clarireedia* spp. and *Microdochium nivale* in plant tissue and *Clarireedia* spp. in commercial seeds;**
3. **Compile a review of the management and potential innovation options of monitoring, warning and control of chafer grubs and leatherjackets on golf courses;**
4. **Provide technology transfer to the golf course industry and disseminate the results from the project through popular and scientific publications and video. To participate in international seminars and meetings, which will provide exchange of knowledge and experience among scientists, superintendents, industry, turfgrass agronomists and consultants.**

This project was a joint collaboration by researchers and greenkeepers from the Nordic countries, Germany, Portugal, the UK, Finland and Russia, suppliers (ICL, Suståne, Aqua-Yield and Syngenta), Golf Federations in Germany and Netherlands and The Danish Environmental Protection Agency.

Tatsiana Espevig
Landvik, 11 January 2024


Photo: Karin Normann


Photo: Tatsiana Espevig


Photo: S. Olsen

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Alternative methods to prevent and control microdochium patch on annual bluegrass predominant putting greens

Field trial at Landvik

Photo: Ove Hetland

1.1 Introduction

Microdochium patch caused by the fungus *Microdochium nivale*, is the economically most impactful disease on turfgrass in the Nordic countries. The use of fungicides against fungal diseases in Europe is strongly restricted. The objective of this study was to find out whether prevention of microdochium patch on greens with high disease pressure can be successfully achieved by integrated turfgrass management without the use of fungicides.

1.2 Materials and methods

A field experiment comparing different non-fungicide vs fungicide strategies to prevent microdochium patch was conducted on annual bluegrass predominated golf greens at the Turfgrass Research Centre Landvik, Grimstad, SE Norway (58°34'N, 8°52'E, 10 m a.s.l.) from May 2020 until May 2022. The two USGA putting greens POA NORTH – with a rootzone mixture of 88% sand and 12% sphagnum peat – and POA NIBLICK – with a rootzone mixture of 90% sand and 10% sphagnum peat (volume percentages) – were used in the study. The experiment included 15 treatments in 2020-21 and 16 treatments in 2021-22 which are presented in Table 1-1.

Treatment	Nitrogen % of control	Short description
1[1]	100[2]	Untreated (negative) control (no specific IPM programme applied)
2[1]	100	Standard fungicide programme[2]
3[1]	100	Standard fungicide programme at 60% of recommended dose
4[1]	100	Standard fungicide programme at 60% recommended dose + adjuvant (NanoPro)
5	100	Fortnightly nitrogen applications with the same products as in TRT1
6[1]	100	Citric acid 1kg ha^{-1} in 1000 L water weekly in Jun-Nov Solution pH matched to T8
2[1]	100	Citric acid 1kg ha^{-1} in 1000 L water weekly in Aug-Nov Solution pH matched to T8
8[1]	100	Iron sulphate 2kg/ha weekly in Aug-Nov
9[1]	100	Iron sulphate 4kg/ha weekly in Aug-Nov
10[1]	100 + 3	Standard nitrogen programme + late-autumn nutrition (LAN) supplying NK only
11	77	Reduced nitrogen rate (72% dose of T1)
12	92	Organic slow release nutrition, Sustâne 5-2-4+Fe
13	92	Mineral nutrition as in TRT1
14[1]	100	Rolling[3] 2 times per week in Sept-Oct
15	77	Rolling[3] 2 times per week in Sept-Oct
16	100	ICL program (in 2021-22 only)[4]

Table 1-1. Treatments investigated in this trial.

[1] Standard nutrient programme with weekly spoon-feeding applications from April to the end of October using either Wallco liquid fertilizer 5-1-4 (Orkla Care, Solna, Sweden), Greenmaster Cold Start 11-5-5 (ICL Speciality Fertilizers, Ipswich, UK) or Greenmaster Zero 14-0-10 (ICL Speciality Fertilizers, Ipswich, UK). N (nitrogen) rate of 292 and 313kg ha^{-1} yr^{-1} in 2020 and 2021, respectively. The higher N rate in 2021 then in 2020 was due to high pressure of anthracnose from July 2020.

[2] Fungicides were applied at recommended dosage three times in total: Delaro® SC 325 in September and October and Medallion® TL in November each trial year.

[3] Double rolling (back and forth) from September 28 to October 29 in 2020, and again from August 23 to October 26 in 2021 using a 378kg golf course putting green roller (Smithco Tournament T4158) consisting of two 91cm wide smoothing rollers 20cm in diameter and roller operator weight of 80kg.

[4] The following products were applied four times in September and October: Greenmaster ProLite Invigorator plus with H2Pro FlowSmart. Greenmaster Liquid was applied two times in September.

The greens were mowed from April to the beginning of November three times per week to 5mm and topdressed with 0.25mm sand weekly from May to October 2020 and 2021. The trial was fertilised always one day before mowing. After each fertiliser application, the greens were irrigated with 5mm water using potable water with a pH of 7.1-7.3. In low precipitation periods, when soil moisture fell below 12% at 0-12 cm depth as measured using TDR, the greens were irrigated to field capacity. Wetting agent H_2O Pro was applied on April 21 in 2020 and in 2021 at a rate of 20g m^{-1}. Additionally, slicing was done four-weekly from May to September using an Allett Aztec (Allett Mowers UK) both growing seasons. To simulate local golf conditions, a self-constructed friction wear drum with golf spikes was used with three passes per week over a period of 20 weeks between May and October in 2020 and 2021, simulating 20,000 rounds of golf per season.

The following parameters were registered monthly: percentage of microdochium patch (from August to May in 2020-21 and 2021-22) and anthracnose (from July to October in 2020 and 2021), visual turfgrass quality (from May to May in 2020-21 and 2021-22): general impression, density, and colour (scales from 1 to 9). Soil pH was measured using ISO 10390:2005 method before the start of the treatments in May 2020 and again in the end of May 2022 in TRT1 (negative control, 100% N), TRT6 (citric acid in June-October), TRT9 (4kg ha^{-1} iron sulphate in August-October) and TRT11 (77% N).

Photo 1-1. Fertilisation – 14-10-2021.

Photo: Karin J. Hesselsøe

1.3 Results and discussion

Microdochium patch first appeared in the middle of September both in 2020 and 2021. The highest percentage of the disease was observed in March both years. The duration of snow cover was 35 days in winter 2020-21 (two periods in January and February) and 60 days in winter 2021-22 (four periods from November to April). On negative control plots microdochium patch amounted to 39% and to 48% in March 2021 and 2022, respectively (Table 1.2 and 1.3).

The only treatments which reduced microdochium patch significantly compared with negative control, were the three fungicide treatments with no significant difference between the recommended and the 40%-reduced fungicide dosage. In an average of three fungicide treatments, the reduction of the disease was 74% and 80% in March 2021 and 2022, respectively. While using NanoPro with 60%-fungicide dosage resulted in a microdochium patch reduction of 74% and 83% compared with negative control plots, the area under disease pressure curve (AUDPC) did not differ from the negative control plots significantly. Non-significant but consistent reduction in microdochium patch both years was observed in the following five treatments: bi-weekly nutrition (TRT5), citric acid in August-October (TRT7), 23%-reduced N (TRT11), organic slow release Suståne 5-2-4+Fe (TRT12), and rolling in combination with 23%-reduced N rate (TRT15). Late autumn nutrition (TRT10) increased microdochium patch by 39% and 12% compared with negative control in March 2021 and 2022, respectively. However, this increase was not statistically significant.

Treatment		Microdochium patch				Anthracnose		Overall impression[1]		Density[1]		Colour[1]	
		% March-21		AUDPC[2]				Scale 1-9					
2	F100	7	g	871	e	333	cd	6.9	a	7.3	a	6.8	abc
4	F60+NanoPro	10	fg	1977	cde	422	cd	6.3	abc	7.1	abc	6.5	bcde
3	F60	13	efg	1475	de	305	cd	6.7	ab	7.3	a	6.7	abcd
15	N77+Rolling	22	defg	1957	cde	849	abc	5.6	def	6.6	cde	6.0	efg
7	N100+CitricAugOct	23	cdefg	2477	bcde	540	bcd	6.1	bcde	7.0	abcd	6.5	bcde
11	N77wkly	30	bcdef	3106	bcd	1079	ab	5.3	f	6.5	de	5.9	g
12	N92OrganicSuståneFe	31	bcde	3099	bcd	1289	a	5.4	ef	6.4	e	6.0	fg
5	N100biwkly	34	abcd	4133	ab	677	bcd	5.4	ef	6.7	cde	6.1	efg
1	N100wkly	39	abcd	3778	abc	370	cd	5.7	cdef	6.9	abcd	6.4	cdefg
9	N100+4kgFeSO4	39	abcd	3238	bcd	236	d	6.3	abcd	7.2	ab	7.2	a
3	N100+CitricJunOct	41	abcd	4183	ab	439	cd	5.6	def	6.9	abcde	6.3	cdefg
13	N92Mineral	43	abc	3957	ab	630	bcd	5.6	cdef	6.8	bcde	6.2	defg
14	N100+Rolling	44	ab	3733	abc	408	cd	5.6	def	6.9	abcde	6.5	cdefg
8	N100+2kgFeSO4	46	ab	4291	ab	347	cd	5.7	cdef	7.0	abc	6.9	ab
10	N100wkly+N3LAN	54	a	5209	a	419	cd	5.4	ef	6.7	cde	6.4	cdefg
	p-value	0.000		0.001		0.025		<.0001		0.0041		<.0001	

Table 1-2. Effects of the 15 treatments on microdochium patch, anthracnose and overall impression on the experimental greens during 2020-21. Treatments sorted according to increasing microdochium patch.

Anthracnose (caused by *Colletotrichum graminicola*) was not a focus of this study. However, the disease, with outbreaks in early July in both years, reduced visual quality of the greens significantly, especially on POA NIBLICK (data not shown). From July to October 2020, the highest percentage of anthracnose (14-25%) was observed in July on the plots with biweekly nutrition (TRT5) and on all plots which received one of four treatments with N rate lower than control: N77 (TRT11 and TRT15) and N92 (TRT12 and TRT13) (data not shown). All these five treatments also resulted in the highest AUDPC for anthracnose above 600 (Table 1-2). Since the total annual N amount was increased by 20% in 2021, the anthracnose disease did not exceed 11% and amounted to 6% in the average for all treatments in August 2021 when anthracnose pressure was highest (data not shown). There were no significant differences in AUDPC among the treatments in 2021 (Table 1-3).

[1] Average from May 2020 to May 2021.

[2] AUDPC – area under disease pressure curve from September to March for microdochium patch and from July to October for anthracnose.

An average overall impression from May 2020 to May 2021 was improved by 1.2 scores on the positive control plots (TRT2) and by 1.0 score on the plots with 40%-reduced fungicide dosage compared with negative control plots (Table 1-2). For the period from June 2021 to May 2022, an average overall impression was 1.8, 1.3, 1.3 0.9, 0.9 and 0.7 scores better on the plots which received TRT 2, 4, 3, 12 and 7, respectively, compared with negative control (Table 1-3). The lower overall impression on the Suståne 5-2-4+Fe plots (TRT12) then on negative control plots in 2020 was most likely due to insufficient N in the beginning of the season. The annual N amount on Suståne plots was evenly distributed throughout the season, making these plots behind the control plots, especially from May to August-September 2020. This also resulted in high anthracnose pressure on Suståne plots in 2020. To avoid this in 2021, the annual N amount on Suståne plots which was 2.9kg per 100m² per year, was distributed according to turfgrass needs during the growing season called precisio fertilisation (Ericsson et al., 2013) as in TRT1 and other treatments. In 2021, anthracnose on Suståne plots was on the same and even lower levels than on plots which received fungicides in the previous autumn, in spite of the fact that Suståne plots received 7% lower annual N compared with negative control in 2020 and in 2021.

Both dosages of iron sulphate of 2 and 4kg ha^{-1} yr^{-1} (TRT 8 and 9) improved turfgrass colour (mean for 2020-21) with 0.8 and 0.5 scores as compared with negative control (Table 1-2). In 2021-22, another iron containing treatment – ICL programme – gave 0.6 scores better colour compared with negative control (Table 1-3). None of the iron containing treatments reduced turfgrass density. Otherwise, the differences in density among the treatments were not consistent in the first and second experimental years.

Treatment		Microdochium patch				Anthracnose		Overall impression[1]		Density[1]		Colour[1]	
		% March-22		AUDPC2[2]				Scale 1-9					
2	F100	7	c	552	d	197	bc	7.0	a	7.2	a	7.0	ab
4	F60+NanoPro	8	c	1228	bcd	380	abc	6.5	ab	7.1	ab	6.7	abcd
3	F60	13	bc	956	cd	350	abc	6.5	ab	7.0	abc	6.8	abcd
11	N77wkly	27	abc	2316	abcd	284	abc	5.6	cde	6.9	abcd	6.4	de
5	N100biwkly	28	abc	2763	abcd	103	c	5.6	cde	6.8	abcd	6.5	cde
15	N77+Rolling	28	abc	3546	abc	554	a	5.0	e	6.6	cd	6.0	e
14	N100+Rolling	31	abc	3385	abc	398	abc	5.3	ed	6.6	cd	6.4	de
12	N92OrganicSuståneFe	32	abc	3056	abcd	210	bc	6.1	cb	7.0	abc	6.6	abcd
6	N100+CitricJunOct	35	abc	3701	abc	204	bc	5.7	cde	7.1	ab	6.5	cde
9	N100+4kgFeSO4	42	ab	3242	abcd	96	c	6.1	bc	7.3	a	7.0	a
8	N100+2kgFeSO4	43	ab	4050	a	164	bc	5.6	cde	7.2	a	6.9	abc
7	N100+CitricAugOct	44	a	3806	ab	138	bc	5.9	bcd	7.0	abcd	6.7	abcd
1	N100wkly	48	a	3928	ab	433	ab	5.2	e	6.5	d	6.4	de
10	N100wkly+N3LAN	53	a	4995	a	423	ab	5.0	e	6.7	bcd	6.4	de
16	ICL	54	a	4043	a	171	bc	5.7	cde	7.2	a	7.0	a
13	N92Mineral	55	a	5017	a	141	bc	5.5	cde	7.1	ab	6.7	abcd
	p-value	0.027		0.056		0.12		<.0001		0.008		0.001	

Table 1-3. Effects of the 16 treatments on microdochium patch, anthracnose and overall impression on the experimental greens in 2021-22. Treatments sorted according to increasing microdochium patch.

[1] Average from June 2021 to May 2022.

[2] AUDPC – area under disease pressure curve from September to March for microdochium patch and from July to October for anthracnose.

On the POA NORTH green, the soil pH decreased from initial pH 6.0 to pH 5.7 on the plots which received 4kg ha^{-1} (TRT9) and to pH 5.8 on the negative control plots (TRT 1), which received either citric acid from June-October (TRT6) or reduced N rate (TRT11). On the POA NIBLICK green, soil pH remained almost the same in the end of the trial (pH 6.4 in May 2020 and pH in May 2022) with no difference among the four treatments.

Photo 1-2. Fertilisation – 13-08-2020. The yellowing on the POA NIBLICK was due to outbreak of anthracnose in July.

Photo: Tatsiana Espevig

1.4 Conclusions on benefits and advice for the golf and turfgrass sector

- **Fungicides were the most effective method for control of microdochium patch disease and provided microdochium patch reduction of 66-85%.**
- **The most effective alternative methods in prevention of microdochium patch were 23%-reduced N rate alone or in combination with rolling, biweekly nutrition and slow-release organic fertiliser from Suståne. But none of them provided a reduction in microdochium patch of more than 43%.**
- **Use of reduced nitrogen rates in prevention of microdochium patch causes high anthracnose severity.**
- **Effects of rolling, citric acid and iron sulphate in this trial were not consistent.**
- **Additional N application in the autumn increased microdochium patch in the spring.**

02

Alternative methods to prevent and control microdochium patch on annual bluegrass predominant putting greens

Field trial at Bingley

Photo: STRI group

2.1 Introduction

Microdochium patch (*Microdochium nivale*) is the most damaging and common disease of golf green turf in the UK. A significant proportion of a turf manager's time and resource is spent preventing or dealing with outbreaks of microdochium patch disease, making it an economically significant disease.

2.2 Materials and methods

A field trial was run over two microdochium patch seasons (2020/2021 and 2021/2022) at the STRI's research facility in Bingley, West Yorkshire (53° 50' 54.274" N, 1° 51' 21.205" W, 180 m a.s.l.). The area consisted of golf green turf grown on a sandy loam soil (78% sand, 11% silt and 11% clay). The area had a mixed sward comprising a blend dominated by browntop bent (*Agrostis capillaris*) and annual meadow-grass (*Poa annua*). Both grasses can become infected with microdochium patch, but annual meadow-grass is particularly susceptible. The green had a long history of suffering from high levels of microdochium patch and had significant levels of thatch.

The trail area was mown regularly, with the frequency dependent on growing conditions. This meant that during the main growing season turf was typically mown three times per week, while into autumn when growth rates slowed this was reduced to twice per week, then over winter this dropped to weekly or as needed. The area was maintained to create conditions that favoured microdochium patch such as allowing thatch to develop. Turf was typically aerated twice a year to relieve compaction with verticutting carried out as needed to clean out the sward and help remove any moss that had developed over the winter months. Nutrient applications were carefully controlled throughout the trials as nutrient inputs were part of the trial. Irrigation was applied as needed during the trial using manually placed impact sprinklers and trial plots were not subject to dew removal to promote leaf humidity and disease development.

The trial area had 12 treatments which corresponded to those at Landvik and as outlined in Table 2-1. The objective of treatments was to apply different IPM based solutions that may help reduce the incidence of microdochium patch, ranging from use of adjuvants to help reduce the dose of fungicide needed to control disease, through to applications of acidifying agents and nutrient programmes providing nitrogen in different quantities and intervals. Treatments were applied to (2 x 1m) plots with each treatment replicated four times. During assessments a 5cm gap was left around the edge of each plot.

In 2020, treatments started in June with most treatments running into November. The LAN and organic based fertiliser programmes extended throughout the winter months of 2020/2021. In 2021, treatments started in late May and ran through into November and as in the previous season, the LAN and organic fertiliser programmes ran through the winter months.

The assessments carried out during the trial included microdochium patch incidence (%), turf quality and colour (both scored on 1-9 scale) and turf density (%). Assessments were carried out monthly. Data were analysed as a one-way ANOVA randomised complete block design using Genstat with a significance level of 5%. Fishers LSD at a 5% significance level was used to identify significant differences among treatments.

Treatment	Nitrogen (kg/ha/yr)	Short description
1*	122	Untreated control (no specific IPM programme applied)
2*	122	Standard fungicide programme #
3*	122	Standard fungicide programme at 60% of recommended dose
4*	122	Standard fungicide programme at 60% recommended dose + adjuvant (NanoPro)
5	122	Fortnightly nitrogen applications
6*	122	Citric acid in Jun-Nov applied 3x a month then weekly from Aug onwards. Solution pH matched to T8
7*	122	Citric acid in Aug-Nov applied weekly. Solution pH matched to T8
8*	122	Iron sulphate at 4kg/ha weekly in Aug-Nov
9*	122	Iron sulphate applied at 8kg/ha weekly in Aug-Nov
10	122 + LAN	Standard nitrogen programme + late-autumn nutrition (LAN) supplying NK only
11	88	Reduced nitrogen rate (72% dose of T1)
12	122 + LAN	Organic slow release nutrition, Sustâne 5-2-4+Fe with applications extending into autumn and winter

Table 2-1. Treatments investigated in this trial.

2.3 Results and discussion

Data have been summarised over each season and then over each year to aid with interpretation. Disease pressure was broadly similar in both 2020/21 and 2021/22, peaking at a maximum area affected of 13%. However, 2020/2021 disease pressure peaked during January and February 2021, whereas in 2021/2022 disease pressure built earlier in autumn (Tables 2-2 & 2-3).

Over both microdochium patch disease seasons, application of fungicides effectively controlled microdochium patch and with disease pressure building slowly, even the 60% dose rate treatment was effective (Tables 2-2 & 2-3). There was no benefit of adding an adjuvant in the form of NanoPro in this trial.

There were differences in the efficacy of treatments between the two disease seasons. In the first season, the only IPM treatment that was effective at reducing disease incidence was application of $FeSO_4$ at 8 kg/ha. The downside of this level of $FeSO_4$ application was the high level of turf darkening associated with the application of the iron. However, in the second year, both $FeSO_4$ treatments reduced disease incidence in autumn. It was interesting to note that the citric acid at similar solution pH did not have the same effect in this trial. It was also evident that treatments that supplied nitrogen in slower release forms (organic based Sustâne) and infrequent fertiliser application all had greater disease incidence compared to the untreated control. Additionally, in winter the reduced nitrogen treatment also had greater disease incidence. This clearly shows that if nitrogen inputs are too low, the consequence is greater susceptibility to microdochium patch, likely as a result of poorer turf health and vigour during growth periods in autumn and winter.

* Standard nutrient programme comprised alternating NPK and NK liquid fertilisers with weekly spoon-feeding applications.

Fungicides were applied in September and November with the first application consisting of a trifloxystrobin based product and the second application a fludioxonil based fungicide.

Treatment	Summer 20	Autumn 20		Winter 20-21		Overall average	
1 Untreated control	1.2	3.0	abcd	7.8	bc	4.8	e
2 Fungicides at 100% dose	1.7	1.8	abc	0.8	a	1.3	abc
3 Fungicides at 60% dose	0.7	0.8	ab	0.6	a	0.7	a
4 Fungicides at 60% dose + NanoPro	0.8	1.7	abc	0.8	a	1.1	ab
5 Fortnightly nitrogen application	1.8	3.8	cd	5.1	abc	3.9	cde
6 Citric acid in Jun-Nov	2.3	3.5	bcd	4.8	abc	3.8	bcde
7 Citric acid in Aug-Nov	2.2	3.1	abcd	8.8	c	5.5	e
8 $FeSO_4$ at 4kg/ha in Aug-Nov	2.2	1.7	abc	4.9	abc	3.3	abcde
9 $FeSO_4$ at 8kg/ha in Aug-Nov	1.5	0.4	a	3.0	ab	1.8	abcd
10 Late Autumn nutrition (LAN)	2.7	5.2	de	7.7	bc	5.8	e
11 Reduced nitrogen input	1.7	3.7	cd	4.0	abc	3.4	abcde
12 Sustâne 5-2-4+Fe + LAN	1.7	7.1	e	3.6	ab	4.2	de
P	NS	<0.001		0.014		0.007	
LSD	–	2.77		4.93		2.88	
d.f.	33	33		33		33	
%c.v.	60.4	64.7		79.3		60.7	

Table 2-2. Microdochium patch incidence (% area affected) in key seasons and throughout 2020 and the early winter months of 2021.

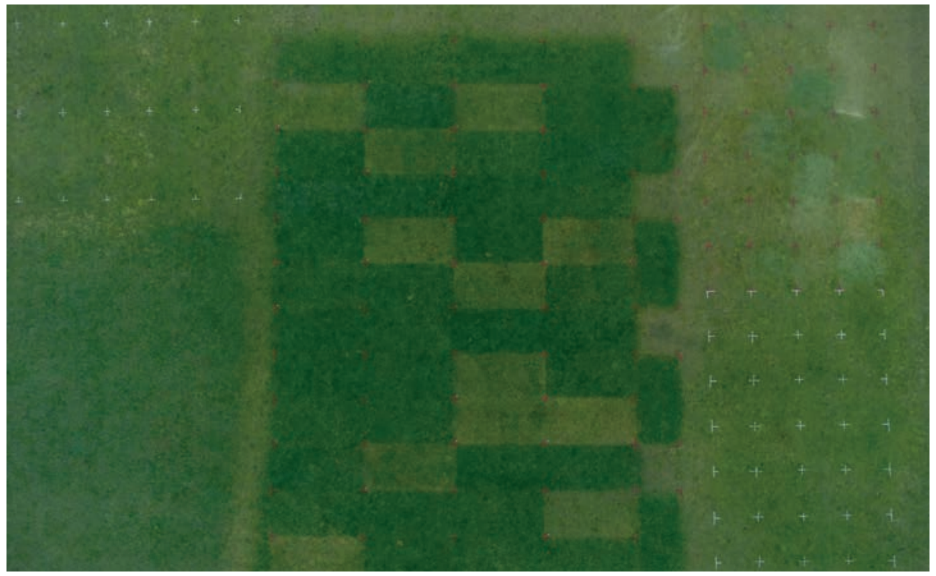


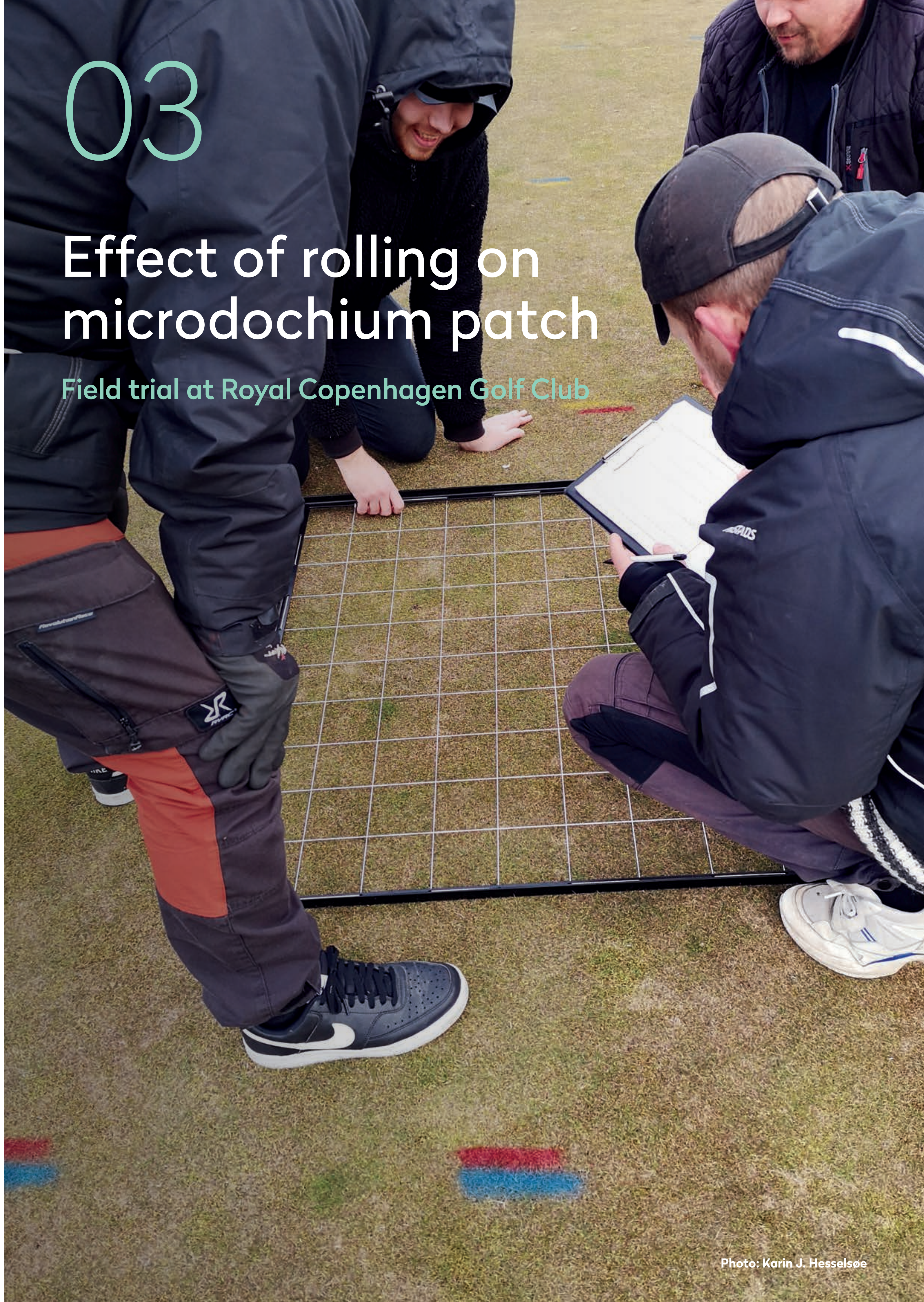
Photo 2-1. Trial plots – 06-01-2022. Plant health and colour responses can be clearly seen.

Treatment	Summer 21		Autumn 21		Winter 21-22		Overall average	
1 Untreated control	2.0	abc	3.4	cd	3.5	ab	2.3	cd
2 Fungicides at 100% dose	1.5	ab	0.6	a	0.9	a	0.8	ab
3 Fungicides at 60% dose	1.2	a	0.5	a	0.8	a	0.6	a
4 Fungicides at 60% dose + NanoPro	1.2	a	1.1	ab	2.5	ab	1.2	abc
5 Fortnightly nitrogen application	2.3	bc	6.9	ef	6.6	cd	4.0	e
6 Citric acid in Jun-Nov	1.9	abc	5.0	de	2.4	ab	2.4	d
7 Citric acid in Aug-Nov	1.8	abc	3.1	bcd	3.4	ab	2.1	cd
8 $FeSO_4$ at4 kg/ha in Aug-Nov	1.6	abc	0.9	ab	3.4	ab	1.4	abcd
9 $FeSO_4$ at8 kg/ha in Aug-Nov	1.3	a	1.0	ab	2.6	ab	1.2	abc
10 Late autumn nutrition (LAN)	1.2	a	2.5	abc	3.4	ab	1.8	bcd
11 Reduced nitrogen input	2.2	bc	4.9	de	8.7	d	3.9	e
12 Suståne 5-2-4+Fe + LAN	2.4	c	7.2	f	4.7	bc	3.7	e
P	0.029		0.001		0.001		0.001	
LSD	0.85		2.16		3.03		1.09	
d.f.	33		33		33		33	
%c.v.	35.1		49.0		59.0		35.9	

Table 2-3. Microdochium patch incidence (% area affected) in key seasons and throughout 2021 and the early winter months of 2022.

2.4 Conclusions on benefits and advice for the golf and turfgrass sector

- **Fungicides were effective tools at controlling microdochium patch disease.**
- **At low disease pressure (less than 13%), lower dose rates of fungicide were effective but there is increased risk that as pressure increases this will not be effective, so extreme caution is needed when reducing dose rate. This is especially important as dose rate is determined as the minimum dose required to provide effective control and not 100% control.**
- **In this trial, where disease pressure was low, use of an adjuvant did not provide additional benefit.**
- **Iron sulphate was effective at reducing disease incidence when used preventatively and during early disease pressure. The dose of iron sulphate that is effective depends on how the disease develops during the disease season.**
- **Citric acid application in this trial did not reduce disease incidence, which indicates that the effect of iron sulphate is perhaps not due to the pH of the spray solution.**
- **Not achieving adequate turf nutrition during the lead up to and into early disease development, either due to nitrogen being in a very slow-release form or too little is applied, is detrimental to reducing disease incidence. Too little turf nutrition will result in less healthy and resilient turf that may be more susceptible to pathogen attack.**
- **The optimal IPM approach is unlikely to rely on one approach. To achieve maximum benefit multiple solutions are likely to be needed. Additionally, as disease pressure develops differently in each year, a prescriptive approach is unlikely to work and a dynamic approach that takes into account plant health and disease pressure will be key.**



03

Effect of rolling on microdochium patch

Field trial at Royal Copenhagen Golf Club

Photo: Karin J. Hesselsøe

3.1 Introduction

Microdochium patch, caused by the fungus *Microdochium nivale*, is the economically most impactful disease on turfgrass in the Nordic countries (Kvalbein et al., 2017; Melbye, 2019). The use of fungicides in Europe is strongly restricted, resulting in a need for alternatives to prevent this fungal disease.

Full NIBIO-report available at: https://hdl.handle.net/11250/3012601

Video on Rolling against microdochium patch at: https://www.youtube.com/watch?v=_nNZn-8Qx6A

3.2 Materials and methods

Field experiments on a red fescue (*Festuca rubra*) dominated golf green at Royal Copenhagen Golf Club in 2020 and 2021, examined the effects of light weight rolling on development of microdochium patch caused by *Microdocium nivale*. The green was rolled two or four times per week for either three or five months ending in November. Two different rollers were used in 2020 and 2021 (Photo 3-1 and 3-2). Assessments of turfgrass quality, coverage of microdochium patch, green colour and soil moisture were done monthly from June to December each year. The percentage of annual bluegrass (*Poa annua)* coverage was evaluated at the end of the trial in March 2022.

3.3 Results and discussion

Results from 2020 (Table 3-1) showed that rolling two times and four times per week caused significantly lower coverage of microdochium patch in December compared to no rolling. These differences were not confirmed the year after as there was very low disease pressure in 2021 on the experimental green.

Regarding turfgrass visual quality, in 2020 there was no difference in the beginning of the trial in June, but from August rolling improved turfgrass visual quality significantly compared to no rolling through the autumn until December. The higher score was mainly due to less moss and lower coverage of microdochium patch.

Photo 3-1 (left): Rolling with Smithco Tournament Ultra 7590 in 2020.

Photo: Karin J. Hesselsøe

Photo 3-2 (right): Rolling with Smithco Tournament Ultra XXL in 2021.

Photo: Martin Nilsson

2020	Turfgrass visual quality Scale 1-9 June	Aug-Oct	Nov-Dec	Microdochium patch % December
Rolling two times per wk	5.0	7.0 a	6.5 a	2.0 a
Rolling four times per wk	5.0	7.0 a	6.0 a	2.3 a
No rolling	5.0	5.0 b	4.0 b	5.0 b
P-value	ns	0.004	0.004	0.003

Table 3-1: Turfgrass visual quality and coverage of microdochium patch as affected by rolling treatments.

No differences in content of soil water were found among the treatments in neither 2020 nor 2021. At the end of the trial in March 2022, no significant differences in the coverage of annual bluegrass were found among the treatments, which suggests that rolling two or four times per week did not increase annual bluegrass percentage.

3.4 Conclusions on benefits and advice for the golf and turfgrass sector

- **Espevig et al., (2020) recommended rolling 3-4 times per week on Scandinavian golf greens that are under dollar spot pressure.**
- **This experiment supports this recommendation and suggests that rolling two times per week from August to November is sufficient to reduce microdochium patch on Scandinavian red fescue dominated golf greens.**

Photo 3-3: The trial area at Royal Copenhagen Golf Club in autumn 2020. From left – Yellow: No rolling; Blue: Rolled four times per week; Red: Rolled two times per week. More moss was found in plots with no rolling.

Photo: Martin Nilsson

Espevig, T., K. Normann, N. Bosholdt, M. Usoltseva, S. Nilsson, A. Olofsson, J.A. Crouch, K. Entwistle, K. Sundsdal, T.O. Pettersen, T.S. Aamlid and T. Torp (2020): Risk assessment, management and control of dollar spot caused by Clarireedia species on Scandinavian golf courses (2017-2020). STERF Final Report. *http://www.sterf.org/Media/Get/3604/final-report-dollar-spot_final-report_2020*

Kvalbein A., W.M. Marie Waalen, L. Bjørnstad, T.S. Aamlid and T. Espevig. 2017. Winter injuries on golf greens in the Nordic countries: Survey of causes and economic consequences. Int. Turfgrass Soc. Res. J. 13:604-609.

Melbye P. 2019. STERF survey on prioritising research areas, 2019. *https://www.survey-xact.dk/report/shared/e8b4947e-fed6-42b8-9705-7b2946efae7d*

04

Effects of UVC radiation on dollar spot and microdochium patch on golf greens

Field trial at Osnabrück Golf Club

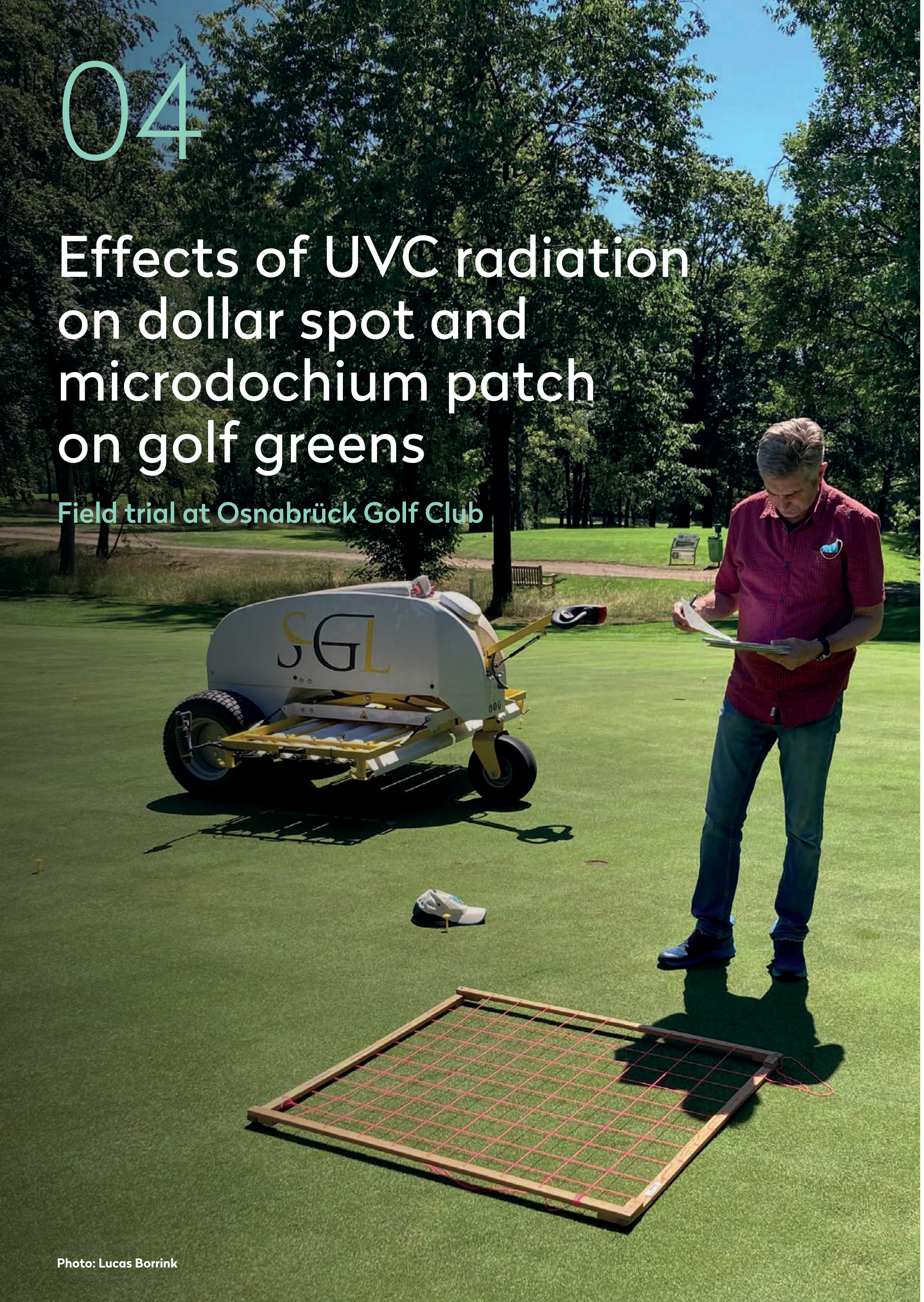


Photo: Lucas Borrink

4.1 Introduction

UV-C is part of ultraviolet radiation (UV) with the shortest wavelength band from 100-280nm (Blatchley III et al., 2023) and highest energy. UV-C radiation does not reach Earth's surface but possesses unique germicidal properties by using UV-C lamps for short time radiation with 254nm (Urban et al., 2016). When plants are exposed to UV-C radiation, it triggers both alteration in gene-expression due to signalling of photoreceptors and occurrence of reactive oxygen species (ROS) inside the cell and its organelles leading to several stress responses (Vanhaelewyn et al., 2016) and otherwise can kill *fungi hyphae* on the surface of plant foliage (Berkelmann-Löhnertz et al., 2015). The aim of the study was to investigate the effectiveness of UV-C radiation on a golf green to suppress or prevent dollar spot and microdochium patch.

Full report available at: http://www.sterf.org/Media/Get/4166/effect-of-uv-c-radiation-and-sustane-on-dollar-spot-and-microdochium-patch-field-trials-v2

Video on UV-C radiation and slow-release fertilisation available at: https://www.youtube.comwatch?v=J3COMqHEAhg

4.2 Materials and methods

The experiments were carried out on a golf green with creeping bentgrass (*Agrostis stolonifera*) and annual bluegrass (*Poa annua*) on the Osnabrück Golf Club, Bissendorf-Jeggen, Germany. The maintenance of the test area was carried out by the Club's greenkeeper team. The cutting height of the test area was 4mm through the vegetation period. In this study, the trials were carried out using the UV-C unit SGL UVC 180 (SGL System) to compare three different dosages of UV-C radiation and an untreated control (Figure 4-1). The dosages were applied by using different operating speeds of the device as a percentage of maximum speed and were specified, set and controlled by the measuring device UVpro Radiometer (Orca GmbH) (Figure 4-2).

The trial was carried out with four treatments in a randomised complete block design with three replications. Trial plots had a size of 6m² (3 x 2m) and the corners of plots were marked with spray and maintained throughout the experiment. The four treatments consisted of TRT 1 – control (untreated), TRT 2 UV-C – dosage 1 with 7-8 mJ/cm² (50% operating speed UV-C unit), TRT 3 UV-C – dosage 2 with 35-40 mJ/cm² (10% operating speed UV-C unit), TRT 4 UV-C – dosage 3 with 70-80 mJ/cm² (10% operating speed UV-C unit, irradiated twice) and were applied three times per week. The UV-C radiation was applied between May 2020 and May 2022 each year from spring to autumn and further on during late autumn and winter, depending on weather conditions in relation to occurrence of microdochium patch. Figure 4-1 shows the SGL UVC 180 unit in the field on the trial plots.

The dollar spot and respective microdochium patch incidence was registered monthly from the beginning of summer to autumn and respectively through winter to spring by visual assessment of the percentage (%) of plot area covered with disease using a frame of one square meter with a graduation of hundred patterns 100 x 100mm at two areas inside the plots. Further monthly assessments of visual turf quality and turfgrass density were taken with a scale 1-9 (1 is very poor/low and 9 is very good/high) and turfgrass colour was evaluated by measuring the NDVI (Natural Difference Vegetation Index) by using the Greenseeker Handheld Crop Sensor (Trimble Inc.).

For statistical analysis the Kruskal-Wallis analysis was used for all parameters to test for significance. The Wilcoxon-Mann-Whitney test was used to test significance in pairs. Differences were marked at a 0.05 probability level. In addition, the area under disease progress curve (AUDPC) was calculated according to Madden et al., (2007) as a quantitative summary of disease severity over time for all treatments and separately as mean values.

Figure 4-1 (left): Application of UV-C radiation with SGL UVC 180 unit on the putting green of Osnabrück Golf Club.

Photo: W. Prämaßing

Figure 4-2 (right): UVpro Radiometer to specify and control UV-C radiation dosages on the turf canopy.

4.3 Results and discussion

The results for turf quality, density and colour are summarised over the whole trial period through summer and winter. The highest ratings for turf quality of all four variants were achieved from July to October in both years. The UV-C dosages 2 and 3 were reached in summer 2021, with rating values from 7 to 8, while control and UV-C dosage 1 showed values between 6 and 7. In winter 2020-2021, lowest values were observed for control and UV-C dosage 1 with values around 2 and values of up to 3 for UV-C dosages 2 and 3. Figure 4-3 shows median turf quality in boxplots of each UV-C treatment. The median turf quality for the variants UV-C dosage 2 and UV-C dosage 3 are higher than for the untreated control and UV-C dosage 1. There were significant ($p<0.05$) differences for the median estimates between the untreated control and UV-C dosages 2 and 3.

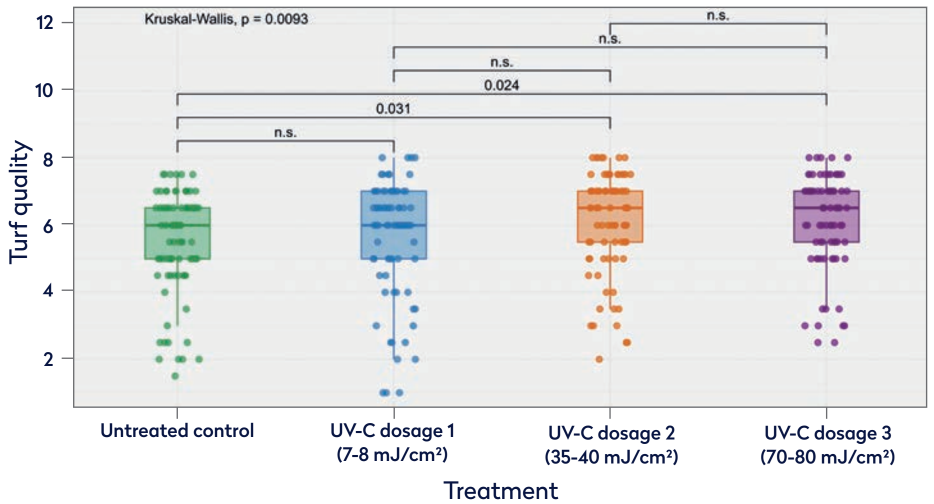


Figure 4-3: Turf quality of the UV-C trial from spring 2020 to spring 2022. There were significant differences between the untreated control and UV-C 2 and 3 using pairwise Wilcoxon-Mann-Whitney rank sum test and p-value ($p<0.05$). The figure shows the adjusted p-values for the significant groups and the Kruskal-Wallis test.

There were no significant differences for turf colour and turf density between the four different UV-C treatments during the trial period. During the winter months, the NDVI decreased down to measurements between 0.5 and 0.6, and the density was estimated to be lower. From spring onwards, the NDVI increased until it peaked in August 2021 with values between 0.8 and 0.85 for all treatments. Density reached its highest value with the grade 8 on UV-C dosages 2 and 3 in September 2021. (Results not shown).

Microdochium patch

Figure 4-4 shows boxplots containing all observed values for the sampling days where microdochium patch was observed in winter 2020-2021 and winter 2021-2022. The untreated control had the highest median percentage of microdochium patch with 2.75%. The UV-C dosages 1 and 2 had the same median microdochium patch coverage with 2%. The UV-C dosage 3 had the lowest microdochium patch infestation with 1%.

The Kruskal-Wallis global test was significant, but after adjusting the p-value according to Bonferroni, no significant differences in median values were found, possibly due to high variability differences in disease severity for the treatments (Figure 4-5). Higher dosages of UV-C radiation led to a reduced variability in disease coverage.

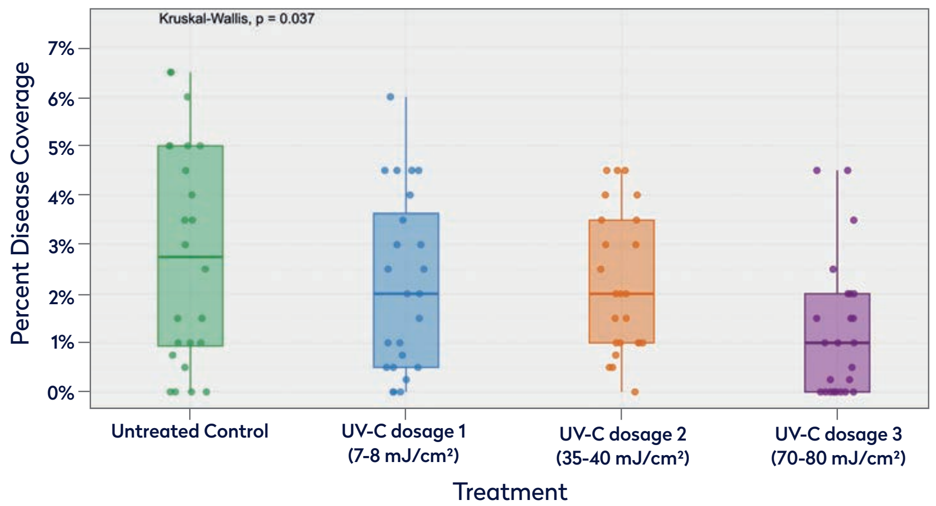


Figure 4-4: Median values for disease coverage in % for all observed values of microdochium patch occurrence in the UV-C trial. There were no significant differences of median values after adjustment according to Bonferroni using pairwise Wilcoxon-Mann-Whitney test ($p<0.05$).

Table 4-1 shows the AUDPC mean values for microdochium patch infestation (% of plot area). The variant UV-C dosage 3 had lower AUDPC values of 224 rsp. 15.67 in both winter periods compared to control and the two other UV-C treatments. It seems evident that an increased radiation with UV-C led to a less severe disease outbreak in this trial, which corresponds with the peak percentage of disease outbreak.

Treatments	AUDPC winter 2020-2021	Percentage peak 25 Feb 2021	AUDPC winter 2021-2022	Percentage peak 20 Oct 2021
Control	474	5.67%	88	2.17%
UV-C dosage 1	379	4.50%	65	0.40%
UV-C dosage 2	335	4.00%	105	1.83%
UV-C dosage 3	224	3.00%	16	0.50%

Table 4-1: Mean values for AUDPC and peak percentage for microdochium patch infestation (% of plot area) in winter 2020-2021 and winter 2021-2022 for different UV-C dosages.

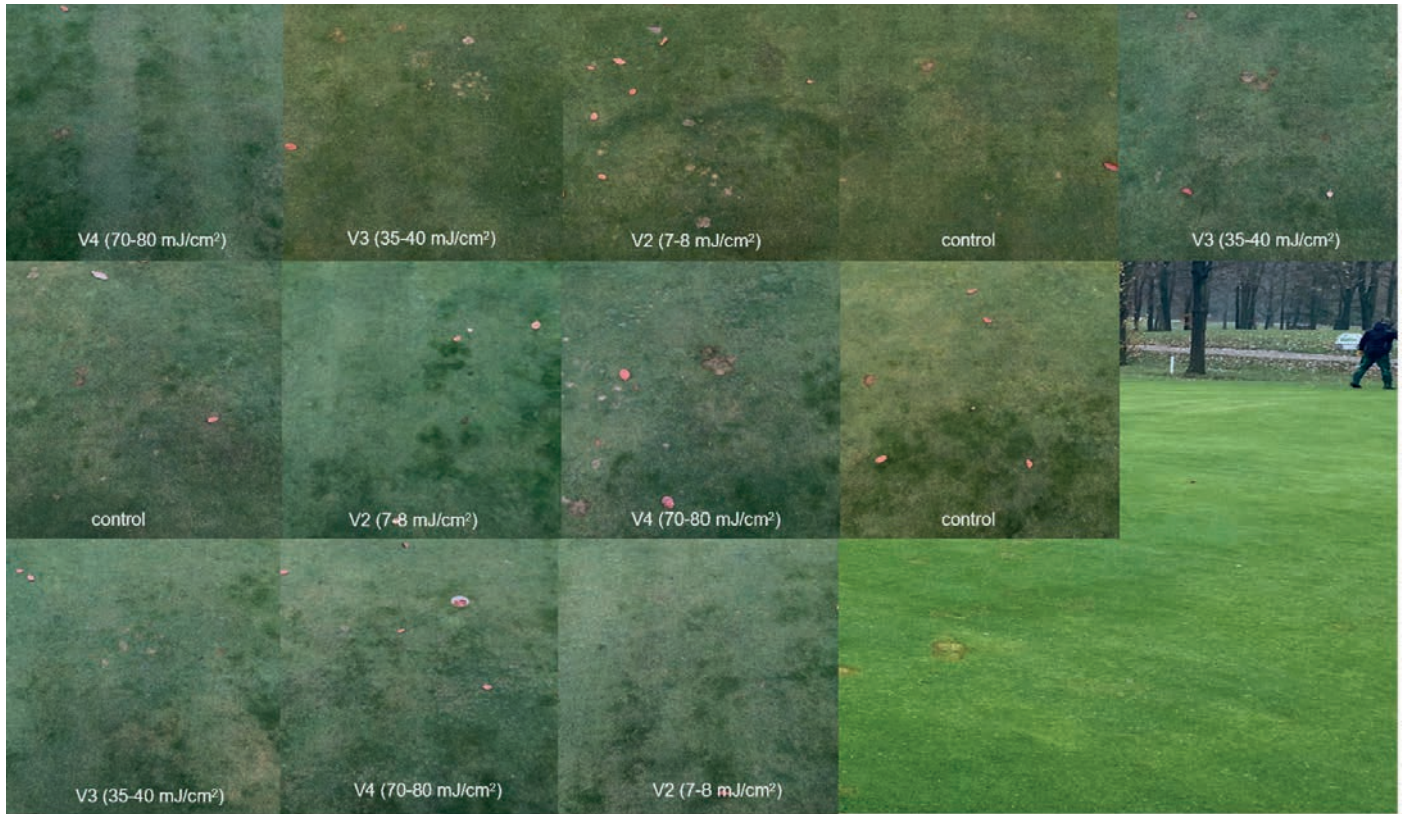


Figure 4-5: Variability of microdochium patch occurrence on trial plots of different UV-C radiation dosages on putting green of Osnabrück Golf Club. *Photo: Borrink – 17-11-2021*

Dollar spot

Figure 4-6 shows boxplots containing all observed values for the sampling days where dollar spot was observed. The untreated control had the highest median percentage of dollar spot during the trial with 2.5%, followed by the UV-C dosage 1 (2.25%) and UV-C dosage 2 (0.75%). The UV-C dosage 3 variant showed the significant lowest median dollar spot infestation (0.75%). Significant differences in the median ranks between the untreated control and UV-C dosage 2 and 3 and between UV-C dosage 1 and 3 were found. It seems evident that an increased treatment of turfgrass with UV-C radiation led to less severity in dollar spot occurrence. Furthermore, higher dosages of UV-C radiation led to a reduced variability in the severity of disease infestation.

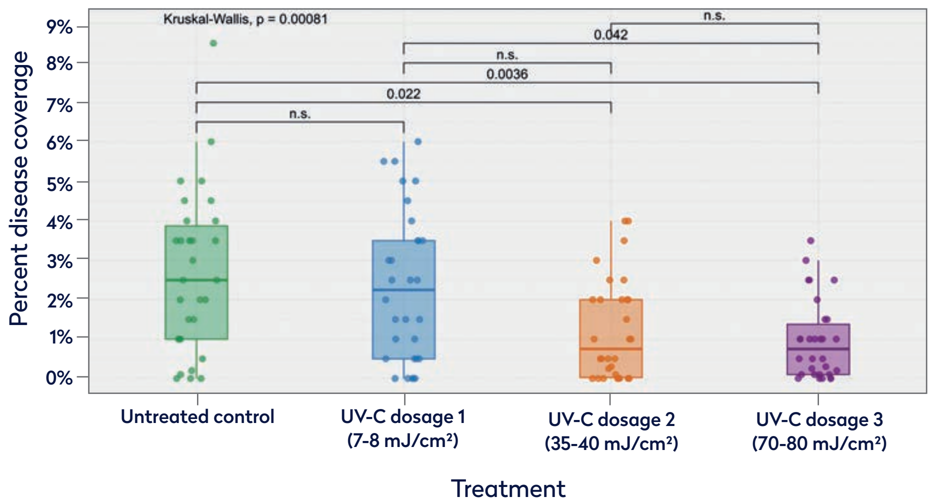


Figure 4-6 Median values for disease coverage in % for all observed values of dollar spot occurrence in the UV-C trial. There were significantly different median values after adjustment according to Bonferroni using pairwise Wilcoxon-Mann-Whitney test ($p<0.05$) for untreated control and UV-C dosage 2 and 3, as well as between UV-C dosage 1 and 3.

Table 4-2 shows the AUDPC mean values for dollar spot infestation (% of plot area). The variant UV-C dosage 3 had lower AUDPC values of 200.72 respectively 48.13 in the summer periods 2020 and 2021, compared to control and UV-C dosage 1 and 2. It seems evident that an increased treatment with UV-C radiation led to a less severe disease outbreak in this trial and this corresponds with the percentage peaks in both years. Figure 4-7 shows different dollar spot severities on a control plot compared to a UV-C dosage 3 plot with lower dollar spot infestation.

Treatments	AUDPC year 2020	Percentage peak 20 Oct 2020	AUDPC year 2021	Percentage peak 19 Aug 2021
Control	402	4.33%	262	5.33%
UV-C dosage 1	375	4.50%	132	4.00%
UV-C dosage 2	230	2.83%	83	1.83%
UV-C dosage 3	201	2.33%	48	1.00%

Table 4-2: Median values AUDPC and peak percentage for dollar spot infestation (% of plot area) in summer 2020 and summer 2021 for different UV-C dosages.

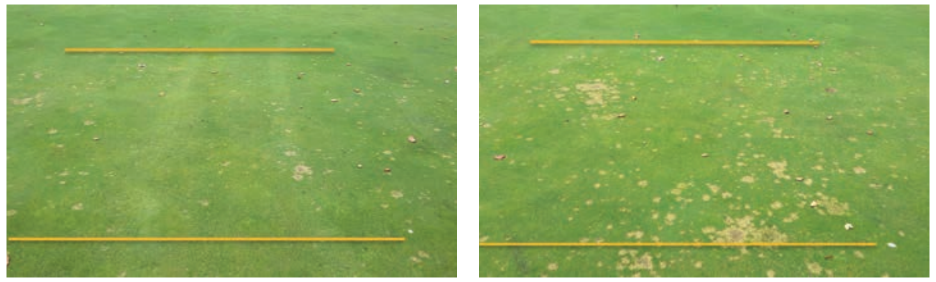


Figure 4-7: Plot receiving UV-C dosage 3 (left) with lower dollar spot incidence compared to a control plot with higher dollar spot severity.

Photo: W. Prämaßing – 03-10-2020

4.4 Conclusions on benefits and advice for the golf and turfgrass sector

- **Higher UV-C dosages 2 and 3 were able to successfully suppress dollar spot infestation in this trial showing both overall smaller median disease infestation and AUDPC values and more compact variability. In addition, it can be stated that an efficient dollar spot disease suppression can be achieved from an UV-C radiation treatment with 35-40 mJ/cm² and higher. The treatment with UV-C dosage 1 (7-8 mJ/cm²) could not efficiently suppress dollar spot occurrence compared to the untreated control in this trial.**
- **Regarding the infestation with microdochium patch, a different pattern could be observed. None of the treatments were able to suppress disease outbreak significantly. While median disease infestation and AUDPC values were lowest for UV-C dosage 3, some plots showed disease coverage values equal to the control leading to the conclusion: that a treatment with UV-C radiation could not fully suppress or control outbreak of microdochium patch in this trial but showed a trend to be helpful.**

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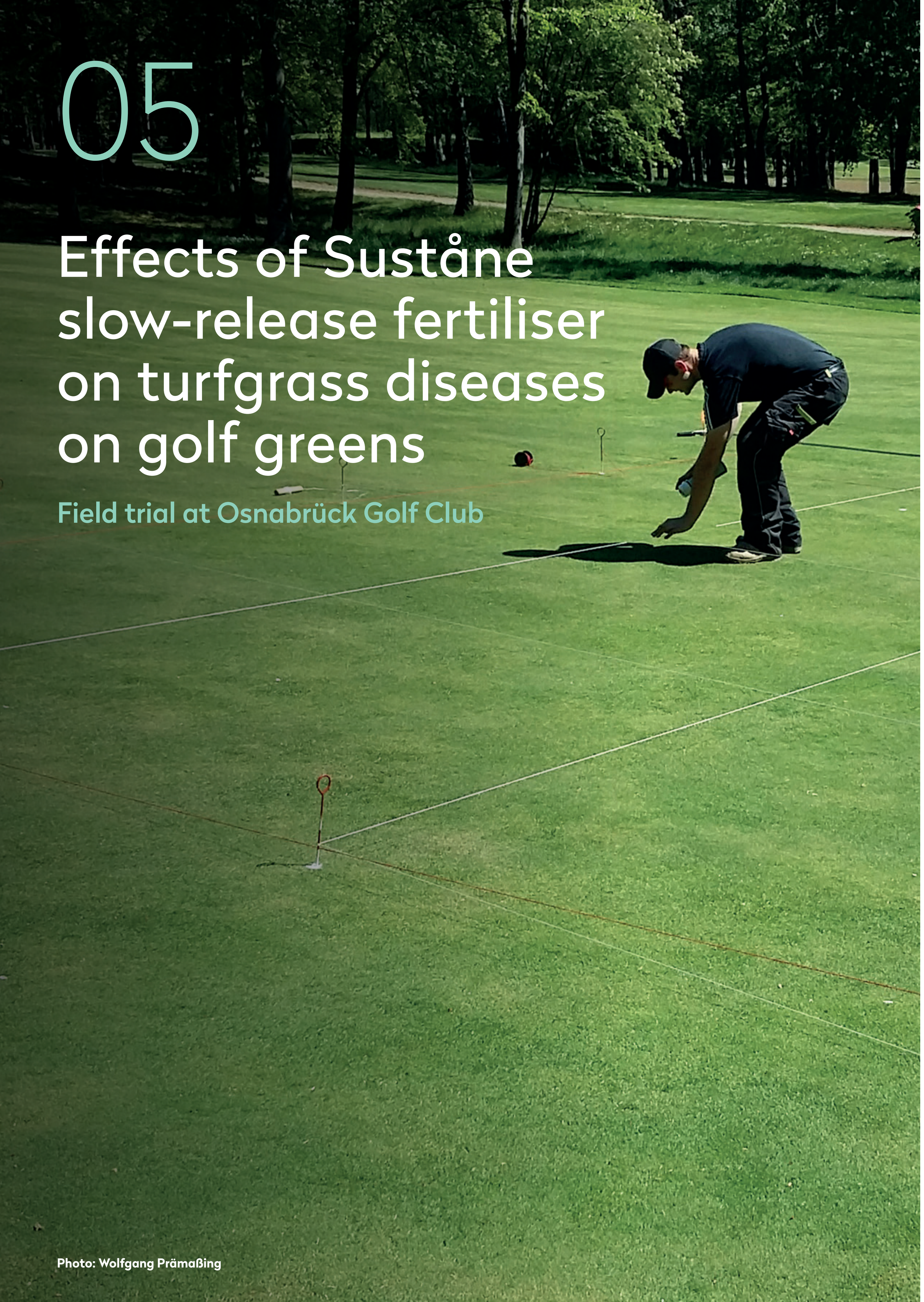


05

Effects of Suståne slow-release fertiliser on turfgrass diseases on golf greens

Field trial at Osnabrück Golf Club

Photo: Wolfgang Prämaßing

R&A

5.1 Introduction

The aim of the study was to investigate effects of two different rates of organic slow-release nutrition Suståne 5-2-4+Fe on dollar spot disease severity.

Full report available at: http://www.sterf.org/Media/Get/4166/effect-of-uv-c-radiation-and-sustane-on-dollar-spot-and-microdochium-patch-field-trials-v2

Video on UVC-radiation and slow-release fertilization available at: https://www.youtube.com/watch?v=J3COMqHEAhg

5.2 Materials and methods

The experiments were carried out on a golf green with creeping bentgrass (*Agrostis stolonifera*) and annual bluegrass (*Poa annua*) at the Osnabrück Golf Club, Bissendorf-Jeggen, Germany. The maintenance of the test area was carried out by the Club's greenkeeper team. The cutting height of the test area was 4mm through the vegetation period.

The fertiliser trial was carried out with three treatments in a randomised complete block design with four replications. Trial plots (Figure 5-1) had a size of 4m² (2 x 2m) and the corners of plots were marked with spray and maintained throughout the experiment. The three treatments consisted of TRT 1 – control, with a nitrogen rate of approximately 130kg N/ha/yr according to greenkeepers individual fertilising programme, TRT 2 – Suståne 5-2-4+Fe with a nitrogen rate of 130kg N/ha/yr and TRT 3 – Suståne 5-2-4+Fe with a nitrogen rate of 70kg N/ha/yr. Treatments 2 and 3 were split into 11 single amounts of 1.18g N/m² for TRT 2 and 0.64g N/m² for TRT 3 for each application. It was applied every two weeks from May to October using a box spreader.

For statistical analysis the Kruskal-Wallis analysis was used for all parameters to test for significance. The Wilcoxon-Mann-Whitney test was used to test significance in pairs. Differences were marked at a 0.05 probability level. In addition, the area under disease progress curve (AUDPC) was calculated according to Madden et al., (2007) as quantitative summary of disease severity over time for all treatments separately.

Figure 5-1: Trial area showing plots with effects of different slow-release fertiliser amounts with differences in green colour on putting green at Osnabrück Golf Club.

Photo: W. Prämaßing.

5.3 Results and discussion

Turfgrass quality and density

No significant differences were found between the three treatments for the visual turfgrass quality and density. During the summer months, turf quality was rated high for all treatments. TRT 2 Sustâne 130kg N/ha/yr showed a trend with the highest rated quality and density with a value of 8.5 in September 2021. All variants were rated 8 at the end of the trial period in summer 2022. Turf quality and density decreased in the winter period 2020-2021 to a value of 2, a lowest rating during the whole trial period. (Results not shown).

Colour intensity

Figure 5-2 shows boxplots for the colour intensity measured as Normalised Difference Vegetation Index (NDVI) of the Sustâne trial treatments over the whole trial period by using the Greenseeker Handheld Crop Sensor (Trimble Inc.). The median NDVI value of the TRT 2 Sustâne 130kg N/ha/yr differs significantly to the observed median of the control according to Kruskal-Wallis rank sum test ($p<0.05$).

The highest NDVI values (0.85) are achieved by the TRT 2 Sustâne 130kg N/ha/yr on 19 August 2021. The minimum (0.57) is also reached by this treatment on 25 February 2021. NDVI measurements were lowest during late winter periods and highest in late summer NDVI values between 0.57 and 0.85.

According to Turgeon and Kaminski (2019), iron (Fe) is important for the formation of chlorophyll in plant cells. The iron content in the fertiliser explains the significant difference between TRT 2 and TRT 1.

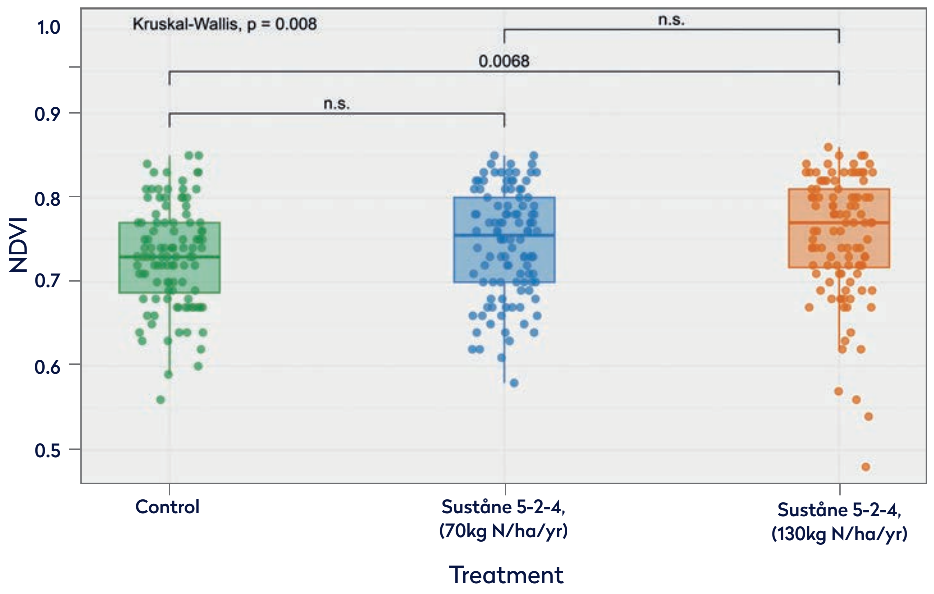


Figure 5-2: Normalised Difference Vegetation Index (NDVI) of the Sustâne (5- 2- 4 + Fe) trial (2). There were significant differences according to Kruskal-Wallis rank sum test ($p<0.05$). Therefore, Wilcoxon-Mann-Whitney test was used pairwise. There were significant differences between the control and Sustâne (130kg N/ha/yr).

Coverage of dollar spot

Figure 5-3 shows boxplots containing all observed values for the sampling days where dollar spot was observed. The control variant had the highest median percentage coverage during the trial with 1%. Followed by the TRT 3 Sustâne 70kg N/ha/yr (0.72 %) and TRT 2 Sustâne 130kg N/ha/yr (0.62 %). The Kruskal-Wallis global test was not significant ($p < 0.05$) thereby not showing any differences between the median disease severities. Higher dosages of Sustâne fertilisation led to increasingly reduced variability in the severity of infestation. The interquartile range (IQR) was highest with 2.00 for the control TRT 1 while TRT 2 Sustâne 130kg N/ha/yr only an IQR of 1.25, thereby showing at least a trend to lesser infestation and spreading of dollar spot.

This corresponds with observations of Turgeon and Kaminski (2019), that a balanced and regular supply of nitrogen and iron can reduce the infestation of dollar spot.

In contrast, the control of TRT 1 was fertilised with an irregular rhythm by the golf course's greenkeeping team and not every two weeks, as with TRT 2 and TRT 3.

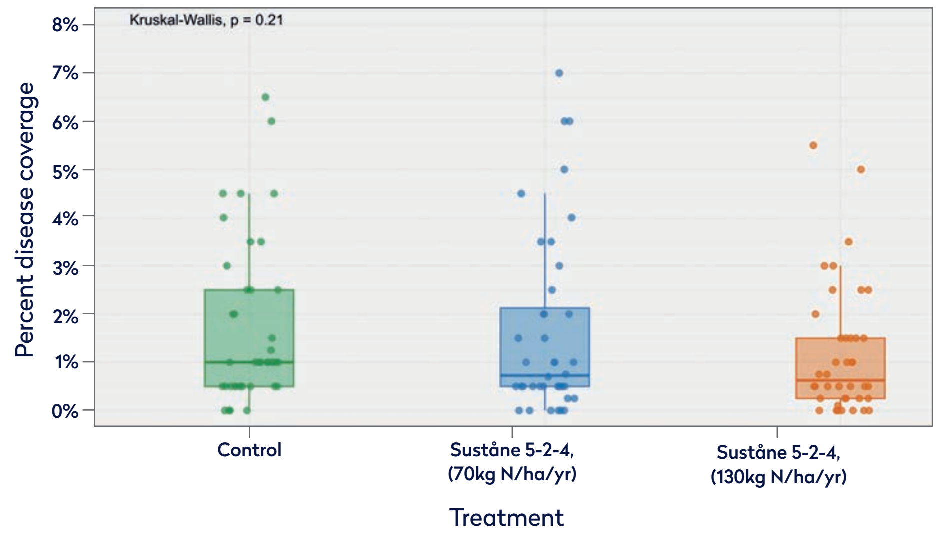


Figure 5-3: Disease coverage in % of the Sustâne trial for dollar spot. There were no significant different median values according to Kruskal-Wallis rank sum test ($p<0.05$).

Table 5-1 shows the AUDPC values for mean dollar spot coverage with a highest value of 380.25 on the control TRT 1 and lowest on TRT 2 with 287.94 in the year 2020. In the year 2021, the dollar spot outbreak was of lesser severity compared to the year 2020.

Treatments	AUDPC year 2020	Percentage peak 20 Oct 2020	AUDPC year 2021	Percentage peak 19 Aug 2021
TRT 1 Control	380	4.75 %	62	1.31 %
TRT 2 Sustâne 130 kg N/ha/yr	288	4.25 %	19	1.00 %
TRT 3 Sustâne 70 kg N/ha/yr	376	3.12 %	43	0.34 %

Table 5-1: AUDPC values for mean dollar spot coverage (% of plot area).

Figure 5-4: Trial plot of TRT 2 (left) with lesser dollar spot infestation compared to a plot of TRT 3 (right) with higher dollar spot percentage in September 2020 on putting green at Osnabrück Golf Club.

Photos: W. Prämaßing

5.4 Conclusions on benefits and advice for the golf and turfgrass sector

- **While higher fertilisation rates with Sustâne 5-2-4 + Fe led to nearly similar dollar spot coverages, peaks of median disease coverages and AUDPC values were lower for the treatment receiving 130kg N/ha/yr in both years of the trial.**
- **As a result, there may be some effect of fertilisation with Sustâne 5-2-4 + Fe compared to the control with a trend to lower dollar spot infestation, but it was not strong enough to efficiently control dollar spot disease outbreak.**

Figure 5-5. Osnabrück Golf Club with location of putting green which was used in the experiments (yellow frame).

Photo: www.ogc.de/platz/platzuebersicht

Madden L.V., Hughes, G., van den Bosch, F. (2007): The study of plant disease epidemics. The American Phytopathological Society, APS Press St. Paul, Minnesota.

Turgeon, A.J. and Kaminski, J.E. (2019): Turfgrass Management, Edition 1.0, Turfpath, LCC. State College Pennsylvania, p. 392.

06

Use of biostimulants in ITM programs on golf greens with microdochium patch pressure

Field trial at Landvik and Bingley

Photo: Tatsiana Espevig

6.1 Introduction

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 microdochium patch affected turf, whilst enhancing turfgrass health, as expressed through visual quality and colour intensity.

Full NIBIO report available at: http://www.sterf.org/Media/Get/4167/evaluation-of-different-integrate-turf-management-programs-to-reduce-microdochium-patch

6.2 Materials and methods

We conducted field trials over the course of two seasons (2020-2022) and in two locations (Landvik, Norway and Bingley, United Kingdom) to investigate the potential of new treatment methods for microdochium patch disease on golf greens. The two locations had comparable composition, being comprised primarily of annual meadow grass (*Poa annua*) with smaller proportions of browntop bent (*Agrostis capillaris*). The trial greens in both locations were treated comparably in terms of fertilisation, nitrogen rates, mowing protocols, irrigation and simulated use, mimicking the natural conditions and treatment protocols of average golf green requirements. Our trial design consisted of four treatments, with one being a control. The first treatment consisted of timely applications of three fungicides (A19188B, A20323D and Medallion) and the colourant Ryder. The second treatment consisted of timely applications of two fungicides (A20323D and Medallion), Ryder and the biostimulant Hicure. The third treatment consisted of timely applications of one fungicide (Medallion), Ryder and Hicure. The trail protocols consisted of a randomised complete block design (RCBD) with four replicates of each treatment, with each replicate plot size being 2.25m² (1.5 x 1.5m) and the registration area in the central 1m². Treatment assessments were conducted to measure visual turfgrass quality, colour intensity and microdochium disease development. The assessments were conducted bi-weekly prior to the first sign of microdochium, then weekly until the last application of the fungicides and finally bi-weekly again unless snow was present.

Turfgrass visual quality, colour intensity and microdochium disease development were modelled using linear regression models, with treatment, season and location as categorical factors. The models were analysed using Type-II ANOVA to identify significant differences, and further compared using Tukey's post-hoc test to identify difference within the factors. Area under disease progress curve (AUDPC) was further calculated from the disease development data. The AUDPC data was calculated for each replicate and then averaged for each treatment and analysed using one-way ANOVA (least squares) and compared with Sidak's post-hoc test.

6.3 Results and discussion

Microdochium patch started to become visible on the turfgrass during early autumn, around September, for both years and locations (Figure 6-1). Initially, the disease progression was about the same for all treatments, but difference between the treatments could be observed around October. Our analyses showed that the effects of the treatments were the same and linked for visual quality and microdochium disease development, in which there were differences between the treatments and the locations. There was no difference in disease prevention for the three and two fungicides with Hicure treatments, showing that the biostimulant can effectively replace the use of one fungicide. The one fungicide treatment was better than the control, yet not as effective as the two previous treatments. The effects of the treatments were more consistent in Bingley than in Landvik, suggesting location-specific variations in the treatment efficiency, which is especially pronounced for Landvik during the 2021-2022 season (Figure 6-2). Large variation for replicates within treatments suggests that some plots inherently experience more infection by microdochium than others, giving rise to non-significant differences in AUDPC for Landvik. However, in Bingley, the within-treatment variation was lower, allowing for significant differences in AUDPC between the fungicide treatments and the control. For the colour intensity, we saw significant improvements with the fungicide treatments containing Ryder and the control, but no difference between the fungicide treatments themselves. This suggests that applying Ryder consistently improves colour intensity.

Treatment of annual bluegrass (*poa annua*) to fungal infection – Microdochium disease development

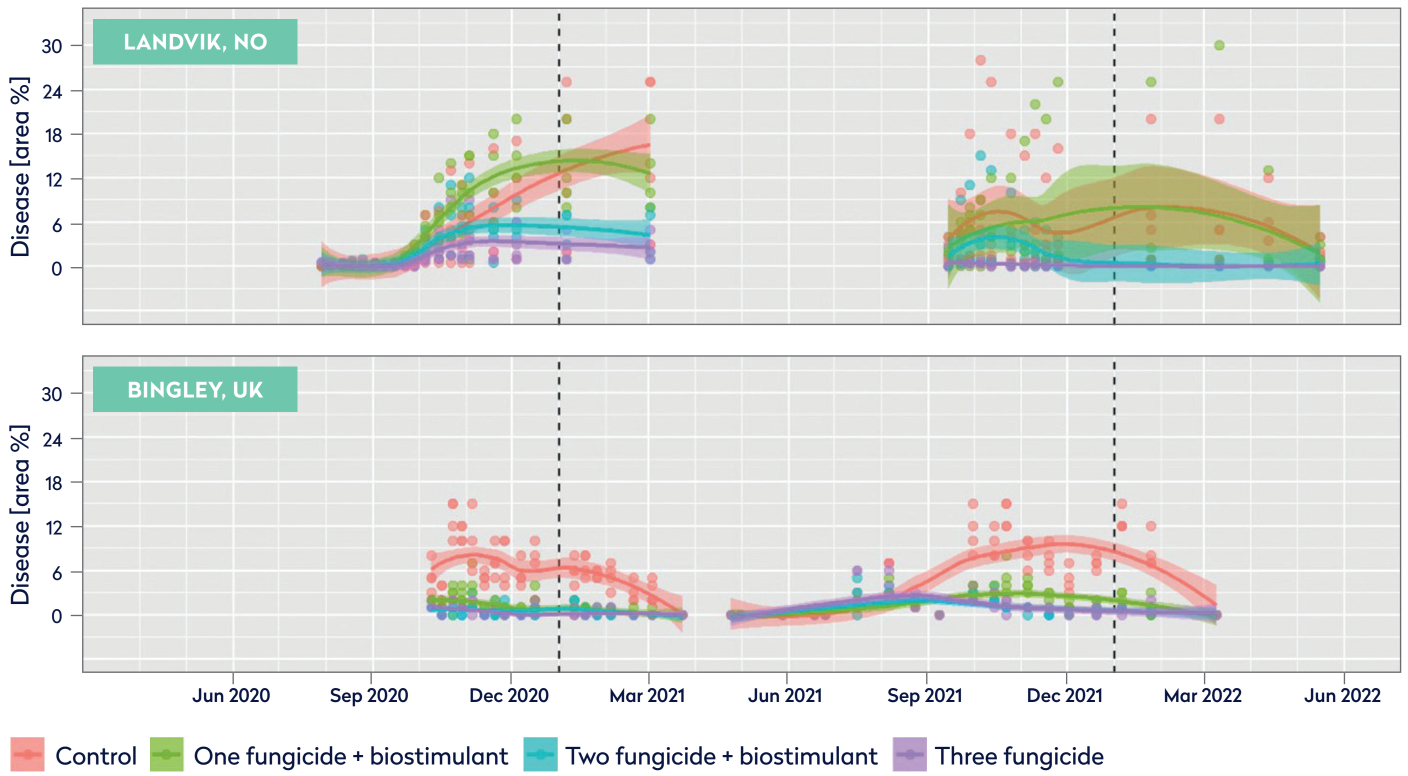


Figure 6-1. Turfgrass microdochium disease development for each treatment (replicates n = 4). The graph includes the 2020-2021 and the 2021-2022 season for Landvik, NO and Bingley, UK. The trend line is a linear smoother using a LOESS-function and 95% confidence intervals around the mean value for each treatment, year and location.

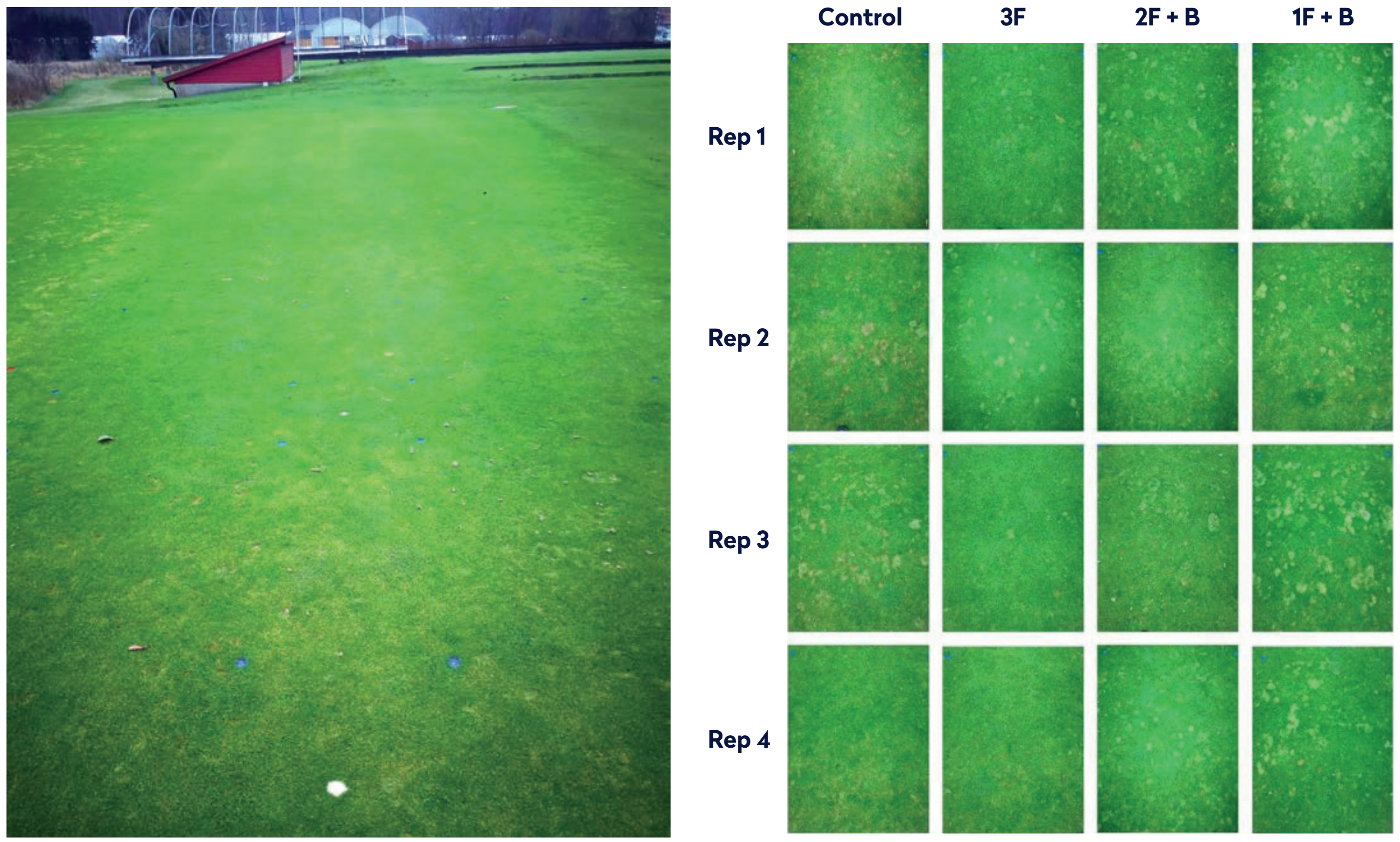


Figure 6-2. Photos of the treatment replicates for each treatment at Landvik – 25-11-2020.

6.4 Conclusions on benefits and advice for the golf and turfgrass sector

- **The biostimulant Hicure can effectively reduce the numbers of fungicides needed to treat microdochium patch disease in turfgrasses while simultaneously improving visual quality.**
- **The colourant Ryder is effective at increasing colour intensity in turfgrasses affected by microdochium patch disease.**

07

Nutrient based IPM programs to prevent dollar spot on a red fescue green

Field trial at Bingley

Photo: STRI group

7.1 Introduction

In the United Kingdom, dollar spot (caused by *Clarireedia* spp.) tends to affect creeping bentgrass (*Agrostis stolonifera*) and red fescue (*Festuca rubra* spp.). Red fescue greens tend to be at high risk of dollar spot due to the low nutrient programmes typically used to promote these grasses in the UK. As a low nutrient disease, an integrated pest management approach would be to use nutrient inputs to minimise the potential for dollar spot infection. The objective of this trial was to assess if nutrient programmes with different nitrogen inputs could reduce the incidence of dollar spot.

7.2 Materials and methods

A field trial was run over two dollar spot seasons (2020 and 2021) at the STRI's research facility in Bingley, West Yorkshire (53° 50' 50.2656" N, 1° 51' 17.3448" W, 180 m a.s.l.). The area consisted of golf green turf grown on a sand-based rootzone (94% sand, 3% silt and 3% clay). The area had a mixed sward comprising a blend of browntop bent (*Agrostis capillaris*), red fescue (*Festuca rubra* spp.) and annual meadowgrass (*Poa annua*). There was a high proportion of dollar spot susceptible slender creeping red fescue (*Festuca rubra* spp. *littoralis*), which had been oversown in previous years to increase the proportion of red fescue in the sward. The area was maintained with low nutrient inputs to create conditions that would favour dollar spot activity.

The field was mown regularly, with the frequency dependent on growing conditions. During the main growing season, turf was typically mown three times per week, and into autumn when growth rates slowed down, twice per week. The area was maintained with a low input management programme to naturally encourage a pathogen population. Turf was aerated twice a year to relieve compaction from vertical cutting – which was carried out intensively during spring each year to clean out the sward and to allow space for new growth of grasses – and to remove surface moss. Nutrient inputs were carefully controlled throughout the trials. Irrigation was applied as needed during the trial using pop-up irrigation and trial plots were not subject to dew removal to help promote leaf humidity.

To boost the pathogen population, the trial area was inoculated with dollar spot using wheat seeds, which were infected with dollar spot once in July in 2020 and twice in 2021 (July and August). This was to help encourage pathogen infection during the trial and to boost the natural dollar spot population.

The trial had 7 treatments (Table 7-1). The objective of treatments was to apply different amounts of nitrogen to turf. Treatments were applied to 2 x 1m plots with each treatment replicated four times. During assessments a 5cm gap was left around the edge of each plot.

The assessments carried out during the trial included dollar spot incidence (%), turf quality and colour (both scored on 1-9 scale) and turf density (%). Assessments were carried out monthly. Data were analysed as a one-way ANOVA randomised complete block design using Genstat with a significance level of 0.05. Fishers LSD at a 0.05 significance level was used to identify significant differences among treatments.

Treatment	Nitrogen (kg/ha/yr)	Treatment description
1	0	Untreated control (no nitrogen applied)
2	86	NPK applied as mineral fertiliser liquids at low rate fortnightly
3	128	NK applied as mineral fertiliser liquids fortnightly
4	137	NPK applied as mineral fertiliser liquids at high rate fortnightly
5	86	Sustâne 5-2-4+Fe organo-based fertiliser at low rate applied fortnightly
6	137	Sustâne 5-2-4+Fe at high rate applied fortnightly
7	128	ICL dollar spot programme which uses different sources of nitrogen, including slow release forms in combination with other elements such as Fe, Ca, Mg and Si, as well as biostimulant compounds

Table 7-1. Treatments investigated during the trial.

7.3 Results and discussion

In the first year of this trial, plots treated with the ICL dollar spot programme (T7) consistently improved turf quality, colour and density as well as reducing dollar spot incidence, when compared to untreated plots (Table 7-2). Plots treated with N and K only at N rate to match ICL programme (T3) and nutrients (NPK) only at high rate (T4) also consistently improved turf quality, colour and density as well as reducing dollar spot, when compared with untreated plots. Nutrients (NPK) only at low rate (T2) also improved turf quality, colour and density when compared with untreated plots but was not as consistently as other treatments. This treatment also reduced dollar spot incidence. Sustâne 5-2-4 + Fe at low rate (T5) and high rate (T6) showed some benefits at points in the trial but these were not observed consistently or to the same level as other treatments.

Treatment	05.06	03.07	10.07	06.08	23.11	08.12
	Pre-treatment	14DAT3	7DAT4	7DAT7	14DAT14	29DAT14
1 Untreated	0.0	3.5 c	4.8 c	3.8 b	0.0	0.0
2 NPK at 86kg N/ha/yr	0.0	1.0 ab	1.8 ab	0.5 a	0.0	0.0
3 NK at 128kg N/ha/yr	0.0	0.3 a	2.0 ab	0.3 a	0.0	0.0
4 NPK at 137kg N/ha/yr	0.0	0.3 a	1.3 a	0.0 a	0.0	0.0
5 Sustâne at 86kg N/ha/yr	0.0	2.0 bc	3.3 bc	1.0 a	0.0	0.0
6 Sustâne at 137kg N/ha/yr	0.0	2.3 bc	3.8 c	1.0 a	0.0	0.0
7 ICL programme at 128 kg N/ha/yr	0.0	0.3 a	1.0 a	0.0 a	0.0	0.0
P	*	0.001	<0.001	<0.001	*	*
LSD	–	1.53	1.61	1.44	–	–
d.f.	18	18	18	18	18	18
%c.v.	0.0	75.7	42.7	104.4	0.0	0.0

Table 7-2: Dollar spot incidence (%) of treated and untreated plots during 2020.

* Insufficient variation for statistical analysis,
NS Not statistically significant.

After inoculation of the trial with dollar spot on 1 July 2020, dollar spot levels increased slightly in untreated plots to just under 5% on 10 July 2020 (Table 7-2). In August, the following month, eight dollar spot levels had begun to decrease again. This was possibly due to the microdochium patch in the area caused by the unusually mild and damp summer, which resulted in competition/antagonism and did not allow for the dollar spot to have as great effect as expected.

This trial continued with the same plots and treatments in 2021. During the second year, plots treated with the ICL dollar spot programme (T7) consistently improved turf quality, colour and density, as well as reducing dollar spot incidence when compared to untreated plots (Table 7-3). Plots treated with N and K only at N rate to match ICL programme (T3) also consistently improved turf quality, colour and density, as well as reducing dollar spot when compared with untreated plots. Nutrients (NPK) only at low rate (T2) also improved turf quality, colour and density, as well as reducing dollar spot when compared with untreated plots, but not as consistently as other treatments.

Plots treated with nutrients (NPK) only at high rate (T4) consistently improved turf quality, colour and density, but was less against dollar spot. Plots treated with nutrients (NPK) only at high rate (T4) consistently improved turf quality, colour and density, but was less against dollar spot.

Treatment	16.07 8DAT5	30.07 1DAT7	12.08 7DAT8	26.08 7DAT10	02.09 14DAT10	09.09 7DAT11	23.09 7DAT13	07.10 7DAT14	08.11 18DAT16	14.12 54DAT16
1 Untreated	2.3 bc	2.8	6.0 d	10.0 f	12.5 e	17.0 c	10.8 cd	7.0 c	2.0 c	1.5 b
2 NPK at 86kg N/ha/yr	2.3 bc	3.0	5.3 cd	8.0 e	10.8 d	9.8 b	6.3 b	3.5 b	1.0 abc	1.0 b
3 NK at 128kg N/ha/yr	0.5 a	2.5	4.5 c	5.8 c	8.5 c	9.0 b	5.8 b	3.5 b	1.0 abc	1.0 b
4 NPK at 137kg N/ha/yr	1.3 ab	2.8	2.8 b	4.3 b	5.0 b	17.8 c	12.3 d	7.3 c	2.0 c	1.5 b
5 Suståne at 86kg N/ha/yr	2.3 bc	3.3	5.0 cd	7.5 de	9.3 cd	11.0 b	7.3 bc	4.5 bc	1.3 bc	1.3 b
6 Suståne at 137kg N/ha/yr	3.3 c	3.8	5.0 cd	5.8 c	8.0 c	10.0 b	6.0 b	3.5 b	0.8 ab	0.8 ab
7 ICL programme at 128kg N/ha/yr	0.0 a	1.0	0.0 a	0.3 a	2.0 a	1.5 a	0.5 a	0.0 a	0.0 a	0.0 a
P	0.003	NS	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.031	0.047
LSD	1.50	–	1.34	1.30	1.59	5.38	4.18	2.87	1.20	0.94
d.f.	27	27	27	27	27	27	27	27	27	27
%c.v.	60.6	–	22.2	14.5	13.4	33.3	40.4	46.1	70.8	63.3

Table 7-3: Dollar spot incidence (%) of treated and untreated plots during 2021.

Plots treated with Suståne (T5 & T6) were successful in reducing dollar spot levels when compared with untreated plots. The higher rate (T6) also significantly improved turf colour on occasion, but not as consistently as other treatments.

In 2021, the trial was inoculated with dollar spot on 30 July and again on 26 August. Dollar spot levels increased across the trial area from just under 3% in untreated plots to 17% by 9 September 2021 (Table 7-3). The onset of dollar spot occurred later in the year than is usually expected across the UK in 2021. Dollar spot is usually seen to become active in late June/early July. The late dollar spot outbreak was possibly due to the cold spring and longer time for turf to recover. From 23 September that same year, dollar spot levels had begun to decrease.

7.4 Conclusions on benefits and advice for the golf and turfgrass sector

- **The use of nutrient inputs, under lower levels of dollar spot pressure, can be successful at reducing disease incidence on red fescue turf.**
- **There was no strong dose rate response with increasing nitrogen application rates and dollar spot incidence. Higher rates of nitrogen typically did not result in significantly less dollar spot, in comparison to lower rates of nitrogen that were tailored to turf demand.**
- **The addition of additional elements and biostimulants, as used in the ICL dollar spot programme, resulted in the lowest incidence of dollar spot, highlighting the benefits of combining different technologies for promoting plant health. The ICL programme kept dollar spot levels to below 2% throughout the trial.**
- **Use of nitrogen and nutrition solutions are a key component of the IPM of dollar spot.**
- **Optimising nutrient inputs also had benefits for turf quality, colour and density, all of which are desirable characteristics for high quality and resilient playing surfaces.**

08

Development of immunoassay for detection of microdochium fungus in grass

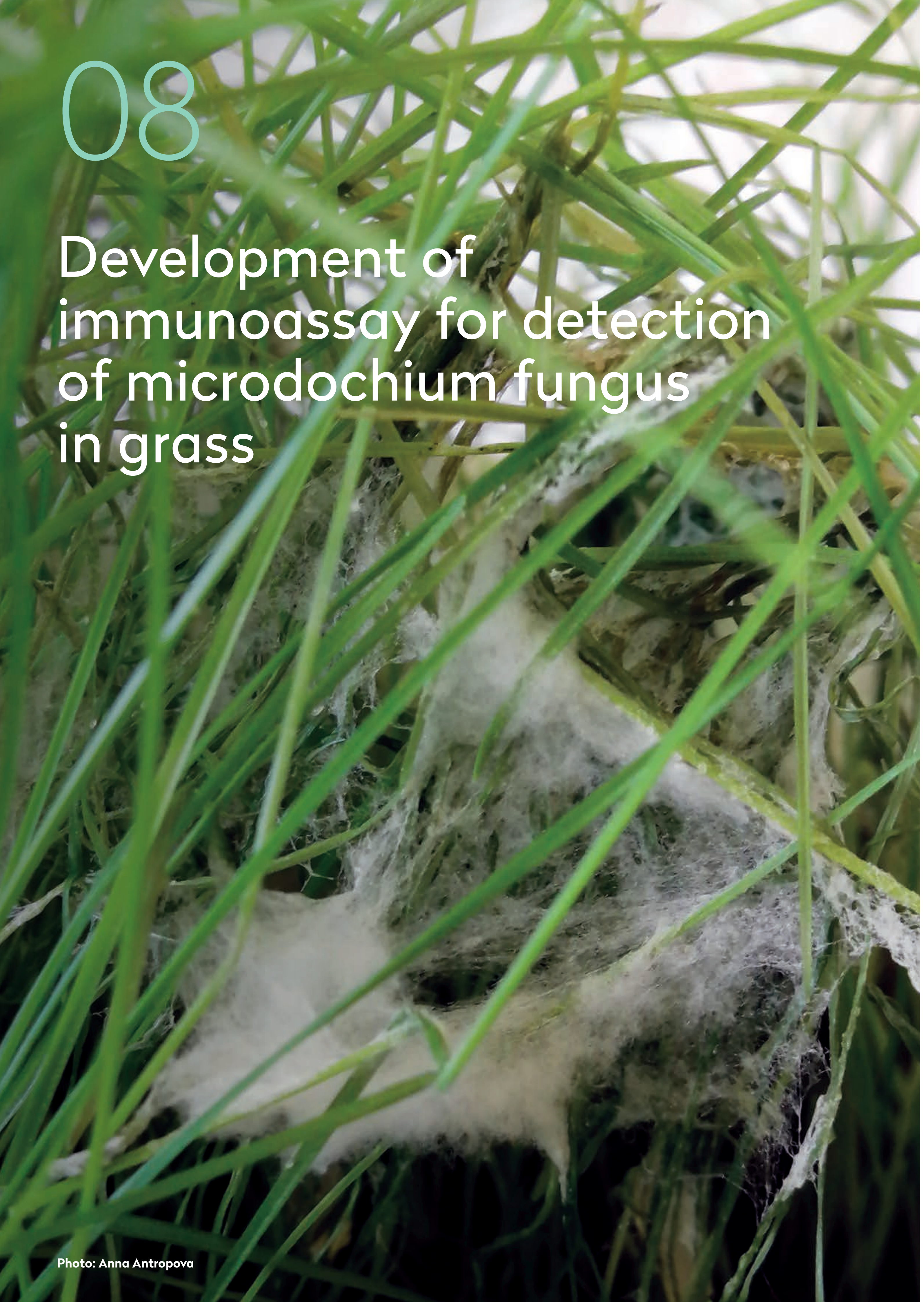


Photo: Anna Antropova

8.1 Introduction

Early and correct identification of diseases in the field is an important step in any IPM program. Early identification of dollar spot using a diagnostic test kit (immunoassay techniques, ELISA) may reduce the number of fungicide applications from eight to five, compared with diagnosis of dollar spot based on symptoms in the field (Baldwin, 1993). Petersen et al., (1989) registered a patent on monoclonal antibodies to *Sclerotinia homoeocarpa*. However, since this patent is species-specific, it is almost useless as there are several clarireedia species which cause dollar spot. There are no successful examples for *Microdochium nivale,* aside from a few on the use of immunoassay for fungi such as *Fusarium* spp. (Gagkaeva et al., 2019). A genus-specific assay for *Clarireedia* spp. and species-specific assay for *Microdochium nivale* could nevertheless become a useful tool for greenkeepers in rapid and early identification of both diseases on golf courses in North and Central Europe, and also for the early implementation of alternatives to fungicides. The aim of this study was to investigate immunoassay for rapid identification of the fungi that cause microdochium patch and dollar spot.

8.2 Materials and methods

Five microdochium strains (two of *M. nivale*, one of *M. majus*, and two of *M. bolleyi*) and eight clarireedia strains (three of *C. jacksonii* and five of *Clarireedia* spp.) were used in the study. Microdochium strains originated from different geographical regions of Russia, isolated from various cereal grasses. Clarireedia strains originated from turfgrass from Denmark, Norway, Sweden and the UK.

Microdochium and clarireedia isolates were cultured on a malt broth 2.5 Balling and potato dextrose broth respectively for 21 days at room temperature. To obtain specific polyclonal antibodies, rabbits were immunised by 1M NaCl fungal extracts with Complete Freund adjuvant. The resultant serum samples were sorted for specific activity by direct binding ELISA, with the best performing serum portions pooled for affinity purifications on antigen adsorbent. The primary antibody preparation was then exhausted on a series of fungal antigen adsorbents. To prepare conjugate, affinity antibodies were labelled by horseradish peroxidase (HRP). Immunisation by clarireedia showed considerable cross-reactivity of immune response towards fungal extracts (*Trichosporon, Aspergillus, Mucorales* etc) with low cross-reactivity to microdochium (Figure 8-1).

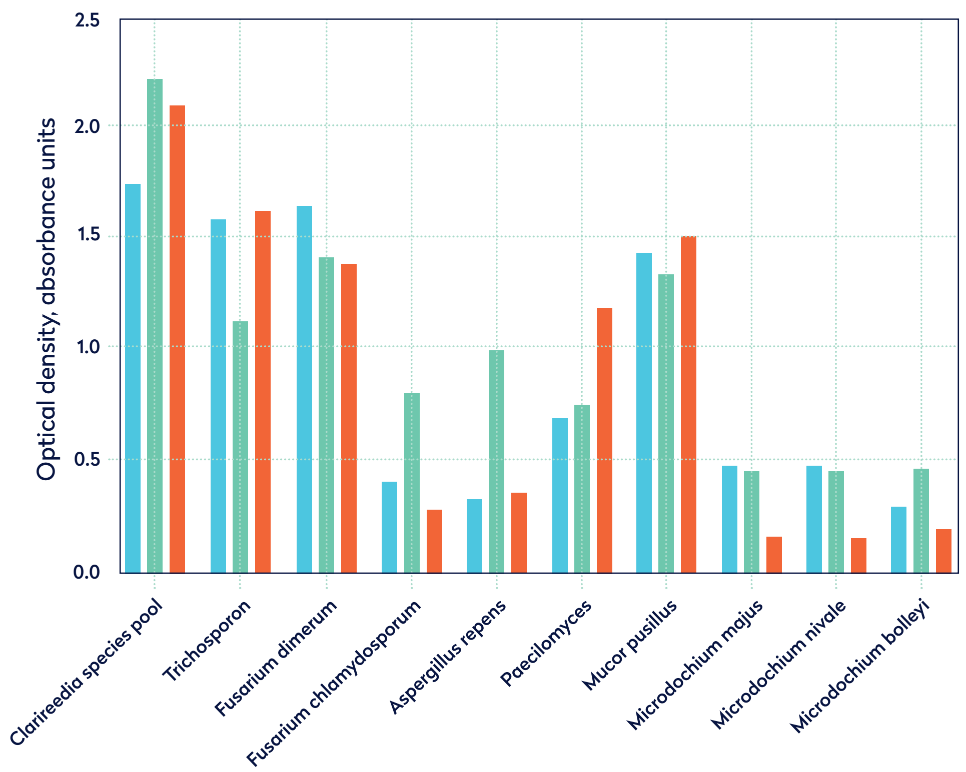


Figure 8-1. Immune response to clarireedia extract: X reactivity to other fungi. Direct binding ELISA, OD data for dilution 1:10,000.

Affinity purified antibodies against clarireedia genus were purified and exhausted by cross-reactive fungi, specific antibody was prepared (Figure 8-2). However, purified antibodies were unable to set up a sandwich ELISA in clarireedia extract suggested by competition for the single epitope.

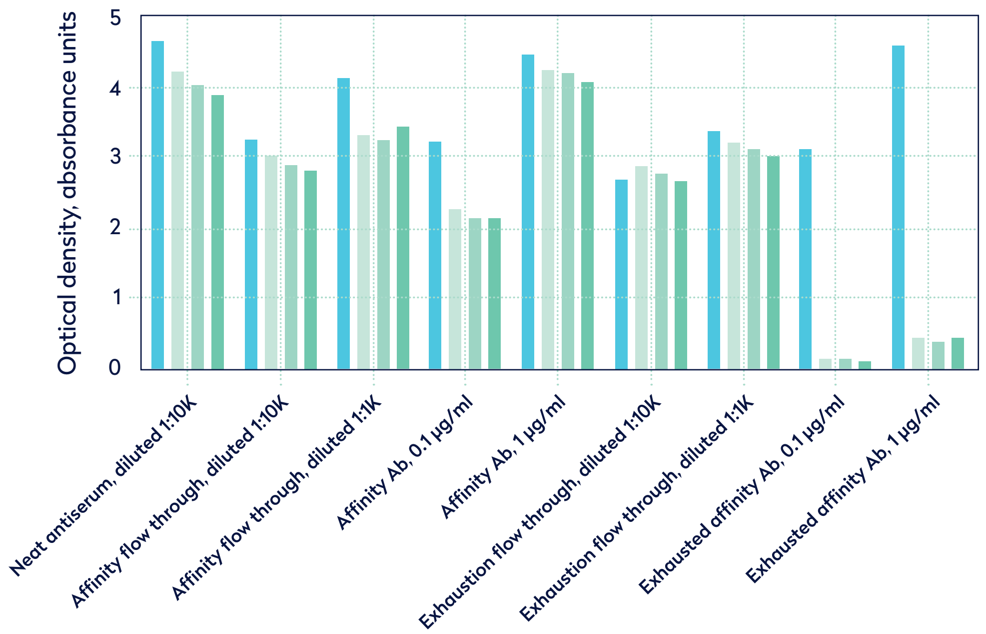


Figure 8-2. Preparation of affinity purified exhausted antibodies against clarireedia. Direct binding ELISA.

The test system for microdochium antigens detection was constructed as a sandwich ELISA. Sample preparation was as follows. Solid samples were mixed with 10 parts of extraction buffer (PBS, pH 7.2-7.4) and homogenised. The resulting extracts were centrifuged at 3000rpm for 15 minutes. Supernatants were used in the analysis immediately. Liquid samples were diluted tenfold in PBS and treated by centrifugation as above. Serial dilutions were performed in both cases if necessary.

Antibody adsorption and calibrator preparation were as follows. Antibodies against microdochium were coated on to polystyrene microplates, blocked by hydrolysed casein and dried. The arbitrary calibrators were prepared by serial dilution of *M. nivale* extract used for immunisation in ELISA buffer (0.1M phosphate buffer containing 0.9% NaCl and 0.1% hydrolysed casein).

In assay run, 100µl of ELISA buffer was added to the wells followed by 20µl of the calibrators or the analysed extracts. The wells were incubated for 30 minutes at +37°C, washed three times with ELISA washing solution and 100µl of HRP-conjugated affinity antibodies prediluted 1:5,000 in ELISA buffer, were added for another 30 minutes at +37°C. After five washings with ELISA washing solution, 100µl of TMB substrate was added. In 15 minutes, reaction was stopped with 100µl of 5% sulphuric acid. Optical density (OD) was measured at 450nm. The calibration curve was constructed by serial dilutions of standard extract and the results were expressed in arbitrary Units/ml.

Microdochium ELISA validation was carried out on pure cultures of fungi (*Microdochium* spp. and non-relevant fungi and oomycetes), in model experiments (analysis of inoculated detached leaves and intact plants of *Poa pratensis* and *Festuca rubra*) and in field experiments (set 1, 28 turfgrass samples with and without signs of microdochium patch were analysed; set 2, 25 turfgrass samples with lesions of different degrees according to visual assessment were analysed; set 3, 24 grass samples with no, latent and active microdochium infection were analysed). Samples in set 1 and 2 were collected from the field at NIBIO Turfgrass Research Centre Landvik, Norway. Samples in set 3 were collected from 17 golf clubs from three different climatic regions of Sweden.

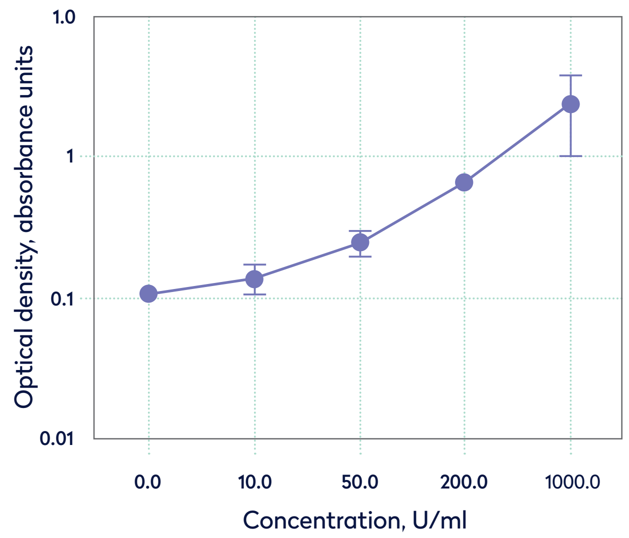


Figure 8-3. Typical calibration curve of microdochium ELISA.

8.3 Results and discussion

The typical calibration curve for microdochium ELISA is shown on Figure 8-3. Limit of detection (LOD) was determined as the sum of average OD of zero calibrator and 1 standard deviation (SD) giving the value of 2.55 U/ml.

Microdochium ELISA efficiently detected all microdochium species in pure cultures with similar sensitivity (Figure 8-4). The extracts of other fungi and oomycetes showed no substantial OD signal (data not shown).

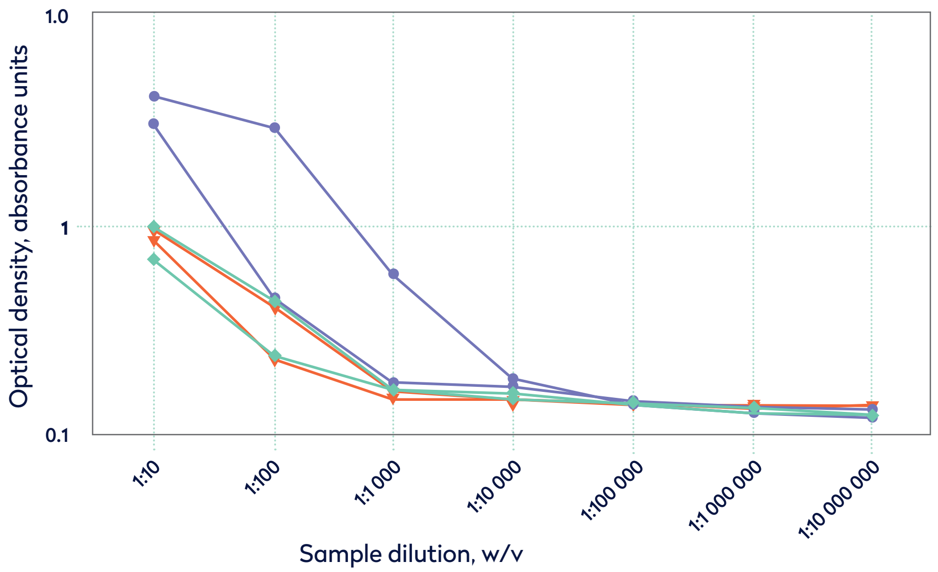
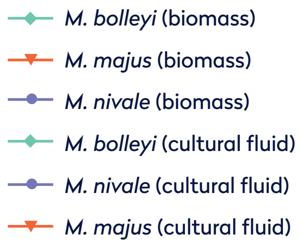
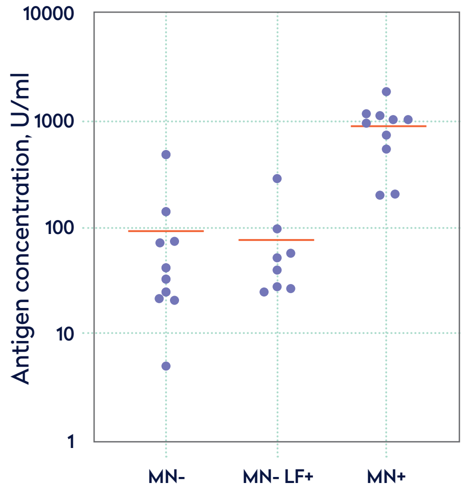


Figure 8-4. ELISA testing in biomass and cultural liquids of three microdochium species: *M. bolleyi, M. nivale, M. majus.*



In laboratory inoculated samples of *P. pratensis* and *F. rubra* microdochium antigen content varied from 417 to 4950U/ml. In field grass samples (set 1 and 2) microdochium antigen concentration varied from 5 to 471U/ml for samples without visible infection and from 39 to 1840U/ml for samples with various degrees of lesions. The mean and median values of antigen concentrations increased as visual signs of infestation increased (Figures 8-5, 8-7). In the field experiments (set 1 and 2), microdochium antigens were detected in both samples with and without visible lesions. It is possible that single or non-viable propagules may have been present in samples without visible signs of infestation, or that the fungus has not yet shown visible signs of infection. A further study (set 3) allowed us to analyse the samples not only visually at the time of collection, but also to distinguish between healthy grass and grass with latent infection.

In healthy grass samples antigen concentration varied from 0 to 6.7U/ml. In grass samples, with latent infection, antigen concentration varied from 0.7 to 19.8U/ml. Finally, in grass samples with visible damage, antigen concentration varied from 47 to 1100U/ml (Figure 8-6). The data obtained allowed to set the threshold value for clear infection as 30U/ml. Application of stricter cutoff below 10U/ml in the absence of false positive reaction caused by cross-reactivity to irrelevant saprophytic fungi, may help to detect the latent infection and introduce specific, timely and efficient fungicidal treatment of infected spots. As some of the potentially infected samples had shown the antigen concentrations close or below the formal LOD of the ELISA set as 2.55U/ml, more sensitive assay modification may be required to reliably detect all latent infection cases.



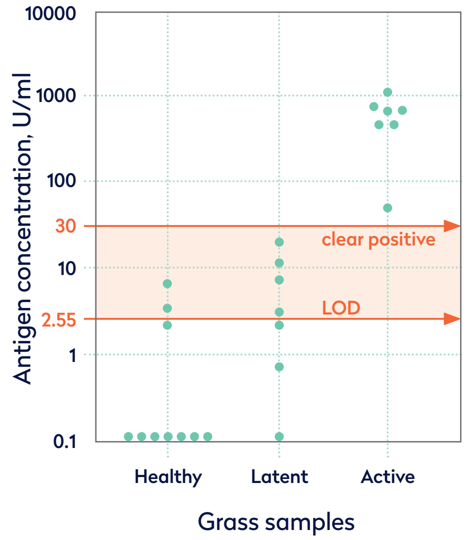


Figure 8-5 (left). Microdochium antigen content in grass samples. 'MN-' no visible *M. nivale* and *L. fuciformis* infection; 'MN- LF+' no visible *M. nivale* infection and visible *L. fuciformis* infection; 'MN+' visible *M. nivale* infection. Red lines show mean concentrations.

Figure 8-6 (right). Microdochium antigen content in grass samples, characterised as with no, latent and active infection. Samples with visible infection were classified as 'active'. Samples without visible infection at the time of collection but showed mould growth after 3-7 days incubation at +4 °C but not at +24 °C, were classified as 'latent'. Samples marked as 'healthy' did not show any symptoms of snow mould at both incubation temperatures.

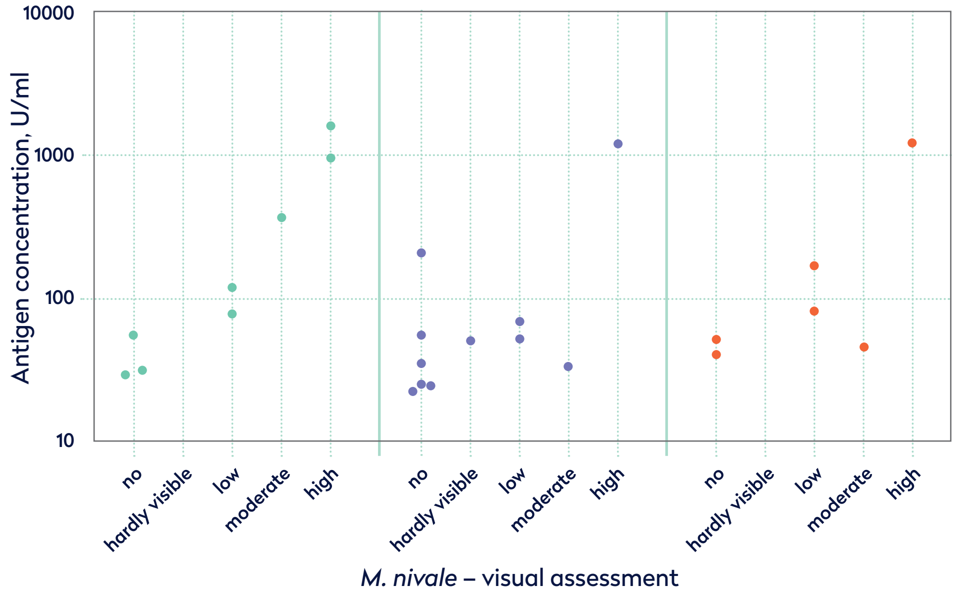
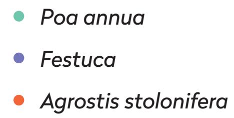


Figure 8-7. Microdochium antigen content in *P. annua, F. rubra, A. stolonifera* samples damaged to varying degrees.



8.4 Conclusions on benefits and advice for the golf and turfgrass sector

- **Microdochium ELISA is a simple and practical method for early detection of latent microdochium infection in turfgrass, which may be integrated into the overall strategy of plant disease control.**
- **Clarireedia affinity polyclonal antibodies do not form sandwich immunoassay and this species requires continued efforts to obtain mouse monoclonal antibodies.**

Baldwin, N.A. 1993. Evaluation of turfgrass disease diagnostic test kits in Europe. Intl. Turfgrass Soc. Res. J. 7:342–347.

Petersen et al., 1989. MONOCLONAL ANTIBODIES TO SCLEROTINIA HOMOEOCARPA United States Patent.

Gagkaeva T., O. Gavrilova, A. Orina, Y. Lebedin, I. Shanin, P. Petukhov, and S. Eremin. 2019. Quantitative determination of toxigenic Fusarium species associated with wheat grain from three regions of Russia: Volga, Ural, and West Siberia. Toxins 11(252):1-15.

09

Causal species for dollar spot in Europe

Photo: Carlos Guerrero

9.1 Introduction

Earlier, the existence of at least two fungal species, which cause dollar spot disease on Scandinavian golf courses, was confirmed (Espevig et al., 2015; Espevig et al., 2017). After sclerotinia was renamed clarireedia in 2018 (Salgado-Salazar et al., 2018), at least 5 clarireedia species are known today. The objective of this study was to find out which clarireedia species are found in Europe.

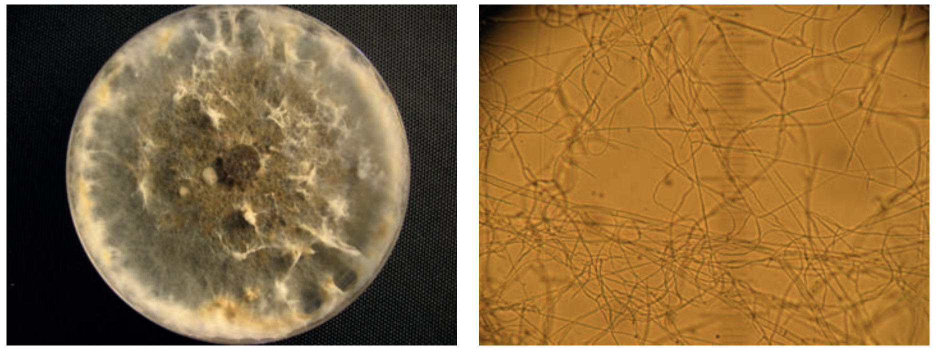


Photo 9-1. Pure culture (left) and mycelium (right) of *Clarireedia* spp.

Photo: Tatsiana Espevig

9.2 Materials and methods

In total, 37 turfgrass samples with dollar spot symptoms and two pure cultures were sent to NIBIO Turfgrass Laboratory (Landvik, Norway) in summer 2020. The turfgrass samples were collected on golf courses in Sweden (6), Denmark (3), Norway (1), the UK (1), Germany (26), Portugal (1) and Spain (1). The pure cultures were one from Portugal and one from Germany (Table 9-1). The fungi from the diseased turfgrass leaves were isolated, and the pure fungal cultures were grown on 50% PDA (potato dextrose agar) and stored in Eppendorf tubes at -80°C prior to molecular identification of target fungi. DNA isolation, amplification and sequencing were conducted at NIBIO Molecular plant biology laboratory (Ås, Norway). DNA was isolated and purified from the pure fungal cultures. Mycelium were ground in liquid nitrogen with a pestle and mortar, and DNA of cultured fungi was isolated using the DNeasy Plant Mini kit (Qiagen) according to the manufacturer's instructions. PCR amplification was performed using three primer pairs – ITS1 (White et al., 1990), ITS2 (ref), and CAL (ref) – and PCR products were submitted to Eurofins (Germany) for Sanger sequencing. Raw sequences were trimmed, assembled and manually checked, and the final sequences were used to support identification of the isolates based on searches in public databases (GenBank and BOLD Systems).

Photo 9-2. Dollar spot on golf greens in Germany (left), Sweden (middle) and Portugal (right), summer 2020.

Photos: Leonhard Anetseder, Marina Usoltseva and Carlos Guerrero

9.3 Results and discussion

The fungi which were identified as dollar-spot-like-fungi morphologically, were recovered from 13 of 37 turfgrass samples which were sent to NIBIO Landvik. The molecular analysis revealed that they belonged to at least two *Clarireedia* spp. – *C. jacksonii* and *C. homoeocarpa* (Table 9-1). *C. jacksonii* with 100% similarity was identified in a total of five cases from Portugal, Spain and Germany. *C. homoeocarpa* with up to 98.7-99% similarity was identified in eight turfgrass samples – from five German and from all three Swedish. The low percentages of similarity of isolates from two German turfgrass samples – NR6 and NR15 with *C. jacksonii* (97.2%) and *C. homoeocarpa* (98%) respectively – could be probably due to the fungi belonging to an unknown *Clarireedia* spp. The fungal species which were isolated from seven turfgrass samples from Denmark, Sweden, the UK and Germany – NR16-NR22 – did not belong to any of *Clarireedia* spp. but to other turfgrass pathogenic fungi: *Ceratobasidium cereale* causing yellow patch, *Fusarium culmorum*, *Fusarium oxysporum*, *Limonomyces roseipellis* causing pink patch, *Microdochium bolleyi* causing basal rot and *Waitea circinata* causing brown ring patch.

NR	ID ÅS	ID Landvik	Country	Turfgrass species	Fungal species	%[1]
1	2021-14	2016	Portugal	NA / Pure culture	*C. jacksonii*	100
2	2021-16	2019	Spain	*Agrostis*	*C. jacksonii*	100
3	2021-28	2045	Germany	*Festuca rubra, Poa annua, Agrostis*	*C. jacksonii*	100
4	2021-29	2046	Germany	*Agrostis, Poa annua*	*C. jacksonii*	100
5	2021-30	2049	Germany	*Poa annua*	*C. jacksonii*	100
6	2021-32	2052	Germany	NA / Pure culture	*C. jacksonii*	97.2
7	2021-25	2033	Germany	*Agrostis, Festuca rubra*	*C. homoeocarpa*	99
8	2021-15	2017	Germany	NA	*C. homoeocarpa*	98.7
9	2021-21	2023	Germany	*Poa annua, Agrostis*	*C. homoeocarpa/C. jacksonii*	98.7/97.2
10	2021-26	2037	Germany	*Poa annua*	*C. homoeocarpa/C. jacksonii*	98.7/97.2
11	2021-27	2039	Germany	*Poa annua, Agrostis*	*C. homoeocarpa/C. jacksonii*	98.7/97.2
12	2021-19	2021	Sweden	*Agrostis, Festuca rubra*	*C. homoeocarpa*	98.7
13	2021-17	2020-2-1	Sweden	*Agrostis, Festuca rubra*	*C. homoeocarpa/C. jacksonii*	98.7/97.2
14	2021-20	2022	Sweden	*Poa annua*	*C. homoeocarpa/C. jacksonii*	98.7/97.2
15	2021-31	2050	Germany	*Agrostis*	*C. homoeocarpa*	98
16	2021-36	2034 D1	Germany	NA	*Ceratobasidium cereale*	100
17	2021-22	2026	Denmark	*Agrostis, Festuca rubra*	*Fusarium culmorum*	100
18	2021-23	2027	Sweden	*Poa annua*	*Fusarium oxysporum*	100
19	2021-33	2053	UK	*Festuca rubra*	*Limonomyces roseipellis*	100
20	2021-24	2031	Germany	*Poa annua, Agrostis*	*Microdochium bolleyi*	100
21	2021-37	2038 B	Germany	NA	*Waitea circinata*	100
22	2021-38	2044 B	Germany	NA	*Waitea circinata*	100

Table 9-1. Blast identity of isolates using ITS1, ITS2 and CAL primers.

[1] Detailed data on % of similarity not shown. The results on which of three primers (ITS1, ITS2 or/and CAL) gave the highest percentage of similarity will be presented in a scientific publication in 2024-25, potentially at the 15th ITSC-2025.

8.4 Conclusions on benefits and advice for the golf and turfgrass sector

- ***Clarireedia jacksonii* and *C. homoeocarpa* are at least main causal species for dollar spot in Europe.**
- **Since dollar spot symptoms can be exchanged with the symptoms of other turfgrass diseases, the use of diagnostic services can be strongly recommended for greenkeepers in order to define a disease correctly and to choose appropriative integrated turf management techniques and strategy.**
- **Future research should focus on practical importance of the differences between/among clarireedia species.**

Figure 9-3. Dollar spot like symptoms on a golf green on a European golf course, 25-9-2023.

Photos: Tatsiana Espevig

Espevig, T., M.B. Brurberg, A. Kvalbein. 2015. First Report of Dollar Spot, Caused by *Sclerotinia homoeocarpa*, of Creeping Bentgrass in Norway. Plant disease 99:287.

Espevig T., Bruberg M.B., Dahl Å., Usoltseva M., Normann K., Crouch J.A., Kvalbein A., Aamlid T.S. Risk assessment, management and control of dollar spot caused by *Sclerotinia homoeocarpa* on Scandinavian golf courses. Annual report. STERF Research and development yearbook 2016. p. 48-49. ***http://www.sterf.org/Media/Get/2779/annual-report-2016***

Salgado-Salazar C, Beirn LA, Ismaiel A, Boehm MJ, Carbone I, Putman AI, Tredway LP, Clarke BB, Crouch JA. Clarireedia: A new fungal genus comprising four pathogenic species responsible for dollar spot disease of turfgrass. 2018. Fungal Biol. 122(8):761-773.

10

Seeds as a source for dollar spot

Photo: Tatsiana Espevig

10.1 Introduction

The increased and rapid spread of dollar spot in the Nordic countries during the past 10 years is not completely understood and can be due to several factors. A warmer climate combined with increased international traffic that brings diseased plant material on golf shoes and golf clubs from one country to another is probably an important reason (Kvalbein, 2016). Riox et al., (2014) using culture-based seed analysis detected presence of dollar spot in commercial seeds and hypothesised that seeds can be a potential source of initial inoculum on golf courses. At Landvik, sporadic dollar spot symptoms on SCANTURF variety trials have been seen on newly established sward in 2011, 2018-19 and in 2020. The objective of this study was to find out whether commercial seeds can be a pathway for dollar spot outbreaks and spread.

Photo 10-1. Dollar spot on newly seeded Kentucky bluegrass in SCANTURF trial at Landvik – 09-08-2019.

Photo: Tatsiana Espevig

10.2 Materials and methods

The commercial seeds of 10 turfgrass varieties were used in this study: Kentucky bluegrass (*Poa pratensis*) 'anisha', 'barserati', 'geisha', 'impact', 'limousine', 'strenuus', chewings fescue (*Festuca rubra commutata*) 'musica', creeping bentgrass (*Agrostis stolonifera*) 'independence' and colonial bentgrass (*Agrostis capillaris*) 'greenspeed'. The seeds of all six varieties of Kentucky bluegrass were taken from the same parties which were used for establishment of SCANTURF in 2019 and on which dollar spot symptoms were recorded on 2-4% of the plots. The surface of the seeds was washed with either 70% ethanol (standard procedure for surface sterilisation) (1) or with azoxystrobin which was diluted to the recommended dosage (2) since dollar spot fungi are known to have low sensitivity to azoxystrobin (Gagkaeva et al., 2022) and were expected to grow if clarireedia was not only inside but also outside the seeds. Ten seeds of each variety were placed into Petri plates filled with water agar. The target fungi were determined based on morphological features using microscopic study and purified by transferring into new Petri plates filled with 50% PDA (potato dextrose agar). The experiment was performed in five replicates, totalling 50 seeds per variety and per surface sterilisation product. The pure cultures were sent for molecular analysis to NIBIO Molecular plant biology laboratory at Ås (Norway). DNA isolation, amplification and sequencing was conducted using the same method as described previously in Chapter 9.

10.3 Results and discussion

At the stage of microscopy and visual studying of morphology of the fungi which grew from the seeds, no fungi which had all clarireedia signs were found. The identified fungi were *Alterternaria* spp., *Cladosporium* spp., *Fusarium* spp. and *Penicillium* spp. In total, morphology of 449 colonies was studied. Only two colonies had some morphological similarity with *Clarireedia* spp. both mycelium and pure culture, see plates 12 and 85 with pure cultures on Photo 10-2. Both were from seeds of 'geisha' treated with either ethanol (12) or azoxystrobin (85). However, no *Clarireedia* spp. were determined using PCR analysis. Some alive non-pathogenic nematodes were also found in the seeds.

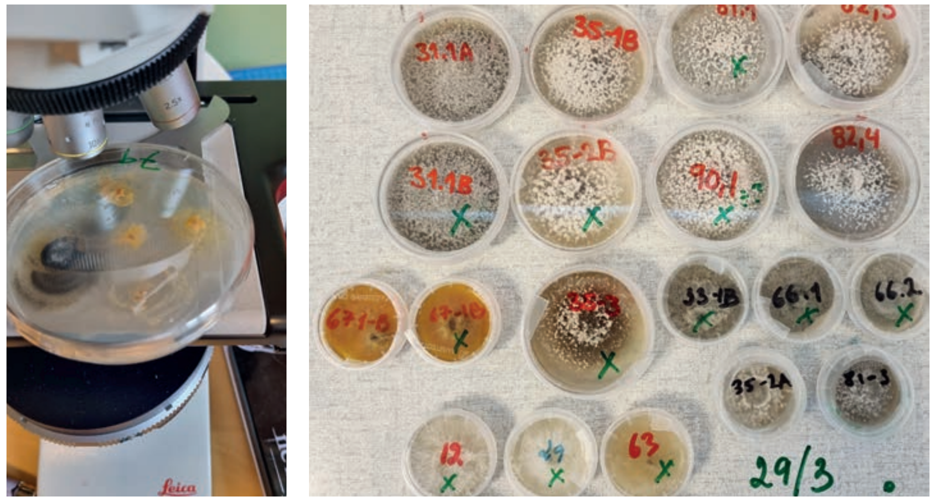


Photo 10-2. Determination of target fungi using microscope (left) and pure cultures which were sent for molecular identification (right).

Photos: Tatsiana Espevig

Ids	ITS1	%	ITS2	%	CAL	%
2022-1	*Neoascochyta desmazieri*	100	*Neoascochyta desmazieri*	100	NA	
2022-2	*Apiospora arundinis*	100	*Apiospora arundinis*	*100*	NA	
2022-3	*Apiospora arundinis*	100	*Apiospora arundinis*	*100*	NA	
2022-4	*Alternaria infectoria*	99,8	*Alternaria infectoria*	100	*Alternaria triticina*	99,2
2022-5	*Alternaria alternata*	100	*Alternaria alternata*	100	*Alternaria arborescens*	100
2022-6	*Alternaria infectoria*	100	*Alternaria infectoria*	100	*Alternaria triticina*	99,5
2022-7	*Alternaria infectoria*	100	*Alternaria infectoria*	*100*	*Alternaria triticina*	99,2
2022-8	*Alternaria infectoria*	100	*Alternaria infectoria*	100	*Alternaria triticina*	99,2
2022-9	*Chaetomium subaffine*	100	*Chaetomium acropullum*	*100*	NA	
2022-10	*Alternaria alternata*	100	*Alternaria alternata*	*100*	NA	
2022-11	*Alternaria alternata*	100	*Alternaria alternata*	*100*	NA	
2022-12	*Epicoccum nigrum*	100	*Epicoccum nigrum*	*100*	NA	
2022-13	*Alternaria infectoria*	100	*Alternaria infectoria*	*100*	NA	
2022-14	*Alternaria infectoria*	100	*Alternaria infectoria*	*100*	NA	
2022-15	*Alternaria infectoria*	100	*Alternaria infectoria*	*100*	NA	
2022-16	*Alternaria infectoria*	100	*Alternaria infectoria*	100	*Alternaria triticina*	99,2
2022-17	*Alternaria infectoria*	100	*Alternaria infectoria*	100	*Alternaria triticina*	99,0
2022-18	*Alternaria infectoria*	100	*Alternaria infectoria*	*100*	*Alternaria triticina*	99,2
2022-19	*Alternaria infectoria*	100	*Alternaria infectoria*	*100*	*Alternaria triticina*	100

Table 10-1. Blast identity of isolates using ITS1, ITS2 and CAL primers.

8.4 Conclusions on benefits and advice for the golf and turfgrass sector

- **Seeds can be a potential source of initial infection of dollar spot and epidemics, but this fact needs more research with bigger seed lots.**

Figure 10-3. Preparing of turfgrass seeds for analysis.

Photo: Ove Hetland (left) and Tatsiana Espevig (right)

Figure 10-4. Grass seeds can be a source for diseases. Infected seeds of colonial bentgrass.

Photo: Ove Hetland

Gagkaeva, T., Orina, A., Gavrilova, O., Usoltseva, M., Crouch, J. A, Normann, K., Entwistle, K., Torp, T., & Espevig, T. (2022). In vitro fungicide sensitivity of Clarireedia, Fusarium, and Microdochium isolates from grasses. International Turfgrass Society Research Journal, 14, 972–980. ***https://doi.org/10.1002/its2.139***

Kvalbein A. 2016. Hygienetiltak på golfbanen. Gressforum 1:30-32.

Rioux, R.A., Shultz, J., Garcia, M., Willis, D.K., Casler, M., Bonos, S., Smith, D. and Kerns, J. (2014) Sclerotinia homoeocarpa overwinters in turfgrass and is present in commercial seed. PLoS One 9, e110897.

11

Chafer grubs and leatherjackets on golf courses

A literature review

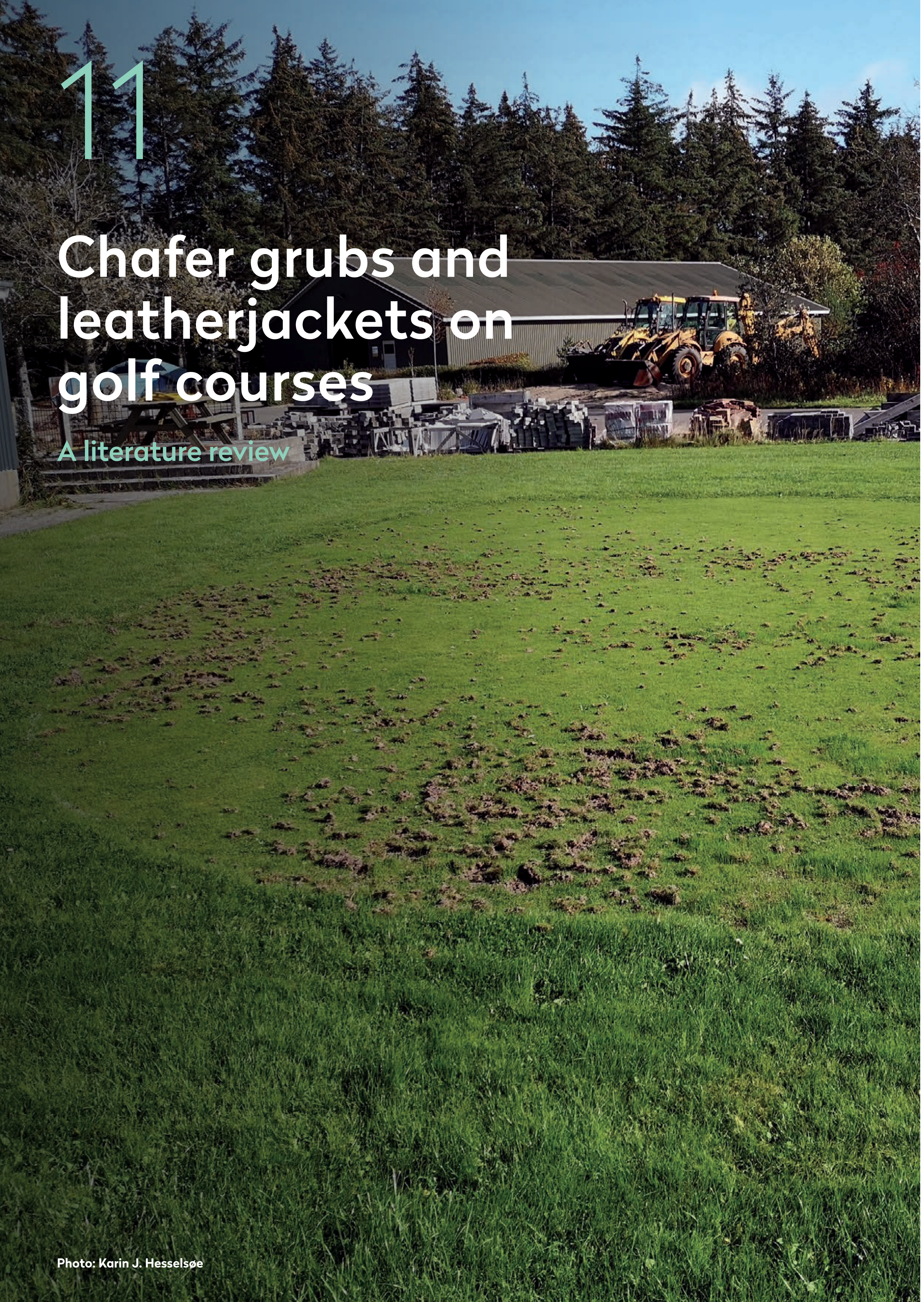


Photo: Karin J. Hesselsøe

11.1 Introduction

Many soil insect pests are long-known problems in European turfgrass, such as *Scarabaeidae larvae* (e.g., the garden chafer (*Phyllopertha horticola)*, the June beetle (*Amphimallon solstitiale)*, the European cockchafer (*Melolontha melolontha*)), click beetle (*Elateridae larvae*) (e.g., wireworms (*Agriotes* spp), cutworms (*Noctuidae larvae*) (e.g., *Agrotis* spp), root moths (*Hepialidae larvae*) (e.g., the common swift moth (*Korscheltellus lupulina*)), or crane fly (*Tipulidae larvae*) (e.g., leatherjackets (*Tipula* spp.) (Toepfer et al., 2009).

Some soil pests, such as the corn rootworm (*Diabrotica virgifera virgifera*), can be considered as emerging pests. Further, for example, *Melolontha melolontha* was nearly eradicated in the middle of the 20th century through broad-spectrum pesticides; however, it is regaining its historic importance as a soil pest. Other scarabeidae that have recently increased their status as pests, include the Welsh chafer (*Hoplia philanthus*) in lawns and gardens and dung beetles (*Aphodius* spp.) in turf and tree nurseries (Toepfer et al., 2009). In addition, along with the leatherjackets, larvae of marsh flies (*Bibionidae*), have occurred in masses in Finnish golf courses (Hokkanen et al., personal communication).

Full NIBIO report available at: http://www.sterf.org/Media/Get/4163/report-chafer-grubs-and-leatherjackets

Updated STERF Factsheets in Danish available at:
Gåsebiller http://www.sterf.org/Media/Get/4157/faktablad-gasebiller-2023-2.pdf
Stankelben http://www.sterf.org/Media/Get/4189/faktablad-stankelben-dansk-2023.pdf

11.2 Current status of insect pests on golf courses in the Nordic countries

No thorough surveys by entomologists have been carried out on golf course pests in the Nordic countries. The surveys that have been conducted have relied mainly on greenkeepers' assessments of the problematic pests, but exact identities of the pest species remain to be determined. Therefore, broad groups of "chafer grubs" and "leatherjackets" are used here.

Chafer grubs: During the past 10-15 years, damage in the Nordic countries has been limited to a few golf courses in each country, with sporadic attacks from year to year. Most damage has been observed on sandy soils.

Leatherjackets: In recent years, more widespread and probably increasing damage has occurred on golf courses, with 5.1 or 20% of courses affected in different countries. Most abundant on moist areas.

Figure 11-1. Close-up of fully developed chafer grubs.

Photo: www.syngentaturf.co.uk

Figure 11-2. Larvae of leatherjackets and marsh flies on a golf course in Finland.

Photo: H. Hokkanen

11.3 Possible control methods and actions

Other than parks, golf courses are among the few remaining nature refuges in urban environments and have the potential to maintain biodiversity in urban environments. Insects play a crucial role in these assets, which with skilful planning can accrue multiple benefits: improved pest control, enhanced biodiversity and beautiful landscapes contributing to player satisfaction and social acceptance of the industry. See Figure 11-3.

After banning the use of chemical pesticides on golf courses, greenkeepers have to become experts in using alternative control strategies. This includes applications with microbiological control agents such as entomopathogenic nematodes, entomopathogenic fungi and strains of the bacterium *Bacillus thuringiensis*. Better monitoring and warning to improve determination on how to use these alternative control strategies correctly is necessary.

Additional non-chemical control methods include irrigation on greens and surroundings for chafer grub control at daytime when beetles are swarming to prevent them laying eggs. An additional measure for leatherjacket management includes setting up nest boxes for starlings and sheeting greens in spring.

Overall, a more comprehensive pest management strategy needs to be developed, based on the principles of ecostacking (stacking of ecosystem services for improved biocontrol in this case). Golf course managers and greenkeepers should add to their skills to increase their expertise in using alternative and biological methods for pest management.

It will be important to improve our knowledge on the utilisation of ecosystem services in particular in respect of improving natural control of pest insects. Very little is known about parasitoids and diseases of the key pests – chafer grubs and leatherjacket – and of ways of supporting their action. Literature states, for example, that “Natural control of European chafer by predators, parasites and pathogens is excellent in Europe” and that several parasitic fly species and two parasitic wasps are important, along with diseases such as a protozoan, a bacterium and rickettsia (East and Willoughby 1983). A PhD-thesis from the UK recorded numerous natural enemies of leatherjackets in Scotland, including viruses (*Tipula* iridescent virus, and a nuclear polyhedrosis virus), intestinal gregarines (*Gregarina longa*, *Hirmocystis ventricose* and *Actinocephalus tipula*) and a mermithid nematode. Further natural enemies identified were a species of parasitic tachinid fly (*Siphona geniculata*) and the predatory carabid beetles (*Nebria brevicollis* and *Pterostichus madius*) (Barbash 1988).

Another study found a high incidence of lethal parasites in the larvae of a *Tipula paludosa* population over two seasons of the study. The proportions of larvae infected with *Tipula* iridescent virus and a tachinid insect were similar to those in previously studied populations, whereas the proportions of larvae infected with *Tipula* nuclear polyhedrosis virus and a spore-forming bacterium were higher. Conservative estimates of mortality due to these four agents were 10.7% in 1977–1978 and 7.7% in 1978–1979 (Carter et al., 1983).

These examples show that there is good potential to utilise the biological control potential of naturally occurring natural enemies of chafer grubs and leatherjackets, utilising the ecostacking approach and techniques (Hokkanen 2017, Hokkanen and Menzler-Hokkanen 2020, Hokkanen and Menzler-Hokkanen 2024, Wang et al., 2024).

Figure 11- 3 Flower edge established at the Hirsala Golf Course in Southern Finland. Flowering plants provide food (pollen and nectar) to a variety of beneficial insects including parasitic wasps (biological control agents) and pollinators, as well as shelter. *Photo: Janne Lehto*

11.4 Conclusions on benefits and advice for the golf and turfgrass sector

- **Since it is not realistic to get Emergency Authorisations to use acelepryn (as in the UK) or other synthetic insecticides to control chafer grubs and leatherjackets in Scandinavia (Pers. Comm. Torben K. Petersen), biological and other alternative products should be used. Good communication with the golfers is therefore essential when a golf course is damaged by chafer grubs or/and leatherjackets, as although the biological methods have been tested, golfers must get used to further damage from insect pests now and in the future. They also must accept that the biological methods are more expensive and less effective than the synthetic insecticides.**
- **For control of the leatherjackets, grubs and other pest insects in turf, more research should be conducted into improving the efficacy and reliability of entomopathogenic nematodes (EPN). Overall, a more comprehensive pest management strategy needs to be developed, based on the principles of ecostacking (stacking of ecosystem services for improved biocontrol in this case). Meanwhile course managers and greenkeepers must improve their skills to become experts in using alternative and biological methods.**

Figure 11-4. Application of entomopathogenic nematodes in Finland.

Photo: H. Hokkanen

Barbash, N. M. (1988). Studies on the biology of Tipula paludosa Meigen (Diptera: Tipulidae) with special reference to mortality factors. University of Glasgow (United Kingdom).

Carter, J. B., Green, E. I., & Kirkham, A. J. (1983). A Tipula paludosa population with a high incidence of two pathogens. Journal of Invertebrate Pathology, 42(3), 312-318.

East, R., & Willoughby, B. E. (1983). Grass grub (Costelytra zealandica) population collapse in the northern North Island. New Zealand journal of agricultural research, 26(3), 381-390.

Hokkanen, H. M.T. & Menzler-Hokkanen I. (2024, in press): The Concept of Ecostacking. Chapter 1 in: Wang JJ et al., (Editors, in press). The Concept of Ecostacking: Techniques and Applications. CABI Ecostacking Series, Volume 1. CABI, Wallingford, UK.

Hokkanen, H.M.T. & Menzler-Hokkanen, I. (2020): Improving the efficacy of biological control by ecostacking. Chapter 1 in: Gao, Y., Hokkanen, H.M.T., Menzler-Hokkanen, I. (eds), Integrative Biological Control. Progress in Biological Control Volume 20, Springer-Nature, Dordrecht, The Netherlands, ISBN 978-3-030-44837-0.

Hokkanen, H.M.T. (2017): Ecostacking: maximising the benefits of ecosystem services. Arthropod-Plant Interactions 11: 471-472. *https://doi.org/10.1007/s11829-017-9575-8*.

Toepfer, S., Enkerli, J., & Kuhlmann, U. (2009). Research needs and promising approaches for the biological control of Diabrotica and other emerging soil insect pests with pathogens or nematodes. Insect Pathog. Insect Parasit. Nematodes IOBC/wprs Bull, 45, 47-58.

Wang JJ, Liu H, Menzler-Hokkanen I, Jiang H (Editors, 2024, in press). The Concept of Ecostacking: Techniques and Applications. CABI Ecostacking Series, Volume 1. CABI, Wallingford, UK.

R&A

The R&A group of companies was formed in 2004 to take on The Royal and Ancient Golf Club of St Andrews' responsibilities for governing the Rules of Golf, staging The Open, golf's original championship, and developing the sport. The World Golf Museum in St Andrews is part of The R&A group.

Together The R&A and the USGA govern the sport of golf worldwide, operating in separate jurisdictions with a commitment to a single code for the Rules of Golf, Rules of Amateur Status and Equipment Standards. The R&A, through R&A Rules Ltd, governs the sport worldwide, outside of the United States and Mexico, on behalf of over 41 million golfers in 144 countries and with the consent of 159 organisations from amateur and professional golf.

The R&A has responsibility for running a series of world class amateur events and international matches in women's and girls' as well as men's and boys' golf. The R&A stages the AIG Women's Open and works with the DP World Tour to stage the Senior Open presented by Rolex.

The R&A is committed to investing £200 million over ten years in developing golf and supports the growth of the sport internationally, including the development and management of sustainable golf facilities. For more information, visit www.randa.org.

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Photo: Leonhard Anetseder