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Testing of alternative plant protection products for the control of *Microdochium nivale* and other diseases on golf greens

Final report from a three year project, Oct. 2011- Sep. 2014

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SCANDINAVIAN TURFGRASS AND ENVIRONMENT RESEARCH FOUNDATION

Reference group visiting trial at Kävlinge GC, Sweden, on 21 March 2013. Photo: Trygve S. Aamlid

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Testing of alternative plant protection products for the control of *Microdochium nivale* and other diseases on golf greens.Final report from a three year project, Oct. 2011- Sep. 2014.

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Summary:

This report presents results from a project testing Turf G+/WPG (fungal products containing *Gliocladium catenulatum*) and Turf S+/WPS (bacterial products containing *Streptomyces* spp.), both from Interagro BIOS AB, and Vacciplant (seaweed product containing laminarine) from Nordisk Alkali AB, for the control of *Microdochium nivale* and other diseases on golf greens. Five field trials were carried out in Denmark, Sweden and Norway from October 2011 to September 2014, and Turf G+/WPG and Turf S+ were tested also *in vitro*.

None of the test-products gave any consistent disease control in the field trials. A significant reduction in *Microdochium nivale* from 3 % of plot area on untreated plots to 2 % on treated plots was seen in one trial, but this was considered to be of little practical relevance. In all other trials with more severe attacks of *Microdochium nivale*, only the fungicide control treatment showed a significant reduction in disease compared with the untreated control. On average for all field trials over three years, the higher rate of Vacciplant, the combination of Turf G+/WPG and Turf S+/WPS, and the fungicide treatment gave, in turn, 22, 24 and 87 % less microdochium patch in the fall, but among these, only the effect of fungicide was significant. The effects of the biological products on pink or gray (*Typhula incarnata*) snow mold after snow melt were even smaller.

In the in vitro trials, Turf S+ provided good control of *Microdochium nivale* at 6 and 16 °C, but Turf G+/WPG was effective only at the higher temperature. However, since these results could not be repeated under field conditions, we have to conclude that none of the test products represent any real alternative to fungicides for control of *M. nivale* or other diseases on Scandinavian golf courses.

Bioforsk Landvik,
1 November 2014
Trygve S. Aamlid
Project leader

Contents

1.Introduction

The most important turfgrass pathogen in Scandinavia is the *Microdochium nivale*. This fungus causes both microdochium patch during the growing season and pink snow mold during or shortly after snow melt. Most golf courses in Norway and Sweden, and quite a few in Denmark, spray their greens routinely with fungicides against this fungus before winter. However, Directive 2009/128/EG of the EU on establishing a framework for Community action on achieving sustainable use of pesticides, calls for a reduction in pesticide use through the introduction of integrated pest management (IPM) and replacement of pesticides with low risk alternatives. The Scandinavian Turfgrass and Environment Research Foundation (STERF) has identified IPM of golf courses as a number one research priority for the period 2011-2015 Thus, the objectives of this project, coordinated by STERF and funded by industrial partners through a grant from the Danish Environmental Protection Agency, were:

1) To provide documentation according to 'Good Experimental Practice' standards for potential registration of Turf S+/WPS (a bacterial product containing *Streptomyces* spp.) and Turf G+/WPG (a fungal product containing *Gliocladium catenulatum*), both from Interagro BIOS AB, and Vacciplant (a seaweed product containing laminarine) from Nordisk Alkali AB, for use on golf courses

2) To find the most optimal way of using these product(s) for the control of *Microdochium nivale* and other turfgrass pathogens and disseminate this knowledge to greenkeepers in the Scandinavian countries

Figure 1. Patch of *Microdochium nivale* on a golf green. Photo: Tatsiana Espevig

2. Field trials

2.1 Protocol

The protocol prescribed field trials according to Good Experimental Practice (GEP). The trials should follow a randomized complete block design with at least three or four replicates. The protocol was developed in October 2011 for the first experimental period 1 Oct. 2011 – 31 May 2012 and revised slightly before the second and third experimental period 1 June 2012 – 31 May 2013 and 1 June 2013-1 Sep. 2014, respectively.

The treatments were:

1. Unsprayed (negative control)
2. Fungicide(s) (positive control). Products, rates and applications intervals varied depending on current labels in each country:
    a. Denmark:
        i. 2011-12 and 2012-13: Folicur EC 250, 1.0 l ha^{-1} = tebuconazole, 250 g a.i. ha^{-1}, two applications four weeks apart in October-November.
        ii. 2013-14: Proline 250 EC, 0.8 l ha^{-1} = prothioconazole, 200 g a.i.ha^{-1}, two applications four weeks apart in October-November.
    b. Norway: Delaro SC 325, 1.0 l/ha = prothioconazole, 175 g a.i. ha^{-1} + trifloxystrobin, 150 g a.i. ha^{-1}, two applications four weeks apart in October-November.
    c. Sweden:
        i. 2011-12: Amistar, 1.0 l/ha = axoxystrobin, 250 g a.i. ha^{-1}, two applications four week apart in October-November.
        ii. 2012-13 and 2013-14: Amistar, 1.0 l/ha = axoxystrobin, 250 g a.i. ha^{-1}, one application in October followed by two applications of Medallion, 3.0 l/ha = fludioxonil, 375 g a.i. ha^{-1}, four weeks apart in November-December.
3. Turf G+ / Turf WPG: A new formulation of *Gliocladium catenulatum* was launched in 2012, hence the protocol was changed during the project period:
    a. 2011-12: Turf G+, 10 l ha^{-1}, applications at four week interval from mid-October until snow cover plus two applications coinciding with day temperatures 5 and 10°C in spring.
    b. 2012-13 and 2013-14: Turf WPG, 1 kg ha^{-1}, applications at four week interval from mid-October until snow cover plus two applications coinciding with day temperatures 5 and 10°C in spring.
4. Turf S+ / WPS: A new formulation *Streptomyces* spp. was launched in 2014, hence the protocol was changed during the project period:
    a. 2011-2013: Turf S+, 1.0 l ha^{-1}, applications at four week intervals during summer, the first application coinciding with day temperature 15°C.
    b. 2014 Turf WPS, 400 g ha^{-1}, applications at four week intervals during summer, the first application coinciding with day temperature 15°C.
5. As treatment 3 + 4.
6. Vacciplant, 1 l ha^{-1} = laminarin, 45 g ha^{-1}, applications at four week intervals from mid-October until snow cover plus two applications coinciding with day temperatures 5 and 10°C in spring.
7. Vacciplant, 2 l ha^{-1} = laminarin, 90 g ha^{-1}, applications at four week intervals from mid-October until snow cover plus two applications coinciding with day temperatures 5 and 10°C in spring.

The project received a temporary approval by the Danish Environmental Protection Agency in mid-October 2011. This was later than optimal, but it was decided to start the field trials in the late fall by condensing the spraying interval from four to three weeks (week 42, 45 and 48, weather permitting). In 2012, 2013 and 2014, the spraying interval was always four weeks. Assessments of disease, turfgrass overall impression (1-9, 9 is highest quality) and turfgrass color (1-9, 1= completely brown/faded, 9 is most intensely green) were made at monthly intervals (before each application).

2.2 Trial at Rungsted GC, Denmark

2.2.1 Materials and methods

2.2.1.1 Experimental site

The trial was established on 20 Oct. 2011 on green no 9 at Rungsted Golfklub, Vestre Stationsvej 16, 2960 Rungsted Kyst, Denmark, GPS coordinates: N: 55.88120, E: 12.52877 (Figure 2). The green was an old push-up green, established approximately 1937. The botanical composition at the start of the trial was 45% *Poa annua*, 45% *Agrostis capillaris* and 10% *Festuca rubra*. Root depth was 5-7 cm.

The trial was discontinued on 16 May 2012 as the golf club decided to spray the entire green with fungicide.

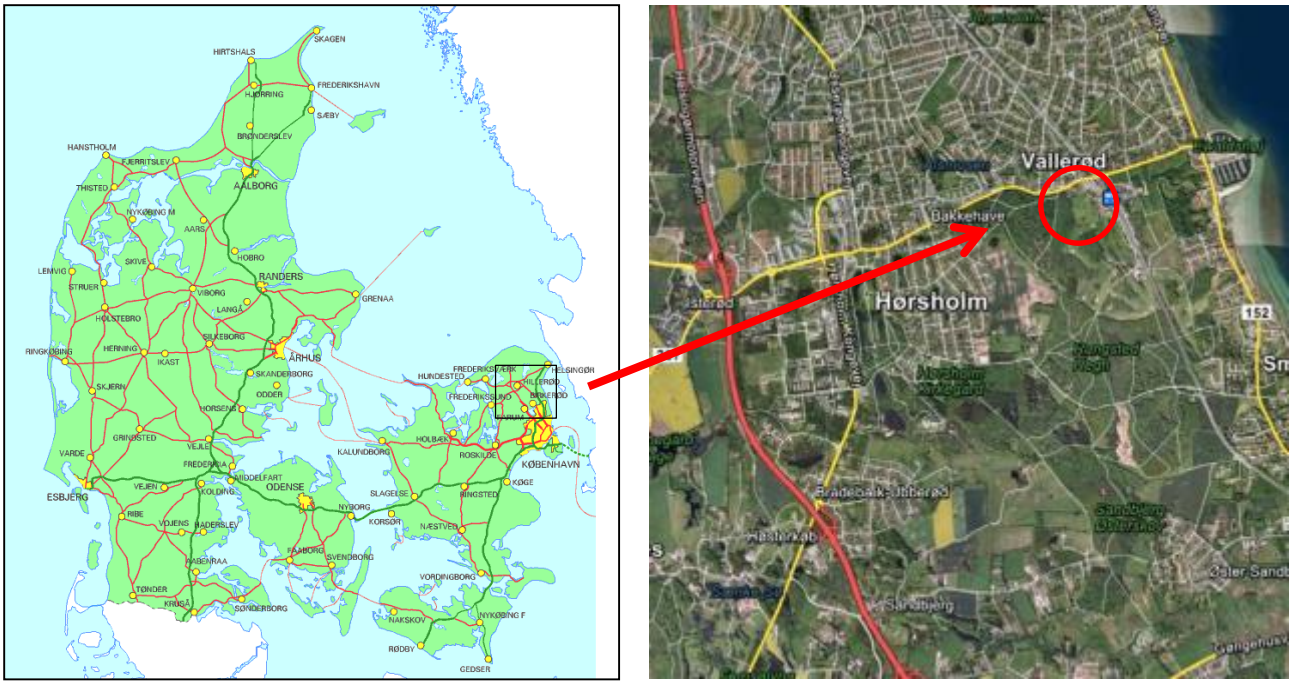


Figure 2 a,b. Maps showing location of trial at Rungsted GC.

2.2.1.2 Turfgrass maintenance

From 30 March to 10 September 2011 the green had received monthly applications of Scotts Invigorator 4-0-8 (NPK), in total 71.5 kg N ha^{-1}. The green had been topdressed at regular intervals and received monthly applications of the wetting agent Revolution, 19 l ha^{-1}, from May to August. Mowing height at the start of the trial on 20 October was 6 mm; this was raised to 7 mm at the last mowing on 12 November 2011. Overseeding was conducted on 19 March and 14 May 2012 with a seed mixture of *Festuca rubra* and *Agrostis capillaris*.

2.2.1.3 Implementation of protocol

Figure 3 gives an overview of the trial area. Plots were 3.5 long and 2.5 m wide. Products were applied using a bicycle track sprayer (Figure 4) with 25 cm distance between nozzles which were of type Hardi F-015-110. The sprayer was equipped with a Lykketronic PX Combi Spray computer and worked at a pressure of 3.0 bar. The spraying volume was 400 l ha^{-1} in all treatments. Application dates are given in Table 1.

Table 1. Applications dates in trial at Rungsted

Date of application	Treatments
20 October 2011	2, 3, 5, 6, 7
10 November 2011	2, 3, 5, 6, 7
30 November 2011	3, 5, 6, 7
22 March 2012	3, 5, 6, 7
18 April 2012	3, 5, 6, 7
16 May 2012	4, 5

Figure 3. Trial on green no 9 at Rungsted ready for first application on 20 October 2011. Photo: Klaus Paaske.

Figure 4. Bicycle track sprayer used in Danish trials. Photo: Klaus Paaske.

2.2.1.4 Weather data

Weather data during the trial period, recorded at the Danish Meteorological Institute's nearest station, are shown in Table 2.

The summer 2011 was warm and very wet and this weather type continued in September. Also October and November were warmer than normal but also much dryer. The warm weather continued until the end of January when it changed dramatically to cold weather. There was no snow during November and December 2011 or January 2012, but the trial was covered with snow from 5 to 19 February with a maximal snow depth of 10 cm. Thereafter it was again mild and no frost.

Table 2. Mean monthly temperature and monthly precipitation compared with 30 year normal values. Data from MET Station 6188 Sjælsmark situated approximately 5.5 km from the trial site at Rungsted GC.

	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.
Temperature, °C								
- 2011/2012	14.2	9.8	6.6	4.2	2.1	-0.8	5.5	6.5
- Average 1961-1990	12.9	9.3	4.8	1.5	-0.2	-0.3	2.0	5.9
Precipitation, mm								
- 2011/2012	59	47	8	57	86	38	14	47
- Average 1961-1990	60	56	61	46	46	30	39	39

2.2.1.5 Statistical analyses

The ARM program (ARM 8, Gylling Data Management Inc.) was used for data management and statistical calculations. Homogeneity of variance was tested by Bartlett's test. In case this test indicated no homogeneity of variance, analysis of variance was performed on transformed data. If still no homogeneity of variance was obtained by the transformation, the statistical analysis should be treated with caution. In case a transformation was made, this is indicated in the tables. The data were subjected to analysis of variance, and treatment means were separated at the 95% probability level using F-test (Student-Newman-Keuls test).

2.2.2 Results

Results from assessments of microdochium patch are shown in Table 3.

When the first application was made on 20 October 2011 no visible symptoms of microdochium patch could be seen on any of the plots (Figure 3). At the second application on 10 November an incipient attack could be found on all plots except those that had been sprayed with Folicur (chemical control). The presence of *Microdochium nivale* was confirmed in samples analysed by Bioforsk Turfgrass Diagnostic Lab. on 17 November (Figure 5a,b). At the assessment on 22 December (Figure 6), the attack had increased to 33.8% on untreated plots. The next assessment was made in February when the snow was gone and this assessment showed no further development of the patches. The disease was still very visible at the last assessment on 16 May 2012.

No significant difference was found between the treatments with Turf G+ or Vacciplant and the untreated control. On average for all assessments from 20 Nov. to 16 May, disease severity was 22% less on plots treated with Vacciplant, 1 l ha^{-1}, than on untreated control plots, but the difference was not significant.

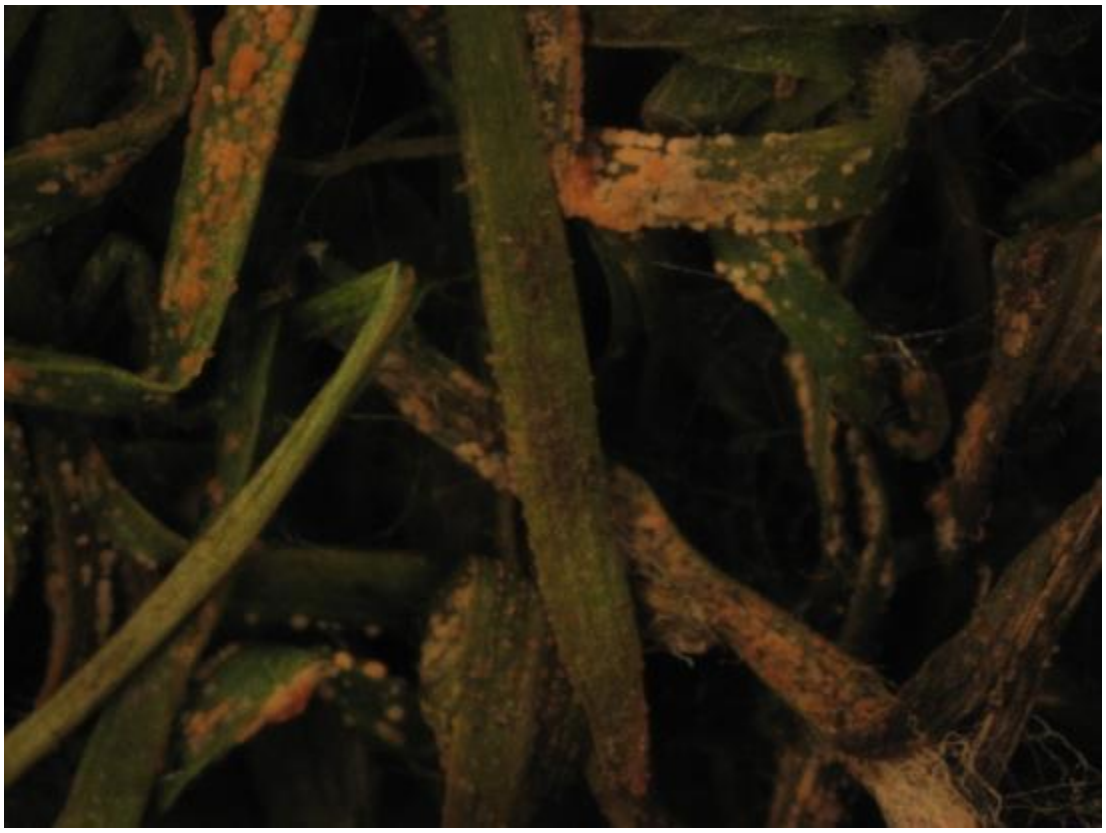
The effect of Turf S+ cannot be evaluated as these plots were untreated until the last assessment.

Phytotoxicity was assessed after each application, but no damage was found on the turf at any time during the trial.

Table 3. Summary of assessments at Rungsted GC.

Treatment	% of plot showing symptoms of *Microdochium nivale*						
	10 Nov. 2011	30 Nov. 2011	22 Dec. 2011	20 Feb. 2012	22 March 2012	18 April 2012	16 May 2012
1. Untreated	8.5 a*	20.5 a*	33.8 a*	32.5 a*	36.3 a*	35.0 a*	30.0 a*
2. Folicur	0 b	0.4 b	1.8 b	1.6 b	2.5 b	2.8 b	0.9 b
3. Turf G+	5.5 a	18.8 a	33.8 a	31.3 a	35.0 a	36.3 a	30.0 a
4. Turf S+	6.0 a	17.3 a	31.3 a	36.3 a	40.0 a	37.5 a	28.8 a
5. Turf G+ / Turf S+	5.5 a	16.3 a	31.3 a	35.0 a	41.3 a	42.5 a	32.5 a
6. Vacciplant 1.0 l	5.8 a	14.3 a	27.5 a	27.5 a	27.5 a	27.5 a	22.5 a
7. Vacciplant 2.0 l	6.5 a	15.8 a	27.5 a	28.8 a	31.3 a	28.8 a	22.5 a

*: Analyses were performed on log(x+1) transformed data. Within each column, means followed by the same letter are not significantly different according to Student Newman Keul's multiple comparison test at *P*=0.05.



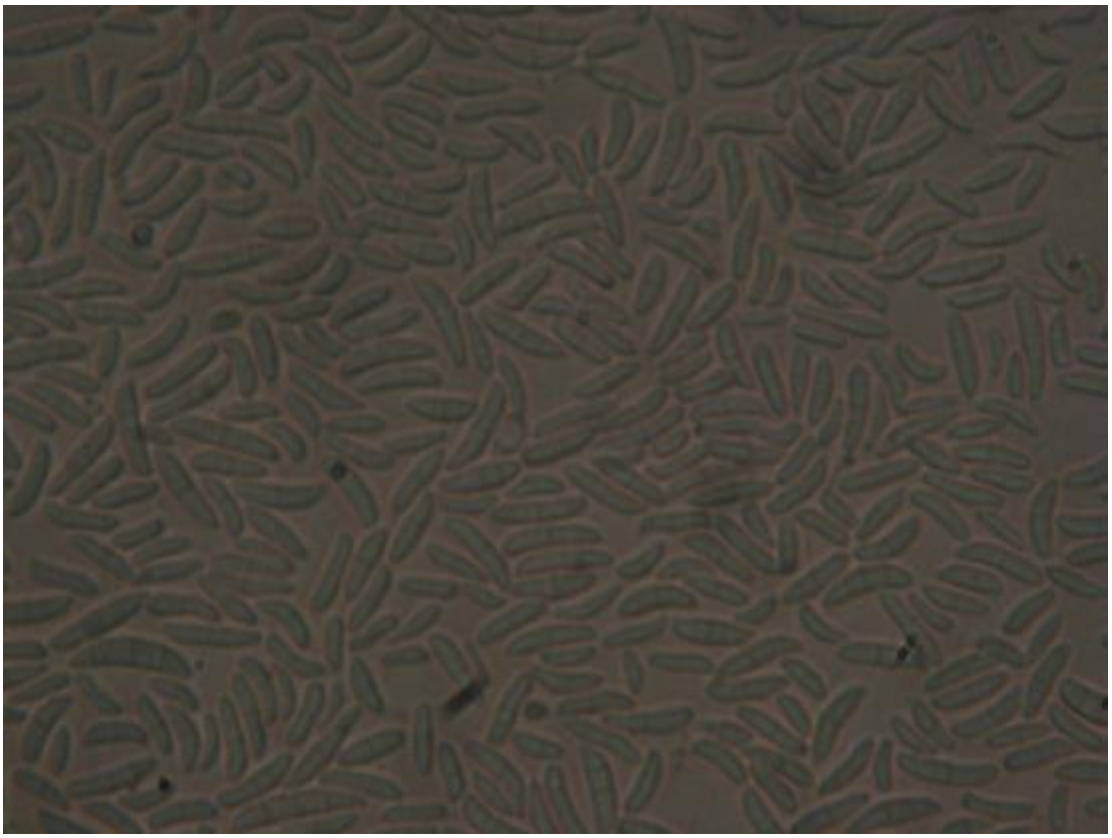


Figure 5 a, b. Sporodochia (top) and spores (bottom) of *Microdochium nivale* in samples taken from trial at Rungsted on 17 November 2011 and analysed in the Bioforsk Turfgrass Diagnostic Lab. Photos: Tatsiana Espevig.

Figure 6 a, b. Various treatments at assessement on 22 Dec. 2011. Photo: Klaus Paaske.

2.3 Trial at Sydsjælland GC, Denmark

2.3.1 Materials and methods

2.3.1.1 Experimental site

The trial was established on 11 July 2012 on green no 1 of the PAR 3 course at Sydsjælland Golfklub, Præstø Landevej 39, Mogenstrup, 4700 Næstved, Denmark, GPS coordinates: N: 55.18462, E: 11.86785 (Figure 7), as a replacement for the trial that had to be discontinued at Rungsted GC.

The experimental green at Sydsjælland had been constructed according to USGA standard in 2005 and seeded / overseeded with a green mixture consisting of *Festuca rubra* and *Agrostis capillaris*, Botanical analyses in October 2012 showed that the ratio between the two species was about 2:1. In addition there was 5-33 % (mean 11 %) *Poa annua*, with the highest amounts on the most eastern plots in block I (treatment 5) and IV (treatment 3, Figure 8).

At the final assessment on 27 Aug. 2014, after a warm and dry summer, the botanical composition was 90 % *Festuca rubra* (variation 80-96 %), 6 % *Agrostis capillaris* (variation 3-10 %) and 4 % *Poa annua* (variation 1-10 %). The experimental treatments had no effect on the proportion of the three species.

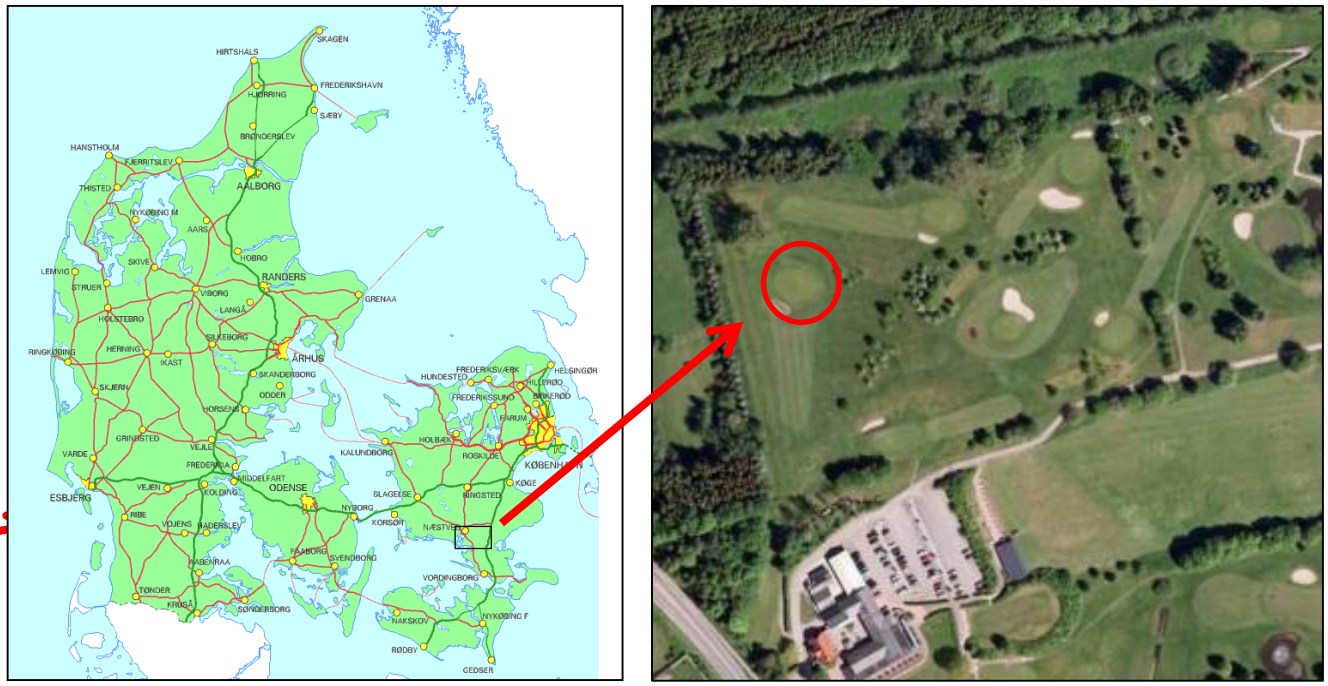


Figure 7 a,b. Maps showing location of trial on green no 1 on the PAR 3 course at Sydsjælland GC.

2.3.1.2 Turfgrass maintenance

The seasonal fertilizer rates in the three growing 2012, 2013 and 2014 seasons were 87-92 kg N, 21-24 kg P and 105-135 kg K per ha. Fertilizers were applied every second week. The last applications before winter were on 17 September 2012 and 27 September 2013.

Mowing with a triplex mower started at 7 mm in spring and was gradually reduced to 4.5 mm. Maintenances such as irrigation, aerification, verticutting, topdressing and overseeding were done according to normal practice on greens.

2.3.1.3 Implementation of protocol

Figure 8 gives an overview of the trial. Plots were 2.5 m and 2.5 m wide and there were four blocks. Products were applied using the same bicycle track sprayer and the same application volume (400 l ha^{-1}) as in the trial at Rungsted GC. Application dates are weather at application are given in Table 4.

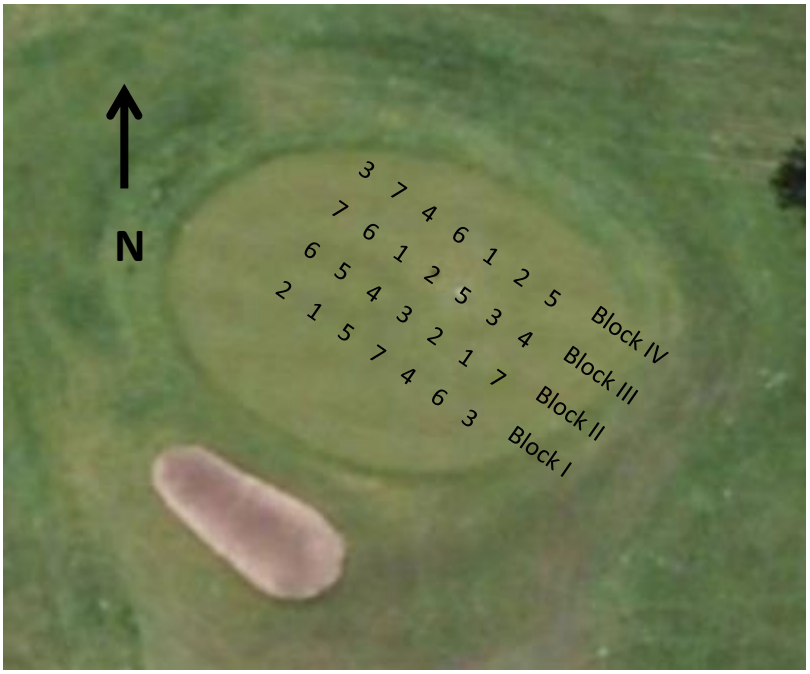


Figure 8. Plot map of trial at Sydsjælland GC.

2.3.1.4 Weather data

Monthly values for temperature and precipitation are given in Table 5. The trial was subjected to higher-than-normal temperature, but normal amounts of rain from establishment until November 2012. December 2012, January 2013, February 2013 and particularly March 2013 were much colder than normal and snow covered the green except for a short period in the first week of March. The last winter in the project was much milder with snow cover only for thirteen days in late January.

Table 4. Applications dates and weather conditions at application in trial at Sydsjælland GC.

Date of application	Treatments	Air temperature,˚C	Relative humidity, %	Wind speed, m s^{-1}
11 July 2012	4, 5	19.2	79	1.5
3 August 2012	4, 5	19.3	66	1.0
29 August 2012	4, 5	23.1	58	0.5
3 October 2012	2, 3, 5, 6, 7	15.3	73	3.0
31 October 2012	2, 3, 5, 6, 7	7.9	80	2.0
28 November 2012	3, 5, 6, 7	5.2	95	0.0
16 April 2013	3, 5, 6, 7	16.8	58	0.0
6 May 2013	3, 5, 6, 7	17.1	58	2.5
3 June 2013	4, 5	15.8	55	2.0
9 July 2013	4, 5	22.1	57	1.5
12 August 2013	4, 5	20.5	59	3.0
12 September 2013	4, 5	14.0	90	0.0
4 October 2013	2, 3, 5, 6, 7	9.8	59	2.5
6 November 2013	2, 3, 5, 6, 7	8.5	90	0.0
2 December 2013	3, 5, 6, 7	3.0	85	0.0
7 January 2014	3, 5, 6, 7	7.0	85	0.5
6 March 2014	3, 5, 6, 7	3.5	82	3.5
15 April 2014	3, 5, 6, 7	9.3	74	2.0
13 May 2014	4, 5	12.4	75	1.5
13 June 2014	4, 5	15.3	66	3.0
10 July 2014	4, 5	25.2	49	3.0

Table 5. Monthly values for air temperature and precipitation for the experimental periods 2012-13 and 2013-14 and for June-August 2014, as well as 30 year normal values for the Danish Meteorological Institute's weather station Brandelev, about 3 km from Sydsjælland GC.

	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Year
Temperature, °C													
2012-13		16.6	17.1	13.5	8.9	6.1	0.5	-0.2	-0.8	-1.0	5.9	12.3	-
2013-14	14.7	18.0	17.5	12.9	10.9	5.9	4.9	1.5	3.9	5.7	8.4	11.8	9.7
2014	14.9	19.6	16.2										-
30 yr normal	15.0	16.2	16.3	13.3	9.5	5.0	1.8	-0.1	0	2.5	6.3	11.5	8.1
Precipitation, mm													
2012-13	-	88	52	75	53	28	56	70	17	17	14	59	-
2013-14	71	35	18	58	64	57	54	64	37	25	30	38	551
2014	39	51	92										-
30 yr normal	49	62	59	56	52	60	53	46	31	38	38	43	587

2.3.2 Results

2.3.2.1 Infection of *Microdochium nivale*

An attack of *Microdochium nivale* started in November 2012 and mycelium growth was found on several plots on 28 November (Table 6). On this date the symptoms in samples from treatment 1, treatment 5 and treatment 7 were all identified as caused by *Microdochium nivale*. On 5 March 2013 the green was without snow, but the grass was withered and it was not possible to distinguish between damage due to frost and damage due to disease (Figure 9). It was also impossible to identify damage due to *Microdochium nivale* at the following assessments in April and May and during the summer and early autumn 2013.

Figure 9. Trial at Sydsjælland GC at assessment on 5 March 2013. Snow covered the green before and after this assessment. Photo: Klaus Paaske.

In the beginning of November 2013 *Microdochium nivale* was found at a low level in all treatments except for the fungicide control treatment. By 7 January 2014 it had developed to 3.3 % of the plot area on untreated plots. On this date, treatments receiving Turf WPG, Turf S+ and/or Vacciplant had significantly less *Microdochium nivale* than the untreated control, but significantly more than the fungicide control. The attack declined over the next two months, but the difference was still significant on 6 March 2014 (Table 6). No diseases were identified during the rest of the project period until 1 September 2014.

Table 6. Summary of assessments of *Microdochium nivale* in trial at Sydsjælland GC.

	% of plot area showing symptoms of *Microdochum nivale*					
Treatment	**28 Nov. 2012**		**6 Nov. 2013**	**2 Dec. 2013**	**7 Jan. 2014**	**6 Mar. 2014**
1. Untreated	1.5 a[1]		0.6 a	0.6 a	3.3 a	1.0 a
2. Folicur EC 250 or Proline 250 EC	0 c		0 b	0 b	0 c	0 c
3. Turf WPG	0.8 b		0.5 a	0.4 a	1.7 b	0.4 b
4. Turf S +	0.5 bc		0.5 a	0.5 a	1.6 b	0.6 b
5. Turf WPG + Turf S+	0.8 b		0.5 a	0.5 a	1.9 b	0.3 bc
6. Vacciplant 1.0 l ha^{-1}	0.5 bc		0.6 a	0.6 a	2.0 b	0.4 b
7. Vacciplant 2.0 l ha^{-1}	0.3 bc		0.4 a	0.4 a	1.6 b	0.5 b

[1]Within each column, means followed by the same letter are not significantly different according to Student Newman Keul's multiple comparison test at P=0.05.

2.3.2.2 Turfgrass color

In the late autumn/early winter 2012 there was a clear positive effect of Folicur (the fungicide control treatment) on turfgrass color (Figure 10). The effects of the biological treatments were mostly insignificant, but Turf S+ (treatment 4) and the higher rate of Vacciplant (treatment 7) caused a significant color improvement over the unsprayed control on 28 November 2012. From December 2012, frost and continuous snow cover eliminated these differences.

Significant color differences reappeared in November 2013 and became more distinct during the last winter in the project. On 6 March, all biological treatments had significantly better color (mean score 4.0) than the unsprayed control treatment (2.8), but not as good at the fungicide control (5.0) (Figure 11).

Differences in turf color in late spring, summer and autumn were not significant in any of the experimental years (Figure 10).

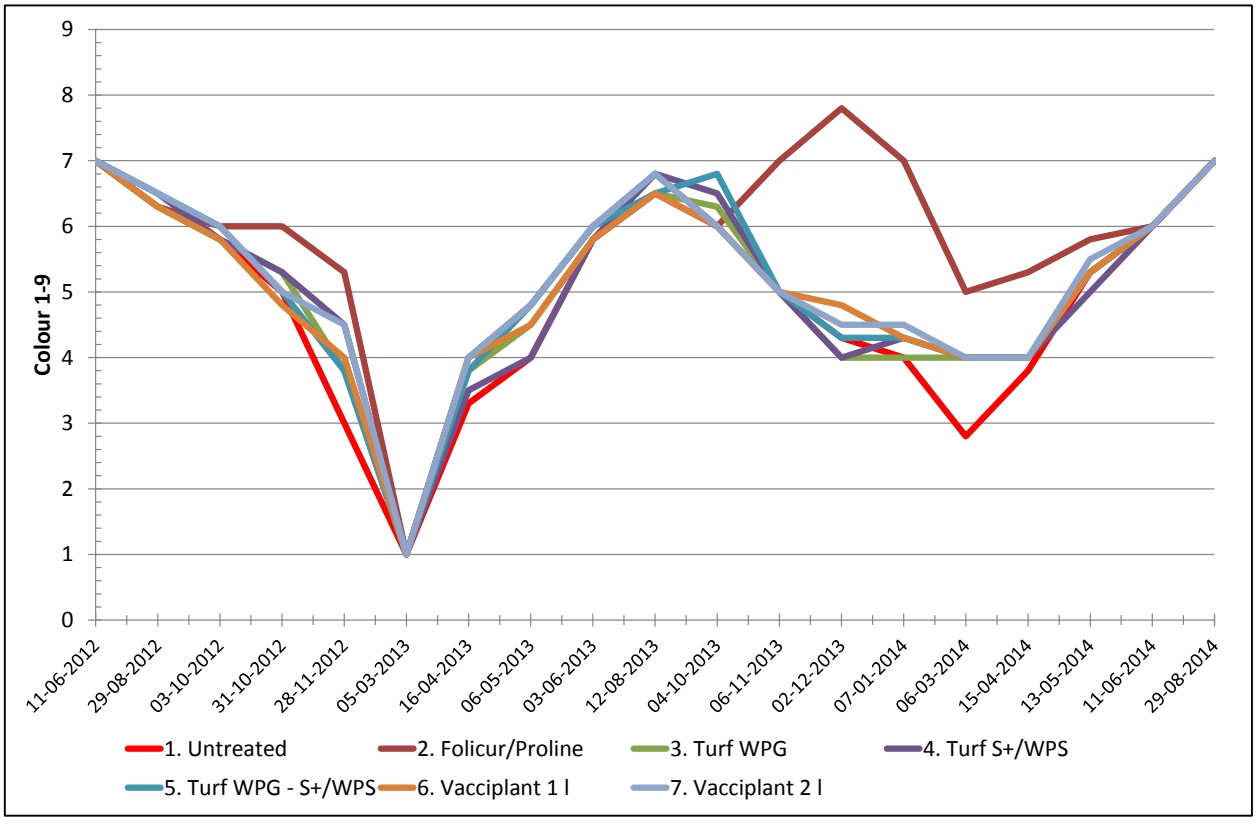


Figure 10. Turfgrass color as affect by various treatments in trial at Sydsjælland GC.

Figure 11. On 6 March 2014 there were significant differences in turf color in the trial at Sydsjælland GC. Photo: Klaus Paaske.

2.3.2.3 Turfgrass overall impression

The rating for turfgrass overall impression (Figure 12) mostly followed the same pattern as for turfgrass color, but differences among treatments were significant on more dates. On average for observations from 31 October 2012 until 6 May 2013, plots receiving the higher rate of Vacciplant had the same overall impression as plots receiving Folicur and significantly better than in the unsprayed control treatment (Table 7). On average for the last winter season (observations from 6 November 2013 to 6 March 2014), none of the biological agents produced the same overall impression as Proline in the fungicide treatment, but the quality of plots receiving both rates of Vacciplant were significantly better than of unsprayed control plots (Figure 12, Table 7).

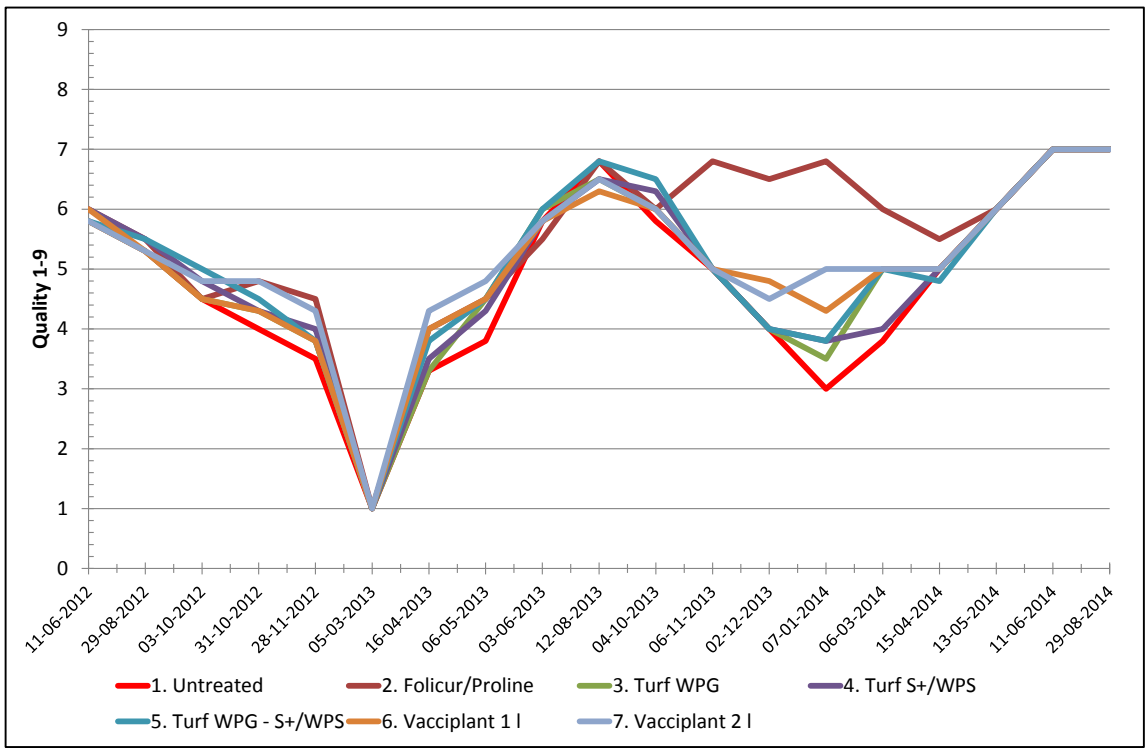


Figure 12. Turfgrass overall impression as affected by treatments in the trial at Sydsjælland GC.

Table 7. Seasonal mean values for turfgrass overall impression (1-9, 9 is best turf) in trial at Sydsjælland GC.

	Summer and early autumn 2012	Late autumn, winter and early spring 2012/13	Late spring, summer and early autumn 2013	Late autumn, winter and early spring 2013/14	Late spring and summer 2014
	(3 obs.)	(5 obs.)	(3 obs.)	(4 obs.)	(4 obs.)
1. Untreated	5.2	3.1 b[1]	6.1	4.0 c	6.3
2. Folicur EC 250 or Proline 250 EC	5.3	3.8 a	6.1	6.5 a	6.4
3. Verdera Turf WPG	5.2	3.4 ab	6.2	4.4 bc	6.3
4. Verdera Turf S+	5.4	3.4 ab	6.2	4.2 bc	6.3
5. Verdera Turf WPG and Verdera Turf S+	5.4	3.5 ab	6.4	4.5 bc	6.2
6. Vacciplant 1.0 l	5.3	3.5 ab	6.0	4.8 b	6.3
7. Vacciplant 2.0 l	5.4	3.8 a	6.1	4.9 b	6.3
P-value	>0.15	0.005	>0.15	<0.0001	0.087

[1]Within each column, means followed by the same letter are not significantly different according to Student Newman Keul's multiple comparison test at *P*=0.05.

2.4 Trial at Kävlinge GC, Sweden

2.4.1 Materials and methods

2.4.1.1 Experimental site

The trial was established on 21 October 2011 on a practice green just outside the clubhouse at Kävlinge Golfklubb, Harrieväg 120-46, 244 91 Kävlinge, Sweden, GPS coordinates: N: 55.790982, E: 13.153429. The practice green was of push-up type, constructed in 1991 and reconstructed by lifting half of the green in 2000. The botanical compostion was 45 % *Poa annua* and 55 % *Agrostis stolonifera* in block I and II and 100 % *Poa annua* in block III and IV. Block III and IV were located on the lower level of the green and were probably more poorly drained than block I and II (Figures 13 and 14).

Figure 13. From the trial at Kävlinge GC. Block I and II (closest to the club house in the background) were located on a higher level of the green than block III and IV (foreground). Photo: Trygve S. Aamlid.

Figure 14. On 11 October 2012, the botanical composition in block I and II was estimated to 55 % *Agrostis stolonifera* and 45 % *Poa annua*. Photo: Trygve S. Aamlid.

2.4.1.2 Turfgrass maintenance

The experimental green at Kävlinge was usually mowed at 3.5 mm, but mowing height was raised to 5 mm in early spring and late fall. The last mowing before winter in 2011 and 2012 was in mid to late October, and in 2013 as late as 15 November. Mowing in spring started in early to mid-March in 2012 and 2014, but as late as 17 April in 2013.

Fertilizers were applied at approximately weekly intervals form mid-March to mid-October in both years. The total inputs were 242 kg N, 18 kg P and 143 kg K ha^{-1} in 2011, 226 kg N, 27 kg P and 294 kg K ha^{-1} in 2012, 200 kg N, 40 kg P and 170 kg K in 2013 and 170 kg N, 23 kg P and 110 kg K per ha in 2014 (until 30 Sep.).

Topdressing was carried out 7 times in 2011, 5 times in 2012, 5 times in 2013 and 4 times in 2014. The total amount of sand varied from 10 to 15 mm per year.

On 26 July 2011, the green received 5 kg ha^{-1} of iron sulfate, 4 l ha^{-1} of soil surfactant and 2 l ha^{-1} of Effekt+ (a pH-lowering liquid containing formic acid 35-45%, propionic acid 20-30% and sodium 15-25%). A new application with the same rates of soil surfactant and Effekt+, but double rate of iron sulfate (10 kg ha^{-1}) was made on 25 August 2011. On 28 September 2011, about three weeks before the start of experimental treatments, the fungicide Amistar was applied to the green at a rate of 1 l ha^{-1} (250 g azoxystrobin ha^{-1}).

No applications of iron sulfate or Effect+ were made after experimental treatments had started in October 2011, but the green was treated occasionally with soil surfactants.

2.4.1.3 Implementation of protocol

Plots were 2.0 m wide and 4.0 m long, and there were four blocks. Products were applied using Agrotop SPRBIC equipment and with application dates as given in Table 8. Throughout the experiment, the application volume was 250 l ha^{-1}.

Figure 15. Practice green at Kävlinge was labelled to explain variation among plots to players. Photo: Per Göran Andersson

Table 8. Applications dates and weather conditions in trial at Kävlinge

Date of application	Treatments	Air temperature, ℃	Relative humidity, %	Wind speed, m s^{-1}
21 October 2011	2,3,5,6,7	8	85	1.0
7 November 20111	2,3,5,6,7	8	100	0.5
08 December 2011	3, 5, 6, 7	5	63	1.5
15 March 2012	3,5,6,7	6	98	0.5
4 May 2012	3,5,6,7	17	65	2.8
25 May 2012	4.5	22	45	3.0
20 June 2012	4.5	23	55	2.8
18 July 2012	4.5	19	60	0.1
14 August 2012	4.5	22	50	4.0
12 September 2012	4.5	17	53	2.0
11 October 2012	2,3,5,6,7	11	69	0.1
06 November 2012	2,3,5,6,7	9	80	2.7
18 December 2012	2,3,5,6,7	1	94	4.0
17 April 2013	3,5,6,7	12	75	2.5
5 May 2013	3,5,6,7	20	49	0.6
31 May 2013	4.5	18	61	0.6
28 June 2013	4.5	17	60	1.5
30 July 2013	4.5	20	85	1.8
29 August 2013	4.5	18	66	*
26 September 2013	4.5	11	73	3.0
15 October 2013	2,3,5,6,7	12	100	2.5
15 November 2013	2,3,5,6,7	9	90	1.8
12 December 2013	2,3,5,6,7	5	72	1.0
6 March 2014	3,5,6,7	6	79	4.4
16 April 2014	3,5,6,7	12	60	0.0
26 May 2014	4.5	19	76	0.0
23 June 2014	4.5	17	76	2.8
21 July 2014	4.5	25	50	1.5
21 August 2014	4.5	22	50	2.1

*weather station out of order

2.4.1.4 Weather data

Monthy values for temperature and rainfall from the start of the trial in October 2011 until August 2014 are shown in Table 9.

During the winter 2011-12 there was no snow cover from November to January, but the green was covered with up to 20 cm snow for a short period in the second half of February.

During the winter 2012-13 snow fell on unfrozen soil in early December but melted again after two weeks. There was also a few days with up to 5 cm of snow around 21 January and from 5 to 15 February. March 2013 had temperatures much lower than the 30 year mean value (Figure 16).

The third winter in the project (2013-14) had 2-3 cm of snow for a few days around 13 January and from 25 January until 5 February. Spring 2014 was early with 3-4°C higher than normal temperatures in February, March and April (Table 9)

Table 9. Monthly values for air temperature and precipitation for the experimental periods 1 October 2011– 31 May 2012, 1 June 2012 – 31 May 2013 and 1 June 2013 – 31 May 2014 as well as 30 year normal values for the Swedish Meteorological Institute's weather station in Lund, about 15 km from Kävlinge GC.

	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Year
Temperature, °C													
2011-12					9.6	6.4	4.2	1.6	-1.4	5.4	6.7	12.9	-
2012-13	13.8	17.0	17.2	13.9	8.7	6.2	0.5	-0.7	-0.6	-1.1	6.2	13.7	7.9
2013-14	15.8	18.8	17.7	13.1	10.8	5.6	4.5	1.1	3.4	5.7	9.2	12.8	9.9
2014	15.8	20.8	17.0										-
30 yr normal	15.4	16.8	16.5	13.1	9.1	4.5	1.1	-0.6	-0.5	2.0	6.0	11.5	7.9
Precipitation, mm													
2011-12					53	10	76	119	43	19	70	62	-
2012-13	133	104	91	42	82	80	56	65	22	30	28	73	893
2013-14	81	17	78	53	84	72	71	61	57	30	28	73	806
2014-	40	57	140										-
30 yr normal	56	70	65	64	60	69	65	54	33	45	40	45	705

Figure 16. Project reference group at Kävlinge on 21 March 2013.
The spring was very late in 2013. Photo: Trygve S. Aamlid

2.4.2 . Results

2.4.2.1 Infection of *Microdochium nivale*

Differences among treatments in per cent of plot area showing symptoms of *Microdochium nivale* were significant in December 2011, from November 2012 to May 2013 and from January to March 2014 (Table 10). However, at all these assessments it was only treatment 2, Amistar or Amistar + Medallion, that had significantly less *Microdochium nivale* than the unsprayed control. The biological agents never resulted in any significant disease control; in contrast, on 31 January 2013, per cent of plot area affected by *Microdochium nivale* was significantly higher on plot receiving the higher rate of Vacciplant (treatment 7) than on untreated control plots (Table 10). On the same date, samples taken from the green and analysed by Botaniska analysgruppen I Gothenburg (M. Usoltseva) confirmed that the symptoms seen on the green were due to *Microdochium nivale*. The laboratory also found *Fusarium* sp. and *Ostracoderma* sp.

Table 10. Per cent of plot area affected by *Microdochium nivale* in the trial at Kävlinge GC.

	First project period, Oct. 2011–May 2012			Second project period, June 2012 – May 2013						Third project period, June 2013 – Sep. 2014			
	5 Dec. 2011[3]	22 Feb. 2012		11 Oct. 2012	6 Nov. 2012	31 Jan. 2013	5 Mar. 2013	17 Apr. 2013	7 May 2013	31 May -16Sep. 2013 (5 obs)	13 Jan. 2014	6 Mar. 2014	16 Apr-16 Sep. 2014 (8 obs.)
1. Unsprayed	42ab[1]	17 a		8 a	33 a	25 b	18 ab	33 a	9 a	0 a	29 a	13 a	0 a
2. Amistar (+ Medallion)	9 c	13 a		7 a	5 b	0 c	0 c	0 b	0 b	0 a	1 b	1 b	0 a
3. Turf G+/WPG	38 ab	17 a		4 a	31 a	36 ab	28 ab	38 a	11 a	0 a	23 a	10 a	0 a
4. Turf S +/WPS	29 b[2]	13 a[2]		4 a	36 a	32 ab	23 ab	37 a	11 a	0 a	23 a	15 a	0 a
5. Turf G+/WPG + Turf S+/WPS	30 b	15 a		3 a	15 ab	36 ab	21 ab	40 a	11 a	0 a	21 a	16 a	0 a
6. Vacciplant, 1 liter ha^{-1}	48 a	18 a		5 a	40 a	40 ab	20 ab	38 a	11 a	0 a	22 a	14 a	0 a
Vacciplant, 2 liter ha^{-1}	32 ab	16 a		6 a	28 a	52 a	35 a	47 a	12 a	0 a	23 a	16 a	0 a

[1] Within each column, means followed by the same letter are not significantly different according to Student Newman Keul's multiple comparison test at *P*=0.05. ANOVA was performed on untransformed data.
[2] Should be regarded as unsprayed control as no treatment had been con
ducted before assessment.
[3] Only blocks III and IV were assessed on 5 December 2011.

2.4.2.2 Turfgrass color and overall impression

Turfgrass color and overall impression were rated regularly only during the last experimental year (2013-2014). On 6 March and 16 April 2014 there was a significant positive effect of Amistar + Medallion on these characters, but the difference from untreated control was no longer significant on 29 April (Table 11). The photo in Figure 17 shows that the fungicides resulted in better color and overall impression even in December 2012, whilst there was no positive effect of the biological agents in comparison with the unsprayed control.

Table 11. Turfgrass color (1-9, 9 is most intensely green) and overall impression (1-9, 9 is best quality) in spring 2014 in trial at Kävlinge.

Treatment		Turfgrass color (1-9)				Turfgrass overall impression (1-9)		
		6 March 2014	16 April 2014	29 April 2014		6 March 2014	16 April 2014	29 April 2014
1.	Unsprayed control	5.8 b	6.5 b	8.3 a		4.3 b	5.0 a	8.0 a
2.	Amistar + Medallion	8.3 a	8.5 a	8.0 a		8.0 a	9.0 b	8.0 a
3.	Turf G+/WPG	5.5 b	6.0 b	8.0 a		5.3 b	5.8 b	8.0 a
4.	Turf S +	5.0 b	5.5 b	8.0 a		4.8 b	5.5 b	8.3 a
5.	Turf G+/WPG + Turf S+/WPS	6.3 b	6.5 b	8.0 a		4.8 b	5.0 b	8.3 a
6.	Vacciplant, 1 liter ha^{-1}	5.5 b	6.5 b	8.5 a		4.5 b	5.3 b	8.8 a
7.	Vacciplant, 2 liter ha^{-1}	5.3 b	5.8 b	8.3 a		4.8 b	5.3 b	8.3 a

[1] Within each column, means followed by the same letter are not significantly different according to Student Newman Keul's multiple comparison test at *P*=0.05.

Figure 17. Differences between treatments in blocks III and IV in trial at Kävlinge GC, 18 December 2012. Photos: Per Göran Andersson.

2.5 Trial at Bioforsk Landvik, Norway

2.5.1 Materials and methods

2.5.1.1 Experimental site

This trial was established on 19 October 2011 on a USGA-spec. green at Bioforsk Landvik, Reddalsveien 215, 4886 Grimstad, Norway, GPS coordinates: N: 58.340071, E: 8.522554. The experimental area had been seeded on 26 July 2011 with *Agrostis stolonifera* 'Independence' along the edges of a green which was otherwise used for the SCANGREEN variety trials (Figure 18).

Figure 18. The experimental area to be used in this project was seeded with *Agrostis stolonifera* 'Independence' on 26 July 2011 and was therefore still covered with white tarp when this photo was taken on 1 Aug. 2011. Photo: Trygve S. Aamlid.

2.5.1.2 Turfgrass maintenance

Information about mowing height, fertilization and other maintenance practises during the four year project is given in Table 12. The green was mowed three times per week with a walk-behind greens mower and fertilized every two weeks, partly with liquid and partly with granular fertilizers. Wear was simulated using a friction wear drum with golf spikes three times per week corresponding to approximately 20.000 rounds of golf per year.

Table 12. Maintenance of trial at Landvik, 2011-2014

	2011	2012	2013	2014
Seasonal rates of N-P-K, kg ha^{-1}	220-44-174	158-12-119	217-22-163	105-13-85
First fertilization in spring	-	23 March	23 April	25 March
Last fertilization in fall	27 Oct.	7 Nov.	8 Nov.	-
Regular mowing height	4 mm	3 mm	3 mm	3 mm
First mowing in spring	-	26 Mar./9 mm	16 May./9 mm	12 Mar./6 mm
Last mowing before winter	11 Nov. /5 mm	17 Oct./ 3 mm	23 Oct./ 5 mm	-
Number of topdressings / total amount of sand	4 / 1.5 mm	21 / 8 mm	21 / 7 mm	23/6 mm
Vertical mowing	0	4	4	0
Areation, 8 mm solid tines	0	5	3	3
Soil surfactant		Aquaduct, 25 l ha^{-1}: 5 times from 9 July to 7 August	Revolution, 19 l ha^{-1} on 18 July	Revolution, 19 l ha^{-1}, on 4 and 24 April. Aquaduct, 25 l ha^{-1} on 6 August

2.5.1.3 Ice damage, reseeding and recovery

In spring 2013, most of the experimental area was dead due to ice encasement during the winter (Figure 19). Assessment on 22 April 2013 showed that the survival of *Agrostis stolonifera* varied from 0 to 20 % (mean 5 %), and that the survival rate was unaffected by the experimental treatments. On 26 April, the trial was reseeded with *Agrostis capillaris*, 50 % 'Barking' + 50 % 'Jorvik' at a seeding rate of 70 kg ha^{-1}. After seeding the trial received the first experimental treatments for the season (Figure 20) and was covered with tarp until 14 May to promote field emergence. During these 18 days the trial was irrigated several times per day, but the recovery was nonetheless slow (Figure 21), and the average coverage on 14 May was only 33 %; field emergence was also unaffected by the experimental treatments. Supplemental seeding and light and frequent irrigation and fertilization was carried out several times during the next weeks (Figure 22), but 100 % turf cover was not achieved until late July.

The reason why *Agrostis capillaris* 'Barking' + 'Jorvik' was used instead of *Agrostis stolonifera* 'Independence' for reseeding was that SCANGREEN project had shown *Agrostis capillaris* to be more susceptible to *Microdochium nivale* (Aamlid et al. 2012). Thus, it was considered that chances for seeing any positive effect of the biological treatments would be greater with *Agrostis capillaris*.

Figure 19. Ninety-five per cent of *Agrostis stolonifera* 'Independece' in the trial at Landvik was dead after the winter 2012-13. This photo of block III was aken on 8 April, shortly after snow melt / ice removal. Photo: Trygve S. Aamlid.

Figure 20. The first application of experimental products in 2013 was conducted immediately after reseeding plots with *Agrostis capillaris* 'Barking' + 'Jorvik' on 26 April. Photo: Trygve S. Aamlid.

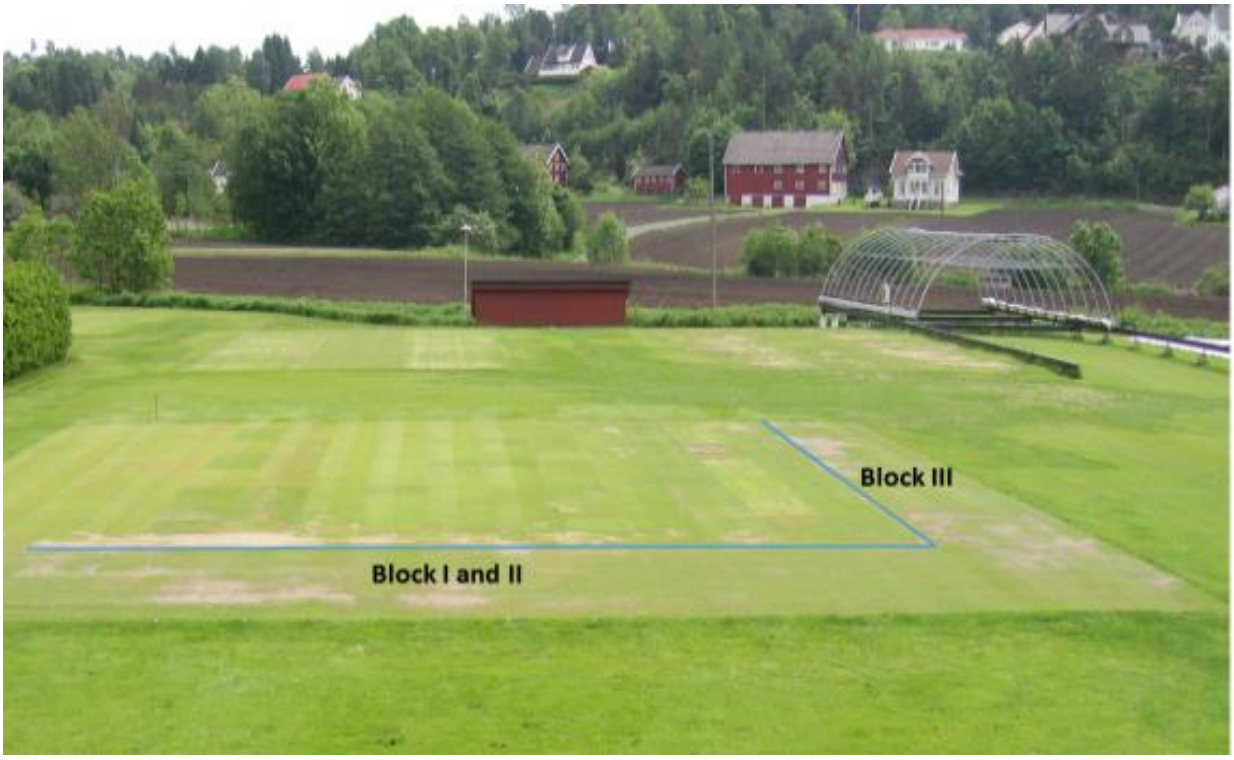


Figure 21. Recovery as of 1 June 2013. Photo: Trygve S. Aamlid.

Figure 22. Supplemental seeding of spots and light and frequent irrigation was necessary as late as in mid-July. Photo: Tatsiana Espevig.

2.5.1.4 Inoculation before winter

Because of very little infection of *Microdochium nivale* during the winter 2011-12, it was decided to inoculate the trial before the winters 2012-13 and 2013-14.

Before the winter 2012-13 this was done in two ways:

- On 21 November 2012, clippings from a nearby green with *Poa annua* infected by *Microdochium nivale* were mixed with topdressing sand and distributed evenly over the entire experimental area.

- On 27 November 2012 inoculum of two isolates of *Microdochium majus* (19/02 and 12/04) was obtained from the fungal collection at the Bioforsk Plant Health Department (inoculum of *Microdochium nivale* was not available in sufficient amounts). The fungus was first grown on PDA on Petri plates. Then PDA plugs containing *Microdochium nivale* were transferred to glass flasks containing Potato Dextrose Broth (PDB; 24 g per liter media). The flasks were incubated at 14 ºC on a shaker at 150 rpm during 12 days. The inoculum was ground using a blender, diluted in water and sprayed evenly over the experimental area at a total rate of 3.9×10^5 cell forming units (CFU) per m^2.

Before the last project winter 2013-14, the trial was inoculated on 11 November 2013 with a suspension of both spores and mycelium of *Microdochium nivale* that had been isolated from the same nearby green with *Poa annua* as used in the previous year. For mycelium suspension, the fungus was cultivated on PDB as described above. In addition to this, the sporodochia of *Microdochium nivale* were scraped from the PDA plates and diluted with water (Figure 23). Mycelium and spore suspension were mixed together, and CFU was measured using a dilution method. The inoculum was sprayed evenly over the experimental green at a total rate of 3.2×10^5 CFU per m^2.

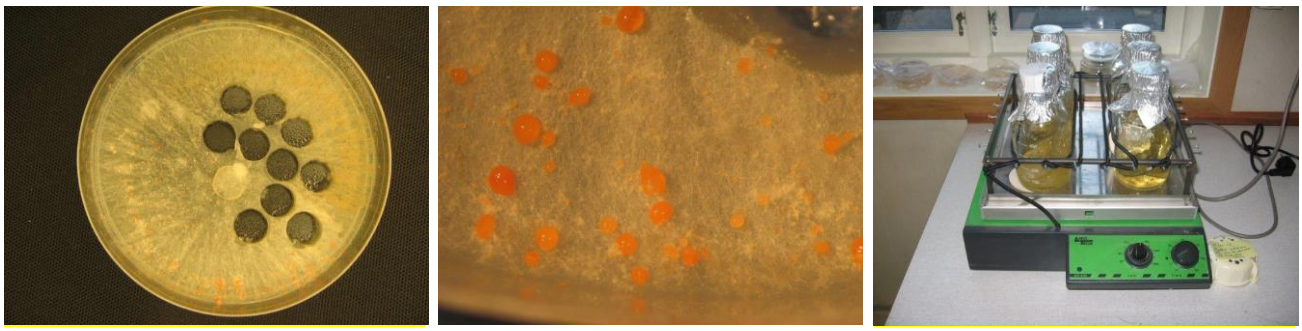


Figure 23 a-c. A Petri plate with pure culture of *Microdochium nivale* (left). Magnification of *M nivale* sporodochia on a Petri plate with a pure culture of the fungus (middle). Cultivation of *Microdochium nivale* on PDB on a shaker at 150 rpm (right). Photos: Tatsiana Espevig.

2.5.1.5 Implementation of protocol

Plots were 1.5 m wide, 2.0 m long and there were three blocks. The experimental products were applied using an experimental backpack plot sprayer (Oxford / LTI) working at 150-200 kPa pressure. The boom had three nozzles spaced 50 cm apart and shields on each side that prevented drift to neighbor plots (Figure 20). This procedure allowed full coverage of the central 1.5m x 1.0m of each plot which was used for assessments. From October 2011 to March 2013 the spraying volume was 250 l ha^{-1} except for Turf G+ that was applied in a volume of 500 l ha^{-1}. From April 2013 the spraying volume was 400 l ha^{-1} in all treatments

Actual (realized) application rates were recorded routinely by weighing the tank before and after spraying. Realized applications rates and weather conditions on the various application dates are given in Appendix Table 1. Deviations from the target rate were usually less than 10 %, which is a common requirement for GEP trials.

2.5.1.6 Weather data

The winters 2011-12 and 2013-14 were mild compared with the 30 year normal temperature at Landvik (Table 13). Snow covered the green only from 21 January to 23 February during the winter 2011-12 and from 13 January to 14 February during the winter 2013-14.

In contrast, the winter 2012-13 was cold with lower-than-normal temperatures from December through April. Snow and ice covered the green from 2 December to 8 April. As already mentioned, the ice cover resulted in severe winter damage.

Table 13. Monthly values for air temperature and precipitation for the experimental periods 2011– 12, 2012-13 and 2013-14 as well as 30 year normal values (1961-90) for the Norwegian Meteorological Institute's weather station Landvik, about 200 m from the trial site.

	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Year
Temperature, °C													
2011-12					8.9	6.5	2.1	0.1	-0.5	6.8	5.2	11.8	-
2012-13	12.9	15.8	15.8	11.6	6.8	4.8	-3.3	-3.1	-2.1	-1.8	3.7	11.5	7.6
2013-14	14.3	17.7	16.0	12.8	8.9	3.4	4.2	0.6	3.0	5.4	8.4	11.7	10.1
2014	15.7	19.6	15.6										-
30 yr normal	14.7	16.2	15.4	11.8	7.9	3.2	0.2	-1.6	-1.9	1.0	5.1	10.4	6.9
Precipitation, mm													
2011-12					74	54	156	144	15	32	136	53	-
2012-13	119	83	107	132	218	239	286	81	26	36	101	134	1562
2013-14	159	12	56	211	173	73	244	288	271	82	47	89	1705
2014	40	37	234										-
30 yr normal	71	92	113	136	162	143	102	113	73	85	58	82	1230

2.5.2 . Results

2.5.2.1 Infection of *Microdochium nivale*

Infection of *Microdochium nivale* was very limited during the experimental years 2011-12 and 2012-13. This most likely reflects a certain degree of resistance to *Microdochium nivale* in *Agrostis stolonifera* 'Independence' (Aamlid et al. 2012). As for the experimental period 2012-13 it is also documented that the aerobic fungus *Microdochium nivale* does not thrive under ice cover (Tronsmo et al. 2013).

During the last experimental period (2013-14) there was severe attack of *Microdochium nivale* in *Agrostis capillaris* 'Barking' + 'Jorvik' (Table 14, Figure 24). Application of Delaro in October and November controlled most of this attack, but none of the biological agents resulted in less disease than on unsprayed control plots.

Table 14. Per cent of plot area affected by *Microdochium nivale* in trial at Landvik.

Treatment		Winter 2011-12			Winter 2012-13		Winter 2013-14		
		9 Dec. 2011	27 Feb. 2012[1]	22 Mar. 2012	29 Nov. 2012	8 Apr. 2013[1]	5 Nov. 2013	3 Dec. 2013	24 Feb. 2014[1]
1	Unsprayed control	0.3	0.7	1.3	0.0	0.2	12.0 a[1]	4.5 ab	8.0 a
2	Delaro	0.0	0.0	0.0	0.0	0.0	0.6 b	0.2 c	0.8 b
3	Turf G+/WPG	0.5	0.8	1.5	0.0	0.2	6.7 ab	2.0 bc	7.0 a
4	Turf S +	0.3	0.4	0.7	0.1	0.2	15.0 a	4.8 a	8.7 a
5	Turf G+/WPG + Turf S+	0.2	0.2	0.5	0.1	0.3	15.0 a	5.3 a	6.7 a
6	Vacciplant, 1 liter ha^{-1}	0.3	0.1	0.3	0.3	0.0	11.7 a	4.5 ab	10.0 a
7	Vacciplant, 2 liter ha^{-1}	0.3	0.4	0.3	0.2	0.0	13.0 a	3.8 ab	8.7 a
	P-value	>0.15	>0.15	0.12	>0.15	>0.15	0.002	0.008	0.007

[1]First observation after longest period of snow cover

[2] Within each column, means followed by the same letter are not significantly different according to Student Newman Keul's multiple comparison test at *P*=0.05.

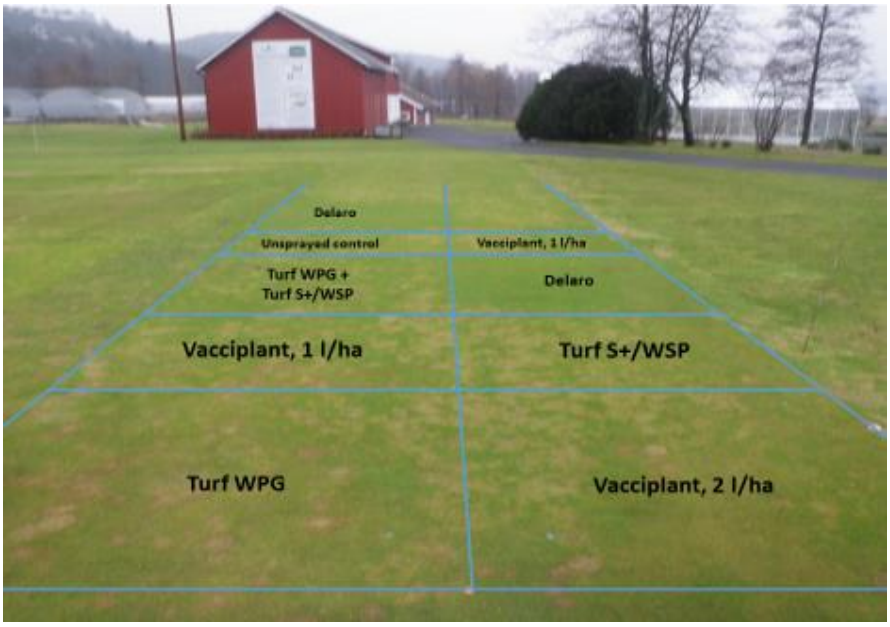


Figure 24. Trial at Bioforsk Landvik on 25 February 2014, after about one month of snow cover. Photo: Trygve S. Aamlid.

2.5.2.2 Other diseases

An outbreak of take-all patch caused by *Gaeumannomyces graminis* occurred in the late summer of 2012 (Figure 25). The patches were less visible after reseeding plots in spring 2013, but they came back in summer 2014. The experimental treatments had no effect on this disease (Table 15).

In August/September 2013, *Leptosphaerulina sp.* was diagnosed as the causal agent for some relatively diffuse patches (Figure 26). This disease was also not affected by the treatments (Table 15).

Table 15. Per cent of plot area affected by *Gaeumannomyces graminis* and *Leptospherulina sp.*in trial at Landvik.

		Gaeumannomyces graminis				*Leptospherulina sp.*
		1 Oct. 2012	5 Nov. 2013	10 Sep. 2014		9 Sep. 2013
1	Unsprayed control	12.0	0.7	5.0		0.7
2	Delaro	10.7	1.0	4.0		1.2
3	Turf G+/WPG	7.2	0.8	6.5		0.7
4	Turf S +	11.0	0.0	3.0		0.1
5	Turf G+/WPG + Turf S+	3.5	0.0	1.8		0.0
6	Vacciplant, 1 liter ha^{-1}	2.7	0.0	0.4		0.2
7	Vacciplant, 2 liter ha^{-1}	3.7	0.0	1.7		0.2
	P-value	>0.15	>0.15	>0.15		>0.15

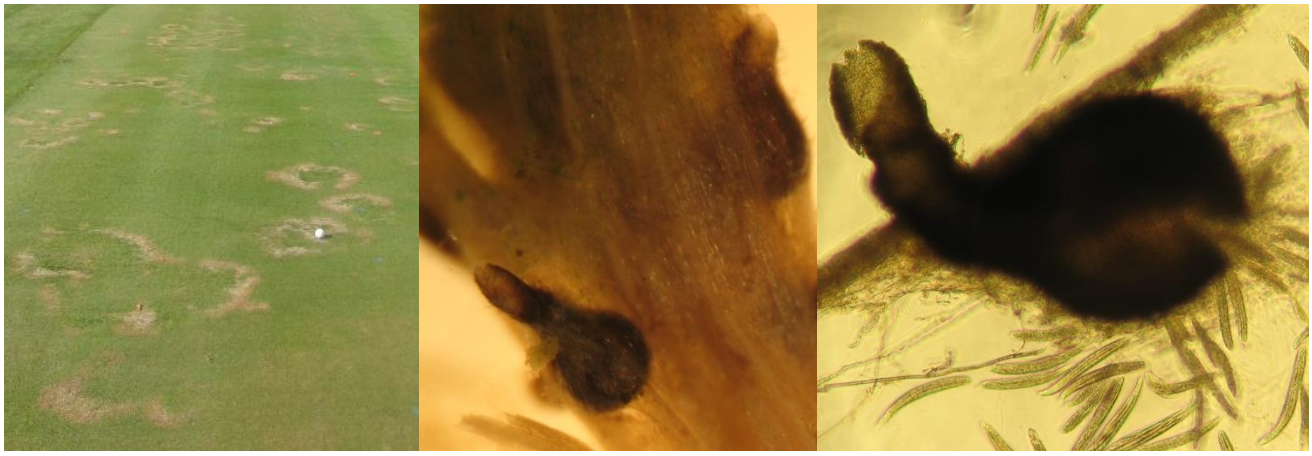


Figure 25. Take-all patch caused by *Gaeumannomyces graminis* on 16 September 2014 *(left)*, perithecia on a grass crown (middle) and perithecium with asci (right) on 3 October 2013 (right). Photos: Tatsiana Espevig.

Figure 26. *Leptospherulina sp.* in trial at Landvik on 20 August 2013: Symptoms on plots (left), asci on leaves (middle) and asci with ascospores at 400 magnification (right). Photos: Tatsiana Espevig.

2.5.2.3 Turfgrass overall impression

Turfgrass overall impression was not affected by the various treatments during the two first experimental periods. From November 2013 to March 2014, turfgrass overall impression was significantly or almost significantly (*P*=0.056) better on plots sprayed with Delaro than in the other treatments. Differences between the biological agents and the unsprayed control were not significant (Table 16).

Table 16. Observations of turfgrass overall impression (scale 1-9, 9 is best visual quality) in trial at Landvik.

	Spring 2012 (2 obs.)	Summer 2012 (4 obs.)	Autumn 2012 (2 obs.)	3 June 2013	14 Aug. 2013	5 Nov. 2013	3 Dec. 2013	24 March 2014	24 April 2014
1. Unsprayed control	6.7	5.9	4.7	4.5	6.2	4.7	3.3 b[1]	3.8 b	6.2
2. Delaro	7.7	6.7	5.2	2.8	6.7	6.7	6.3 a	7.0 a	6.7
3. Turf G+/WPG	7.0	6.6	5.3	3.3	5.2	4.7	4.8 ab	4.0 b	5.2
4. Turf S +	7.0	5.9	4.2	3.5	6.8	4.8	3.5 b	3.5 b	6.8
5. Turf G+/WPG + Turf S+	7.2	7.1	6.1	3.5	6.2	4.5	3.3 b	3.8 b	6.2
6. Vacciplant, 1 liter ha-1	7.4	6.6	5.6	3.7	5.3	4.3	3.0 b	3.5 b	5.3
7. Vacciplant, 2 liter ha-1	7.3	6.7	5.7	3.3	6.5	5.5	4.0 b	2.8 b	6.5
P value	>0.15	>0.15	>0.15	>0.15	>0.15	0.056	0.018	0.0003	>0.15

[1]Within each column, means followed by the same letter are not significantly different according to Student Newman Keul's multiple comparison test at *P*=0.05.

2.5.2.4 Botanical composition

Although *Agrostis stolonifera* was mostly dead in spring 2013, some plants recovered, and assessment of species composition in November 2013 and September 2014 showed 16 and 22 % of this species, respectively. *Poa annua* also germinated in some of the plots, but the species never contributed more than 4 % of plot area (Figure 27). The botanical composition was not affected by the experimental treatments.

Figure 27. Up to 4 % of plot area was contaminated with *Poa annua* in the summer 2014, but the contamination was not affected by the experimental treatments. Photo: Trygve S. Aamlid.

2.6 Trial at Arendal GC, Norway

2.6.1 Materials and methods

2.6.1.1 Experimental site

The trial was laid out on 20 October 2011 on a nursery green at Arendal og omegn Golf Course (Figure 28). Arendal GC is located in Nesgrenda, NO-4900 Tvedestrand, about 40 km north-east of Bioforsk Landvik. Situated about 5 km from the coast, this site usually has a longer snow cover than Landvik. The turfgrass species was *Agrostis stolonifera*.

Figure 28. Nursery green at Arendal GC at the start of the trial on 20 October 2011.
Photo: Trygve S. Aamlid.

2.6.1.2 Turfgrass maintenance

Although established as a nurserygreen, the turf was maintained as a foregreen with two weekly clippings at 10 mm. It received four applications of granular fertilizer per year, the first in mid-April to early May depending on year and the last in early to mid-September. The total nitrogen rate was 128 kg ha^{-1} in 2012, 145 kg ha^{-1} in 2013 and 113 kg ha^{-1} in 2014 (until 1 September 2014)

2.6.1.3 Implementation of protocol

Plots were 1.5 m wide and 3.0 m long, and there were three blocks. The products were applied using the same experimental backpack plot sprayer and the same application volumes as at Landvik. Application dates, weather conditions and realized rates of the different products at each application are given in Appendix Table 2.

2.6.1.4 Weather data

The weather during the three experimental periods was similar to that at Landvik except that the winters were colder (Table 17).

During the first experimental year, the trial was covered with snow for a short period around Christmas / New year 2011/12 and then from Mid-January to mid-March 2012.

During the second experimental year there was almost 5 months of snow cover from 1 December 2012 to 25 April. However, unlike the situation at Landvik there was no winter-kill due to ice.

During the third experimental year 2013-14, there was a very high precipitation in winter and the trial was covered with a thick layer of snow form about 10 January to about 25 March. Like in the previous year, there was no formation of ice.

June and especially July 2014 had much higher temperatures than the 30 year normal values (Table 17).

Table 17. Monthly values for air temperature and precipitation for the experimental periods 2011– 12, 2012-13 and 2013-14 as well as 30 year normal values (1961-90) for the Norwegian Meteorological Institute's weather station Nelaug, about 30 km north of the trial site.

	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Year
Temperature, °C													
2011-12					7.9	5.2	0.5	-1.5	-1.7	5.9	4.2	11.4	-
2012-13	12.3	15.8	15.6	10.7	5.2	3.5	-5.4	-5.0	-4.3	-3.3	2.7	11.1	4.9
2013-14	14.0	17.8	15.6	11.6	7.4	2.1	2.7	-1.4	1.6	4.3	7.2	11.3	7.9
2014	15.4	19.4	15.1										
30 yr normal	14.0	15.5	14.5	10.5	6.7	1.6	-1.9	-3.7	-3.4	0.0	3.9	9.7	5.6
Precipitation, mm													
2011-12					79	55	165	143	13	21	141	63	-
2012-13	122	43	114	117	204	206	264	60	22	29	70	172	1423
2013-14	158	7	89	112	140	88	239	353	303	80	40	68	1677
2014	37	66	298										-
30 yr normal	78	108	109	139	164	138	96	108	72	82	60	90	1244

2.6.2 Results

2.6.2.1 Infection of diseases

The first disease to be diagnosed in the trial at Arendal GK was *Typhula incarnata* (Table 18, Figure 29). The symptoms of gray snow mold caused by this fungus were also clearly visible in spring, especially on 26 April 2013 after almost five months of snow cover. Although differences were not statistically significant, Photo 30 shows that only fungicide control plots were practically without this disease, whilst plots treated with Turf G+/WPG, Turf S+ and Vacciplant had as much gray snow mold as in the unsprayed control treatment.

Red thread (*Laetisaria fuciformis*) was observed in fall 2012 and 2013. On 12 November 2013 this disease was controlled by the application of Delaro one month earlier, but not by Turf WPG, Turf S+ or Vacciplant (Table 18).

Occasional symptoms of superficial fairy ring were identified in fall 2012 and occasional symptoms of *Drechslera* leaf spot in fall 2013, but neither of these diseases were significantly influences by the experimental treatments.

The presence of *Microdochium nivale* was confirmed only once in this trial. That was on 9 October 2013, and the small attack was visible only on unsprayed plots. The effect was almost significant (*P*=0.074, Table 18).

Figure 29. Close-up of *Typhula incarnata*, 8 November 2011. Photo: Trygve S. Aamlid

Table 18. Per cent of plot area with symptoms of gray snow mold (*Typhula incarnata*), red thread (*Laetisaria fuciformis*), superficial fairy ring, microdochium patch (*Microdochium nivale*) and *Drechslera* sp. during the course of the trial at Arendal GK, Norway.

	T. incarnata 14 Dec. 2011	*L.fuciformis* (4 obs in fall 2012)	Superficial fairy rings (2 obs. in fall 2012	*T. incarnata* (2 obs. in spring 2013)	*M. nivale* 9 Oct. 2013	*L. fuciformis* 12 Nov. 2013	*Drechslera* sp. 12 Nov. 2013	*T. incarnata* (31 Mar. 2014)
1. Unsprayed control	1.3	0.5	0.2	26	0.5	0.8 a	0.1	7
2. Delaro	0.0	0.5	0.0	1	0.0	0.0 b	0.0	1
3. Turf G+/WPG	4.0	0.7	0.9	24	0.0	0.7 ab	0.5	12
4. Turf S +/WPS	2.3	0.5	1.4	25	0.0	0.5 ab	0.7	7
5. Turf G+/WPG + Turf S+/WPS	0.0	0.6	0.0	32	0.0	1.0 a	0.7	6
6. Vacciplant, 1 l ha^{-1}	2.3	0.5	0.0	27	0.0	0.6 ab	0.7	11
7. Vacciplant, 2 l ha^{-1}	2.3	0.8	0.1	33	0.0	0.7 ab	2.0	9
P-value	>0.15	>0.15	>0.15	>0.15	0.074	0.040	0.13	>0.15

2.6.2.2 Turfgrass overall impression

The mean values for turfgrass overall impression differed significantly among treatments in fall 2012, spring 2013, fall 2014 and spring 2014. In most cases only plots sprayed with the fungicide Delaro could be separated statistically from the unsprayed control treatment. However, in fall 2013, the highest scores were recorded on plots sprayed with Turf S+ (Table 19).

Figure 30. Trial at Arendal GC on 26 April 2013, shortly after snow melt. The predominant disease was gray snow mold caused by *Typhula incarnata*. Plots sprayed with Delaro were easy to identify. Photo: Trygve S. Aamlid.

Table 19. Seasonal mean values for tuffgarss overall impression (1-9, 9 is best turf) in trial at Arendal GC.

	Fall 2011 (2 obs)	Spring 2012 (2 obs)	Summer 2012 (4 obs)	Fall 2012 (3 obs)	Spring 2013 (2 obs)	Summer 2013 (4 obs)	Fall 2013 (2 obs.)	Spring 2014 (2 obs)	Summer 2014 (3 obs)
1. Unsprayed control	5.3	5.2	7.3	6.0 b	3.8 b	7.5	6.5 b	4.4 b	6.3
2. Delaro	6.1	5.5	7.5	6.6 a	6.5 a	7.7	7.1 ab	6.5 a	5.7
3. Turf G+/WPG	5.2	4.8	7.0	6.0 b	3.8 b	7.7	7.0 ab	4.8 b	6.0
4. Turf S +/ WPS	5.2	5.0	7.3	5.8 b	3.5 b	7.7	7.3 a	4.1 b	5.3
5. Turf G+/WPG + Turf S+/WPS	5.2	4.2	6.5	5.6 b	3.3 b	7.4	6.6 b	4.5 b	6.0
6. Vacciplant, 1 l ha^{-1}	5.4	5.0	7.2	6.1 b	3.5 b	7.7	7.1 ab	4.2 b	5.9
7. Vacciplant, 2 l ha^{-1}	5.1	4.5	6.8	5.5 b	3.2 b	7.5	7.1 ab	4.6 b	5.0
P-value	>0.15	>0.15	>0.15	0.002	0.006	0.107	0.017	0.003	0.079

2.7 Mean values for *Microdochium nivale* in field trials

Results from calculations of mean values for the infection of *Microdochium nivale* before and after snow cover in all field trials are shown in Figure 31. Two applications (in Sweden three applications in two out of three experimental years) of fungicides from late October to early December gave 87 % disease control both before and after snow cover, and this was the only treatment that was significantly different form the unsprayed control.

None of the test products showed any tendency to have an effect on the infection of pink snow mold after snow cover (Figure 31b). However, on average for observations, 24 and 22 % control of microdochium patch in the late fall was obtained after regular application of Turf G+/WPG and Turf S+/WPS and with the highest rate of Vacciplant, respectively. A separate analysis of variance on log-transformed data show the probability level for the difference between the unsprayed control treatment and the combination of Turf G+/WPG and Turf S+/WPS to be *P*=0.13.

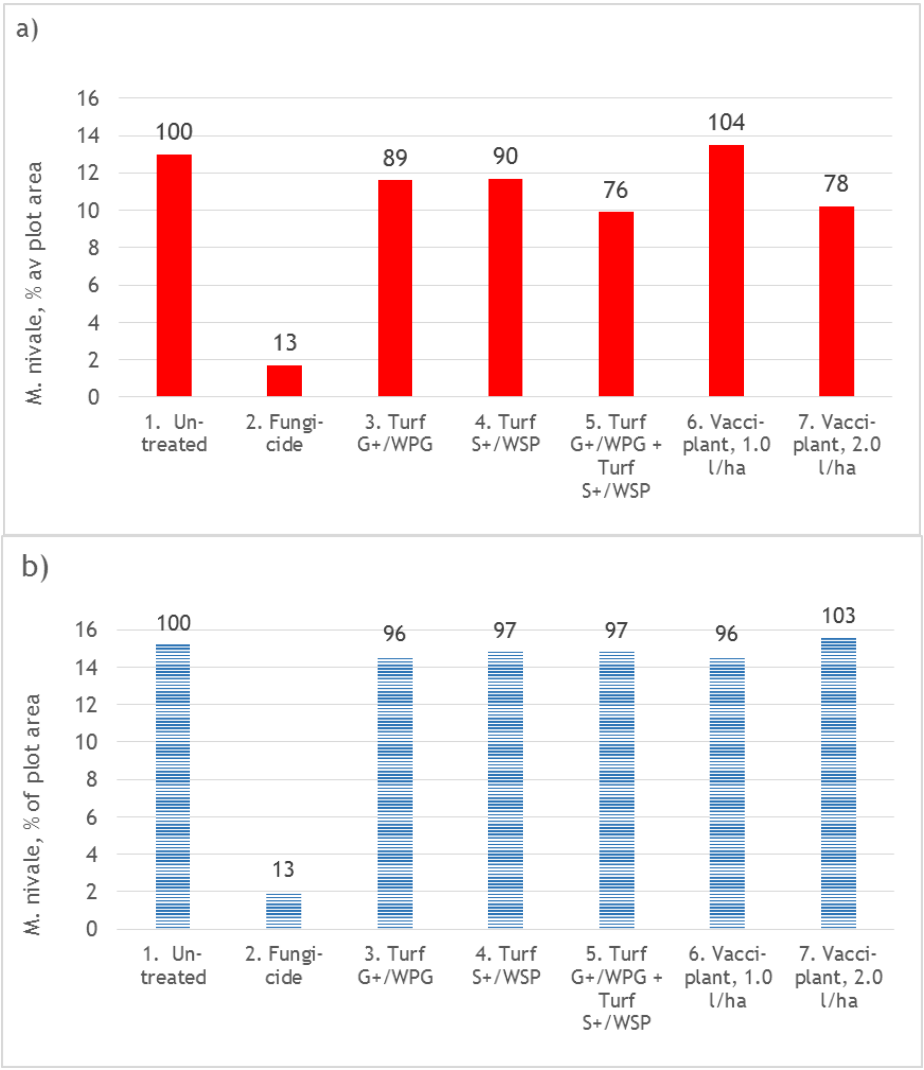


Figure 31. Effect on fungicide and biological agents on per cent of plot area showing symptoms of *Micordochium nivale* (a) at the latest observation before snow cover in late fall/early winter (mean of five trial with a total of ten observations) and (b) at the first observation after snow melt in early spring (mean of five trials with a total of eight observations). The duration of snow cover varied from approximately two weeks to approximately five months. Numbers above bars indicate infection levels relative to the unsprayed control treatment.

3. Evaluation of Turf G+/ WPG and Turf S+ for control of *Microdochium nivale in vitro*

3.1 Rationale

A former STERF project suggested that *in vitro* evaluation could be a useful indicator for the efficacy of microbial agents to control *Microdochium nivale* (Hofgaard et al. 2009). The fungal products Turf G+/WPG and the bacterial product Turf S+ were therefore also tested *in vitro*. The *in vitro* study was conducted in two steps – a pilot study and a main study. The objective of the main study was to test the efficacy of microbiological agents and selected fungicides to reduce mycelial growth of *Microdochium nivale in vitro*, while the objective of the pilot study was to find optimal concentrations of growth medium and biological agents to be used in the main study. The pilot study was run in July 2012 with microbial agents received from Interagro BIOS AB in spring 2012, and the main study in March 2013 with new batches received in November 2012.

Testing of microbiological agents *in vitro* is a useful indicator for their efficacy but such tests are usually performed at temperatures which are optimal for growth of antagonists. Thus, in this project the efficacy of selected products was evaluated at low temperature which favor attack by *Microdochium nivale* in the field.

3.2 Pilot study

3.2.1 Materials and methods

A four-factorial experiment was set up according to a fully randomized design with 3 replicates:

Factor 1. Products
- 1.1. Control (no microbial agent)
- 1.2. Turf G+ (*Gliocladium catenulatum*)
- 1.3. Turf S+ (*Streptomyces* spp.*)*
- 1.4. Combination of Turf G+ and Turf S+

Factor 2. Concentrations of growth medium:
- 2.1. 50-% potato dextrose agar (PDA) (Fluka Analytical, Buchs SG, Switzerland)[1]
- 2.2. 10 % PDA
- 2.3. 1 % PDA;

Factor 3. Concentrations of the agents in medium:
- 3.1. Recommended (full) dose (1/1) (Table 20)
- 3.2. 1/10 of the recommended dose
- 3.3. 1/100 of the recommended dose

Factor 4. Temperature for incubation:
- 4.1. 6°C
- 4.2. 16°C.

[1] 1 l of 100 % PDA contains 4 g potato extract, 20 g dextrose and 15 g agar. The final pH is 5.4.

The PDA-media of different concentrations were autoclaved at 121°C for 15 minutes and cooled down to 50°C. The microbial agents were first dissolved in sterile water and then added to the media and stirred carefully. The amount of media per Petri plate was 16.7 ml. The final concentrations of the agents in the plates with media were recommended dose, 1/10 of recommended dose or 1/100 of recommended dose. After the media had solidified, a PDA plug containing *Microdochium nivale* was placed in the center of each Petri plate. The fungus had been isolated from an annual bluegrass golf green at Arendal GC in July 2012 and cultivated on 50 % PDA prior to the study. After inoculation with *Microdochium nivale* all Petri plates were incubated at either 6°C or 16°C for 13 days. The diameter of *Microdochium nivale* colonies was measured on day 3, 8 and 13, and the data expressed as fungal radial growth per day in percentage of control (no microbial agent) at each temperature.

The number of cell forming units (CFU) of *Gliocladium catenulatum* and *Streptomyces* spp. per one milliliter of Turf G+ and Turf S+, respectively, was determined from one additional Petri plate per combination of PDA-concentration, microbial agent concentration and temperature. These additional plates were not inoculated with *Microdochium nivale*. Counting went well for *Gliocladium catenulatum*, but the number of colonies of *Streptomyces* spp. was too high to be counted. The presence of *Streptomyces* spp. was therefore registered using a scale from 0 (no colony) to 6 (infinite number). According to the manufacturer (Verdera Oy, Espo, Finland) Turf G+ should contain spores and mycelium of *Gliocladium catenulatum* at a minimum of 10^7 CFU ml^{-1} whereas Turf S+ should contain at least 10^8 CFU ml^{-1} of *Streptomyces* spp.

Table 20. Dosage of microbiological products in 100% treatment in pilot study (recommendation from Verdera).

	Recommended dosage		Tank concentration corresponding to recommended dose (1/1), %
	Agent, l/ha	Water, l/ha	
Turf G+	10.0	500	2.0
Turf S+	1.0	250	0.4
Turf G+ & Turf S+			2.0+0.4

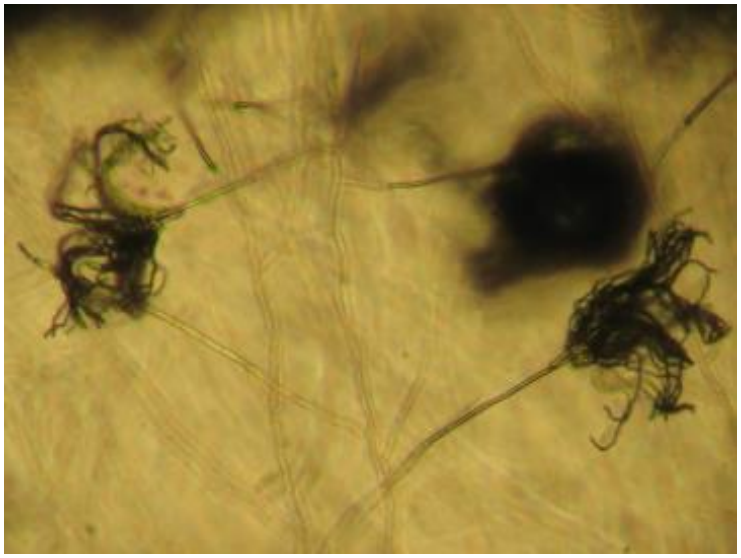


Figure 32. Sporangia of *Gliocladium catenulatum*. Photo: Tatsiana Espevig

3.2.2 Results

3.2.2.1 Cell forming units of *Gliocladium catenulatum* and *Streptomyces* spp.

On average for 1 %, 10 % and 50 % PDA, the number of CFU of *Gliocladium catenulatum* (Turf G+) was only 1.3 x 10^3 and 1.5 x 10^3 at 6ºC and 16ºC, respectively. This was much lower than the CFU of 10^7 reported by the manufacturer. A possible reason for this low CFU of *Gliocladium catenulatum* could be anaerobic conditions for spore germination and mycelial growth since the product was blended into the media. Thus, in the main study microbiological agents should be added to the agar surface and distributed uniformly with a Drigalski spatula. Another reason for the low CFU of *Gliocladium catenulatum* could be that the microbial agent Turf G+ was contaminated with bacteria since a large amount of two bacterial species was observed in Petri plates inoculated with Turf G+ only (Figures 33 and 34). The bacterial contaminants were not identified.

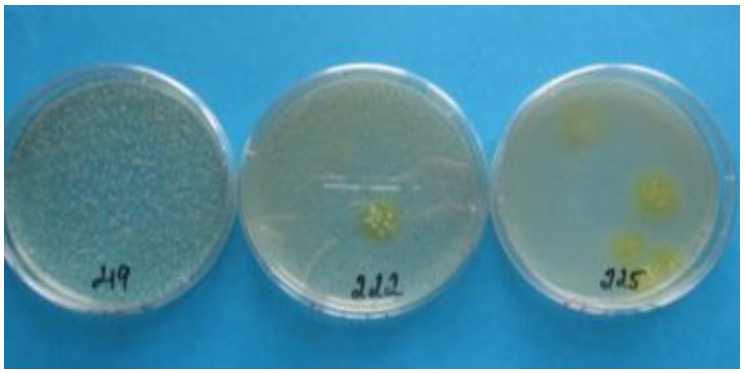


Figure 33. From left to right: 1 %, 10 % and 100 % of Turf G+ on 50 % PDA. Green colonies in dish 222 and 225 are colonies of *Gliocaldium catenulatum*. The light small colonies in the Petri plates are bacteria contamination (unknown species). Photo: Tatsiana Espevig

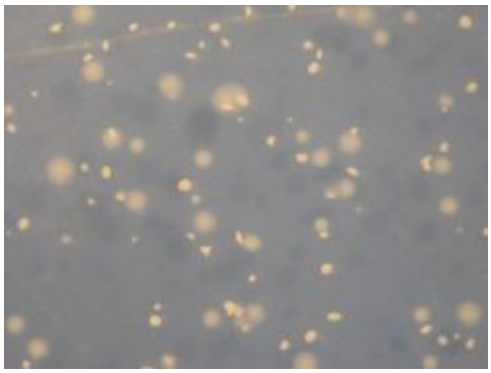


Figure 34. Scaled up Petri plate nr. 219 from Figure 33. The colonies of two bacteria species are visible. Photo: Tatsiana Espevig

The effect of PDA-concentration on bacterial numbers from Turf S+ was inconsistent due to high density and insufficiency of the scale (data not shown). It appears that a parallel determination of CFU in both bacterial and fungal agents by classical dilution method could be useful and should be conducted as a part of the main study for better interpretation of the results.

The effect of temperature on growth of both *Gliocladium catenulatum* and on *Streptomyces* spp. (data not shown) was clear. The higher temperature of 16°C significantly enhanced the number of microbes as opposed to 6°C.

3.2.2.2 Growth of *Microdochium nivale* on control plates without *Gliocladium catenulatum* or *Streptomyces* spp.

Radial growth of *Microdochium nivale* was significantly slower at 6 °C vs. 16 °C and on 50 % PDA vs. 10 and 1 % PDA (Table 21). This suggested that 50 % PDA should be used in the main study. Otherwise the fast radial growth of *Microdochium nivale* in control Petri plates could limit the duration of the experiment as the fungus would grow over the plate border.

Table 21. Radial growth of *Microdochium nivale* in control Petri plates: main effects of temperature and PDA concentration.

Experimental factor	Level	Radial growth, mm/day
Temperature	6°C	1.9 b
	16°C	4.2 a
PDA concentration	1%	3.4 a
	10%	3.4 a
	50%	2.5 b
	ANOVA	
Temp.		***
PDA		**
Temp. x PDA		NS

3.2.2.3 Inhibition of growth of *Microdochium nivale*

The bacterial agent Turf S+ was more efficient in reducing growth of *Microdochium nivale* than the fungal agent Turf G+ (Table 22). Turf S+ was the predominant partner when the two agents were mixed on the same plate.

The efficacy of both microbial agents and their mixture on *Microdochium nivale* was higher at 16°C than at 6°C (Table 22). At 6°C, Turf G+ stimulated the growth of *Microdochium nivale* instead of reducing it.

Table 22. Effects of microbiological agents and their combination on radial growth of *Microdochium nivale* (average for three concentrations of agent and three concentrations of PDA).

Agent	Radial growth of *M. nivale*, % of control	
	At 6°C	At 16°C
Turf G+	131	48
Turf S+	59	36
Turf G+ + S+	57	33

3.3 Main study

3.3.1 Materials and methods

The biological agents in the main study were the same as in the pilot study, but the liquid formulation Turf G+ was replaced with the granular formulation Turf WPG. The main study also included the fungicides Delaro (Bayer) and Medallion TL (Syngenta) labelled for control of *Microdochium nivale* on golf courses in Norway and Sweden, respectively (Table 23). The fungicides were added in amounts necessary to obtain the recommended (full) dose and 1/10 and 1/100 of the recommended dose in 50 % PDA which had been autoclaved at 121°C for 15 minutes and then cooled down to 50 °C. The agar with each fungicide was divided among Petri plates (9 cm diameter) at 16.7 ml per plate. Each microbial agent and their mixture were spread on the solidified pure 50 % PDA with a Drigalski spatula in amounts necessary to obtain the recommended dose and 1/10 and 1/100 of the recommended dose.

Table 23. Microbiological agents and selected fungicides used in main study.

Product	Active microbe / compound	Concentration of active ingredient	Recommended dose in 300 l water per ha	Concentration of product in PDA (full dose)
Turf WPG	*Gliocladium catenulatum*	>1*10^7 CFU[1] g^{-1}	1.0 kg	0.33 %
Turf S+	*Streptomyces spp.*	>1*10^8 CFU ml^{-1}	1.0 l	0.33 %
Turf WPG + Turf S+	Two above			0.33 % + 0.33 %
Delaro	Prothioconazole + trifloxystobin	175 g/l + 150 g/l	1.0 l	0.33 %
Medallion TL	Fludioxonil	125 g/l	3.0 l	1.0 %

[1]CFU – Colony forming units (spores or/and mycelium)

After all media had solidified, 10-mm-diameter plugs from the margin of a *Microdochium nivale* colony grown on PDA was placed at the center of each Petri plate. The *Microdochium nivale* was of the same isolate as used in the pilot study. The Petri plates were incubated either at 6°C or at 16°C in the dark for 12 days. On day 4 and 12, the diameter of the *Microdochium nivale* colonies was measured in two directions and averaged prior to statistical analyses. In control plates with PDA only, the diameter of *Microdochium nivale* colonies was measured on day no 4 at 16 °C and on day no 12 at 6 °C, and the daily growth rate was calculated.

The efficacy (E) of the agents was expressed as reduction in daily growth rate in percentage of control and calculated as follows: $\boldsymbol{E\ \%} = \frac{\emptyset\ control\ per\ day - \emptyset\ treatment\ per\ day}{\emptyset\ control\ per\ day}\ x\ 100\%$

The data were analyzed using ANOVA for a four-factorial experiment arranged according to a randomized complete three-block design. The experimental factors were: agents, dosages, temperatures and incubation periods.

The number of CFU of the batches of Turf WPG and Turf S+ used the main study were determined by classical dilution method. A dilution of 10^{-5} and dilution of 10^{-6} were used for counting CFU from Turf S+ and Turf WPG, respectively.

3.3.2 Results

3.3.2.1 Cell forming units of *Gliocladium catenulatum* and *Streptomyces* spp.

The CFU for per gram of Turf WPG and per ml of Turf S+ were and 2.1 x 10^7 and 5.6 x 10^6 respectively. Neither Turf WPG nor Turf S+ were contaminated with other microbes. The results also showed that the use of Turf WPG and Turf S+ together led to an 82-% reduction in the CFU from Turf WPG (Figure 35, data not shown in tables).

Figure 35. From left to right: Turf WPG + Turf S+, Turf WPG and Turf S at 10^{-6} dilution grown on 50% PDA for 1 week at room temperature. Photo: Tatsiana Espevig.

3.3.2.2 Inhibition of growth of *Microdochium nivale*

The growth rate of *Microdochium nivale* in control plates was 12.8 and 5.0 mm per day at 16°C and 6°C, respectively. Both fungicides completely suppressed the fungal growth regardless of dose and temperature (Table 24, Figure 36). After establishment of the antagonists during the first 4 days at 16 °C, the efficacy of the recommended dose of Turf S+ during day number 4-12 was 11 % less than that of fungicides, and the efficacy of Turf S+ also decreased with decreasing dose. The recommended dose and 1/10 of recommended dose of Turf S+ had the same effect at 6 and 16 °C, but 1/100 dose of Turf S+ had lower efficacy at 6 °C than that at 16 °C.

From day no 4 to day no 12 all rates of Turf WPG had almost the same efficacy as fungicides at 16 °C (Table 24, Figure 36). However, at 6 °C the suppressive effect of WPG completely disappeared. This significant decrease in the antagonistic activity of *Gliocaldium catenulatum* from Turf WPG at 6°C was most likely due to a significant reduction in number of microbes, and thus reduction of the efficiency of the agent at 6 °C compared with 16 °C. The stimulating effects of 1/100 and 1/10 dose of Turf WPG on *Microdochium nivale* at 6 °C is, however, unclear.

The vital activity and antibiotic production of *Streptomyces* spp. from Turf S+ appeared to persist at 6 °C. In the mixture of Turf WPG and Turf S+, suppression of *Microdochium nivale* mostly resembled the suppression by Turf WPG at 16 °C and by Turf S+ at 6 °C.

Table 24. Effects of microbiological agents and selected fungicides on radial growth of *Microdochium nivale in vitro*.

		Temperature and incubation period							
		16 °C				6 °C			
Agent	Dose	0-4 D		4-12 D		0-4 D		4-12 D	
		Reduction in radial growth of *M. nivale*, % of control							
Turf WPG	1/100	51	lm1	95	abc	46	lmno	-162[2]	s
	1/10	71	hijk	97	ab	43	mnop	-16[2]	s
	1[3]	80	efgh	100	a	53	l	3	r
Turf S+	1/100	45	lmno	66	jk	40	nop	30	q
	1/10	81	defg	63	k	66	jk	65	k
	1	87	cde	89	bcd	69	ijk	89	bcde
Turf WPG + Turf S+	1/100	48	lmn	98	ab	34	pq	38	opq
	1/10	85	def	99	a	75	ghij	70	ijk
	1	81	defg	100	a	82	defg	77	fghi
Delaro	1/100	100	a	100	a	100	a	100	a
	1/10	100	a	100	a	100	a	100	a
	1	100	a	100	a	100	a	100	a
Medallion TL	1/100	100	a	100	a	100	a	100	a
	1/10	100	a	100	a	100	a	100	a
	1	100	a	100	a	100	a	100	a

[1] The means followed by the same letter are not significantly different according to Fisher's protected LSD-test (α=0.05).
[2] Stimulated radial growth
[3] Recommended dose

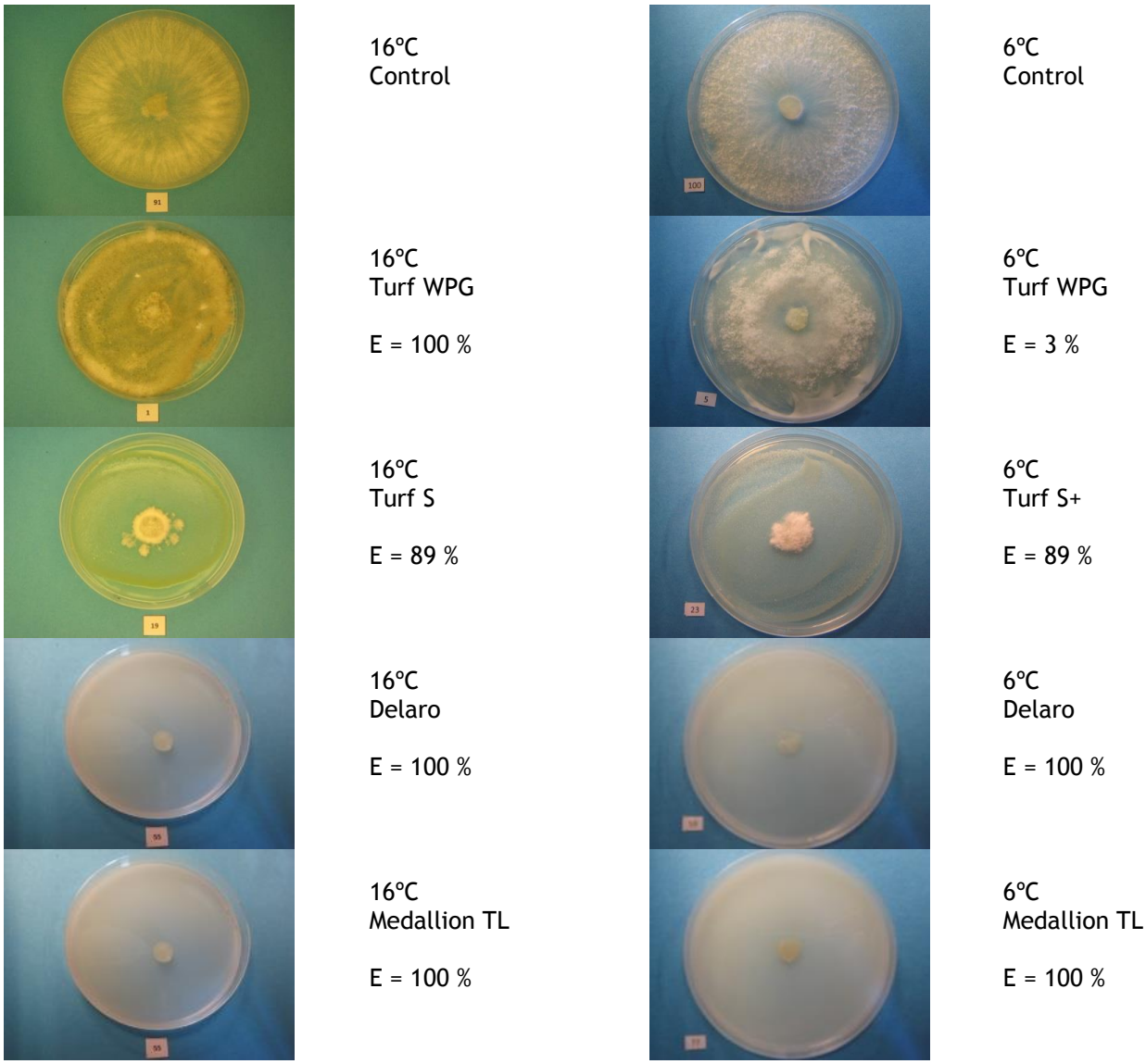


Figure 36. Efficacy (E) of the recommended dose of microbiological agents and selected fungicides on *in vitro* radial growth of *Microdochium nivale* after 12 days. Photos: Tatsiana Espevig.

4.Discussion and conclusion

The biocontrol products tested in this project were of very different nature. Vacciplant is a seaweed product containing the oligosaccharide laminarine. It is supposed to be taken up by the plant and act as a vaccine, thus eliciting the plant's own defense mechanisms. Vacciplant has so far been used primarily in the production of fruits and berries, and its potential to control *Microdochium nivale* or other turfgrass diseases has not been proved previously. The application of another seaweed product which also contains laminarine, Golf Algin, resulted in significantly more *M nivale* than in the unsprayed control treatment in an earlier Scandinavian project (Aamlid & Hanslin 2009).

In contrast to Vacciplant, the Turf G+/WPG and Turf S+/WPS are not supposed to be taken up by the plants but to protect the roots by providing a microbial flora that is antagonistic to *Microdochium nivale* and other pathogenic fungi. Turf G+/WPG contains the fungus *Gliocladium catenulatum* whose main antagonistic mechanism is hyperparasitism (McQuilken et al. 2001), a complex process by which microorganisms parasite on other microorganisms (Nelson 1997). *Gliocladium catenulatum* may also contribute to the production on chitinolytic enzymes and compete with pathogentic fungic for living space and nutrients (Anonymous 2004), but unlike *Gliocladium virens* (Whilhite et al. 1994) it is not considered to produce antibiotic substances (Anonymous 2004). *Gliocladium catenulatum* has been reported to suppress *Fusarium* spp. and *Sclerotinia sclerotiorum* (Huang 1978, Teperi et al. 1998), but significant control on *Microdochium nivale* has not been documented before to the best of our knowledge. *Gliocladium catenulatum* has, however, many of the same characteristics as *Trichoderma harzianum and Trichoderma polysporum* which are already approved for control of *Microdochium nivale* on golf courses in Scandinavia (commercial products Binab TF WP and Trianum-P).

Unlike *Gliocladium catenulatum*, Turf S+/WPS contains bacteria within genus *Streptomyces* whose main mode of action is the production of antifungal antibiotics (Trejo-Estrada et al. 1998). *Streptomyces* spp. has been shown to control *Microdochium nivale* and several other turfgrass diseases in greenhouse experiments (Trejo-Estrada et al. 1998, Chamberlain & Crawford 1999), but its persistency in turfgrass thatch is limited. (Mercier 2006).

Gliocladium catenulatum and *Streptomyces* spp. are already approved in horticultural productions, especially in the greenhouse industry where they are marketed as Mycostop and Prestop, respectively (Anonymous 2013 a,b). In Finland, where authorities have different practices for registration of alternative plant protection products, Turf G+/WPG and Turf S+/WPS are already used on about 15 % of the golf courses (K. Laukkanen, Finnish Golf Union, personal communication).

Common to Vacciplant, Turf G+/WPG and Turf S+/WPS is that they must be applied prophylactically before any symptom of disease. This is especially the case for the microbial products which may require applications over several months (or even years) to build up sufficient antagonistic activity in the rootzone. For these products it was therefore expected that the control of *Microdochium nivale* would increase during the three year project period. Unfortunately, such a long-term effect could not be evaluated in the Danish trials due to change of experimental site or in the Norwegian trials due to change of turfgrass species at Bioforsk Landvik and virtually no attack of *Microdochium nivale* at Arendal GC. However, in the trial at Kävlinge GC, Sweden, in which the same plots and were followed over the three year period, there was no indication of improved microbial control as time went by. Based on the observations between 1 November and 1 May presented in Table 10, it can be calculated that the combination of Turf G+/WPG and Turf S+/WPS controlled 24 % of the disease in the first year but only 12 % of the disease in the third year of the study.

The total microbial population on sand-based putting greens has been reported to vary from 250 000 to more than 100 million (10^8) cell forming units (CFU) per gram soil, with the highest numbers in the rhizosphere and thatch/mat layer (e.g. Mancino et al. 1993, Guertal & Elliott 2004, Aamlid et al. 2009). According to Nelson (1997), populations of biocontrol organisms must stay at numbers higher than 1 million CFU per gram soil in order to be effective against diseases. If the first batches of Turf G+ and Turf S+ used in these trials had contained the number of CFU declared by the manufacturer (10^7 and 10^8 per ml, respectively), the initial applications (10 l/ha and 1.0 l/ha, respectively) would have added about 1000 (10^3) CFU of each of the two microbes per cm^2 (or per g soil if we assume that the microbes were distributed only in the 8-10 mm thatch/mat layer). In other words, even if the products had lived up their specifications, the applications would have added less than 1/1000 of the number required to see any biological effect (Nelson 1997). Fungal or bacterial antagonism is always a 'a number's game' (Horvath & Vargas 2000), and needless to say, it did not make things better that the CFU of *G. catenulatum* in the first batch of Turf G+ was only of magnitude 10^3 as opposed to 10^7 as declared by the manufacturer. This concentration was adjusted in later deliveries and it may well argued that higher rates would have improved the efficacy of Turf G+/WPG and Turf S+/WPS as it, in summary for all trials, tended to do for Vacciplant. Regardless of this, the critical question that remains unanswered is to what extents the microbial inoculants were able to multiply in the rhizosphere and colonize the roots in the field trials.

Low soil temperature during fall, winter and spring is perhaps the most important factor limiting the potential for biological control in Scandinavian climates (e.g. Hjeljord & Tronsmo 2003). In the *in vitro* trials, the recommended rate of *Streptomyces* spp. was, however, equally efficient in inhibiting growth of *Microdochium nivale* at 6 and 16°C, and this was perhaps due to a similar reduction in growth of the two species at the lower temperature. Such an interpretation is substantiated by the pilot study showing radial growth rate of *Microdochium nivale* to be more than two times higher at 16 compared with 6°C, and with Årsvoll (1975) and who found the optimal temperature for growth of *Microdochium nivale* to be as high as 21 °C. The poor effect of *Gliocladium catenulatum* at 6°C was unexprected based on the Verdera's recommendation to apply this product only at temperatures of 10 °C and lower, but it is in line the general experience that hyperparasitism by *Trichoderma* sp. is more efficient at higher soil temperatures (Harman & Lo 1996). Even *Trichoderma atroviride*, a hyperparasite isolated from soils in Alaska, grew five times faster at 20 than at 7 °C (McBeath & Adelman 1991).

All in all, our *in vitro* trials suggest that Verdea's current guidelines for use of Turf G+/WPG ought to be changed so that *Glicladium catenulatum* is applied during the growing season, and not only from October to May. The fact that the blend of Turf WPG + Turf S+ gave the same control as Turf S+ at 6 °C and approximately the same control as Turf G+ at 16 °C suggests that there is no risk in applying the two products at the same time of the year, but this will have to be verified under field conditions.

The only field trial showing a significant positive effect of any of the test products was the green dominated by *Festuca rubra* at Sydsjælland GC. This green had, however, a very moderate attack of *Microdochium nivale,* and the practical significance of the reduction in disease from 3 to 2 % of plot area can probably be questioned. The far more severe attacks of *Microdochium nivale* on the greens dominated by *Poa annua* at Rungsted GC and Kävlinge GC, and on the green dominated by *Agrostis capillaris* at Bioforsk Landvik were never controlled by any of the test products; in a few cases there were even a tendency to the opposite effect. Most notably, on average for all trials, there was no indication for any of the products to control either *Microdochium nivale* or *Typhula incarnata* after snow cover. We therefore have to conclude that Turf G+/WPS, Turf S+/WPS or Vacciplant cannot replace fungicides on Scandinavian golf greens.

One possibility that remains open is if the test products may be able to reduce the number of fungicide applications from two or three to only one application per year. This question was not addressed in these trials as our main objective was to evaluate the products for registration in Scandinavian countries. From the producers their Scandinavian representatives, it may, however, be argued that Turf G+/WPS, Turf

S+/WPS or Vacciplant were only meant to replace fungicides only at moderate attacks of *Microdochium nivale*, and they will have to combined with fungicides if the disease becomes more severe. In the latter case microbial products and fungicides shall not be tank-mixed, but the effect of Turf G+/WPG or Turf S+/WPS will not be impeded if the product is applied three to four days before or after the fungicide (N. J. Grönholkm and M.-L. Lahdenpera, pers.comm, Oct. 2014). This is also in agreement with experimental evidence showing surprisingly small effects of a range of fungicides on soil microbial communities (Harman et al. 2006, Aamlid et al. 2009).

If Nordisk Alkali AB and/or Interagro BIOS AB, despite the negative conclusion of this report, decide to apply for registration of Vacciplant, Turf G+/WPG and/or Turf S+/WPS in Denmark, Sweden and/or Norway, we recommend that a possible registration is followed up with new trials in which the products are tested in a sequential application program with the fungicides that are currently approved in the respective countries.

5.References

Aamlid, T.S, T. Espevig, B. Molteberg, A. Tronsmo, O.M. Eklo, I.S. Hofgaard, G.H. Ludvigsen & M. Almvik 2009. Disease control and leaching potential of fungicides on golf greens with and without organic amendment to the sand-based root zone. International Turfgrass Research Journal 11: 903-917.

Aamlid, T.S, & H.M. Hanslin 2009. Evaluation of organic fertilizers and biostimulants on sand-based golf greens and football piches under Scandinavian climate conditions. International Turfgrass Research Journal 11: 919-931.

Aamlid, T.S., G. Thorvaldsson, F. Enger & T. Pettersen 2012. Turfgrass species and varieties for Integrated Pest Management of Scandinavian putting greens. Acta Agriculturae Scandinavica, Section B, Soil & Plant Science 62 (Supplement 1): 10-23.

Anonymous 2004. *Gliocladium catenulatum*: Appendix 1. Identity and biological properties. European Commission, SANCO/10383/2004 rev. 4.

Anonymous 2013a. Mycostop biofungicide in integrated pest management. www.verdera.fi. (Accessed 20 March 2013).

Anonymous 2013b. Prestop biofungicide in integrated pest management. www.verdera.fi. (Accessed 20 March 2013).

Årsvoll, K. 1975 Fungi causing winter damage on cultivated grasses in Norway. Scientific Reports of The Agricultural University of Norway 54: 1-49

Chamberlain, K. & D.L. Crawford 1999. *In vitro* and *in vivo* antagonism of pathogenic turfgrass fungi by *Streptomyces hygroscopicus* strains YCED9 and WYE53. Journal of Industrial Microbiology & Biotechnology 23: 641-646.

Guerthal, E.A. & M.L. Elliott 2004. Microbial populations in USGA putting greens. USDA Turfgrass and Environmental Research Online 3(3): 1-10.

Harman, G.E. & C-T. Lo 1996. The first registered biological control product for turf disease: Bio-Trek 22G. TurfGrass TRENDS 5(5): 8-14.

Harman, G.E., E.B. Nelson and K.L. Ondik 2006. Fungicide application effects on non-target microbial populations of putting greens. USGA Turfgrass and Environmental Research Online 5(7): 1-6.

Hjeljord, L.G. & A. Tronsmo 2003. Effect of germination initiation on competitive capacity of *Trichoderma* P1 conidia. Phytopathology 93: 1593-1598.

Horvath, B. & Vargas, J. jr. 2000. Biological control: It's a numbers game. Golf Course Management 68(6): 55-58.

Huang, H.C. 1978: *Gliocladium catenulatum*: hyperparasite of *Sclerotinia sclerotiorum* and *Fusarium* species. Canadian Journal of Plant Pathology 56: 2243-2246.

Manciono, C.F., M. Barakat & A. Maricic 1993. Soil and thatch microbial populations in an 80% sand: 20% peat creeping bentgarss putting green. HortScience 28: 189-191.

McBeath, J.H. & M. Adelman 1991. Taxonomy of a new *Trichoderma* found in Alaska. Phytopathology 81: 1151 #128.

McQuilken M.P., J. Gemmell, and M.-L. Lahdenperä 2001: *Gliocladium catenulatum* as a potential biological control agent of damping-off in bedding plants. Journal of Phytopathology 149: 171-178.

Mercier, J. 2006. Dynamics of foliage and thatch populations of introduced *Pseudomonal fluorescens* and *Streptomyces* sp. on fairway turf. BioControl 51: 323-337.

Nelson, E.B. 1997. Microbial inoculants for the control of turfgrass diseases. International Turfgrass Society Research Journal 8: 971-811.

Teperi E., M. Keskinen, E. Ketoja & R. Tahvonen 2001. Screening for fungal antagonists of seedborne *Fusarium culmorum* on wheat using *in vivo* tests. European Journal of Plant Pathology 104: 243-251.

Trejo-Estrada, S.R., I. Rivas Sepulveda & D.L. Crawford 1998. *In vitro* and *in vivo* antagonism of *Streptomyces violaceusniger* YCED9 against fungal pathogens of turfgrass. World Journal of Microbiology & Biotechnology 14: 865-872.

Tronsmo, A., T. Espevig, L. Hjeljord & T.S. Aamlid 2013. Evaluation of freezing tolerance and susceptibility to *Microdochium nivale* of velvet bentgrass cultivars in controlled environments. International Turfgrass Society Research Journal 12: 69-80.

Whilhite, S.E., R.D. Lumsden & D.C. Straney 1994. Mutational analysis of gliotoxin production by the biocontrol fungus *Gliocaldium virens* in relation to suppression of *Phytium* damping off. Phytopathology 84: 816-821.

6.Appendix Tables

Appendix Table 1. Applications dates, realized rates and weather conditions at application in trial at Bioforsk Landvik

Date	Spraying, time of day (hours)	Treatment no / Product applied	Target rate, per ha	Realized rate, per ha	Weather at application			Hours before rainfall
					Air temp.˚C	Relative humidity, %	Wind, m/s	
19 Oct. 2011	1200-1330	2. Delaro	1000 ml	973 ml	8.8	64	1.4	>12
		3. Turf G+	10000 ml	9392 ml				
		5. Turf G+	10000 ml	10150 ml				
		6. Vacciplant	1000 ml	1137 ml				
		7. Vacciplant	2000 ml	1967 ml				
9 Nov. 2011	1200-1330	2. Delaro	1000 ml	1067 ml	6.9	87	1.4	11
		3. Turf G+	10000 ml	9925 ml				
		5. Turf G+	10000 ml	9883 ml				
		6. Vacciplant	1000 ml	1092 ml				
		7. Vacciplant	2000 ml	2170 ml				
15 Dec. 2011	1200-1330	2. Delaro	1000 ml	1060 ml	5.5	87	1.6	6
		3. Turf G+	10000 ml	9300 ml				
		5. Turf G+	10000 ml	9200 ml				
		6. Vacciplant	1000 ml	1137 ml				
		7. Vacciplant	2000 ml	2087 ml				
22 Mar. 2012	0900-1000	3. Turf G+	10000 ml	9533 ml	14.3	59	2.2	>12
		5. Turf G+	10000 ml	10567 ml				
		6. Vacciplant	1000 ml	1077 ml				
		7. Vacciplant	2000 ml	2113 ml				
17 Apr. 2012	1130-1230	3. Turf G+	10000 ml	9317 ml	6.8	40	2.4	>12
		5. Turf G+	10000 ml	10483 ml				
		6. Vacciplant	1000 ml	1123 ml				
		7. Vacciplant	2000 ml	2187 ml				
23 May 2012	1000-1030	4. Turf S+	1000 ml	1093 ml	20.5	58	1,3	>12
		5. Turf S+	1000 ml	1117 ml				
19 June 2012	0830-0900	4. Turf S+	1000 ml	1063 ml	14.0	59	1.6	>12
		5. Turf S+	1000 ml	1107 ml				
13 Jul. 2012	0830-0900	4. Turf S+	1000 ml	1067 ml	15.5	75	2.5	>12
		5. Turf S+	1000 ml	1200 ml				
1 Aug. 2012	0830-0900	4. Turf S+	1000 ml	1120 ml	17.2	62	3.2	9
		5. Turf S+	1000 ml	1153				
5 Sep. 2012	0930-1000	4. Turf S+	1000 ml	1200 ml	14.1	56	1.8	>12
		5. Turf S+	1000 ml	1000 ml				
5 Oct. 2012	1000-1100	2. Delaro	1000 ml	Records lost	9.5	87	1.2	>12
		3. Turf WPG	1000 g					
		5. Turf WPG	1000 g					
		6. Vacciplant	1000 ml					
		7. Vacciplant	2000 ml					
1 Nov. 2012	0900 -1000	2. Delaro	1000 ml	1100 ml	8.5	85	1.7	5
		3. Turf WPG	1000 g	1100 g				
		5. Turf WPG	1000 g	1100 g				
		6. Vacciplant	1000 ml	1067 ml				
		7. Vacciplant	2000 ml	2200 ml				

Appendix Table 1 (continued). Applications dates, realized rates and weather conditions at application in trial at Landvik.

Date	Spraying, time of day (hours)	Treatment no / Product applied	Target rate, per ha	Realized rate, ml per ha	Weather at application			Hours before rainfall
					Air temp. °C	Relative humidity, %	Wind, m/s	
26 April 2013	1200-1300	3. Turf WPG	1000 g	9396 g	11.0	44	2.1	>12
		5. Turf WPG	1000 g	1004 g				
		6. Vacciplant	1000 ml	996 ml				
		7. Vacciplant	2000 ml	994 ml				
14 May 2013	1300-1400	3. Turf WPG	1000 g	1017 g	9.0	77	5.0	1.5
		5. Turf WPG	1000 g	1029 g				
		6. Vacciplant	1000 ml	996 ml				
		7. Vacciplant	2000 ml	992 ml				
12 June 2013	1230-1300	4. Turf S+	1000 ml	1000 ml	12.8	75	3.6	3
		5. Turf S+	1000 ml	958 ml				
17 July 2013	1400-1430	4. Turf S+	1000 ml	1000 ml	24.4	45	1.8	>12
		5 Turf S+	1000 ml	1042 ml				
14 Aug. 2013	1230-1300	4. Turf S+	1000 ml	958 ml	19.3	49	0.7	>12
		5 Turf S+	1000 ml	977 ml				
11 Sep. 2013	1015-1045	4. Turf S+	1000 ml	990 ml	24.1	49	0.1	>12
		5 Turf S+	1000 ml	994 ml				
3 Oct. 2013	1245-1345	2. Delaro	1000 ml	1000 ml	13.1	54	1.8	>12
		3. Turf WPG	1000 g	1042 g				
		5. Turf WPG	1000 g	958 g				
		6. Vacciplant	1000 ml	958 ml				
		7. Vacciplant	2000 ml	2000 ml				
6 Nov. 2013	1200-1300	2. Delaro	1000 ml	1008 ml	5.8*	79	0.4	>12
		3. Turf WPG	1000 g	1017 g				
		5. Turf WPG	1000 g	981 g				
		6. Vacciplant	1000 ml	1015 ml				
		7. Vacciplant	2000 ml	1984 ml				
3 Dec. 2013	1245-1330	3. Turf WPG	1000 g	1000 g	9.0**	78	3.3	>12
		5. Turf WPG	1000 g	1000 g				
		6. Vacciplant	1000 ml	958 ml				
		7. Vacciplant	2000 ml	2000 ml				
8 Jan. 2014	1230-1300	3. Turf WPG	1000 g	1083 g	8.1	89	2.5	2
		5. Turf WPG	1000 g	1000 g				
		6. Vacciplant	1000 ml	958 ml				
		7. Vacciplant	2000 ml	2167 ml				

*Soil temperature 1.0°C. **Soil frozen at 2-12 cm depth.

Appendix Table 1 (continued). Applications dates, realized rates and weather conditions at application in trial at Landvik

Date	Spraying, time of day (hours)	Treatment no / Product applied	Target rate, per ha	Realized rate, per ha	Weather at application			Hours before rainfall
					Air temp.,°C	Relative humidity, %	Wind, m/s	
25 Feb. 2014	0815-0845	3. Turf WPG	1000 g	988 g	4.5	87	0.6	11
		5. Turf WPG	1000 g	1002 g				
		6. Vacciplant	1000 ml	1025 ml				
		7. Vacciplant	2000 ml	2063 ml				
24 Mar. 2014	1230-1300	3. Turf WPG	1000 g	1019 g	8.4	64	1.4	>12
		5. Turf WPG	1000 g	1027 g				
		6. Vacciplant	1000 ml	1025 ml				
		7. Vacciplant	2000 ml	2004 ml				
24 Apr. 2014	0800-0830	4. Turf WPS	400 g	406 g	6.8	54	2.1	>12
		5. Turf WPS	400 g	408 g				
22 May 2014	1030-1100	4. Turf WPS	400 g	388 g	19.2	74	1.2	7
		5 Turf WPS	400 g	389 g				
19 June 2014	0815-0845	4. Turf WPS	400 g	394 g	15.0	58	1.2	6
		5. Turf WPS	400 g	401 g				
22 July 2014	1200-1230	4. Turf WPS	400 g	376 g	27.3	49	1.3	>12
		5 Turf WPS	400 g	376 g				

Appendix Table 2. Applications dates, realized rates and weather conditions at application in trial at Arendal GC.

Date	Spraying, time of day (hours)	Treatment no / Product applied	Target rate per ha	Real rate	Weather at application			Hours before rainfall
					Air temp. °C	Relative humidity, %	Wind, m/s	
20 Oct. 2011	1330-1515	2. Delaro	1000 ml	960 ml	7.0	57	1,6	>12
		3. Turf G+	10000 ml	8533 ml				
		5. Turf G+	10000 ml	9956 ml				
		6. Vacciplant	1000 ml	960 ml				
		7. Vacciplant	2000 ml	1920 ml				
17 Nov. 2011	1345-1500	2. Delaro	1000	1031	2.0	85	0.3	>12
		3. Turf G+	10000	10133				
		5. Turf G+	10000	9778				
		6. Vacciplant	1000	1138				
		7. Vacciplant	2000	2276				
20 Mar. 2012	1300-1430	3. Turf G+	10000	9533	14.8	59	2.5	>12
		5. Turf G+	10000	10567				
		6. Vacciplant	1000	1102				
		7. Vacciplant	2000	2444				
24 Apr. 2012	1100-1200	3. Turf G+	10000	11556	10.4	82	1.4	12
		5. Turf G+	10000	9926				
		6. Vacciplant	1000	1138				
		7. Vacciplant	2000	1896				
24 May 2012*	1130-1200	4. Turf S+	1000	1102	25.2	37	2.0	
		5. Turf S+	1000	1126				
30 May 2012	0930-1000	4. Turf S+	1000	1031	13.3	66	1.4	>12
		5. Turf S+	1000	1067				
21 June 2012	1430-1500	4. Turf S+	1000	1120	18.4	64	1.5	>12
		5. Turf S+	1000	1170				
13 Jul. 2012	1200-1230	4. Turf S+	1000	1173	18.6	66	3.0	>12
		5. Turf S+	1000	1156				
9 Aug. 2012	1130-1200	4. Turf S+	1000	1031	17.4	58	2.0	>12
		5. Turf S+	1000	1156				
6 Sep. 2012		4. Turf S+	1000	1031	13.7	51	2.2	5
		5. Turf S+	1000	1067				
11 Oct. 2012	1130-1300	2. Delaro	1000	1102	7.6	70	1,5	>12
		3. Turf WPG	1000g	1031				
		5. Turf WPG	1000g	1037				
		6. Vacciplant	1000 m	1102				
		7. Vacciplant	2000 ml	2074 ml				
8 Nov. 2012	1130-1230	2. Delaro	1000 ml	1102 ml	7.8	80	2,0	>12
		3. Turf WPG	1000 g	1102 g				
		5. Turf WPG	1000 g	1037 g				
		6. Vacciplant	1000 ml	1013 ml				
		7. Vacciplant	2000 ml	2134 ml				
26 April 2013	1400-1500	3. Turf WPG	1000 g	922 g	15.6	32	0,2	
		5. Turf WPG	1000 g	972 g				
		6. Vacciplant	1000 ml	1000 ml				
		7. Vacciplant	2000 ml	1907 ml				

*Application was repeated on 30 May because the turf was mown by a mistake shortly after application on 24 May

Appendix table 2 (continued) . Applications dates, realized rates and weather conditions at application in trial at Arendal GC

Date	Spraying, time of day (hours)	Treatment no / Product applied	Target rate per ha	Realized rate per ha	Weather at application			Hours before rainfall
					Air temp. °C	Relative humidity %	Wind, m/s	
7 May 2013	1130-1220	3. Turf WPG	1000 g	933 g	18.5	61	0.5	
		5. Turf WPG	1000 g	944 g				
		6. Vacciplant	1000 ml	978 ml				
		7. Vacciplant	2000 ml	2000 ml				
12 June 2013	1000-1445	4. Turf S+	1000 ml	889 ml	16.0	68	1.7	
		5. Turf S+	1000 ml	981 ml				
17 July 2013	1200-1300	4. Turf S+	1000 ml	911 ml	27.0	49	0.0	
		5. Turf S+	1000 ml	944 ml				
15 Aug. 2013	1030-1100	4. Turf S+	1000 ml	1111 ml	21.4	54	0.7	
		5. Turf S+	1000 ml	944 ml				
12 Sep. 2013	1045-1115	4. Turf S+	1000 ml	956 ml	16.6	84	0.1	
		5. Turf S+	1000 ml	1037 ml				
9 Oct. 2013	1100-1200	2. Delaro	1000 ml	944 ml	13.4	78	0.6	
		3. Turf WPG	1000 g	978 g				
		5. Turf WPG	1000 g	926 g				
		6. Vacciplant	1000 ml	978 ml				
		7. Vacciplant	2000 ml	1944 ml				
12 Nov. 2013	1200-1300	2. Delaro	1000 ml	1044 ml	8.5	82	0.1	
		3. Turf WPG	1000 g	1067 g				
		5. Turf WPG	1000 g	1056 g				
		6. Vacciplant	1000 ml	1067 ml				
		7. Vacciplant	2000 ml	1956 ml				
8 Jan. 2014	1200-1300	3. Turf WPG	1000 g	1000 g	5.7*	82	0.0	
		5. Turf WPG	1000 g	1019 g				
		6. Vacciplant	1000 ml	1000 ml				
		7. Vacciplant	2000 ml	2074 ml				
31 Mar. 2014	1350-1430	3. Turf WPG	1000 g	1000 g	11.8	39	0.7	
		5. Turf WPG	1000 g	926 g				
		6. Vacciplant	1000 ml	1044 ml				
		7. Vacciplant	2000 ml	2037 ml				
29 April 2014	1030-1100	4. Turf WPS	400 g	351 g*	21.2	42	1.2	
		5. Turf WPS	400 g	400 g				
28 May 2014	1030-1100	4. Turf WPS	400 g	360 g*	17.5	48	1.3	
		5. Turf WPS	400 g	393 g				
1 July 2014	1215-1245	4. Turf WPS	400 g	436 g	19.8	75	0.2	
		5. Turf WPS	400 g	393 g				
30 July 2014	1230-1300	4. Turf WPS	400 g	418 g	26.5	41	1.1	
		5. Turf WPS	400 g	415 g				

*New product Turf WPS blocked filters