

Winter hardiness and management of velvet bentgrass (*Agrostis canina*) on golf greens in the Nordic climate

Vinter herdighet og skjøtsel av hundekvein (*Agrostis canina*)
på golf greener i nordiske klima

Philosophiae Doctor (PhD) Thesis
Tatsiana Espevig

Arable Crops Division
Norwegian Institute for Agricultural and
Environmental Research

Department of Plant and
Environmental Sciences
Norwegian University of Life Sciences



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PhD supervisors

Anne Marte Tronsmo
Department of Plant and Environmental Sciences
Norwegian University of Life Sciences
P.O. Box 5003
NO-1432 Ås, Norway

Trygve S. Aamlid
Research Centre Landvik
Arable Crops Division
Norwegian Institute for Agricultural and Environmental Research
Reddalsveien 215
NO-4886 Grimstad, Norway

Arne Tronsmo
Department of Chemistry, Biotechnology and Food Science
Norwegian University of Life Sciences
P.O. Box 5003
NO-1432 Ås, Norway

Bingru Huang
Department of Plant Biology and Pathology
Rutgers, The State University of New Jersey
Dudley Road 59
NJ 08901 New Brunswick, USA

PhD evaluation committee

James A. Murphy
Department of Plant Biology and Pathology
Rutgers, The State University of New Jersey
Dudley Road 59
NJ 08901 New Brunswick, USA

Birte Boelt
Research Centre Flakkebjerg
Department of Genetics and Biotechnology
Faculty of Agricultural Sciences
Aarhus University
Forsøgsvej 1
DK-4200 Slagelse, Denmark

Lars Olav Brandsæter
Department of Plant and Environmental Sciences
Norwegian University of Life Sciences
P.O. Box 5003
NO-1432 Ås, Norway

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ABSTRACT

Major concerns for the introduction of velvet bentgrass (*Agrostis canina*) on Nordic golf courses are whether current cultivars have sufficient winter hardiness, and if it is possible to control the rapid thatch formation in this species. This thesis is a part of the project 'Velvet Green' funded by the Scandinavian Turfgrass and Environment Research Foundation (STERF) and the Norwegian Research Council and running from 2007 to 2010. The first part of the thesis includes screening of velvet bentgrass cultivars for winter hardiness under controlled environmental conditions and evaluation of effects of metabolic changes induced by cold acclimation on winter hardiness. The second part of the project covers field trials at two locations in Norway with different climatic conditions. The field trials focused on effects of rootzone composition, irrigation regime, and key management practices on turfgrass visual quality, playability, winter survival, and thatch formation.

Up to six velvet bentgrass cultivars Avalon, Greenwich, Legendary, Villa, Venus, and Vesper, and creeping bentgrass 'Penn A-4' in nonacclimated and acclimated state were tested under controlled environmental conditions for freezing tolerance, susceptibility to *Microdochium nivale*, and tolerance to anoxia under simulated ice cover. The experiments were carried out in 2007, 2008, and 2009 in University of Life Sciences and Norwegian Institute for Agricultural and Environmental Research (Bioforsk). Differences in freezing tolerance between velvet bentgrass and creeping bentgrass and among velvet bentgrass cultivars were nonsignificant, but as a species velvet bentgrass tended to be more susceptible to pink snow mold than creeping bentgrass. Acclimation significantly improved freezing tolerance, susceptibility to *Microdochium nivale*, and tolerance to anoxia. Freezing tolerance increased in the order: nonacclimated turf < acclimated at 2°C for 2 wk and 16 h photoperiod < acclimated at 2°C for 2 wk and 16 h photoperiod with additional subzero acclimation at -2°C for 2 wk in darkness < acclimation in the field during fall.

The freezing tolerance of velvet bentgrass 'Greenwich' and creeping bentgrass 'Penncross' was further studied under controlled environments in collaboration with Rutgers University and University of Massachusetts (USA) in 2009 to determine crown carbohydrate and protein changes at different stages of cold acclimation and assess their relationship to freezing tolerance. Similar freezing tolerance in velvet bentgrass and creeping bentgrass was associated with similar levels of sucrose in crown tissue of acclimated plants. Significantly higher crown fructan content in creeping bentgrass than in velvet bentgrass had no significant

impact on LT₅₀ and suggested negligible direct contribution of fructans to freezing tolerance. Increased freezing tolerance in response to cold acclimation was associated with enhanced amino acid synthesis, since serine hydroxymethyltransferase and methionine synthase were up-regulated by acclimation. The first acclimation stage caused more changes in the crown protein composition than subzero acclimation.

Effects of nitrogen (75 or 150 kg ha⁻¹ yr⁻¹), topdressing (0.5 or 1.0 mm sand biweekly), and mechanical (grooming, vertical cutting, spiking) / biological ('Thatch-less™') treatments on turfgrass visual quality, playability, winter survival, and thatch formation were evaluated on USGA greens at a coastal (Landvik, 58°N) and a continental (Apelsvoll, 61°N) location in Norway in 2007-2010 and 2007-2009, respectively. Velvet bentgrass required at least 150 kg N ha⁻¹ yr⁻¹ and heavy topdressing during the first year after establishment. From the second year, 75 kg N ha⁻¹ yr⁻¹ and heavy topdressing were key elements in maintenance of velvet bentgrass with acceptable turf visual and playing quality and adequate percentage of organic matter in the mat. Monthly spiking improved water infiltration rate by more than 50%, but led to softening of the green surface. Monthly vertical cutting resulted in better visual quality and reduced the content of organic matter in the mat. We concluded that monthly verticutting and spiking once or twice per year can be recommended as standard mechanical treatments for a mature velvet bentgrass green.

The last field trial was conducted to clarify the effects of rootzone composition (straight sand vs. sand amended with 20% v/v garden compost) and irrigation regime (light and frequent vs. deep and infrequent) on turfgrass visual quality, playability, thatch formation, root development, and nutrient leaching. The study was carried out from August 2007 to October 2009 on a USGA-green at a coastal location in Norway (58°N). Neither rootzone composition nor irrigation regime affected the thickness or percentage of organic matter in the mat. Amendment with compost showed clear advantages in the form of higher visual quality, less snow mold caused by *Microdochium nivale*, longer irrigation intervals, and less risk for development of soil water repellency. Infrequent irrigation to the field capacity was a better irrigation strategy on velvet bentgrass greens than frequent irrigation, except for the first year after sowing.

Key words: Acclimation, first stage, second stage, freezing tolerance, simulated ice cover, *Microdochium nivale*, snow mold, simulated snow cover, metabolic changes, USGA green, thatch, rootzone, compost, irrigation, fertility, grooming, vertical cutting, spiking, 'Thatch-less™', topdressing.

SAMMENDRAG

Før hundekvein (*Agrostis canina*) kan anbefales for golfgreener i Norden må to viktige spørsmål avklares. Har tilgjengelige sorter tilstrekkelig vinterherdighet? Kan vi kontrollere den store mengden filt som hundekvein produserer? Denne avhandlingen er en del av prosjektet "Velvet Green" finansiert av Scandinavian Turfgrass and Environment Research Foundation (STERF) og Norges forskningsråd og som varte fra 2007 til 2010. Den første delen av avhandlingen handler om vurderingen av vinterherdighet av hundekveinsorter basert på forsøk i kontrollert klima og effekten av metaboliske endringer ved herding på vinterherdighet. Den andre delen av avhandlingen presenterer tre feltforsøk på to steder med ulikt klima. Disse forsøkene viser hvordan visuell kvalitet, spillekvalitet, vinteroverlevelse og filtdannelse påvirkes av ulike vekstmedier, vanningsregime og skjøtselstiltak.

Inntil 6 ulike sorter av hundekvein; Avalon, Greenwich, Legendary, Villa, Venus og Vesper, og krypkvein (*Agrostis stolonifera*) Penn A-4, ble testet under kontrollerte klimaforhold for frosttoleranse, mottagelighet for rosa snømugg *Microdochium nivale* og toleranse for oksygenmangel under simulert isdekke. Forsøkene ble utført i perioden fra 2007 til 2009 ved Universitetet for Miljø og Biovitenskap og Bioforsk Øst Landvik. Forskjellene i frosttoleranse mellom hundekveinsortene og krypkvein var ikke signifikante, men arten hundekvein var mer mottakelig mot *M. nivale* enn krypkvein. Herding forbedret frosttoleranse, reduserte mottakelighet for *M. nivale* og økte toleransen for anaerobe forhold. Frosttoleranse økte i rekkefølge: ikke-herdet gress < herdet ved 2°C i 2 uker og 16 timers daglengde < herdet ved 2°C og 16 timers daglengde i 2 uker med påfølgende -2°C for 2 uker i mørke < herdet i felt om høsten.

Frosttoleransen til hundekvein 'Greenwich' og krypkvein 'Penncross' ble testet under kontrollerte klimaforhold i samarbeid med Rutgers University og University of Massachusetts (USA) i 2009. Forsøket ble designet for å kartlegge endringer i sammensetningen av karbohydrater og proteiner i kroner av gresset ved ulike stadium under herding, og å vurdere stoffenes forhold til frosttoleranse. Tilsvarende frosttoleranse mellom hundekvein og krypkvein ble assosiert med like nivå av sukrose i kroner av herdete planter. Det betydelige høyere kronenes fruktaninnholdet i krypkvein hadde ingen signifikant påvirkning på LT₅₀ og dette antyder at fruktaner ikke har noen direkte påvirkning på frosttoleranse for kveinartene. Økt frosttoleranse etter herding ble forklart med økt aminosyresyntese da serinhydroxymetyltransferase og methioninsyntase ble oppregulert ved herding. Den første

fasen av herding førte til flere endringer i kronenes protein sammensetning enn ved tillegsherdingen på -2 °C.

Feltforsøkene ble utført på greener bygget etter USGA sine anbefalinger på to steder: kystnært på Landvik i Grimstad (58°N) i 2007-2010 og i innlandsklima på Apelsvoll på Toten (61°N) i 2007-2009. Det første forsøket vurderer effektene av nitrogengjødsling (75 eller 150 kg ha⁻¹ år⁻¹), toppdressing (0,5 eller 1,0 mm sand annenhver uke), mekaniske (grooming, vertikal skjæring, stikklufting) og biologiske ('Thatch-lessTM') behandlinger på visuell kvalitet, spillekvalitet, vinteroverlevelse og oppbygging av filt. Hundekvein krevde minimum 150 kg N ha⁻¹ år⁻¹ og største mengde toppdressing første året etter etablering. Fra det andre året gav 75 kg N ha⁻¹ år⁻¹ og største mengde toppdressing best visuelt inntrykk og spillekvalitet og passe mengde organisk materiale i filtlaget. Månedlig stikklufting forbedret infiltrasjonshastigheten for vann med mer enn 50%, men førte til mykere overflate. Månedlig vertikalskjæring førte til bedre visuell kvalitet og reduserte organisk materiale i filt laget. Vi konkluderer med at månedlig vertikalskjæring og stikklufting en eller to ganger i året kan anbefales som standard mekanisk behandling på en moden hundekveingreen.

Det siste felt forsøket ble utformet for å kartlegge effektene av vekstmedium (ren sand eller sand tilført 20 volumprosent hagekompost) og vanningsregime (lite og ofte eller mye og sjeldent) på visuell kvalitet, spillekvalitet, filtutvikling, rotutvikling og avrenning fra USGA-green. Forsøket ble utført på Landvik fra august 2007 til oktober 2009. Verken vekstmedium eller vanningsregime påvirket tykkelsen på filtlaget eller innhold av organisk materiale. Innblanding av kompost ga klart bedre visuell kvalitet, mindre angrep av rosa snømugg, mindre behov for vanning og mindre risiko for utvikling av lokale tørrflekker pga hydrofobisk vekstmasse. Med unntak av første året etter såing gav sjelden vanning til feltkapasitet det beste resultatet på hundekvein greener.

Nøkkelord: Herding, første fasen, andre fasen, frosttoleranse, simulert is dekke, *Microdochium nivale*, snømugg, simulert snødekke, metaboliske endringer, USGA green, filt, rotsone, kompost, vanning, gjødsling, grooming, vertikal skjæring, stikklufting, 'Thatch-lessTM', toppdressing.

АННОТАЦИЯ

Татьяна Эспеви́г. Зимостойкость и технологии возделывания полевицы собачьей (*Agrostis canina*) на гринах в условиях Скандинавии

Главными вопросами при введении полевицы собачьей (*Agrostis canina*) на скандинавские гольф поля являются: обладают ли ныне существующие сорта достаточной зимостойкостью и возможно ли контролировать чрезвычайно быстрое накопление органического вещества (тач) в верхнем слое почвы? Настоящая диссертационная работа входит в состав проекта "Velvet green", который осуществлялся в 2007-2010 гг. при финансовой поддержке Scandinavian Turfgrass and Environment Research Foundation (STERF) и Norwegian Research Council. Первая часть работы посвящена оценке зимостойкости сортов полевицы собачьей в условиях теплицы и эффектам метаболических изменений в процессе закаливания на различные аспекты зимостойкости. Вторая часть работы представляет результаты трех полевых исследований, которые проводились в двух климатических зонах Норвегии. Полевые исследования были направлены на изучение влияния почвы, режимов полива и наиболее используемых приемов по уходу за грином на его качество, зимостойкость и накопление таха.

Закаленные и незакаленные сорта полевицы собачьей Avalon, Greenwich, Legendary, Villa, Venus и Vesper, и сорт полевицы побегообразующей (*Agrostis stolonifera*) Penn A-4 были испытаны на морозостойкость, устойчивость к *Microdochium nivale* и анаэробным условиям под искусственно созданной ледяной коркой. Серии исследований проводились в 2007-2009 гг. в Университете естественных наук и Научно-исследовательском институте сельского хозяйства и охраны окружающей среды. Сорта полевицы собачьей не отличались между собой по морозостойкости, так же как и не было обнаружено разницы в морозостойкости между полевицей собачьей и полевицей побегообразующей. Закаливание растений улучшило морозостойкость, устойчивость к *Microdochium nivale* и устойчивость к анаэробным условиям. Морозостойкость закаленных растений возросла в следующей последовательности: незакаленные < закаленные в течение 2 недель при 2°C и 16-часовой длине дня < закаленные в течение 2 недель при 2°C и 16-часовой длине дня и затем в темноте в течение 2 недель при -2°C < закаленные в полевых условиях в течение осеннего периода.

Дальнейшие исследования по морозостойкости полевицы собачьей (Greenwich) и полевицы побегообразующей (Penncross) проводились в 2009 году при сотрудничестве университета Rutgers и Университета Массачусетс (США). Исследования проводились с целью выявления изменений в составе углеводов и белков в узлах кущения на различных стадиях закаливания и оценки влияния этих изменений на морозостойкость полевиц. Отсутствие

разницы в морозостойкости между полевицами было связано со сходным содержанием сахарозы в узлах кущения после закалки. Однако в узлах кущения полевицы побегообразующей содержалось значительно большее количество фруктанов, что наводит на предположение о том, что фруктаны не принимают непосредственного участия в морозостойкости полевиц. Возросшая морозостойкость полевиц после закаливания могла быть связана с возросшей концентрацией аминокислот, об увеличении синтеза которых говорит возросшее содержание таких ферментов, как серингидрокси метилтрансфераза и метионинсинтетаза. Первая стадия закаливания вызвала большие изменения в составе белков нежели вторая стадия закаливания.

Полевые исследования по влиянию азота (75 или 150 кг га⁻¹ в год), топдресинга (0,5 или 1,0 мм песка раз в две недели), механических (грумминг, вертикальная резка, спайкинг) и биологических ('Thatch-less™') методов в предотвращении накопления тача на качество и зимостойкость гринов проводились в 2007-2010 гг. на Ландвике (южный морской климат, 58° северной широты) и в 2007-2009 гг. в Апельсволе (континентальный климат, 61° северной широты). Минимум 150 кг га⁻¹ азота в год и усиленный топдресинг были необходимы для полевицы собачьей в течение первого года после посева. Во второй год 75 кг га⁻¹ азота было достаточно для приемливого качества и небольшого количества органического вещества (тача) при условии усиленного топдресинга. Спайкинг раз в месяц улучшил инфильтрацию более чем на 50%, но привел к мягкости гринов. Вертикальная резка улучшила качество поверхности и уменьшила процент органического вещества в верхнем слое почвы. Со второго года после посева стандартными механическими обработками на гринах полевицы собачьей могут быть вертикальная резка раз в месяц и спайкинг 1-2 раза в год.

Третье полевое исследование проводилось в 2007-2009 на Ландвике. В его задачу входило изучение влияния почвы (песок или песок с 20% компоста по объему) и режима полива (частый и редкий, оба до полевой влажности) на качество грин. Накопление тача не зависело ни от почвы, ни от режима полива. На песке, обогащенном компостом, возросло качество грин, был сокращен полив, наблюдалось подавление развития розовой снежной плесени и не развивалась гидрофобность. Редкий режим полива был лучшим приемом из двух изученных.

Ключевые слова: Закаливание, первая фаза, вторая фаза, морозостойкость, симулированная ледяная корка, *Microdochium nivale*, снежная плесень, симулированное снежное покрытие, метаболические изменения, USGA грин, тач, почва, компост, полив, удобрения, грумминг, вертикальная резка, спайкинг, 'Thatch-less™', топдресинг.

LIST OF PAPERS

This thesis is based on the following papers:

- I. Espevig, T., M. DaCosta, L. Hoffman, T. S. Aamlid, A. M. Tronsmo, B. B. Clarke, and B. Huang. 2011. Freezing tolerance and carbohydrate changes of two *Agrostis* species during cold acclimation. *Crop Science* 51:May-June.
- II. Espevig, T., C. Xu, M. DaCosta, T. S. Aamlid, and B. Huang. Proteomic analysis of velvet bentgrass in response to cold acclimation. (Manuscript).
- III. Tronsmo, A., T. Espevig, L. Hjeljord, and T. S. Aamlid. Evaluation of the winter hardiness of velvet bentgrass cultivars in controlled environments. (Manuscript).
- IV. Espevig, T., B. Molteberg, A. M. Tronsmo, A. Tronsmo, and T.S. Aamlid. Thatch control and winter survival of newly established velvet bentgrass greens in northern environments. (Manuscript).
- V. Espevig, T., and T. S. Aamlid. Effects of rootzone composition and irrigation regime on performance of velvet bentgrass golf greens. (Manuscript).

1. INTRODUCTION

Approximately 2.5 to 5.5% of the Scandinavian population plays golf. In Norway there are about 174 golf courses including courses with 18 or less holes. The area around each hole is covered with turf which is mowed very closely to enhance ball roll. This entire area around the hole is called a golf green. The most commonly seeded turfgrasses for golf greens in Norway are either mixtures of red fescue (*Festuca rubra* L.) and colonial bentgrass (*Agrostis capillaris* L.) (ca. 60% of golf greens) or pure creeping bentgrass (*Agrostis stolonifera*) (ca. 40%).

Among bentgrass species, velvet bentgrass (*Agrostis canina* L.) is the most fine-textured and dense. It is reported to exhibit better tolerance to several biotic and abiotic stresses compared to the more widely used creeping bentgrass (Brilman, 2003; Chakraborty et al., 2006; DaCosta and Huang, 2006b). Despite the desirable characteristics, velvet bentgrass has not been widely used in part due to difficulties in seed production in early velvet bentgrass cultivars, but also due to the limited information available on optimal management, especially thatch control, in this species (Skogley, 1975; Brilman and Meyer, 2000; Koeritz and Stier, 2009).

In the perspective of integrated pest management, velvet bentgrass seems to meet the demand in North America and Europe for well-adapted turfgrass species requiring less water, pesticides, and fertilizers. During the last decade, there has been a strong resurgence in the interest for velvet bentgrass in North America (Rutgers University, Cornell University, University of Wisconsin, University of Massachusetts, Michigan State University), Canada (University of Guelph), and Europe (University of Hohenheim). A breeding program is underway at Rutgers University, and this has so far resulted in cultivars such as Greenwich, Legendary, Venus, Vesper, and Villa.

In Norway, the benefits of velvet bentgrass were discovered through a variety evaluation project under green conditions from 2003 to 2006 where velvet bentgrass exhibited better winter survival and overall impression compared with other turfgrass species (Aamlid et al., 2006). Because winter injury in the field can be caused by many different stresses (freezing temperatures, ice encasement, crown dehydration, and/or low temperature fungal diseases), the superior winter survival of velvet bentgrass needs further research.

The winter survival of a turfgrass species depends on its acclimation ability and on the sufficiency of cold acclimation which activates structural, biochemical, and metabolic

changes in plants (Livingston, 1991; Tronsmo et al., 1993; Dionne et al., 2001a, 2001b; Hoffman et al., 2010). Two consecutive stages of cold acclimation have been suggested in winter cereals and temperate grasses (Tumanov, 1940). Although much work has been devoted to plant responses to cold acclimation, a full understanding of the cellular and molecular mechanisms underlying the first stage that occurs at from 2 to 5°C, and, especially the second stage which occurs at sub-zero temperatures, has not been reached yet. The winter survival of turf also depends on weather conditions and management, particularly nutrition (Smith et al., 1989).

Besides winter survival, the biggest challenge in management of velvet bentgrass on putting greens is to control thatch (organic matter) accumulation in the upper soil layer. Velvet bentgrass accumulates more thatch than other turfgrass species (Aamlid et al., 2011). Among the problems caused by excessive thatch on golf greens are reduced water infiltration and increased risk for disease injury, scalping, and dry spots, all resulting in poor playing quality (Jordan, 2008). Stimulation of biological thatch degradation is often a difficult task due to lignin, which enters into the thatch composition along with other organic polymers, and which is very resistant to degradation (Kirk, 1971; Ledebøer and Skogley, 1967; Crawford and Crawford, 1980; Couillard and Turgeon, 1997). Testing of the products containing biological thatch decomposers or their enzymes and claiming to improve thatch degradation ought to be tested under realistic field conditions. Thatch can be controlled by restricted fertilization and irrigation avoiding excessive plant growth. However, our knowledge on optimal nitrogen and irrigation inputs to velvet bentgrass greens is very sparse. Nitrogen rates varying from 48 to 342 kg N ha⁻¹ yr⁻¹ were compared by Skogley (1975), Boesch and Mitkowski (2007), and Koeritz and Stier (2009), but limited information on thatch formation is available from those studies.

A proper irrigation schedule (amount and frequency) will not only result in good visual quality and playing quality, but also minimize nutrient leaching from putting greens (Mancino and Troll, 1990; Frank et al., 2005; Paré et al., 2006; Soldat and Petrovic, 2008; Steinke et al., 2009). Soil water repellency has been reported to result in fingered flow and thus increased risk for leaching, especially from straight sand greens with no organic amendment. Velvet bentgrass has been reported to require less irrigation water than other bentgrass species (DaCosta and Huang, 2006a, 2006b), but the optimal irrigation management of this species on rootzones varying in organic matter content has not been determined so far.

Thatch can be also diluted with topdressing sand or reduced mechanically (Smith, 1979; Carrow et al., 1987; Murphy et al., 1993a; McCarty et al., 2005, 2007; Barton et al., 2009).

Because velvet bentgrass seems to have poor recuperative capacity (Boesch and Mitkowski, 2007), more research is needed on timing and frequency of mechanical treatments on velvet bentgrass greens.

The objectives of the present project were:

(1) To compare the tolerance of available cultivars of velvet bentgrass to freezing temperatures, ice cover, snow cover, and resistance to pink snow mold (*Microdochium nivale*) with creeping bentgrass under controlled conditions;

(2) To determine levels of nonstructural carbohydrates at different stages of cold acclimation and assess their relationship to winter hardiness for velvet bentgrass and creeping bentgrass;

(3) To reveal some mechanisms underlying the first and the second stages of cold acclimation using protein analysis and determine the impact of these changes on freezing tolerance of velvet bentgrass;

(4) To compare direct and indirect methods for estimation of freezing injury in bentgrasses;

(5) To determine the effects of nitrogen rates, topdressing levels, and mechanical / biological treatments on turf quality, thatch formation, and winter survival of velvet bentgrass golf greens in a coastal and a continental region of Scandinavia;

(6) To clarify the effects of rootzone composition and irrigation regime on turfgrass visual quality, playability, root development, thatch formation, and nutrient leaching from velvet bentgrass golf green.

2. LITERATURE REVIEW

2.1. Velvet bentgrass: origin, importance, and use to present day

Velvet bentgrass is a native species to northern and central Europe (Brilman, 2003). After being brought to North America during the emigration period, New England golf superintendents realized that velvet bentgrass produced beautiful greens like a ‘velvet carpet’. In 1927, R.A. Jones, on behalf of the United States Golf Association (USGA) Advisory Committee, stated that ‘velvet bent produces the finest and most beautiful turf of any of the northern grasses’ (Brilman & Meyer, 2000).

In the 1960’s and 1970’s velvet bentgrass fell out of favor on North-American golf courses. As fertilizers and pesticides were introduced, creeping bentgrass and annual bluegrass (*Poa annua* L.) became the predominant species on putting greens. Since then, increasing environmental awareness has raised the quest for well-adapted turfgrass species requiring less water, pesticide, and fertilizer use. In this context, velvet bentgrass seems to have a potential in North America and Europe.

During the last decade, there has been a strong resurgence in the interest for velvet bentgrass in North America. In New England and New York, velvet bentgrass is perceived as the ideal species for integrated pest management (IPM) of putting greens (Grant and Rossi, 2004). In addition to its very fine surface texture, velvet bentgrass has better shade tolerance (Reid, 1933), needs less irrigation water (DaCosta and Huang, 2006a, 2006b), and exhibits lower leaching of nitrates (Paré et al., 2006) than other bentgrass species. It is more resistant to dollar spot (*Sclerotinia homoeocarpa*) and brown patch (*Rhizoctonia* spp.) (Brilman and Meyer, 2000), tolerates as much or even more compaction and wear stress (Murphy et al., 2009; Samaranayake et al., 2009), and competes better against annual bluegrass infestation (Samaranayake et al., 2009) than creeping bentgrass. Despite these desirable characteristics, the use of velvet bentgrass on golf courses is limited. Among the causes are seed production problems which more or less have been overcome (Brilman, 2003), and sparse knowledge on optimal maintenance. In the Nordic countries, velvet bentgrass is used on about 10% of the golf courses in Finland. In Norway, Sweden, and Denmark, less than 3% of the golf courses have velvet bentgrass on greens.

The benefits of velvet bentgrass under Nordic climate conditions were rediscovered through a variety evaluation project at Bioforsk Landvik and Apelsvoll from 2003 to 2006

(Aamlid et al., 2005, 2006). In that project, 43 cultivars of creeping bentgrass, velvet bentgrass colonial bentgrass, red fescue, and annual bluegrass were compared. The most conspicuous result was the outstanding performance of velvet bentgrass at both locations. Velvet bentgrass not only produced the densest, finest and most even turf, but also had better winter hardiness than any other species (Photo 1). Because winter injury in the field may be caused by one or more stresses and since winter hardiness depends on acclimation ability, additional controlled environment studies are required to determine potential causes for superior winter survival of velvet bentgrass.



Photo 1. Only velvet bentgrass (green plots to the left) came out of the winter 2004-05 with hardly any winter damage at Apelsvoll. To the right: Creeping bentgrass. (Photo in May 2005 by Bjørn Molteberg)

2.2. Winter survival – the biggest challenge for turfgrasses in northern climates

Winter injury of temperate grasses used for turf is a significant problem in northern climatic regions. About 70% of Scandinavian courses suffer from winter damage every year (STERF, 2009). During the winter months, turfgrasses may be exposed to various low temperature related stresses (Levitt, 1980; Sakai and Larcher, 1987; Stier and Fei, 2008). These different stresses may occur individually or in combination and result in significant decreases in turfgrass function and/or playability.

2.2.1. Winter hardiness and acclimation stages

Winter hardiness is a complex phenomenon including tolerance to freezing temperatures, ice encasement, hypoxia, and/or resistance to low temperature fungal diseases (Humphreys, 1989; Ergon et al., 1998; Bertrand et al., 2009a; Castonguay et al., 2009). Winter hardiness is significantly affected by a period of cold acclimation or cold hardening, whereby a number of physical, biochemical, and physiological changes contribute to enhanced cellular stability under different winter stresses (Levitt, 1980; Steponkus et al., 1990; Guy, 1999; Thomashow, 1999; Rajashekar, 2006).

Two consecutive stages of cold acclimation have been suggested in winter cereals and temperate grass species (Tumanov, 1940). The first acclimation stage occurs at temperatures above freezing (approximately 2 to 5 °C) and is characterized by several changes including accumulation of osmolytes (e.g. carbohydrates, proline and other amino acids), antifreeze proteins, and reserve carbohydrates, increases in antioxidant production, and alterations in phospholipids and fatty acids (Anchordoguy et al., 1987; Livingston, 1991; Tronsmo et al., 1993; Dionne et al., 2001a, 2001b; Zhang et al., 2009; Hoffman et al., 2010).

The second stage is referred to as sub-zero acclimation (SZA) and leads to acquisition of additional freezing tolerance (Tumanov, 1940; Livingston, 1996; Herman et al., 2006). Exposure to sub-freezing temperatures (-2 to -5 °C) is commonly associated with induced ice formation in the apoplast and dehydration of plant cells (Steponkus, 1989; Herman et al., 2006). The required duration of the second acclimation stage is still controversial and its impact on winter hardiness of turfgrasses not sufficiently studied.

2.2.2. Evaluation of freezing tolerance using direct and indirect methods

Freezing tolerance has been shown to be a major component of winter hardiness of perennial grasses (Larsen, 1994; Humphreys and Eagles, 1988; Humphreys, 1989; Xiong and Fei, 2006; Hulke et al, 2008). Freezing tolerance tested under controlled conditions can be evaluated by direct (whole plant survival) and indirect (e.g., electrolyte leakage) methods. Because assessment of plant survival following freeze tests may take several days or weeks, faster indirect methods may be useful if sufficiently correlated with plant survival. Crown survival is crucial for the survival of the grass plant. The crown apical meristem (upper region) and vascular transition zone (lower region) have been shown to respond differently to freezing in winter wheat (*Triticum aestivum* L.) (Tanino and McKersie, 1985). Therefore indirect methods using grass crowns are not always consistently correlated with survival, possibly due

to the heterogeneous structure of crown tissues (Tanino and McKersie, 1985; Shashikumar and Nus, 1993; Livingston et al., 2005).

Electrolyte leakage (EL) tests have been used for evaluation of freezing injury of leaves, roots, and crowns of winter cereals (Chen et al., 1983) and turfgrasses (Gusta et al., 1980; Rajashekar et al., 1983). In addition to EL, 2,3,5-triphenyltetrazolium chloride (TTC) reduction has also been used for detection of freezing injury of different plant tissues (Steponkus and Lanphear, 1967; Lindstöm and Mattsson, 1989; Guo et al., 2006). This method is based on the capacity of living plant cells to reduce 2,3,5-triphenyltetrazolium chloride (TTC) to formazan by the dehydrogenase enzyme system (Knievel, 1973; Rachmilevitch et al., 2006). While many studies have utilized one of these methods (EL or TTC reduction), their sensitivities for estimation of lethal temperature for 50% survival of the test population (LT_{50}) relative to LT_{50} determined by whole-plant survival are not well documented in turfgrasses.

2.2.3. Role of carbohydrates

Low temperatures in fall trigger changes in net carbon metabolism in plants (Huner et al., 1993). The level of sucrose in herbaceous plants increases in response to inhibition of plant growth. This results in accumulation of storage carbohydrates, in cool season grasses primarily fructans. The role of non-structural carbohydrates in freezing tolerance in winter cereals and forage grasses has been extensively evaluated (Tumanov, 1940; Levitt, 1980; Livingston, 1991, 1996).

Sucrose has been identified as an important cryoprotectant. It defends plants from freeze-induced dehydration, reduces ice formation by increasing the intracellular solute concentration, and inhibits liposome fusion during freezing (Anchordoguy et al., 1987). Soluble sugars may also delay freezing by direct inhibition of ice crystal growth in the apoplast (Olien, 1967; Livingston et al., 2009). As with fructans, there is increasing evidence for the role of sucrose in coordinating plant responses to oxidative stress (Parvanova et al., 2004; Van den Ende and Valluru, 2009). Although the importance of fructans as reserve carbohydrates in cool-season grasses is widely accepted (Pollock and Cairns, 1991), their role as cryoprotectants is somewhat controversial (Olien and Clark, 1993; Livingston, 1996; Livingston and Henson, 1998). A potential role of fructans as inhibitors of ice crystal formation was described by Olien (1967). More recently, studies have shown that fructans can directly interact with cell membranes to improve membrane stability during dehydration-

related stresses (Hincha et al., 2002; Valluru and Van den Ende, 2008). Carbohydrates have also been reported to contribute to resistance to snow molds (*Typhula incarnata*, *T. ishikariensis*, *Microdochium nivale*, and *Coprinus psychromorbidus*) in winter wheat (Yoshida et al., 1998) and annual bluegrass (Bertrand et al., 2009a).

Compared with the number of investigations in winter cereals (Olien and Clark, 1993; Livingston and Henson, 1998; Gusta et al., 1996) and forage grasses (Tronsmo et al., 1993, Hisano et al., 2004), research is limited regarding the role of carbohydrates in freezing tolerance of cool-season turfgrasses. In particular, it is unclear which specific changes occur in turfgrasses during the two stages of cold acclimation. Dionne et al. (2001a) found that carbohydrate concentrations increased during cold acclimation in three ecotypes of annual bluegrass, but variations in the individual carbohydrate fractions did not account for differences in freezing tolerance among the three ecotypes. However, upon inspection of a larger collection of annual bluegrass ecotypes (a total of 42), the authors determined a strong correlation between the accumulation of high molecular weight fructans and freezing tolerance (Dionne et al., 2001a). Hoffman et al. (2010) reported that the freezing tolerance of perennial ryegrass (*Lolium perenne* L.) accessions was associated with the accumulation of water soluble carbohydrates in crowns during cold acclimation at 2 °C. Additional research is necessary to evaluate carbohydrate changes of cool-season turfgrasses during the first and the second stages of cold acclimation, and to determine the role of carbohydrates in relation to inter- and intraspecific differences in freezing tolerance.

2.2.4. Proteomic response

Proteomics offer a powerful approach to reveal mechanisms underlying different aspects of winter hardiness. Many studies have been carried out with *Arabidopsis thaliana* (Jaglo-Ottosen et al., 1998; Le et al., 2008), but this species is not necessarily representative as a model for cold-induced responses at the molecular, cellular, or whole-plant level in perennial grasses (Livingston et al., 2007). Only few studies have been performed on grasses. Synthesis of soluble proteins and their expression is more pronounced in acclimated and freezing tolerant species than in freezing sensitive species (Perras and Sarham, 1989; Dionne, 2001b). Direct protection from freezing is one of the reported functions of cold-regulated proteins (COR). Antifreeze proteins adhere to the surface of ice crystals and inhibit their growth through thermal hysteresis (Duman and Olsen, 1993; Griffith et al., 1997). Antifreeze proteins may also inhibit ice re-crystallisation (Sandve et al., 2008) and protect thylacoid membranes

against freeze-thaw damage (Sieg et al., 1996). Small dehydrins, usually rich in lysine and induced by abscisic acid, have not only been associated with drought stress, but also with cold acclimation and freezing tolerance (Close et al., 1989; Puhakainen et al., 2004; Patton et al., 2007).

2.2.5. Low temperature fungal diseases

Pink snow mold, gray snow mold, and speckled snow mold caused by the fungi *Microdochium nivale*, *Typhula incarnata*, and *T. ishikariensis*, respectively, are the most damaging low-temperature diseases of turfgrasses and winter cereals in Europe, North America, Japan, and other temperate and boreal regions (Årsvoll, 1973, 1975; Smith et al., 1989; Smiley et al., 2005; Tompkins et al., 2004; Matsumo, 2009; Bertrand et al., 2009b).

Snow mold pathogens are difficult and costly to control. On most golf courses in the United States control of these diseases relies on preventive fungicide applications (Chang et al., 2006). As of 1 Jan. 2011, only five fungicides (active ingredients) are allowed against snow mold on turfgrasses in Norway, and even fewer products are permitted in some of the other Nordic countries (STERF, 2010). Difficulties in choice of active chemical substance and estimation of required dose are associated with difficulties in prediction of disease injury.

2.2.5.1. Some aspects of biology and ecology of snow molds

M. nivale (teleomorph *Monographella nivalis* (Schaffnit) E. Müller) and *Typhula* spp. belong to different fungal classes – Ascomycota and Basidiomycota, respectively, and they have different life cycles. The biology and ecology of these fungi are well described in the literature (Smith et al., 1989; Hsiang, 1999; Tronsmo et al., 2001; Matsumo, 2009).

The optimal mycelial growth of *M. nivale* and *T. ishikariensis* occurs at 18-20 °C and 10 °C (2 °C for some strains), respectively (Bennett, 1933 cited in Smith 1989; Hoshino, 1998; Snider, 2000). The low competitive ability of the fungi during the growing season results in ‘avoidance of antagonism by escaping to the under-snow habit’ (Matsumoto, 2009). The growth of *Typhula* spp. (with the exception of *T. canadensis*) at low temperatures is facilitated by lipolytic enzyme activity (Hoshino, 1997) and by production of antifreeze-like proteins and their ability to inhibit intra- and extracellular ice formation (Newsted et al., 1994; Snider et al., 2000; Hoshimo et al., 2001). In contrast to *Typhula* spp., *M. nivale* does not grow at subfreezing temperatures *in vitro* (Snider et al., 2000). Incidence and severity of snow molds varies depending on presence and duration of snow cover (Årsvoll, 1973). Matsumo

(1994) divided snow molds into obligate (e.g., *Typhula* spp.) and facultative (e.g., *M. nivale*) regarding their dependence on winter conditions.

2.2.5.2. Resistance to snow molds

No turfgrass species has absolute resistance to snow molds, but the susceptibility to low-temperature diseases varies among species and cultivars (Smith et al., 1989; Hofgaard et al., 2003; Smiley et al. 2005; Casler et al., 2006, 2007; Chang et al., 2006, 2007; Latin, 2007). Compared with winter cereals and forage grasses, studies on snow mold resistance in turfgrasses are limited. Old and recent studies show variation in susceptibility to low-temperature Basidiomycota and *M. nivale* in creeping bentgrass, colonial bentgrass, Kentucky bluegrass, red fescue, and sheep's fescue collected from North America and Europe (Smith, 1975; Casler et al., 2001; Wang, 2005; Chang et al., 2006). Fine fescue and colonial bentgrass were shown to have better resistance to snow molds than creeping bentgrass (Casler, 2001). So far, our knowledge regarding disease resistance in velvet bentgrass is restricted to grey snow mold (*Typhula incarnata*), dollar spot (*Sclerotinia homoeocarpa*), brown patch (*Rhizoctonia* spp.), and copper spot (*Gloeocercospora sorghi*) (DeFrance et al., 1952; Brilman and Meyer, 2000; Brown and Jung, 2010). Chang et al. (2007) reported that velvet bentgrass was more susceptible to *Typhula incarnata* than creeping bentgrass and colonial bentgrass under controlled environmental conditions.

Similar to freezing tolerance, resistance to snow molds can be enhanced by acclimation (Ergon, 1998; Tronsmo et al., 2001; Hofgaard et al., 2006; Tronsmo et al., 2008), but freezing tolerance and snow mold resistance were reported to have different mechanisms in cereals and forage grasses (Tronsmo, 1985; Yoshida et al., 1998). Reduced water potential and increased carbohydrate levels in acclimated crowns of winter cereals and perennial grasses explain the resistance pattern only to a certain extent (Tronsmo, 1986; Yoshida et al., 1998). The expression of pathogenesis-related proteins (PR-proteins) has been demonstrated under cold acclimation in inoculated grasses (Ergon et al., 1998; Hofgaard et al., 2006) and cereals (Gaudet et al., 2000; Muthukrishnan et al., 2001).

Field trials such as the National Turfgrass Evaluation Program (NTEP) in USA and Canada (www.ntep.org), the Sport Turf Research Institute (STRI) variety trials in UK (www.stri.co.uk), and the Scandinavian Turfgrass and Environment Research Foundation variety trials in the Nordic countries (sterf.se), play an important role in finding turfgrass varieties that are less susceptible to diseases in different geographical regions. Still, there is a

need for studies under controlled environmental conditions to save time, test a large number of plants and exclude other factors occurring in the field.

2.3. Rootzone composition influence turf performance

Turf performance on golf greens greatly depends on rootzone composition (Joo et al., 2001; Murphy et al., 2004; Bigelow et al., 2004; Aamlid, 2005). Many greens in United States, Europe and elsewhere are built according to the most widely used method of putting green construction developed by the United States Golf Association (USGA) Green Section Staff (USGA Green Section Staff, 2004). Focusing on rootzone physical properties, particularly on macroporosity and hydraulic conductivity, the method restricts compaction and provides a good combination of drainage and water retention. These physical properties are obtained with sand-based rootzones containing from 90 to 100% (w/w) sand of specified grain size distribution. McCoy (1992) and Murphy et al. (1993b) considered that critical maximal values for organic matter content in rootzones were 3.5 % and 4.5 % (w/w), respectively. The inclusion of organic matter in putting green rootzones is not required by the USGA Green Section Staff (2004) construction, but the benefits of using proper organic amendments are not in question (Murphy, 2007). Among them are better nutrient and water retention, improved cation exchange capacity, and increased soil microbial activity (Engelsjord et al., 2004; Murphy et al., 2004; Kaminski et al., 2004; Aamlid et al., 2005). In spite of these benefits, many greens have been constructing with 100% sand mostly to save costs. It has been claimed that the organic matter content in the entire rootzone will increase over time, but research shows that this happens mostly in the 5-cm upper layer (Liu, 2004; Murphy, 2007). A common phenomenon on sand-based rootzones is soil water repellency which causes localized dry spots, fingered flow and unnecessary nutrient losses from golf courses (Bauters et al., 1998; Dekker et al. 2001; Larsbo et al., 2008; Aamlid et al., 2009). Although soil surfactants (wetting agents) help to overcome soil water repellency (Aamlid et al., 2009), the phenomenon is still a problem, especially on straight sand rootzones.

Instead of peat which is a non-renewable resource, sand-based rootzones may be amended with compost. The physical, chemical, and biological quality of compost varies depending on the source and on the composting process (Murphy, 2007). Although there are some data available on the utilization of composts on American putting greens (Murphy et al., 2004; Liu, 2004), the increased interest among Scandinavian greenkeepers for garden

compost ought to be followed up by research beyond the preliminary data provided by Aamlid (2005). As composts usually have pH of 7.2 or higher, a pertinent question is if this amendment will meet the requirement of velvet bentgrass which is traditionally regarded as being adapted to acid soils (pH<7) (Torello, undated).

The use of compost is also interesting from the perspective of integrated pest management (IPM). Suppressive effects of compost on the development of *Microdochium nivale* and *Typhula ishikariensis* were reported by Boulter *et al.* (2002a).

2.4. Irrigation schedules and water conservation

Globally there is a focus on water conservation, and much work has been devoted to effects of reduced irrigation on turf quality and health (Qian and Fry, 1996; DaCosta and Huang, 2006a,b; McCann, 2008). The optimal irrigation schedule (amount and frequency) for a certain turfgrass area depends on the species' water requirement, rootzone water holding capacity, and turfgrass evapotranspiration which again depends on radiation, temperature, relative humidity, and wind (Meyer and Gibeault, 1986; Aronson *et al.*, 1987; Huang, 2006; Aamlid *et al.*, 2008).

Few data are available on the water requirement of velvet bentgrass. DaCosta and Huang (2006a) reported that velvet bentgrass maintained under fairway conditions performed better at 60% and 80% (deficit irrigation) than at 100% evapotranspiration replacement. They concluded that velvet bentgrass has lower water requirements and more capacity for osmotic adjustment than creeping bentgrass and colonial bentgrass. The investigators also demonstrated that velvet bentgrass exhibited lower soil water depletion, higher water use efficiency, and lower carbon isotope discrimination than other bentgrass species (DaCosta and Huang, 2006b).

Fry and Huang (2004) introduced the terms 'field capacity-based' and 'wilt-based' turfgrass irrigation. Field capacity-based irrigation implies a light and frequent irrigation pattern always keeping the rootzone close to field capacity. Conversely, wilt-based irrigation implies a deep and infrequent irrigation pattern which allows the rootzone to become depleted for water at certain intervals. The effects of these contrasting irrigation strategies on turfgrass visual quality, playability, thatch formation, root development, and nutrient leaching have not been sufficiently investigated. Deeper rooting as a result of infrequent irrigation has been shown to enhance turfgrass survival during dry periods (Qian and Fry, 1996; Jordan *et al.*,

2003; Fu and Dernoeden, 2009b). However, in spite of a mostly lower soil water content, deep and infrequent irrigation is often considered to cause more drainage and nutrient leaching than light and frequent irrigation (Starett et al., 1995; Kenna and Snow, 2000; Fry and Huang, 2004; Barton and Colmer, 2006). On straight sand, this phenomenon is often referred to fingered flow which develops due to great fluctuations in soil water content and soil water repellency (Bauters et al., 1998; Nektarios et al., 1999; Larsbo et al., 2008). This situation may, however, be different in a coastal climate where natural rainfall often results in oversaturation and thus drainage from the turfgrass rootzone.

2.5. Thatch control – a central issue in management of velvet bentgrass

Thatch is defined as "an intermingled organic layer of dead and living shoots, stems, and roots of grasses that develops between the turf canopy of green vegetation and the soil surface" (Beard, 2002). The term *mat* is used for the layer which is formed when thatch is intermixed with sand in the case of topdressing (Beard, 2002). Excessive thatch layers develop when thatch accumulation exceeds thatch degradation (Beard, 2002). As already mentioned, McCoy (1992) and Murphy et al. (1993b) considered critical values for organic matter content in turfgrass rootzones to be 3.5 % and 4.5 %, respectively, and these limits have also been used for the mat layer (Carrow, 2004).

Velvet bentgrass accumulates more thatch than other turfgrass species especially if the velvet bentgrass is maintained as creeping bentgrass (Rinehart et al., 2005; Aamlid et al., 2010) (Photo 2).



Photo 2. Thatch accumulation 15 months after sowing of red fescue Calliope, colonial bentgrass Bardot, creeping bentgrass Penn G6 and velvet bentgrass Greenwich. (Photo: Trygve S. Aamlid)

Among the problems caused by excessive thatch on golf greens are reduced water infiltration and increased risk for disease injury, scalping, dry spots, and poor playing quality (Jordan, 2008).

Thatch control can be grouped into (1) prevention of excessive plant growth and shoot density, e.g. by confined fertilizer inputs and irrigation (2) thatch dilution by sand, (3) mechanical thatch removal, and (4) enhancement of microbial thatch degradation.

2.5.1. Nitrogen fertilization

Excessive plant growth leading to thatch can be minimized by appropriate nitrogen fertilization. Velvet bentgrass is considered to require less nitrogen than creeping bentgrass (Brilman and Meyer, 2000; Torello, undated). There are, however, few studies comparing fertility programs on either newly-established or mature greens of velvet bentgrass. A 5 year study by Skogley (1975) starting on a 3 year old green with velvet bentgrass 'Kingstown' on a fine sandy loam in Rhode Island (nitrogen rate during establishment not stated) showed that 146 kg N ha⁻¹ yr⁻¹ led to better performance over time than 244 and 342 kg N ha⁻¹ yr⁻¹. Thirty-two years later Boesch and Mitkowski (2007), working at the same university, reported acceptable turf quality from nitrogen rates varying from 48 to 146 kg ha⁻¹ yr⁻¹ on greens sodded with velvet bentgrass 'SR 7200' (European name 'Avalon') on a silt loam soil. However, these authors also concluded that velvet bentgrass required at least 196-243 kg N ha⁻¹ yr⁻¹ during the first two years following nine months of establishment from seeds on a sand root zone amended with 20-30 % (v/v) *Sphagnum* peat. Recently, Koeritz and Stier (2009) suggested that velvet bentgrass response to nitrogen rate was cultivar specific. They indicated that a nitrogen rate of 146 kg N ha⁻¹ yr⁻¹ on a sand-based root zone was sufficient for newly established 'Vesper', but not for 'SR7200' ('Avalon'). Among these studies, only Skogley (1975) reported data regarding thatch accumulation. The investigator showed 11.4-12.5% (w/w) organic matter in velvet bentgrass mats, but, surprisingly, these numbers were not affected by nitrogen rate under the given experimental conditions.

Carrow et al. (1987) stated that thatch will increase with increasing nitrogen input at both deficient and excessive fertility levels, but remain constant with increasing nitrogen rate at an intermediate level. This assumption was based on his data showing no significant increase in the percentage of mat organic matter in the mat layer with an increase in nitrogen rate from 98 to 296 kg N ha⁻¹ yr⁻¹ in a 3-yr study on a bermudagrass [*Cynodon dactylon* (L.) Pers. x *C. transvaalensis* (Burt-Davis)] home lawn. Skogley (1975) also found that eight

years with fertilizer rates varying from 146 to 342 kg N ha⁻¹ did not influence the percentage of organic matter in mat samples from a velvet bentgrass green. There are, however, conflicting reports showing higher nitrogen rates to exacerbate thatch problems in various turfgrass species (e.g. Meinhold et al., 1973; Potter et al., 1985; Davis and Dernoeden, 2002).

2.5.2. Topdressing and mechanical treatments

Numerous studies have demonstrated effects of topdressing and mechanical treatments on thatch formation. Topdressing (Murphy, 1983; White and Dickens, 1984; Smith, 1979; McCarty et al., 2005, 2007) or return of soil from hollow tine coring (Murphy et al., 1993a; Fu et al., 2009) will usually decrease the content of organic matter in mat by dilution, but at the same time, these treatments also increase mat depth. Based on microscopic observations, Ledeborer and Skogley (1967) reported enhanced thatch degradation in old velvet bentgrass sod that had been dressed regularly compared with sod that had not received topdressing for nearly 20 years. Still, the contribution of topdressing to microbial thatch degradation remains controversial (Murphy, 1983; Carrow et al., 1987; Couillard et al., 1997; McCarty, 2005).

Vertical cutting and hollow tine coring usually reduce mat depth due to direct thatch removal (Smith, 1979; McCarty, 2005). However, the effect of coring (Carrow et al., 1987; McCarty et al., 2005, 2007; Barton et al., 2009), vertical cutting (Carrow et al., 1987; McCarty et al., 2005, 2007) or spiking (Murphy et al., 1993a) on the percentage of organic matter in the mat layer is often small unless combined with topdressing. Depending on timing and frequency, mechanical treatments are sometimes disruptive to turfgrass surfaces (White and Dickens, 1984; Carrow et al., 1987; McCarty et al., 2005; Fu et al., 2009), and this may be particularly harmful in velvet bentgrass because of the poor recuperative capacity of this species (Boesch and Mitkowski, 2007).

2.5.3. Biological thatch control

Thatch is composed mainly of cellulose, hemicellulose and lignin (Ledebor and Skogley, 1967; Couillard and Turgeon, 1997). Lignin is a complex aromatic polymer that is extremely resistant to degradation (Kirk, 1971; Crawford and Crawford, 1980). Biodegradation of lignin is mainly accomplished by a few species of fungi (Martin and Dale, 1980; Blanchette, 1991, Sidhu et al. 2010), but bacteria (Vicuña, 1988; Zimmermann, 1990), especially actinomycetes (Crawford, 1978), have also been reported as lignin degraders. Degradation of thatch is essentially an aerobic process and the degradation rate depends on turf age (Shi et al., 2006),

soil temperature, soil moisture content (Donnelly et al., 1990; Pastor and Post, 1986), soil pH (Martin and Beard, 1975), and the carbon-to-nitrogen (C:N) ratio (Raun et al., 1998; Henriksen and Breland, 1999). It has been claimed that application of products containing fungi, bacteria, enzymes or other bioactive ingredients will enhance thatch degradation (Crawford, 1978; Martin and Dale, 1980; Roudsari et al., 2008), however the efficiency of such products under field conditions remains controversial (Chamberlain and Crawford, 2000; McCarty et al., 2005, 2007).

3. MAIN RESULTS AND DISCUSSION

3.1. Winter hardiness of velvet bentgrass

3.1.1. Metabolic changes during cold acclimation and tolerance to freezing

As determined by whole plant survival after 3 weeks of recovery in the greenhouse the freezing tolerances velvet bentgrass turf were not significantly different from that of creeping bentgrass turf receiving the same acclimation treatments (Paper I). Moreover, the freezing tolerance did not vary among velvet bentgrass cultivars (Paper III). Gusta et al. (1980) previously reported that creeping bentgrass plants that had been acclimated under field conditions were able to survive temperatures as low as -35 °C (LT₅₀). Taken together, this would suggest that the better winter survival of velvet bentgrass compared to creeping bentgrass in Scandinavian variety trials (Aamlid et al., 2006) was not due to differences in freezing tolerance.

Freezing tolerance of both bentgrass species was enhanced by acclimation treatments (Paper 1, 3) as was previously showed in winter cereals, forage grasses, and annual bluegrass (e.g. Veisz and Sutka, 1989; Livingston, 1991; Tronsmo et al., 1993; Livingston et al., 2009). The LT₅₀ based on whole plant survival was significantly reduced by exposure of plants to 2 °C and light for 2 weeks (-12.7 °C) or 4 weeks (-14.5 °C) (Paper I).

Cold acclimation resulted in changes in nonstructural carbohydrates in both bentgrass species (Paper I). The major nonstructural carbohydrates in crown tissues of velvet bentgrass and creeping bentgrass were fructans (35 and 58% of total nonstructural carbohydrates, respectively) followed by sucrose (27 and 13%, respectively). Increased freezing tolerance

after 2 weeks at 2°C was associated with 2-fold and 3-fold increases in crown sucrose content and 4-fold and 3-fold increases in crown fructan contents in velvet bentgrass and creeping bentgrass, respectively, compared with nonacclimated plants. These findings are in line with earlier studies reporting cold-induced increases in the content of sucrose and fructans in winter cereals, forage grasses, and annual bluegrass (Livingston, 1991; Olien and Clark, 1993; Tronsmo et al., 1993; Dionne et al., 2001a). While the content of fructans increased with acclimation time (5-fold increase in velvet bentgrass and 4-fold in creeping bentgrass after 4 weeks at 2°C compared with nonacclimated plants), the sucrose content after 4 weeks at 2°C just slightly increased in velvet bentgrass and remained at the same level as after 2 weeks at 2°C in creeping bentgrass. It is also worth noting that the sucrose contents in turfgrass crowns were similar in acclimated plants of velvet bentgrass and creeping bentgrass (27 and 24 g kg⁻¹ DW, respectively), while creeping bentgrass had a higher content of fructan than velvet bentgrass both initially (31 vs. 15 g kg⁻¹ DW) and after acclimation (99 vs. 63 g kg⁻¹ DW).

No differences in freezing tolerance between velvet bentgrass and creeping bentgrass may have been due to similar levels of sucrose in the two species after the first acclimation stage. A high correlation between levels of mono- and disaccharides and freezing tolerance was found in winter wheat by Fowler et al. (1981) and Yoshida et al. (1998). In contrast, a direct contribution of fructans to freezing tolerance in the bentgrass species seems negligible since the considerable difference in crown fructan content between velvet bentgrass and creeping bentgrass had no significant impact on the LT₅₀ of the two species (Paper I).

Additional freezing tolerance was acquired after 2 weeks of subzero acclimation in darkness following 2 weeks at 2 °C and high light intensity (Paper III), but not after 4 weeks at 2°C) (Paper I). The sufficiency of artificial acclimation depends on light intensity, temperature, day length (Huner et al., 1993; Tepperman et al., 2001), and duration of the first (Veisz and Sutka, 1989; Vágújfalvi et al., 1999) and second (Herman et al., 2006) stages of cold acclimation. The negligible effect of subzero acclimation in Paper I could be due to the four week duration of the first acclimation stage in contrast to only two weeks in Paper III. Unlike Veisz and Sutka (1989) and Vágújfalvi et al. (1999) who found a negative effect of prolongation of the first acclimation stage on freezing tolerance of wheat (from 7 to 8 weeks and from 5 to 6 weeks, respectively), and Dionne et al. (2001a) who found no effect of prolongation of acclimation at 2°C (light) from 2 to 4 weeks in annual bluegrass, Paper I shows significantly better freezing tolerance after 4 than after 2 weeks of acclimation at 2°C in velvet bentgrass and creeping bentgrass. Another explanation could be that the effect of subzero acclimation was not sufficiently expressed in Paper I as plants were exposed to each

freezing temperature for only 2 h in contrast to 24 h in Paper III. Dionne et al. (2001a) found an additional effect of subzero acclimation even with only 1 h exposure to various freezing temperatures in annual bluegrass, but this may well be different in the more winter-hardy bentgrass species. In any case, the distinction between the first and second stage of cold acclimation becomes less apparent under field conditions with fluctuating temperatures. In the Paper III it is, indeed, noteworthy, that the highest freezing tolerance was achieved after outdoor acclimation, despite the fact that the average light intensity in the field from 1 Oct. to 1 Jan. was significantly lower than in the indoor acclimation chamber.

The effect of subzero acclimation on carbohydrate metabolism in turfgrasses has been poorly studied. A decrease in fructans in velvet bentgrass (only a tendency) and creeping bentgrass after subzero acclimation (Paper I) agreed with previous studies in rye (*Secale cereale* L.) (Olien and Lester, 1984), barley (*Hordeum vulgare* L.) and oat (*Avena sativa* L.) (Livingston, 1996), and annual bluegrass (Dionne et al., 2001a). In contrast to annual bluegrass showing an increase in sucrose, glucose, and fructose as a result of fructan depletion in response to subzero acclimation (e.g. Dionne et al., 2001a), velvet bentgrass and creeping bentgrass only showed a slight increase in reducing sugars (fructose and glucose) (Paper I). A cryoprotective role of fructose was earlier reported in rye (Olien and Clarke, 1993). Further research is underway to clarify to what extent the increase in freezing tolerance after subzero acclimation was associated with hydrolysis of fructans to mono- or disaccharides.

Both acclimation at 2 °C for 4 weeks and subsequent subzero acclimation led to quantitative changes in crown protein content of velvet bentgrass (Paper II). Currently, there is little evidence regarding protein changes in crown tissue of cool-season turfgrasses in response to the first and second stages of acclimation. Similar to findings in wheat (Herman et al., 2006), differences in protein expression in crown tissue between nonacclimated plants and plants subjected to the first acclimation stage (4 weeks at 2°C) were more pronounced than differences between plants exposed to 4 weeks at 2°C and plants exposed to 4 weeks at 2°C followed by subzero acclimation. The cold-responsive proteins identified in our study had metabolic, energy, and defence functions. Among proteins up-regulated after 4 weeks at 2°C were methionine synthase, serine hydromethyltrasferase, aconitase, UDP-D-glucuronate decarboxylase, and a putative glycine rich protein. The increased freezing tolerance might be associated with enhanced amino acid synthesis, since serine hydroxymethyltrasferase and methionine synthase were up-regulated by acclimation. Large and small subunits of rubisco, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and putative peroxidase were down-regulated. Our data on the expression of enzymes involved in the Calvin cycle (rubisco) and

glycolysis (aldolase, GAPDH) suggest that acclimation of velvet bentgrass at low non-freezing and sub-freezing temperatures occurs under suppressed photosynthesis and respiration.

3.1.2. Evaluation of freezing tolerance by indirect methods

Results indicated that triphenyltetrazolium chloride (TTC) reduction slightly underestimated freezing tolerance compared with whole plant survival (Paper I). This might reflect heterogeneous crown tissue as has been shown earlier (Tanino and McKersie, 1985). The meristematic crown region is small compared to surrounding tissues, and much of the tissue that was injured by frost in our study was probably non-meristematic and thus not responsible for recovery. Nonetheless, the correlation between LT_{50} values estimated by TTC reduction and those estimated by whole plant survival was very high ($r=0.97$, $p<0.05$), which suggests that the TTC technique is sensitive enough to detect treatment and/or genotype differences over a wide range of LT_{50} values. This method should be further explored as a rapid method to screen freezing tolerance of grasses.

In contrast to those obtained by TTC reduction, the LT_{50} values determined by electrolyte leakage were not significantly correlated with those determined by whole plant survival ($r=0.34$) (Paper I). Electrolyte leakage clearly underestimated freezing survival as was previously reported for perennial ryegrass (Ebdon et al., 2002).

3.1.3. Resistance to pink snow mold and tolerance to ice cover

Acclimation for 2 weeks at 2°C significantly improved resistance to pink snow mold (Paper III). This finding is in accordance with earlier investigations showing cold acclimation to enhance resistance to pink snow mold in winter cereals and forage grasses (Tronsmo, 1984; Hofgaard et al., 2006). Resistance to pink snow mold did not vary among velvet bentgrass cultivars, but velvet bentgrass was more susceptible to *M. nivale* than creeping bentgrass 'Penn A-4'. As snow mold resistant cultivars of winter wheat have been shown to accumulate higher levels of polysaccharides and metabolize them at slower rates than susceptible cultivars (Yoshida et al., 1998), it might be speculated that the higher fructan levels in creeping bentgrass could contribute to snow mold resistance. The small differences in snow mold resistance in velvet bentgrass were probably due to little genetic diversity as most cultivars came from the same breeding program (S. Bonos, pers. Comm. 2011). This is different from annual bluegrass where Aamlid et al. (2008) and Bertrand et al. (2009b)

reported genetic variability in resistance to pink snow mold among ecotypes naturally selected in areas with various duration of snow cover. Simulated snow cover for 6 and 12 weeks reduced survival of acclimated velvet and creeping bentgrass plants which had been inoculated with *M. nivale* by 44% and 41%, respectively, compared with uncovered plants showing 100% survival in darkness at 0.5 - 1.0 °C (Paper III). The results suggest that even acclimated plants of velvet bentgrass and creeping bentgrass that have been optimally acclimated under field conditions are not able to completely withstand snow mold invasion in the presence of snow cover. In agreement with investigations into forage grasses (Årsvoll, 1973) prolongation of the snow cover from 6 to 12 weeks only insignificantly decreased survival of plants inoculated with *M. nivale*.

Simulated ice cover for 12 weeks caused the largest damage regardless of acclimation and/or inoculation with snow mold (only 9% survival). In contrast, 99% of acclimated plants and 58% of nonacclimated plants survived 6 weeks of simulated ice cover regardless of inoculation with the snow mold (Paper III). The development of anaerobic conditions and accumulation of toxic gases under the simulated ice cover (Levitt, 1980; Aamlid, 2008) probably inhibited both snow mold and turfgrass growth. The prolonged ice cover may also have resulted in the loss of hardiness as was demonstrated in annual bluegrass and creeping bentgrass by Tompkins et al. (2004). Our results suggest that the critical duration of anoxic conditions for survival of velvet bentgrass and creeping bentgrass lies between 42 and 84 days. Aamlid et al. (2008), using a similar technique to that in our experiment, found critical ice encasement periods of 25-30 days in annual bluegrass and 42-47 days in creeping bentgrass.

3.1.4. Winter survival in the field

In the first spring after sowing at Landvik, less pink snow mold was observed on plots with the higher (150 kg N ha⁻¹ yr⁻¹) than with the lower (75 kg N ha⁻¹ yr⁻¹) nitrogen rate. An effect of topdressing was expressed only at the low nitrogen rate where light topdressing led to less injury than heavy topdressing (Paper IV). The fact that less pink snow mold was observed on plots with 150 kg N ha⁻¹ yr⁻¹ than with 75 kg N ha⁻¹ yr⁻¹ does not contradict the general opinion that snow mold development on turfgrass is enhanced by excessive nitrogen application in the fall (Smith et al., 1989). Indeed, our highest nitrogen rate was not excessive for immature turf, and nitrogen inputs in the fall were always adjusted to ensure good acclimation. On the other hand, the lowest nitrogen rate was probably insufficient to ensure

optimal plant growth prior to the first winter. Within reasonable limits, there is substantial evidence that increasing fertilizer inputs will improve turfgrass winter survival and spring growth rather than suppress it (e.g. Carow et al., 2001; Lloyd, 2009).

Better turfgrass performance on compost-amended ('Green Mix') rootzones compared with straight sand rootzones was observed in the spring and fall at Landvik and reflected less injury by *M. nivale* (Paper V). These results are substantiated by earlier reports showing suppressive effects of compost on soil-borne turfgrass diseases (Boulter et al., 2002b; Nelson and Boehm, 2002; Tilston et al., 2002). Boulter et al. (2002a) found a reduction in the development of *M. nivale* and *Typhula ishikariensis* on a 4-5 year old creeping bentgrass green after topdressing with compost. They also observed a quicker green-up and recovery from dormancy on compost-amended plots.

In continental climate at Apelsvoll, the effects of nitrogen and topdressing on velvet bentgrass winter survival were less clear-cut than at Landvik (Paper IV). All experimental plots at Apelsvoll were reseeded in spring after the first winter, and the whole experiment had to be discontinued after the second winter. This was due to the green at Apelsvoll having a continuous layer of ice for nearly four months during the first winter and being even more injured by melting water during the second winter. In contrast, the green at Landvik was never covered by ice or water for more than a couple of weeks. Although velvet bentgrass at Apelsvoll showed poor winter survival in our study, a parallel variety evaluation trial on the same experimental green confirmed our previous findings (Aamlid et al., 2006) that winter survival of velvet bentgrass is mainly better than of creeping bentgrass under Nordic climate conditions (Molteberg et al., 2010).

3.2. Management of newly established velvet bentgrass greens

3.2.1. Turf visual quality, root development, soil water repellency, and leaching

Rootzone composition, irrigation regime, nitrogen rate, topdressing level, and mechanical treatments had significant impacts on velvet bentgrass performance during the first and the second year after sowing (Paper IV, V).

In spite of lower fertilizer inputs, velvet bentgrass performed generally better on the 80 sand: 20 garden compost (v/v) rootzone than on the straight sand rootzone (Paper V). Nitrogen losses in the form of nitrate/nitrite were eight and three times higher from straight

sand plots compared with compost-amended plots in the sowing year and in the first year after sowing, respectively. This is in accordance with earlier investigations showing amendment with compost to improve the water holding and nutrient retention capacities and enhance turf quality on sand-based rootzones (McCoy, 1992; Brauen and Stahnke, 1995; Gibbs et al., 2000; Joo et al., 2001; Murphy et al., 2004; Aamlid, 2005). As already discussed in relation to winter survival, the reduction in turfgrass overall impression and shoot density on straight sand plots was more noteworthy in spring and fall than during summer. In the second year after sowing, differences in nitrate/nitrite leaching rate from the two rootzones were no longer significant (Paper V).

Straight sand plots showed strong and severe water repellency at most investigated depths in contrast to compost-amended plots which were wettable or only slightly water repellent. The higher water repellency on straight sand plots corroborates earlier studies showing water repellency to be an important property of sand which develops when the soil water content is depleted below a certain critical threshold (Bauters et al., 1998; Dekker et al., 2001; Larsbo et al., 2008). Our results suggest that development of water repellency on straight sand plots was delayed but not totally prevented by light and frequent irrigation. In the last year of the study we even found that a decrease in turf quality in response to light and frequent irrigation on straight sand plots was associated with stronger water repellency in the mat layer and lower water infiltration rate than with deep and infrequent irrigation (Paper V). We have no good explanation for this phenomenon but suggest that a high moisture in the mat layer could lead to microbial water repellency (Wilkinson and Miller, 1977; Hallet et al., 2001) or repellency induced by the products of organic matter decomposition or by the exudates from grass roots (Doerr et al., 2000).

Although turf visual quality was generally lower, there were always more roots at 6- to 10-cm and 10- to 20-cm depth on straight sand than on compost-amended plots (Paper V). The higher root density in the lower layer most likely maximized water and nutrient uptake due to the lower soil moisture content (Huang et al., 1997; Leinauer et al., 1997). Light and frequent irrigation provided better overall impression during the first year after sowing compared with deep and infrequent irrigation, but the effect of irrigation frequency was reversed on straight sand plots by the end of the second year (Paper V) (Photo 3). This is in line with earlier research (Jordan et al., 2003; Fu and Dernoeden, 2009a). Fu and Dernoeden (2009a, 2009b) showed that deep and infrequent irrigation resulted in higher turfgrass chlorophyll content, but also led to deeper root system and thus in better adaptation of creeping bentgrass to wilt stress than light and frequent irrigation. We also found that deep

and infrequent irrigation led to more roots at a depth of 10-20 cm in the straight sand rootzone by the end of the study. A similar tendency was observed even in the compost-amended rootzone, but this effect was not significant.

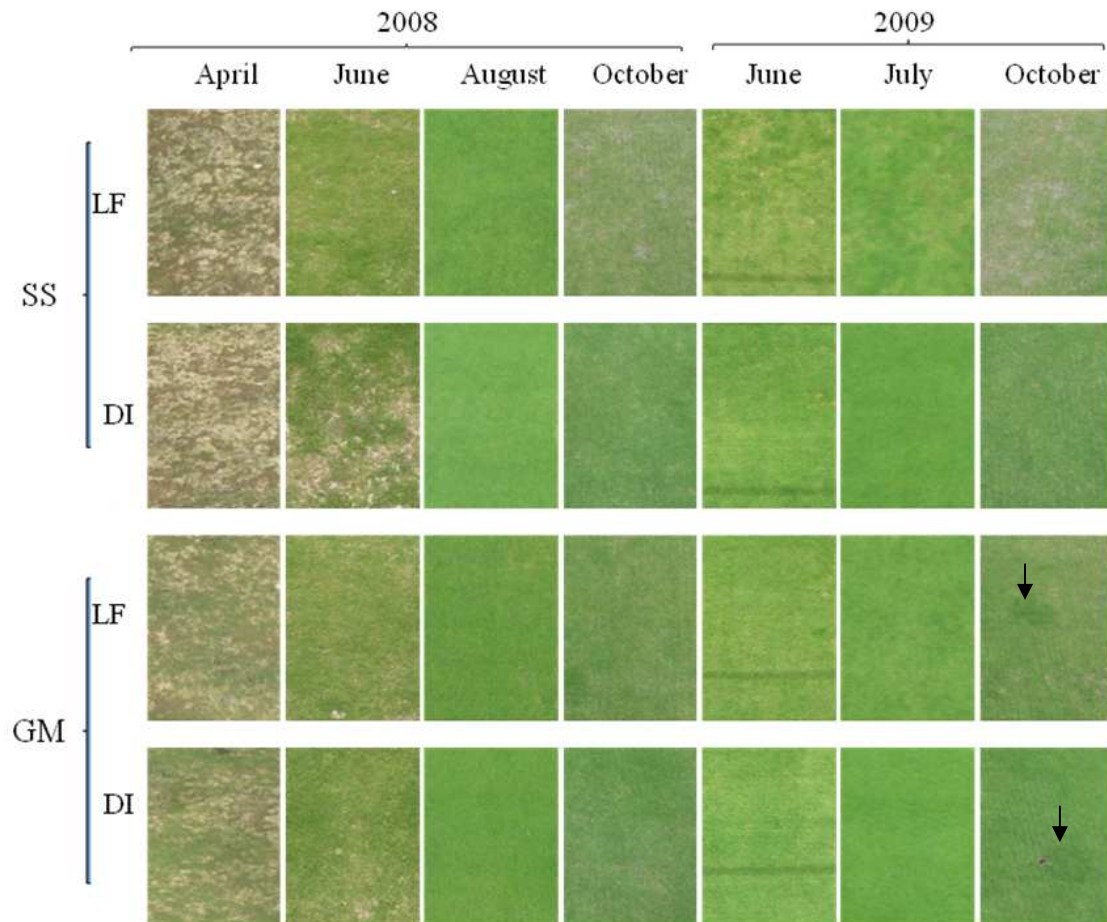


Photo 3. Effects of rootzone composition and irrigation regime on performance of velvet bentgrass during 2008 and 2009. The green was seeded in June 2007. Arrows show irregular patches on GM appeared first in August 2009. Abbreviations: SS, straight sand; GM, ‘Green Mix’; LF, light and frequent irrigation; DI, deep and infrequent irrigation.

Infrequent irrigation to field capacity on established velvet bentgrass greens was a better strategy than frequent irrigation also because infrequent irrigation caused lower drainage volumes than light and infrequent irrigation (significantly in the end of the second year after sowing) (Paper V). This happened as frequently irrigated plots were usually closer to field capacity and therefore had less space to absorb natural rainfall than infrequently irrigated plots. In contrast, infrequent irrigation was earlier shown to cause higher drainage volumes and often higher nutrient losses. This was often related to fingered flow or inadequate timing of irrigation and/or fertilizer inputs (Starrett et al., 1995; Bauters et al.,

1998; Kenna and Snow, 2000; Fry and Huang, 2004; Barton and Colmer, 2006; Nektarios et al., 2007).

Irrespective of type and amount of organic matter in the rootzone, a nitrogen rate of 150 kg ha⁻¹ yr⁻¹ gave acceptable overall impression and shoot density during the first year after sowing (Paper IV) (Photo 4). By contrast, the low nitrogen rate of 75 kg ha⁻¹ yr⁻¹ became sufficient to more mature turf in the second year after establishment. Our results suggest that well-established greens with velvet bentgrass can be maintained at lower nitrogen rates than reported by Skogley, (1975), Boesch and Mitkowski (2007), and Koeritz and Stier (2009) for other velvet bentgrass cultivars.

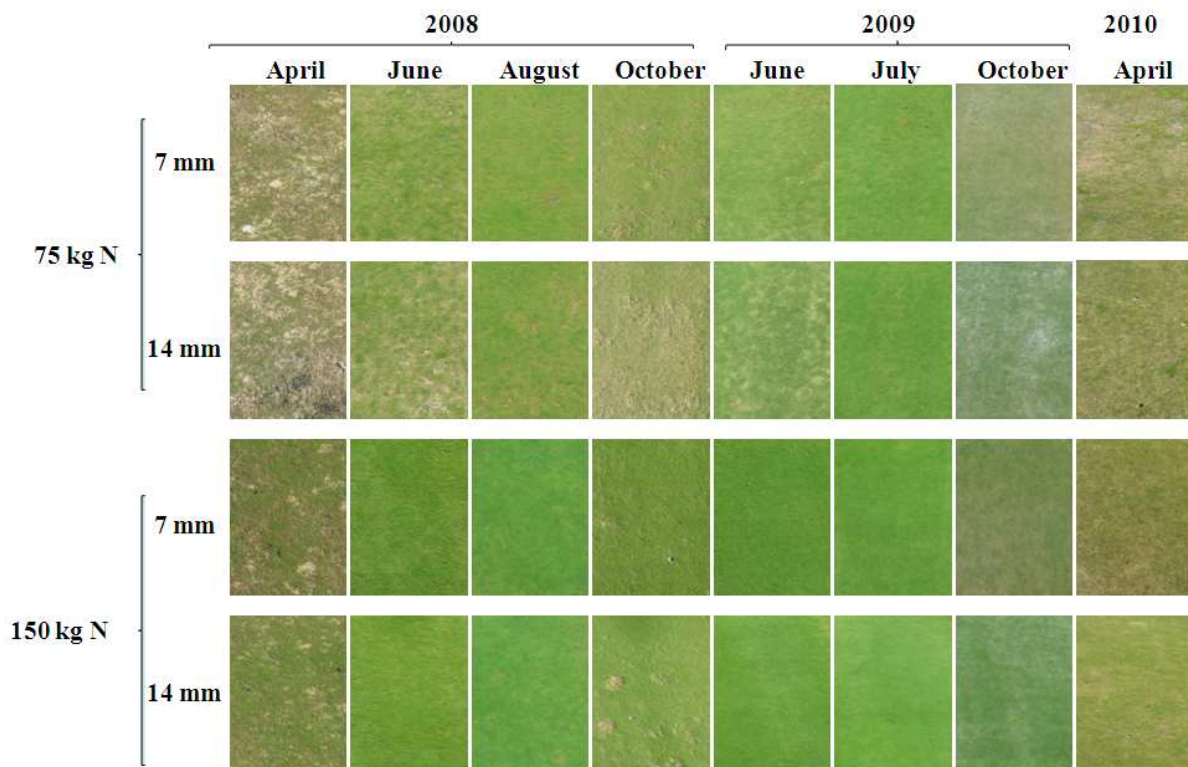


Photo 4. Effects of nitrogen (75 or 150 kg ha⁻¹ yr⁻¹) and topdressing (0.5 or 1.0 mm sand biweekly) on performance of velvet bentgrass plots receiving grooming in 2008-2010 at Landvik. The green was seeded in June 2007.

Immature turf performed better under light (7 mm yr⁻¹) than under heavy topdressing (14 mm yr⁻¹), but, as with irrigation frequency and nitrogen rate, the response was reversed from two years after sowing (Paper IV). Even so, light topdressing cannot be recommended in combination with 150 kg ha⁻¹ yr⁻¹ as it led to an unacceptable percentage of organic matter in the mat layer (8.1%).

An undesirable infestation of moss (*Bryum* spp.) was observed when *Sphagnum* peat was used as organic amendment at Landvik (Paper IV). Less competition from the turf and

more competition from the moss occurred at the low nitrogen rate, especially when topdressing was also low. The heavy topdressing probably resulted in a dryer surface. It is well documented that moss infestation will be most pronounced under wet conditions and at low fertilizer inputs (e.g. Brauen et al., 1986; Hummel, 1994; Cook et al., 2002). Borst et al. (2009) also found topdressing to be helpful in providing long-term moss control on putting greens.

Compared with nitrogen rate and topdressing level, the effect of mechanical treatments on turfgrass visual quality was less pronounced except for the initial coring and spiking which significantly decreased overall impression of the immature greens at Landvik and Apelsvoll, respectively (Paper IV). Later, vertical cutting resulted in better overall impression than the other mechanical treatments at Apelsvoll, and the same tendency was observed at Landvik. As compared with the other mechanical treatments, vertical cutting probably led to better incorporation of topdressing sand (Pease, 2009), and provided a smoother surface with better mowing quality and less risk for scalping.

3.2.2. Playability

Turfgrass ball roll distance was 6-17% longer on plots receiving 75 kg N ha⁻¹ yr⁻¹ compared with 150 kg N ha⁻¹ yr⁻¹ (Paper IV), but this character was not influenced by topdressing level (Paper IV), mechanical/biological thatch control treatments (Paper IV), rootzone composition (Paper V) or irrigation regime (Paper V). The negligible effect of rootzone is in accordance with earlier investigations (Baker and Richards, 1995; Baker et al., 1999; Gibbs et al., 2000).

At Landvik, surface hardness was improved by the low N rate (vs. high) and heavy topdressing level (vs. light). Among mechanical treatments, spiking in combination with grooming led to the softest green surface. The biological product 'Thatch less™' slightly increased hardness of plots receiving spiking and grooming treatments. At Apelsvoll, surface hardness was lower than at Landvik and was significantly affected by topdressing level and mechanical treatment. Heavy topdressing increased the surface hardness of plots that received spiking in combination with grooming, but had no effect on plots that received grooming only or vertical cutting in combination with grooming (Paper IV). Irrigation regime did not influence surface hardness (Paper V). Newly established playing surfaces were 20% harder on straight sand than on compost-amended plots, (Paper V), but in accordance with earlier investigations (Baker and Richards, 1995; Gibbs et al., 2000; McCarty, 2005, 2007), this difference was no longer significant in the second year after establishment. Similar mat depth

and equal content of organic matter in the may layer at various combinations of rootzone and irrigation regime treatments resulted in the minimal differences in hardness on the mature green.

3.2.3. Thatch formation and thatch control

The content of organic matter in the mat varied from 3.6 to 8.7% among the 16 combinations of nitrogen rate, topdressing level, and mechanical / biological treatments, but remained stable for each combination from 16 to 28 months after sowing (Paper IV). Nitrogen rate and topdressing level had the highest impact on thatch formation and we also found an interaction between these factors. Both at Landvik and Apelsvoll, the content of organic matter in the mat increased by nearly 2 percentage points with an increase in N rate from 75 to 150 kg ha⁻¹ yr⁻¹ under light topdressing, but it was low and virtually unaffected by nitrogen rate under heavy topdressing (Photo 5). This suggests that topdressing contributed not only to thatch dilution, but also to thatch degradation. The heavy topdressing provided adequate oxygen concentration in the mat where more nitrogen not only stimulated turfgrass growth but also microbial degradation of organic matter through amplification of soil microbial communities (Blagodatskaya and Kuzyakov, 2008) and/or a lower C:N ratio (Berndt et al., 1992; Henriksen and Breland, 1999; Roudsari et al., 2008). This effect has sometimes been referred to as the “*added nitrogen interaction*” (Jenkinson et al., 1985) or the “*positive priming effect*” (Kuzyakov et al., 2000).

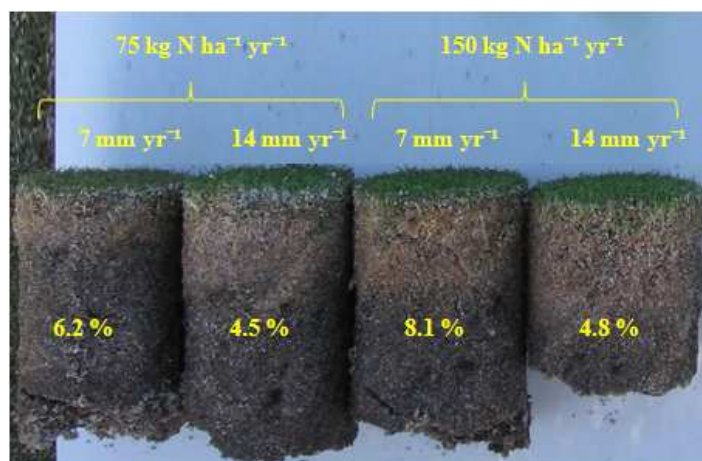


Photo 5. Effects of nitrogen rate and topdressing level on thatch formation (expressed as organic matter percentage) on velvet bentgrass green.

Mechanical treatments had no significant effect on the percentage of organic matter in the mat by the end of first year after sowing at Landvik and Apelsvoll (Paper IV). However,

by the end of the second year at Landvik, grooming plus vertical cutting, i.e. the only treatment where organic matter was physically removed from the green, had reduced organic matter in the mat by 1.1 percentage point compared with grooming only. Earlier studies also showed a negligible effect of mechanical treatments on greens that were either young (McCarty et al., 2005; Barton et al., 2009) or had an initial content of organic matter in the mat layer lower than 3.5% (McCarty et al., 2007). In contrast, Carrow et al. (1987) reported that vertical cutting twice per year reduced organic matter in the mat from 15.3% to 14.1% even without topdressing on a bermudagrass home lawn.

In contrast to nitrogen, topdressing, and vertical cutting, rootzone composition and irrigation regime had negligible effect on the content of organic matter in the mat (Paper V). In contrast, Fu and Dernoeden (2009a) reported 24.7% and 20.0 % organic matter in the mat on a creeping bentgrass green compared after two years with light and frequent and deep and infrequent irrigation, respectively, but in our study, any possible effect of irrigation on organic matter accumulation was probably masked by our frequent topdressing program. On average for treatments we detected a reduction in the organic matter content in the mat from 9.7% to 8.3% from the first year to the second year, and this was most likely mediated by an increase in the topdressing level from 4 to 9 mm yr⁻¹ and a concomitant reduction in the N rate from 192 to 131 kg ha⁻¹ yr⁻¹ on straight sand plots and from 131 to 108 kg ha⁻¹ yr⁻¹ on compost-amended plots. This observation underscores the predominant effect of nitrogen and topdressing on thatch formation shown in Paper IV.

As with organic matter percentage, mat depth was significantly affected by nitrogen rate and topdressing level (Paper IV), but not by rootzone composition or irrigation regime (Paper V). From 16 to 28 months after establishment mat depth increased by 65-98% depending on nitrogen rate and topdressing level in our first field study (Paper IV) and doubled regardless of rootzone composition in our second field study (Paper V). Grooming plus vertical cutting reduced mat depth by 2.3 mm compared to plots with grooming only, but neither this effect nor the effects of spiking or ‘Thatch less™’ were significant (Paper IV).

The average depth of the mat layer by the end of the first growing season was lower at Apelsvoll than at Landvik (10.7 mm vs. 13.8 mm, $p=0.006$), while the average content of organic matter was higher at Apelsvoll than at Landvik (6.9 % vs. 5.9 %, $p=0.019$). This was most likely due a colder summer with less degradation of organic matter and to a shorter growing season with therefore fewer events of topdressing and mechanical treatments at Apelsvoll than at Landvik.

In the second year after sowing, irregular patches with enhanced shoot growth were observed on some compost-amended plots at Landvik. Inspection of the mat layer indicated that the dark-colored thatch, most likely lignin, had been degraded, and microscopic observation suggested that it could be caused by a fungi belonging to Basidiomycota, probably white-rot fungi contained in the compost (Tuomela et al., 2000).

4. MAIN CONCLUSIONS AND FUTURE PERSPECTIVES

The superior winter survival of velvet bentgrass in the variety trials from 2003 to 2006 (Aamlid et al., 2006) has not be fully explained in this thesis. In the test series under controlled environmental conditions, the species showed the same freezing tolerance, with no difference between cultivars, as creeping bentgrass, and velvet bentgrass was in fact more susceptible to *Microdochium nivale* than creeping bentgrass. Even with optimal acclimation, neither velvet bentgrass nor creeping bentgrass were able to resist snow mold infection sufficiently under simulated snow cover. This fact, unfortunately, reduces the potential of the species on Nordic golf courses. On golf greens that normally suffer from pink snow mold, today's varieties of velvet bentgrass or creeping bentgrass will be not able to provide excellent turf in spring without fungicide use. For both species, the most efficient way to improve snow mold resistance is probably to emphasize this aspect in the breeding program.

Acclimation improved freezing tolerance, susceptibility to pink snow mold, and tolerance to anoxia. Simulated ice cover caused the largest damage regardless of acclimation and inoculation with *M. nivale*. This supports common observations of winter damage on Norwegian golf courses. The critical duration of anoxia for survival of bentgrasses was between 42 and 84 days. This ought to be validated in the field as the critical ice duration can differ from that under the simulated conditions. The mechanisms underlying the tolerance to ice damage also need further research.

Similar freezing tolerance of velvet bentgrass and creeping bentgrass was associated with similar crown sucrose content. By contrast, crown fructan content was considerably higher in creeping bentgrass than in velvet bentgrass. Apparently, fructans did not directly contribute to freezing tolerance. Additional studies are needed to find out whether the lower fructan accumulation in velvet bentgrass reflects lower rates of metabolic processes in the species and how this influences other aspects of the overwintering ability, particularly,

resistance to snow molds, tolerance to ice covers and anoxia, deacclimation ability, and the recuperative capacity in spring.

Velvet bentgrass and creeping bentgrass showed superior freezing tolerance when acclimated under the field conditions with low light intensity, temperature fluctuations, and no distinction between the first and second stages of cold acclimation. This suggests that weather conditions in fall provide good acclimation and acquirement of winter hardiness. Along with research on the mechanisms underlying cold acclimation, additional studies are required to identify factors affecting loss of acquired winter hardiness during either winter or early spring.

While this investigation showed that establishment and maintenance of velvet bentgrass is possible on both straight sand and compost-amended rootzones, the use of the latter showed clear advantages in the form of higher visual quality, less pink snow mold, longer irrigation intervals, and less risk for development of soil water repellency. Further analysis of microbial composition and testing of compost amendments in the field are required to assess whether the presence of fungi with lignin degradation ability will provide advantages or disadvantages on golf greens in the long run.

Compared with light and frequent irrigation, deep and infrequent irrigation resulted in better overall impression, lower drainage volumes and improved root development in the 10- to 20-cm soil layer in the second year after sowing. This suggests infrequent irrigation to the field capacity to be a better irrigation strategy on velvet bentgrass greens than frequent irrigation, except for the first year after sowing. Research is needed to explore deficit irrigation as an alternative irrigation strategy on velvet bentgrass golf greens.

The present work showed that velvet bentgrass requires much care regarding thatch control. Thatch formation in velvet bentgrass is very rapid but can be controlled by proper cultural management starting from the green establishment. Due to the limited recuperative capacity of velvet bentgrass and the low soil temperatures during most of the season in Scandinavia, mechanical and biological treatments to control thatch are of secondary importance compared with appropriate fertilization and topdressing programs during the first year after sowing. Apart from the presowing application, an optimal nitrogen rate of at least 150 kg N ha⁻¹ can be recommended during the first year after sowing. More nitrogen during the first year provided better turf quality, better winter survival, and better competition against moss. But it is extremely important to reduce the nitrogen rate as the turf becomes mature. From the first year after sowing, topdressing should be maintained at a level of 10-14 mm per year to keep thatch formation within acceptable level. From the second year after sowing,

monthly vertical cutting reduced thatch, contributed to the better sand incorporation into the dense turf surface, and led to generally better overall impression. Monthly spiking improved infiltration rate by more than 50%, but reduced surface hardness. We conclude that from the second year after sowing vertical cutting can be applied monthly, but spiking cannot be recommended more than once or twice per year. Biological thatch control products warrant further investigation as there were indications that that may improve surface hardness.

5. REFERENCES

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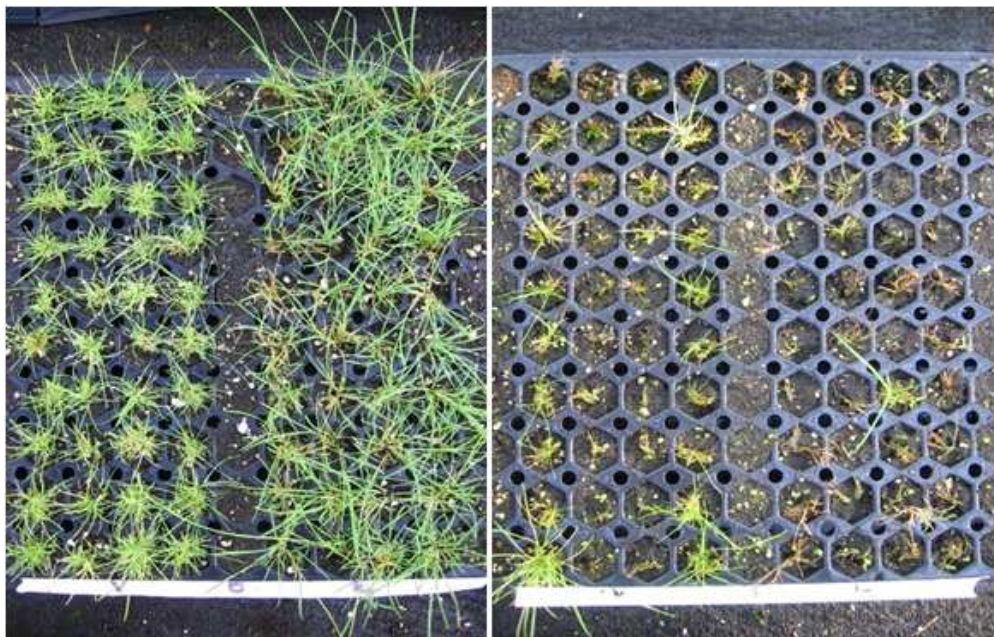
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PAPER I



Freezing Tolerance and Carbohydrate Changes of Two *Agrostis* Species during Cold Acclimation

Tatsiana Espevig, Michelle DaCosta,^{*} Lindsey Hoffman, Trygve S. Aamlid, Anne Marte Tronsmo, Bruce B. Clarke, and Bingru Huang

ABSTRACT

Field trials at two locations in Norway previously demonstrated differences in winter survival between two *Agrostis* species used for turf, velvet bentgrass (VB; *A. canina* L.) and creeping bentgrass (CB; *A. stolonifera* L.). The objectives of this study were to compare freezing tolerance and crown carbohydrate composition of VB and CB. We also compared a direct and two indirect methods of measurements of freezing tolerance. Treatments consisted of: (i) nonacclimated (NA); (ii) acclimation at 2°C for 2 wk (A2); (iii) acclimation at 2°C for 4 wk (A4); and (iv) acclimation at 2°C for 4 wk plus subzero acclimation at -2°C for 2 wk (A4+SZA2). Crowns were harvested for determination of carbohydrates and freezing tolerance. Freezing tolerance (lethal temperature for 50% of the test population [LT₅₀]) was based on whole plant survival (WPS), 2,3,5-triphenyltetrazolium chloride (TTC) reduction, and electrolyte leakage (EL). There were no significant difference in freezing tolerance between VB and CB. The LT₅₀ based on WPS was significantly lower for plants exposed to A2 (-12.7°C), A4 (-14.5°C), and A4+SZA2 (-14.6°C) compared to the NA control treatment (-8.4°C). The concentrations of fructans and sucrose were significantly higher in A2 compared to NA plants of both species, but only fructans continued to increase at A4. The LT₅₀ based on TTC reduction showed better correlation with LT₅₀ based on WPS compared to LT₅₀ values based on EL.

T. Espevig and T.S. Aamlid, Norwegian Institute for Agricultural and Environmental Research, Bioforsk Landvik, Grimstad, N-4886, Norway; T. Espevig and A.M. Tronsmo, Dep. of Plant and Environmental Science, Norwegian Univ. of Life Sciences, Ås 1432, Norway; M. DaCosta and L. Hoffman, Dep. of Plant, Soil, and Insect Sciences, Univ. of Massachusetts, Amherst, MA 01003; B.B. Clarke and B. Huang, Dep. of Plant Biology and Pathology, Rutgers Univ., New Brunswick, NJ, 08901. ^{*}Corresponding author (mdacosta@psis.umass.edu).

Abbreviations: A2, acclimation at 2°C for 2 wk; A4, acclimation at 2°C for 4 wk; A4+SZA2, acclimation at 2°C for 4 wk plus subzero acclimation at -2°C for 2 wk; CB, creeping bentgrass; DW, dry weight; EL, electrolyte leakage; LT₅₀, lethal temperature for 50% of the test population; MDS, total mono- and disaccharides; NA, nonacclimated; PPFD, photosynthetic photon flux density; PVC, polyvinyl chloride; SC, total storage carbohydrates; TNC, total nonstructural carbohydrates; TTC, 2,3,5-triphenyltetrazolium chloride; VB, velvet bentgrass; WPS, whole plant survival.

AMONG *Agrostis* species used for turf, velvet bentgrass (VB; *A. canina* L.) is characterized by an extremely fine-textured and dense canopy and is reported to exhibit better tolerance to several biotic and abiotic stresses compared to creeping bentgrass (CB; *Agrostis stolonifera* L.) (Brilman, 2003; Chakraborty et al., 2006; DaCosta and Huang, 2006). Despite these desirable characteristics, VB has not been as widely utilized as CB, in part due to the limited information available on optimal management of this species (Skogley, 1976; Brilman and Meyer, 2000; Koeritz and Stier, 2009). In cultivar evaluation trials at two locations in Norway from 2003 to 2006, VB exhibited better winter survival and turf quality characteristics compared to CB (Aamlid et al., 2006; Molteberg et al., 2008). Because winter injury in field trials may

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be caused by one or more stresses (freezing temperatures, ice encasement, crown hydration, and/or low temperature fungal diseases), additional controlled environment studies are necessary to determine potential causes for differences in winter survival among VB and CB.

Freezing tolerance has been shown to be an important component of winter hardiness of perennial grasses (Humphreys and Eagles, 1988; Humphreys, 1989; Xiong and Fei, 2006; Hulke et al., 2008). Freezing tolerance of plants is significantly enhanced following a period of cold acclimation or cold hardening, whereby a number of physical, biochemical, and physiological changes contribute to enhanced cellular stability at low temperatures (Levitt, 1980; Rajashekar, 2006). Two consecutive phases of cold acclimation have been suggested in winter cereals and temperate grass species (Tumanov, 1940). The first acclimation phase occurs at temperatures above freezing (approximately 2 to 5°C) and is characterized by several changes including accumulation of antifreeze and cryoprotective proteins, carbohydrates, and other compatible solutes (Livingston, 1991; Livingston et al., 2009). The second phase of acclimation occurs at temperatures below freezing (approximately -2 to -5°C) and is commonly referred to as subzero acclimation (Tumanov, 1940). Exposure to subfreezing temperatures induces additional cellular modifications, including further dehydration of plant cells and an increase in the stability of plasma membranes, which altogether contribute to increased stability at freezing temperatures (Steponkus, 1984; Sakai and Larcher, 1987; Herman et al., 2006).

The role of carbohydrates in freezing tolerance of grasses and cereals has been extensively evaluated (Tumanov, 1940; Levitt, 1980; Livingston 1991, 1996). Soluble sugars, such as sucrose and fructans, are reported to protect plants from freeze-induced dehydration and reduce ice formation by increasing the intracellular solute concentration (Steponkus, 1984). Soluble sugars may also delay freezing by direct inhibition of ice crystal growth in the apoplast (Olien, 1967; Livingston et al., 2009). Although the importance of fructans as reserve carbohydrates in cool-season grasses is widely accepted (Pollock and Cairns, 1991), their role as cryoprotectants is somewhat controversial (Livingston and Henson, 1998). In addition to aiding in cellular stability at low temperatures, carbohydrates have also been reported to play an important role in resistance to snow molds (*Typhula incarnata*, *T. ishikariensis*, *Microdochium nivale*, and *Coprinus psychromorbidus*) in winter wheat (*Triticum aestivum* L.) (Yoshida et al., 1998) and annual bluegrass (*Poa annua* L.) (Bertrand et al., 2009).

Compared with the number of investigations in winter cereals (Olien and Clark, 1993; Livingston and Henson, 1998; Gusta et al., 1996) and forage grasses (Tronsmo et al., 1993, Hisano et al., 2004), research is limited as to the role of carbohydrates in freezing tolerance of cool-season turfgrasses. In addition, it is unclear as to the specific changes that

occur in turfgrasses during the second phase cold acclimation. Dionne et al. (2001) found that carbohydrate concentrations increased during cold acclimation for three ecotypes of annual bluegrass; however, variations in the individual carbohydrate fractions did not account for differences in freezing tolerance among the three ecotypes. Upon inspection of a larger collection of annual bluegrass ecotypes (total of 42), however, authors determined a strong correlation between the accumulation of high molecular weight fructans and freezing tolerance (Dionne et al., 2010). Hoffman et al. (2010) also recently reported that freezing tolerance of different perennial ryegrass accessions were associated with higher accumulation of water soluble carbohydrates in crowns during cold acclimation at 2°C. Additional research is necessary to evaluate carbohydrate changes of cool-season turfgrasses during the first and second phases of cold acclimation and to determine the role of carbohydrates in relation to inter- and intraspecific differences in freezing tolerance.

Freezing tolerance tested under controlled conditions can be evaluated by direct (plant survival) and indirect (e.g., electrolyte leakage) methods. Assessment of plant survival following freeze tests may take several days or weeks, and therefore indirect methods are typically faster and useful if sufficiently correlated with plant survival. Electrolyte leakage (EL) tests have been used for evaluation of freezing injury of leaves, roots, and crowns of winter cereals (Chen et al., 1983) and turfgrasses (Gusta et al., 1980; Rajashekar et al., 1983). In addition to EL, 2,3,5-triphenyltetrazolium chloride (TTC) reduction has also been used for detection of freezing injury of different plant tissues (Steponkus and Lanphear, 1967; Lindström and Mattsson, 1989). In some cases, however, the use of EL and TTC reduction methods with grass crowns were not consistently correlated with survival, possibly due to the heterogeneous structure of crown tissues (Tanino and McKersie, 1985; Shashikumar and Nus, 1993; Livingston et al., 2005). While many studies have utilized one of these methods (EL or TTC reduction), their sensitivities for estimation of LT_{50} relative to LT_{50} determined by whole plant survival (WPS) after freezing are not well documented.

Winter injury of some temperate turfgrass species can be a significant problem in northern climatic regions. Predicted future increases in temperatures may further predispose these grasses to winter injury since elevated temperatures during summer and autumn months could prolong growing seasons and negatively impact the capacity of plants to acclimate before low temperatures. Therefore, it is important to identify turfgrass species and/or cultivars that will persist under present and future climatic conditions. This will require a better understanding of how different grasses acclimate to low temperatures and how these responses can be estimated reliably and efficiently. Therefore, the objectives of this study were (i) to compare freezing tolerance of VB and CB at different stages of cold

acclimation, (ii) to quantify carbohydrates at different stages of cold acclimation and assess their relationship to changes in freezing tolerance for VB and CB, and (iii) to compare assessments of freezing tolerance of *Agrostis* using a direct and two indirect methods for estimation of LT_{50} .

MATERIALS AND METHODS

Plant Materials

Mature sods (10 cm diam. and 1 cm deep) of VB cv. Greenwich and CB cv. Penncross were taken from field plots at Rutgers University (North Brunswick, NJ) and transplanted into polyvinyl chloride (PVC) tubes (10 cm diam. and 25 cm height) filled with straight sand. The bottom of each PVC tube was covered with a nylon screen to allow for drainage. Plants were maintained in a growth chamber at 18/12°C (day/night temperatures) with a 16 h photoperiod and photosynthetic photon flux density (PPFD) of $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ for 5 wk. Pots were irrigated daily, hand clipped three times per week to 3 mm, and fertilized once per week with 100 mL of a complete nutrient solution containing $0.11 \text{ g L}^{-1} \text{ N}$, $0.02 \text{ g L}^{-1} \text{ P}$, $0.10 \text{ g L}^{-1} \text{ K}$, and micronutrients.

Treatments

The experiment consisted of four treatments representing different phases of cold acclimation: (i) nonacclimated (NA) plants maintained at 18/12°C (day/night); (ii) plants acclimated at 2°C for 2 wk (A2); (iii) plants acclimated at 2°C for 4 wk (A4); and (iv) plants acclimated at 2°C for 4 wk plus subzero acclimation at -2°C for 2 wk (A4+SZA2). For acclimation treatments at 2°C, plants were maintained in a growth chamber with a 10 h photoperiod and PPFD of $250 \mu\text{mol m}^{-2} \text{s}^{-1}$. Acclimation at -2°C was performed in darkness.

Sampling

Following each of the acclimation treatments, individual crowns were harvested (stems and roots removed) for carbohydrate analysis and determination of LT_{50} using EL and TTC reduction techniques. For determination of LT_{50} based on WPS, intact plants with leaves and roots were used. Plants subjected to A4+SZA2 were thawed overnight (24 h) at 4°C to facilitate sampling as described by Dionne et al. (2001).

LT_{50} Determined from Whole Plant Survival

For each test temperature, one replicate consisted of ten groups of four to five plants (total of 40–50 plants per replicate). These 10 plant groups were wrapped in a moistened paper towel to ensure ice nucleation and placed into a freezer bag according to the methods previously described by Ebdon et al. (2002). During harvest, the bags were temporarily stored at 4°C until all plant material had been sampled. Freezing tests were conducted using a programmable freeze chamber (ScienTemp Corp., Adrian, MI). The freezer was cooled in a stepwise fashion at a rate of 2°C h^{-1} to the desired temperature and held at the each test temperature (4, -6 , -9 , -12 , -15 , -18 , and -21°C) for 2 h. Bags were removed from the freezer after each test temperature and thawed at 4°C for a minimum of 12 h.

After thawing, tillers were replanted into cell trays filled with a commercial potting medium (Pro-Mix; Griffin

Greenhouse and Nursery Supplies, Tewksbury, MA) and placed in a greenhouse at approximately 20°C. Recovery was assessed following a 3 wk regrowth period and percent WPS was calculated as $\text{WPS (\%)} = (\text{no. plants survived}/\text{total no. plants}) \times 100$. The LT_{50} was determined mathematically by curve fitting percent survival to temperature using a four-parameter sigmoid model in Sigma Plot (SPSS, 1997) as previously described by Ebdon et al. (2002).

Carbohydrate Determination

Crowns were dried in an oven at 70°C for determination of total mono- and disaccharides (MDS) (glucose, fructose, and sucrose) and total storage carbohydrates (SC) (starch and fructans). Carbohydrate extraction was performed according to the procedure described by Fu and Dernoeden (2009) with modifications. Briefly, 50 mg of dried and ground crown tissues were extracted in a 2.0 mL microtube containing 1 mL of 92% ethanol and placed in a water bath at 80°C. Microtubes were then shaken and centrifuged at 14,000 rpm for 10 min, and the supernatant was transferred to a 10-mL test tube. The extraction was repeated a total of three times and the pooled supernatant diluted to 10 mL using distilled water. After extraction, the microtubes were placed in an oven at 70°C to evaporate any residual ethanol. The residue was saved for starch and fructan analysis.

For analysis of reducing sugars (glucose and fructose), a 0.2-mL aliquot of solution was transferred into a volumetric test tube with 0.8 mL of distilled water and 1.25 mL ferricyanide reagent as described by Ting (1956). The solution was placed in a water bath at 100°C for 10 min and then cooled to room temperature. A 2.5-mL aliquot of 1 M H_2SO_4 was added, tubes were vigorously shaken, and 1.0 mL arsenomolybdate solution was added when gas evolution ceased. Test tubes were vortexed and diluted to 10 mL with distilled water. The absorbance of the solution was measured at 515 nm using a spectrophotometer (GENESYS 2; Spectronic Instruments Inc., Thermo Electron Corp., Waltham, MA). The carbohydrate content in the solution was calculated based on reference to a glucose standard curve and expressed as grams per kilogram dry weight.

For sucrose hydrolysis, a 2-mL sample of supernatant (containing sucrose and reducing sugars) was mixed with 2 mL of 4% H_2SO_4 , vortexed, and then placed in a water bath at 100°C for 15 min. The solution was cooled and neutralized with 1.0 mL of 1 M NaOH. One milliliter of the hydrolyzed solution was transferred to volumetric test tube and 1.25 mL of ferricyanide reagent was added. This extract was used for quantification of reducing sugars as described above, and the content of sucrose was calculated as the difference between MDS and reducing sugars.

For starch and fructan analyses, 0.5 mL distilled water was added to each microtube containing the recovered residue. For quantification of starch, microtubes were sealed and placed in a water bath at 100°C for 10 min and then cooled to room temperature. To each microtube, 0.4 mL of 200 mM acetate buffer (pH 5.1) and 0.1 mL of enzyme solution were added for final enzyme concentrations of 0.2 units of amyloglucosidase (Sigma A1602; Sigma Chemicals, St. Louis, MO) and 40 units of α -amylase (Sigma A2643; Sigma Chemicals) per microtube. Tubes were then capped, vortexed, and incubated at 55°C for 16 to 24 h. At the end of the incubation period, microtubes were centrifuged at 14,000 rpm for 10 min, and the extract was

diluted 10 times using distilled water (1:10, v/v). This extract was used for quantification of reducing sugars as described above, and represented starch content.

For fructan quantification, 0.9 mL extract was added to a 2.0 mL microtube with 0.1 mL of 0.5 M H₂SO₄ and placed in a water bath at 100°C for 15 min. Microtubes were then cooled to room temperature and neutralized with 0.1 mL of 1 M NaOH. This extract was used for quantification of reducing sugars as described above, and represented total storage carbohydrate content. The content of fructans was calculated as the difference between total storage carbohydrates and starch.

LT₅₀ Determined from 2,3,5-Triphenyltetrazolium Chloride Reduction

Crown viability was estimated from measurements of dehydrogenase activity using the TTC reduction technique originally described by Knievel (1973) with modifications. Crowns (3 replicates of 10 crowns for each species × acclimation combination, except A2 due to lack of plant material) were sampled and subjected to freezing temperatures as described above. Following each of the test temperatures bags were removed from the freezer and placed at 4°C for 18 to 24 h. Crowns were transferred into test tubes (10 crowns per tube) with 6.25 mL 0.6% TTC solution in 0.05 M phosphate buffer (pH 7). Test tubes were placed at 37°C in a water bath and incubated in the dark for 24 h. Crowns were rinsed with distilled water, blotted dry, and then transferred into test tubes with 5 mL of 95% ethanol. Test tubes were incubated at 60°C in a water bath for 4 h. Following incubation, tubes were vortexed, and the absorbance of the solution was read at 490 nm using a spectrophotometer (Genesys 2; Spectronic Instruments Inc., Rochester, NY). Crowns were removed from solution and blotted dry and then placed in an oven at 70°C for 72 h. Total crown dry weight was determined individually from each tube and used for calculating TTC reduction per unit dry weight. Lastly, percent crown viability for each temperature treatment was expressed relative to TTC reduction for nonfrozen control plants (4°C). The LT₅₀ based on TTC reduction was then determined using the same curve-fitting model as described for WPS.

LT₅₀ Determined from Electrolyte Leakage

Conductivity of cell sap leaked from cells injured by low temperature was determined by methods described by Marcum (1998) with modifications. For measurement of EL, 10 crowns from each pot were wrapped in moistened paper towels and placed into freezer bags as described above. Following each of the freezing temperatures, bags (3 replicates for each species × acclimation combination, except A2 due to lack of plant material) were removed from the freezer and placed at 4°C to thaw for approximately 3 h to avoid electrolyte losses into the surrounding paper towel. Crowns were transferred into 50 mL test tubes (10 crowns per tube) with 20 mL distilled water and placed on a shaker for approximately 6 h at room temperature. The initial electrical conductivity of the solution (EC_i) was measured using a conductivity meter (Orion 3-Star; Thermo-Electron Corp., Waltham, MA). Next, crowns were autoclaved at 132°C for 20 min and placed on the shaker for approximately 16 to 20 h. The maximal electrical conductivity (EC_m) of the

solution was measured, and percent EL was determined as: EL (%) = (EC_i/EC_m) × 100. The LT₅₀ based on EL was determined using the same curve-fitting technique as described for WPS.

Experimental Design and Statistical Analyses

The experiment was conducted as completely randomized design with four replications for WPS and carbohydrate analysis and three replications for EL and TTC reduction. Statistical analyses were performed using the software packages STATISTICA (Version 6.0; StatSoft, 2001) (Hill and Lewicki, 2007) and Statistical Analysis System v. 9.1 (SAS Institute, 1990). Two species, three (LT₅₀ determined by EL and TTC reduction) or four (carbohydrates and LT₅₀ determined by WPS) acclimation treatments, and their interaction were tested as fixed effects against the pooled interaction with pot (replication) number, which was considered the only random variable in the experiment. For a comparison of LT₅₀ values estimated by WPS, TTC reduction, EL, and their interactions with species and acclimation treatments, the results were also analyzed in a three-factorial ANOVA and by simple correlation analyses. Significant differences among treatments were identified by LSD test at the 5% probability level. Although the main effect of species and the interactions between species and other experimental factors were usually not significant, results will be presented separately for VB and CB. This is due to the fact that LT₅₀ estimates for VB have not, to the best of our knowledge, been previously reported.

RESULTS

Freezing Tolerance of Velvet Bentgrass and Creeping Bentgrass

The LT₅₀ of VB and CB (as determined by WPS after 3 wk of recovery in the greenhouse) showed that the baseline freezing tolerance of NA plants was not significantly different for both species (−8.6 and −8.4°C, respectively) (Fig. 1). All cold acclimation treatments resulted in improved freezing tolerance (lower LT₅₀) compared to NA. Acclimation of plants for 4 wk at 2°C (A4) reduced the LT₅₀ to approximately −14.5°C for both species; however, subzero acclimation did not provide additional freezing tolerance compared to A4. Overall, there were no significant differences in LT₅₀ between VB and CB at any of the acclimation treatments.

Carbohydrate Changes

The major total nonstructural carbohydrates (TNC) in crown tissues of the two bentgrass species were fructans, followed by sucrose (Fig. 2). When averaged across acclimation treatments, fructans accounted for approximately 53 and 66% of TNC and sucrose accounted for 26 and 17% of TNC in crowns of VB and CB, respectively (Table 1). In general, VB exhibited 15% higher MDS content compared to CB (averaged across all treatments). In contrast, CB exhibited 55% higher SC content compared to VB.

Exposing plants to A2 resulted in significant increases in fructans and sucrose for both VB and CB compared to NA plants, whereas no significant trends were detected for

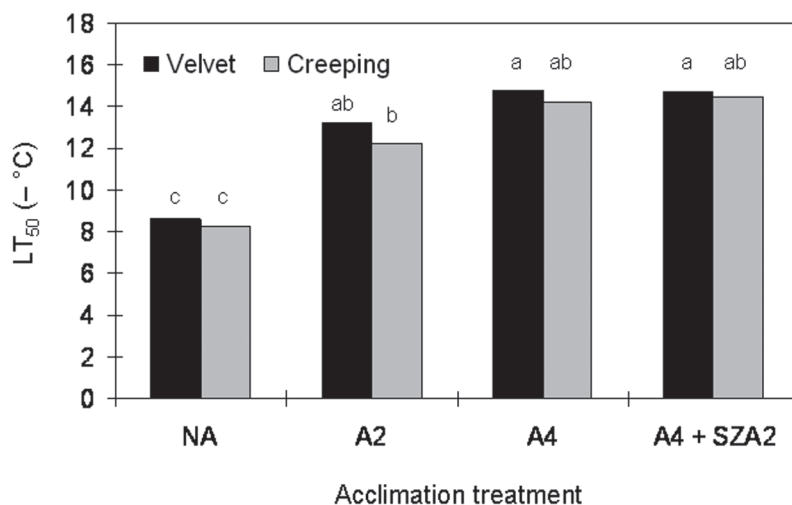


Figure 1. Effects of cold acclimation treatments on freezing tolerance of velvet bentgrass and creeping bentgrass reported as lethal temperature for 50% of the test population (LT_{50}) based on whole plant survival. Treatments consisted of nonacclimated (NA) plants, acclimation at 2°C for 2 wk (A2), acclimation at 2°C for 4 wk (A4), and acclimation at 2°C for 4 wk following by subzero acclimation at -2°C for 2 wk (A4+SZA2). Bars followed by the same letter indicate no significant differences in LT_{50} across species and treatments based on Fisher's protected LSD test ($\alpha = 0.05$).

reducing sugars and starch (Fig. 2). Although there were no significant differences in sucrose content between VB and CB following A2, CB did exhibit significantly higher accumulation of fructans (approximately 89 g kg⁻¹ dry weight [DW]) compared to VB (approximately 52 g kg⁻¹ DW). Prolonged acclimation at 2°C for 4 wk (A4) resulted in further increases in fructan content for both species, whereas an increase in sucrose content during A4 was only significant for VB. There were no significant differences in reducing sugar or starch content between the two species following A4.

Compared to A4, there was no further increase in sucrose content detected for either species after exposing plants to subzero temperatures (A4+SZA2). In addition, both species exhibited similar sucrose concentrations (approximately 29 g kg⁻¹ DW). In contrast, the fructan content in crowns of CB decreased in response to A4+SZA2 (approximately 92 g kg⁻¹ DW) compared to that observed at A4 (approximately 108 g kg⁻¹ DW). A similar trend was observed for VB, although the decrease in fructans was not statistically significant. Although there were little changes in reducing sugars and starch contents in response to A2 and A4, there was an increase in these carbohydrate fractions for both VB and CB when plants were exposed to A4+SZA2.

LT₅₀ Determined from 2,3,5-Triphenyltetrazolium Chloride Reduction and Electrolyte Leakage

On average for VB and CB, LT_{50} for the A4 and A4+SZA2 treatments estimated by TTC reduction were 4.5°C and 4.4°C lower than for the NA treatment (Table 2). Differences in LT_{50} between the two acclimation treatments A4 and A4+SZA2 and between species were not significant. A high correlation ($r = 0.97$ and $p < 0.05$) existed between LT_{50} determined by WPS and by TTC reduction, but on

average for both species the LT_{50} determined by TTC reduction was 1.1, 2.7, and 2.9°C higher than LT_{50} determined by WPS for plants acclimated at NA, A4, and A4+SZA2, respectively.

Determination of LT_{50} from EL indicated that there was no significant difference between the A4+SZA2 and NA acclimation treatments (Table 2). The highest freezing tolerance was found for plants acclimated at 2°C only (A4). On average for both species, the LT_{50} for A4 and A4+SZA2 determined by EL were 4.6 and 7.0°C higher than the corresponding values determined by WPS. The correlation between LT_{50} determined by WPS and LT_{50} determined by EL was not significant ($r = 0.34$).

The three-factorial ANOVA for comparison of LT_{50} values determined by WPS, TTC reduction, and EL and their interactions with species and acclimation treatments showed a significant interaction between method for LT_{50} determination and acclimation treatment. This can be explained by the fact that EL estimated a significantly higher LT_{50} for plants exposed to A4+SZA2 than for plants exposed to A4 only. In contrast, LT_{50} after A4 and A4+SZA2 exposure were on the same level when determined by WPS and TTC reduction.

DISCUSSION

Freezing Tolerance of Velvet Bentgrass and Creeping Bentgrass at Different Stages of Cold Acclimation

Cool-season grasses have the ability to acclimate to cold temperatures and survive freezing. Gusta et al. (1980) previously reported that LT_{50} of CB plants acclimated under field conditions were able to survive to temperatures as low as -35°C. Estimates of LT_{50} for VB have not been

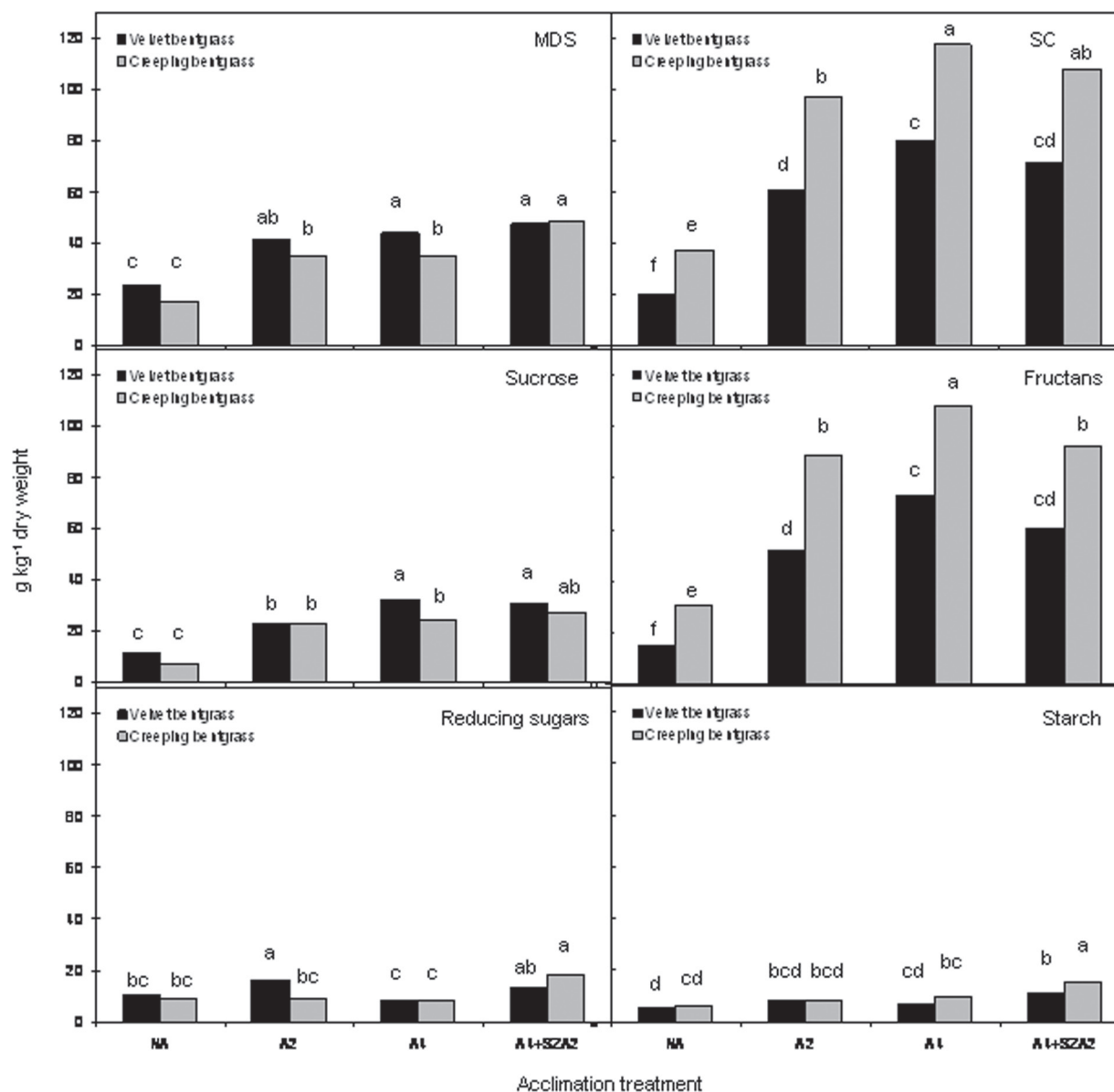


Figure 2. Effects of cold acclimation treatments on concentrations of total mono- and disaccharides (MDS), sucrose, reducing sugars (glucose + fructose), total storage carbohydrates (SC) (fructans and starch), fructans, and starch in crowns of velvet bentgrass and creeping bentgrass. Treatments consisted of nonacclimated (NA) plants, acclimation at 2°C for 2 wk (A2) or 4 wk (A4), and acclimation at 4 wk at 2°C following by 2 wk at -2°C (A4+SZA2). Bars followed by the same letter indicate no significant difference in carbohydrate content across species and treatments based on Fisher's protected LSD test ($\alpha = 0.05$).

reported, but it has been shown that various cultivars of VB (Avalon, Greenwich, Legendary, and Villa) acclimated at 2°C in a controlled environment had similar freezing tolerance as CB cv. Penn A-4 (Tronsmo et al., 2008). In the present study, there were also no significant differences freezing tolerance detected between VB cv. Greenwich and CB cv. Penncross, regardless of acclimation treatment. Taken together, these studies suggest that previously observed variability in winter survival between VB and CB under field conditions (Molteberg et al., 2008) is not necessarily due to differences in the initial rate and/or capacity to acclimate to low temperatures. Additional studies are required to identify underlying factors

affecting winter survival among these bentgrass species, including differences in overwintering physiology and deacclimation potential. Species variation in freezing tolerance may also vary with the cultivars used for comparison, and therefore additional cultivars should be compared to make further conclusions on *Agrostis* species differences in freezing tolerance. Acclimation conditions (controlled vs. natural conditions) could also be included as a factor that could affect the cold acclimation response.

Different periods of optimal duration of the first and second phase of cold acclimation in winter cereals and cool-season grasses have been reported (Olien, 1967; Andrews and Pomeroy, 1975; Dionne et al., 2001). In our study,

prolonged duration of the first acclimation phase from 2 to 4 wk significantly improved freezing tolerance (Table 2). In contrast, exposure of plants to subfreezing temperature did not improve freezing tolerance of bentgrasses in our study, which differs from results in earlier studies with winter cereals (Tumanov, 1940; Livingston, 1996) and annual bluegrass (Dionne et al., 2001). The observed differences in plant response to subzero temperatures may be due either to differences in the duration of the first acclimation phase (Vágújfalvi et al., 1999) or to a short deacclimation under crown sampling before freezing tests.

Changes in Carbohydrates

When averaged over acclimation treatments, fructans accounted for the majority of TNC in crowns of both VB (53%) and CB (66%), followed by sucrose content (26 and 17% of TNC for VB and CB, respectively). Compared to NA plants, significant increases in both fructans and sucrose were detected in response to cold acclimation treatments. In addition, significant increases in both fructans and sucrose were detected in response to cold acclimation treatments. This is in broad agreement with studies in other cool-season grasses and cereals (Livingston, 1991; Olien and Clark, 1993; Tronsmo et al., 1993; Dionne et al., 2001). There were some species differences in the accumulation of different TNC fractions. Specifically, CB exhibited significantly higher crown fructan content, regardless of acclimation treatment. It is also worth noting that VB maintained a higher ratio of MDS to SC compared to CB. While these differences had no significant impact on LT_{50} in our study (both species exhibited similar freezing tolerance), the balance of MDS and SC may be important for the overwintering ability and recuperative capacity of these species in the spring (Molteberg et al., 2008), which warrants further investigation.

Changes in different TNC fractions during the first and second phases of cold acclimation and their impact on freezing tolerance have been discussed by Levitt (1980) and Rajashekar (2006). In our study, the increases in fructans and sucrose in response to different acclimation treatments were accompanied by increases in freezing tolerance for both species, suggesting a central role for these carbohydrates in cellular stability. A potential role of fructans as inhibitors of ice crystal formation was described by Olien (1967), but their role as cryoprotectants has been controversial since they may be hydrolyzed during the second acclimation phase (Olien and Clark, 1993; Livingston, 1996). More recently, studies have shown that fructans can directly interact with cell membranes and improve membrane stability during dehydration-related stresses (Hinch et al., 2002; Valluru and Van den Ende, 2008), and a role for fructans in oxidative stress defense has also been proposed (Parvanova et al., 2004). In addition, sucrose has been identified as an important cryoprotectant that prevents desiccation and inhibits liposome fusion during freezing (Steponkus, 1984; Ancho doguy et al., 1987).

Table 1. Comparison of carbohydrate concentrations for velvet bentgrass and creeping bentgrass averaged over acclimation treatments.

Species	MDS [†]	Sucrose	Reducing	SC [‡]	Fructans	Starch
			sugars			
—g kg ⁻¹ dry wt.—						
Velvet	36.3 a [§]	24.1 a	12.2 a	57.9 b	50.1 b	7.8 a
Creeping	31.5 b	20.2 b	11.3 a	89.9 a	80.1 a	9.8 a

[†]MDS, total mono- and disaccharides (glucose, fructose, and sucrose).

[‡]SC, total storage carbohydrates (fructans and starch).

[§]Means within columns followed by the same letter indicate no significant difference based on Fisher's protected LSD test ($\alpha = 0.05$).

Table 2. Mean lethal temperature for 50% of the test population (LT_{50}) averaged for velvet bentgrass and creeping bentgrass as determined by whole plant survival (WPS), 2,3,5-triphenyltetrazolium chloride (TTC) reduction, and electrolyte leakage (EL) from crowns. Treatments consisted of nonacclimated (NA) plants, acclimation at 2°C for 2 wk (A2), acclimation at 2°C for 4 wk (A4), and acclimation at 2°C for 4 wk following by subzero acclimation at -2°C for 2 wk (A4+SZA2).

Acclimation treatment [†]	WPS	TTC reduction	EL
	—°C—		
NA	-8.4 c [†]	-7.3 b	-7.9 b
A2	-12.7 b	—	—
A4	-14.5 a	-11.8 a	-9.9 a
A4+SZA2	-14.6 a	-11.7 a	-7.6 b

[†]Means within columns followed by the same letter indicate no significant difference based on Fisher's protected LSD test ($\alpha = 0.05$).

As with fructans, there is increasing evidence on the role of sucrose in coordinating plant responses to oxidative stress (Van den Ende and Valluru, 2009).

A decrease in fructan and concomitant increase in sucrose, glucose, and fructose during acclimation at subzero temperature was previously shown for barley (*Hordeum vulgare* L.), oat (*Avena sativa* L.) (Livingston, 1996), and annual bluegrass (Dionne et al., 2001). In our study, the decrease in fructans after A4+SZA2 occurred in both bentgrass species, but there was no increase in sucrose content detected. Compared to A4, the concentrations of reducing sugars and starch were enhanced by exposure to A4+SZA2; however, these changes did not influence LT_{50} for either species.

LT_{50} determined from 2,3,5-Triphenyltetrazolium Chloride Reduction and Electrolyte Leakage

Living plant cells have the capacity to reduce TTC to formazan by the dehydrogenase enzyme system. Therefore, staining by TTC followed by extraction of formazan by ethanol is an easy and widespread method to estimate injury of plant tissues (Knievel, 1973; Rachmilevitch et al., 2006). The TTC method has been used for estimation of cold injury of leaves and stems of *Hedera helix* L. (English ivy) (Steponkus and Lanphear, 1967), roots of Norway spruce [*Picea abies* (L.) H. Karst.] seedlings (Lindström and Mattsson, 1989), and cultured cells from alpine Blue

mustard (*Choripora bungeana* Fisch. & C.A. Mey) (Guo et al., 2006). There are also some studies on use of TTC reduction for evaluation of freezing injury within crowns of perennial grasses or cereals (Tanino and McKersie, 1985; Shashikumar and Nus, 1993). While crown survival is crucial for the survival of the grass plant, difficulties with the use of TTC reduction on crowns have been related to the heterogeneity of crown tissue. For example, the apical meristem (upper region) and vascular transition zone (lower region) have been shown to respond differently to freezing in winter wheat (Tanino and McKersie, 1985). Plant recovery after freezing depended on survival of both regions but was more limited by the viability of the vascular transition zone in acclimated plants and by the apical meristem in unacclimated plants.

Results from our study indicated that TTC reduction slightly underestimated freezing tolerance, as shown by the higher LT_{50} determined by this method than by WPS (Table 2). This might reflect that the meristematic crown region was small compared to surrounding tissues and that much of the tissue that was injured by frost was probably nonmeristematic and thus not responsible for recovery. Consequently, it is possible that the underestimation of survival could reduce the sensitivity of detecting genotypic differences when the range of LT_{50} values is small. Nonetheless, the correlation between LT_{50} values estimated by TTC reduction and those estimated by WPS was very high ($r = 0.97$ and $p < 0.05$), which suggests that the TTC technique is sensitive enough to detect treatment and/or genotype differences over a larger range of LT_{50} values. This method should be further explored as a rapid method for screening freezing tolerance of grasses.

Determination of EL from plant tissues after freezing has been used as a test for hardiness in cool-season turfgrasses due to its correlation with whole plant survival (Gusta et al., 1980; Rajashekhar et al., 1983). However, some investigators reported that EL overestimated freezing tolerance (Chen et al., 1983; Maier et al., 1994), while others reported that EL underestimated actual freezing survival (Ebdon et al., 2002). In our study the LT_{50} values determined by EL underestimated freezing survival and were not significantly correlated with those determined by WPS ($r = 0.34$). In addition, plants exposed to A4+SZA2 exhibited higher EL compared to A4 plants (suggesting lower freezing tolerance). Palta et al. (1977) reported that EL from onion (*Allium cepa* L.) cells frozen to -4 and -11°C were significantly higher immediately after thawing compared to EL from the nonfrozen control, even though all frozen cells were later found to be alive and not injured by freezing. Later Palta and Li (1980) suggested that enhanced EL from frozen cells could be due to membrane rupture during freezing injury or to loss of membrane semipermeability. Therefore, a possible explanation for the higher ion leakage from A4+SZA2 plants could be that subzero acclimation

or the subsequent thawing and handling of crowns during harvest may have caused some membrane injury without impacting plant regrowth capacity.

CONCLUSIONS

This investigation demonstrated that VB and CB did not vary in freezing tolerance (LT_{50}) in response to different stages of cold acclimation. Acclimation at temperatures above 0°C was accompanied by an accumulation of TNC, particularly fructans and sucrose, and was associated with enhanced plant freezing tolerance. Acclimation at 2°C for 4 wk was more effective than for 2 wk and resulted in higher concentrations of fructans. Additional subzero acclimation at -2°C resulted in a reduction in fructans and higher concentration of reducing sugars; however, there was no additional improvement in freezing tolerance. Crown viability determined by reduction of TTC showed close correlation with LT_{50} values based on whole plant survival, while EL of crowns produced less consistent results.

Acknowledgments

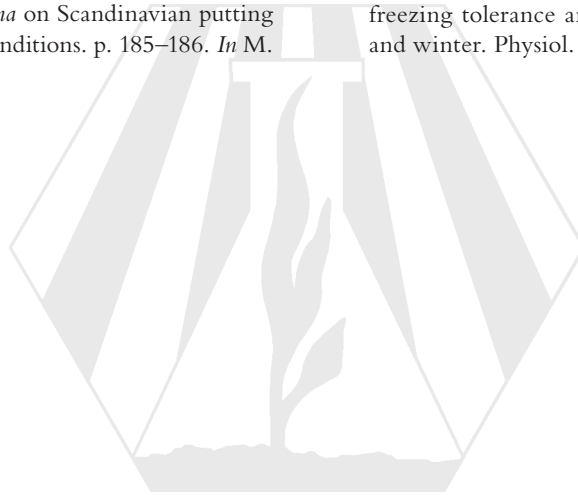
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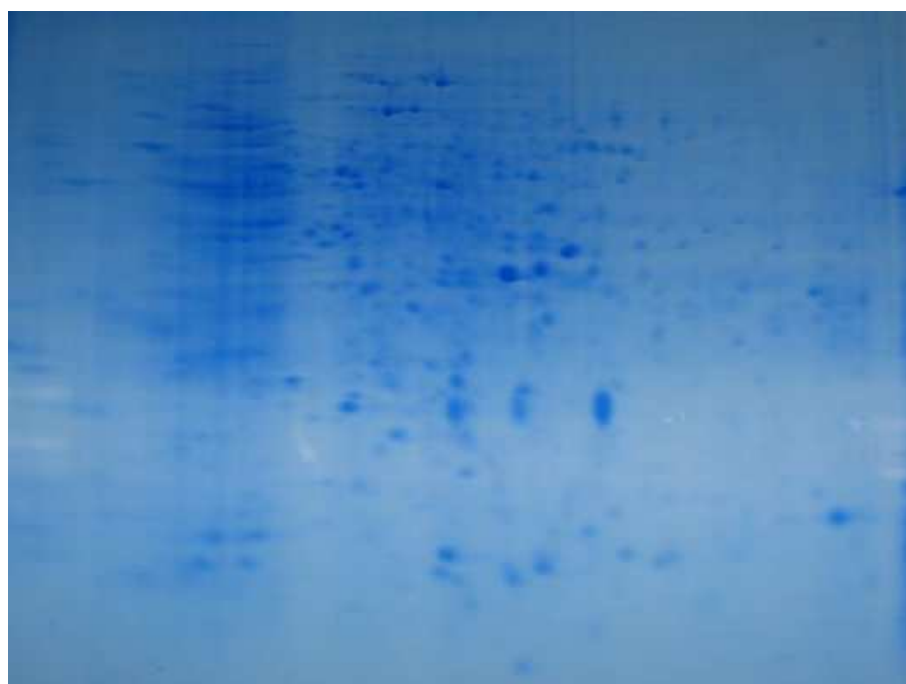
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PAPER II



Proteomic analysis of velvet bentgrass in response to cold acclimation

Tatsiana Espevig, Chenping Xu, Michelle DaCosta, Trygve S. Aamlid,
and Bingru Huang*

T.Espevig and T.S. Aamlid, Norwegian Institute for Agricultural and Environmental Research, Bioforsk Landvik, Grimstad, N-4886, Norway; T. Espevig, Dept. of Plant and Environmental Science, Norwegian Univ. of Life Sciences, Ås 1432, Norway; M. DaCosta, Dept. of Plant, Soil, and Insect Sciences, Univ. of Massachusetts, Amherst, MA 01003, USA; C. Xu and B. Huang, Dept. of Plant Biology and Pathology, Rutgers Univ., New Brunswick, NJ, 08901, USA.

ABSTRACT

Field trials at two locations in Norway previously demonstrated superior winter survival of velvet bentgrass (*Agrostis canina* L.). The objectives of this study were to determine protein changes in crowns of velvet bentgrass during the first and second stages of cold acclimation and to define their potential role in freezing tolerance of this species. Treatments consisted of: (i) nonacclimated (NA) plants maintained at 18/12°C (day/night); (ii) plants acclimated at a constant 2°C for 4 wk with a 10 h photoperiod (A4); and (iii) plants acclimated at a constant 2°C for 4 wk with an additional sub-zero acclimation at a constant -2°C for 2 wk in darkness (A4+SZA2). Acclimation of plants for 4 wk at 2°C (A4) significantly increased freezing tolerance, but additional SZA had no further effect. Nineteen protein spots differentially expressed by acclimation were chosen for identification and 13 of them were identified. Among proteins up-regulated after A4 were methionine synthase, serine hydromethyltransferase, aconitase, UDP-D-glucuronate decarboxylase, and putative glycine rich protein. Rubisco large and small subunit, glyceraldehyde-3-phosphate dehydrogenase, and putative peroxidase were down-regulated. Differences in protein composition were more pronounced between NA and A4 than between A4 and A4+SZA.

Abbreviations: LT₅₀, lethal temperature for 50% of the test population; SZA, sub-zero acclimation.

*Corresponding author (huang@AESOP.Rutgers.edu)

INTRODUCTION

Among turfgrass species, velvet bentgrass (*Agrostis canina* L.) is characterized by an extremely fine-textured and dense canopy, and it is reported to have lower water and pesticide requirements and good tolerance to several biotic and abiotic stresses (Brilman, 2003; Chakraborty et al., 2006; DaCosta and Huang, 2006; Murphy et al., 2009). The use of velvet bentgrass on golf courses in Europe and North America is, nonetheless, limited due to scarce information on its optimal maintenance requirements (Skogley, 1976; Koeritz and Stier, 2009). The use of the species in boreal and temperate regions also requires knowledge on its winter survival, but only few data are currently available regarding winter hardiness of velvet bentgrass. In cultivar evaluation trials at two locations in Norway from 2003 to 2006, velvet bentgrass exhibited better winter survival and turf quality characteristics compared with other turfgrass species (Aamlid et al., 2006). This suggests the potential use of this species as a low input alternative for northern climates.

Winter injury in field environments may be caused by one or multiple stresses, some of which include direct low temperature kill, ice encasement, hypoxia, crown hydration, and/or low temperature fungal diseases (Humphreys, 1989; Bertrand et al., 2009; Castonguay et al., 2009). Although specific resistance strategies for each of these stresses may differ, it has been demonstrated that freezing tolerance is a major component of winter hardiness of perennial grasses (Humphreys and Eagles, 1988; Xiong and Fei, 2006). The development of freezing tolerance is dependent on a period of cold acclimation, with two consecutive stages of cold acclimation suggested in winter cereals and temperate grass species (Tumanov, 1940). The first acclimation stage occurs at temperatures above freezing (approximately 2 to 5 °C) and contributes to accumulation of cryoprotectants, particularly osmolytes and antifreeze proteins, reserve carbohydrates, alterations in phospholipid and fatty acid composition, which enhance cellular stability when freezing occurs (Levitt, 1980; Livingston, 1991; Tronsmo et al., 1993; Guy, 1999; Thomashow, 1999; Dionne et al., 2001a, 2001b; Rajashekar, 2006; Hoffman et al., 2010). Recently, we demonstrated that acclimation at 2°C for 2 and 4 wk enhanced freezing tolerance of velvet bentgrass and was associated with 2-fold and 3-fold increase in sucrose and 4-fold and 5-fold increase in fructans, respectively, compared with non-acclimated plants (Espevig et al., Paper 1).

There are significant changes in protein metabolism in response to low temperatures, which include selective up- and down-regulation of protein synthesis as well as modifications of existing proteins. Transcriptome and proteome analyses have indicated considerable

similarity across species for induction of cold-responsive proteins, including late embryogenesis abundant (LEA) proteins, antifreeze proteins (AFP), heat shock proteins (HSP), and detoxification enzymes (Zhang et al., 2009; Janská et al., 2010). Although the functions of many cold-responsive proteins are unknown, these proteins can generally be classified as serving cryoprotective or antifreeze functions (Guy et al., 1999; Thomashow, 1999; Griffith and Yaish, 2004). For example, an increase in freezing tolerance due to accumulation of dehydrins (LEA D-11 family proteins) has been attributed to their function as chaperones by protecting proteins from denaturation and aggregation as well as stabilization of membranes, which would be important under conditions of freeze-induced dehydration stress (Close, 1996). In addition to dehydrins, the accumulation of AFP can improve freezing tolerance by adhering to the surface of ice crystals and inhibiting their growth through thermal hysteresis (Duman and Olsen, 1993; Griffith et al., 1997). Antifreeze proteins may also inhibit ice re-crystallization (Sandve et al., 2008) and protect thylakoid membranes against freeze-thaw damage (Sieg et al., 1996). As a result, the synthesis and expression of these proteins is more pronounced in acclimated and freezing tolerant species than in freezing sensitive species (Perras and Sarham, 1989; Dionne, 2001b; Puhakainen et al., 2004; Patton et al., 2007).

The second stage of acclimation occurs at temperatures below freezing (-2 to -5°C). It is commonly referred to as sub-zero acclimation (SZA) and leads to acquisition of additional freezing tolerance (Tumanov, 1940; Livingston, 1996; Herman et al., 2006). Exposure to sub-freezing temperatures is commonly associated with induced ice formation in the apoplast and dehydration of plant cells (Steponkus, 1989). The high intracellular concentration of solutes along with its increase during desiccation will reduce initial and further loss of intracellular water, respectively, and prevent ice formation inside cells (Herman et al., 2006). Winter wheat (*Triticum aestivum* L.) was shown to undergo the second acclimation stage during a shorter period of time compared with the first stage (Tumanov, 1940). Herman et al. (2006) demonstrated that morphological changes in crowns of winter wheat occurred already after a few hours of exposure to SZA, while maximal acclimation was achieved after 3 days. In contrast, maximal freezing tolerance in annual blue grass (*Poa annua* L.) was acquired after 2 wk of SZA following 2 wk of acclimation at 2°C (Dionne et al., 2001a).

Experimental evidence regarding structural, biochemical, and metabolic changes during SZA in winter cereals are restricted to few studies (Livingstone, 1996; Herman et al., 2006) and in perennial grasses is limited. Many studies have been carried out with *Arabidopsis thaliana* (Jaglo-Ottosen et al., 1998; Le et al., 2008), but this species is not necessarily representative for cold-induced responses at the molecular, cellular, or whole-plant level in

perennial grasses (Livingston et al., 2007). Mechanisms underlying different aspects of winter hardiness in perennial grasses can be revealed using novel genomic and proteomics approaches. Thus, the identification of cold-regulated proteins may provide more information about numerous metabolic processes and their activity under cold acclimation. The objectives of our study were to determine protein changes under the first and the second stages of cold acclimation and define their potential role in freezing tolerance of velvet bentgrass.

MATERIALS AND METHODS

Plant Material and Growing Conditions

Detailed information regarding plant material and experimental procedures were described previously (Espevig et al., Paper 1). Briefly, mature sods of velvet bentgrass cv. Greenwich were taken from field plots at Rutgers University (North Brunswick, NJ, USA), and transplanted into polyvinyl chloride (PVC) tubes filled with sand. Plants were maintained in a growth chamber at 18/12°C (day/night temperatures) with a 16-h photoperiod and photosynthetic photon flux density (PPFD) of 500 $\mu\text{mol m}^{-2}\text{s}^{-1}$ for 5 wk. Pots were irrigated daily, hand-clipped 3 times per wk to 3 mm, and fertilized once per wk with 100 mL of a complete nutrient solution containing 0.11 g L⁻¹ nitrogen, 0.02 g L⁻¹ phosphorus, 0.10 g L⁻¹ potassium and micronutrients.

Treatments

The experiment consisted of three treatments: (i) non-acclimated (NA) plants maintained at 18/12°C (day/night) with a 10-h photoperiod and PPFD of 500 $\mu\text{mol m}^{-2}\text{s}^{-1}$; (ii) plants acclimated at a constant 2°C for 4 wk with a 10-h photoperiod and PPFD of 250 $\mu\text{mol m}^{-2}\text{s}^{-1}$ (A4); and (iii) plants acclimated at a constant 2°C for 4 wk with an additional sub-zero acclimation at a constant -2°C for 2 wk in darkness (A4+SZA2).

Determination of Freezing Tolerance

Following each acclimation treatment, intact plants with leaves and roots were used for determination of freezing tolerance based on whole plant survival. Plants acclimated at -2°C (A4+SZA2) were thawed overnight (24 h) at 4°C to facilitate sampling as described by Dionne et al. (2001a). For each test temperature, 10 plant groups (4-5 plants per group) were

washed for each of 4 replicates, wrapped in a moistened paper towel to ensure ice nucleation, and placed into a freezer bag according to the methods previously described by Espevig et al. (Paper 1). During harvest, the bags were temporarily stored at 4°C until all plant material had been sampled. Freezing tests were conducted using a programmable freeze chamber (ScienTemp Corp., Adrian, MI). The freezer was cooled in a stepwise fashion at a rate of 2°C h⁻¹ to the desired temperature and held at the each test temperature (4, -6, -9, -12, -15, -18, and -21°C) for 2 h. Bags were removed from the freezer after each test temperature and thawed at 4°C for a minimum of 12 h.

After thawing, tillers were re-planted into cell trays filled with a commercial potting medium (Pro-Mix, Griffin Greenhouse and Nursery Supplies, Tewksbury, MA) and placed in a greenhouse at approximately 20°C. Recovery was assessed following a 3 wk regrowth period and percent whole plant survival (WPS) was calculated as: $WPS (\%) = (\text{no. plants survived} / \text{total no. plants}) \times 100$. The lethal temperature for 50 % of the test population (LT₅₀) was determined mathematically by curve fitting percent survival to temperature using a four-parameter sigmoid model (Sigma Plot, SPSS, Chicago, IL) as previously described by Ebdon et al. (2002).

Proteomic analysis

Following each acclimation treatments, approximately 0.5 g (fresh weight) of crown tissues (stems and roots were removed) were harvested for each of 3 replicates, immediately frozen in liquid nitrogen, and stored at -80°C for protein analysis.

Protein extraction was performed according to the trichloroacetic acid (TCA)/acetone method described by Xu et al. (2008). Briefly, crowns were homogenized with liquid nitrogen and incubated with 10 mL of precipitation solution (10% TCA and 0.07% 2-mercaptoethanol in acetone) overnight at -20°C. After centrifugation at 10,000 rpm for 15 min at 4°C, the pellets were rinsed twice with ice-cold 0.07% 2-mercaptoethanol in acetone to remove pigments and lipids, then vacuum-dried, resuspended in rehydration solution (8 M urea, 2 M thiourea, 2% CHAPS, 1% dithiothreitol (DTT), and 1% pharmalyte), and sonicated to provide transition of proteins from the pellet to the solution. After centrifugation at 10,000 rpm for 15 min at 4°C, the supernatants were removed, and protein concentrations were determined according to Bradford (1976) using a commercial dye reagent (Bio-Rad Laboratories, Hercules, CA) with bovine serum albumen as a standard.

First-dimension (isoelectric focusing, IEF) and second-dimension (sodium dodecyl sulphate-polyacrilamide gel electrophoresis, SDS-PAGE) separation of proteins was performed according to a procedure described by Xu et al. (2008). Briefly, immobilized pH gradient (IPG) strips (pH 3.0-10.0, linear gradient, 13 cm) were filled with 250 μ L rehydration buffer (8 M urea, 2 M thiourea, 2 % CHAPS, 1% DTT, 1% pharmalyte, and 0.002% bromophenol blue) containing 200 μ g of proteins, and rehydrated at room temperature in IPGPhor apparatus (GE Healthcare, Piscataway, NJ) at 50 V for 12 h. Following IEF for a total 94.5 kVh, IPG strips were denatured with 10 mL of equilibration buffer (50 mM tris-HCl pH 8.8, 6 M urea, 30% glycerol, 2% sodium dodecyl sulphate, and 0.002% bromophenol blue) containing 1% DTT for 20 min. Then the strips were incubated twice with 10 mL of the same buffer containing 2.5% iodoacetamide for 15 min. The second-dimension electrophoresis was performed on 12.5% SDS-polyacrylamide gel using a Hoefer SE 600 Ruby electrophoresis unit (GE Healthcare, Piscataway, NJ). The gels were stained with coomassie brilliant blue-250 (Newsholme et al., 2000) and scanned using a Personal Densitometer (GE Healthcare, Piscataway, NJ). Image analysis was performed using Progenesis SameSpots software (Nonlinear Dynamics, Durham, NC) including ANOVA.

Protein digestion and identification were performed as described previously (Xu and Huang, 2008). The gel spots were excised and washed with 30% acetonitrile in 50 mM ammonium bicarbonate prior to DTT reduction and iodoacetamide alkylation. Trypsin was used for digestion at 37 °C overnight. The resulting peptides were extracted with 30 μ l of 1% trifluoroacetic acid followed by C₁₈ Ziptip desalting. For the MS analysis, the peptides were mixed with 7 mg mL⁻¹ α -cyano-4-hydroxy-cinnamic acid matrix in a 1:1 ratio and spotted onto a matrix assisted laser desorption/ionization (MALDI) plate. The peptides were analyzed on a 4800 MALDI TOF/TOF analyzer (Applied Biosystem, Framingham, MA). Mass spectra (m/z 880-3,200) were acquired in positive ion reflector mode. Twenty-five most intense ions were selected for subsequent MS/MS sequencing analysis in 1 kV mode. Protein identification was performed by searching the combined MS and MS/MS spectra against the green plant NCBI database using a local MASCOT search engine (V.1.9) on a GPS (V. 3.5, ABI) server. A protein containing at least two unique peptides with Confidence Interval (C. I.) values no less than 95% was considered being identified.

Experimental Design and Statistical Analyses

The experiment was conducted as completely randomized design with four replications for LT₅₀ based on whole plant survival and three replications for protein analysis. The ANOVA for LT₅₀ was performed using the software packages STATISTICA (Version 6.0, 2001) (Hill and Lewicki, 2007). Significant differences among treatments were identified by Fisher's least significant difference (LSD) test at the 5 % probability level.

RESULTS

Freezing Tolerance of velvet bentgrass

Changes in freezing tolerance (LT₅₀) of velvet bentgrass determined by whole plant survival is shown in Table 1. Acclimation of plants for 4 wk at 2 °C (A4) resulted in higher freezing tolerance (LT₅₀ of -14.5 °C) compared to NA plants (LT₅₀ of -8.6°C). Additional acclimation at sub-zero temperature (SZA) did not result in enhanced freezing tolerance since the LT₅₀ after A4 and A4+SZA were not significantly different.

Table 1. Effect of acclimation on freezing tolerance of velvet bentgrass expressed as mean lethal temperatures for 50% of test population (LT₅₀). Abbreviations for treatments: NA, non-acclimated plants; A4, acclimation at 2°C for 4 wk; and A4+SZA2, acclimation at 2°C for 4 wk followed by sub-zero acclimation at -2°C for 2 wk.

Acclimation treatment	LT ₅₀ °C
NA	-8.6 b†
A4	-14.8 a
A4+SZA	-14.7 a
<i>p</i>	< 0.001

§ The same letter indicates no significant difference based on Fisher's protected LSD test.

Proteomic responses to cold acclimation

Among 375 separated protein spots, approximately 86 spots exhibited response to at least one acclimation treatment. Nineteen protein spots differentially expressed by acclimation were chosen for identification and 13 of them were identified (Fig. 1). The identified proteins were

divided into the functional categories described by Bevan et al. (1998) (Table 2). The expression of the six non-identified proteins is shown in Figure 2.

The proteins belonging to the category ‘metabolism’ were methionine synthase (spots 1-3) and serine hydroxymethyltransferase (spot 4). They were up-regulated by A4 and A4+SZA, but differences between the two acclimation treatments were non-significant.

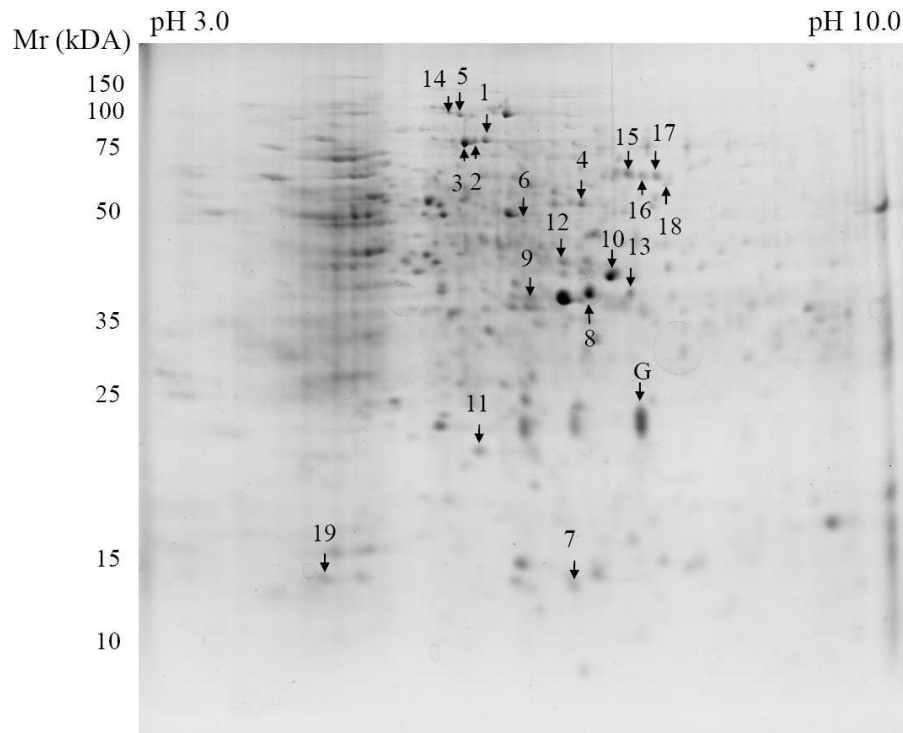
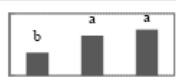
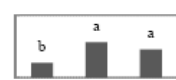

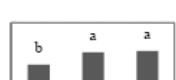
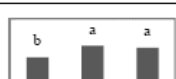


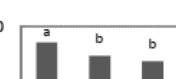
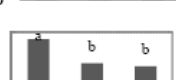
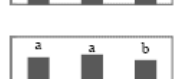
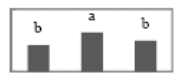
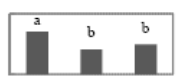
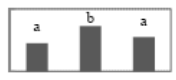


Figure 1. Coomassie stained 2D-PAGE gel of separated proteins from crowns of velvet bentgrass acclimated at 2°C for 4 wk following by sub-zero acclimation at -2°C for 2 wk. The numbered spots were affected by acclimation treatments. Germin (G) was identified but was not regulated by acclimation.

Some proteins from the category ‘energy’ showed different responses to acclimation treatments. Aconitase (spot 5) was up-regulated to similar level by A4 and A4+SZA acclimation. Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) large and small subunits (spots 6 and 7, respectively) and glyceraldehydes-3-phosphate dehydrogenase (spots 8 and 9) were down-regulated to similar level by A4 and A4+SZA acclimation. Fructose-bisphosphate aldolase (spot 10) was down regulated only under the A4+SZA treatment. A putative glycine-rich protein belonging to the category ‘disease/defense’ was up-regulated by A4, but its production after SZA returned to the same level as in non-acclimated plants. The initially high level of a putative peroxidase in non-acclimated crowns was reduced after A4 and A4+SZA to a similar level.

Table 2. Cold-regulated proteins from crowns of velvet bentgrass divided into functional groups according to Bevan et al. (1998). Abbreviations for treatments: NA, non-acclimated plants; A4, acclimation at 2°C for 4 wk; and A4+SZ A2, acclimation at 2°C for 4 wk followed by sub-zero acclimation at -2°C for 2 wk. The same letter indicates no significant difference based on Fisher's protected LSD test.

Spot nr.	Protein name [species]	Accession nr.	Protein Score	Total Ion Score	Theoretical protein MW, kDa	Theoretical protein pI	Average normalised volumes		
							NA	A4	A4+SZ A
Functional category 01: Metabolism									
1	Methionine synthase [<i>Hordeum vulgare</i>]	gi 8134570	304	274	84.8	6.1	15000		
2	Methionine synthase [<i>Hordeum vulgare</i>]	gi 8134570	340	303	84.8	6.1	15000		
3	Methionine synthase [<i>Hordeum vulgare</i>]	gi 50897038	1.13	989	84.5	5.68	50000		
4	Serine hydroxymethyltransferase [<i>Arabidopsis thaliana</i>]	gi 11762130	235	203	51.8	7.12	15000		
Functional category 02: Energy									
5	Aconitase [<i>Lycopersicon pennellii</i>]	gi 29027432	254	225	98.1	6.07	15000		
6	Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) large subunit [<i>Agrostis capillaris</i>]	gi 41056380	799	632	51.8	6.13	50000		
7	Rubisco small subunit [<i>Avena clauda</i>]	gi 6573202	610	486	18.8	8.6	50000		
8	Glyceraldehyde-3-phosphate dehydrogenase, cytosolic [<i>Oryza sativa subsp. indica</i>]	gi 120680	316	242	36.5	6.67	170000		
9	Glyceraldehyde-3-phosphate dehydrogenase, cytosolic [<i>Oryza sativa subsp. indica</i>]	gi 120668	309	257	33.2	6.2	50000		
10	Fructose-bisphosphate aldolase, cytosolic [<i>Oryza sativa subsp. japonica</i>]	gi 218157	555	457	38.7	6.56	70000		
Functional category 11: Disease / defence									
11	Putative glycine-rich protein [<i>Triticum aestivum</i>]	gi 40363759	90	82	19.2	5.63	25000		
12	Putative peroxidase [<i>Oryza sativa subsp. japonica</i>]	gi 115436084	124	116	37.9	6.46	25000		
Functional category 20: Secondary metabolism									
13	UDP-D-glucuronate decarboxylase [<i>Hordeum vulgare</i>]	gi 50659026	411	344	38.9	7.1	30000		

Within the category ‘secondary metabolism’, the enzyme UDP-D-glucuronate decarboxylase was up-regulated by A4, but its level decreased to the same level as in non-acclimated crowns after 2 wk sub-zero acclimation.

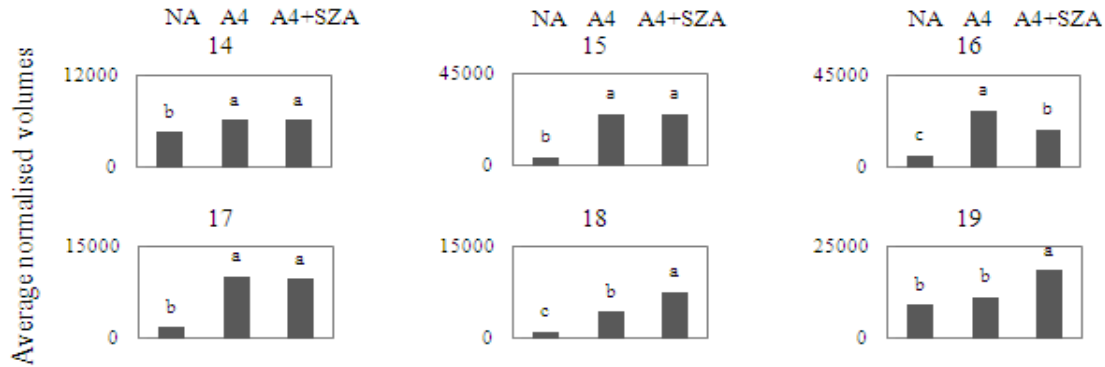


Figure 2. The expression of unidentified cold-regulated proteins from crowns of velvet bentgrass. Abbreviations for treatments: NA, non-acclimated plants; A4, acclimation at 2°C for 4 wk; and A4+SZA2, acclimation at 2°C for 4 wk followed by sub-zero acclimation at -2°C for 2 wk. The same letter indicates no significant difference based on Fisher’s protected LSD test.

DISCUSSION

Acclimation of plants for 4 wk at 2 °C (A4) significantly increased freezing tolerance, but the following sub-zero acclimation did not provide additional freezing tolerance. In contrast, sub-zero acclimation was shown to improve freezing tolerance in cereals and grasses as compared with acclimation at low non-freezing temperatures only (Tumanov, 1940; Livingston, 1996; Dionne et al., 2001a; Herman et al., 2006). The sufficiency of artificial acclimation depends on light intensity, temperature, day length (Huner et al., 1993; Tepperman et al., 2001), and duration of the first (Veisz and Sutka, 1989; Vágújfalvi et al., 1999) and second (Herman et al., 2006) stages of cold acclimation. As previously discussed by Espevig et al. (Paper 1), the negligible effect of SZA in our study could be due to the long duration of the first or second acclimation stages. Thus, Veisz and Sutka (1989) and Vágújfalvi et al. (1999) demonstrated a negative effect of prolongation of the first stage of acclimation on freezing tolerance in wheat (from 7 to 8 weeks and from 5 to 6 weeks, respectively). Dionne et al. (2001a) found no effect of prolongation of acclimation at 2°C (light) from 2 to 4 weeks in annual bluegrass. However, we previously showed a significantly better freezing tolerance after 4 than after 2 weeks of hardening at 2°C in velvet bentgrass (Espevig et al., Paper 1). Herman et al. (2006), for instance, reported negative effect of prolonged subzero acclimation. The investigators

reported that acclimation of winter wheat crowns at -3°C under controlled conditions for periods longer than 3 d led to reduced survival after freezing. But it appears that negligible effect of subzero acclimation in our study could be due to the effect of subzero acclimation was not sufficiently expressed as plants were exposed to each freezing temperature only for 2 h. Dionne et al. (2001a) found an additional effect of subzero hardening even with only 1 h exposure to various freezing temperatures in annual bluegrass, but this situation may well be different in a more winter-hardy species such as velvet bentgrass.

Results showed that both acclimation treatments led to quantitative changes in crown protein content. Currently, there are very few reports on protein changes in crown tissue in grasses in response to cold acclimation. Similar with cold acclimated wheat (Herman et al., 2006), differences in protein expression in crown tissue between non-acclimated plants and plants exposed to the first acclimation stage (A4) were more pronounced than differences between plants exposed to A4 and A4+SZA. The cold-responsive proteins identified in our study had metabolic, energy, and defense functions.

Metabolism. Quantitative changes in amino acids in plants in response to cold acclimation have been previously reported, but their role in freezing tolerance remains unclear (Naidu et al., 1991; Dionne et al., 2001b). In our study, both serine hydroxymethyltransferase and methionine synthase involved in amino acid synthesis were up-regulated. The major reaction catalyzed by serine hydroxymethyltransferase is the interconversion of serine and glycine resulting in generation of one-carbon unit for the biosynthesis of many organic compounds, including nucleotides, methionine, thymidylate, choline, etc. (Schirch and Szebenyi, 2005). Methionine synthase catalyzes the terminal step of methionine biosynthesis, notably the transfer of a methyl group from N^5 -methyl-tetrahydrofolate to homocysteine (Ravanel et al., 1998). Up-regulation of both enzymes by cold acclimation suggests an increase in the formation of amino acids including methionine, serine, and glycine. Naidu et al. (1991) reported higher levels of glycine in leaves of winter wheat after 5 d at 4°C , but no changes in serine and methionine. The possible increase in methionine in our study might refer to a general increase in protein synthesis since the formation of a peptide chain always begins from methionine. Methionine is also involved in ethylene and polyamines biosynthesis (Wang et al., 2002). Ethylene plays a role in tolerance to various biotic and abiotic stresses. Heat (Hays et al., 2007), drought (Apelbaum and Yang, 1981), and hypoxia (Huang et al., 1997) were shown to induce production of ethylene. Ethylene was reported to increase protein production in the apoplast and induce antifreeze activity in non-acclimated winter rye leaves

(Yu et al., 2001). Macháčková et al. (1989), however, reported a decrease in ethylene production at low temperature.

Energy. Since photosynthesis and respiration are temperature dependent processes (Huner et al., 1993; Guy, 1999; Klimov, 2009), significant changes in proteins, particularly, enzymes involved in carbon metabolism in response to cold acclimation have been reported by many investigators (Herman et al., 2006; Rapacz et al., 2008; Kosmala et al., 2009). Similar to our results, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) production was down-regulated during cold acclimation in rice (*Oryza sativa* L.) (Hahn and Walbot, 1989) and winter wheat (Herman et al., 2006). Moreover, the carboxylase activity of the enzyme was suggested to be more suppressed by cold than oxygenase activity (Sawada and Miyachi, 1974; Graham and Patterson, 1982). In contrast, Vu et al. (1995) reported that the large subunit of Rubisco was up-regulated, but the small unit down-regulated, in response to cold acclimation in citrus.

Aldolase was down-regulated only after SZA in our study. The metabolic function of cytosolic fructose-bisphosphate aldolase is cleavage of fructose 1,6-bisphosphate (F-1,6-BP) into glyceraldehyde 3-phosphate and dihydroxyacetone phosphate during glycolysis. In addition, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which catalyzes the subsequent conversion of glyceraldehyde 3-phosphate to glycerate 1,3-bisphosphate, was also down-regulated. Although there is limited information on the regulation of these two proteins during cold acclimation, our results indicate a suppression of respiration at low temperatures. In contrast to other studies generally showing increase in GAPDH production (mostly in roots) and respiration rates in response to different stresses such as heat, hypoxia, or salt (Yang et al., 1993; Chang et al., 2000; Xu et al., 2010). Our data on expression of the enzymes involving either in Calvin cycle (rubisco) or glycolysis (aldolase, GAPDH), suggests that adaptation of velvet bentgrass to low non-freezing and sub-freezing temperatures occurred under suppressed photosynthesis and respiration.

There is limited information regarding the physiological role of cytoplasmic aconitase in plants. The enzyme appears to be involved in the glyoxylate cycle (Courtois-Verniquet and Douce, 1993) which converts lipid to sucrose. Although this enzyme was up-regulated in our study, the contribution of the glyoxylate cycle to the considerable increase in sucrose content during cold acclimation in velvet bentgrass seems marginal compared with the role of the photosynthetic pathway. Like mitochondrial aconitase, which catalyzes the isomerization of citrate to isocitrate in the tricarboxylic acid cycle, cytoplasmic aconitase was reported to be inhibited by hydrogen peroxide (H₂O₂) (Verniquet et al., 1991). So, probably, the

concentration of H₂O₂ after A4 was lower than in NA crowns, though the concentration of H₂O₂ is expected to increase during chilling and cold acclimation (Kocsy et al., 2001).

Disease/defence. The generation of H₂O₂ or other reactive oxygen species (ROS) is commonly associated with abiotic and biotic stress conditions (Neill et al., 2002). According to the current understanding of the mechanisms underlying cold acclimation in grasses (Huner et al., 1993; Sandve et al., 2011), the generation of ROS is associated with an imbalance between rates of photochemical reactions vs. thermochemical reactions (CO₂ fixation and Calvin cycle), leading to an over-excitation of Photosystem II (PSII). In our study the only protein which could potentially contribute to detoxification of H₂O₂, was peroxidase. The enzyme was indeed down-regulated after A4, but up-regulated again after SZA. Gaudet et al. (2000) also reported peroxidase levels were down-regulated in crowns of winter wheat following 7 d of exposure to cold acclimation at 2°C, but up-regulated again following two more weeks at given conditions. The potentially low peroxidase level in crowns of A4 plants in our study could be due to quick recovery from potential photoinhibition (Mullineaux et al., 2006). Okuda et al. (1991) found that elevated H₂O₂ in leaves of wheat returned to the normal level within 15-20 min after exposure to cold treatment.

A putative glycine-rich protein was up-regulated in response to A4, but its production was found to return to the same level as in non-acclimated plants after SZA. This protein had 2-sequence similarity with cold shock protein (CSP) from wheat. Plant CSP with high sequence similarity with bacterial CSP, which is up-regulated by cold and is capable of binding nucleic acids, were found in barley (*Hordeum vulgare* L.) (*blt 801*) (Dunn et al., 1996) and winter wheat (WCSP1). Chaikam and Karlson (2008) showed that CSP in rice (*OsCSP*) was more expressed in reproductive tissues and tissues with high meristematic activity. Similar to our study, Karlson et al. (2002) observed that the level of WCSP1 was low in NA plants, but then it was gradually increased during 18 d of cold acclimation.

The up-regulation of UDP-D-glucuronate decarboxylase in response to cold acclimation has not been reported previously. The enzyme catalyzes synthesis of UDP-D-xylose from UDP-D-glucuronate and is suggested to influence cell wall composition in barley (Zhang et al., 2005). Cell walls most likely contribute to maintenance of cell integrity during desiccation caused by freezing similarly to that caused by drought (Neale et al., 2000).

In summary, acclimation of plants for 4 wk at 2 °C (A4) significantly increased freezing tolerance, but following sub-zero acclimation did not provide additional freezing tolerance. The increased freezing tolerance might be associated with enhanced amino acid synthesis, since serine hydroxymethyltransferase and methionine synthase were up-regulated by

acclimation. Aconitase, UDP-D-glucuronate decarboxylase and glycine rich protein were also up-regulated after A4 acclimation, while rubisco large and small subunit, glyceraldehyde-3-phosphate dehydrogenase and peroxidase were down-regulated. The differences in protein composition were more pronounced between NA and A4 than between A4 and SZA.

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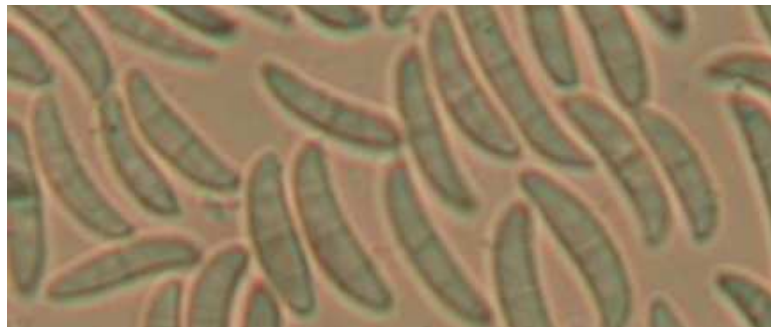
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PAPER III



Evaluation of the winter hardiness of velvet bentgrass cultivars in controlled environments

Arne Tronsmo¹, Tatsiana Espevig^{2,3}, Linda Hjeljord¹, and Trygve S. Aamlid³

¹ Department of Chemistry, Biotechnology and Food Sciences. University of Life Sciences, Ås 1432, Norway .

²Department of Plant and Environmental Science, Norwegian University of Life Sciences, Ås 1432, Norway

³Norwegian Institute for Agricultural and Environmental Research, Bioforsk Landvik , Grimstad, N-4886, Norway.

ABSTRACT

Up to six velvet bentgrass cultivars Avalon, Greenwich, Legendary, Villa, Venus and Vesper and creeping bentgrass ‘Penn A-4’ were tested under controlled environmental conditions for freezing tolerance and susceptibility to *Microdochium nivale* under different simulated winter covers (uncovered, snow, and ice) for 6 and 12 weeks. Without cold hardening, plants of all cultivars failed to survive -6°C, whereas plants cold hardened in a growth chamber at 2 °C for 2 weeks survived -6°C, but failed to survive lower temperatures. Turf that had been hardened for two additional weeks at -2 °C survived -9 °C, and to a certain extent -12 °C. Only turf that had been hardened under natural outdoor conditions from August to January survived -15 °C. Cold hardening at 2 °C also increased survival after inoculation with *M. nivale*. After 6 weeks of simulated winter conditions, the plants covered by artificial snow showed more winter injury than those under ice, but after 12 weeks, ice cover caused the largest damage. Cold hardened plants without cover were fairly resistant to infection by *M. nivale*. Differences in freezing tolerance between velvet bentgrass and creeping bentgrass ‘Penn A-4’ and among velvet bentgrass cultivars were generally not significant, but ‘Penn A-4’ was more resistant to *M. nivale* than velvet bentgrass cultivars.

Key words: acclimation, *Agrostis canina*, cold hardening, freezing tolerance, golfgreen, simulated ice cover, *Microdochium nivale*, snow mold, simulated snow cover

INTRODUCTION

Winter injury of temperate grasses used for turf is a significant problem in northern climatic regions. At present, about 70 % of Scandinavian golf courses suffer from winter damage every year (STERF, 2009), and the use of winter-hardy species and cultivars in the Nordic countries is necessary. In cultivar evaluation field trials at two locations in Norway (one continental, 61°N, and one coastal, 58°N) from 2003 to 2006, velvet bentgrass (*Agrostis canina*) showed better winter survival than creeping bentgrass (*A. stolonifera* L.) (Aamlid et al., 2006; Molteberg et al., 2008). However, the differences between velvet bentgrass cultivars regarding ability to survive winter damage from frost, snow, ice cover, and snow mold are not known.

Velvet bentgrass is known for producing excellent playing surfaces without the need for high inputs of pesticides, fertilizers, or irrigation water. The species also has better tolerance to several abiotic and biotic stresses than creeping bentgrass (Brillmann, 2003; Da Costa and Huang, 2006; Murphy et al., 2009)

Because winter injury in field trials may be caused by one or more stresses (freezing temperatures, ice encasement, crown dehydration, and/or fungal diseases) (Humphreys, 1989; Ergon et al., 1998; Bertrand et al., 2009a; Castonguay et al., 2009), controlled environment studies are needed to determine possible differences in winter survival among velvet bentgrass cultivars and to determine the causes for differences in winter survival between velvet bentgrass and creeping bentgrass.

Winter hardiness is significantly affected by a period of cold acclimation or cold hardening, during which a number of physical, biochemical, and physiological changes contribute to enhanced cellular stability under different winter stresses (Levitt, 1980; Guy, 1999; Thomashow, 1999; Rajashekar, 2006). Two consecutive stages of cold hardening have been suggested in winter cereals and temperate grass species (Tumanov, 1940). The first hardening stage occurs at temperatures above freezing (approximately 2 to 5°C) and is characterized by several changes including accumulation of osmolytes (e.g. nonstructural carbohydrates, amino acids) and antifreeze proteins, reserve carbohydrates, increases in antioxidant production, and alterations in phospholipids and fatty acids (Livingston, 1991; Tronsmo et al., 1993; Dionne et al., 2001a, 2001b; Hoffman et al., 2010; Paper 1). The second stage, referred to as sub-zero hardening, leads to acquisition of additional freezing tolerance (Tumanov, 1940; Livingston, 1996; Herman et al., 2006). Exposure to sub-freezing temperatures (-2 to -5°C) is commonly associated with induced ice formation in the apoplast

and dehydration of plant cells (Steponkus and Lynch, 1989; Herman et al., 2006). The minimum necessary duration of the second hardening stage is still controversial.

Freezing tolerance is a major component of winter hardiness of perennial grasses (Humphreys and Eagles 1988; Xiong and Fei, 2006; Hulke et al, 2008). Recently, we found no differences in freezing tolerance between ‘Greenwich’ velvet bentgrass and ‘Penncross’ creeping bentgrass (Paper 1). However, the freezing tolerance of different velvet bentgrass cultivars has not yet been investigated.

Pink snow mold caused by the fungus *Microdochium nivale* is one of the most damaging low-temperature diseases of turfgrasses in northern Europe, northern USA, and other temperate and boreal regions (Årsvoll, 1973, 1975; Smith et al., 1989; Smiley *et al.*, 2005; Matsumo, 2009; Bertrand et al., 2009b). Snow mold pathogens are difficult and costly to control. While the optimal mycelial growth for most low-temperature fungi occurs at temperatures above 10 °C (Bennett, 1933 cited in Smith et al. 1989; Snider et al., 2000), their low competitive ability results in ‘avoidance of antagonism by escaping to the under-snow habit’ (Matsumoto, 2009). Plant survival during winter months and severity of snow mold depend on the presence or absence of winter covers, particularly snow or ice (Årsvoll, 1973; Dionne et al., 1997; Tompkins et al., 2004; Aamlid et al., 2008). However the critical duration of winter covers and their effects on susceptibility to snow molds in turfgrasses are not well documented.

Similar to freezing tolerance, resistance to snow molds can be enhanced by cold hardening (Tronsmo, 1984; Hofgaard et al., 2006), but the mechanisms underlying freezing tolerance and resistance to snow molds are not completely understood. No turfgrass species has absolute resistance to snow molds, but susceptibility to low-temperature diseases can vary among plant species and cultivars (Smith et al., 1989; Hofgaard et al., 2003; Smiley et al. 2005; Latin, 2007). In contrast to winter cereals and forage grasses, studies on snow mold resistance in turfgrasses are limited (Smith, 1989; Casler et al., 2001; Wang, 2005; Chang et al., 2007; Casler et al., 2006, 2007). The knowledge regarding disease resistance in velvet bentgrass is restricted to grey snow mold (*Typhula incarnata*), dollar spot (*Sclerotinia homoeocarpa*), brown patch (*Rhizoctonia* spp.), and copper spot (*Gloeocercospora sorghi*) (Chang et al., 2007; Brilman and Meyer, 2000; Brown and Jung, 2010). Chang et al. (2007) reported that velvet bentgrass was more susceptible to *Typhula incarnata* than creeping bentgrass and colonial bentgrass under controlled environmental conditions.

The aim of the present study was to compare velvet bentgrass cultivars and creeping bentgrass ‘Penn-A4’, with respect to freezing tolerance, survival under different simulated winter conditions and resistance to pink snow mold, after different periods of cold hardening.

MATERIALS AND METHODS

Plant material and hardening conditions

Up to six velvet bentgrass (*Agrostis canina*) cultivars, Avalon, Greenwich, Legendary, Venus, Vesper, and Villa, and the creeping bentgrass (*A. stolonifera*) cultivar Penn A-4 were included in three independent trials. Seeds were sown at rate of 6.7 g/m² in 10 x 10 x 7.5 cm pots with USGA-specifications growth medium containing 0.5% (w/w) organic matter (Baskarpsand, Sweden). Unhardened plants were grown for 9 and 10 weeks in a greenhouse in 2006 and 2007, respectively, or for 10 weeks in a growth chamber in 2008, at 18/12°C (day/night temperature) with a 16h photoperiod and photosynthetic photon flux density (PPFD) of 150 µmol m⁻² s⁻¹. For hardening, plants were grown under the same conditions for 8 weeks in 2006, and 9 weeks in 2007 and 2008, and then hardened in a growth chamber at 2°C for 2 weeks and 16 h photoperiod with a PPFD of 250 µmol m⁻² s⁻¹. In 2008, an additional hardening treatment was also included, with 2 weeks of additional subzero hardening at -2 °C in darkness following the 2-week hardening at 2°C under light. The plants were watered regularly and fertilized once a week with 25 mL per pot of a complete nutrient solution containing micronutrients and 0.31 g liter⁻¹ nitrogen (N), 0.05 g liter⁻¹ phosphorus (P) and 0.36 g liter⁻¹ potassium (K). The turf was mowed to a height of 5-7 mm three times per week using a hand-held electric grass cutter (Gardena).

For evaluation of cold hardening in the field, the cultivars were sown at 6.7 g/m² on a green at Landvik 21 Aug. 2008. The plots were mowed to 3 mm three times per week until the last week of September, when mowing height was increased to 4 mm. The last mowing was performed in mid-October. The plots were irrigated regularly and fertilized at biweekly intervals until late October. Four-month-old sods of the cultivars (10 x 10 x 7.5 cm) were taken from the green and potted the day before the freezing test. The temperature and light intensities for the natural acclimatization (or hardening) are shown in Fig. 1.

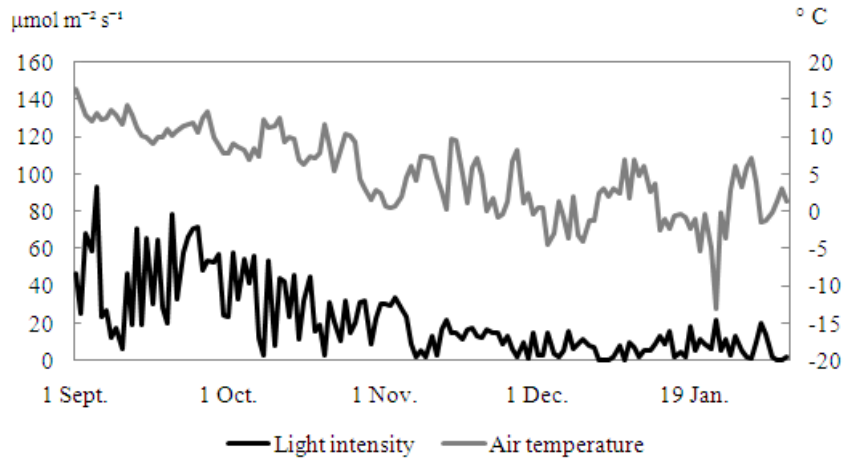


Fig. 1. Mean diurnal temperature and mean diurnal light intensity (PPFD averaged over 24 h) at Landvik in autumn and early winter 2008-09 (Bioforsk AgroMetBase).

Freezing tests

For the freezing tests, unhardened and cold hardened plants were transferred to controlled climate chambers and incubated for 12 h at 2°C. The temperature was then reduced at a rate of 1°C/h to -6, -9, -12, or -15°C and maintained at these levels for 24 h. The temperature was then raised by 1°C/h and maintained at -2°C for periods ranging from 5 to 23 h before being increased at a rate of 1°C/h to 2°C. The pots were transferred to the greenhouse at 18/12 °C day/night temperatures and 16 h day length (150 μmol m⁻² s⁻¹). Freezing tolerance was assessed visually after 14 and 26 days as turfgrass survival, i.e. the percentage of the pot covered with healthy grass.

Susceptibility to *Microdochium nivale* and effects of snow and ice cover

Evaluation of susceptibility to *M. nivale* with or without simulated winter covers was performed in 2006 only. A Norwegian isolate of snow mould fungi, *M. nivale* 3/98, originally isolated from perennial ryegrass, was cultured in Potato Dextrose Broth (PDB) for 14 days at 9 °C in darkness (Tronsmo, 1993). Fungal mycelium was harvested by filtration through cheesecloth. Inoculum was prepared by homogenizing the mycelium in distilled water using a Ultra Turrax homogenizer (Janke & Kunke). The final inoculum was adjusted to an optical density of OD₄₃₀ = 0.48, which is roughly equivalent to 10⁵ colony forming units mL⁻¹. To evaluate the effect of artificial snow or ice cover on snow mold infection, unhardened and hardened plants were sprayed with approximately 2 mL mycelial suspension/pot with a high-pressure-sprayer. Control pots were sprayed with water. The pots were incubated for 6 or 12

weeks in darkness at 0.5 - 1.0 °C, either uncovered (simulating winter conditions with no snow or ice cover), enclosed in air-tight vacuum bags using a food vacuum sealer (Vuomatic VM 360, Bernardi, Italy) (simulating anaerobic conditions under ice cover; Aamlid et al., 2008) or covered with a sheet of wet cotton and wrapped in plastic (simulating snow cover; Årsvoll, 1977; Tronsmo, 1993). In all cases, plant responses were evaluated as turf survival (percentage of pot covered with healthy grass) after two weeks of recovery in the greenhouse under the same conditions as described above.

Statistical analyses

The experiments were conducted according to completely randomized designs with three replications for each combination of all experimental treatments. Analyses of variance were performed using the software package STATISTICA (Version 6.0, Hill and Lewicki, 2007). There were three experimental factors in the freezing tests (cultivar, hardening, and freezing temperature) and five experimental factors (cultivar, hardening, inoculation with pink snow mold, simulated cover, and duration of winter treatment) in the winter survival tests. Significant differences among treatments were identified by Fisher's least significant difference (LSD) test at the 5 % probability level.

RESULTS

Freezing tests

The freezing test in 2006 revealed slightly lower freezing tolerance of creeping bentgrass 'Penn A-4' compared with velvet bentgrass cultivars (Figure 2). Among velvet bentgrass cultivars, 'Greenwich' had the highest freezing tolerance in the unhardened state. The repeated experiments in 2007 and 2008 showed no significant differences in freezing tolerance between creeping bentgrass and velvet bentgrass (also among cultivars; data not shown).

In all experiments, unhardened plants barely survived any of the freezing temperatures (results from 2006 and 2008 are shown in Figure 3). All cold hardening treatments significantly improved freezing tolerance of velvet bentgrass and creeping bentgrass. Plants hardened at 2°C for two weeks under light had an average of 89, 8, 0, and 0% surviving plant coverage after freezing to -6, -9, -12 and -15°C, respectively. Additional sub-zero hardening

significantly enhanced tolerance to -9 °C, -12 °C and -15°C, as the plant coverage was 60, 57 and 30%, respectively. Plants hardened in the field survived all freezing temperatures (Figure 4).

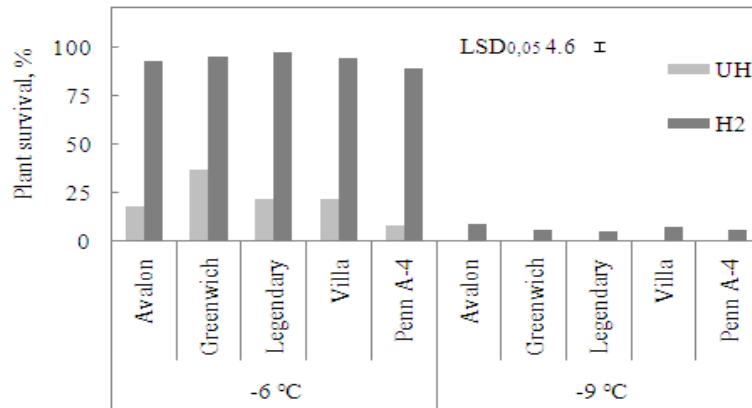


Figure 2. Survival of unhardened (UH) and cold hardened (H2) cultivars of velvet bentgrass and creeping bentgrass 'Penn A-4' after freezing test (H2 = hardening for 2 wk at 2 °C and 16 h photoperiod with PPFD of 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$; experiment 2006).

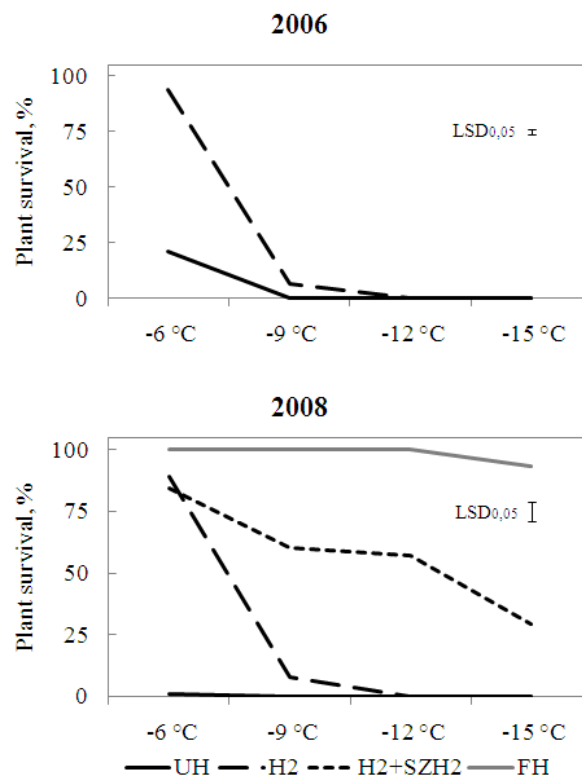


Figure 3. Effects of cold hardening treatments on turfgrass survival 14 days after exposure to different freezing temperatures in the 2006 and 2008 experiments. Treatments: UH, unhardened plants; H2, hardened at 2°C for 2 wk; H2+SZH2 hardened at 2 wk at 2°C followed by 2 wk at -2°C, and FH, cold hardened under field conditions (mean of creeping bentgrass 'Penn A-4' and velvet bentgrass cultivars).

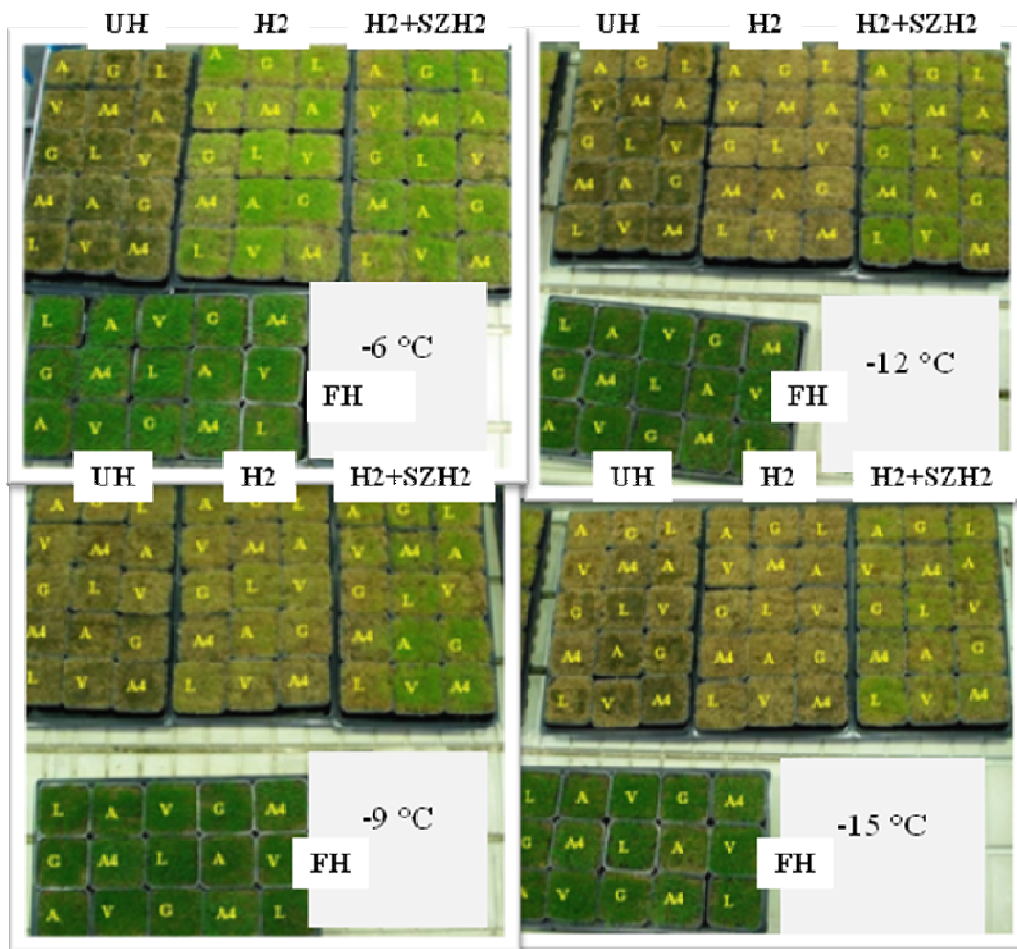


Fig 4. Recovery of plants 26 days after exposure to the freezing temperatures of -6, -9, -12, and -15°C (2008 experiment). The three trays in the top of each photo: unhardened plants (UH), hardened 2 weeks at 2°C (H2), and hardened 2 weeks at 2°C followed by 2 weeks at -2°C (H2+SZH2); Pots at the bottom of each photo are plants hardened in field (FH). A=Avalon, G=Greenwich, L=Legendary, V=Villa, A4=Penn A-4. (Photo: Arne Tronsmo).

Susceptibility to *Microdochium nivale* and effects of snow and ice cover

Since most of the two- and three-way interactions among hardening, simulated ice and snow covers, duration of treatment, and inoculation with *M. nivale* were significant, turfgrass survival following various combinations of these factors is shown in Figure 5. Under the given conditions, uncovered plants survived better (mean value 85 %) than plants maintained under simulated ice or snow cover (both 44% survival). Nearly 100% of the hardened uncovered plants survived regardless of inoculation with *M. nivale* and duration of treatment (Fig. 5).

Only 57% of the unhardened plants survived 6 weeks under simulated ice cover. Hardening at 2°C for 2 weeks resulted in nearly 100% survival under these conditions. However, when the duration of ice cover was prolonged from 6 to 12 weeks, plant survival only amounted to 9% on average for both unhardened and hardened plants. Inoculation with *M. nivale* had no effect on survival of hardened or unhardened plants after simulated ice cover for 6 or 12 weeks.

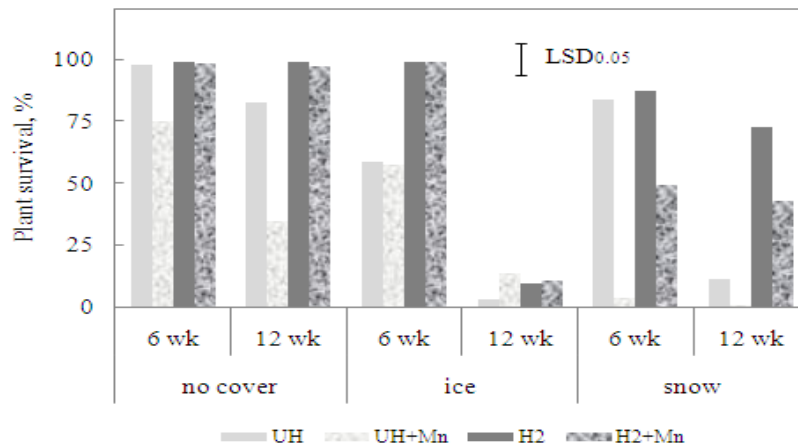


Figure 5. Effects of hardening and duration of simulated winter covers on survival of noninoculated plants and plants inoculated with *Microdochium nivale* (Mn) (mean of creeping bentgrass ‘Penn A-4’ and velvet bentgrass cultivars). H2 = hardening for 2 wk at 2 °C and 16 h photoperiod with PPFD of 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$; experiment 2006.

Simulated snow cover significantly lowered survival of plants which had been inoculated with *M. nivale* compared with noninoculated plants. Unhardened inoculated plants did not survive even 6 weeks under simulated snow cover in contrast to hardened plants, which showed 46% survival (Fig. 5).

As compared with 6 weeks, 12 weeks of simulated snow cover only led to a small decrease in plant survival of hardened noninoculated (14 percentage points) or inoculated plants (6 percentage points). By contrast, prolongation of the simulated snow cover resulted in 73 percentage points decrease in the survival of unhardened and noninoculated plants.

Hardening significantly improved survival of inoculated plants in all treatments except for the plants maintained for 12 weeks under simulated ice cover (Fig. 5, Fig. 7).

The differences among velvet bentgrass cultivars were small and inconsistent except for ‘Avalon’, which, on average for all treatments, had lower survival than the other cultivars. The velvet bentgrass cultivars were more susceptible than creeping bentgrass ‘Penn A-4’ to

M. nivale (two interaction significant at $p < 0.01$; Figs. 6 and 7). Interactions among cultivar and ice or cover or duration of exposure were nonsignificant.

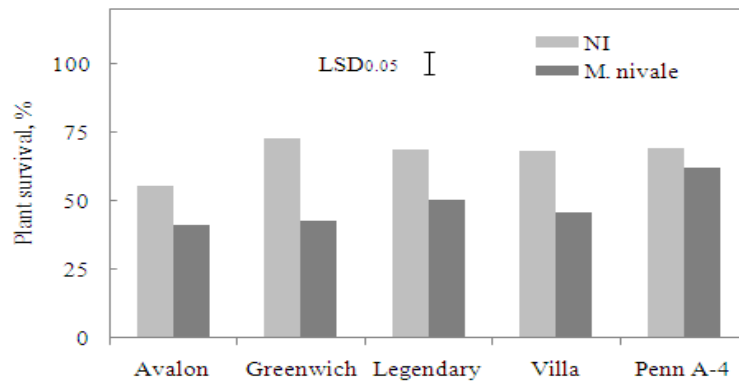


Figure 6. Effect of inoculation with *Microdochium nivale* on survival of cultivars of velvet bentgrass and creeping bentgrass 'Penn A-4' (average for all treatments).



Fig 7. Symptoms of *Microdochium nivale* on unhardened (left) and hardened (right) turf after 12 weeks without ice or snow cover. Photo taken after two weeks recovery in the greenhouse. Cultivars: A = Avalon, V = Villa, G = Greenwich, L = Legendary, A4 = Penn A-4. (Photo: Katarina Gundsø Jensen).

DISCUSSION

Little is known about winter hardiness of velvet bentgrass. Recently, we demonstrated that velvet bentgrass 'Greenwich' and creeping bentgrass 'Penncross' did not differ in freezing tolerance (Paper 1). Creeping bentgrass was previously reported to survive -35°C (LT_{50}) when hardened in the field (Gusta et al., 1980). Although unhardened plants of 'Greenwich'

survived exposure to -6 °C better than the other cultivars in one of the present trials, our overall results showed no differences in freezing tolerance among velvet bentgrass cultivars.

Freezing tolerance of velvet bentgrass and creeping was enhanced by the first stage of cold hardening as was previously shown in winter cereals and forage grasses (Veisz and Sutka, 1989; Livingston, 1991; Tronsmo et al., 1993; Livingston et al., 2009). We also found that hardening in light at 2 °C followed by sub-zero hardening at -2 °C for 2 weeks led to additional freezing tolerance in both bentgrass species. This is in agreement with Dionne et al. (2001a), who showed the same phenomenon in annual bluegrass (*Poa annua*). However, in our previous study freezing tolerance of velvet bentgrass and creeping bentgrass was not improved by subzero hardening at -2 °C in darkness after hardening at 2 °C for 4 weeks (Paper 1 and 2). The lack of additional freezing tolerance after the second hardening stage was most likely due to the effect of subzero hardening was not sufficiently expressed in our previous study as plants were exposed to each freezing temperature only for 2 h in contrast to 24 h in the present study. Dionne et al. (2001a) found an additional effect of subzero hardening even with only 1 h exposure to various freezing temperatures in annual bluegrass, but this situation may well be different in a more winter-hardy species such as velvet bentgrass and creeping bentgrass. In any case, the distinction between the first and second stage of cold hardening becomes less apparent under field conditions. In the present study it is, indeed, noteworthy, the highest freezing tolerance was achieved outdoor, despite the fact the average light intensity from 1 Oct. to 1 Jan. was significantly lower than in the indoor hardening chamber. Better freezing tolerance of plants hardened under natural outdoor conditions can be explained by the longer hardening period with temperature fluctuations and also by the exposure to subfreezing temperatures in early January.

Hardening significantly improved survival of bentgrass which had been inoculated with *M. nivale*. This is in accordance with earlier investigations showing cold hardening to enhance resistance to pink snow mold in winter cereals and forage grasses (Tronsmo, 1984; Hofgaard, 2006). Among possible mechanisms for the cold-induced resistance in cereals and grasses are reduced water potential (Tronsmo, 1986) and production of pathogenesis-related proteins (PR-proteins) (Ergon et al., 1998; Gaudet et al., 2000; Muthukrishnan et al., 2001; Paper 2). The composition of nonstructural carbohydrates, which accumulate in crowns during cold hardening, is also thought to contribute to snow mold resistance. Snow mold-resistant cultivars of winter wheat have been suggested to accumulate higher levels of nonstructural carbohydrates and metabolize them at slower rates than susceptible cultivars (Yoshida et al., 1998). Previously, we demonstrated that both unhardened and hardened creeping bentgrass

accumulated more fructan than velvet bentgrass, while sucrose was maintained at similar levels in both species (Paper 1). Apparently, fructans did not contribute to freezing tolerance, which did not differ between the two species, but it may have contributed to snow mold resistance, because inoculated creeping bentgrass ‘Penn A-4’ had generally higher survival than velvet bentgrass cultivars in both unhardened and hardened state. This would suggest that freezing tolerance and pink snow mold resistance in the bentgrass species have different mechanisms, as was previously proposed for timothy (regarding resistance to *Typhula ishikariensis*) (Tronsmo, 1985) and natural infection by different snow mold fungi in winter wheat (Yoshida et al., 1998).

As for freezing tolerance, the small differences in resistance to pink snow mold among velvet bentgrass cultivars was probably due to limited genetic diversity among the cultivars included in this study. ‘Avalon’ was originally released from the University of Rhode Island, but the other varieties all originated at Rutgers University (L. Brilman, pers. comm., April 2010), where the breeding program has focused on other characters than overall winter hardiness or snow mold resistance (Brown and Ung 2010, S. Bonos, pers. comm. 2011). This situation is very different from naturally selected ecotypes of annual bluegrass showing genetic variability in resistance to pink snow mold (Aamlid et al., 2008; Bertrand et al., 2009b) or freezing tolerance (Dionne et al., 2010). The latter investigators also reported positive correlation between resistance to *M. nivale* and duration of snow cover on green-type annual bluegrass.

Additionally, as a species, velvet bentgrass tended to be more susceptible to pink snow mold than creeping bentgrass. Earlier, velvet bentgrass was reported to be more susceptible to grey snow mold caused by *Typhula incarnata* than creeping bentgrass under controlled environmental conditions (Chang et al., 2007).

Pink snow mold reduced survival of hardened plants after 6 and 12 weeks under simulated snow cover by 44% and 41%, respectively, compared with hardened non-inoculated plants. In contrast, inoculation with *M. nivale* had no effect on uncovered hardened plants, in spite of the fact that snow cover is not necessary for the development of pink snow mold (Årsvoll, 1973; Matsumoto, 2009). Our results suggest that at given conditions (0.5 - 1.0 °C, darkness) even hardened plants of velvet bentgrass and creeping bentgrass are not able to completely withstand snow mold infection in the presence of snow cover.

The duration of snow cover has different effects on the incidence and severity of various snow molds (Årsvoll, 1973). In our study, the survival of plants inoculated with *M. nivale* was only insignificantly lower after 12 weeks compared with 6 weeks duration of simulated snow

cover. This is in agreement with Årsvoll (1973), who observed pink snow mold after only 30 days, with only insignificant increases after 90 days of snow cover on forage grasslands.

Simulated ice cover for 12 weeks caused the largest damage, regardless of hardening or inoculation with snow mold. This is in agreement with observations of winter damage on Norwegian golf courses. The lack of effect of snow mold fungi under ice cover can be explained by inhibition of the fungi by anaerobic conditions and accumulation of toxic gases (Levitt, 1980), which, in our experiment, probably reached a critical level somewhere between 6 and 12 weeks of exposure. Aamlid et al. (2008), using a simulation technique similar to that in our experiment, found critical ice encasement periods of 25-30 days in annual bluegrass (*Poa annua*) and 42-47 days in creeping bentgrass. Other investigators have found that creeping bentgrass can tolerate up to 120 days of ice cover under field conditions, but this depends on how the ice is formed and how compact it is (Beard, 1964; Hamilton, 2001; Thompkins et al., 2004). Studies under Norwegian field conditions suggest that velvet bentgrass may be more tolerant to long-lasting ice cover than creeping bentgrass (Aamlid et al. 2006), but this aspect needs further investigation as it could not be confirmed under the simulated conditions used in this study.

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PAPER IV



Thatch control and winter survival of newly established velvet bentgrass greens in northern environments

Tatsiana Espevig^{1,3*}, Bjørn Molteberg², Anne Marte Tronsmo³, Arne Tronsmo⁴, and

Trygve S. Aamlid^{1*}

¹Norwegian Institute for Agricultural and Environmental Research, Bioforsk Landvik, Grimstad, N-4886, Norway; ²Norwegian Institute for Agricultural and Environmental Research, Bioforsk Apelsvoll, Kapp, N-2849, Norway, present address: Strand Unikorn, Moelv, N-2390, Norway; ³Department of Plant and Environmental Science, Norwegian University of Life Sciences, Ås 1432, Norway; ⁴Department of Chemistry, Biotechnology and Food Sciences, University of Life Sciences, Ås 1432, Norway

ABSTRACT

The use of velvet bentgrass (*Agrostis canina* L.) on golf greens is limited by sparse knowledge on optimal maintenance. Our objective was to determine the effects of nitrogen (75 or 150 kg N ha⁻¹ yr⁻¹), topdressing (0.5 or 1.0 mm biweekly), and mechanical/biological treatments (grooming, vertical cutting, spiking, ‘Thatch-less™’) on turfgrass visual quality, playability, winter survival, and thatch formation. The study was conducted on USGA greens seeded with velvet bentgrass ‘Legendary’ at a coastal (Landvik, 58°N) and a continental (Apelsvoll, 61°N) location in Norway in the period 2007-10 and 2007-09, respectively. Velvet bentgrass required at least 150 kg N ha⁻¹ yr⁻¹ and heavy topdressing during the first year after sowing. The N rate of 150 kg ha⁻¹ yr⁻¹ reduced moss and winter injuries compared with 75 kg N ha⁻¹ yr⁻¹, but lowered surface hardness by 21% and ball roll distance by 6-14%. On older green, the lower N rate and heavy topdressing were key elements in maintenance of velvet bentgrass with acceptable turf visual and playing quality and adequate percentage of organic matter in the mat. Compared with grooming only, grooming + vertical cutting significantly reduced mat organic matter from 6.4 to 5.3 % at Landvik and resulted in better visual quality at Apelsvoll. Grooming + spiking improved water infiltration rate by 51% at Landvik and 253% at Apelsvoll compared with grooming only, but reduced surface hardness. ‘Thatch-less™’ increased hardness of the otherwise soft plots receiving grooming + spiking, but had no effect on mat depth or organic matter content.

* Corresponding authors (trygve.aamlid@bioforsk.no, tanja.espevig@bioforsk.no)

INTRODUCTION

Velvet bentgrass (*Agrostis canina* L.) is a native species in northern and central Europe (Brilman, 2003). After being brought to North America during the emigration period, New England golf superintendents realized that velvet bentgrass produced beautiful greens like a 'velvet carpet'. In addition to very fine surface texture, velvet bentgrass has better shade (Reid, 1933) and drought (DaCosta and Huang, 2006) tolerance than other bentgrass species. It is more resistant to dollar spot (*Sclerotinia homoeocarpa*) and brown patch (*Rhizoctonia* spp.) (Brilman and Meyer, 2000), tolerates as much or even more compaction and wear stress (Murphy et al., 2009; Samaranayake et al., 2009), and competes better against annual bluegrass (*Poa annua* L.) infestation (Samaranayake et al., 2009) than creeping bentgrass (*Agrostis stolonifera* L.). In the Nordic countries, the benefits of velvet bentgrass were rediscovered through a variety evaluation project in which velvet bentgrass had better winter survival than any other species on a putting green where no pesticides were used (Molteberg et al., 2008). However, as Espevig et al. (Paper 1) found freezing tolerance to be equal in velvet bentgrass and creeping bentgrass under controlled conditions, we hypothesize that the superior winter survival of velvet bentgrass in the variety evaluation project was due to other traits than freezing tolerance per se. Thus, winter survival of velvet bentgrass warrant further investigation under various climatic conditions.

In the 1960's and 1970's velvet bentgrass fell out of favor on North-American golf courses. As fertilizers and pesticides were introduced, creeping bentgrass and annual bluegrass became the predominant species on putting greens. Since then, increasing environmental awareness has raised the quest for well-adapted turfgrass species requiring less water, pesticide and fertilizer use. In this context, velvet bentgrass seems to have a potential in North America and Europe, but its acceptance among golf course superintendents depends on guidelines for optimal maintenance, and especially thatch management, which is considered to be one of the biggest problems in this species.

According to Beard (2002), *thatch* is "an intermingled organic layer of dead and living shoots, stems, and roots of grasses that develops between the turf canopy of green vegetation and the soil surface". When topdressing is used, thatch is intermixed with sand and a layer called *mat* is formed. Excessive thatch layers develop when thatch accumulation exceeds thatch degradation (Beard, 2002). Thatch control can be grouped into (1) prevention of excessive plant growth and shoot density, (2) enhancement of microbial thatch degradation, (3) thatch dilution by sand, and (4) mechanical thatch removal.

Excessive plant growth leading to thatch can be minimized by an appropriate nitrogen fertilization program. Carrow et al. (1987) stated that thatch will increase with increasing nitrogen input at both deficient and excessive fertility levels, but remain constant with increasing nitrogen within an optimal threshold interval. Nitrogen rates varying from 48 to 342 kg N ha⁻¹ yr⁻¹ to velvet bentgrass greens were compared by Skogley (1975), Boesch and Mitkowski (2007), and Koeritz and Stier (2009), but limited information on thatch formation is available from those studies. Skogley (1975) reported 11.4-12.5% organic matter in velvet bentgrass mats, but, surprisingly, these numbers were not affected by N rate under his experimental conditions.

Numerous studies have demonstrated effects of topdressing and mechanical treatments on thatch formation. Topdressing (Murphy, 1983; White and Dickens, 1984; Smith, 1979; McCarty et al., 2005, 2007) or return of soil from hollow tine coring (Murphy et al., 1993a ; Fu et al., 2009) will usually decrease the content of organic matter in mat by dilution, but at the same time, these treatments will also increase mat depth. The contribution of topdressing to microbial thatch degradation has been controversial (Murphy, 1983; Ledebøer and Skogley, 1967; Carrow et al., 1987; Couillard et al., 1997; McCarty et al., 2005). Vertical cutting and hollow tine coring usually reduce mat depth due to direct thatch removal (Smith, 1979; McCarty et al., 2005), but the effect of coring (Carrow et al., 1987; McCarty et al., 2005, 2007; Barton et al., 2009), vertical cutting (Carrow et al., 1987; McCarty et al., 2005, 2007), and spiking (Murphy et al., 1993a) on the percentage of organic matter in mat is often small unless combined with topdressing. Depending on timing and frequency, mechanical treatments are sometimes disruptive to turfgrass surfaces (White and Dickens, 1984; Carrow et al., 1987; McCarty et al., 2005; Fu et al., 2009), and this may be particularly harmful in velvet bentgrass because of the poor recuperative capacity of this species (Boesch and Mitkowski, 2007).

Stimulation of thatch degradation is often a difficult task. Thatch is composed mainly of cellulose, hemicelluloses, and lignin (Ledebøer and Skogley, 1967; Couillard and Turgeon, 1997). Lignin is a complex aromatic polymer that is extremely resistant to degradation (Kirk, 1971; Crawford and Crawford, 1980). Biodegradation of lignin is mainly accomplished by a few species of fungi (Martin and Dale, 1980; Blanchette, 1991, Sidhu et al. 2010), but bacteria (Vicuña, 1988; Zimmermann, 1990), especially actinomycetes (Crawford, 1978), have also been reported as lignin degraders. Degradation of cellulose and lignin is essentially an aerobic process and the degradation rate depends on turf age (Shi et al., 2006), temperature and soil moisture (Donnelly et al., 1990; Pastor and Post, 1986), pH (Martin and Beard,

1975), available nitrogen, and the carbon-to-nitrogen (C:N) ratio (Raun et al., 1998; Henriksen and Breland, 1999). It has been claimed that application of products containing fungi, bacteria, enzymes or other bioactive ingredients will enhance thatch degradation (Crawford, 1978; Martin and Dale, 1980; Roudsari et al., 2008), however the efficiency of such products under the field conditions remains controversial (Chamberlain and Crawford, 2000; McCarty et al., 2005, 2007).

Based on the literature cited above, we hypothesized that moderate nitrogen inputs and heavy topdressing would be key elements to maintenance of velvet bentgrass greens with good playability and aesthetic quality, acceptable content of organic matter in the mat layer, and little winter damage. Due to the limited recuperative capacity of velvet bentgrass and the low soil temperatures during most of the season in Scandinavia, we also hypothesized that mechanical and biological treatments to control thatch would be of secondary importance compared with appropriate fertilization and topdressing programs. The objective of this research was to determine the effects of nitrogen level, topdressing levels and mechanical / biological treatments on turf quality, thatch formation and winter survival of velvet bentgrass greens in a coastal and a continental region of Scandinavia.

MATERIALS AND METHODS

Sites, Soil, and Weather Conditions

Velvet bentgrass ‘Legendary’ was seeded at a rate of 6 g m⁻² on experimental greens at Bioforsk Landvik (coastal location, 58 °N lat., 12 m a.s.l.) and Bioforsk Apelsvoll (inland location, 61 °N lat., 250 m a.s.l.) on 30 May and 26 June 2007, respectively. The rootzones were constructed according to USGA-specifications (USGA Green Section Staff, 2004) with a 30 cm layer of sand amended with 15% (v/v) of *Sphagnum* peat at Landvik and 20 % (v/v) garden compost (‘Green Mix’, Høst AS, Grimstad, Norway) at Apelsvoll. Soil samples taken in April 2008 showed lower pH and lower contents of phosphorus, potassium, magnesium and calcium in the rootzone at Landvik than at Apelsvoll (Table 1).

The monthly precipitation and mean monthly temperature for the entire experimental period at both sites are shown in Table 2. The total precipitation on both sites was higher than the 30-yr normal during all growing seasons. Unusually wet months were July 2007, August 2008, and July 2009. A long drought period during the growing season 2008 was observed at

Landvik with no rainfall from 2 May till 13 June. A total of 23, 70, and 104 days of snow cover were observed at Landvik during the winters 2007-08, 2008-09, and 2009-10, respectively, but the green was never covered by ice or water for more than a couple of weeks. By contrast, the green at Apelsvoll had a continuous layer of ice for nearly four months during the winter 2007-08 and was even more injured by melting water during the winter 2008-09. Therefore, unlike the situation at Landvik, all experimental plots at Apelsvoll were reseeded in spring 2008, and the whole experiment was discontinued in spring 2009.

Table 1. Chemical characteristics of soil samples taken to 20 cm depth at Landvik and Apelsvoll prior to the start of the study (April 2008).

Sites	pH	P-AL	K-AL	Mg-AL	Ca-AL
		————— mg per 100 g dry soil —————			
Apelsvoll	7.1	5.5	4.0	4.1	60
Landvik	6.3	1.0	2.0	1.7	15

Experimental Treatments and Plot Maintenance

Experimental treatments. At both sites a three-factorial experiment was arranged according to a split-plot design with three blocks (replicates). At Landvik, each block contained eight main plots with combinations of a low or a high nitrogen level (75 or 150 kg N ha⁻¹ yr⁻¹) with one of four mechanical/biological treatments: 1) Weekly grooming, 2) Weekly grooming + monthly verticutting, 3) Weekly grooming + monthly spiking; or 4) Weekly grooming + monthly spiking + monthly application of the biological product ‘Thatchless’ (Novozymes Biologicals, France) (Table 3). Due to limited area available at Apelsvoll, only the three strictly mechanical treatments (1, 2, and 3) were included there, giving six main plot combinations (Table 3). At both sites each main plot was split into two subplots that received either light or heavy topdressing (0.5 mm or 1 mm of pure sand with no organic matter; grain size 0.2-0.8 mm; Baskarp, Sweden) every second week. Due to different durations of the growing season, these rates corresponded for the entire growing season to 7 and 14 mm sand at Landvik and 4.5 and 9 mm sand at Apelsvoll. Topdressing sand was usually applied on Fridays before weekends with irrigation but no mowing of the greens. Subplots were 2 m by 1.5 m and main plots 2 m by 3 m at both sites.

Table 2. Mean monthly air temperature and monthly precipitation at Landvik and Apelsvoll during the experimental period compared with normal values.

Month	Air temperature					Total precipitation				
	2007	2008	2009	2010	Normal†	2007	2008	2009	2010	Normal
	° C					mm				
	<u>Landvik</u>									
January	-	2.8	0.5	-5.4	-1.6	-	358	178	44	113
February	-	4.3	-2.2	-6	-1.9	-	82	65	83	73
March	-	2.3	2.7	1.4	1	-	184	116	62	85
April	-	6	7.9	6.2	5.1	-	91	28	33	58
May	10.2	11.9	11.3	-	10.4	107	20	76	-	82
June	15.9	14.7	14.8	-	14.7	109	75	54	-	71
July	15.5	17.3	16.8	-	16.2	213	101	244	-	92
August	16.2	15.6	15.9	-	15.4	132	250	99	-	113
September	12	11.6	13	-	11.8	59	137	79	-	136
October	7.7	7.9	6.1	-	7.9	53	153	252	-	162
November	3.6	3.7	5.3	-	3.2	69	123	295	-	143
December	0.9	0.6	-1.7	-	0.2	155	80	218	-	102
May-Sept.	14	14.2	14.4	-	13.7	620	583	552	-	494
Year						-	1653	1703	-	1230
	<u>Apelsvoll</u>									
January	-	-1.5	-4.3	-	-7.4	-	71	70	-	37
February	-	-0.4	-8.5	-	-7	-	27	64	-	26
March	-	-1.2	-0.7	-	-2.5	-	52	47	-	29
April	-	4.7	5.9	-	2.3	-	42	67	-	32
May	9.7	10.2	-	-	9	49	64	-	-	44
June	15.6	14.1	-	-	13.7	82	43	-	-	60
July	14.8	16.7	-	-	14.8	118	82	-	-	77
August	14.7	13.6	-	-	13.5	77	140	-	-	72
September	9.3	9.3	-	-	9.1	73	47	-	-	66
October	5.1	5.7	-	-	4.6	6	57	-	-	64
November	-0.3	0	-	-	-1.3	45	64	-	-	53
December	-4	-4.4	-	-	-5.3	210	36	-	-	40
May-Sept.	12.8	12.8			12	399	376	-	-	319
Year						-	726	-	-	600

† Reference period 1961-90.

Table 3. Schedule for mechanical and biological treatments at Landvik and Apelsvoll.

Mechanical/biological treatment†	Depth	Frequency	Treatments per year	
			Landvik (2008 /2009)	Apelsvoll (2008)
Grooming	0.1-0.2 mm	Weekly	21	15
Vertical cutting	2 mm	Monthly	6/5	3
Spiking	6 cm	Monthly	6/5	3
'Thatch less™'	-	Monthly	8	-

† The following treatments and their combinations were compared in the study: 1) grooming alone, 2) grooming + vertical cutting, 3) grooming + spiking, and 4) grooming + spiking + 'Thatch less™' (only at Landvik).

Fertilization. Including a presowing application of organic fertilizer, the total input of N, P, and K during grow-in in 2007 (before start of experimental treatments) amounted to 134, 18, and 108 kg ha⁻¹, at Landvik and 100, 20, and 88 kg ha⁻¹ at Apelsvoll, respectively.

During the first experimental period August – October 2007, the two nitrogen levels at Landvik equalled 34 and 69 kg N ha⁻¹, the corresponding amounts at Apelsvoll being 17 and 34 kg N ha⁻¹, respectively. In 2008 (Landvik and Apelsvoll) and 2009 (Landvik only), the total N inputs were 75 or 150 kg N ha⁻¹ at both sites as indicated in the experimental plan. At Landvik, granular Arena® products were applied at two week intervals except for four applications of liquid fertilizer Arena® Crystal (Yara International ASA, Norway) in 2008. At Apelsvoll (Table 1), granular Arena® products were supplemented with ammonium sulfate 21-0-0 (Yara International ASA, Norway) due to the high pH level (Table 1). Inputs of P, K, and other nutrients varied proportionately with the N input. Thus, the relative N:P:K rate was overall 6:1:6.

The mechanical treatments were started on 31 July and 28 Aug. 2007 at Landvik and Apelsvoll, respectively. Weekly grooming was performed using John Deere grooming attachment mounted on a John Deere 220A walk-behind mower (Moline, IL) and adjusted to a depth of 0.1-0.2 mm from the surface. Monthly vertical cutting was performed to 2 mm depth using Aztec verticutter pod (Allett mowers LTD, Arbroath) mounted on a Aztec drive unit (Allett mowers LTD, Arbroath). Except for the first treatment using 6 mm hollow tines (i.e. actually a coring treatment) at Landvik, monthly spiking was performed at both sites with 8 mm solid tines mounted on a John Deere Aerator 800 (Moline, IL) to a working of 6 cm. As the first spiking (coring) was rather disruptive at both sites, we suspended all mechanical

treatments for the rest of 2007. The treatments were resumed on 25 June 2008 at Landvik and 9 July 2008 at Apelsvoll. In 2009, the treatments at Landvik were applied from 26 May to 20 Aug.

The biological product ‘Thatch less™’ contained 0.04% microbial cultures (*Bacillus licheniformis* 1.35×10^8 cfu/mL and *B. subtilis* 1.65×10^8 cell forming units/mL) and 24.10% cellulase enzymes derived from *Trichoderma reesei* (700 endo-glucanase units /g). It was applied at 10 L ha^{-1} at 10 day interval in the beginning of each growing season and then monthly just after spiking.

Mowing. The greens were mowed with John Deere 220A or Allett walk-behind green’s mowers three times a week to 3 mm apart from May-June and September-October when mowing heights were raised to 4.5 mm at Landvik and 5 mm at Apelsvoll.

Rolling and artificial wear. The experiment at Landvik was exposed to artificial wear from pulling a friction wear roller with soft spikes over the plots three times per week. This treatment corresponded to 20 000 rounds of golf per year.

Irrigation. In addition to light irrigation (5-7 mm) after fertilization, topdressing, and application of ‘Thatch less™’, both trials received approximately 7 mm of irrigation water at 10 mm water deficit as determined by an open evaporation pan.

Pesticides and growth regulators. Due to severe infestation of *Pythium* spp. and *Microdochium nivale* in the late autumn 2007, the trial at Landvik was sprayed with Amistar Duo, 1.0 L ha^{-1} (azoxystobin, $200 \text{ g a.i. ha}^{-1}$ + propconazole, $125 \text{ g a.i. ha}^{-1}$) on 17 Oct. 2007. No other pesticides or plant growth regulators were used in the trial.

Registrations and Statistical Data Analyses

Turfgrass Visual Quality and Winter Survival. Visual assessments of turf quality were started on the 2-months old green plots and were conducted biweekly for turfgrass overall impression (scale from 1 = uneven and very poor turf, to 9 = even and very good turf; acceptable level = 5) and monthly for shoot density (scale from 1 = very thin to 9 = very dense), color (scale from 1 = very light to 9 = very dark), diseases (% of plot covered with diseased turf), moss (% of plot covered with moss), and turf coverage (% of plot covered with healthy uninjured turf of the sown species). Assessments were always conducted prior to mechanical treatments. The last observations were accomplished in the spring of 2009 at Apelsvoll and in the spring of 2010 at Landvik.

Playing quality at Landvik was recorded monthly and assessed as surface hardness and ball roll distance. These observations were always performed prior to mechanical treatments and one day after mowing. Surface hardness was recorded as gravity units (G) using the Clegg Soil Impact Tester (2.25 kg hammer, Lafayette Instrument Co., Lafayette, IN). Readings were taken after each of two successive blows by the hammer from 0.46 m height at two places per plot. Ball roll distance was determined using a stimpmeter modified for research plots (Gaussoin et al., 1995). The stimpmeter had its ball release notch 38 cm rather than 76 cm from the bevelled end, and measurements were always taken in two directions. At Apelsvoll, playability was recorded only in late September and October 2008.

For each experimental year, data for visual observations and playing quality were pooled into four consecutive periods each containing 2-6 registrations: spring (March and April), summer before mechanical treatments (from early May until verticutting and spiking started), summer after mechanical treatments (till 1 September), and fall (September and October).

Thatch assessments. Mat depth and content of organic matter in the mat layer were determined from 4 and 2 uncompressed soil cores (2.4 mm in diameter), respectively, taken from each plot in September at both sites. For determination of organic matter content, foliage above the mat layer and roots below it were removed. The weight of organic matter (ignition loss) was determined as the difference between sample dry weight (105 °C for 48 h) and sample ash weight (550°C for 3 h). The content of organic matter in mat was calculated individually for each core as: organic matter (%) = (sample ignition loss / sample dry weight) x 100 %; the data were averaged for each plot prior to variance analyses. The net accumulation of organic in the mat layer was calculated as: organic matter (g m⁻²) = sample ignition loss / surface area of taken soil core.

Water Infiltration Rates were measured each year in September-October using a double ring infiltrometer with an outer ring diameter of 12.8 cm and an inner ring diameter of 4.5 cm. The infiltrometer was inserted 2 cm into the turf after the soil had been saturated by irrigation or natural rainfall. Water levels in the inner ring were measured at two sites per plot after three minutes of infiltration. The infiltration rate was expressed as mm pr hour.

The data were analysed by the SAS procedure PROC ANOVA using statements providing an analysis for a split-plot design separately for each year (SAS Institute, 2008). Special analyses were performed to compare the mat depth and organic matter percentage at Landvik and Apelsvoll in 2008 (site effect) and to compare the mat depth and net accumulation of organic matter in 2008 and 2009 at Landvik (year effect). Site and year

effects were tested individually against replicates nested within either “site” or “year”. The Fisher’s least-significant-difference (LSD) was used for the testing of statistical hypothesis at the 5% probability level.

RESULTS

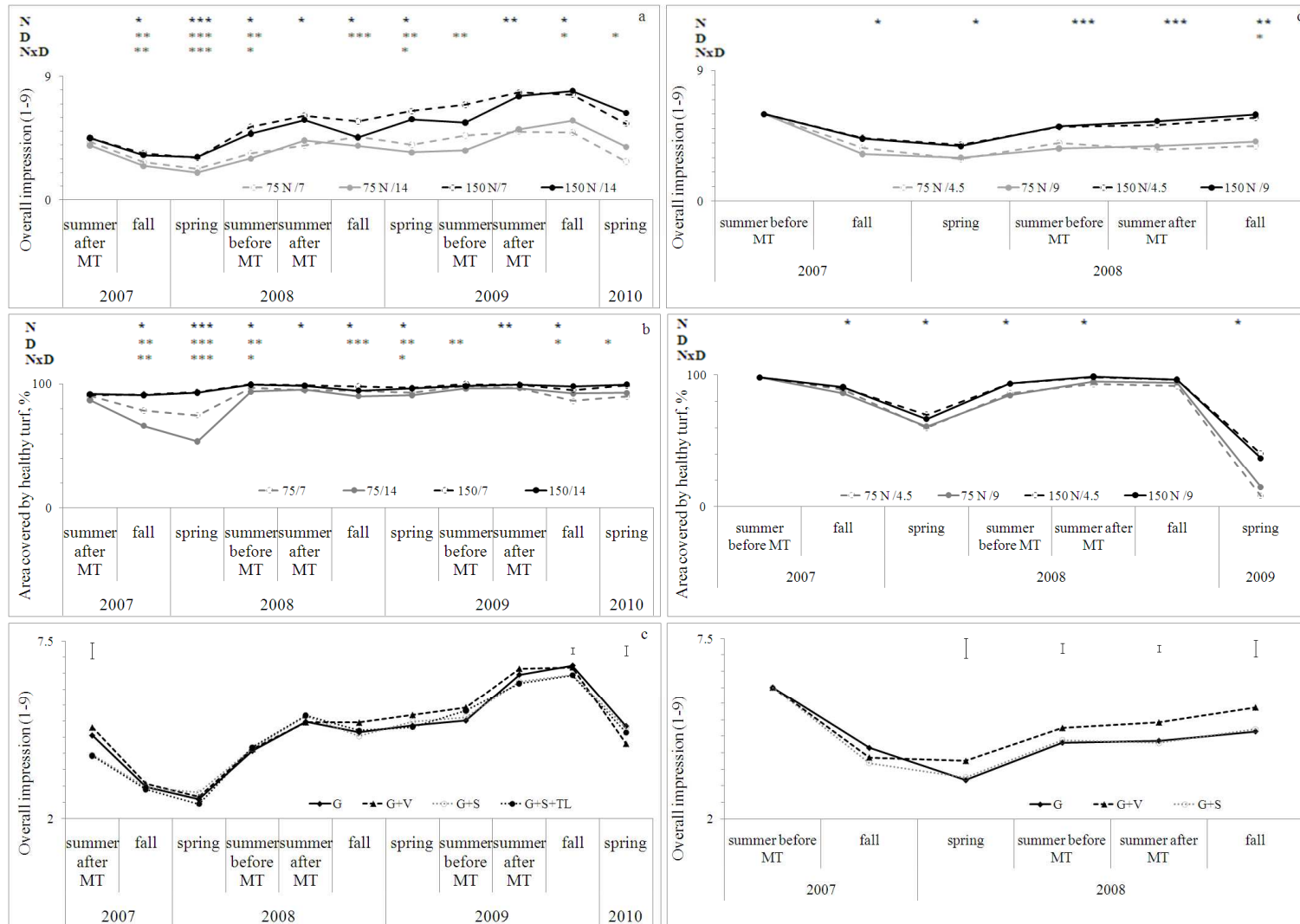
Turfgrass Visual Quality, and Winter Survival

At *Landvik*, nitrogen and topdressing rates had the highest impact on velvet bentgrass visual performance. Regardless of mechanical/biological treatments and topdressing levels, 150 kg N ha⁻¹ yr⁻¹ led to a significantly better overall impression (Fig. 1a) and significantly higher shoot density (data not shown) than 75 kg N ha⁻¹ yr⁻¹ throughout the entire experimental period. Plots receiving 150 kg N ha⁻¹ yr⁻¹ were characterized by a rapid recovery after the first disruptive mechanical treatments in the summer of 2007 and a quick green-up in spring. Plots receiving 75 kg N ha⁻¹ yr⁻¹ did not achieve acceptable overall impression (level 5) until in the summer of 2009.

The light topdressing led to a better overall impression (Fig. 1a) and a higher shoot density (data not shown) at the low nitrogen rate (significant interaction) until the summer of 2008 and regardless of nitrogen rate from the summer of 2008 until the summer of 2009. A reversion in response to topdressing amount occurred in the summer of 2009. After that, the heavy topdressing tended to provide better overall impression (Fig. 1a) and shoot density (data not shown) at both nitrogen rates.

A significant interaction between nitrogen and topdressing rates was observed in response to an outbreak of pink snow mold (*Microdochium nivale*) in the spring of 2008. Regardless of topdressing level, the area covered with diseased turf only amounted to 7% on plots receiving 150 kg N ha⁻¹ yr⁻¹ (Fig. 1b), but heavy topdressing led to more injury (46 %) than light topdressing (25 %) on plots receiving 75 kg N ha⁻¹ yr⁻¹.

Mechanical or biological treatments had no significant effect on turfgrass visual quality except at the beginning and end of the experiment (Fig. 1c). The initial coring caused a significant decrease turfgrass overall impression in the late summer of 2007 (Fig. 1c).



Landvik

Apelsvoll

Figure 1. Overall impression and percent of plot area covered with healthy velvet bentgrass as affected by nitrogen rate (75 vs. 150 kg N ha⁻¹ yr⁻¹) and topdressing level (7 vs. 14 mm D yr⁻¹) (*a* and *b* at Landvik, and *d* and *e* at Apelsvoll), and overall impression as affected by mechanical/biological treatments: grooming (G), vertical cutting (V), spiking (S), biological thatch control agent ‘ThatchlessTM’ (TL) (*c* at Landvik and *f* at Apelsvoll). The growing seasons were divided into four consecutive periods with 2-6 registrations each: early spring (March-April), summer before mechanical treatments (MT) started (on 24 June 2008 and 26 May 2009 at Landvik and on 9 July 2008 at Apelsvoll), summer after MT (from start of MT till 1 September), and fall (September-October). Notes for statistical significance for graphs a, b, d, e: * significant at 0.05 probability level; ** significant at 0.01 probability level; *** significant at 0.001 probability level; the lack of a note when nonsignificant (for all graphs). Vertical bars on graphs *c* and *f* represent LSD ($\alpha=0.05$).

In the fall of 2009, plots that received spiking also had a lower overall impression (Fig. 1c) and shoot density (data not shown) than plots that received only grooming or grooming in combination with vertical cutting. While there was an insignificant tendency for vertical cutting to improve the visual impression from the fall of 2008 until the fall of 2009, this treatment also led to a significant decrease in the same character in the spring of 2010.

Differences in turf color in response to experimental factors first became obvious in the summer of 2008 (data not shown). Plots that received 150 kg N ha⁻¹ yr⁻¹ were significantly darker than plots that received 75 kg N ha⁻¹ yr⁻¹. At the higher nitrogen rate there were no differences in color between plots receiving 7 or 14 mm topdressing per year. However, at the low nitrogen rate, plots receiving light topdressing were darker than plots receiving heavy topdressing from the summer of 2008 until the summer of 2009. In the summer of 2009, the effect of topdressing on color was completely reversed: plots with heavy topdressing became darker than plots with light topdressing under the low nitrogen rate (data not shown). An undesirable infestation of moss (*Bryum* spp.) was observed at Landvik in 2009 and 2010 (Fig. 2). Less competition from the turf and more competition from the moss were indicated at the low nitrogen rate, especially when topdressing was also low.

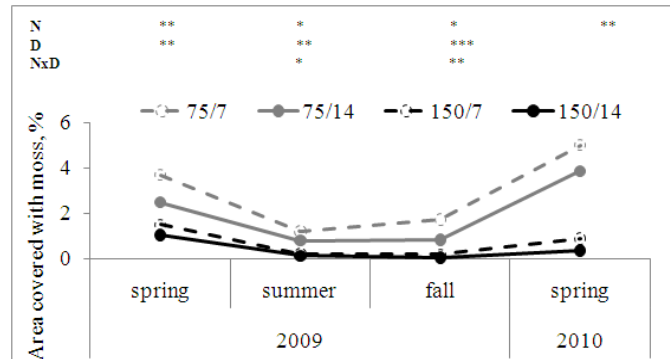


Figure 2. Effects of nitrogen rate (75 vs. 150 kg N ha⁻¹ yr⁻¹) and topdressing level (7 vs. 14 mm D yr⁻¹) on moss development on velvet bentgrass' plots at Landvik. The growing season 2009 was divided into three periods: spring (March-April), summer (May-August), and fall (September-October). Notes for statistical significance: * significant at 0.05 probability level; ** significant at 0.01 probability level; * significant at 0.001 probability level; and without note when NS.**

Apelsvoll. Unlike topdressing, nitrogen fertilization had a strong effect on velvet bentgrass overall impression, winter survival, and recovery throughout the study (Fig. 1d-e). On plots with low and high nitrogen rates, the winter injury caused by snow mold, frost and/or ice resulted in 39 and 32% damage in the spring of 2008, and 88% and 61 % damage in the spring of 2009, respectively (Fig. 1e). Plots that received 150 kg N ha⁻¹ yr⁻¹ achieved an

acceptable overall impression in June of 2008, but the overall impression on plots with 75 kg N ha⁻¹ yr⁻¹ remained poor (scores from 3.6-4.1) from June to October 2008. In 2008, shoot density was also significantly higher (mean score 6.6 vs. 4.7) and turfgrass color significantly darker (mean scores 5.9 vs. 4.1) on plots receiving 150 kg N ha⁻¹ yr⁻¹ than on plots receiving 75 kg N ha⁻¹ yr⁻¹ (data not shown).

Following the first mechanical treatment on 28 Aug. 2007, turfgrass overall impression at Apelsvoll decreased from 6.0 to 4.3 and from 6.0 to 3.5 on plots with high and low N rates, respectively (Fig. 1d). In 2008 plots that received grooming in combination with vertical cutting had higher overall impression than plots that received grooming alone or in combination with spiking (Fig. 1f).

Playability

At *Landvik*, the ball roll distance was overall 6-17% longer on plots receiving 75 compared with 150 kg N ha⁻¹ yr⁻¹ (Fig 3). The main effects of topdressing rate or mechanical/biological treatments on this character were not significant.

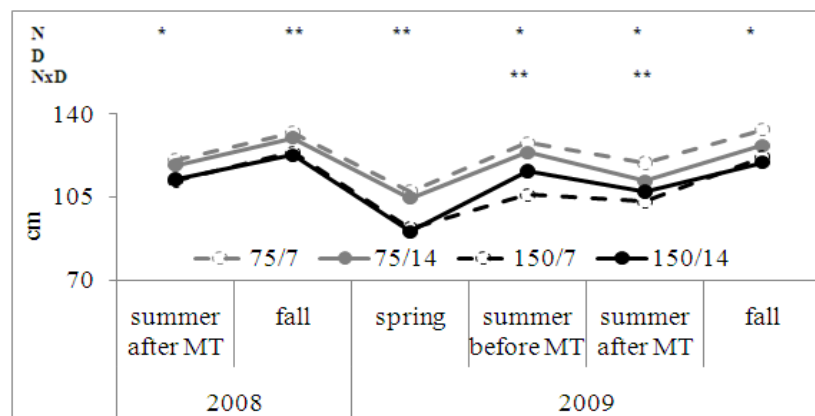


Figure 3. Ball roll distance as affected by nitrogen rate (75 vs. 150 kg N ha⁻¹ yr⁻¹) and topdressing level (7 vs. 14 mm D yr⁻¹) at *Landvik*. The growing seasons were divided into four consecutive periods with 2-6 registrations each: spring (March-April), summer before mechanical treatments (MT) (from early May till MT started – on 24 June 2008 and 26 May 2009), summer after MT (from start of MT till 1 September), and fall (September-October). Notes for statistical significance: * significant at 0.05 probability level; ** significant at 0.01 probability level; *** significant at 0.001 probability level; and without note when NS.

Surface hardness was significantly affected by all three experimental factors, but the interactions were not significant (Table 4). The low nitrogen rate enhanced surface hardness by 8% in 2008 and by 16% in 2009 compared with the high. The effect of topdressing was less conspicuous, but in 2009, plots received 14 mm topdressing were 4% harder than plots received 7 mm. Among mechanical treatments, spiking in combination with grooming led to

the softest green surface, however, in summer and fall 2009, the biological product ‘Thatch less™’ increased hardness of plots also receiving spiking + grooming treatment.

At *Apelsvoll* the ball roll distance in the fall of 2008 was 8 cm longer on plots with the low N rate (129 cm) than with high (121 cm) (data not shown). No other factors affected ball roll distance significantly. Surface hardness was significantly affected by topdressing level, mechanical treatments, and their interaction (Table 4). Heavy topdressing increased the surface hardness of plots that received spiking in combination with grooming, but had no effect on the hardness of plots that received grooming only or vertical cutting in combination with grooming (Fig. 4).

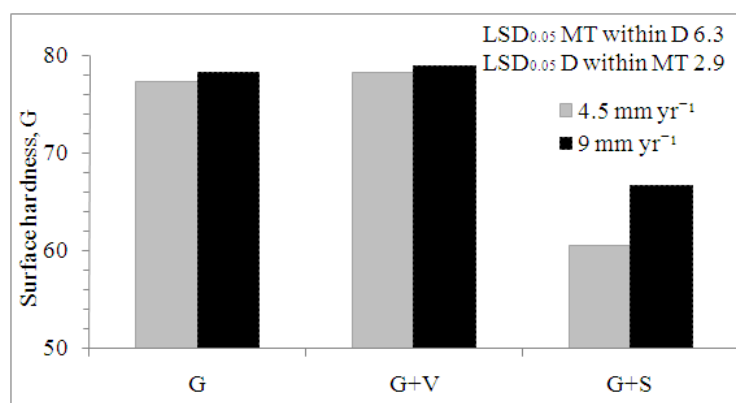


Figure 4. Effect of topdressing (D, 4.5 and 9 mm yr⁻¹) and mechanical treatments (MT: G, grooming; V, vertical cutting; S, spiking) in surface hardness (gravities) as measured using Clegg Soil Impact Tester in September 2008 at *Apelsvoll*.

Thatch assessments

Landvik - The content of organic matter in mat varied from 3.6% to 8.7% among the 16 treatment combinations of (data not shown). These levels remained stable from 2008 to 2009, but mat depth increased by 65-98% ($p < 0.001$) (Table 5). On average for topdressing levels, and mechanical/biological treatments, doubling the fertilizer rate from 75 to 150 kg N ha⁻¹ yr⁻¹ increased mat thickness by 4.4 mm by the end of 2008 and 9.8 mm by the end of 2009. The main effect of doubling the annual topdressing level from 7 to 14 mm was less pronounced with an increase in mat thickness of 2.9 mm by the end of 2008 and 6.9 mm by the end of 2009. By the end of 2009, vertical cutting had reduced mat depth by an average of 2.3 mm compared to plots with grooming only, but neither this effect nor the effects of spiking or ‘Thatch less™’ were significant.

Table 4. Effects of nitrogen rates, topdressing levels and mechanical/biological treatments (MBT) on surface hardness measured as gravities using a Clegg Soil Impact Tester at Landvik and Apelsvoll.

Source of variation	Level	Landvik						Apelsvoll		
		2008			2009			2008		
		df	Summer before MBT	Summer after MBT	Fall	Summer before MBT	Summer after MBT	Fall	df	Fall
		Gravities							Gravities	
N rate (N), kg ha ⁻¹ yr ⁻¹	75	77	85	82	76	88	89		75	
	150	73	79	71	68	73	70		72	
Topdressing (D), mm yr ⁻¹	7 (4.5) [†]	75	81	75	70	79	79		72	
	14 (9)	75	83	78	74	81	81		75	
Mechanical / biological treatments (MBT)	Grooming (G)	75	85	80	72	81	81		78	
	G+Vertical cutting	76	83	77	73	82	83		79	
	G+Spiking (S)	75	80	74	71	77	76		64	
	G+S+'Thatch less TM '	75	82	76	71	80	79		-	
	<i>LSD_{0.05} MBT</i>	-	2.7	3.5	-	1.9	2.5		4.4	
		ANOVA								
N		1	NS	*	***	*	**	***	1	NS
D		1	NS	NS	*	**	**	**	1	**
N x D		1	NS	NS	NS	NS	NS	NS	1	NS
MBT		3	NS	*	*	NS	**	**	2	**
N x MBT		3	NS	NS	NS	NS	NS	NS	2	NS
D x MBT		3	NS	NS	NS	NS	NS	NS	2	*
N x D x MBT		3	NS	NS	NS	NS	NS	NS	2	NS

* Significant at 0.05 probability level.

** Significant at 0.01 probability level.

*** Significant at 0.001 probability level.

[†] Topdressing amount in brackets is concern of Apelsvoll

Table 5. Thatch/mat characteristics measured as mat depth and percentage of organic matter (OM), and infiltration rates as affected by nitrogen rates, topdressing levels, and mechanical/biological treatments at Landvik and Apelsvoll.

Source of variation	Level	df	Landvik						Apelsvoll					
			Mat depth		OM in mat		OM per m ²		Infiltration rate	Mat depth		OM	OM per m ²	Infiltration rate
			2008	2009	2008	2009	2008	2009	2009	df	2008	2008	2008	2008
			mm		%		g		mm hr ⁻¹	mm	%	g	mm hr ⁻¹	
N rate (N), kg ha ⁻¹ yr ⁻¹	75		11.6	21.1	5.3	5.3	630	1129	34	10.8	6.4	548	27	
	150		16.0	30.9	6.5	6.4	854	1441	60	10.6	7.5	531	23	
Topdressing (D), mm yr ⁻¹	7 (4.5)†		12.3	22.5	7.1	7.1	769	1335	36	10.0	8.1	551	26	
	14 (9)		15.2	29.4	4.7	4.6	715	1235	58	11.4	5.7	529	25	
	Grooming (G)		14.1	27.4	6.3	6.4	791	1451	39	10.8	7	555	13	
Mechanical / biological treatments (MBT)	G+Vertical cutting		13.8	25.1	5.7	5.3	745	1201	27	10.8	6.9	560	17	
	G+Spiking (S)		13.6	25.9	5.8	5.9	713	1222	59	10.5	6.9	503	46	
	G+S+‘Thatch less™’		13.5	25.6	5.8	5.8	720	1265	62	-	-	-	-	
	<i>LSD_{0.05} MBT</i>		-	-	-	0.54	-	-	13.3	-	-	-	11.6	
<u>ANOVA</u>														
N		1	*	*	**	*	*	**	NS	1	NS	*	NS	NS
D		1	***	***	***	***	NS	NS	*	1	*	**	NS	NS
N x D		1	NS	NS	***	***	NS	*	NS	1	NS	**	NS	NS
MBT		3	NS	NS	NS	*	NS	NS	***	2	NS	NS	NS	**
N x MBT		3	NS	NS	NS	NS	NS	NS	NS	2	NS	NS	NS	NS
D x MBT		3	NS	NS	NS	NS	NS	NS	NS	2	NS	NS	NS	NS
N x D x MBT		3	NS	NS	NS	NS	NS	NS	NS	2	NS	NS	NS	NS

* Significant at 0.05 probability level.

** Significant at 0.01 probability level.

*** Significant at 0.001 probability level.

† Topdressing amount in brackets is concern of Apelsvoll.

Percentage of organic matter in the mat layer was significantly influenced by nitrogen rate, topdressing level, and their interaction (Tables 5, 6). By the end of 2009, percentage of organic matter increased by 1.9 units with an increase in N rate from 75 to 150 kg ha⁻¹ yr⁻¹ under light topdressing, but was virtually unaffected by nitrogen rate under heavy topdressing. By the same time, an increase in topdressing from 7 to 14 mm yr⁻¹ led to a 3.4 percentage points decrease in the content of mat organic matter on plots receiving 150 kg N ha⁻¹ yr⁻¹ as opposed to a mere 1.7 percentage points decrease on plots receiving 75 kg N ha⁻¹ yr⁻¹. The main effect of mechanical and biological treatments on percentage of organic matter was not significant in 2008, but by the end of 2009, the content was 1.1 percentage points lower on plots receiving vertical cutting plus grooming than on plots receiving grooming only (Table 5).

Table 6. Effect of nitrogen rate (N) and topdressing level (D) on the amount of organic matter (OM) in the mat layer.

		Landvik						Apelsvoll	
		OM in mat				OM per m ²		OM in mat	
		2008		2009		2009		2008	
D, mm yr ⁻¹	→	7	14	7	14	7	14	4.5	9
		———— % ————				———— g m ⁻² ————		———— % ————	
N, kg ha ⁻¹ yr ⁻¹	75	6.2	4.4	6.2	4.5	1114	1143	7.2	5.5
	150	8.1	5.0	8.1	4.7	1556	1326	9.0	6.0
LSD _{0.05}		0.64		0.95		143.6		1.39	
	N rates within D level	0.64		0.95		143.6		1.39	
LSD _{0.05}		0.57		0.77		184.3		0.61	
	D levels within N rate	0.57		0.77		184.3		0.61	

From 2008 to 2009, the accumulated dry weight of organic matter in the mat layer per unit area increased by an average of 73% ($p < 0.001$; Table 5). The main effect of topdressing level on this character was not significant. However, by the end of 2009, a significant interaction between nitrogen rate and topdressing level revealed, firstly, that the increase in mat organic matter dry weight with nitrogen rate was less dramatic under heavy topdressing than under light topdressing; and secondly, that the heavy topdressing reduced organic matter dry weight only at the high nitrogen rate (Table 6). The main effect of mechanical or biological treatments on mat organic matter dry weight per unit area was not significant.

At *Apelsvoll*, the average depth of the mat layer by the end of the growing season 2008 was 10.7 mm vs. 13.8 mm at Landvik ($p = 0.006$) and the average content of organic matter

was 6.9 % vs. 5.9 % at Landvik ($p=0.019$). Percentage of organic matter in the mat layer by the end of 2008 was significantly affected by nitrogen rate, topdressing level, and their interaction (Table 5): Under light topdressing, percentage of organic matter increased by 1.8 percentage points with a doubling of the nitrogen rate from 75 to 150 kg ha⁻¹ yr⁻¹, but under heavy topdressing, the increase was nonsignificant (Table 6). Correspondingly, an increase in the topdressing level from 4.5 to 9 mm caused a 3.0 percentage points decrease in the organic matter content at 150 kg N ha⁻¹ yr⁻¹ in contrast to a mere 1.7 points decrease at 75 kg N ha⁻¹ yr⁻¹. There was no effect of mechanical treatments on percentage of organic matter in the mat layer at Apelsvoll. Moreover, the total content of organic matter in the mat layer was not significantly affected by any of the experimental treatments at this continental site (Table 5).

Water Infiltration Rate

Compared with grooming alone, spiking in combination with grooming enhanced the infiltration rate by 51% at Landvik and 254% at Apelsvoll. Addition of ‘Thatch less™’ to the grooming plus spiking treatment caused no further increase at Landvik. As a main effect, doubling the topdressing level significantly improved infiltration by 61% at Landvik, but had no effect at Apelsvoll. Fertilizer rate had no significant effect on infiltration rate at any site.

DISCUSSION

Nitrogen and topdressing

Turfgrass visual quality and playability. Velvet bentgrass is considered to require less nitrogen than creeping bentgrass (Brilman and Meyer, 2000; Torello, 2001). There are, however, few studies comparing fertility programs on either newly-established or mature greens of velvet bentgrass. A 5 year study by Skogley (1975) starting on a 3 year old green with velvet bentgrass ‘Kingstown’ on a fine sandy loam in Rhode Island (N rate during establishment is unknown) showed that 146 kg N ha⁻¹ yr⁻¹ led to better performance over time than 244 and 342 kg N ha⁻¹ yr⁻¹. Thirty-two years later Boesch and Mitkowski (2007), working at the same university, reported acceptable turf quality from nitrogen rates varying from 48 to 146 kg ha⁻¹ yr⁻¹ on greens sodded with velvet bentgrass ‘SR 7200’ (European name ‘Avalon’) on a silt loam soil. However, these authors concluded that velvet bentgrass required at least 196-243 kg N ha⁻¹ yr⁻¹ during first two years following nine months of

establishment from seeds on a sand root zone amended with 20-30 % (v/v) *Sphagnum* peat. Recently, Koeritz and Stier (2009) suggested that velvet bentgrass's response to nitrogen rate was cultivar specific. They indicated that a nitrogen rate of 146 kg ha⁻¹ yr⁻¹ on a sand-based root zone was sufficient for newly established 'Vesper', but not for 'SR7200' ('Avalon').

For grow-in of creeping bentgrass greens, White (2003) recommended a presowing application of 50 kg N ha⁻¹ followed by inputs of 15 to 30 kg N ha⁻¹ every five days, adding up to 200-300 kg N ha⁻¹ during the first two months after sowing. To what extent this recommendation is relevant for velvet bentgrass is unknown, however, our experiments were initiated two months after sowing on greens that had received only 100-134 kg N ha⁻¹ including the presowing application. Although plant cover was almost 100% and the visual quality acceptable at the start of experimentation, the low turfgrass overall impression in the fall of 2007 and the spring of 2008 was most likely caused by the early start of mechanical treatments at both sites. During 2008 and 2009, turfgrass overall impression gradually increased, but just like 'Vesper' in the study by Koeritz and Stier (2009), 'Legendary' in our study always performed better under the high than under the low nitrogen rate until the summer of 2009, approximately two years after sowing. However, as time went by, 150 kg N ha⁻¹ yr⁻¹ led to excessive thatch formation and a reduction in turfgrass ball roll distance and surface hardness. When combined with light topdressing, the high nitrogen rate also resulted in the highest content of organic matter in the mat layer (8.1%). By comparison, the low nitrogen rate of 75 kg ha⁻¹ yr⁻¹ considerably delayed establishment and recovery of velvet bentgrass, but once the turf became mature about two years after sowing, the rate was sufficient to provide adequate turfgrass overall impression. We therefore conclude that the optimal nitrogen rate for newly established velvet bentgrass greens was closer to 150 than to 75 kg N ha⁻¹ yr⁻¹, but with respect to thatch formation and playing quality, 75 kg N ha⁻¹ yr⁻¹, gave the best result in the long run.

The negative effect of heavy topdressing on the visual quality of the immature green at Landvik can be explained by the turf not being able to absorb sufficient amounts of sand. Incorporation of sand into the green surface can be a big hurdle in high density turfgrass varieties (e.g. Kauffman et al. 2009; Pippin 2010), and this problem may be even more accentuated in velvet bentgrass, especially if topdressing is not combined with mechanical treatments (Pease, 2009). Towards the end of the experimental period, the high topdressing level not only improved surface hardness, but also resulted in darker turfgrass color, higher shoot density, and better overall impression, especially at the low nitrogen rate. Better turfgrass quality with a high topdressing level was also reflected by less infestation of moss.

Besides the direct effect on turfgrass competitive ability, the heavy topdressing probably resulted in a dryer surface due to less organic matter in the mat layer, and this probably reduced the competitive ability of the moss. It is well documented that moss infestation will be most pronounced under wet conditions and at low fertilizer inputs (e.g. Brauen et al., 1986; Hummel, 1994; Cook et al., 2002). Borst et al. (2009) also found topdressing to be helpful in providing long-term moss control on putting greens.

Winter survival. The fact that less pink snow mold was observed on plots with 150 kg N ha⁻¹ yr⁻¹ than with 75 kg N ha⁻¹ yr⁻¹ at Landvik does not contradict the opinion that snow mold development on turfgrass is enhanced by excessive nitrogen application in fall (Smith et al., 1989). Neither in 2008 nor in 2009 was our highest nitrogen rate excessive for immature turf, and nitrogen inputs in the fall were adjusted to ensure good acclimation. Within reasonable limits, there is substantial evidence that increasing fertilizer inputs will improve turfgrass winter survival and spring growth rather than suppress it (e.g. Carow et al., 2001; Lloyd, 2009).

The less clear-cut effects of nitrogen, topdressing and their combination on winter survival at Apelsvoll than at Landvik during the winter of 2007-08, was probably due to differences in turf stand prior to winter, weather conditions, and injury pattern. At Landvik visible symptoms of pink snow mold only developed during a few days of snow cover after a sudden snow fall in late March. At Apelsvoll, severe injury on all plots was caused by nearly four months of ice cover, and the winter damage was primarily abiotic. Moreover, the combination of a short growing season and mechanical treatments as late as 28 Aug. 2007 probably left the turf with limited time to recover and acclimate before the winter at Apelsvoll. Although velvet bentgrass at Apelsvoll was set severely back during the winter 2007-08 and mostly died due to submersion in melting water during the winter 2008-09, a parallel variety evaluation trial on the same experimental green confirmed our previous findings (Molteberg et al., 2008) that winter survival of velvet bentgrass is mostly better than of creeping bentgrass under Nordic conditions (Molteberg et al., 2010).

Thatch. Based on rootzone physical properties (macroporosity and hydraulic conductivity), McCoy (1992) and Murphy et al. (1993b) considered that critical values for organic matter content in rootzone were 3.5 % and 4.5 %, respectively. The limit is also used for the mat layer (Carrow, 2004). Compared with a two year old creeping bentgrass green, which showed equally low content of organic matter in mat either undressed (1.38 %) or topdressed (1.30 %) (McCarty et al., 2005), the percentage of organic matter in our velvet bentgrass mats were relatively high (3.6-8.7 %) already by the end of the first experimental

year. One percentage point higher organic matter content in the mat at Apelsvoll than at Landvik could be due to a colder summer with less degradation of organic matter, but the main reason for the thinner layer with more organic matter was probably less frequent topdressing at the former site.

The fact that percentage of organic matter in the mat layer increased with increasing nitrogen rate under light topdressing, but remained low under heavy topdressing suggests that topdressing contributed not only to thatch dilution, but also to thatch degradation. This is in line with microscopic observations showing more thatch degradation in samples from old velvet bentgrass sod receiving regular soil topdressing compared with sod that had not received topdressing for nearly 20 years (Ledeborer and Skogley 1967). Skogley (1975) also found that an increase in annual fertilizer rate from 146 to 342 kg N ha⁻¹ did not influence percentage organic matter in mat samples from an eight year old velvet bentgrass green. At the same time, there are numerous reports showing increasing nitrogen rates to exacerbate thatch problems in various turfgrass species (e.g. Meinhold et al. 1973; Potter et al. 1985; Davis and Dernoeden 2002). Our data suggest that one reason for these contradictory results might be that topdressing rates were sufficient to provide optimal conditions for thatch degradation in the studies by Skogley (Ledborer and Skogley, 1967; Skogley, 1975) but not in other studies. Provided that the oxygen concentration in the mat is adequate, more nitrogen will not only stimulate turfgrass growth but also microbial degradation of organic matter through amplification of soil microbial communities (Blagodatskaya and Kuzyakov, 2008) and/or a lower C:N ratio (Berndt et al., 1992; Henriksen and Breland, 1999; Roudsari et al., 2008;). This effect has sometimes been referred to as the “*added nitrogen interaction*” (Jenkinson et al., 1985) or the “*positive priming effect*” (Kuzyakov et al., 2000).

Mechanical and biological thatch control

Probably due to the initially low content of thatch on the newly established green, the effect of mechanical treatments on thatch formation in the mat layer did not become significant until the end of 2008. At this point, percentage of organic matter in the mat layer on plots receiving grooming plus vertical cutting was significantly lower than on plots receiving grooming only, with plots receiving grooming and spiking, with or without ‘Thatch less™’, in an intermediate position. Earlier studies also showed a negligible effect of vertical cutting on greens that were either young (McCarty et al., 2005; Barton et al., 2009) or had an initial content of organic matter in mat layer lower than 3.5% (McCarty et al., 2007). In contrast, vertical cutting twice

per year significantly reduced organic matter in mat from 15.3% to 14.1% even without topdressing on a bermudagrass home lawn (Carrow et al., 1987). The fact that vertical cutting resulted in the lowest content of organic matter in the mat layer at Landvik is not surprising as this was only treatment where organic matter was actually removed from the green. No effect of mechanical treatments on percentage of organic matter in the mat layer after one year at Apelsvoll can probably be explained by the low number of treatments compared to Landvik.

The first coring at Landvik and spiking at Apelsvoll was more disruptive than beneficial. This was probably due to the low stability (Carrow et al., 2001), softening and scalping of the immature turf (McCarty et al., 2005), and/or the low recuperative capacity of velvet bentgrass (Boesch et al., 2007). In contrast, aerification improved turf quality on a three to six year old creeping bentgrass green (Murphy et al., 1993a), reduced scalping with no effect on turf quality on a mature bermudagrass green (White and Dickens, 1984), and had beneficial effect on spring green-up of a bermudagrass homelawn (Carrow et al., 1987).

The reason why grooming plus vertical cutting resulted in better winter survival than the other mechanical treatments at Apelsvoll was probably that this treatment provided a smooth surface with better mowing quality and less risk for scalping before the winter than grooming only or grooming plus spiking. On the other hand, the slightly reduced quality in the spring of 2010 on plots that had been verticut the previous season can be explained by slow recovery after the treatment being conducted as late as 20 Aug. 2009 due to growth cessation and the unusually low temperatures in October 2009. In Scandinavia, it is commonly observed that velvet bentgrass has a more rapid growth cessation in the fall than other turfgrass species.

Although spiking resulted in softer green surface, it significantly improved infiltration rate. A similar effect, but of coring, was reported by McCarty et al. (2005). Conversely, Murphy et al. (1993a) found no differences in field infiltration rates on mature creeping bentgrass green exposed to spiking or coring, compared with an untreated control.

In the summer and fall of 2010, the 4 % increase in hardness by ‘Thatch less™’ applications on plots receiving spiking in combination with grooming was probably due to enhanced thatch degradation. However, as we observed no significant difference in the organic matter content between the corresponding treatments, it might be speculated that surface hardness is a more sensitive character reflecting thatch degradation than is organic content per se. One explanation may be that cellulase enzymes derived from *Tricoderma reesei* in ‘Thatch less™’ stimulated degradation of soft and extensible microfibrils of cellulose, but had little or no effect on the degradation of lignin (Sidhu et al., 2010) which probably contributes more to surface hardness. McCarty et al. (2005) found that application

two times per year of a biological thatch control product, which contained selected microorganisms, bioactive ingredients and some nutrients, did not reduce thatch on a newly seeded creeping bentgrass green.

CONCLUSION

This research has confirmed our hypothesis that moderate nitrogen inputs and heavy topdressing rates are key elements in maintenance of velvet bentgrass golf greens. Apart from the presowing application, an optimal N rate of at least 150 kg N ha⁻¹ can be conjectured during the first year after sowing once grow-in is completed; the nitrogen rate can be gradually reduced for mature turf. Once the green is established, topdressing rates should be maintained at a level of 10-14 mm per year to keep thatch formation within acceptable level. Weekly grooming, monthly verticutting, and spiking once or twice per year can be recommended as standard maintenance program for a mature velvet bentgrass green.

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PAPER V



Effects of rootzone composition and irrigation regime on performance of velvet bentgrass golf greens

Tatsiana Espevig^{1,2*} and Trygve S. Aamlid^{1*}

¹Norwegian Institute for Agricultural and Environmental Research, Bioforsk Landvik, Grimstad, N-4886, Norway; ²Department of Plant and Environmental Science, Norwegian University of Life Sciences, Ås 1432, Norway

ABSTRACT

The use of velvet bentgrass (*Agrostis canina* L.) on golf greens is limited by sparse knowledge on optimal maintenance. The objectives of this study were to clarify the effects of rootzone composition (SS; straight sand, or GM; sand amended with 20% v/v garden compost) and irrigation regime (LF; light and frequent, or DI; deep and infrequent) on turfgrass visual quality, playability, thatch formation, root development, and nutrient leaching. A project was conducted from August 2007 to October 2009 on a USGA green seeded in June 2007 with velvet bentgrass 'Legendary' at a coastal location in Norway (Landvik, 58°N). Better turf performance on GM than on SS was associated with 88% lower nitrogen loss in the form of nitrate/nitrite during the first year after sowing, less injury caused by *Microdochium nivale* and quicker recovery during both spring periods, and less water repellency at most investigated depths. Compared with light and frequent irrigation, deep and infrequent irrigation resulted in better overall impression, lower drainage volumes, and improved root development in the 10- to 20-cm soil layer in the second year after sowing. A decrease in turf visual quality on SS receiving light and frequent irrigation in the second year was associated with strong water repellency in the mat layer and low infiltration rate. Neither thickness nor content of organic matter in the mat layer were significantly affected by the treatments. A 20% higher surface hardness on SS vs. GM persisted only during the first year of the study.

Abbreviations: DI, deep and infrequent; GM, organic amendment 'Green Mix'; LF, light and frequent; SS, straight sand.

*Corresponding authors (trygve.aamlid@bioforsk.no, tanja.espevig@bioforsk.no)

INTRODUCTION

Due to water, fertilizer and pesticide restrictions, there is increasing interest for velvet bentgrass (*Agrostis canina* L.) as an ideal species for integrated pest management of golf greens in USA, Canada, and Europe. Velvet bentgrass performs better at low nitrogen rates (Skogley, 1976, Espevig et al. 2009), is more resistant to dollar spot (*Sclerotinia homoeocarpa*) and brown patch (*Rhizoctonia* spp.) (Brilman and Meyer, 2000), needs less irrigation water (DaCosta and Huang, 2006a, 2006b) and exhibits lower leaching of nitrates (Paré et al., 2006) than other bentgrass species. This very fine-textured turfgrass is also more tolerant to shade (Reid, 1933) and wear stress (Murphy et al., 2009), and it competes better against annual bluegrass (*Poa annua* L.) (Samaranayake et al., 2009) than creeping bentgrass (*Agrostis stolonifera* L.).

Despite these advantages, the use of velvet bentgrass on golf greens is limited by few experimental data on optimal maintenance of this turfgrass species. A special concern is thatch control. *Thatch* is defined as "an intermingled organic layer of dead and living shoots, stems, and roots of grasses that develops between the turf canopy of green vegetation and the soil surface", while *mat* is used for the layer which is formed when thatch is intermixed with sand in the case of topdressing (Beard, 2002). Among the problems caused by excessive thatch on golf greens are reduced water infiltration and increased risk for disease injury, scalping, dry spots, and poor playing quality (Jordan, 2008). We previously showed that the combination of a low N input and heavy topdressing resulted in acceptable visual turf quality and less than 5% organic matter in the mat of a 2-yr old velvet bentgrass green (Espevig et al., 2009). However, effects of rootzone composition and irrigation management in relation to thatch control on velvet bentgrass golf greens have not been investigated before.

Globally there is focus on water conservation, and much work has been devoted to effects of reduced irrigation on turf quality and plant health (Qian and Fry, 1996; DaCosta and Huang, 2006a,b; McCann, 2008). Deeper rooting as a result of infrequent irrigation was shown to enhance turfgrass survival during dry periods (Qian and Fry, 1996; Jordan et al., 2003; Fu and Dernoeden, 2009b). Irrigation influences leaching and runoff of nutrients and pesticides from golf greens (Mancino and Troll, 1990; Brauen and Stahnke, 1995; Starrett et al., 1995, 2000; Barton et al., 2006). Many studies showed that infrequent irrigation may lead to development of soil water repellency causing fingered flow and leaching from sandy soils (Bauters et al., 1998; Nektarios et al., 1999; Larsbo et al., 2008). Therefore, in spite of a mostly lower soil water content, deep and infrequent irrigation is often considered to cause

more drainage and nutrient leaching than light and frequent irrigation (Kenna and Snow, 2000; Barton and Colmer, 2006). This situation may, however, be different in a coastal climate where natural rainfall often results in oversaturation and thus drainage from the turfgrass rootzone.

The most widely used guidelines for putting green construction developed by United States Golf Association (USGA) does not require organic amendments to the sand-based rootzone (USGA Green Section Staff, 2004). Some authors therefore advocate the use of straight sand (SS) root zones as they believe that sand will be amended with organic matter over time (Hurdzan, 2004). Research, however, shows that this happens essentially at the 5-cm upper layer (Liu, 2004; Murphy, 2007). The low water holding capacity and poor nutrient retention of SS rootzones may lead to high leaching losses, especially on young greens (Brauen and Stahnke, 1995; Aamlid, 2005). Compost or other organic amendments contribute to less drainage and improved quality of sand-based rootzones (Petrovic, 1995; Engelsjord and Singh, 1997; Bigelow et al., 2000; Murphy et al., 2004; Wu et al, 2007; Aamlid et al., 2005). It has been proved that amendment with compost increases microbial numbers in sand-based root zones (Aamlid et al. 2009). However, the studies regarding the specific effect of compost on thatch formation are limited (Liu, 2004).

The objective of this study was to clarify the effects of rootzone composition and irrigation regime on turfgrass visual quality, playability, root development, thatch formation, and nutrient leaching from velvet bentgrass golf green. Our hypotheses were: 1) Velvet bentgrass greens can be maintained at higher turf quality levels, with less thatch formation, and with longer irrigation intervals if compost is included in the rootzone; and 2) In temperate coastal climates with high annual precipitation, deep and infrequent, as opposed to light and frequent irrigation, will provide better turfgrass quality, deeper root development, and reduced leaching losses on velvet bentgrass greens.

MATERIALS AND METHODS

Site and Weather Conditions

The experiment was conducted from 21 Aug. 2007 to 1 Oct. 2009 on a USGA green (USGA Green Section Staff, 2004) containing 1 m x 2 m stainless steel lysimeters, earlier described by Aamlid et al. (2009), at Bioforsk Øst Landvik, Norway (58° 9' N; 8° 30' E, 5 m a.s.l.). The

surface area of each plot including lysimeter and boundary area was 6 m². In the spring of 2007, the sod from all plots was removed and replaced with a new 4 cm top layer with slightly finer texture but the same type and amount of organic matter as in the initial root zone. The green was seeded with velvet bentgrass ‘Legendary’ at a rate of 6 g m⁻² on 8 June 2007 and observations and irrigation treatments started once plant coverage was close to 100 % in late August.

Being located on the Norwegian south coast, the experimental site has a moderate climate with fairly mild winters and relatively high annual precipitation. Twenty-three and 70 days of snow cover were registered during the winters 2007-08 and 2008-09, respectively. The mean monthly temperature, monthly precipitation, and monthly evaporation (Thorsrud 2500 evaporation pan; Riley and Berentsen, 2009) for the grow-in period and the experimental period are shown in Table 1. In 2007 and 2008, the total precipitation for June-August were, in turn, 178 mm and 169 mm higher than the 30 yr normal value. The highest evaporation values were recorded in June 2008 and 2009 (87 mm and 89 mm respectively). In 2009 a mobile rainout shelter was constructed; this came into operation on 1 July, but unfortunately malfunctioned during a heavy rainfall on 18 and 19 July. From 20 July 2009 the rainout shelter worked well for the rest of the experiment. Apart from the period covered by the rainout shelter, the longest continuous period without rainfall was from 2 May till 13 June 2008.

Treatments and Experimental Design

The experimental treatments were arranged in a completely randomized block design with three blocks. Each block contained four lysimeter-plots representing the four combinations of rootzone and irrigation regime. The *rootzones* were either straight sand (SS) or SS amended with 20% v/v garden compost (GM, ‘Green Mix’, Høst AS, Grimstad, Norway).

Chemical analyses in March 2008 and October 2009 indicated higher pH and higher content of plant available P, K, Mg and Ca in the GM than in SS rootzone (Table 2). Soil physical data from undisturbed soil cores taken at 13-50 and 150-187 mm depth in October 2007 are given in Table 3. Assuming a root depth of 20 cm, the field capacity was estimated to 25.7 mm on the SS rootzone and 41.9 mm on the GM rootzone. *Irrigation treatments* were either light & frequent (LF) or deep & infrequent (DI). In the LF regime, plots were irrigated back to field capacity once water deficit exceeded 5 mm (19.5 % depletion of field capacity) in SS plots and 10 mm (23.9 % depletion of field capacity) in GM plots.

Table 1. Mean monthly air temperature, monthly precipitation and monthly (only for the irrigation period) pan evaporation at Landvik during the grow-in period (8 June – 21 Aug. 2007) and experimental period (21 Aug. 2007 – 1 Oct. 2009) compared with 30-yr normal values.

Month	Air temperature				Total precipitation				Total pan evaporation			
	2007	2008	2009	Normal†	2007	2008	2009	Normal†	2007	2008	2009	Normal‡
	° C				mm				mm			
January	-	2.8	0.5	-1.6	-	358	178	113	-	-	-	
February	-	4.3	-2.2	-1.9	-	82	65	73	-	-	-	
March	-	2.3	2.7	1	-	184	116	85	-	-	-	
April	-	6	7.9	5.1	-	91	28	58	-	-	-	
May	-	11.9	11.3	10.4	-	25	70	82	-	69	48	65
June	15.9	14.7	14.8	14.7	109	75	59	71	-	87	89	84
July	15.5	17.3	16.8	16.2	213	129	55§	92	-	75	73	88
August	16.2	15.6	15.9	15.4	132	241	Rain out shelter	113	28	68	54	70
September	12	11.6	13	11.8	59	129	Rain out shelter	136	50	31	38	40
October	7.7	7.9	-	7.9	53	153	-	162	-	-	-	
November	3.6	3.7	-	3.2	69	123	-	143	-	-	-	
December	0.9	0.6	-	0.2	155	80	-	102	-	-	-	
Year		8.2		6.9		1670		1230				

† Reference period 1961-1990.

‡ Reference period 1968-2007.

§ Rainfall on 18-19 July due to malfunctioning of the rainout shelter. The total precipitation in July was 244 mm

Table 2. Chemical analyses of soil samples taken in March 2008 and in October 2009.

Treatment	pH	P-AL	K-AL	Mg-AL	Ca-AL
----- mg per 100 g dry soil -----					
2008					
SS	6.5	1.0	1.0	1.0	5.5
GM	7.2	2.5	2.3	3.3	40.8
<i>p</i>	0.006	0.014	0.015	0.003	0.004
2009					
SS	6.0	1.2	4.2	2.8	15.5
GM	6.6	4.2	5.0	5.0	59.0
<i>p</i>	0.002	<0.001	0.455	<0.001	<0.001

† SS, straight sand; GM, 'Green Mix'; LF, light and frequent irrigation; DI, deep and infrequent irrigation.

The corresponding thresholds for DI irrigation were 10 mm (39.0 % depletion of field capacity) and 20 mm (47.8 % depletion of field capacity), respectively. Accumulated water deficits for each of the four treatments were calculated five days per week using daily rainfall and values from the evaporation pan. Each year the soil water status prior to the initiation of irrigation treatments corresponded to field capacity. Individual plots were irrigated very precisely using a wagon with drip nozzles at 20 cm x 20 cm distance, the ordinary sprinkler system only being used for application of 3 mm water to the whole experiment after fertilizer inputs every second week. Irrigation treatments were carried out from 21 Aug. to 1 Oct. 2007 and from 1 May to 1 Oct. 2008 and 2009. Until the rainout shelter was installed on 1 July 2009, the trial was open for natural precipitation.

Daily rainfall and evaporation, irrigation amounts, soil water deficits, and soil moisture values are illustrated in Fig.1, while seasonal number of irrigations and irrigation amounts are summarized in Table 4.

Plots' Maintenance

During the period from 21 Aug. to 1 Oct. 2007, all plots received 45, 7, and 44 kg ha⁻¹ of N, P, and K, respectively. To equalize growing conditions on the two different rootzones, NPK inputs on SS plots were higher than on GM plots in 2008 and 2009. The total input of

Table 3. Physical characteristics of soil cores from the SS and GM root zones at two soil depths, sampled on 15 Oct. 2007.

Rootzone†	Depth mm	Capillary porosity,						Field capacity, 0-20 cm‡	Soil density g cm ⁻³	Ignition loss %	Hydraulic conductivity mm h ⁻¹
		Total porosity	Air-filled porosity	Unavailable water, pF>4.2	Plant-available water						
					pF 1.5- 3.0	pF 3.0- 4.2	Total				
		----- % (v/v) -----									
SS	13-50	37.4 b§	17.9 b	0.6 b	17.4 a	1.5 b	18.9 a	24.8 b	1.58 a	0.60 b	226
	150-187	36.4 b	25.2 a	0.4 b	9.4 b	1.4 b	10.8 b		1.56 a	0.48 b	237
GM	13-50	42.7 a	18.1 b	3.3 a	15.0 a	6.2 a	21.3 a	41.8 a	1.44 b	2.44 a	200
	150-187	44.0 a	20.8 b	2.4 a	15.1 a	5.7 a	20.8 a		1.39 b	1.84 a	220

† SS, straight sand; GM, 'Green Mix'.

‡ The calculation of field capacity was based on the fact that the 4 cm top layer had been replaced with new sand of finer texture. The given depths correspond to the depths of TDR probes used to measure soil water content.

§ Mean values followed by the same letter in the same column are not significantly different based on Fisher protected LSD test ($\alpha=0.05$).

Table 4. Seasonal number of irrigations and total water use in various treatments. Figures include irrigation of the whole experiment after fertilizer application every second week. Treatments' abbreviations: SS, straight sand; GM, 'Green Mix'; LF, light and frequent irrigation; DI, deep and infrequent irrigation.

Month	Number of irrigations			Total irrigation water		
	SS+LF	GM+LF	GM+DI	SS+LF	GM+LF	GM+DI
	----- mm -----					
Total 2007†	10	5	2	50	45	25
Total 2008‡	45	23	16	239	184	174
Total 2009‡	49	29	18	227	213	173

† 21 Aug – 1 Oct.

‡ 1 May – 1 Oct.

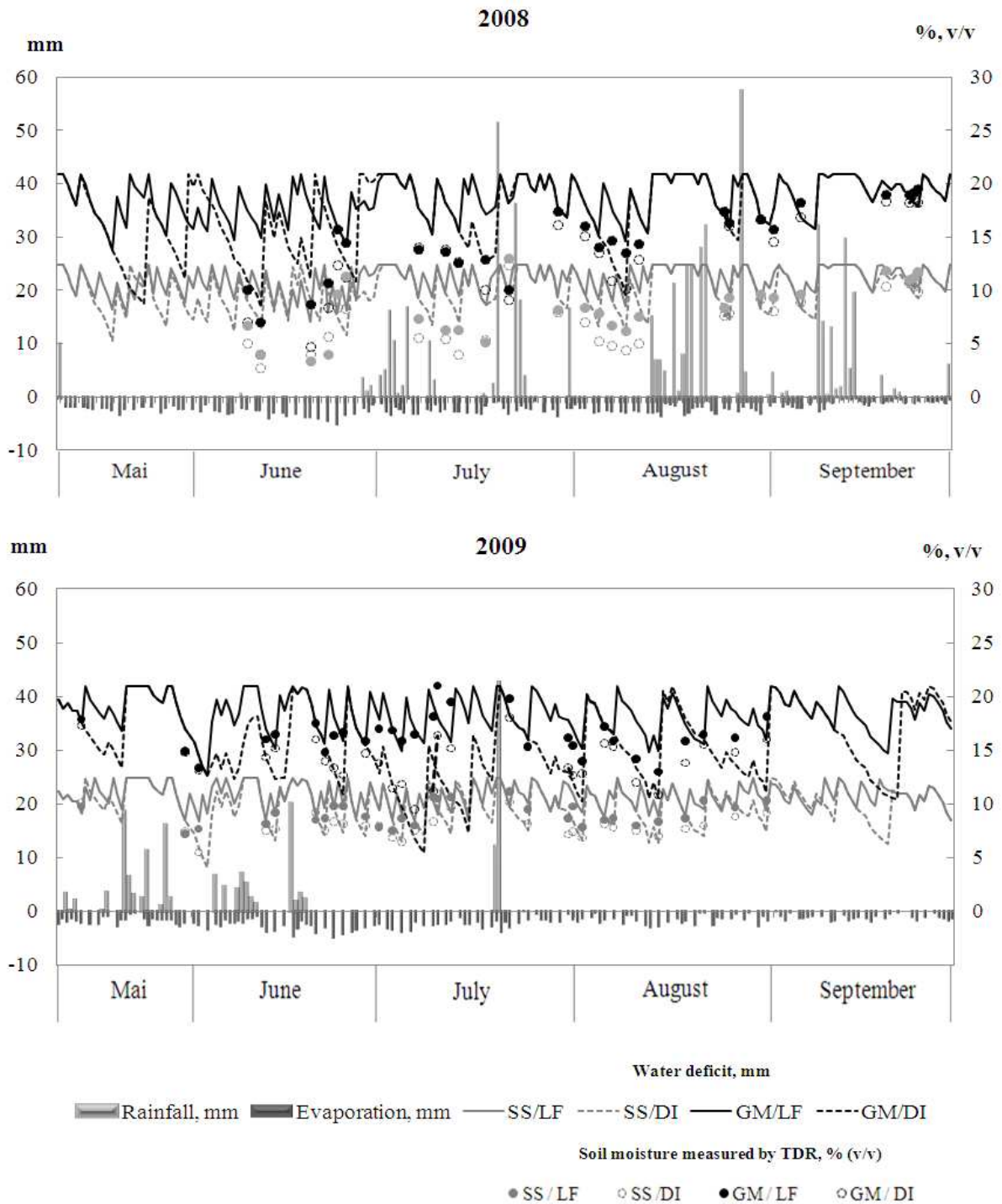


Figure 1. Daily soil water deficit as calculated from the difference between rainfall and pan evaporation in 2008 and from 1 May to 20 July 2009, and from pan evaporation after 20 July 2009 (rain out shelter installed). The plots were irrigated to field capacity each time the calculated soil water deficit exceeded 5 mm, 10 mm and 20 mm in the treatments SS+LF, SS+DI & SS+LF and GM+DI, respectively. Volumetric soil moisture content in the 0-20 cm rootzone shown with circles and was measured with a time-domain reflectometer (TDR) prior to irrigation of at least one of the four treatments. SS = *Straight sand*; GM = *'Green Mix'*; LF = *Light and frequent irrigation*; DI = *Deep and infrequent irrigation*.

N, P, and K on GM plots in 2008 (15 Apr. - 1 Nov.) amounted to 131, 14, and 117 kg ha⁻¹ and in 2009 to 108, 20, 138 kg ha⁻¹, respectively. The corresponding amounts of NPK on SS plots were 192, 28, and 185 kg ha⁻¹ in 2008 and 130, 30, and 206 kg ha⁻¹ in 2009. Fertilization interval was always two weeks. Except for four applications of liquid fertilizer Arena® Crystal (Yara International ASA, Norway) in the fall of 2008, fertilizers were mostly given as inorganic granular Arena® products. Due to high pH (Table 2) ammonium sulfate 21-0-0 (Yara International ASA, Norway) or Anderson 13-2-13 (Andersons Lawn Fertilizer Division Inc., Maumee, OH) was also included in the fertilization plan on GM plots. The green was mowed using a John Deere 220A walk-behind mower (Moline, IL) three times a week at 3 mm except for the periods April-June and September-October when mowing height was raised to 3.5-4.5 mm. Starting in July 2008, the green was groomed with a groomer attached to the mower once a week and brushed twice a week. In 2008 and 2009, monthly vertical cutting was performed to 2 mm depth using an Aztec verticutter pod mounted on an Aztec drive unit (Allett mowers LTD, Arbroath, Scotland). On 12 June 2008 the green was aerated with a John Deere Aerator 800 using 8 mm solid spikes to 8 cm depth. At the end of each season, the green was core-aerated using 6 mm hollow tines to 8 cm depth. From May to October 2008 and 2009, the green was exposed to artificial wear from a friction drum with golf spikes corresponding to 20000 rounds of golf per year. As a result of topdressing every one to two weeks, the green received 4 mm and 9 mm of sand (no organic matter; grain size 0.2-0.8 mm; Baskarp, Sweden) in 2008 and 2009, respectively.

Registrations and Statistical Data Analyses

Visual assessments were conducted from 26 Aug. to 30 Oct. 2007 and from 15 March to 15 Oct. 2008 and 2009. The assessments were conducted every second week for turfgrass overall impression (scale from 1=uneven and very bad turf to 9=even and very good turf; acceptability level = 5) and monthly for tiller density (scale from 1=very thin to 9 =very dense), colour (scale from 1=very light to 9=very dark), diseases (% of plot covered with diseased turf) and turf coverage (% of plot covered with undiseased turf of the sown species).

Playing quality was recorded monthly and assessed as surface hardness and ball roll distance. Surface hardness was measured from 23 Apr. until 16 Sept. 2008 and from 30 Apr. until 16 Oct. 2009 using a Clegg Soil Impact Tester (Lafayette Instrument Co., Lafayette, IN). Readings were taken after each of two successive blows by the 2.25-kg hammer from 0.46-m height at two places per plot. Ball roll distance was determined from 22 July until 15

Oct. 2008 and from 29 Apr. until 16 Oct. 2009 using a stimpmeter modified for research plots (Gaussoin et al., 1995). Measurements were always conducted the day after mowing. The stimpmeter had its ball release notch 38 cm rather than 76 cm from the bevelled end, and measurements were always taken in two directions.

For visual assessments and playing quality, data from each experimental year were pooled into three consecutive periods with 2-5 registrations each: spring (March-May), summer (June-August), and fall (September-October).

Soil moisture was measured on all plots (three measurements per plot) prior to irrigation of at least one of four treatments using a portable time-domain reflectometer (TDR) 'HydroSence™' (Campbell Scientific, Ltd., Thuringowa, Australia) with 20 cm long probes except in September 2009 due to mechanical rupture of one probe.

Thatch/mat assessments. Thatch/mat depth was determined from four cores taken from each plot in September 2008 and 2009. Two of the four samples were also used for determination of organic matter content. The foliage above the mat layer and roots below it were removed. The organic matter content was calculated individually for each core as: Organic matter (%) = (sample ignition loss / sample dry weight) x 100 %. Sample ignition loss was determined as the difference between sample dry weight (105 °C for 48 h) and sample ash weight (550°C for 3 h). The data were averaged for each plot prior to analyses of variance.

Root assessments. On 13 June (by the end of the six week drought period) and 8 Sep. 2008 and 10 Oct. 2009, a soil core was taken from each plot using a root auger, 30 cm long and 5 cm in diameter. The cores were separated into the following layers: 0-6 cm, 6-10 cm, and 10-20 cm. The depths were measured from the green surface, and the mat layer removed from the upper samples. Roots from each layer were washed free from soil and dried for 48 h at 105 °C. Root densities were calculated by dividing weight of dry roots by the volume of the sample taken at a certain rootzone depth.

Water infiltration rates were measured on 12 June 2008 and 12 Oct. 2009 at two sites per plot using a double ring infiltrometer with an outer ring diameter of 12.8 cm and an inner ring diameter of 4.5 cm. The infiltrometer was inserted 2 cm into the turf after the rootzone had been saturated. Water levels in the inner ring were measured after three minutes of infiltration. The infiltration rate was expressed as mm per hour.

Potential soil water repellency was determined on 13 June 2008 and 20 Sept. 2009 using the water drop penetration time (WDPT) test (Dekker et al., 2001). One soil sample (11 x 12 x 2 cm) was taken from each plot with a spade sampler. After 48 h of air drying in the

laboratory, three drops of water were placed at 0.5-cm depth (in mat), just under the mat (at 1-cm depth in 2008 and 2-cm depth in 2009), and at 3-, 5- and 10-cm depth from the green surface, and the times until drops had penetrated were measured. The following classification was used for interpretation of results: Wettable (not water repellent) if $WDPT < 5s$, slightly water repellent if $5s \leq WDPT < 60s$, strongly water repellent if $60s \leq WTPT < 600s$, and severely water repellent if $600s \leq WDPT$ (Dekker et al., 2001).

Leaching water from the lysimeters was collected monthly during the following periods: August-September 2007, May-September 2008, and May-June 2009. Leaching in May and June 2008 were pooled due to little rainfall. Samples were analysed individually for nitrate/nitrite (standard EN ISO 13395) and total N (standard EN ISO 13395), P (standard EN ISO 15681-2), and K (standard NS EN ISO 11885) concentrations at AnalyCen laboratory (Norway). The total nutrient leakage was calculated based on monthly concentrations and amount of leachate.

The data were analyzed by the SAS procedure PROC ANOVA using statements providing one-way analysis for a block design (SAS Institute, 1990). In the case of water drop penetration times, ANOVA was performed on data transformed to $\ln(y+1)$ due to deviation from normal distribution. The Fisher's protected least-significant-difference (LSD) at the 5% probability level was used to identify significant differences among treatments.

RESULTS

Turf overall impression, color, shoot density, and diseases

Regardless of irrigation treatment, velvet bentgrass produced significantly better overall impression (Fig. 2a and 3) and higher shoot density (Fig. 2b) on GM plots than on SS plots in the spring of 2008 and 2009. A similar tendency ($p = 0.08$) was observed the fall of 2007. The effect of rootzone composition on turf quality was less conspicuous during the summer and fall of 2008. Light and frequent irrigation produced slightly, although not significantly, better overall impression than DI irrigation on both rootzones in 2008. Starting in the spring of 2009, DI irrigation produced better overall impression and higher shoot density than LF irrigation on the SS rootzone, and this effect became highly significant by the end of the experiment. Color was improved during the first year after sowing from a score of 5.2 in the fall of 2007 to 6.2 in the fall of 2008. There were no differences in color among the

treatments except for the higher score (6.4) on SS DI-irrigated plots in the summer of 2008. In 2009, the color trends for the treatments were similar to those for overall impression (data not shown).

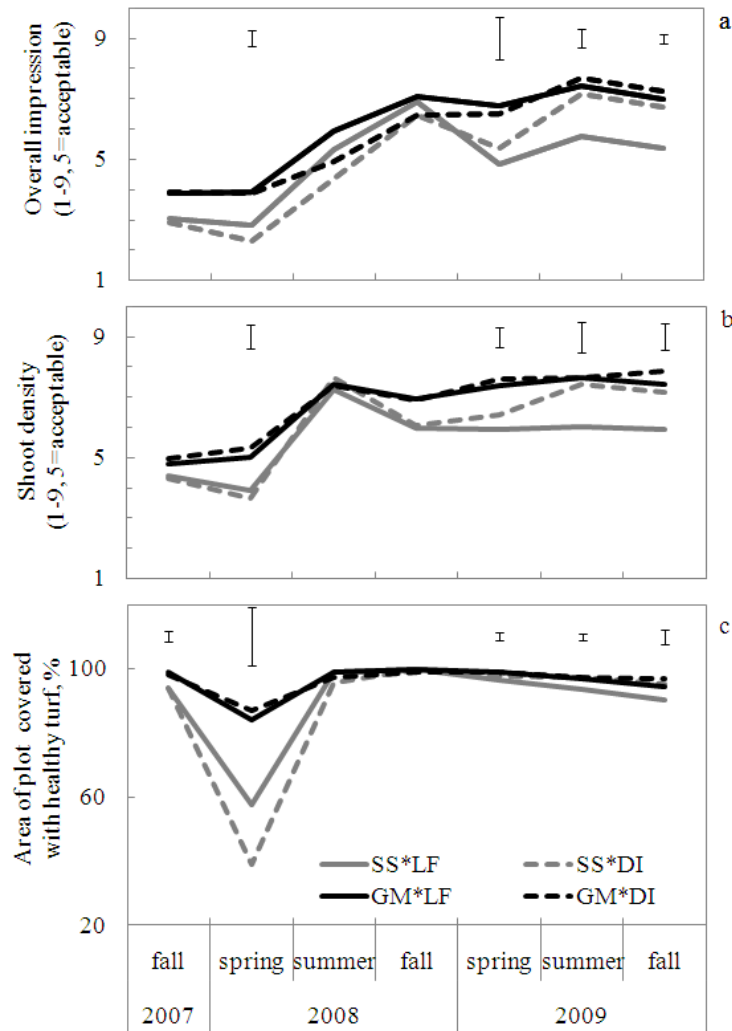


Figure 2. Effects of rootzone composition and irrigation regimes on visual quality of velvet bentgrass green in 2007, 2008, and 2009. Vertical bars are LSD values indicating significant differences between treatments at 5 % probability level. SS = Straight sand; GM = ‘Green Mix’; LF = Light and frequent irrigation; DI = Deep and infrequent irrigation

A severe snow mold attack, caused by *Microdochium nivale*, was observed on the SS rootzone in the spring of 2008 (Fig. 2c). On that rootzone, light and frequent irrigation led to 30% less injury than DI irrigation. The area covered by diseased turf was lower (14%) and did not depend on irrigation treatments on GM plots. In the spring of 2009, snow mold attack was much lower than in 2008, but again GM plots had less injury (<1%) than SS plots (3%); no significant effect of irrigation treatment could be detected in this case. In September 2009,

DI irrigation reduced a sudden outbreak of *M. nivale* compared with LF irrigation on both rootzones. For LF and DI irrigation the affected area was 14 vs. 5 % on the SS rootzone and 8 vs. 4% on the GM rootzone (data not shown).

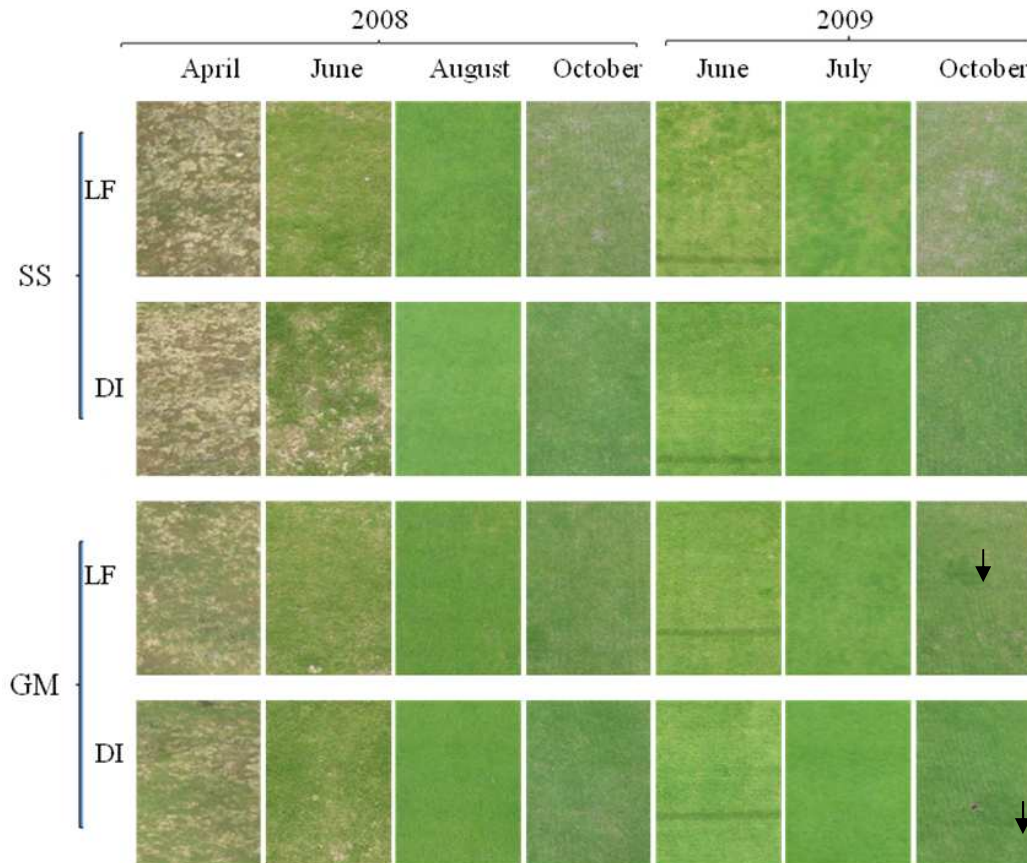


Figure 3. Effects of rootzone composition and irrigation regime on performance of velvet bentgrass during 2008 and 2009. The green was seeded in June 2007. Arrows show irregular patches on GM appeared first in August 2009. SS = *Straight sand*; GM = *'Green Mix'*; LF = *Light and frequent irrigation*; DI = *Deep and infrequent irrigation*.

In August 2009, irregular patches with enhanced shoot growth were observed on some GM plots independent of irrigation treatments (Fig. 3, arrows). Inspection of the mat layer indicated that the dark-colored thatch, most likely lignin, had been degraded, and microscopic observation suggested that it could be caused by a fungi belonging to Basidiomycota, probably white-rot fungi contained in the compost (Tuomela et al., 2000).

Playing quality

Ball roll distance increased during summer and fall in 2008 and 2009, but was never significantly affected by treatments (Fig. 4a). As indicated by the first blow with the Clegg

hammer, playing surfaces were on average 20% harder on SS plots than on GM plots in 2008 (Fig. 4b). This difference became even more conspicuous in the second blow at the same position (Fig. 4c). From April to October 2009, differences between the two rootzones were significant in the second blow only.

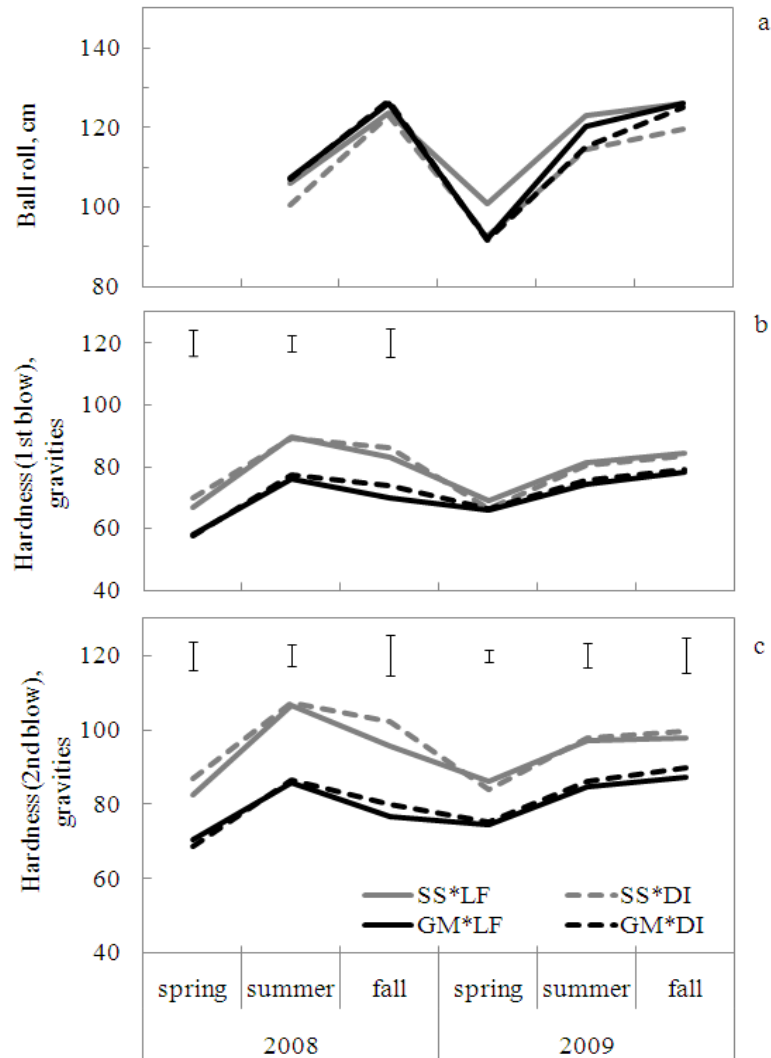


Figure 4. Effects of rootzone composition and irrigation regimes on playing quality of velvet bentgrass green in 2007, 2008, and 2009. Vertical bars are LSD values indicating significant differences between treatments at 5 % probability level. *SS* = *Straight sand*; *GM* = *'Green Mix'*; *LF* = *Light and frequent irrigation*; *DI* = *Deep and infrequent irrigation*.

Soil Moisture

Volumetric soil water content measured by TDR was generally lower on DI-irrigated plots than on LF-irrigated plots (Fig. 1), but differences were significant only for the GM rootzone. On average for measurements just before irrigation events in 2008 and 2009, soil moisture in the 0-20 cm layer amounted to 8.3% under LF irrigation vs. 6.7% under DI irrigation in the

SS rootzone, and 14.5% under LF irrigation vs. 9.9% under DI irrigation in the GM rootzone (data not shown).

Thatch / mat assessments

Neither thickness nor content of organic matter in the mat layer were significantly affected by treatments in any year (Table 5). On average for all treatments mat thickness doubled from 10 mm in the fall of 2008 (16 months old green) to 20 mm in the fall of 2009 (28 months old green), while the average content of organic matter in mat layer dropped from 9.8% to 8.3%.

Table 5. The effect of rootzone composition and irrigation regime on mat characteristics of velvet bentgrass green

Treatment†	September 2008		September 2009	
	Mat depth cm	Organic matter in mat % (w/w)	Mat depth cm	Organic matter in mat % (w/w)
SS+LF	1.0	10.3	2.0	8.0
SS+DI	0.9	9.1	1.9	7.9
GM+LF	1.0	10.1	2.2	8.8
GM+DI	1.0	9.7	2.0	8.4
<i>p</i>	0.812	0.735	0.631	0.610

†SS, straight sand; GM, 'Green Mix'; LF, light and frequent irrigation; DI, deep and infrequent irrigation.

Root density

The average root dry weight per unit area was 1.4, 1.6, and 2.0 times higher on SS than on GM plots in June 2008, September 2008, and October 2009, respectively (Table 6). The effect of irrigation frequency on the total root dry weight was inconsistent within each rootzone.

Root densities at different depths are shown in Fig. 5. The highest root density was always recorded in the upper 4.0-5.5 cm under the mat layer, but differences in root density between rootzones were not consistent in 2007, 2008, and 2009. The decrease in root density from upper to lower layers occurred more rapidly on GM plots compared with SS plots. Significant effects of irrigation regime only appeared in October 2009. On SS plots, DI irrigation almost doubled root density in the 10-20 cm layer, but reduced root density by 30% in the 6-10 cm layer compared with LF. On GM plots, DI irrigation reduced root density by

about 50% in the 2 cm topsoil layer (just under the mat), but increased root density about four fold at 10-20 cm depth.

Table 6. Effect of rootzone composition and irrigation regime on total weight of dry roots per one square meter.

Treatment†	June 2008	September 2008	October 2009
	----- g m ⁻² -----		
SS+LF	376 a	295	497 a
SS+DI	329 ab	278	515 a
GM+LF	229 c	235	274 b
GM+DI	263 bc	129	238 b
<i>p</i>	0.036	0.072	<0.001

†SS, straight sand; GM, 'Green Mix'; LF, light and frequent irrigation; DI, deep and infrequent irrigation.

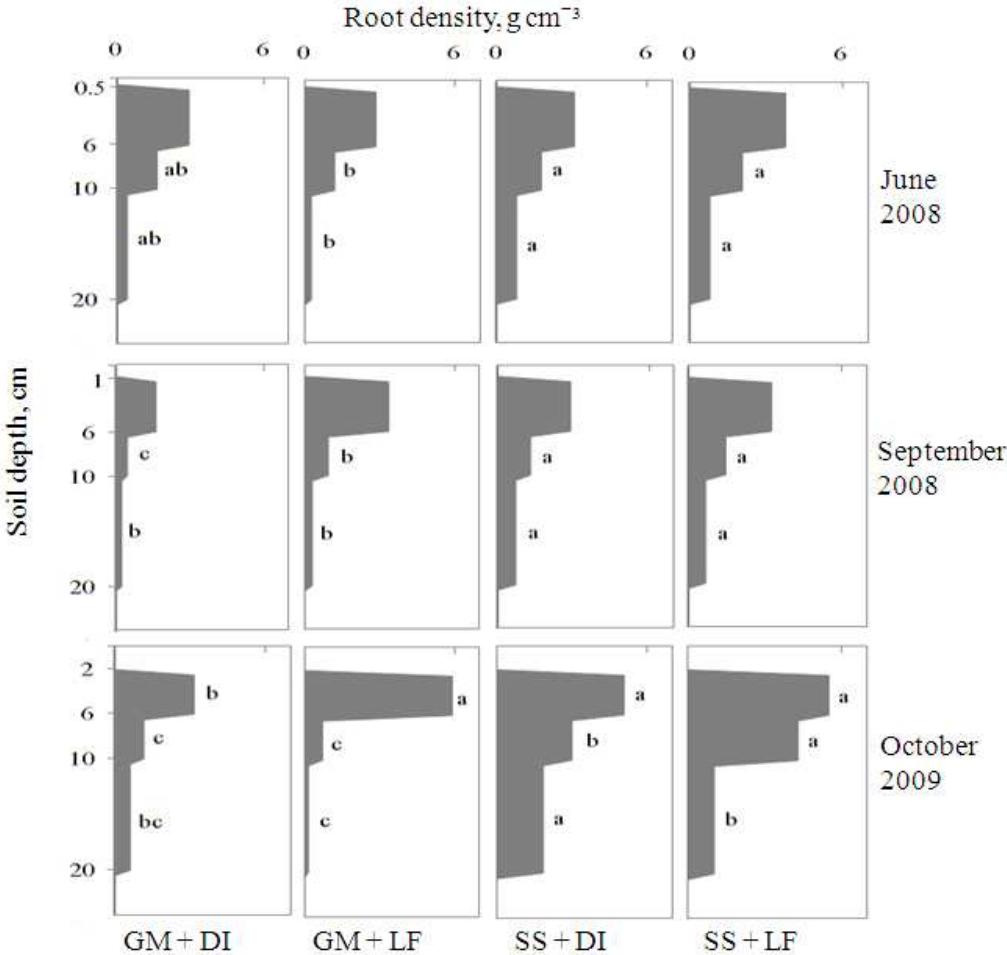


Figure 5. Root density at different soil depths on velvet bentgrass green with rootzone compositions Straight Sand (SS) or 'Green Mix' (GM) and irrigation regime Light and Frequent (LF) or Deep and Infrequent (DI), measured by the end of dry period in 2008 and the end of both experimental years. Mean values followed by the same letter within the same depth and measured at the same time are not significantly different based on Fisher protected LSD test ($\alpha=0.05$).

Water infiltration rate

The lowest infiltration rate had SS plots receiving LF irrigation. Deep and infrequent irrigation improved the infiltration rate by 26% and 449% on SS plots in June 2008 and in September 2009, respectively (Table 7). Effects of irrigation on GM plots were not significant.

Table 7. Effects of rootzones and irrigation regimes on infiltration rates measured by double ring infiltrometer.

Treatment†	June	
	2008	September 2009
	———— mm hr ⁻¹ ————	
SS+LF	82 b	63 b
SS+DI	103 a	343 a
GM+LF	104 a	267 ab
GM+DI	99 a	333 a
<i>p</i>	0.003	0.051

† SS, straight sand; GM, 'Green Mix'; LF, light and frequent irrigation; DI, deep and infrequent irrigation.

‡ Mean values followed by the same letter in the same column have no significant difference based on Fisher protected LSD test ($\alpha=0.05$).

Potential soil moisture repellency

In general, GM plots were wettable or slightly water repellent at all investigated depths except for the mat layer on plots receiving LF irrigation in 2008 (Table 8). Both in June 2008 and September 2009, the mat layer was more water repellent under LF than DI irrigation. At 5- and 10-cm depth, water drop penetration time (WDPT) was generally higher under DI (WDPT varied from 11 to 19 s) than under LF irrigation (WDPT varied from 3 to 6 s). In contrast to GM plots, SS plots showed strong and severe water repellency at certain depths.

In 2008, DI irrigation resulted in higher water repellency immediately under the mat layer and at 1 cm under the mat layer than LF irrigation. In 2009, strong water repellency was observed at these depths regardless of irrigation regime. Deep and infrequent irrigation led to the higher repellency at the 10-cm depth in 2008 and at 5- and 10-cm depth in 2009. Like on GM plots, measurement in September 2009 showed that the mat layer on SS plots was potentially more water repellent under LF irrigation than under DI irrigation.

Table 8. Water drop penetration time (WDPT) at different depths of SS and GM plots and LF or DI irrigation, measured after drying samples at room temperature for 48 h in 2008 and 2009.

Treatment†	June 2008					September 2009				
	In mat	Immediately under mat	1 cm under mat	5 cm from green surface	10 cm from green surface	In mat	Immediately under mat	1 cm under mat	5 cm from green surface	10 cm from green surface
	WDPT, s									
SS+LF	74 a	35 a	9 ab	5 ab	6 a	111 d	76 b	123 b	11 b	6 b
SS+DI	217 c	208 b	1282 c	1 a	192 b	31 c	79 b	136 b	24 c	69 c
GM+LF	107 b	7 a	5 a	4 bc	5 a	17 b	8 a	9 a	6 a	3 a
GM+DI	51 a	11 a	14 b	12 c	19 a	12 a	7 a	9 a	11 b	11 b
<i>p</i>	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

† SS, straight sand; GM, 'Green Mix'; LF, light and frequent irrigation; DI, deep and infrequent irrigation.

Leaching

During the periods of the leachate collection in 2007, 2008 and 2009, SS plots with LF and DI irrigation received totally 362 mm and 292 mm irrigation water, respectively. The corresponding amounts on GM plots were 292 mm and 222 mm. The total rainfall for the collection periods amounted to 790 mm. Because leachate was collected during periods of different duration in 2007, 2008 and 2009, leaching data are presented on a daily basis (Table 9).

Table 9. Effects of the treatments on leaching rates during the water collection periods in 2007, 2008, and 2009.

Treatment†	20 Aug. – 1 Oct.	1 May – 1 Oct.	1 May – 1 July
	2007	2008	2009
	mm day ⁻¹		
SS+LF	1.7 b	3.1	1.1 d
SS+DI	1.5 ab	2.7	0.8 b
GM+LF	1.5 ab	2.8	1.0 c
GM+DI	1.3 a	2.7	0.7 a
<i>p</i>	0.023	0.105	< 0.001

† SS, straight sand; GM, 'Green Mix'; LF, light and frequent irrigation; DI, deep and infrequent irrigation.

‡ Mean values followed by the same letter in the same column have no significant difference based on Fisher protected LSD test ($\alpha=0.05$).

In 2009, LF irrigation caused significantly higher leakage rates than DI irrigation from both SS plots and GM plots. In 2007 and 2008, differences showed similar trends but were not significant.

Significant differences in concentrations and average daily loss of nutrients occurred between treatments, but they were usually due to rootzone rather than irrigation frequency (Table 10). The highest concentrations were measured of potassium (12.6-31.7 mg L⁻¹) followed by total nitrogen (0.7-3.0 mg L⁻¹) and phosphorus (0.01-0.19 mg L⁻¹).

On averaged for irrigation treatments the loss of nitrate/nitrite through the drainage system was eight and three times higher on SS plots compared with GM plots in 2007 and 2008, respectively. In 2009, differences in nitrate/nitrite leaching rate from the two rootzones were no longer significant. By contrast, total nitrogen leaching was higher from GM plots than from SS plots in 2009 only. Leaching of phosphorus was eight, six, and eleven times higher on GM plots compared with SS plots in 2007, 2008 and 2009, respectively. Potassium

leaching from GM plots was two times higher than from SS plots in 2007, but it did not differ between rootzones in 2008 and 2009.

Table 10. Nutrient concentrations in leachate and total nutrient losses from velvet bentgrass green as affected by root zone type and irrigation regime and expressed for 2007, 2008, and 2009 on a daily basis.

Treatment	20 Aug. – 1 Oct. 2007		1 May – 1 Oct. 2008		1 May – 1 July 2009	
	Concentration, mg L ⁻¹	Leaching rate, mg m ⁻² day ⁻¹	Concentration, mg L ⁻¹	Leaching rate, mg m ⁻² day ⁻¹	Concentration, mg L ⁻¹	Leaching rate, mg m ⁻² day ⁻¹
NO₂⁻/NO₃⁻						
SS+LF	1.8 b	3.3 b	0.7 b	2.3 ab	0.5	0.6
SS+DI	2.2 b	3.3 b	1.5 a	4.1 b	0.5	0.4
GM+LF	0.3 a	0.5 a	0.3 b	0.9 a	0.9	0.9
GM+DI	0.3 a	0.3 a	0.4 b	1.0 a	0.7	0.5
<i>p</i>	0.020	0.030	0.028	0.052	0.811	0.639
Nitrogen (N)						
SS+LF	3.0	5.2	2.0	6.4	0.7 a	0.9 a
SS+DI	2.5	3.9	2.7	7.3	0.7 a	0.6 a
GM+LF	1.8	2.8	2.7	7.6	2.4 b	2.4 b
GM+DI	1.9	2.5	3.0	7.9	2.0 ab	1.3 ab
<i>p</i>	0.476	0.293	0.165	0.630	0.057	0.052
Phosphorus (P)						
SS+LF	0.02 a	0.04 a	0.02 a	0.07 a	0.01 a	0.01 a
SS+DI	0.02 a	0.03 a	0.03 a	0.07 a	0.01 a	0.01 a
GM+LF	0.19 b	0.30 b	0.16 b	0.44 b	0.15 b	0.15 b
GM+DI	0.19 b	0.26 b	0.15 b	0.41 b	0.15 b	0.10 b
<i>p</i>	0.001	0.004	<0.001	<0.001	0.003	0.002
Potassium (K)						
SS+LF	14.4 a	25.1 a	12.6	39.5	14.3	16.5 b
SS+DI	12.7 a	19.3 a	14.7	39.4	16.8	13.6 ab
GM+LF	31.7 b	48.2 c	14.4	39.7	17.1	16.7 b
GM+DI	29.2 b	39.1 b	13.5	35.9	15.4	10.4 a
<i>p</i>	<0.001	0.001	0.251	0.821	0.548	0.076

† SS, straight sand; GM, 'Green Mix'; LF, light and frequent irrigation; DI, deep and infrequent irrigation.

‡ Mean values followed by the same letter in the same column have no significant difference based on Fisher protected LSD test ($\alpha=0.05$).

As compared with DI irrigation, LF irrigation resulted in more leaching of potassium from GM root zones in 2007 and 2009. Effects of irrigation regime on nutrient losses from SS

rootzones were never significant, but a significantly higher concentration of nitrate/nitrite was detected in leachate from plots with DI than from plots with LF irrigation in 2008.

DISCUSSION

Turf quality in the sowing year and during spring periods. The lower overall impression and shoot density on SS vs. GM plots in fall 2007 and during both spring periods is in accordance with earlier investigations (Gibbs et al., 2000; Joo et al., 2001). Nitrogen loss in the form of nitrate/nitrite from the 2-4 months old green was eight times higher from SS plots than from GM plots and corresponded, in turn, to 4.5 % and 0.5 % of total N applied in fertilizer. Our results are in agreement with earlier studies showing amendment with compost to improve the water holding and nutrient retention capacities, and thus turf quality of sand-based rootzones (McCoy, 1992; Brauen and Stahnke, 1995; Murphy et al., 2004; Aamlid, 2005). Better turfgrass performance on GM compared with SS plots in the spring of 2008 and 2009 was also due to less injury caused by *Microdochium nivale*. This was later confirmed by a sudden outbreak of the *Microdochium* patch in September 2009; again, the affected area was lower on GM plots compared with SS plots. These results are substantiated by earlier reports showing suppressive effects of compost on soil-borne turfgrass diseases (Boulter et al., 2002b; Nelson and Boehm, 2002; Tilston et al., 2002). Boulter et al. (2002a) found a reduction in the development of *M. nivale* and *Typhula ishikariensis* on a 4-5-yr old creeping bentgrass green after topdressing with compost. They also observed a quicker green-up and recovery from dormancy on compost-amended plots.

In our study, the period from 2 May to 13 June 2008 was without any natural rainfall, and the turf obviously performed better and recovered more quickly from the disease with LF than with DI irrigation. Better turf performance of young greens under more frequent irrigation was reported also by Fu and Dernoeden (2009a).

Turf quality in summer and fall. The rapid improvement in turfgrass overall impression and shoot density on SS plots in the summer and fall of 2008 was facilitated by a 46% higher nitrogen input on SS than on GM plots. After achieving maximum scores in the fall of 2008, turfgrass overall impression declined in response to LF irrigation on SS plots. This is in accordance with earlier research by Jordan et al. (2003) and Fu and Dernoeden (2009a). Thus, Fu and Dernoeden (2009a, 2009b) reported better turf quality with DI irrigation than with LF irrigation by the end of a 2-yr study on creeping bentgrass green (97% sand, 1% silt, 2% clay,

and 10 mg g⁻¹ organic matter), and they attributed this to, among other things, a higher chlorophyll content and better adaptation to wilt stress after DI irrigation.

Root development. In our study, DI irrigation led to an increase in root density at a depth of 10-20 cm, but the effect was significant only when measured in October 2009 on SS plots. At the same time, root density was reduced in the upper layers on both rootzones. Most likely, effects of irrigation on root development in deeper layers in 2007 and 2008 were masked by uncontrolled natural precipitation. Jordan et al. (2003) also reported no differences in rooting on a creeping bentgrass green in response to irrigation at one, two, or four day intervals during the first year of a 2-yr study under natural rainfall. In our study, the high water holding capacity of the GM rootzone probably also contributed to negligible effect of reduced irrigation frequency on turfgrass root development.

Although several studies showed an increase in root length, root number or root density at lower depths in response to limited soil moisture in the upper layer (Huang et al., 1997; Jordan et al. 2003; Fu and Dernoeden, 2009b), effects on root distribution in the upper layer have been inconsistent. Huang et al. (1997) reported a decrease in root length of most cultivars of four warm-season turfgrasses in the upper 20 cm soil in response to drying. By contrast, Fu and Dernoeden (2009b) observed an increase in the total root length and total root surface even in the in the upper 6 cm soil when irrigating at leaf wilt compared with LF irrigation. Jordan et al. (2003) also showed a higher root density at 1-7.5 cm depth on creeping bentgrass green after irrigating every 4 days than every 1 or 2 days, but only in the end of a 2-yr study.

Until construction of the rainout shelter in July 2009, root development was more affected by root zone composition than by irrigation treatment. Although turf visual quality was generally lower, there always were more roots at 6-10 and 10-20 cm depth on SS than on GM plots. The higher root density in the lower layer most likely maximized water and nutrient uptake due to the lower soil moisture content in SS than in GM rootzones soil moisture (7.8% vs. 13.9% in 2008 and 8.5% vs. 15.2% in 2009) (Huang et al., 1997; Leinauer et al., 1997).

Characteristics of the thatch/mat layer. The content of organic matter in the mat varied from 9.1% to 10.3% already by the end of 2008, i.e. considerably higher than the optimal organic matter content in a sand medium which is considered not to exceed 4% (McCoy, 1992; Murphy et al., 1993). This was not surprising as far as velvet bentgrass possesses high thatch accumulation (Rinehart et al., 2005; Aamlid et al., 2006). As the nitrogen input was reduced 27% on SS plots and 21% on GM plots, the doubling of mat thickness from 2008 to

2009 must have been due to an increase in topdressing rate from 4 mm to 9 mm. In accordance with earlier investigations (Murphy, 1983; McCarty et al., 2005, 2007), this increase in topdressing also led to a 23 % decrease in the content of organic matter in the mat layer (average for all treatments) from 2008 to 2009 ($p=0.006$). In contrast to our results showing no significant effect of treatments on thatch/mat characteristics, Fu and Dernoeden (2009a) found that DI irrigation reduced the content of organic matter in the mat on a creeping bentgrass green compared with LF irrigation by the end of their 2-yr study (24.7% under LF irrigation vs. 20.0% under DI irrigation).

By the end of our study the inferior turfgrass visual quality on SS plots that had received LF irrigation, could be due to the more or less continuous wetness of the mat layer on those plots. This, in turn, may have led to oxygen deficiency and N losses due to denitrification from these plots (Mancino et al., 1988; Engelsjord et al., 2004). A more compact thatch layer with less favourable conditions for aerobic bacteria may also be indicated by the strongly reduced infiltration rate (Table 7) and by the more severe outbreak of *Microdochium* patch on these plots than on other plots in September 2009.

The higher water repellency on SS plots compared with GM plots at most of the investigated depths corroborates earlier studies showing water repellency to be an important property of sand which develops when the soil water content is below a certain critical threshold (Bauters et al., 1998; Dekker et al. 2001; Larsbo et al., 2008). Our results suggest that development of water repellency on SS plots was delayed but not totally prevented by LF irrigation. However, contrary to our expectation, higher soil water repellency in the mat layer on the GM rootzone in June 2008 and on both rootzones in September 2009 was observed on plots receiving LF irrigation as opposed to DI irrigation. We have no good explanation for this phenomenon but suggest that moisture or/and oxygen conditions provided by LF irrigation could derive microbial water repellency (Wilkinson and Miller, 1977; Hallet et al., 2001) or repellency induced by the products of organic matter decomposition or by the exudates of grass roots (Doerr et al., 2000).

Playing quality. The negligible effect of rootzone in our study is in accordance with earlier investigations showing that ball roll depends on the quality of the immediate green surface and not on rootzone composition (Baker and Richards, 1995; Baker et al., 1999; Gibbs et al., 2000). The measurement of surface hardness in our study was important both from a player's perspective and since it reflected thatch accumulation by the species. Similar to earlier investigations, our results showed that the higher surface hardness on SS vs. GM plots persisted only during the first year of the study (Baker and Richards, 1995; Gibbs et al.,

2000; McCarty, 2005, 2007). Most probably the thick but similar mat depth and equal content of organic matter among our treatments resulted in the minimal differences in hardness between rootzones on the mature green.

Leaching. Infrequent irrigation (DI) did not lead to increased drainage as reported by Bauters et al., 1998; Kenna and Snow, 2000; Fry and Huang, 2004; Barton and Colmer, 2006; Nektarios et al., 2007). Starrett et al. (1995, 1996, 2000) also found more drainage from fine-loamy soil columns with Kentucky bluegrass turf receiving DI irrigation (4 applications of 25.4 mm) than LF irrigation (16 applications of 6.4 mm). They also showed more nitrogen (Starrett et al., 1995), pesticide (Starrett et al., 1996), and herbicide leaching (Starrett et al., 2000) with DI vs. LF irrigation. In our study, the higher amounts of leachate with LF than with DI irrigation within each rootzone were caused, firstly, by the generally higher amounts of irrigation water received by LF- than by DI-irrigated plots. Secondly, plots with LF irrigation were mostly closer to field capacity and therefore had less space for natural rainfall than plots with DI irrigation.

The overall leakage of nutrients in our study was low as has previously been reported from other studies with well-established and properly maintained turf (Mancino and Troll, 1990; Frank et al., 2005; Paré et al., 2006; Soldat and Petrovic, 2008; Steinke et al., 2009). Thus, the nitrate/nitrite concentrations in leachate were 95, 98, and 99 % lower on SS plots and 99 %, 99 %, and 98 % lower on GM plots than the current regulatory standard of 50 mg of nitrates per litre groundwater (The EU Nitrates Drinking Water Directive, 2010).

In 2007 the nitrogen inputs on SS and GM plots was the same and amounted to 34 kg N ha⁻¹ for the period of leachate collection. Leaching of nitrogen as nitrate/nitrite was, nonetheless, significantly higher from SS than from GM plots. That is not surprising due to lower immobilization, absence of thatch and lower organic matter content in the SS rootzone (Brauen and Stahnke, 1995). The higher leakage of organic N (difference between total N and nitrate/nitrite) from GM than from SS plots can be ascribed to more 'conversion of mineral fertilizer to leachable organic form' (Paré et al., 2006) or to an initially higher content of organic nitrogen in the GM rootzone. Unlike Djodjic et al. (2004) who found no relationship between soil P content and P losses, differences in P level between soil types in our study were huge and caused more P leaching from the GM than from SS rootzone. The amount of potassium applied to both rootzones could be reduced as suggested by total leaching of this nutrient.

CONCLUSIONS AND PRACTICAL RECOMMENDATIONS

While this investigation showed that establishment and maintenance of velvet bentgrass is possible on both SS and GM root zones, the use of GM showed clear advantages in the form of higher visual quality, less disease, longer irrigation intervals, and less risk for development of soil water repellency. Disadvantages of using the compost-amended root zone were a softer green surface during the first year after establishment and greater risk for nitrogen and phosphorus leakage from established turf. Although the effect of root zone was mostly dominant to the effect of irrigation, this investigation showed a shift in optimal irrigation strategy from light and frequent irrigation during the first 18 months after the two months grow-in period to deeper and more infrequent irrigation on more mature velvet bentgrass greens. Under Scandinavian climatic conditions, neither rootzone composition nor the intervals between irrigation to field capacity showed significant effects on mat thickness or organic matter content. On the GM root zone, with an initial organic matter content of 2%, it is noteworthy that mature velvet bentgrass maintained acceptable turfgrass quality if irrigated to field capacity only at 20 mm deficit, i.e. at approximately weekly intervals.

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