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

Results from the experimental period 1 October 2011 – 1 March 2013

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Reference group visiting trial at Rungsted GC, Denmark, on 2 March 2012. Photo: Trygve S. Aamlid

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Summary:

This progress report presents preliminary results from a project providing data for potential registration of Turf S+ (bacterial product containing *Streptomyces*) and Turf G+/WPG (fungal product containing *Gliocladium catenulatum*), 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. Field trials with all three products are carried out on greens in Denmark, Sweden and Norway from 2011 to 2014, and Turf S+ og Turf G+/WPG are also tested *in vitro*.

Half way through the project, none of the test-products have shown any consistent control of *M. nivale* or any other disease. In the trials at Bioforsk Landvik and Arendal GK, Norway, there was little attack of *M. nivale* and no significant effect of any treatment, while in the trials at Rungsted GC, Denmark and Kävlinge GC, Sweden, there were massive attacks, but a significant reduction in disease only in the fungicide control treatments. The fifth trial, at Sydsjælland GC, Denmark, had more healthy turf just before snow cover in late November 2012 on plots that had been sprayed the test products, especially with Turf S+ or Vacciplant than on unsprayed control plots, although the turf quality was not as good as in the fungicide control treatment. The first *in vitro* trial with the microbial agents suggested better effect of both *G. catenulatum* and *Streptomyces* sp. on *M. nivale* at 16 than at 6°C. Possible implications of this for the protocol will be discussed with the manufacturer.

The experimental work continues until the summer of 2014.

Bioforsk Landvik,
12 April 2013
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, are:

1) To provide documentation according to 'Good Experimental Practice' standards for potential registration of Turf S+ (a bacterial product containing Streptomyces) 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 the product(s) for the control of *M. nivale* and other turfgrass pathogens and disseminate this knowledge to greenkeepers in the Nordic countries

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

2. Field trials

2.1 General protocol

The experimental protocol prescribes field experiments conducted according to Good Experimental Practice (GEP) Standards. The trials should follow a completely randomized block design with at least three, preferably four, replicates. The protocol was developed in October 2011 for the first experimental period 1 Oct. 2011 – 31 May 2012 and revised slightly for the second experimental period 1 June 2012 – 31 May 2013.

2.1.1 Treatments

1. Unsprayed (negative control)
2. Fungicide(s) (positive control). Products, rates and applications intervals varied depending on current labels in each country:
    a. Denmark: Folicur EC 250, 1.0 l/ha = tebuconazole, 250 g a.i. ha^{-1}, two applications at four week interval in October-November.
    b. Norway: Delaro SC 325, 1.0 l/ha = protioconazole, 175 g a.i. ha^{-1} + trifloxystrobin, 150 g a.i. ha^{-1}, two applications at four week interval in October-November.
    c. Sweden: Medallion (fludioxinil) was approved for turfgrass use and added to the protocol in 2012. Thus:
        i. 2011-12: Amistar, 1.0 l/ha = axoxystrobin, 250 g a.i. ha^{-1}, two applications at four week interval in October-November.
        ii. 2012-13: Amistar, 1.0 l/ha = axoxystrobin, 250 g a.i. ha^{-1}, one application in October followed by two or three applications of Medallion, 3.0 l/ha = 375 g a.i. ha^{-1}, fludioxonil, at four week intervals.
3. Turf G+ / Turf WPG: A new formulation of *Gliocladium catenulatum* was launched in 2012, hence the protocol was different in the two years:
    a. 2011-12: Turf G+, 10 l ha^{-1}, two or three applications at four week interval in October-December plus two applications coinciding with day temperatures 5 and 10°C in spring.
    b. 2012-13: Turf G+, 1 kg ha^{-1}, two or three applications at four week interval in October-December plus two applications coinciding with day temperatures 5 and 10°C in spring.
4. Turf S+, 1.0 l ha^{-1}, applications at four week intervals during summer time, the first coinciding with day temperature 15°C.
5. As treatment 3 + 4.
6. Vacciplant, 1 l ha^{-1} = laminarin, 45 g ha^{-1}, two to four applications at four week intervals in October – December.
7. Vacciplant, 2 l ha^{-1} = laminarin, 90 g ha^{-1}, two to four applications at four week intervals in October – December.

The project received a temporary approval by the Danish Environmental Protection Agency in mid-October 2011. This was later than optimal, but it was nevertheless 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, the spraying interval in autumn was four week (week 40, 44 and 48) according to product labels.

2.2 Experiment at Rungsted GC, Denmark, 20 October 2011 – 16 May 2012

2.2.1 Materials and methods

2.2.1.1 Experimental site

The trials 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 (Fig. 1). The green was an old push-up green, established approximately 1937. The botanical compostion at the start of the trial was 45% *Poa annua*, 45% *Agrostis capillaris* and 10% *Festuca rubra*. Root depth was 5-7 cm.

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

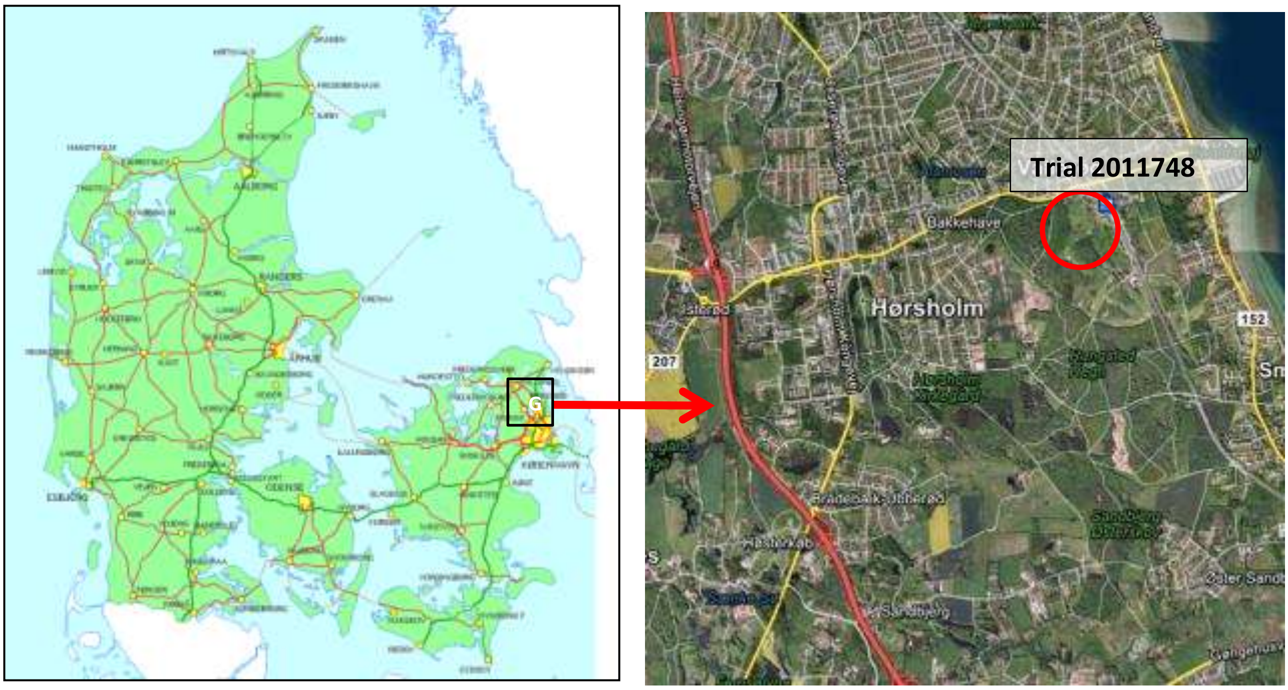


Figure 2 a,b. Maps showing location of trial at Rungsted GC.

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 experimentation (20 Oct.) 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 fescue/bentgrass seed mixture.

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.

Table 1. Applications dates in trial at Rungsted

Planned timing according to protocol	Date of application	Treatments
Week 42	20 October 2011	2, 3, 5, 6, 7
Week 45	10 November 2011	2, 3, 5, 6, 7
Week 48	30 November 2011	3, 5, 6, 7
Spring, day temperature 5°C	22 March 2012	3, 5, 6, 7
Spring, day temperature 10°C	18 April 2012	3, 5, 6, 7
Spring, day temperature 15°C	16 May 2012	4, 5

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

Figure 4. Bicycle track sprayer used in trial at Rungsted GC. Photo: Klaus Paaske.

2.2.1.4 Weather data

Meteorological data during the trial period, measured by the nearest station operated by the Danish Meteorological Institute, are shown in Table 3 and Figure 5.

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 very cold weather. There was no snow during November and December 2011 or January 2012, but the experiment 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 3. 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								
- Actual 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								
- Actual 2011/2012	59	47	8	57	86	38	14	47
- Average 1961-1990	60	56	61	46	46	30	39	39

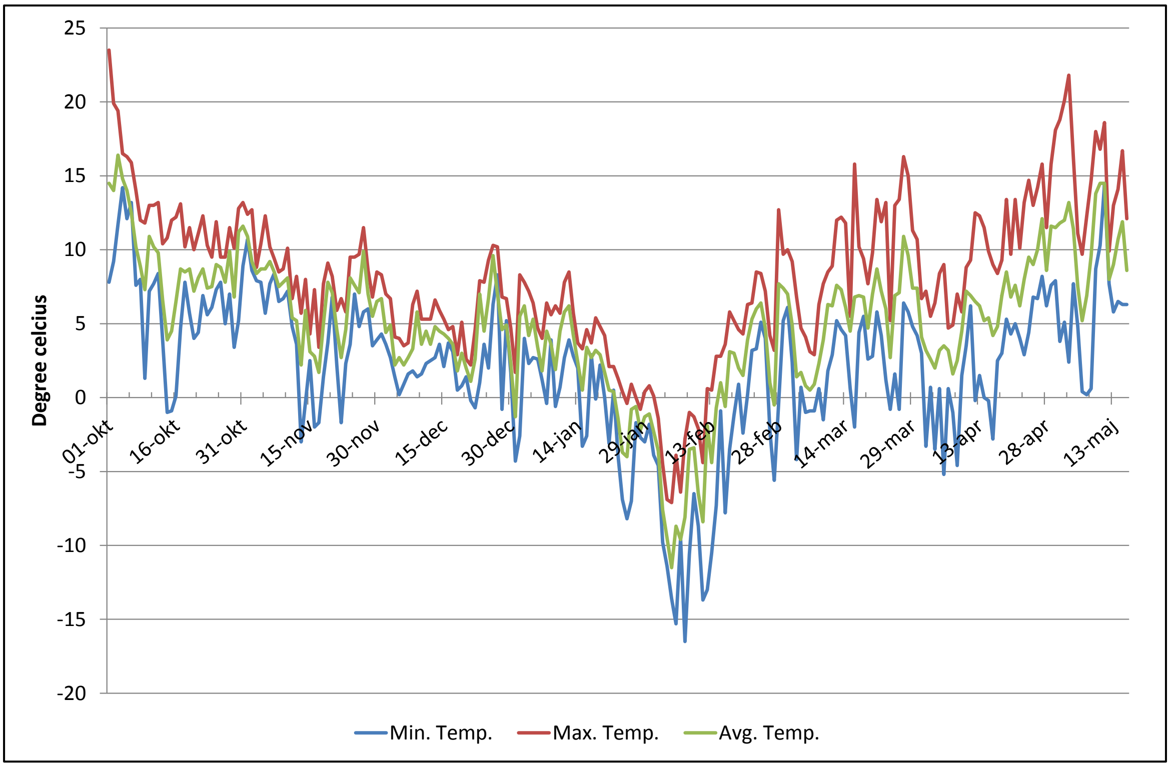


Figure 5. Minimum, maximum and average temperature from 1 October 2011 to 16 May 2012

2.2.1.5 Statistical analysis

For data management and statistical calculations the ARM program (ARM 8, Gylling Data Management Inc.) was used. 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 as follows: TL = LOG(x+1), TA = ARCSIN(SQR(X/100)) or TS = SQR(X+0.5). 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 and discussion

Results from assessments of microdochium patch are shown in Table 4.

When the first application was made on 20 October 2011 no visible sign of microdochium patch was found on any of the plots (Figure 3). At the second application on 10 November an incipient attack could be found in 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 6a,b). At the assessment on 22 December (Figure 7a.b), the attack had increased to 33.8% on untreated plots. The next assessment was made in February when the snow was gone and this showed no further development of the patches. At the last assessment on 16 May the disease incidence was decreasing due to the regrowth of the grass and the effect of the overseeding on 19 March.

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 this plot was 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 4. Summary of assessments at Rungsted GC.

Treatment	% attack of microdochium patch						
	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

*: Analysis was 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.

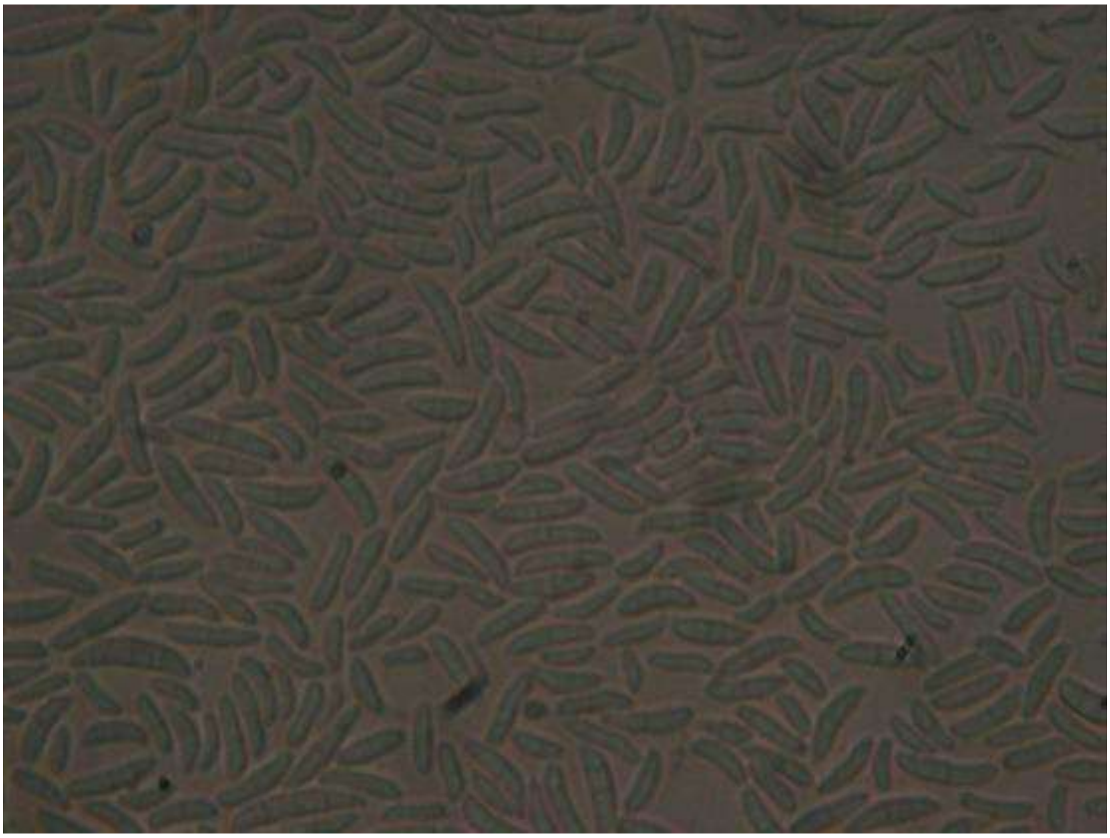
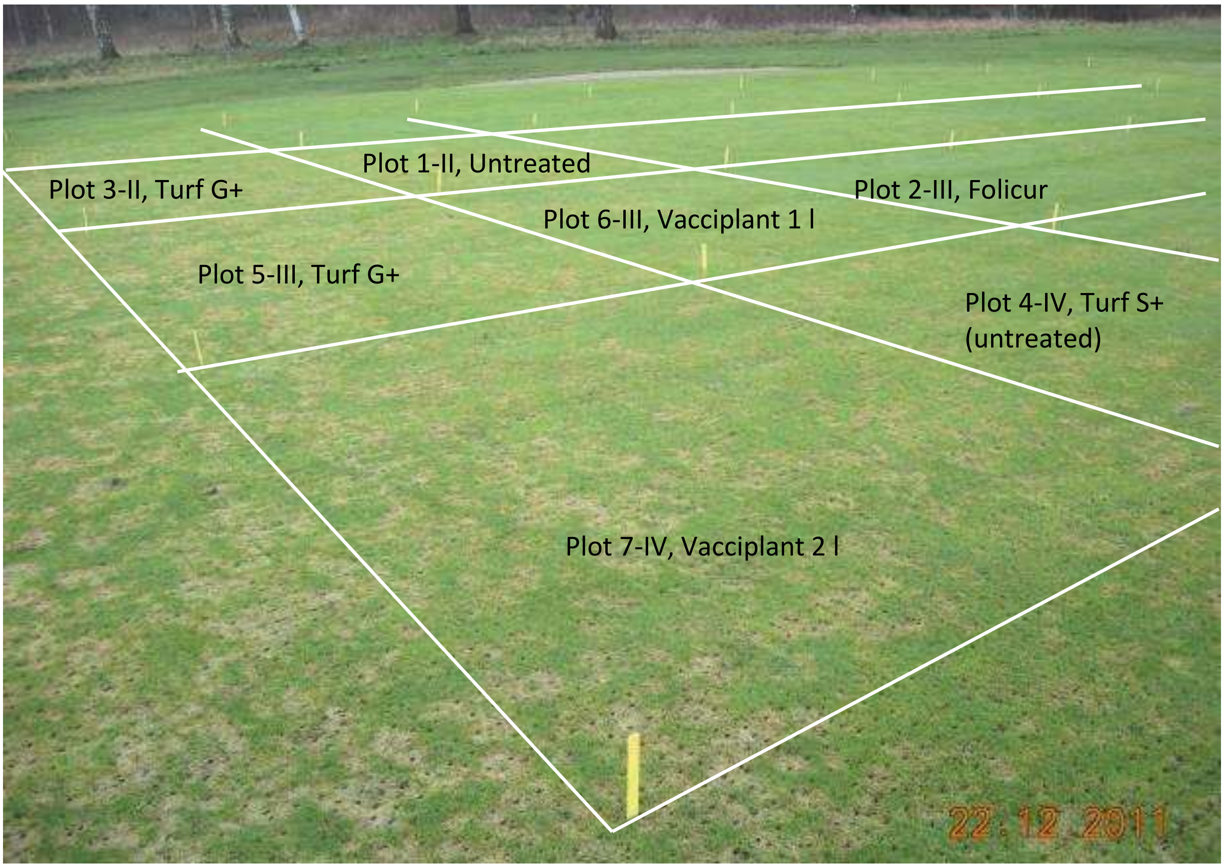


Figure 6 a,b. Sporodochia (top) and spores (bottom) of *M.nivale* in samples from trial at Rungsted analysed in Bioforsk Turfgrass Diagnostic Lab, 17 November 2011. Photos: Tatsiana Espevig.



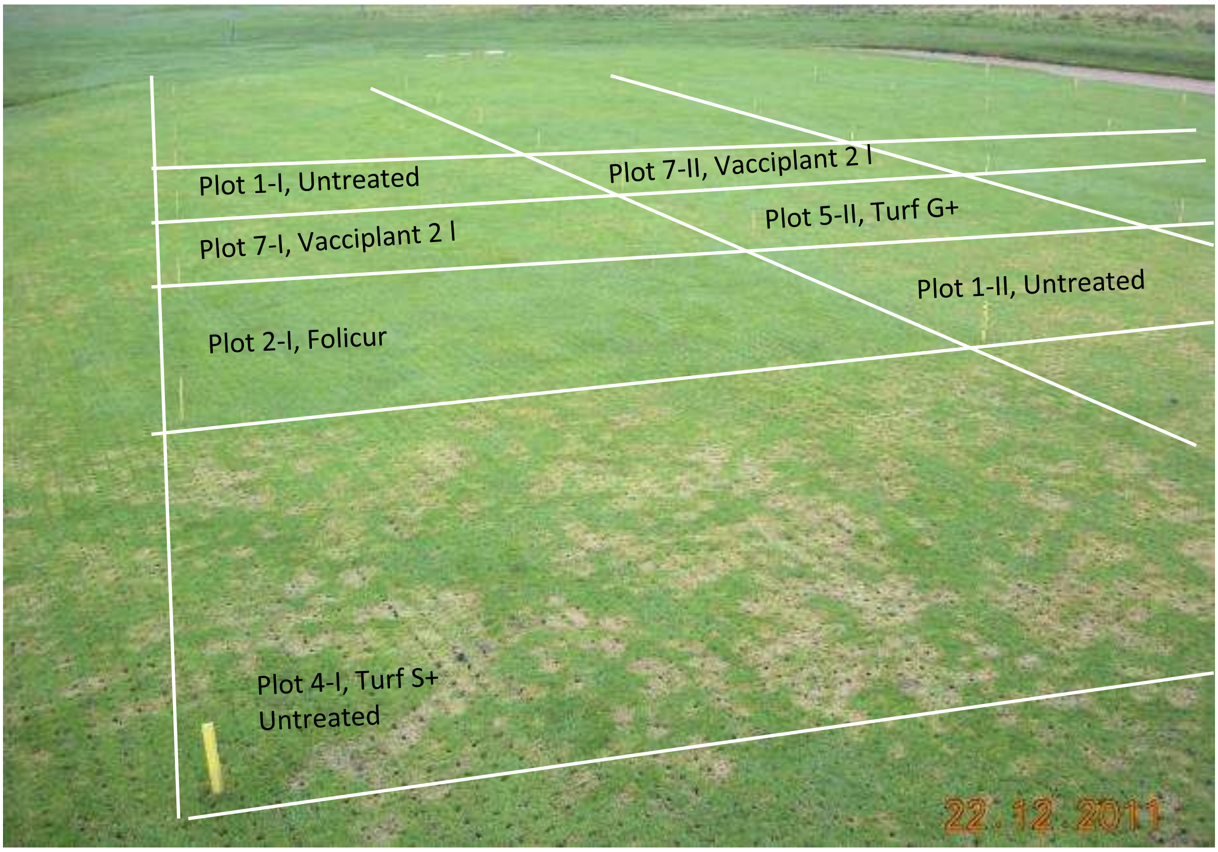


Figure 7 a, b. Various treatments at assessement on 22 Dec. 2011. Photo: Klaus Paaske.

2.3 Experiment 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 course at Sydsjælland Golfklub, Præstø Landevej 39, Mogenstrup, 4700 Næstved, Denmark, GPS coordinates: N: 55.18462, E: 11.86785 (Figure 8), 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. The botanical compostion was 5-15 % (mean 11 %) *Poa annua* except for the most eastern plots in block I (treatment 5) and IV (treatment 3, Figure 9) that had 33 % *Poa annua*. The rest was made up of equal amounts of *Festuca rubra* and *Agrostis capillaris*.

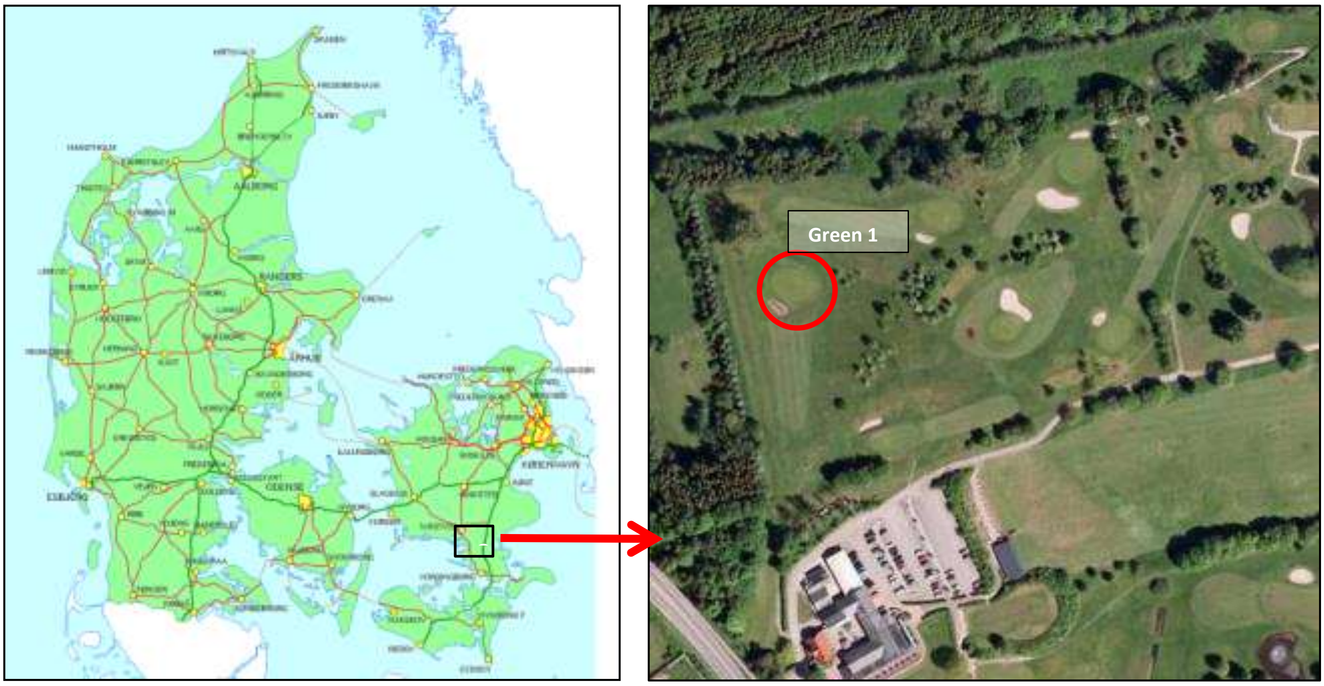


Figure 8 a,b. Maps showing location at trial at Rungsted GC.

2.3.1.2 Turfgrass maintenance

The total fertilizer rate in 2012 was 92 kg N, 24 kg P, 135 kg K per ha. The last application before winter was given on 17 September.

Figure 9. Plot map of trial at Sydsjælland GC.

2.3.1.3 Implementation of protocol

Figure 9 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 given in Table 5.

Table 5. Applications dates in trial at Sydsjælland as of 14 March 2013.

Planned timing according to protocol	Date of application	Plots treated
At monthly intervals during summer	11 July 2012 3 August 2012 29 August 2012	4, 5 4, 5 4, 5
Week 40	3 October 2012	2, 3, 5, 6, 7
Week 44	31 October 2012	2, 3, 5, 6, 7
Week 48	28 November 2012	3, 5, 6, 7

2.3.1.4 Weather data

Monthly values for temperature and precipitation are given in Table 6 and daily mean temperatures for the experimental period in Figure 10. As of 14 March 2012 the green has been covered by snow since 7 Dec. except for a few days in early March. The ground has mostly been frozen under the snow.

Table 6. Monthly values for air temperature and precipitation for the experimental periods 1 July 2012 - 28 Feb 2013 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		16.2	17.1	13.5	8.9	6.1	0.5	-0.2	-0.8				
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				
30 yr normal	49	62	59	56	52	60	53	46	31	38	38	43	587

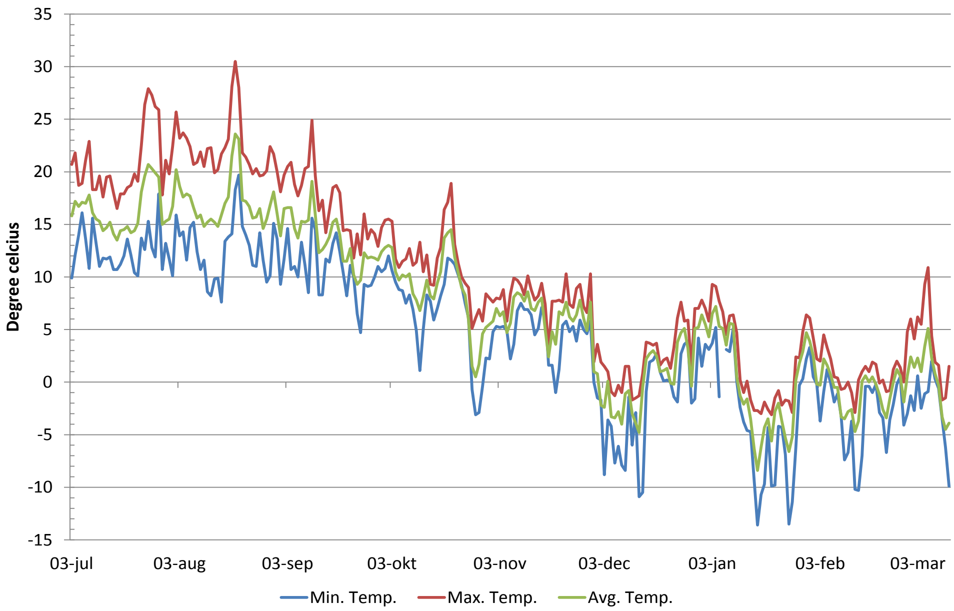


Figure 10. Minimum, maximum and daily mean temperature for the period 3 July 2012 to 12 March 2013 at DMI's station Brandelev, about 3 km from Sydsjælland GC.

2.3.2 Results

Assessments made on 11 July, 3 August, 29 August and 3 October 2012 showed no visible differences among treatments. On 28 November (Table 7) mycelium growth was found in several plots and samples were taken from treatments 1 (untreated), treatment 5 (Turf WDG/Turf S+) and treatment 7 (Vacciplant). The samples were diagnosed and the presence of *Microdochium nivale* was confirmed. Most treatments had significantly less brownish surface and dead turf than the untreated control. Apart from the fungicide Folicur, the highest quality was obtained with Turf S+ and Vacciplant.

At the assessment on 5 March (Figure 11) it was not possible to distinguish between turf surface that has been killed by frost or by *Microdochium nivale*. This distinction has to await regrowth of the grasses.

Table 7. Results from assessment on 28 November 2012.

Treatment	No. of treatments before this assessment	% of turf surface brownish	% of turf surface dead
1. Untreated	-	42.5 a[1]	1.5 a
2. Folicur EC 250	2	11.3 d	0 c
3. Turf WPG	2	32.5 bc	0.8 b
4. Turf S+	3	27.5 c	0.5 bc
5. Turf WPG +	2	37.5 ab	0.8 b
Turf S+	3		
6. Vacciplant 1.0 l	2	31.3 bc	0.5 bc
7. Vacciplant 2.0 l	2	27.5 c	0.3 bc

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

2.4 Experiment at Kävlinge GC, Sweden

2.4.1Materials 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 no I and II and 100 % *Poa annua* in block no III and IV. Block III and IV were located at a lower level of the green and were probably more poorly drained than block I and II (Figures 12 and 13).

Figure 12. 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 13. 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 mown at 5 mm. The last mowing before winter was in mid-October 2011 and in late October 2012.

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 and 226 kg N, 27 kg P and 294 kg K ha^{-1} in 2012. In both years Mg, Fe, Ca, S and micronutrients were also applied several times.

Aeration was carried out 19 times in 2011 and 15 times in 2011, and topdressing 7 times in 2011 and 5 times in 2012.

On 26 July 2011, the green received 5 kg of iron sulphate, 4 liter of wetting agent and 2 liter 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 weeting agent and Effekt+, but double rate of iron sulphate (10 kg) was made on 25 August 2011. On 28 Sep. 2011, about three weeks before the start of experimental treatments, the fungicide Amistar was applied to the experimental area at a rate of 1 liter (250 g azoxystrobin) per ha. No applications of wetting agents, iron sulfate or pesticides were made during the experimental period.

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. Application volume was 250 l ha^{-1}.

Table 8. Applications dates in trial at Kävlinge as of 14 March 2013.

Planned timing according to protocol	Date of application	Treatments
Week 42	21 October 2011	2, 3, 5, 6, 7
Week 45	7 November 2011	2, 3, 5, 6, 7
Week 48	8 December 2011	3, 5, 6, 7
Spring, day temperature 5°C	15 March 2012	3, 5, 6, 7
Spring, day temperature 10°C	4 May 2012	3, 5, 6, 7
At monthly intervals during summer	25 May 2012	4, 5
	20 June 2012	4, 5
	18 July 2012	4, 5
	14 August 2012	4, 5
	12 September 2012	4, 5
Week 40	11 October 2012	2, 3, 5, 6, 7
Week 44	6 November 2012	2, 3, 5, 6, 7
Week 48	18 December 2012	2, 3, 5, 6, 7

2.4.1.4 Weather data

Monthy temperature and rainfall from the start of the experiment in October 2011 until February 2013 are presented 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 in the second half of February.

During the winter 2012-13 snow fell on non-frozen soil in early December but melted again after two weeks. There was also about two weeks of snow cover in late January.

Table 9. Monthly values for air temperature and precipitation for the experimental periods 1 October 2011– 31 May 2012 and 1 June 2012 – 28 Feb 2013 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	16.1	17.2	16.6	14.4	9.6	6.4	4.2	1.6	-1.4	5.4	6.7	12.9	9.1
2012-13	13.8	17.0	17.2	13.9	8.7	6.2	0.5	-0.7	-0.6				
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	78	163	144	56	53	10	76	119	43	19	70	62	792
2012-13	133	104	91	42	82	80	56	65	22				897
30 yr normal	56	70	65	64	60	69	65	54	33	45	40	45	666

Figure 14. Practice green at Kävlinge was labelled to explain variation among plots to players.
Photo: Per Göran Andersson

2.4.2 . Results

Infection of *M. nivale* was always more severe in block III and IV that had an almost pure stand of *Poa annua* than in block I and II that had a mixed stand of *Agrostis stolonifera* and *Poa annua* (Table 10). During both project periods, the difference depending on species composition was more pronounced in autumn before snowfall than after snow melt in spring or during mild periods in winter. Samples taken on 31 January 2013 and analysed by the laboratory Botaniska analysgruppen (M. Usoltseva) confirmed that the symptoms seen on the green were due to *Microdochium nivale*. The laboratory also found *Fusarium* sp. and *Ostracoderma* sp.

On average for all four blocks, differences among treatments were significant except for the assessments on 22 Feb. 2012 (first project period) and 11 Oct. 2012 (before the first treatment with fungicide, Turf WPG and Vacciplant in the autumn of the second project period). However, it was only treatment 2, fungicide control, that had significantly less Microdochium than the unsprayed control. Assessments on 18 Dec. 2012, 31 Jan. 2013 and 5 Mar. 2013 in fact showed more M. nivale on plots treated with alternative products than on untreated control plots (Table 10). Separate analyses for the two blocks with almost pure *Poa annua* showed this negative impact of treatment 5 (Turf WPG & Turf S+) and 7 (Vacciplant, 2 l ha^{-1}) to be significant on 18 Dec. 2012 and on 31 Jan. 2013. This can also be seen in Figure 15.

Table 10. Per cent microdochium patch in trial at Kävlinge.

		First project period, Oct. 2011 – May 2012			Second project period, June 2012 – March 2013				
		5 Dec. 2011	22 Feb. 2012		11 Oct. 2012	6 Nov. 2012	18 Dec. 2012	31 Jan. 2013	5 Mar. 2013
Block I and II: 55% *A. stolonifera*, 45% % *Poa annua*		-[3]	11.7		1.0	17.9	24.3	23.9	17.8
Block III and IV: 99% *Poa annua*		31.3	19.6		9.9	35.3	40.1	39.3	23.2
Treatment (mean of four blocks)									
1.	Unsprayed control	41.8 ab[1]	17.4 a		7.9 a	32.5 a	31.6 a	25.0 b	17.6 ab
2.	Amistar (+ Medallion)	8.8 c	12.5 a		7.4 a	4.5 b	0.8 b	0.0 c	0.0 c
3.	Turf G+/WPG	37.5 ab	16.9 a		4.1 a	30.5 a	32.8 a	36.3 ab	27.5 ab
4.	Turf S +	28.8 b[2]	12.8 a[2]		4.3 a	36.3 a	36.4 a	32.0 ab	22.8 ab
5.	Turf G+/WPG + Turf S+	30.0 b	15.3 a		3.0 a	15.0 ab	35.4 a	36.3 ab	21.3 ab
6.	Vacciplant, 1 liter ha^{-1}	47.5 a	18.1 a		5.4 a	40.0 a	39.5 a	40.0 ab	19.8 ab
7.	Vacciplant, 2 liter ha^{-1}	31.8 ab	16.4 a		6.0 a	27.5 a	49.1 a	51.8 a	34.6 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 conducted before assessment.
[3] Only blosck III and IV be regarded as unsprayed control as no treatment had been conducted before assessment.

Figure 15 a,b. Differences between treatments in blocks III and IV (pure *Poa annua*) at Kävlinge on 18 Dec. 2012. Photos: Per Göran Andersson

2.5 Experiment 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 an 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 variety trials (Figure 16).

Figure 16. The experimental area to be used in this project was seeded with *Agrostis stolonifera* on 26 July 2011 was therefore still covered when this photo was taken on 1 Aug. 2011. Photo: Trygve S. Aamlid.

2.5.1.2 Turfgrass maintenance

The green was mowed three times per week with a walk-behind mower. In 2011, the lowest mowing height was 4 mm, while the last mowing was to 5 mm on 11 November. In 2012, the first mowing was to 9 mm on 26 March; this was lowered step by step until the standard height of 3 mm was reached on 16 May and practiced throughout the rest of the growing season until the last mowing on 17 Oct.

The total fertilizer rate in the grow-in year 2011 was 220 kg N, 44 kg P and 174 kg K per ha. This includes 70 kg N, 28 kg P and 56 kg K of organic fertilizer raked into the seedbed before sowing. Fertilization after field mergence included weekly applications, partly of liquid and partly of granular fertilizers, during the first month after seeding, and then at two week intervals until the last application on 27 October.

In 2012, fertilizers were always given at two week intervals, the total fertilizer rate equaling 158 kg N, 12 kg P and 119 kg K per ha. The first input was on 23 March and the last input on 7 November. The experimental area was verticut four times, aerated with 6 mm solid spikes to 3-4 cm depth four times, and topdressed 18 times (almost every week), the total amount of straight sand equaling 6.5 mm. There was no regular wear or compaction from players on the green, but this was compensated by several weekly passes with as friction wear roller with golf spikes, the total amount of wear corresponding to 20.000 rounds of golf. Deep aeration with 16 mm solid spikes was performed on 15 Nov. 2012.

From July 2012 a severe problem on the green was take-all patch (*Gaeumannomyces graminis*) that also contributed to localized dry spots (Figure 18b). To reduce dry spots the green was sprayed with the soil surfactant Aqueduct at a rate 25 l ha^{-1} five times from 9 July to 7 Aug. To reduce take-all patch it was also sprayed with Yaravita Mantrac Optiflo, 1 l = 0.274 kg Mn ha^{-1}) on 17 October 2012.

2.5.1.3 Inoculation before winter

Because of very little infection of *M. nivale* during the winter 2011-12, it was decided to inoculate the green before the winter 2012-13. This was done in two ways:

1. On 21 November 2012, clippings from a nearby green with *Poa annua* infected by *M. nivale* was mixed with topdressing sand and distributed evenly over the entire experimental area.
2. On 27 November 2012 inoculum of two isolates of *Microdochium majus* was obtained from the fungal collection at Bioforsk Plant Health Department (inoculum of M. nivale was not available in sufficient amounts). The inoculum was diluted in water and sprayed evenly over the experimental area at a total rate of 3.9 x 10^5 cell forming units per m^2.

2.5.1.4 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 17). This procedure allowed full coverage of the central 1.5m x 1.0m of each plot which was used for assessments. The spraying volume was 250 l ha^{-1} except for Turf G+ that was applied in a volume of 500 l ha^{-1} in autumn 2011 and spring 2012. Actual application rates were recorded by weighing the tank before and after spraying. Realized applications rates and weather conditions on the various application dates are given in Table 11.

Figure 17.
Experimental plots sprayer used in trial at Bioforsk Landvik, Norway. Photo: Trygve S. Aamlid.

Table 11. Applications dates, realized rates and weather conditions in trial at Landvik as of 20 March 2013.

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

2.5.1.5 Weather data

The autumn 2011 and winter 2011-12 at Landvik was much milder than the 30 year normal temperature (Table 12). Snow covered the experimental green only from 21 January to 23 February 2012. March 2012 was exceptionally warm with a monthly mean of 6.8 °C and a maximum of 23.1 °C on 27 March. May was rather dry, but the rest of the growing season was conducive to grass growth with no drought periods. The winter 2012-13 has so far been rather cold, with snow covering the green from 2 December to the present day (2 April). After a mild period around 1 January there is a 2 cm ice layer under the snow, and we are concerned what consequences this might have for turf survival and for *Microdochium nivale/majus*.

Table 12. Monthly values for air temperature and precipitation for the experimental periods 1 Oct. 2011– 31 May 2012 and 1 June 2012 – 28 Feb. 2013 as well as 30 year normal values 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	14.8	17.0	15.5	12.9	8.9	6.5	2.1	0.1	-0.5	6.8	5.2	11.8	8.4
2012-13	12.9	15.8	15.8	11.6	6.8	4.8	-3.3	-3.1	-2.1				
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	104	157	189	235	74	54	156	144	15	32	136	53	1338
2012-13	119	83	107	132	218	239	286	81	26				
30 yr normal	71	92	113	136	162	143	102	113	73	85	58	82	1230

2.5.2 . Results

Throughout the experimental period there were small attacks of *M .nivale* on the bentgrass green (Figure 18a) but these attacks were not significantly affected by any of the experimental treatments (Table 13). The only treatment with a trend to less microdochium patch / pink snow mold at the first two observations after snow melt in February 2012 was the chemical control.

From mid-summer 2012, there was an increasing attack of take-all patch (*Gaeumannomyces graminis*) especially in block no III (Figure 18b). The spots appeared randomly on the green, and the difference in mean values shown in Table 13 were far from significant. This was also the case for traces of red thread (*Laetisaria fuciformis*) seen at the last observation shortly before snow fall in November 2012.

I addition to diseases, turfgrass overall impression was also assessed at monthly intervals throughout the experimental period. Seasonal mean values from these observations are shown in Table 14. Differences were not significant, and in summer and fall 2013 they mostly reflected the random distribution of take-all patch.

Table 13. Per cent of plot area affected by microdochium patch or pink snow mold caused by *Microdochium nivale*, take-all patch caused by *Gaeumannomyces graminis*, and red thread caused by *Laetisaria fuciformis*.

	Microdochium nivale								*Gaeumannomyces graminis*			*L. fuci-formis*
	18 Oct. 2011	9 Nov. 2011	9 Dec. 2011	27 Feb. 2012	22 Mar. 2012	17 Apr. 2012	21 May 2012	29 Nov. 2012	1 Aug. 2012	1 Oct. 2012	29 Nov. 2012	29 Nov. 2012
Unsprayed control	0.0	1.0	0.3	0.7	1.3	1.8	2.5	0.0	3.8	12.0	9.5	0.0
Delaro	0.3	0.2	0.0	0.0	0.0	0.0	0.0	0.0	2.2	10.7	8.0	0.0
Turf G+/WPG	0.0	0.7	0.5	0.8	1.5	1.8	3.0	0.0	2.7	7.2	7.0	0.0
Turf S +	0.0	0.7	0.3	0.4	0.7	1.0	1.8	0.1	4.3	11.0	12.0	0.0
Turf G+/WPG + Turf S+	0.0	0.7	0.2	0.2	0.5	0.7	1.2	0.1	1.7	3.5	1.7	0.2
Vacciplant, 1 liter ha^{-1}	0.3	0.5	0.3	0.1	0.3	0.5	1.3	0.3	3.5	2.7	3.7	0.2
Vacciplant, 2 liter ha^{-1}	0.3	1.0	0.3	0.4	0.3	0.3	1.3	0.2	1.7	3.7	3.8	0.1
P-value	> 0.20	> 0.20	> 0.20	> 0.20	0.12	0.12	> 0.20	> 0.20	> 0.20	> 0.20	> 0.20	> 0.20

Figure 18 a, b. From trial at Landvik on 9 Dec. 2011 (left) and 1 September 2012 (right). Photos: Trygve S. Aamlid.

Table 14. Means of seasonal observations for turfgrass overall impression (scale 1-9, 9 is best visual quality) in trial at Landvik.

	Autumn 2011 (1 obs.)	Spring 2012 (2 obs.)	Summer 2012 (4 obs.)	Autumn 2012 (2 obs)
1. Unsprayed control	6.7	6.7	5.9	4.7
2. Delaro	7.3	7.7	6.7	5.2
3. Turf G+/WPG	7.5	7.0	6.6	5.3
4. Turf S +	7.2	7.0	5.9	4.2
5. Turf G+/WPG + Turf S+	6.7	7.2	7.1	6.1
6. Vacciplant, 1 liter ha^{-1}	6.7	7.4	6.6	5.6
7. Vacciplant, 2 liter ha^{-1}	7.0	7.3	6.7	5.7
P value	>0.20	>0.20	>0.20	>0.20

2.6 Experiment at Arendal GC, Norway

2.6.1 Materials and methods

2.6.1.1 Experimental site

The trial was laid out on 20 Oct. 2011 on a nursery green at Arendal GC (Figure 19). Arendal GC is located in Nesgrenda, NO-4900 Tvedestrand, about 40 km north-east of the other Norwegian location 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 19. Nursery green at Arendal GC at the start of experimentation 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. In 2012 it received four applications of granular fertilizer, the first application on 15 April and the last application on 18 September. The total N rate was 128 kg ha^{-1}.

Table 15. Applications dates in trial at Arendal GK as of 14 March 2013.

Date	Spraying, time of day (hours)	Treatment no / Product applied	Target rate, mL or g per ha	Real rate, mL/ha	Weather at application			Hours before rainfall
					Air temp. °C	Relative humidity, %	Wind, m/s	
20 Oct. 2011	1330-1515	2. Delaro	1000	960	7.0	57	1,6	>12
		3. Turf G+	10000	8533				
		5. Turf G+	10000	9956				
		6. Vacciplant	1000	960				
		7. Vacciplant	2000	1920				
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				

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

Table 15 continued

Date	Spraying, time of day (hours)	Treatment no / Product applied	Target rate, mL or g per ha	Real rate, mL/ha	Tempera -ture, °C	Relative humidity %	Wind, m/s	Hours before rainfall
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	1102				
		7. Vacciplant	2000	1037				
8 Nov. 2012	1130-1230	2. Delaro	1000	1102	7.8	80	2,0	>12
		3. Turf WPG	1000g	1102				
		5. Turf WPG	1000g	1037				
		6. Vacciplant	1000	1013				
		7. Vacciplant	2000	1067				

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. Details from applications are given in Table 15.

2.6.1.4 Weather data

The weather during both evaluation seasons was similar to that at Landvik except that winters were slightly colder (Table 16). The green was covered by snow for a short period around Christmas / New year 2011/12 and then from Mid-January to mid-March 2012. As of 20 March 2013, the green has been covered by snow since 1 December 2012.

Table 16. Monthly values for air temperature and precipitation for the experimental periods 1 October 2011– 31 May 2012 and 1 June 2012 – 28 Feb 2013 as well as 30 year normal values for The Norwegian Meteorological Institute's weather station at Nelaug, about 30 km north of the experimental site.

	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Year
Temperature, °C													
2011-12	15.2	16.5	15.0	12.3	7.9	5.2	0.5	-1.5	-1.7	5.9	4.2	11.4	7.6
2012-13	12.3	15.8	15.6	10.7	5.2	3.5	-5.4	-5.0	-4.3				
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	102	227	208	255	79	55	165	143	13	21	141	63	1472
2012-13	122	43	114	117	204	206	264	60	22				
30 yr normal	78	108	109	139	164	138	96	108	72	82	60	90	1244

2.6.2 Results

The first diseases to be diagnosed in the trial at Arendal GK were superficial fairy rings and *Typhula incarnata* (Figures 20 and 21) Both appeared in the late fall, *Typhula* mostly in 2011 and fairy rings mostly in 2012. In fall 2012 there were also a certain infection of red tread. None of these diseases were significantly affected by any of the experimental treatments (Table 17).

Figure 20. Superficial fairy rings and *Typhula incarnata* in trial at Arendal GK, 8 Nov. 2011. Photo: Trygve S. Aamlid

Figure 21. Close-up of *Typhula incarnata*, 8 Nov. 2011.
Photo: Trygve S. Aamlid

Table 17. Mean values for infection of gray snow mold (*Typhula incarnata*), red thread (*Laetisaria fuciformis*) and superficial fairy ring, as well as mean seasonal values for turfgrass overall impression in trial at Arendal GK, Norway.

	% of plot area				Turfgrass overall impression (1-9)			
	Typhula incarnata 14 Dec. 2011	*Laetisaria fuciformis* (4 obs in fall 2012)	Superficial fairy rings (2 obs. in fall 2012		Autumn 2011 (2 obs)	Spring 2012 (2 obs)	Summer 2012 (4 obs)	Autumn 2012 (3 obs)
1. Unsprayed control	1.3	0.5	0.2		5.3	5.2	7.3	6.7
2. Delaro	0.0	0.5	0.0		6.1	5.5	7.5	7.3
3. Turf G+/WPG	4.0	0.7	0.9		5.2	4.8	7.0	6.9
4. Turf S +	2.3	0.5	1.4		5.2	5.0	7.3	6.6
5. Turf G+/WPG + Turf S+	0.0	0.6	0.0		5.2	4.2	6.5	6.2
6. Vacciplant, 1 liter ha^{-1}	2.3	0.5	0.0		5.4	5.0	7.2	6.9
7. Vacciplant, 2 liter ha^{-1}	2.3	0.8	0.1		5.1	4.5	6.8	6.4
P-value	>0.20	>0.20	>0.20		>0.20	>0.20	>0.20	0.006
LSD 5%								0.5

Seasonal averages for turfgrass quality shows that highest overall turfgrass impression was usually found on control plots treated with Delaro and that the alternative products did not produce any better quality than the unsprayed control. However, the difference between the fungicide control treatment and the other treatments was only significant in autumn 2012.

3. Evaluation of Turf G+/ WPG and Turf S+ for control of M. nivale *in vitro*[1]

3.1.1 Rationale

A former STERF project suggested that *in vitro* evaluation might be a useful indicator for the efficiency of microbial agents to control *M. nivale* (Hofgaard et al. 2009). The two microbiological products tested in the field trials are therefore also included in an *in vitro* study: the fungal agent Turf G+ (Verdera, Esbo, Finland) and the bacterial agent Turf S+ (Verdera, Esbo, Finland). The study has been run into two steps – a pilot study and a main study. The objective of the pilot study was to find optimal conditions (concentration of growth medium and sufficient concentrations for biological agents) for the main study. This study was run in July 2012 with microbial agents received from Interagro BIOS AB in spring 2012 and results are reported here. The objective of the main study is to test the efficiency of microbiological agents and selected fungicides to reduce mycelial growth of *M. nivale* in vitro. The main study is being conducted from 4 to 23 March 2013 and the results will be shown in the final project report.

3.1.2 Materials and methods

The 4-factorial experiment was set up according to a fully randomized design with 3 replicates:

Factor 1. Products (Table 18):

1.1. Control
1.2. Turf G+
1.3. Turf S+
1.4. Turf G+ + S+ (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)[2]
2.2. 10-% PDA
2.3. 1-% PDA;

Factor 3. Concentrations of the agents in medium:

3.1. Recommended dosage (100 % concentration) (Table 19)
3.2. 10 % of recommended concentration
3.3. 1 % of recommended concentration

Factor 4. Temperature for incubation:

4.1. 6°C
4.2. 16°C.

According to Verdera, Turf G+ contains spores and mycelium of *Gliocladium catenulatum* (Figure 22) at a minimum of 10^7 CFU (Colony forming units per ml), whereas Turf S+ contains at least 10^8 CFU of *Streptomyces* spp.

The PDA media of different concentrations were autoclaved at 121°C for 15 minutes and cooled down to 50°C.The microbial agents were dissolved in sterile water in amounts necessary to get the final concentrations of 100%, 10% or 1% of the products in the media. The microbial agents were added to the media and carefully stirred. Hundred milliliter media was divided among six Petri plates. After the media

[1] *In vitro* (lat.) - in an artificial environment
[2] 1 L of 100 % PDA contains 4 g potato extract, 20 g dextrose and 15 g agar, with final pH 5.4.

had solidified, one replicate was inoculated with agents and 3 control replicates were inoculated with *M. nivale* (the fungus had been isolated from an annual bluegrass green at Arendal and Omegn GC in July 2012) at the center of the Petri plate. All Petri plates were then incubated at either 6°C or 16°C for 13 days.

Table 18. Recommended dosage of microbiological products according to Verdera.

	Recommended dosage		100% tank concentration,
	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

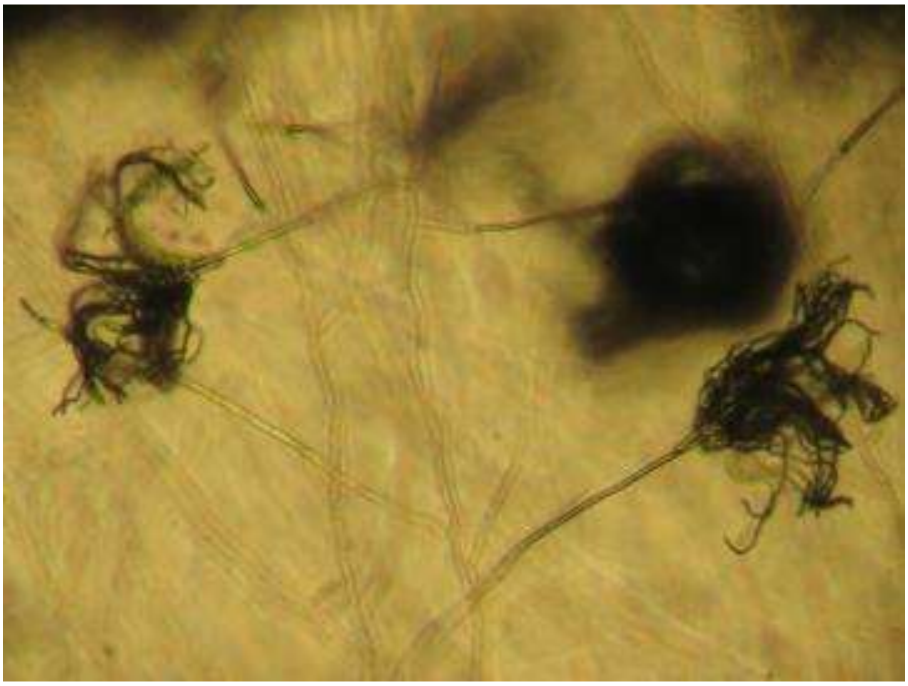


Figure 22. Sporangia of *G. catenulatum*.
Photo: Tatsiana Espevig

The number of *G. catenulatum* colonies was determined on Petri plates with Turf G+ and with Turf G+ and Turf S+, and the CFU of Turf G+ was calculated. The number of bacterial colonies was too high to be counted, and it was instead registered using a scale from 0 (no colony) to 6 (infinite number). The diameter of *M. nivale* colonies was measured on day 3, 8 and 13, and the data expressed as fungal radial growth per day in percentage of control at each temperature.

3.1.3 Results and discussion

The number of CFU of *G. catenulatum* (Turf G+) was on average for 1 %, 10 % and 50 % PDA and both temperatures only 10^3 (Table 19). This was much lower than the CFU of 10^7 reported by the manufacturer. Among possible reasons for such low CFU of *G. catenulatum* could be anaerobic conditions for spore germination and mycelial growth since the product was blended into the media. A better method to determine the CFU of Turf G+ will be to add the culture to the agar surface and distribute it uniformly with a Drigalski spatula. Another reason for the low CFU could be a strong bacterial contamination of Turf G+ since a large amount of two bacterial species was observed in Petri plates inoculated with Turf G+ only (Figures 23 and 24). The bacterial contaminants were not identified. The reason for the big difference in CFU between 1 % and 10 %/50 % PDA is unknown.

Table 19. CFU of *G. catenulatum* averaged for 100%, 10% and 1% Turf G+.

	Temperature for incubation	
PDA concentrations	6 °C	16 °C
1 %	3852	4458
10 %	16	31
50 %	36	67

Table 20. Effects of microbiological agents and their combination on radial growth of M. nivale (average for 3 concentrations of agent and 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

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 test-study on determination of CFU in both bacterial and fungal agents 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 both *G. catenulatum* growth (Table 19) and on *Streptomyces* growth (data not shown) was clear. The higher temperature of 16°C significantly enhanced the number of the microbes as opposed to 6°C. The efficiency of both microbial agents and their combination was also higher at 16°C as compared with 6°C (Table 20). The bacterial agent Turf S+ had higher efficiency to reduce radial growth of *M. nivale* than the fungal agent Turf G+ and the efficiency of Turf S+ was also persistent in the presence of Turf G+ (Table 20). These results on *M. nivale* suppression by microbiological agents are preliminary and based on only one replicate. As we are now using new inoculum of the new formulation Turf WPG, results may well be different in the main study.

The slower radial growth of *M. nivale* on 50 % PDA (Table 21) suggests using this PDA concentration in the main study. Otherwise the fast radial growth of *M. nivale* in control Petri plates can limit the duration of the experiment as the fungus will grow over the plate border. The argument against using 50 % PDA is that fungus experiences much lower nutrient concentration on the leaf surface than on 50 % PDA.

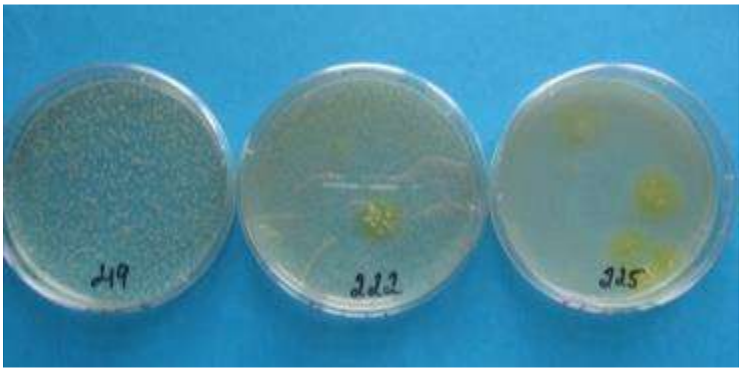


Figure 23. From left to right: 1 %, 10 % and 100 % of Turf G+ on 50 % PDA. Green colonies in dish 222 and 225 are colonies of *G. catenulatum*. Petri plates were also contaminated by bacteria. Photo: Tatsiana Espevig

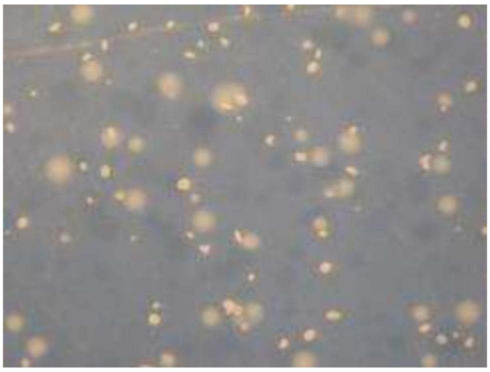


Figure 24. Scaled up Petri plat nr. 219 from Figure 1. The colonies of two bacteria species are visible. Photo: Tatsiana Espevig

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

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

4. Discussion

The biocontrol products tested in this project are 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 is not specific for turf and has so far been used primarily in the production of fruits and berries. On 19 Dec. 2012, Vacciplant was registered for control of *Botrytis* in strawberry production in Denmark, but its effect of *Microdochium nivale* has not been proved previously. The application of another seaweed product which also contains laminarine, Golf Algin, resulted in significantly more *M. nivale* than on the unsprayed control plots in an earlier Norwegian project (Aamlid et al. 2009).

In contrast to Vacciplant, the fungal product Turf G+/WPG (contains *Gliocladium catenulatum*) and bacterial product Turf S+ (contains *Streptomyces* sp.) are not supposed to be taken up by the plant but to protect by providing a microbial flora that is antagonistic to *M. nivale* and other pathogenic fungi. At their website, the Finnish manufacturer Verdera OY highlights the direct protection of the root system (Anonymous 2013a,b), but as reviewed by Nelson (1997), there may be other mechanisms as well. *G. catenulatum* and *Streptomyces* sp. 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+ are even used by many greenkeepers, usually in combination with fungicides (K. Laukkanen, Finnish Golf Union, personal communication).

Common to Vacciplant, Turf G+/WPG and Turf S+ 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 thatch/rootzone. It was therefore unfortunate that the acceptance of the project was delayed so that the first application of the test products could not start until c. 20 October 2011. At all test sites, October, November and December 2011 and January 2012 were, however milder that the 30 year normal values, and with three applications before winter, it could be expected that at least Vacciplant, which does not require any build-up of microbial populations, would have certain effect on *M. nivale* already in the first year. However, this did not occur, in fact one of the observations at the *Poa annua* dominated green at Kävlinge suggested less *M. nivale* on unsprayed control plots than after application of the test products. This resembles some of our earlier experiences with Golf Algin (Aamlid et al. 2009).

It seems to be a general experience that populations of biocontrol organisms must stay at numbers higher than 1 million cells per gram of soil to be effective against diseases (Nelson 1997). Fungal or bacterial antagonism is, as stated by Horvath & Vargas (2000), 'a number's game'. As documented by the *in-vitro* pilot study, it was therefore unfortunate that the number of cell forming units (CFU) of *G. catenulatum* in the batch of Turf G+ delivered in spring 2012 was only of magnitude 10^3 as opposed to 10^7 stated by the manufacturer. If this was the case even for the batch delivered in October 2011, it would explain the generally poor effect of Turf G+ so far in the project. Quality assurance of products delivered for experimental purposes is of course the responsibility of the manufacturer, and preliminary data from the second *in-vitro* study in March 2013 (not included in this report) shows that CFU in the batch of Turf WPG delivered in January 2013 was according to the label.

Another interesting finding in the *in-vitro* pilot study is that *Streptomyces* spp. was more efficient than *G.catenulatum* in controlling *M. nivale*. *G. catenulatum* had no effect on *M. nivale* growth at 6°C. This may not be surprising given the fact that the optimum temperature for growth of *M. nivale* is as high as 21°C (Årsvoll 1975, Hofgaard et al. 2009) and our own observations that microdochium patches may develop even during wet summers (Espevig, 2012). Hofgaard et al. (2009) reported that the prevalence of *M. nivale* in leaves and stems of grasses was usually reduced during the growing season, but they also concluded that the fungus survived from year to year within the same locations on greens and foregreens. While the manufacturer recommends the use of *G. catenulatum* in early spring and late autumn (coinciding with day temperatures 5 and 10 ºC) and *Streptomyces* during summer, our observations suggest that *G. catenulatum* would be more efficient if applied also during the growing season, and that there is no antagonism between the two agents *in vitro*. If the fungus and the bacterium compete for the same sites along plant roots, applications during summer may perhaps alternate between the two products instead of giving them in tank mixture. In the greenhouse industry, *G.catenulatum* is primarily recommended for the control of *Pythium*, especially at the seedling stage, while *Streptomyces* is considered more efficient against *Fusarium* (Anonymous 2013 a,b). The possibility of changing the protocol so that *G. catenulaum* is applied even during summer months will be discussed with the manufacturer.

The only field trial suggesting a certain positive effect of the test products so far is the trial at Sydsjælland GC. On 28 Nov. 2012 this trial showed more brownish and/or dead turf on unsprayed control plots than on plots treated with the test products and samples diagnosed at Århus University indicated that these differences in turf quality could be due to *M. nivale*. As earlier research has shown *M. nivale* at Sydsjælland GC to be partially resistant to tebuconazole (Paaske 2012), the level of brownish turf in the fungicide control treatment also suggests that this discoloration was mostly caused by *M. nivale*. We have previously observed that M. nivale develops different and perhaps more diffuse patches in *F. rubra* than in *Agrostis* sp. and *Poa annua*.

Apart from the fact that populations of *M. nivale* were probably different, it is hard to explain why the test products were more efficient in controlling *M. nivale* at Sydsjælland GC than at Rungsted GC and Kävlinge GC. As there was more *F. rubra* and less *Poa annua* at Sydsjælland, it might be speculated that the products were efficient in protecting roots of *F. rubra* (at Sydsjælland) than of Poa annua (at Rungsted and Kävlinge). While it does not explain differences between Rungsted and Sydsjælland, a notable difference in the protocols between the trials in Denmark and the trials in Sweden and Norway is that the application volume for all products was 400 l/ha in Denmark as opposed to 250 l/ha in Sweden and Norway. This difference in application volume was accepted by Interagro BIOS by the start of the project, although they usually recommend at an application volume of 300-400 l/ha (http://ny.interagrobios.se). The need for high application volumes of biocontrol agents was mentioned also by Nelson & Craft (1999) who recommended up to 800 l/ha for some products. Starting with the first applications in spring 2013, the protocols have now been changed so that all products are applied at a rate of 400 l/ha in all trials.

As the trials at Denmark, Sweden and Norway are now experiencing a longer snow cover than during the winter 2011/12, it will be very interesting to observed any effects of the test products on *M. nivale* in spring 2013. The project is further scheduled to continue with a third experimental period from 1 June 2013 to 31 May 2014, and with a final report to be delivered by the end of 2014. An important objective of the project is to observe if product efficacy will become more consistent when Vacciplant is applied earlier in the autumn and when *G. catenulatum* and *Streptomyces* sp. are allowed to build up over a longer period. While may not be realistic to expect that the products can offer as good control as fungicides, a lot would be gained if Vacciplent, Turf G+/WPG and/or Turf S+ could replace at least some of the annual fungicide applications on many golf courses.

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