Dehardening resistance of six turfgrasses used on golf greens

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Abstract

Winter injuries on golf greens cause big economic losses in Scandinavia. Dehardening resistance and rehardening capacity are important traits for survival of low freezing temperatures following warm spells during winter and early spring. Our objective was to determine plant hardiness at the start of winter and dehardening resistance of six cool-season turfgrass species/subspecies commonly used on golf greens. Plant material was collected on an experimental green at Bioforsk Landvik, SE Norway in late November 2011 and 2012 and subjected to six or twelve days of dehardening at 10 °C in a growth chamber. The ranking order for freezing tolerance (measured as lethal temperature for 50 % plants (LT_{50})) of turfgrasses taken from the field in late November was: annual bluegrass (*Poa annua* L.) (-13…-14 ˚C) < colonial bentgrass (*Agrostis capillaris* L.) (-18…-20 ˚C) ≤ slender creeping fescue (*Festuca rubra trichophylla* L.) (-19 ˚C) ≤ chewings fescue (*Festuca rubra commutate* L.) (-21 ˚C) < velvet bentgrass (*Agrostis canina* L.) (-23…-27 ˚C) ≤ creeping bentgrass (*Agrostis stolonifera* L.) (<-30 ˚C). The main dehardening occurred during the first 6 day at 10 ˚C and dehardening rates increased in the order: slender creeping fescue < chewings fescue < colonial bentgrass < annual bluegrass < creeping bentgrass. The dehardening rate of velvet bentgrass was inconsistent in the two years. An additional rehardening treatment at 2 °C for 23 days was included in 2012. None of the species were able to reharden to their original freezing tolerance after 12-d dehardening at 10 ˚C. Low overall freezing resistance and less capacity to reharden in annual bluegrass than in the other species was associated with more leaf growth during both hardening and dehardening. The results indicate that hardening ability and dehardening resistance are not necessarily positively correlated and that the turfgrass studied have developed different strategies to survive the winter.

Keywords: Cold acclimation, de-acclimation, re-acclimation, freezing tolerance, LT_{50}, perennial grasses, *Agrostis*, *Festuca*, *Poa*, daily height increment

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Abbreviations: LT_{50}, lethal temperature for 50% plants; PPFD, photosynthetic photon flux density

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1. Introduction

Winter damage is a major problem on Scandinavian sport fields. Reseeding in spring is time consuming, labour intensive, and leads to a delay of the playing season and to economic losses. Winter injuries can be caused by low freezing temperatures, frost heave, ice, flooding, photoinhibition (abiotic factors) and/or snow molds (biotic factors) alone or in combination (Kvalbein et al., 2013). The extent and origin of winter injuries vary from year to year depending on the interaction between turfgrass genotype, turf management and the environmental conditions during fall and winter.

Freezing tolerance – the ability of the plant to withstand low temperatures – has been shown to be a major component of winter hardiness of perennial grasses (Larsen, 1994; Hulke et al, 2008; Gusta and Wishniewski, 2012). Cold hardening, also known as cold acclimation, refers to an increase in freezing tolerance over time in response to inductive conditions. Hardening is a long process starting in late summer and peaking in January (White and Smithberg, 1980). Two alternate 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 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; Espevig et al. 2011, 2012). The second stage is referred to as sub-zero hardening and leads to acquisition of additional freezing tolerance (Tumanov, 1940; Livingston, 1996; Tronsmo et al., 2013). The second hardening stage is commonly associated with induced ice formation in the apoplast and dehydration of plant cells (Steponkus, 1989; Herman et al., 2006).

Dehardening, or deacclimation, refers to a loss of freezing tolerance (Kalberer et al., 2006) and occurs much faster than hardening (Gay and Eagles, 1991). Research on dehardening of winter cereals and forage grasses shows that temperature is the main factor which triggers loss of freezing tolerance and that the rate of dehardening increases both with the temperature *per se* and with the duration of the mild period (Gusta and Fowler, 1976b; Jørgensen et al., 2010; Hoffman et al., 2014). The rate of dehardening may also vary from year to year and among plant species and cultivars. Jørgensen et al. (2010) showed that dehardening of timothy (*Phleum pratense* L.) ’Engmo’ under controlled condtions varied significantly in spite of the same freezing tolerance gained in January in two experimental years in the field. Jørgensen et al. (2010) also documented that a hardier cultivar of timothy dehardened faster than a less hardy one. Similarly, Hoffmann et al. (2014) recently showed that at higher temperature (8 °C and 12 °C) the more winter hardy creeping bentgrass (*Agrostis stolonifera* L.) dehardened more than the less hardy annual bluegrass (*Poa annua* L.). However, at the lower temperature of 4 °C annual bluegrass exhibited a greater loss in freezing tolerance than creeping bentgrass, indicating a *genotype* x *temperature* interaction on dehardening resistance.

Dehardening can be completely reversible, partly reversible or completely irreversible depending on the temperature and duration of the dehardening period (Pomeroy et. al, 1975; Gusta and

Fowler, 1976a and 1976b; Rapaz, 2002). Rehardening capability refers to the plants' ability to increase its freezing tolerance after a mild spell. Mechanisms for dehardening and rehardening are not completely understood, and reversibility has been shown to depend on water content and distribution, carbohydrate metabolism, photosynthesis, antioxidants, proteins and gene expression (Kalberer et al., 2006). Only few studies have been conducting on dehardening of cool season turfgrasses in the field (Hoffman et al., 2014) or under controlled environmental conditions (Tompkins et al., 2000) and these studies seem to be limited to creeping bentgrass and annual bluegrass and except for Tompkins et al. (2000) we are not aware of there is no literature on rehardening capacities of turfgrasses.

Dehardening in response to spells of mild temperature conditions in winter and spring followed by rapid temperature drop leading to freezing injury, is claimed to be a major reason for winter kill in perennial plants including turf grasses. Due to coastal climate in the south and west of Norway, warm spells may occur any time of the winter, also in mid-winter. According to scenarios for climate change in Norway, mild spells may appear more frequently and for longer periods of time (Hansen-Bauer et al, 2009), and plants may be more exposed to temperature fluctuations due to reduced snow cover and snow duration (Thorsen and Höglind, 2010). Moreover, the global warming would lead to a warmer fall and, thus, to an incomplete hardening (Jørgensen et al., 2010, Thorsen and Höglind, 2010). Thus, our primary objective was to study over two years the resistance to dehardening of six turfgrass species/subspecies (in the following referred to as 'species') commonly used of golf greens during mild periods in winter. In the second year, the species' capacity to reharden after such dehardening was also studied.

2. Materials and methods

2.1. Site and weather data

Plant material was collected from an experimental green at the Bioforsk Turfgrass Research Centre Landvik (south coast of Norway, 58° N latitude, 12 m above sea level). Weather data from the local weather station are shown in Figures 1 and 2 (http://lmt.bioforsk.no/). The fall 2012 was colder than the fall 2011. The average daily air temperatures in September, October, and November (until samplings dates) were 12.9 ˚C, 8.9 ˚C, 6.3 ˚C in 2011 and 11.6 ˚C, 6.8 ˚C, and 5.8 ˚C in 2012, while 30-yr normal temperatures for the corresponding moths are 11.8 ˚C, 7.9 ˚C, and 3.2 ˚C. Monthly precipitation in September, October and November were, in turn, 103 mm lower, 144 mm higher and 173 mm higher in 2012 than in 2011. In spite of more rainfall the light conditions almost did not differ between the two years. The average photosynthetic photon flux density (PPFD) for the light hours in September (13.5 h), October (11 h) and November (until samplings dates) (9 h) amounted to 399, 256, and 102 μmol m^{-2} s^{-1} in 2011 and to 437, 268, and 107 μmol m^{-2} s^{-1} in 2012, respectively.

2.2. Plant material and general maintenance

The experimental green, from which plant material was collected, had been established in June 2011 from 4-yr-old sods of the following species: chewings fescue (*Festuca rubra* L. ssp. *commutata* (Thuill.) Nyman) 'Musica' (RAGT, France), slender creeping red fescue (*Festuca rubra* L. ssp. *litoralis* (G.F.W. Meyer) Auquier 'Viktorka' (Barenbrug, originally from Czech Republic), velvet bentgrass (*Agrostis canina* L) 'Villa' (Rutgers University, USA), colonial bentgrass (*Agrostis capillaris* L) 'AberRoyal' (British Seed Houses, UK), and blend of creeping bentgrass ‘Penn A1’, ‘Penn G6’ and ‘Independence’ (Penn State University and Rutgers University, USA). Annual bluegrass (unspecified seed imported from USA as *Poa annua* L. ssp. *reptans* (Hausskn.) Timm.) was seeded in July 2011 and 2012 at a rate of 10 g m^{-2}. The root zone was constructed according to the United State Golf Association specifications (USGA Green Section Staff, 2004) in 2007 and consisted of 80 % (v/v) straight sand and 20 % (v/v) garden compost.

The experimental plots of 1 m^2 each were maintained to create optimal conditions for the individual species. The total annual input of nitrogen (N), phosphorus (P) and potassium (K) on plots with velvet bentgrass, colonial bentgrass and red fescues amounted to 86, 13 and 76 kg ha^{-1} in 2011 and to 104, 8 and 74 kg ha^{-1} in 2012, respectively. N, P, and K rates on plots with creeping bentgrass and annual bluegrass were 173, 26 and 153 kg ha^{-1} in 2011 and 160, 12 and 114 kg ha^{-1} in 2012, respectively. The last fall application of fertilizer was made on November 2011 15th and November 7th 2012. The bentgrasses and annual bluegrass were mowed to 3 mm and red fescues to 5 mm three times a week using John Deere 220A (Moline, IL) or Allett (Allett Mowers LTD, Arbroath, U.K.) walk-behind green’s mowers. The last mowing in fall was made on October 26th 2011 and October 17th 2012. The experimental green was irrigated to the field capacity each time moisture content in the upper 12 cm became lower than 12 % (v/v), as measured with a TDR probe (Delta T devices, Cambridge, UK). Topdressing was applied 2-4 times per month at a total amount of 3 mm per year. In 2012, a friction wear drum with soft spikes was pulled over the plots 1-3 times a week corresponding to total wear of 7000 rounds of golf.

On November 11th 2011 and on October 5th 2012 at first appearance of the symptoms of microdochium patch caused by *Microdochium nivale* (Fr.) Samuels & Hallett the trial was treated with Delaro SC 325 (Bayer CropScience AG, Monheim, Germany) at a rate of 1 L ha^{-1} (trifloxystrobin, 150 g a.i. L^{-1} and protioconazole 175 g a.i. L^{-1}).

2.3. Dehardening and rehardening treatments

Turfgrass cores of 10-cm diameter and 7-cm depth were taken from the field on November 24th 2011 and on November 22nd 2012 and were inserted into pots of the same size. Because the mean temperatures during the last two weeks before sampling was as high as 5.0 °C in 2011 and 6.3 °C in 2012, the pots were transferred to a cold store for additional four days at -2 °C in darkness to ensure optimal hardening before the onset of dehardening treatments (Tronsmo et al., 2013). After thawing at 2 °C for 48 hours in darkness the control plots went to freezing tests (see below) while other plots were transferred to climate chambers at Bioforsk Særheim for dehardening at 10 °C and 10-h photoperiod with a PPFD of 50 µmol m^{-2} s^{-1} (Osram Lumilux T8 58W Cool White fluorescent lamps) for either 6 or 12 days. In 2012, an additional rehardening treatment at 2 °C and 6-h photoperiod with a PPFD of 150 µmol m^{-2} s^{-1} (Philips SON-T 400W high pressure sodium lamps) was conducted for 23 days after 12-day dehardening.

2.4. Freezing tests and regrowth

Short-term freezing tests were conducted according to the method described by Höglind et al. (2010) with modifications. Briefly, 10 plant groups, each consisting of 3-6 tillers, were collected for one replicate of each species and each freezing temperature and one non-freezing temperature (4 ˚C), and there were four replicates per species in each freezing test. The tillers were washed free from soil in cold water, dried between paper towels and wrapped into slightly moist paper towels. Prior to the wrapping, the roots were truncated to 3-4 cm. Plant material wrapped in paper towels was placed in the middle of plastic trays of 17x17x6 cm filled with slightly moist sand. The trays were located in the middle of a programmable freezing chamber. After the temperature had been lowered from 2 °C to -2 °C at a rate of 2 °C h^{-1}, the plants were kept at -2 °C overnight to ensure ice nucleation. Then, the temperature was lowered from -2 °C to -12 °C at a rate of 2 °C h^{-1} and from -12 °C to -30 °C in 2011 and from -12 °C to -36 °C in 2012 at a rate of 3 °C h^{-1}. Plants were removed from the freezer after they had experienced up to 7 test temperatures at 3 °C intervals. The predetermined test temperatures chosen for each species depended on their expected freezing tolerance. During freezing tests, the temperature was monitored at the plant level in the sand.

After thawing at 2 °C overnight, the groups of 3-6 tillers were planted into trays filled with sand. Non-frozen plants were included to ensure that damage was not due to separation of the tiller groups and they were planted after separation. The survival of the plants was recorded as “dead” or “alive” after 3-wk regrowth in a greenhouse. The conditions in the greenhouse were 18 °C (day) and 12 °C (night) and 16-h photoperiod with a PPDF of 150-200 µmol m^{-2} s^{-1}. The natural daylight was supplemented with Philips SON-T 400W high pressure sodium lamps to ensure a minimum of 150 µmol m^{-2} s^{-1} the average PPFD over the 3 weeks was 175 µmol m^{-2} s^{-1}. Freezing tolerance of the species was expressed as a lethal temperature for 50 % of the tiller groups (LT_{50}).

2.5. Turfgrass daily height increment

In 2012, the heights of the grasses were measured using a ruler from the soil level to the mean height of the leaves prior to the first freezing test on November 28th and prior to the third freezing test on December 10th (after 12-d dehardening). Plant growth during hardening was expressed as average daily height increment since the last mowing on October 17th, and plant growth during dehardening as daily height increment from November 28th to December 10th.

2.6. Statistical data analysis

The experiment was conducted according to a randomized complete 4-block design (RCBD) in which the layout in the field experiment was followed during sampling, dehardening/rehardening treatments, freezing tests, and regrowth in the greenhouse. The data were analysed using the procedure PROC ANOVA (version 9.2, SAS Institute Inc., Cary, NC, USA) with species and dehardening/rehardening treatments as fixed factors. LT_{50}s were calculated by PROBIT analysis using the logistic distribution in SAS.

3. Results

3.1. Dehardening and rehardening

The freezing tolerance by the end of November varied among the grass species (Figure 3). The hardiest species was creeping bentgrass with LT_{50} lower than -30 °C in 2011 (exact LT_{50} could not be determined as 85 % of creeping bentgrass plants survived the lowest test temperature of -30 °C) and -31 °C in 2012. Next to creeping bentgrass velvet bentgrass was the most freezing tolerant species with LT_{50} -23 °C in 2011 and -27 °C in 2012. The LT_{50} of chewings fescue, slender creeping red fescue and colonial bentgrass was between -18 °C and -21 °C both years. The least freezing tolerant species was annual bluegrass with LT_{50} -13 °C in 2011 and -14 °C in 2012. While the freezing tolerance of velvet bentgrass, colonial bentgrass and annual bluegrass was appropriately 4 °C, 2 °C, and 1 °C lower in the last week of November 2012 than in 2011, the freezing tolerance of the fescue subspecies was similar in 2011 and 2012.

The main dehardening occurred during the first 6 days at 10 °C, irrespective of species (Figure 3, Table 1). The rate of dehardening during this period varied among the grasses and on average for the species it was 1.5 °C greater in 2012 compared with 2011. Creeping bentgrass and annual bluegrass dehardened faster than the other species in both years: an absolute loss of freezing tolerance in creeping bentgrass was more than 6.2 °C in 2011 and 8.6 °C in 2012, whereas the loss inn annual bluegrass was 5.2 °C in 2011 and 6 °C in 2012 (Table 2). The most dehardening resistant species were velvet bentgrass in 2011 and slender creeping red fescue in 2012 with only 2.0-2.5 °C loss of the freezing tolerance during first 6 days at 10 °C.

All species, except annual bluegrass, dehardened more in 2012 than in 2011. On average for the bentgrasses and fescues, further dehardening for additional six days at 10 °C led to 0.6 °C and 2.8 °C loss of the freezing tolerance in 2011 and 2012, respectively (Table 2). The freezing tolerance of annual bluegrass was decreased by 1.4 °C in 2011 but showed no further change (0.2 °C) in 2012 (0.2 °C). After either 6-d or 12-d dehardening at 10 °C, creeping bentgrass remained the hardiest species but differences from velvet bentgrass became insignificant (except after 6-d of dehardening in 2011), followed by fescues, colonial bentgrass, and annual bluegrass (Figure 3, Table 1).

In 2012 all species rehardened to some extent at 2°C for 23 days, but never back to their initial LT_{50} levels before dehardening. The highest rehardening capacity of 4.1-4.4°C had the bentgrasses, followed by fescues (3.3-3.4°C), and annual bluegrass (only 1.5°C)

3.2. Turfgrass daily height increment

The daily height increments during hardening varied among the grasses (Table 3). Annual bluegrass had the highest daily height increment of 0.167 mm day^{-1}, followed by fescues and colonial bentgrass (0.030-0.042 mm day^{-1}), creeping bentgrass (0.012 mm day^{-1}), and velvet bentgrass (no detectible growth). During 12-d dehardening at 10 °C growth rates of creeping bentgrass, colonial bentgrass, annual bluegrass, and chewings fescue were increased 10.5, 5.3, 4.4, and 1.4 times, respectively. No increase in growth was recorded in slender creeping red fescue. The growth rate of velvet bentgrass during dehardening was significantly lower than for the other bentgrasses.

4. Discussion

In Norway, forage grasses and turfgrasses are usually most freezing tolerant from December through January (Thorsen and Höglind, 2010; Espevig et al., 2013). In continental Canada in the end of November, LT_{50}s as low as -40 °C and -20 °C have been reported for creeping bentgrass and annual bluegrass, respectively (Tompkins et al., 2000), but in our study the LT_{50} was hardly below -30 °C for creeping bentgrass and -14 °C for annual bluegrass. Thus, even though they were exposed to additional subzero hardening for four days in a freezing chamber, the grasses in our study were likely hardened incompletely by the end of November. The ranking order for freezing tolerance in late November, annual bluegrass < colonial bentgrass ≤ slender creeping fescue ≤ chewings fescue < velvet bentgrass ≤ creeping bentgrass, is however, in good agreement with the results of White and Smithberg (1980) and with the general ranking order found in most American textbooks (e.g. Beard 2002; Turgeon, 2005). It is also important to keep in mind that significant differences in hardening potential may exist among cultivars within each species depending of the temperature and day length conditions at the place of origin. A noteworthy example is 'AberRoyal' which in variety testing has been documented to be less winter hardy than most other cultivars of colonial bentgrass commonly used in Scandinavia (Aamlid et al., 2012).

We observed that creeping bentgrass dehardened faster than annual bluegrass during 6 days at 10 °C as revealed by a greater absolute loss in freezing tolerance in creeping bentgrass than in annual bluegrass. This is in agreement with DaCosta and Hoffman (2010) who showed that after cold hardening in controlled environment the loss of freezing tolerance during 5 days at 12 °C was greater in creeping bentgrass 'L-93' (from -19.2 °C to -8.5 °C) than in annual bluegrass (Canadian biotype) (from -14.3 °C to -6.1 °C). A field study with creeping bentgrass 'Penncross' and annual bluegrass (biotype from Minnesota) in late winter and early spring also showed that creeping bentgrass was more vulnerable to dehardening than annual bluegrass (Tompkins et al., 2000). In that study, the loss of freezing tolerance (measured as LT_{50}) in creeping bentgrass from the end of March to the end of April was far greater (from -31 °C to -10 °C) than in annual bluegrass (from -14 °C to -13 °C). If the snow was removed in late March, the freezing tolerance of creeping bentgrass declined even faster as temperature became warmer (varied from 2 °C to 14 °C) as compared with that under snow (constant near 0 °C) (Tompkins et al., 2000). Studying forage grasses, Jørgensen et al. (2010) also found that the more winter hardy timothy ’Engmo’ dehardened more rapidly than less winter hardy ’Grindstad’.

In spite of the greater absolute dehardening in creeping bentgrass than in annual bentgrass, the relative loss in freezing tolerance in creeping bentgrass was lower than in annual bluegrass. If freezing tolerance of nonhardened creeping bentgrass and annual bluegrass is assumed to be -9.3 °C and -8.3 °C, respectively (Hoffmann et al., 2014), the hardening capacity of creeping bentgrass was more than 20 ˚C vs. 5 ˚C in annual bluegrass. Thus, the loss of freezing tolerance relative to hardening capacity of creeping bentgrass (in our study would amount to 27 % and 38 % in 2011 and 2012, respectively, vs. more than 100 % in annual bluegrass. Thus, the consequences of dehardening for turfgrass winter survival will usually be more critical in annual bluegrass than in creeping bentgrass. Of particular importance is that creeping bentgrass seems to be more resistant than annual bluegrass to dehardening at temperatures as low as 4 °C (Hoffman et al., 2014). Future studies on dehardening should include measurements of freezing tolerance of non-hardened plants to be able to determine the relative loss of their hardiness.

In addition to creeping bentgrass and annual bluegrass, our investigation included four species and subspecies for which little information exist about hardening or/and dehardening characteristics (Espevig et al., 2011; Blombäck et al., 2012; Tronsmo et al. 2013). Very little information is available on the freezing tolerance of fine fescues (Blombäck et al. 2012) although it generally considered that chewings fescue is more winter-hardy than slender creeping red fescue (Kvalbein and Aamlid, 2012). In our study, this was confirmed by better freezing tolerance of ‘Musica’ than of ‘Viktorka’ by the end of November in both years (-21 °C vs. -19 °C, respectively). On the other hand, the data again show that the less winter hardy slender creeping type was more resistant to loss of freezing tolerance than the more winter hardy chewings type; the loss after 12 days at 10 °C was 3.6 °C vs. 4.3 °C in 2011 and 5.4 °C vs. 6.7 °C in 2012, respectively.

In our study the freezing tolerance in late November was either on the same level (fescues) or better (colonial bentgrass and velvet bentgrass) in 2012 than in 2011, but dehardening occurred faster after a colder fall in 2012 than after a warmer fall in 2011 for all species except annual bluegrass. The higher dehardening rate in colonial bentgrass and velvet bentgrass in 2012 vs. 2011 may have been associated with a higher loss of carbohydrates and other freezing protective compounds since their freezing tolerance was better in 2012 than in 2011 (Espevig et al., 2011, 2012). If this is correct, our results are opposite to those of Jørgensen et al. (2010) who found that timothy plants that had low carbohydrate contents after a mild fall were more susceptible to dehardening than plants with a higher initial carbohydrate content after a cold fall.

We observed that the main dehardening in the all the studied turfgrass species occurred during the first 6-d exposure to 10 °C, and that the subsequent 6-d exposure reduced freezing tolerance to a lesser degree. For comparison, Hoffman et al. (2014) observed that the main dehardening in creeping bentgrass and annual bluegrass occurred already during the first day at 4-12 °C and that a full loss of freezing tolerance required either longer exposure to the same temperature or exposure to a higher temperature (DaCosta and Hoffman, 2010). In our study, the loss of freezing tolerance at 10 °C was substantial, but did not lead to completely kill of unhardened plants. Further research is needed to establish critical combinations of temperature and duration for complete dehardening in the different species. Another issue is that LT_{50}, which is a measure for plants' ability to cope sudden frosts of short duration and mainly refers to injury from intracellular ice formation, may not always be the best expression for freezing tolerance under field conditions. At least in the early winter, the LD_{50}, i.e. the lethal duration of a moderately low freezing temperature, is probably a better expression for winter hardiness than LT_{50} as it also include a prolonged desiccation injury due to extracellular ice formation (Gusta et al., 1997; Waalen et al., 2011; Espevig, 2013).

All in all, our results are in agreement with the conclusion by Hoffman et al. (2014) that cold hardening capacity and susceptibility to dehardening are separate traits with different genetic inheritance. More research is needed to identify how dehardening resistance/susceptibility develops during winter and whether there is a relationship between dehardening and the plants' hardening status, namely, whether dehardening rates differ in completely versus incompletely hardened plants and what the mechanisms are.

After 12 days of dehardening at 10 °C none of the species in our study were able to reharden to their original pre-dehardening freezing tolerance acquired in the field in November. This could probably be related to the relatively long duration of the dehardening treatment and its stimulating effect on leaf growth. Pomeroy et al. (1975) showed that dehardening of winter barley (*Hordeum vulgare* L.) and winter wheat (*Tritcium aestivum* L.) was not completely reversible if it lasted more than 3 and 24 hours, respectively. That study also showed that winter hardy winter wheat seedlings which had been dehardened no longer than 24 h at 20 °C/15 °C were able to reharden during 5 days to the original freezing tolerance gained during 6-wk hardening at 2 °C/-2 °C. Similarly, exposure to 2 °C for two weeks was enough to promote complete rehardening in a study of *Lolium* and *Festulolium* that had been dehardened for 8 days at 9 °C (Höglind et al, unpublished results). Conversely, Rapacz (2002) demonstrated that dehardening of oilseed rape was not completely reversible if plant growth had been induced by warm temperature. Our results are in agreement with the latter findings since a significant induction of leaf growth in response to 10 °C for 12 days was recorded in all grass species except for slender creeping red fescue. The fact that the lowest loss in freezing tolerance during 12 weeks of dehardening in 2012 was observed in slender creeping red fescue may be compatible with the observation that this was the only species not showing an increase in height growth during dehardening.

In perennial grass species there is often a positive correlation between early growth cessation and winter hardiness including freezing tolerance, although this relationship is not always strict (Brummer et al, 2000). In this study, the leaf growth of creeping bentgrass, chewings fescue, slender creeping red fescue, colonial bentgrass, and annual bluegrass under hardening in the fall 2012 was reduced approximately 98.3 %, 97.6 %, 96.2 %, 96.2 %, and 72.2 %, respectively, compared to their normal growth rate in summer (Aamlid and Molteberg 2011; Aamlid et al., 2012). This can be compared with the freezing tolerance that these species achieved namely -31 °C, -21 °C, -19 °C, -20 °C, and -14 °C, respectively. These findings would indicate a positive correlation between degree of growth reduction in fall and freezing tolerance also for the turfgrass species studied here. Conversely, among species the ability to initiate leaf growth during mild periods seems to be negatively correlated with the species' resistance to dehardening.

In conclusion, this study indicates that hardening ability and dehardening resistance of turfgrasses are not necessarily correlated and that the cool-season grasses used on golf greens have developed different strategies to survive the winter. Creeping bentgrass usually develops superior freezing tolerance in the late fall; in comparison with other species this favourable starting point will normally more than compensate for its relatively high vulnerability to deharden and initiate new growth during extended periods with mild temperatures during winter and early spring. Velvet bentgrass is usually slightly less freezing tolerant than creeping bentgrass at the onset of winter but also more resistant to dehardening; for practical purposes, we therefore consider the freezing tolerance of velvet bentgrass to be on the same level as creeping bentgrass. Chewings fescue, slender creeping red fescue, and colonial bentgrass are commonly seeded in mixtures on golf greens; common to these species is that they only develop moderate freezing tolerance in response to hardening conditions in the fall, but for the fescues this is partly compensated by relatively slow dehardening and initiation of growth during mild periods. Annual bluegrass is in class of its own in that the freezing tolerance obtained in fall is too low to be compensated by a moderate rate of dehardening during mild periods. Compared with other species, the best indication of the poor winter tolerance of annual bluegrass is probably its high growth rate under both hardening and dehardening conditions.

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FIGURE LEGENDS (Figures are attached as JPEG files)

Figure 1. Daily air temperature and precipitation during the fall 2011 and 2012 at Landvik prior to dehardening treatments. Dotted lines show normal temperature for the reference period 1961-90. Bars following the daily air temperature show maximal and minimal air temperatures.

Figure 2. Photosynthetic photon flux density (PPFD) during the fall 2011 and 2012 at Landvik prior to dehardening treatments as averaged for either 24 hours or for only hours with positive radiation balance

Figure 3. Freezing tolerance of six turfgrass species after hardening in the field by November 24th 2011 and November 22nd 2012 (FH) and after subsequent dehardening at 10 °C during either 6 days (DH6) or 12 days (DH12), and after reharding at 2 °C during 23 days (RH). Bars show standard errors of the means.

Table 1. Main effects of *species* and *dehardening/rehardening treatments* on freezing tolerance as determined by LT$_{50}$, and results from analysis of variance (ANOVA) of experiments in 2011 and 2012.

Source of variation	Level	LT$_{50}$ 2011 (°C)	LT$_{50}$ 2012 (°C)
Species	Creeping bentgrass blend of ‘Penn A1’, ‘Penn G6’ and ‘Independence’	(<) -26 a^{ab}	-24 a
(*Spp*)	Velvet bentgrass 'Villa'	-22 b	-23 a
	Chewings fescue 'Musica'	-19 c	-17 b
	Slender creeping red fescue 'Viktorka'	-17 d	-17 b
	Colonial bent 'AberRoyal'	-15 e	-15 c
	Annual bluegrass	-9 f	-10 d
De-/ Rehardening (DH/RH)^{c}	Field hardening	-21 A	-22 A
	Dehardening 6 days^{c}	-17 B	-16 B
	Dehardening 12 days (DH12)	-16 C	-14 C
	Rehardening 23 days after DH12^{c}	-	-18 B

	ANOVA			
	df	*p*	df	*p*
Spp	5	0.000	5	0.000
DH/RH	2	0.000	3	0.000
Spp x DH/RH	10	0.001	15	0.339

[a] Lethal temperature for 50% plants (LT$_{50}$) of creeping bentgrass was lower than -30 °C after hardening in the field and could not be determined exactly as the lowest test temperature was -30 °C.

[b] Means that do not share a letter are significantly different as tested using Fisher LSD (alpha = 0.05).

[c] Plants were dehardened at 10 °C and 10-h photoperiod with 50µmol m^{-2} s^{-1} PPFD and rehardened at 2 °C and 6-h photoperiod with 150µmol m^{-2} s^{-1} PPFD.

Table 2. Absolute loss in lethal temperature for 50 % plants (LT$_{50}$) of six turfgrass species/subspecies after dehardening at 10 °C for 6 days (DH6) (compared with hardening in the field (FH)), after dehardening at 10 °C for 12 days (DH12) and after rehardening at 2 °C for 23d (RH).

	2011		2012		
	FH-DH6 (°C)	DH6-DH12 (°C)	FH-DH6 (°C)	DH6-DH12 (°C)	DH12-RH (°C)
Creeping bentgrass blend of‘Penn A1’, ‘Penn G6’ and ‘Independence’	>6.2[a]	0.9	8.6	3.2	4.4
Velvet bentgrass 'Villa'	2.5	+0.6	6.2	2.3	4.4
Chewings fescue 'Musica'	3.5	0.8	4.6	2.1	3.4
Slender creeping red fescue 'Viktorka'	3.0	0.6	2.0	3.4	3.3
Colonial bent 'AberRoyal'	3.9	1.5	5.0	2.9	4.1
Annual bluegrass	5.2	1.4	6.0	0.2	1.5

[a] LT$_{50}$ of creeping bentgrass was lower than -30 °C after hardening in the field and could not be determined exactly as the lowest test temperature was -30 °C.

Table 3. Daily heights increment of six turfgrass species/subspecies during hardening period (from the last mowing on October 17th 2012 until the first freezing test on November 29th) (FH) and during 12-d deacclimation period at 10 °C (from November 29th (FH) until the third freezing test on December 11th) (FH-DH12).

Species		FH (mm)		FH-DH12 (mm)
Creeping bentgrass blend of ‘Penn A1’, ‘Penn G6’ and ‘Independence’		0.012 ab[a]		0.125 ab
Velvet bentgrass 'Villa'		0.000 a		0.063 a
Chewings fescue 'Musica'		0.030 ab		0.042 a
Slender creeping red fescue 'Viktorka'		0.042 b		0.042 a
Colonial bentgrass 'AberRoyal'		0.036 ab		0.188 b
Annual bluegrass (unspeciefied)		0.167 c		0.729 c
		ANOVA		
	df	*p*	df	*p*
Species	5	0.000	5	0.000

[a] Means that do not share a letter are significantly different as tested using Fisher LSD (alpha = 0.05).