Report from the project "Improved Strategy for Control of *Microdochium nivale* on Golf Courses" (2006-2008), funded by STERF

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Content

Project summary:

The most important and widespread disease on golf courses is *Microdochium nivale.* Attempt to control the disease is mainly by prophylactic spraying with fungicides in fall. The aim of this project has been to understand how inoculum of *M. nivale* survives from spring to fall and to clarify the efficiency of selected fungicides.

Snow mould symptoms and the occurrence of *M. nivale* in leaves and stems of grasses sampled from golf greens and foregreens was reduced during the growth season. We also found that *M. nivale* could be isolated from locations without visible symptoms. Despite a lower isolation rate in autumn, *M. nivale* was again isolated in some of the originally locations, the following spring. The *M. nivale* isolation rate was similar from sites located on greens compared to foregreens, and from greens located at more sunny sites compared to more shadowy located greens. We conclude that this fungus seem to survive from year to year within the same locations on greens and foregreens.

A significant correlation was found between mycelial growth rate of *M. nivale* isolates at 2°C compared to growth at 20°C. At 20°C, a greater variation in growth rate was registered between strains isolated right after snow melt, compared to strains isolated in spring, summer, autumn or prior to snowfall. No clear picture emerged in growth rate differences between groups of *M. nivale* strains isolated at different time points throughout the year. Significant reduction in mycelial growth rate of *M. nivale* was registered on agar added low concentrations of all the fungicides tested.The products were: Acanto Prima (cyprodinil, pikoksystrobin), Amistar (azoksystrobin), Amistar duo (azoksystrobin, propikonazol), Baycor (bitertanol), Bumper (propikonazol), Comet (pyraklostrobin), Proline (protiokonazol), Rovral 75WG/Chipco Green 75WG (iprodion), Sportak EW (prokloraz), Stratego 250 EC (propikonazol, trifloksystrobin), Topsin WG (tiofanatmetyl). It was large variation among the fungicides in the effect on fungal growth rate. Sportak, Stratego and Topsin were the most efficient products; 90-100% reduction in mycelial growth rate was registered on agar added 0.1% of the fungicide concentration recommended for disease control on golf greens. Acanto Prima, Bumper, Comet and Stratego were also tested for their effect to reduce snow mould damage on golf greens. Due to severe water damage on the greens the second season, only results from one season of the fungicide field trial could be used. Acanto prima was ranked as the best product, significantly reducing winter injury on average from 21% (in control plots) to 6% in the treated plots.

Introduction

The most important and widespread disease on golf courses is *Microdochium nivale* (Tronsmo et al 2001, Mann and Newell 2005). It is a psycrotrophic fungal plant pathogen that is the main cause of biotic winter injury in grasses in the temperate and sub-arctic climates, both with and without snow cover. During the growing season, this fungus is the cause of "Fusarium patch" on turfgrasses, causing stem rot and leaf blotch (Smiley et al 2005). It is an opportunistic pathogen, with the ability to attack plants under a wide range of environmental conditions. Even though its optimum for growth *in vitro* is around 20 ºC (Årsvoll 1975), in the Nordic countries *M. nivale* is mainly regarded as a snow mould pathogen. It is however, not clear if the same strains of the fungus are responsible for both snow mould and Fusarium patch. It is feasible that there exist strains with different temperature preferences, and hence, other "individuals" would dominate in summer than in winter. A large variation in both host preference and aggressiveness among isolates has been documented (Hageskal et al.1999, Simpson et al 2000, Hofgaard et al 2006). It is speculated that these traits as well as competition between islolates may be dependent on temperature (Simpson et al. 2004).

The fungus is believed to be spread by infected seeds (McBeath et al. 1993) and inoculum (mycelia, conidia and ascospores) from infected plants or debris (Domsch et al. 1980). In absence of seed transmitted inoculum, however, it is not clear whether the primary inoculum source is wind dispersed ascospores or soilborne/plant debris borne inoculum (Tronsmo et al 2001). Wind borne ascospores has been claimed to be the main inoculum source (Parry 1995, Mahuku 1998), but it is also reported that *M.nivale* has good saprophytic ability (Domsch et al.1980), hence the inoculum source could be conidia and hyphae. If wind borne ascospores are the main source of inoculum in autumn, leading to snow mould injury, the climatic conditions influencing perithecia development and ascospore release will be decisive parameters in estimation of the risk of dispersal of inoculum and infection. Soil borne inoculum does not necessary depend on perithecia formation, and are therefore less dependent on climatic factors.

Most green keepers attempt to control the disease by prophylactic spraying with fungicides in fall. The fungicides, which are permitted for this purpose, vary between the Nordic countries (Lilleby 2005, Green bladet 2005), and the effect of these against *M. nivale* is not satisfactory documented. It is therefore probable that many golf courses are sprayed with fungicides without the desired effect. A preventive application of fungicides against snow mould is independent of the actual risk of infection and disease development. The risk is related to the inoculum potential, the aggressiveness of the fungal strain, the resistance of the plants and the weather conditions. Since most of these factors varies from year to year, a large proportion of the fungicide applications on golf courses are unnecessary or wasted. For several major plant diseases, disease-forecasting systems have been developed (VIPS 2005), but such a system is not yet available for *M. nivale*.

The aim of the present project was get more insight in to what is the source of primary inoculum for snow mould caused by *M. nivale*; to understand how inoculum of *M. nivale* survives from spring to fall, and from year to year, to understand how climatic conditions affects the potential inoculum by monitoring symptoms on plants, occurrence of the fungus

and growth characteristics *in vitro* of strains sampled from snow melt and through summer and autumn. To obtain such knowledge, surveys and sampling on selected golf courses was conducted. Profound understanding of the biology and the ecology of the pathogen is essential for development of a disease forecasting system in the future. The project also aimed at clarifying if the fungicides permitted to use for control of snow moulds in the Nordic countries are effective against *M. nivale*.

1. Golf course surveys for Microdochium nivale inoculum

Material and methods

Golf courses; Locations, plant material and sampling

Three golf courses with different climatic and biologic conditions were selected: **Bogstad** (Oslo) with *Poa annua* greens, **Mørk** with *Agrostis* greens and **Sorknes** with *Agrostis* greens and continental climate with long winters (Table 1). On each golf course snow mould injury was assessed on two greens, one with shadow and one mainly free of shadow (Figure1). From snow mould patches, infested grasses were sampled in early spring. The same sites were sampled 4-5 times during the growth season until late autumn (snow or frost). Identification of location of sites for revisits are shown if figure 2. Visible patches were assessed and documented by photography (Figures x-y). On one location (Mørk), the same sites were sampled from after snow melt 2006 until after snow melt 2008. At Bogstad, samples were taken during the first year (2006). The greens at Bogstad were sprayed with fungicides before last sampling late autumn 2006. In summer 2007 they started rebuilding the golf course, and could therefore not longer be part of the project.

Table 1. Facts about the different golf courses:

Name og the golf course	Location	Duration of snow cover	Grass species and varieties	Surveys and sampling	Comments
Mørk golf	Spydeberg	3 months	Greens: *Agrostis stolonifera* 'Penncross' and 'Norgreen' Forgreen: *Festuca (and Poa)*	2006 spring-snow 2007 spring-snow 2008 spring	Supplementary sowing at least once each year
Oslo golfklubb, Bogstad	Bogstad, Oslo	3 months	*Poa annua*	2006 spring-autumn 2007 spring	Annual sowing Sprayed with fungicides late autumn 2006 Rebuilding of golf course started 2007
Sorknes golf	Rena	4-6 months	*Agrostis stolonifera*	2006 spring 2007 spring	

Bogstad Green 12 (Sun) Sept 2006

Bogstad Green 11 (Shadow) Sept 2006

Mørk Green 18 (Sun) June 2006

Mørk Green xtra (Shadow) June 2006

Sorknes Green 9 (Sun) April 2006

Sorknes Green 6 (Shadow) April 2006

Figure 1. Surveyed Greens at Mørk, Oslo and Sorknes Golf

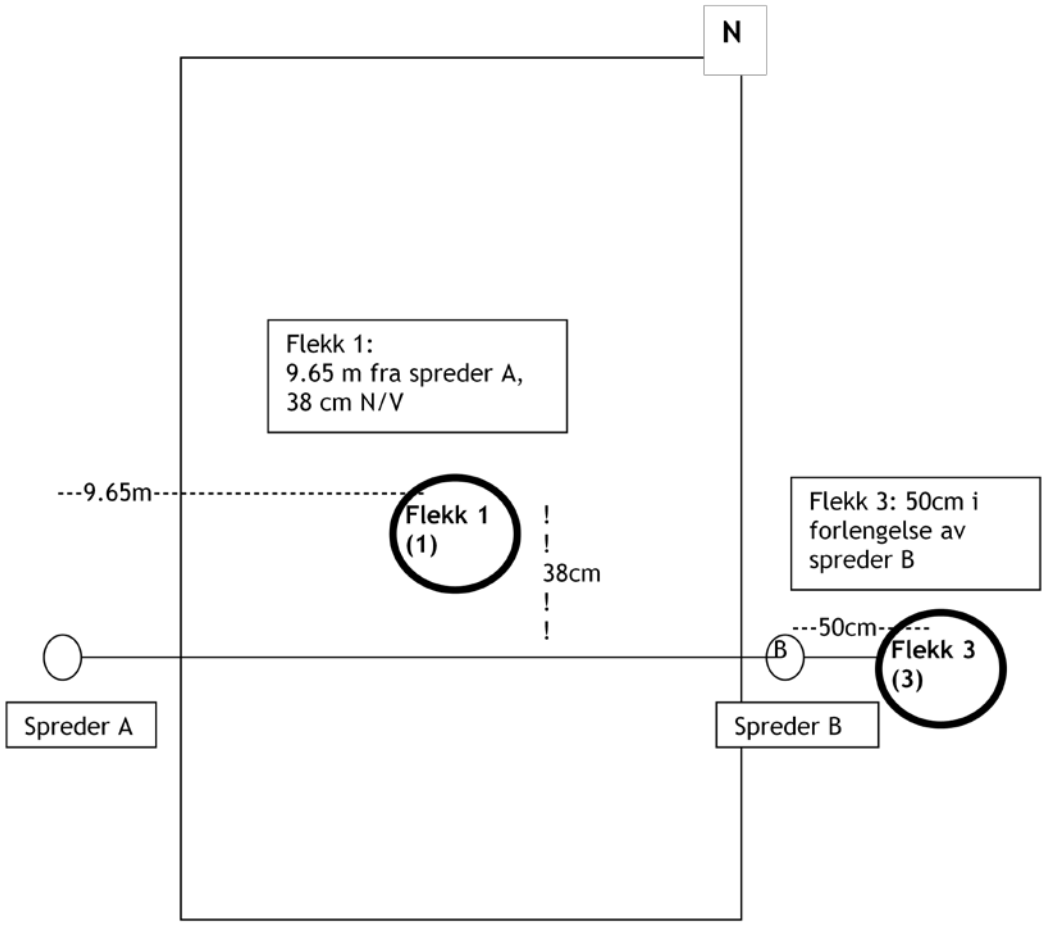

Figure 2. Illustration of sampling sites (spots) at Mørk Green and forgreen No 18 (Sun)

Isolation of the fungal strains

Fungal strains were isolated from plants, plant debris and roots/soil from the samples collected during spring 2006. Leaf and root samples of grasses were +/- surface disinfected and placed on nutrient medium, Potato Dextrose Agar (PDA) or Water Agar (VA) for further work to get the isolate in pure culture. Two different incubation regimes were used in the first isolations, 9°C in darkness and 20°C in 12h light+NUV. Different media for *M. nivale* growth was tested: PDA, VA, acidic PDB+ streptomycin agar and oat meal agar.

As the samples from roots and soil more often was contaminated with bacteria and *Mucor/Rhizopus*, less successful isolations of *M. nivale* were performed on root samples compared to pieces of leaves and leaf stems. Placing the plant material on VA gave a higher rate of *M. nivale* isolations, compared to initially placing the plant samples on PDA.
More efficient (less incubation time, same output) *M. nivale* isolations were registered when the leaves were incubated at 20°C compared to 9°C. Poor growth of *M. nivale* was registered on acidic PDB+ streptomycin agar. Growth of *M. nivale* on oat meal agar was similar to growth on PDA, but due to the white/beige colour of the oat meal agar, this agar was not suited for observation of the white-salmon coloured *M. nivale* colonies. Sporulation of *M. nivale* was registered more often on PDA compared to oat meal agar.

Based on the experience from 2006 the following procedure was used:

- The sample (grass with roots and soil) were stored in a cold room until isolation was performed. Each sample was placed in a separate plastic bag with moist paper towel or coffee filter
- Individual plants with symptoms or leaved with a clear zone between healthy and diseased area were selected and placed in a dish with clean water (Dish 1).
- The plants were rinsed until all sand and soil were removed. The plants were cut into small pieces while in water, and roots and dead leaves removed.
- Plant pieces displaying both diseased and healthy area were transferred to a new dish with clean water (Dish 2), and rinsed further.
- The plant tissue pieces were placed on filter paper to drain off the water.
- For each sample, 4-5 pieces were plated on each of 4-5 Water agar plates.
- The plates were incubated at 20°C +12h light+NUV in plastic bag (closed with tape).
- After 3-4 days, fungal growth was recorded, mycelium from the actively growing margin were transferred to PDA plates. (1st transfer).
- Growth and possible contamination was recorded after one week at 20°C +12h light+NUV.
- After one week, white and salmon colored mycelium were transferred to new PDA plates (2nd transfer) and incubated at 20°C and light for further growth and identification of the fungus before transfer to new PDA plates and then stored at – 80°C.

Results and discussion

Observations in the fields

In 2006, the recorded and sampled spots at Mørk and Bogstad showed typical symptoms of *M. nivale* infection (Figs 3 an4). The symptoms faded through summer, but could in many cases be seen again the next spring following year (Figs 3 and 4). No clear difference was observed between sun exposed greens and greens located in an area with more shadow. The recorded and sampled spots at Sorknes contained also typical *M. nivale* symptoms, but the symptomatic plans were intermingles with plant showing other winter injury symptoms.

Fungal isolations

The results from the isolations performed in 2006 are presented in table 2. The first sampling date after snow melt 2006, *M. nivale* was isolated from all samples from Mørk and Bogstad. None of the April samples from Sorknes revealed *M. nivale* infection. The only positive *M. nivale* finding from Sorknes all together, was from a sun exposed green sampled early may 2006. After several attempts to isolate the fungus from these greens and forgreens, no further samples was taken from this golf course in 2006. At Mørk and Bogstad, *M. nivale* was isolated from most sites in May and June. Sampling in September, however, identified *M. nivale* at only one site at Mørk. However, these samples revealed *Fusarium sambucinum* from one site at Mørk and 3 sites at Bogstad. In soil there is a continuous competition between the microorganisms (Alabouvette et al. 2004, 2006, Janvier et al. 2007) that will affect the survival of a psycrotrophic fungus as *M. nivale* during the summer period and hence its ability to infect plants. Our finding may indicate that the two pathogens are occupying in the same ecological niche, and may be antagonists. Competitive interactions between *M. nivale* varieties and *Fusarium* species *in planta* has also been documented and shown to be affected by temperature (Simpson et al. 2004). The last sampling before snow, in November 2006, revealed *M. nivale*, *Pythium* and an unidentified

Fusarium species from Mørk. At Bogstad, the greens were sprayed with fungicides late autumn, hence, November sapling was not performed.

The results from isolations performed in 2007 are presented in table 3. At sampling immediately after snow melt, two of the four sites at Mørk revealed *M. nivale*. There were no differences between sun exposed and less sun exposed greens. At Bogstad, none of the original sites revealed *M. nivale*, most probably due to spraying with fungicides prior to snow fall 2006. *M. nivale* was isolated from sites outside the green area. Due to rebuilding of the golf greens at Bogstad, no further samples were collected there. At Sorknes, none of the leaf samples taken after snow melt in May 2007 contained *M. nivale*. Sampling from this location therefore discontinued. Sampling at Mørk continued throughout 2007 and through early spring 2008. May sampling at Mørk revealed *M. nivale* in samples from sun exposed greens. *M. nivale* was not found in the autumn sapling.

Table 4 shows the results from sampling and isolations from Mørk in spring 2008. *M. nivale* was found in samples from the same site on the sun-exposed forgreen as in 2007 and 2006 (Figs 3 and 4). Samples from other sites revealed *Fusarium dimerum* and *Pythium*. This is again an indication of that several fungi occupy the same niche, and that competitive ability of the fungus affects the survival of inoculum in soil.

Table 2. Sampling and isolation of Microdochium nivale from golf greens 2006

Year	Sampling time	Golf course	Date	Who	Green no	sample no	*M. nivale* isolated from leaf or stem	*Comments*
2006	1	Mørk	19/4	Ingerd/Birgitte	Green 18 sol, green	01	1	
2006	1	Mørk	19/4	Ingerd/Birgitte	Green 18 sol, forgreen	03	1	
2006	1	Mørk	19/4	Ingerd/Birgitte	ekstragreen, green	09	1	
2006	1	Mørk	19/4	Ingerd/Birgitte	ekstragreen, forgreen	12	1	
2006	1	Bogstad	19/4	Anne Marte	Green 12 sol, green	15	1	
2006	1	Bogstad	19/4	Anne Marte	Green 12 sol, forgreen	17	1	
2006	1	Bogstad	19/4	Anne Marte	Green 11, green	18	1	
2006	1	Bogstad	19/4	Anne Marte	Green 11, forgreen	20	1	
2006	1	Sorknes	22/4	Anne Marte	Green 9 sun, green	31	0	
2006	1	Sorknes	22/4	Anne Marte	Green 9 sun, forgreen	32	0	
2006	1	Sorknes	22/4	Anne Marte	Green 6, green	34	0	
2006	1	Sorknes	22/4	Anne Marte	Green 6, forgreen	35	0	
2006	2	Mørk	8/5	Ingerd	Green 18 sun, green	01	1	
2006	2	Mørk	8/5	Ingerd	Green 18 sun, forgreen	03	1	
2006	2	Mørk	8/5	Ingerd	ekstragreen, green	09	1	
2006	2	Mørk	8/5	Ingerd	ekstragreen, forgreen	10	1	
2006	2	Bogstad	9/5	Ingerd	Green 12 sun, green	15	1	
2006	2	Bogstad	9/5	Ingerd	Green 12 sun, forgreen	17	1	
2006	2	Bogstad	9/5	Ingerd	Green 11, green	18	0	
2006	2	Bogstad	9/5	Ingerd	Green 11, forgreen	20	1	
2006	2	Sorknes	ca 8/5	Rena	Green 9 sun, green	31	1	
2006	2	Sorknes	ca 8/5	Rena	Green 9 sun, forgreen	32	0	
2006	2	Sorknes	ca 8/5	Rena	Green 6, green	34	0	
2006	2	Sorknes	ca 8/5	Rena	Green 6, forgreen	35	0	
2006	3	Mørk	14/6	Ingerd	Green 18 sun, green	01	1	
2006	3	Mørk	14/6	Ingerd	Green 18 sun, forgreen	03	1	
2006	3	Mørk	14/6	Ingerd	ekstragreen, green	09	1	
2006	3	Mørk	14/6	Ingerd	ekstragreen, forgreen	10	0	
2006	3	Bogstad	13/6	Ingerd	Green 12 sun, green	15	1	
2006	3	Bogstad	13/6	Ingerd	Green 12 sun, forgreen	17	0	
2006	3	Bogstad	13/6	Ingerd	Green 11, green	18	1	
2006	3	Bogstad	13/6	Ingerd	Green 11, forgreen	20	0	
2006	4*	Mørk	26/9	Ingerd	Green 18 sun, green	01	0	
2006	4	Mørk	26/9	Ingerd	Green 18 sun,forgreen	03	0	*F. sambucinum*
2006	4	Mørk	26/9	Ingerd	ekstragreen, green	09	1	
2006	4	Mørk	26/9	Ingerd	ekstragreen, forgreen	10	0	
2006	4	Bogstad	28/9	Ingerd	Green 12 sun, green	15	0	
2006	4	Bogstad	28/9	Ingerd	Green 12 sun, forgreen	17	0	*F. sambucinum*
2006	4	Bogstad	28/9	Ingerd	Green 11, green	18	0	*F. sambucinum*
2006	4	Bogstad	28/9	Ingerd	Green 11, forgreen	20	0	*F. sambucinum*
2006	5	Mørk	7/11	Ingerd	Green 18 sun, green	01	0	*F. sambucinum*
2006	5	Mørk	7/11	Ingerd	Green 18 sun, forgreen	03	1	
2006	5	Mørk	7/11	Ingerd	ekstragreen, green	09	0	*Pythium sp*
2006	5	Mørk	7/11	Ingerd	ekstragreen, forgreen	10	0	*Fusarium*
					Sum isolates 2006		23	

Table 3. Sampling and isolation of Microdochium nivale from golf greens 2007

Year	Sampling time	Golf course	Date	Who	Green nr	Sample no	*M. nivale* isolated from leaf or stem	*Comments*
2007	**1**	Mørk	19.03	Ingerd	Green 18 sol, green	01	0	
2007	**1**	Mørk	19.03	Ingerd	Green 18 sol, forgreen	03	1	in all samples
2007	**1**	Mørk	19.03	Ingerd	ekstragreen, green	09	1	In most samples
2007	**1**	Mørk	19.03	Ingerd	ekstragreen, forgreen	10	0	*T. incarnata*
2007	**1***	Bogstad	28.03	Ingerd	Green 12 sol, green	15	0	*Pythium*
2007	**1***	Bogstad	28.03	Ingerd	Green 12 sol, forgreen	17	0	
2007	**1***	Bogstad	28.03	Ingerd	Green 12 sol, forgreen	14-	1-New spot	M. nivale symptoms
2007	**1***	Bogstad	28.03	Ingerd	Green 11, green	18	0	Pythium
2007	**1***	Bogstad	28.03	Ingerd	Green 11, forgreen	20	0	
2007	**1**	Sorknes	15.05	Tilsendt fra Rena golf	Green 9 sol, green	31	0	probably Pythium
2007	**1**	Sorknes	15.05	----------	Green 9 sol, forgreen	32	0	Pythium?
2007	**1**	Sorknes	15.05	----------	Green 6, green	34	0	Pythium?
2007	**1**	Sorknes	15.05	----------	Green 6, forgreen	35	0	Pythium?
2007	**2**	Mørk	16.05	Ingerd	Green 18 sol, green	01	1	In only on of the samples
2007	**2**	Mørk	16.05	Ingerd	Green 18 sol, forgreen	03	1	in all samples
2007	**2**	Mørk	16.05	Ingerd	ekstragreen, green	09	0	
2007	**2**	Mørk	16.05	Ingerd	ekstragreen, forgreen	10	0	
2007	**2**	Mørk	16.05	Ingerd	ekstragreen, forgreen	12	0	
			HØST					
2007	**3**	Mørk	24.09	ingerd	Green 18 sol, green	01	0	
2007	**3**	Mørk	24.09	ingerd	Green 18 sol, forgreen	03	0	
2007	**3**	Mørk	24.09	ingerd	ekstragreen, green	09	0	
2007	**3**	Mørk	24.09	ingerd	ekstragreen, forgreen	10	0	*F. dimerum*
2007	**3**	Mørk	24.09	ingerd	ekstragreen, forgreen	12	0	
2007	**4**	Mørk		November	No sampling			**
					Sum isolates 2007		5	

(*)Fungicides was applied prior to snowfall 2006 (*M. nivale* was isolated outside the green area).

Table 4. Sampling and isolation of Microdochium nivale from golf greens 2008

Year	Sampling time	Golf course	Date	Who	Green nr	Sample no	*M. nivale* isolated from leaf or stem	*Comments*
			SPRING					
2008	**1**	Mørk	17.04	Ingerd	Green 18 sol, green	01	0	*F. dimerum*
2008	**1**	Mørk	17.04	Ingerd	Green 18 sol, forgreen	03	1	M niv in 3 of 4 plates
2008	**1**	Mørk	17.04	Ingerd	Green 18 sol, ny flekk	x	0	New spot
2008	**1**	Mørk	17.04	Ingerd	ekstragreen, green	09	0	*F. dimerum og Pythium*
2008	**1**	Mørk	17.04	Ingerd	ekstragreen, forgreen	10	0	*F. dimerum*
2008	**1**	Mørk	17.04	Ingerd	ekstragreen, forgreen	12	1	M niv in 2 of 4 plates. Pink patch

Summary and conclusions from surveys over the years 2006-2008

Source of primary inoculum

Occurrence of *M. nivale* on grass leaves and stems from spots in greens and forgreens was observed in selected spots right after snow melt in 2006 until after snow melt in spring 2008. In 2006, grass samples were collected from four different spots at 3 different golf courses. From Sorknes golf, hardly any strains of *M. nivale* were isolated, and we decided not to take further samples from this golf course. Right after snow melt and in late spring 2006, *M. nivale* was isolated from leaves and stems in most of the selected spots at Mørk and Oslo golf course (Table 5).

Table 5: Overview of areas on the green/forgreen where *M. nivale* was isolated (1= M. nivale isolated, 0=not isolated) at different time points during the year. Bold types indicate that these samples are from sun exposed greens. A very distinct spot in the sampled area of the green is marked +. Greens with only small areas of dead plants or thin turf stand is marked +-. No distinct spot on the green is marked -. M= Mørk, O= Oslo, S= Sorknes golf. G=green, FG= forgreen. (*)Fungicides were applied on the greens prior to snowfall 2006 (*M. nivale* was isolated outside the green area), (**…….**) indicates that no samples were taken. Pictures taken from spots with sample_ID: M-G18-01 and M-FG18-03 are presented in Figure 3 and 4.

		2006					2007			2008	SUM
Golf Field	Sample_ID	After snowmelt	Spring	Summer	Autumn	Prior to snowfall	After snowmelt	Spring	Autumn	After snowmelt	M-% positive
Mørk	**M-G18-01**	**1+**	**1+**	**1-**	**0-**	**0-**	**0+-**	**1-**	**0-**	**0+-**	44%
	M-FG18-03	**1+**	**1+**	**1-**	**0-**	**1-**	**1+**	**1-**	**0-**	**1+-**	78%
	M-GX-09	1+	1+	1+-	1+-	0-	1+-	0+-	0-	0+-	55%
	M-FGX-10/12	1+	1+	0+-	0+-	0-	0+	0-	0+-	1+	33%
	M-% positive	100%	100%	75%	25%	25%	50%	50%	0%	50%	
Oslo	**O-G11-18**	**1+**	**0+**	**1+-**	**0-**	…….	**0*+-**	…….	…….	…….	40%
	O-FG11-20	**1+**	**1+**	**0-**	**0-**	…….	**0*+-**	…….	…….	…….	40%
	O -G12-15	1+	1+	1+-	0-	…….	0*+-	…….	…….	…….	60%
	O -FG12-17	1+	1+	0-	0+-	…….	0*+-	…….	…….	…….	40%
	O -% positive	100%	75%	50%	0%		0%*				
Sorknes	**S-G6-34**	**0+**	**0**	…….	…….	…….	…….	**0**	…….	…….	0%
	S-FG6-35	**0+**	**0**	…….	…….	…….	…….	**0**	…….	…….	0%
	S-G9-31	0+	1	…….	…….	…….	…….	0	…….	…….	33%
	S-FG9-32	0+	0	…….	…….	…….	…….	0	…….	…….	0%
	S-% positive	0%	25%					0%			

The occurrence of *M. nivale* was reduced during summer. In autumn, *M. nivale* was hardly isolated from leaves and stems from any of the selected spots. In 2007/2008 we focused the study to only one golf course, Mørk. Oslo golf course was discontinued since fungicides had been applied prior to snowfall 2006, and was in addition closed in 2007 for rebuilding the greens. In 2007 the same tendency was registered at Mørk golf as in 2006: The occurrence of *M. nivale* in leaves and stems was reduced from spring to autumn (Figure 1 and 2).

Despite a lower isolation rate in autumn 2006 and 2007, *M. nivale* was again isolated in 2 out of the originally 4 spots the following spring. The *M. nivale* isolation rate was similar from spots located on greens and on forgreens, and from spots on greens located at more sunny sites compared to more shadowy located greens. We conclude that although *M. nivale* was seldom

isolated from leaves and stems in late summer, this fungus seem to survive from year to year within the same spots in a golf green since *M. nivale* was isolated from some of the spots the following spring.

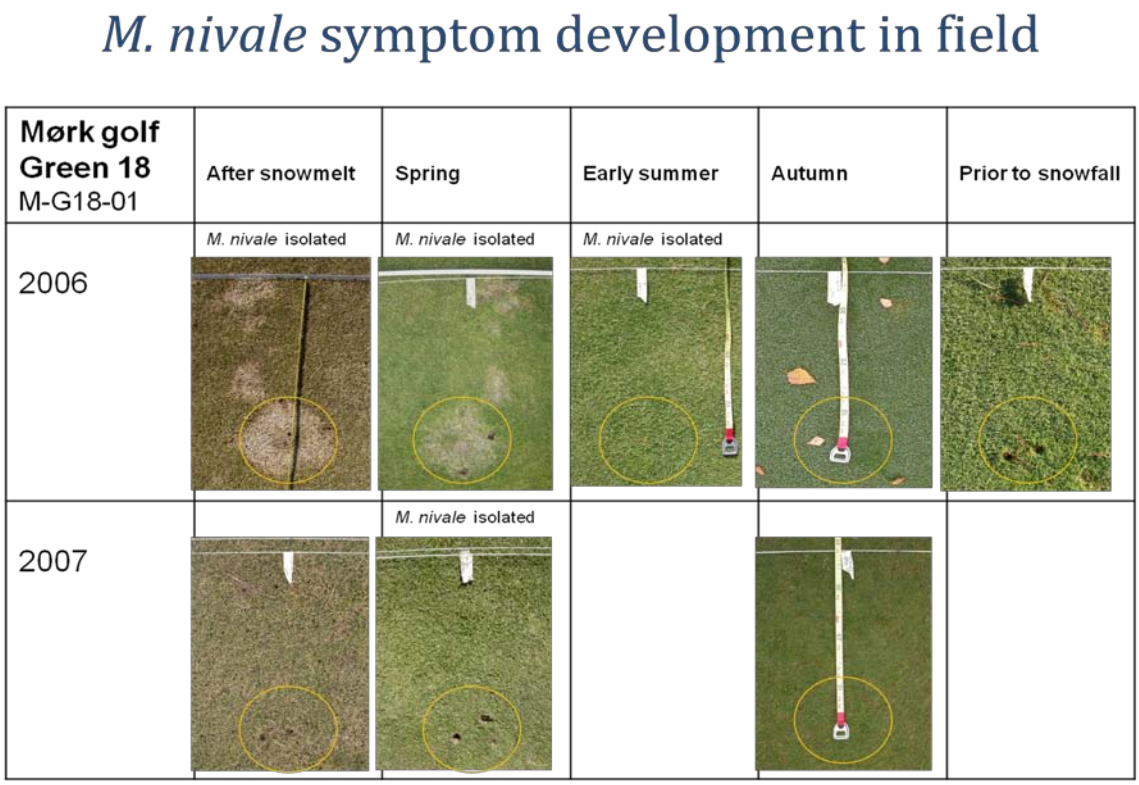

Figure 3: Symptoms observed at selected spots at Mørk golf course, green no. 18, spot no M-G18-01, from 2006-2008

M. nivale symptom development in field

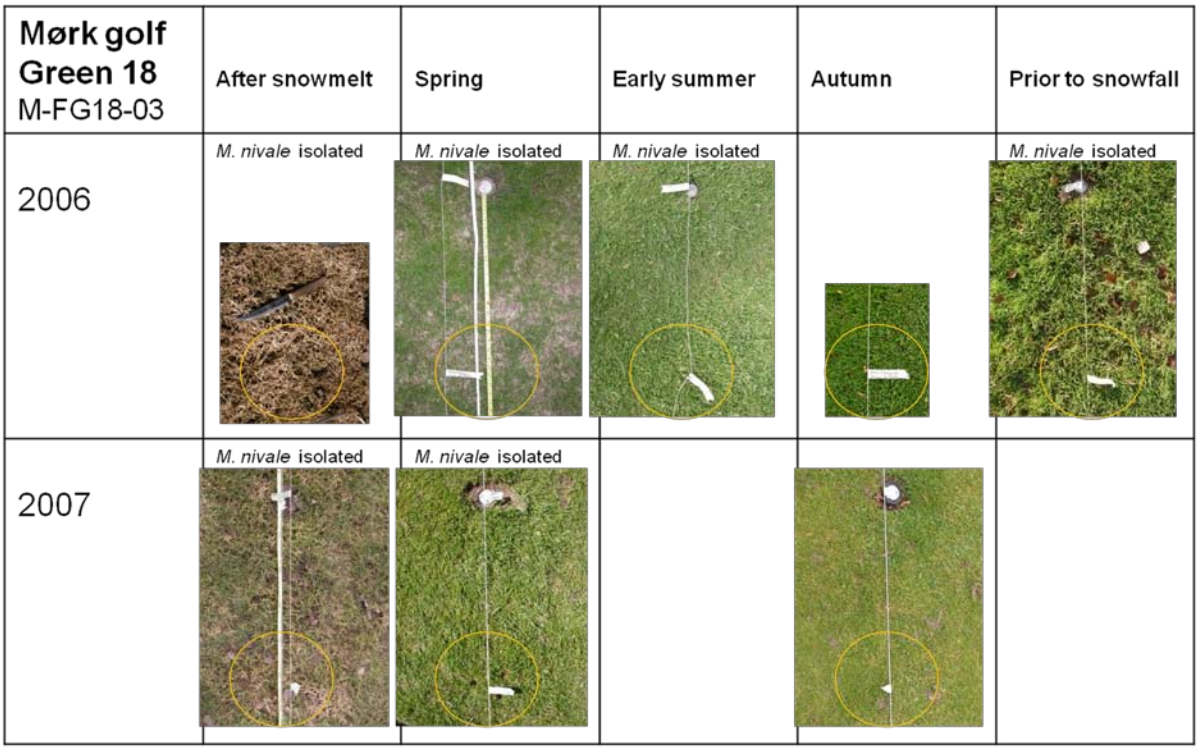

Figure 4: Symptoms observed at selected spots at Mørk golf course, green nb. 18, spot nb M-G18-03, from 2006-2008

2. Growth characteristics of *M. nivale* strains isolated from surveyed sites at the golf courses

Material and methods

In vitro growth rate of *M. nivale* on PDA was measured at two different temperatures (2 and 20°C). The 32 *M. nivale* strains isolated from greens and forgreens at the surveyed sites, and 8 strains isolated after snow melt in the fungicide trials were included in this study. For each strain, a 0,5 cm "biscuit" from the actively growing margin of the fungal culture was placed in the centre of a 9 cm petri dish with PDA (potato dextrose agar). The plates (3 plates of each isolate at each temperature) were incubated at 2 and 20°C. Radial growth rate (mm/day) was calculated after measurements of the mycelial diameter at 1 and 4 days after start of incubation at 20°C, and at 4 and 15 days after start of incubation at 2°C. The experiment was repeated two times (3 replicates). The growth rate was calculated as the average growth rate calculated from the 3 replicate experiments.

Results

At 2°C, most isolates had a growth rate between 1.3 and 1.6 mm per day (average growth rate of 1.4 mm per day). At 20°C a fungal growth rate between 7.5 and 8.5 mm per day was registered for most of the isolates (average growth rate of 7.7 mm per day was calculated) (Fig 5). A significant correlation (Pearson correlation 0.738, P=0.000) was found between growth at 2°C and growth at 20°C (Fig 5). When studying isolates from Mørk and Oslo golf course, a greater variation in growth rate was registered at 20°C between the strains isolated right after snow melt (sampling time 1), compared to the strains isolated in spring, summer, autumn or prior to snowfall (Fig. 6). Besides that, no obvious differences emerged regarding growth rates between groups of *M. nivale* strains isolated at different time points throughout the year.

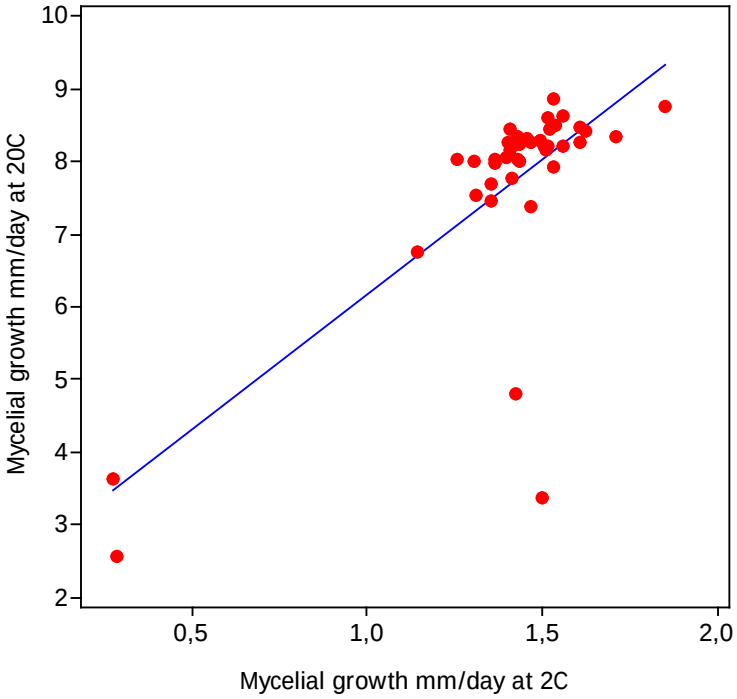

Figure 5: Growth rate on PDA (mm day-1) of M. nivale isolates at 2°C vs 20°C.

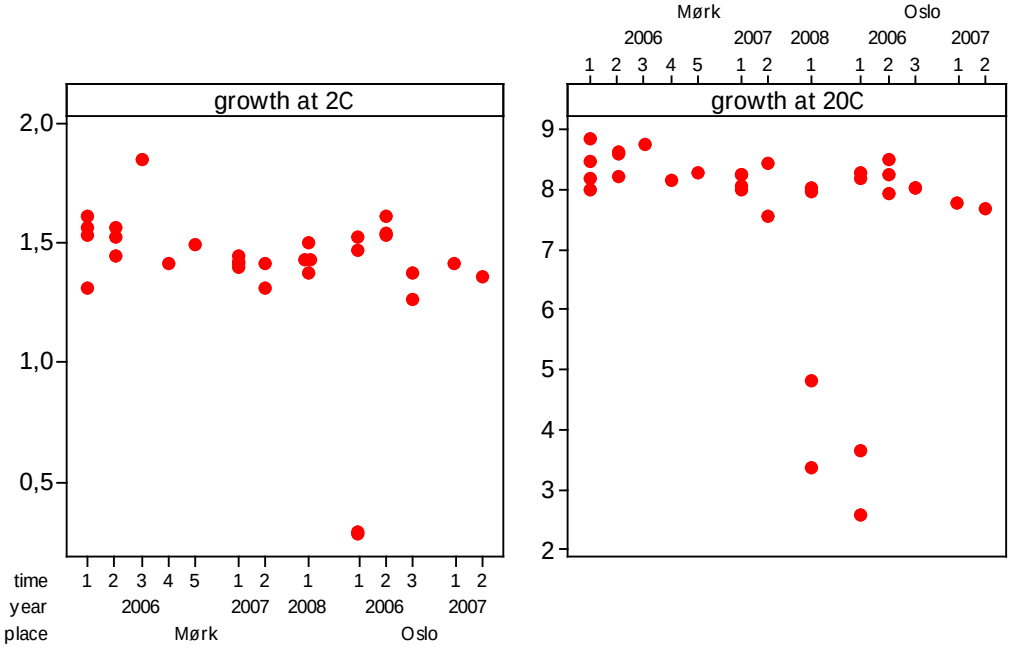

Figure 6a: Mycelial growth rate on PDA (mm day-1) at 2 and 20° of *M. nivale* isolates from Mørk and Oslo golf courses. Isolates are separated according to the year, place and time point of isolation. The numbers 1-5 indicate at what time of year the plant was taken from the green (1:after snow melt, 2:spring, 3:summer, 4:autumn, 5:prior to snowfall.

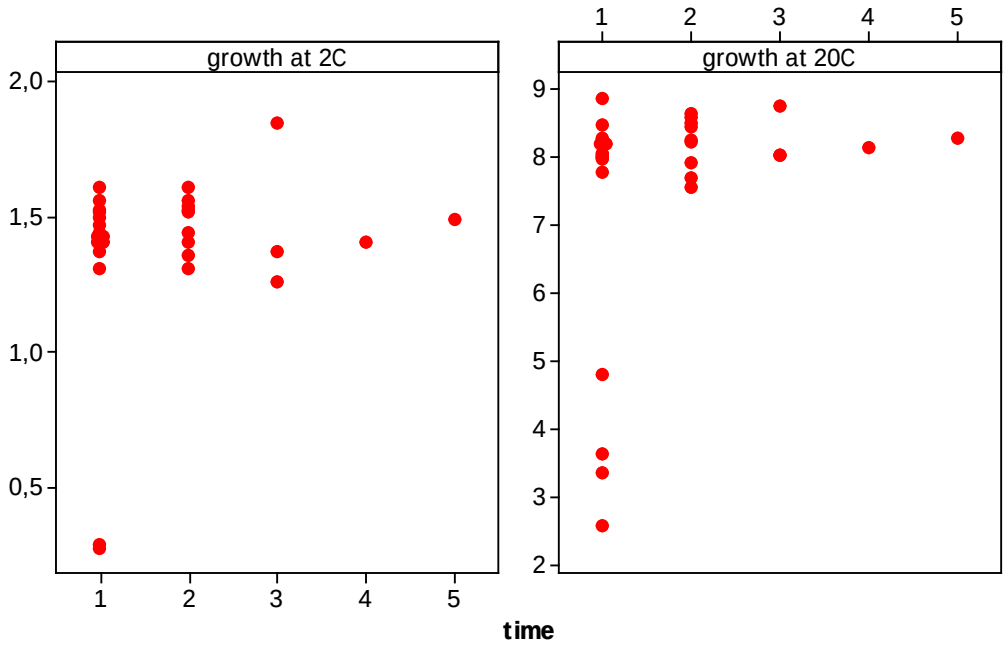

Figure 6b: Mycelial growth rate on PDA (mm day-1) at 2 and 20°C of *M. nivale* isolates from Mørk and Oslo golf courses. The numbers 1-5 indicate at what time of year the sample was taken from the green (1:after snow melt, 2:spring, 3:summer, 4:autumn, 5:prior to snowfall.

Variation in aggressiveness

Experiment for assessment of aggressiveness was started in March 2008. Grass plants were grown in greenhouse prior to the planned date of inoculation. Unfortunately the last week prior to *M. nivale* inoculation, more than 50% of the plants were severely affected with *Pythium* spp. The inoculation experiment was therefore not accomplished.

3. Effect of selected fungicides against *M. nivale*

3.1. Effect of fungicides on mycelia growth of *M. nivale* in vitro

Material and methods

Fungicides used for control of snow mould in Scandinavia were selected and tested *in vitro* for their efficiency to reduce mycelial growth of *M. nivale* (Table 6). The following procedure was used: Each fungicide was suspended in the growth medium (PDA), in concentrations varying from 100% to 0.01% of recommended dosage (see table 6 for details). In each experiment four parallel plates of the medium were inoculated with *M. nivale*, and growth rate (mm day^{-1}) assessed after incubation at 9°C in darkness. Four plates with no product added were used as control. The experiment was performed 4 times. The fungicide concentrations varied between the experiments. In the first experiment, concentrations 100%, 50% and 10 % were tested, in experiment 2, concentrations 10%, 1% and 0,1% were used, and in experiment 3 and 4 the concentrations were 1%, 0.1% and 0.01% of recommended dosage in field. Acanto prima was only included in experiment 3 and 4.

Table 6 Fungicides tested for effect on mycelia growth of M. nivale in vitro

Trade name	Active compunds	100% Cons. of recommended dosage (amount/daa, based on 25 L liquid per daa)
Acanto prima	Picoksystrobin +cyprodinil	6g/L (150g/daa)
Aliette	Fosetyl aluminium	16g/L (400g/daa)
Amistar	Azoksystrobin	4ml/L (100ml/daa)
Amistar Duo	Azoksystrobin+ propikonazol	4ml/L (100 ml /daa)
Baycor 25W	Bitertanol	6g/L (150g/daa)
Bumper 250 EC	Propiconazol (=Tilt)	2ml/L (50ml/daa)
Comet	Pyraklostrobin	4ml/L (100ml/da)
Proline	Protiokonazol	3.2ml/L (80ml/daa)
Inulex	Plant extract	
Resistim	Foilage fertilizer	50ml/L
Rovral 75WG	Iprodion (= Chipco Green)	6,8g/L (170g/da)
Sportak EW	Prokloraz (= Key EW)	8ml/L (200 ml /da)
Stratego 250 EC	Propikonazol + trifloksystrobin	4ml/L (100ml/da)
Topsin	Tiofanametyl	1,6g/L (40g/da)

Results

A significant reduction in *M. nivale* growth rate was registered at low concentrations of all the fungicides used in these experiments (compared to growth rate on control plates where no fungicide was added to the PDA). Acanto prima, Sportak, Stratego and Topsin were the most efficient ones; 90-100% reduction in mycelial growth was registered at 0.1% of the concentration recommended for disease control on golf greens (Figure 6). Amistar were the least efficient product tested, whereas the effect of Rovral 75WG was clearly reduced at concentrations 0.1 and 0.01%.

3.2. Effect of fungicides on *M. nivale* winter injury in field

Material and methods

The products Acanto Prima (cyprodinil, pikoksystrobin), Bumper(propikonazol), Comet (pyraklostrobin) and Stratego (propikonazol, trifloksystrobin) were tested for their efficacy in reducing snow mould injury on golf greens. The fungicide field trials were performed in two following seasons (2006/2007 and 2007/2008), in two golf courses/fields near Bioforsk, Apelsvoll research station (Apelsvoll forsøksgreen, Mjøsen golf and Toten golf). Each of the selected fungicides was sprayed, in the recommended dosage, on three plots once in 24th or 25th October 2006 and 30th October or 1st November 2007. Plot size varied between the three locations and the years. Grass cover and fungal injury (if any) were assess at spraying. Percentage winter injury and grass coverage was registered (estimation by visual observation) twice the following spring: immediately after snow melt and after a period of spring regrowth. The percentage winter injury and spring regrowth for each treatment was calculated as an average of the two observations.

Table 6: Fungicide treatment of golf greens in autumn 2006

	Compund	Innhold	Dosage	Mengde /daa	Time for treatment
1	Control (Untreated)	----------	---	---	----
2	Stratego 250 EC	*Trifloxystrobin + Propikonazole*	1/1	100 ml/daa	24.10 or 25.10
3	Bumper 25 EC	P*ropikonazole*	1/1	50 ml/daa	24.10 or 25.10
4	Comet	*Pyraklostrobin 250 g/l*	1/1	100 ml/daa	24.10 or 25.10
5	Acanto Prima	*Picoksystrobin+cyprodinil*	1/1	150 g/daa	24.10 or 25.10

Results

In the first season 2006/2007, a reduced winter injury was observed for all of the products tested. Acanto prima was ranked as the best product, significantly reducing winter injury from 21% (control) to 6 % (p= 0.026, Turkey simultaneous tests, Minitab) (Figure 7). In the second season 2007/2008, more than 95% water injury was registered in one of the two golf courses, and winter injury was unevenly distributed on the second green. Therefore no clear effect of the fungicides could be found from the two field trials 2007/2008 (results not shown).

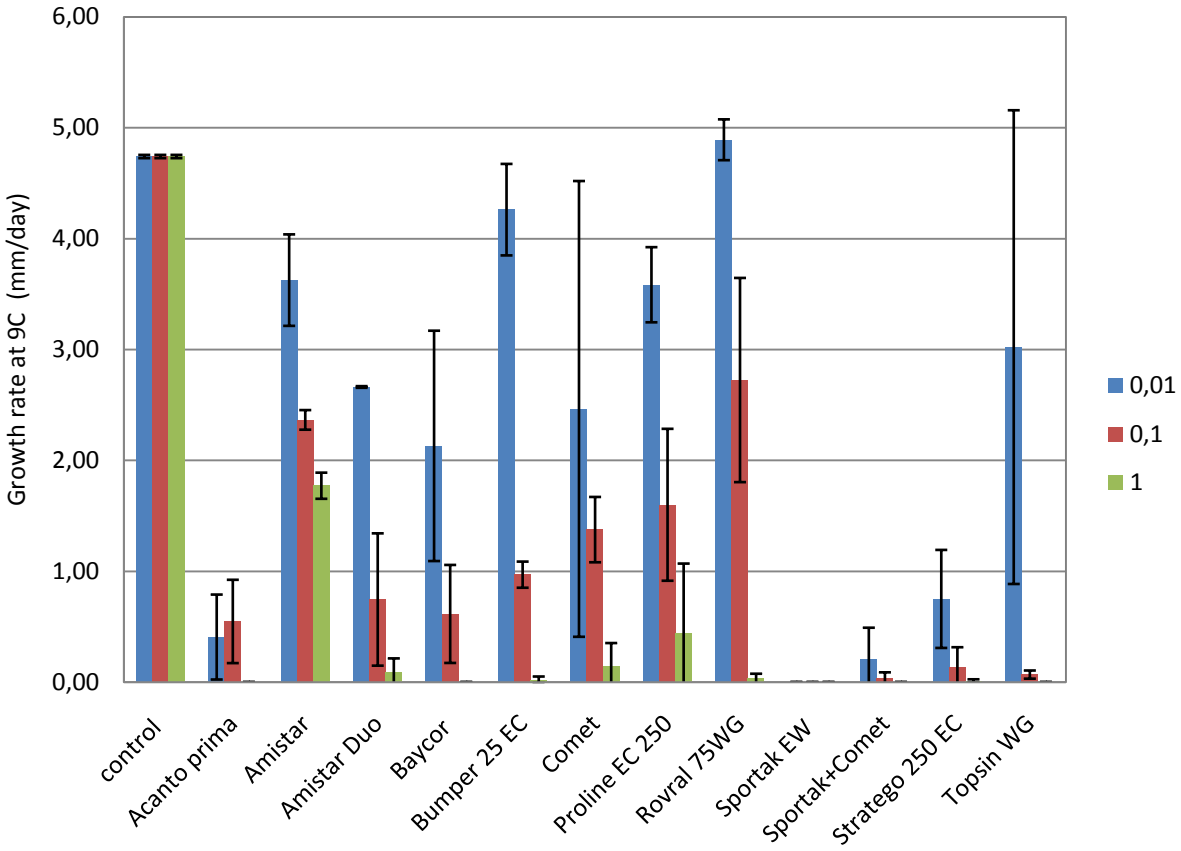

Figure 7: Effect of different fungicides on *M. nivale* growth rate on PDA at 9°C. The concentration of the different fungicides is presented as percentage of the concentration (full dose) recommended for use on golf greens (0.01, 0.1 and 1%). Results from two replicates of the experiment are presented in the figure.

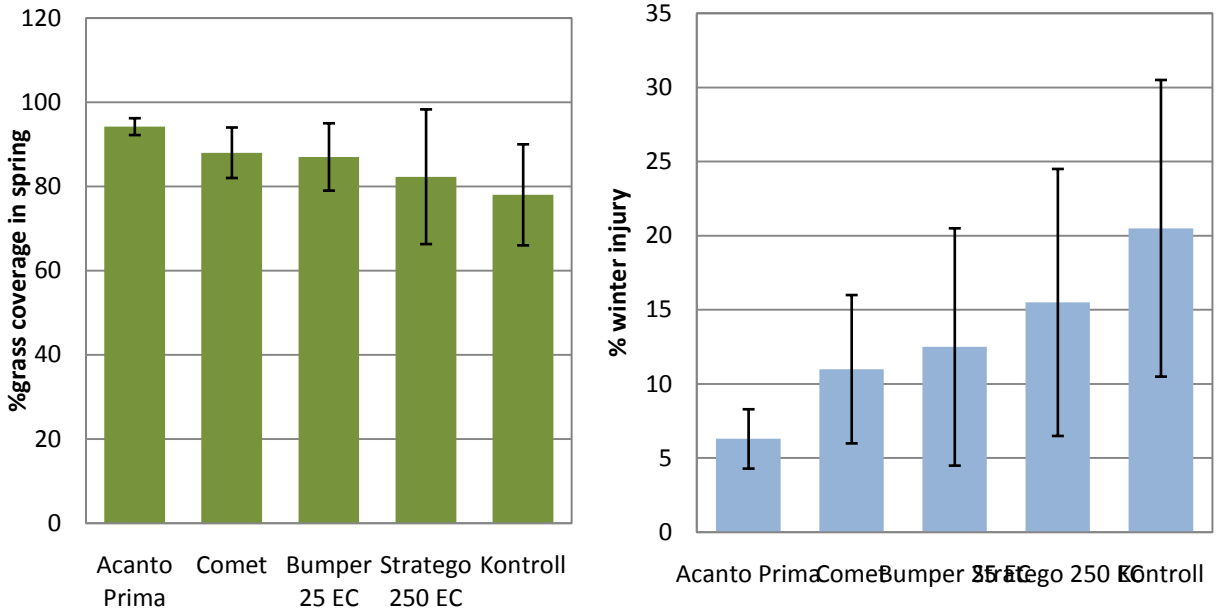

Figure 8: Effect of different fungicides on snow mould damage in field, presented as % winter injury and % grass coverage in spring 2007. The fungicides were sprayed in late autumn 2006, prior to snowfall, on greens located at two separate golf courses. The percentage winter injury and spring regrowth presented for each treatment is calculated as an average of registrations at both trials after two observations of winter injury and spring regrowth at each site.

Figure 9a. Toten golf October 2007

Figure 9b. Apelsvoll 30. March 2007

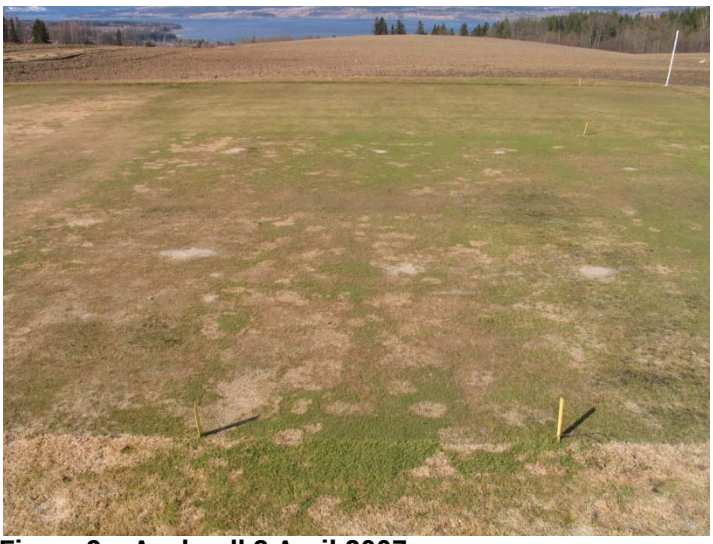

Figure 9c. Apelsvoll 3 April 2007

Summary of the results and conclusions

The occurrence of *Microdochium nivale* in leaves and stems of grasses sampled from golf greens and forgreens was reduced during the growth season, from spring to fall. Despite a lower isolation rate in autumn, *M. nivale* was again isolated in some of the originally locations, the following spring. This same tendency was observed during two seasons. The M. nivale isolation rate was similar from sites located on greens compared to forgreens, and from greens located at more sunny sites compared to more shadowy located greens. We conclude that although *M. nivale* was seldom isolated from leaves and stems of grasses in late summer, the fungus seem to survive from year to year within the same locations on greens and forgreens.

A significant correlation was found between mycelial growth rate of *M. nivale* isolates at 2°C compared to growth at 20°C. At 20°C, a greater variation in growth rate was registered between strains isolated right after snow melt, compared to strains isolated in spring, summer, autumn or prior to snowfall. No clear picture emerged in growth rate differences between groups of *M. nivale* strains isolated at different time points throughout the year. Tests for aggressiveness on grass of these *M. nivale* isolates were not accomplished due to severe *Pythium spp*. infection of the grass plants cultivated in the greenhouse.

Significant reduction in mycelial growth rate of *M. nivale* was registered on agar added low concentrations of all the fungicides tested.The products used were: Acanto Prima (cyprodinil, pikoksystrobin), Amistar (azoksystrobin), Amistar duo (azoksystrobin, propikonazol), Baycor (bitertanol), Bumper (propikonazol), Comet (pyraklostrobin), Proline (protiokonazol), Rovral 75WG/Chipco Green 75WG (iprodion), Sportak EW (prokloraz), Stratego 250 EC (propikonazol, trifloksystrobin), Topsin WG (tiofanatmetyl). Sportak, Stratego and Topsin were the most efficient products; 90-100% reduction in mycelial growth rate was registered on agar added 0.1% of the fungicide concentration recommended for disease control on golf greens.

Acanto Prima, Bumper, Comet and Stratego were also tested for their effect to reduce snow mould damage on golf greens. Acanto prima was ranked as the best product, significantly reducing winter injury on average from 21% (in control plots) to 6% in the treated plots. Due to severe water damage on the greens the second season, only results from one season of the fungicide field trial could be used.

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