Norwegian University of Life Sciences
Faculty of Veterinary Medicine and Biosciences
Department of Plant Sciences

Philosophiae Doctor (PhD)
Thesis 2015:88

Winter hardening of Norwegian perennial forage crops in a prolonged growing season

Vinterherdighet av flerårige norske engvekster i en forlenget vekstsesong

Sigríður Dalmannsdóttir

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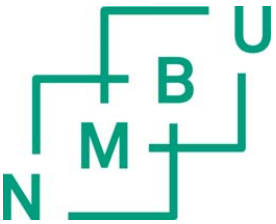

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This work is dedicated to the farmers in Northern Norway

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Compete with yourself and collaborate with others

Sigríður Dalmannsdóttir

Tromsø, 1 October 2015

Content

ABSTRACT

Current climate change models predict that the temperature increase due to global warming will be greatest at higher northern latitudes. Most probably, temperature will increase more during autumn and winter than summer, with more of the precipitation falling as rain. The question is whether perennial plants will be able to cold acclimate sufficiently during late summer and autumn in future climate. Current northern-adapted cultivars of timothy are becoming less competitive compared with cultivars adapted to regions further south in Scandinavia and new breeding efforts are needed. Timothy (*Phleum pratense* L.) is the most common forage grass species in Northern Norway today, but with extended growing season perennial ryegrass (*Lolium perenne* L.) may be introduced. The possibility to introduce new, and more productive perennial crops in high latitude areas, will most likely still be dependent on the winter survival.

The effect of climatic conditions during autumn on cold acclimation of Norwegian perennial forage crops was investigated, focusing on future climate scenarios. The study was based on pre-breeding material and cultivars, which were a part of the material selected for use in the VARCLIM project. Northern and southern-adapted populations of perennial ryegrass, timothy, red clover (*Trifolium pratense* L.) and Festuca-Lolium hybrids (×*Festulolium*) were tested. Freezing tolerance, photochemical activity and growth cessation were measured as responses to temperature, daylength/irradiance and water-logging treatments. Plants were studied both in field experiments, in outside pot experiments and under controlled conditions in the phytotron.

In a phytotron study we tested the effect of pre-acclimation temperatures (9°, 12°, 15°C) on cold acclimation of perennial ryegrass, timothy and red clover under constant controlled light conditions. A rise in pre-acclimation temperature decreased both freezing tolerance and photoacclimation, results being species dependent. In another phytotron study the effect of natural light conditions during autumn, in a combination with different temperature treatments, was tested on perennial ryegrass and timothy. Plants were first pre-acclimated at 12°, 6° and 9/3°C (day/night) and then cold acclimated at 2°C. This procedure was repeated at three different periods in autumn (Sept., Oct., Nov.) with decreasing sums of irradiance and daylengths. Plants cold acclimated during the lowest irradiance/shortest daylength had the lowest freezing tolerance and photosynthetic activity, especially in combination with higher temperature. Northern-adapted populations were more sensitive to shorter daylength and higher temperature treatments compared to southern-adapted.

Aiming to understand better the growth cessation process, a field experiment was established in Bodø (67°N) and on the northwest coast, Fureneset (61°C). Leaf elongation and chlorophyll fluorescence of perennial ryegrass, Festulolium and adapted meadow fescue cultivars were measured once a week during two autumns. Results indicated a stronger photoperiodically-controlled growth cessation within the northern-adapted populations compared to the southern-adapted. Using fluorescence recording might be a valuable tool for early selection of non-native grasses for high latitude regions.

Water-logging effects on cold acclimation during autumn were tested on red clover and timothy in outside-pot experiments both at Holt (69°N) and Fureneset (61°N). Treatments with a totally water-logged soil or soil at field capacity affected the level of freezing tolerance and carbohydrate concentration less than the differences in climate conditions between locations and years, in addition to species and population differences. Red clover was in general less freezing tolerant compared to timothy, but less sensitive to climatic differences between locations. In timothy, water-logged soil enhanced the freezing tolerance at the northern site (Holt) with lower autumn temperatures, but reduced the freezing tolerance at the southern site (Fureneset) where autumn temperatures were higher. Prolonged water-logging may negatively affect the cold acclimation and freezing tolerance at future elevated temperatures, timothy being more affected compared to red clover.

Results from all studies indicate that the projected climate change in the north may delay hardening in autumn, reduce photoacclimation and decrease freezing tolerance in grasses and clover. Climate change with warmer and wetter autumns could cause inadequate cold acclimation and winter survival at higher latitudes in non-native grass species that have been introduced to Scandinavian grassland in recent decades. The present forage species and cultivars may therefore have to be replaced by species and cultivars which are able to acclimate adequately under new light × temperature combinations, combinations which are unique in the global context for agricultural production.

Keywords: Cold acclimation, perennial forage crops, freezing tolerance, photochemical activity, growth cessation, anoxia, breeding populations

SAMMENDRAG

Fremtidige klimaprojeksjoner indikerer størst økning i temperatur ved nordlige breddegrader. Mest sannsynlig vil temperaturen øke mer om høsten og vinteren en om sommeren, og nedbøren kommer mer i form av regn enn snø. Spørsmålet er om flerårige fôrvekster kan oppnå tilstrekkelig vinterherdighet i løpet av sensommeren og høsten i det framtidige klimaet. Nordlig adapterte sorter er ikke lenger like konkurransedyktige sammenlignet med sorter som er adaptert til forhold lenger sør i Skandinavia, vi behøver derfor mer fokus på foredling. Timotei (*Phleum pratense* L.) er den mest brukte fôrveksten i Nord-Norge i dag, men i en forlenget vekstsesong vil økt bruk av raigras (*Lolium perenne* L.) være mulig. Muligheten til å innføre nye, mer produktive engvekster i nord som følge av klimaendringer, vil mest sannsynligvis fortsatt være begrenset av overvintringsevnen.

Som en del av prosjektet VARCLIM har vi undersøkt effekten av høyere temperatur og vannmettet jord om høsten på plantenes innvintring. Vi har målt fysiologiske og morfologiske karakterer knyttet til vekstavslutning og herding, både fotosyntese, bladvekst og frosttoleranse. Vi testet sorter og populasjoner av flerårig raigras, timotei, rødkløver (*Trifolium pratense* L.) og raisvingel (×*Festulolium*) som er foredlet for nordlige eller sørlige forhold. Forsøk ble gjort både i felt, som semi-felt forsøk med potteplanter og under kontrollerte klimaforhold i fytotron.

I fytotron testet vi effekter av økt temperatur (9°, 12°, 15°C) tidlig i herdingsfasen hos raigras, timotei og rødkløver under kontrollerte lysforhold. Resultatene viste at plantene hadde mindre frosttoleranse hvis de var herdet ved høyere temperatur. Det var tydelige artsforskjeller, og grasartene viste økt fotoinhibering ved høyere temperatur tidlig i herdinga. Vi testet også effekter av temperatur og fotoperiode på herding hos raigras og timotei. Frøplanter ble etablert under kontrollerte lysforhold og deretter herdet under naturlig dagslys ved tre forskjellige temperaturer; 12°, 6° og 9/3°C (dag/natt) i fire uker, og til slutt i ei uke ved 2°C. Denne prosessen ble gjentatt tre ganger utover høsten ved forskjellig fotoperiode (sept., okt., nov.) for å teste effekten av temperatur under avtakende daglengde og lysinnstråling. Plantene som ble herdet under kortest fotoperiode (nov.) hadde lavest frosttoleranse. Høyere herdingstemperatur (12°C) førte også til redusert frosttoleranse. Det nordlige materialet viste bedre frosttoleranse, unntatt ved behandling med høy temperatur (12°C) og kortest fotoperiode, der det ikke var noen forskjell på nordlig og sørlig materiale. Plantenes fotosyntetiske aktivitet ble redusert når fotoperioden ble kortere. Lengdevekst av blad ble påvirket mer av temperatur enn av fotoperiode, og høyere temperatur ga mer vekst.

For å forstå bedre selve vekstavslutningen om høsten, utførte vi et feltforsøk med raigras og raisvingel på to lokaliteter, Bodø, Vågønes (67°17′N) og Fjaler, Fureneset, (61°34′N). Lengdeveksten ble målt på enkeltblad en gang i uken utover høsten i tillegg til måling av fotosyntetisk aktivitet ved hjelp av klorofyllfluorescens. Resultatene viste forskjellig samspill mellom målte parametre og lokalitet. I sør (Fjaler) var det en positiv korrelasjon mellom fotosyntetisk aktivitet på høsten og vinteroverlevelse følgende vår. I nord (Bodø) var korrelasjonen mellom fotosyntetisk aktivitet og vinteroverlevelse svakt negativ. Det var en sterk sammenheng med redusert fotosyntetisk aktivitet på høsten og økt vinteroverlevelse i nordlig adaptert gras i feltet i Bodø.

Effekten av vannmettet jord under herding ble testet hos rødkløver og timotei. Utendørs potteforsøk på Holt (69°N) og på Fureneset (61°N) ble foretatt to etterfølgende år. Behandling med fullstendig vannmettet jord ble sammenlignet med jord som hadde vannprosent som under vanlige dyrkingsforhold. Forsøkslokalitet og klimaforskjeller mellom år hadde større effekt på frost toleransen og karbohydratsammensetningen enn vannbehandlingen. Artene reagerte forskjellig, rød kløver hadde sannelig mindre frosttoleranse enn timotei, men den var mindre sensitive mot klimaforskjeller mellom lokaliteter. Timotei viste økt frost toleranse når den ble herdet i nord (Holt) ved lavere temperaturer, men frost toleransen ble svekket når den ble herdet lenger sør ved høyere temperaturer (Fureneset). Resultatene tyder på at lengere perioder med vannmettet jord om høsten ved høyere temperaturer kan svekke herdigheten og frosttoleransen, hvor timotei kan være mer sensitiv enn rødkløver.

Våre resultater indikerer at forventede klimaendringer i nord kan føre til en forsinket herdingsprosess som vil foregå ved kortere daglengde, noe som kan redusere frosttoleranse hos gras. Arter og foredlete sorter/populasjoner responderer forskjellig på temperatur og fotoperiode, og sørlig tilpasset materiale mangler egenskapen til å starte vekstavslutning tidlig nok for å kunne oppnå tilstrekkelig herding. Nordlig materiale derimot, responderer på temperatur og daglengde og reduserer fotosyntetisk aktivitet tidlig på høsten. I et fremtidig klima kan vi trenge sorter som opprettholder fotosyntetisk aktivitet utover høsten og har lav respirasjon ved lite lys slik at de kan dra nytte av forlenget vekstsesong, men i tillegg bli godt nok herdet før vinteren.

Nøkkelord: Herding, forvekster, frost toleranse, fotosyntetisk aktivitet, vekstavslutting, anoxia, foredlingspopulasjoner

LIST OF PAPERS

This thesis is based on the following papers:

I. Dalmannsdottir S., Rapacz M, Jørgensen M., Østrem L., Larsen A., Rognli O.A. Temperature before cold acclimation affects cold tolerance and photoacclimation in timothy (*Phleum pratense* L.), perennial ryegrass (*Lolium perenne* L.) and red clover (*Trifolium pratense* L.). Journal of Agronomy and Crop Science 2015, doi:10.1111/jac.12149

II. Dalmannsdottir, S., Jørgensen, M., Rapacz, M., Østrem, L., Larsen, A., Rognli, O.A. Interaction of Temperature and Autumn Light Conditions Affecting Cold Tolerance of Timothy (*Phleum pratense* L) and Perennial Ryegrass (*Lolium perenne* L.). (Submitted to Physiologia Plantarum, in review)

III. Østrem L., Rapacz M., Larsen A., Dalmannsdottir S., Jørgensen M., 2014: Influences of growth cessation and photoacclimation on winter survival of non-native *Lolium-Festuca* grasses in high-latitude regions. Environ. Exp. Bot. 111, 21-31.

IV. Dalmannsdottir, S., Østrem, L., Larsen, A., Jørgensen. M., Rognli, O.A. Effect of water-logging on winter survival of red clover (*Trifolium pratense* L.) and timothy (*Phleum pratense* L.). (Manuscript)

1. BACKGROUND FOR THE PROJECT

This PhD project is a part of a larger project established to respond to the need for developing new and improved cultivars of forage grasses and legumes adapted to the future projected climate conditions in Norway.

Improving adaptation of high-yielding forage crops creates substantial economic value. In Norway, compensatory grants to farmers because of winter damaged fields have amounted to about 150 million NOK totally the last 20 years (Statistics Norway). These payments go to farmers who have to re-establish their meadows after a harsh winter with complete winter kill. However, there are substantial differences between years with 1995, 1998, 2010 and especially 2013 being the most damaging winters in the country during the last two decades.

Farmers in Norway, especially in the north and on the northwest coast, are demanding new cultivars, better adaptable to the current climatic conditions. Current northern-adapted cultivars of timothy are becoming less competitive compared with cultivars adapted to regions further south in Scandinavia according to the results of national variety testing (Larsen and Rognli 2007).

Expected climate changes may further change the conditions for perennial forage plants, especially at in high latitude regions of the north. In Northern Norway, i.e. Nordland, Troms and Finnmark counties, future climate changes are expected to extend the growing season by 1-4 weeks from the period 1961-1990 to 2021-2050 (Uleberg et al. 2014). This may increase the potential biomass production of forage grasses in these areas substantially, and increase the number of harvests per year. Farmers in Northern Norway already report that the grazing season has been prolonged due to increased temperatures, especially in the autumn (Uleberg et al. 2014). This is consistent with weather data showing that warming during autumn has been stronger than during spring the last 30 years (Hansen-Bauer et al. 2009). The current northern-adapted cultivars are adapted to long day light conditions at high latitudes and low temperature during summer, followed by period with fast declining daylength during late summer/early autumn when the cold hardening process starts taking place. The ongoing climate change with later onset of autumn is changing the temperature/daylength relationship, and this may interact and hamper the cold acclimation of northern-adapted cultivars. Replacing the northern-adapted cultivars by more southern-adapted does not solve the problem since southern-adapted cultivars are not winter hardy enough; they are not adapted to the daylength in the north and do not strop growth early enough to prepare sufficiently for winter. In addition to the temperature increase, the amount of precipitation during autumn is

increasing, especially in coastal regions of the country. This is resulting in heavily waterlogged soils, additionally changing the conditions for perennial plants during cold acclimation.

Warmer and longer growing season may open up possibilities for increased use of more southern-adapted species than timothy, which currently is the most used perennial forage grass species in Norway. Perennial ryegrass and red clover are important forage crop species in Central and Western Europe and might have a better chance of surviving in future climate of the north compared to today.

This PhD project is part of the research project VARCLIM – 'Understanding the genetic and physiological basis for adaptation of Norwegian perennial forage crops to future climates (2010-2014)', funded by the Research Council of Norway. Important traits involved in adaptation were studied in pre-breeding and breeding populations, and cultivars, including adapted and exotic germplasm of timothy, perennial ryegrass, Festulolium, red clover and Lucerne, made by Graminor AS, the national breeding company in Norway. Increasing robustness of perennial forage crops for changed climatic conditions in Norway is also a contribution to the goal of The Food and Agricultural Organisation (FAO) for a global food security under climate change (Schmidhuber and Tubiello 2007).

Though this project used breeding material from the Norwegian breeding company Graminor and focused on the climatic conditions within Norway, it is also a contribution to development of new plant material suitable for high northern latitudes with similar climate conditions as in Norway. Plant breeding is a time consuming activity and collaboration between countries is therefore very important.

During the last decades breeding has been calculated to contribute to approximately 50% of the world`s crop production (Parry et al. 2012) following the `green evolution`. Selection and breeding has broadened the climate adaptation of many species from beyond their geographical origin.Thus, there is no doubt that plant breeding will be an important factor of the bioeconomy in future climates.

1.1 Objectives

The VARCLIM research project focused on pre-breeding objectives for future climate conditions and the overall aim is mentioned above. The aim of this particular PhD project was to investigate how the physiological processes of photoacclimation, cold acclimation and growth cessation are affected by the different climatic factors such as temperature, daylength and soil water content. Emphasis has been on how the rapid ongoing and future climate

change is affecting the acclimation process, especially in high latitude regions where the light and temperature combination is unique.

Furthermore, northern- and southern-adapted plant material of timothy, perennial ryegrass and red clover was compared in order to identify target traits for winter survival in existing breeding populations and cultivars. Two main hypotheses have been tested in this thesis:

- Higher fall and winter temperatures combined with normal short photoperiods and more rain will intensify or pose new types of stresses for winter survival of forage plants, especially in the north.
- Different genotypes have different physiological strategies to meet these challenges, and their plasticity can be investigated using physiological test methods.

2. INTRODUCTION

2.1 Forage production in Norway

Agriculture in Norway is mainly based on forage from perennial forage crops, mainly grasses. Norway is 385.230 km^2 and characterised by mountains and an extensive coastline stretching from 57°57´30″N in the south to 71°10´21 ″N in the north. Only about 3 % of the country is cultivated and estimated arable land is about the double of that cultivated today (Strand and Bekkhus 2008). Large mountain and forest areas, shallow soils and cold climate are among reasons for this low proportion of cultivated land compared to other European countries. The most commonly sown forage grass species in Norway is timothy (*Phleum pratense* L.) because of its good persistence, high yields and high feed quality. It is usually sown in mixtures with meadow fescue (*Festuca pratensis* Huds.) and red clover (*Trifolium pratense* L.) or white clover (*Trifolium repens* L.). Because of insufficient winter hardiness, the use of perennial ryegrass (*Lolium perenne* L.) is limited mainly to the southern and South-Western parts of the country. Huge climatic differences within the country influence the timing and number of harvests within the different regions. A long growing season in the southern and eastern part of the country allows farmers to harvest 3-4 times during summer whereas the short growing season traditionally allows only 1-2 harvests in the northernmost counties.

Agriculture in Northern Norway is the northernmost active agriculture in the world today with 24 hour daylength in mid-summer and fast diminishing daylength in the autumn after the fall

equinox. Nowhere else in the world is agricultural activity performed under such climate conditions, these are unique combinations of daylength, light intensity and quality, and temperature. Agriculture in Northern Norway is at its margin climatically and winter survival is the far most important trait for perennial crops. Current northern adapted cultivars are based on germplasm introduced from regions further south in Norway and Northern Europe. Farmers and the agricultural sector are increasingly demanding cultivars with better adaptation, especially in the northernmost regions of Norway (Uleberg et al. 2014). The current northern-adapted cultivars are performing sub-optimally, while certain southern cultivars are performing progressively better, probably due to the higher temperatures and milder winters experienced in the last decades (Larsen and Rognli 2007).

2.2 Climatic conditions in Norway

2.2.1 *Temperature and precipitation*

As a consequence of its geographical structure and position, climate varies considerably within Norway, from south to north and from cost to inland, in addition to local climatic difference due to topography. Because of the North Atlantic Current, the climate in Northern Norway is considerably milder than at corresponding latitude elsewhere (Stubsjøen 1994). Coastal areas are characterised by fluctuating temperature around 0°C and unstable snow cover during winter. The lower southern and northern inland areas have very low mean temperatures in winter, with the lowest mean temperature (-15°C) in the northernmost county, Finnmark (Norwegian Metrological Institute). The growing season, defined as the number of days with average temperature ≥ 5°C (Growing degree-days, GDDs), extends to about 220 days in the south and only 120 days in the most northern part and mountain regions of the country (Skjelvåg 1998). Mean summer temperature is about 17°C in Oslo and 10°C in Tromsø, and the mean winter temperature at these two locations is approximately -4°C and -3°C respectively. The highest annual precipitation in the country is along the West coast with approximately 3500-5000 mm, most of it as rain. The driest areas are in the eastern part of south Norway and in the interior of Finnmark, with only about 250 mm annual precipitation. In inland areas and further north in the country, a large proportion of the precipitation is in the form of snow during winter.

2.2.2 *Daylength, light quantity and quality*

Huge differences in daylength between north and south (Fig. 1) affect the seasonal adaptation of plants. Between the fall (September) and spring (March) equinoxes, days are shorter than nights at the northern hemisphere. North of the Arctic Circle, (66°33′45.9″N), the annual variation in daylength is between 0 and 24 hours. In Tromsø (69.68°N) for example, 24 hours daylight from 20 May to 22 July and a polar night from 27 November to 13 January. The rate of change in daylength during autumn increased with latitude, e.g. the daylength decreases with nearly 3 hours during the first week after the midnight sun has disappeared in Tromsø (Nilsen 1985).

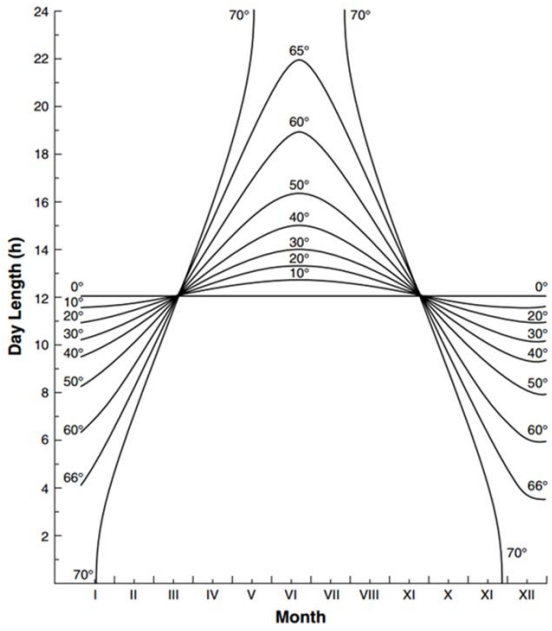

Figure 1. Seasonal changes in photoperiod at different latitudes. The variation in annual daylength is largest at higher latitudes. Because the seasonal climatic changes are often more pronounced at higher versus lower latitudes, photoperiodism is more common among high versus low latitude species. From Nelson et al. (2002) with permission from Cambridge University Press.

Solar irradiation is even more important for plant growth than daylength (Lawrence et al. 1973). Fig. 2 shows the insolation (available sun irradiation) at different latitudes. The yearly variation is least around equator, but increases at higher latitudes. The actual irradiance reaching the plant leaves is further affected by the atmospheric conditions such as humidity

and cloudiness, which are reducing the emittance of irradiation (Mcgregor and Porteous 1994).

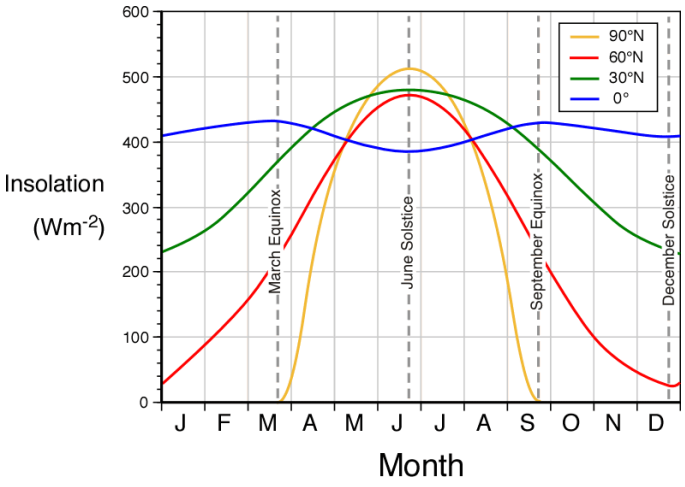

Figure 2. Monthly values of available insolation in Wm^{-2} for the equator, 30, 60, and 90° North. From Pidwirny (2006) with permission.

The light quality in form of spectral energy distribution is also different at higher latitudes compared to more southern latitudes (Fig. 3). This is mainly seen as the change in ratio between red (R) and far red (FR) light. Red light (660-680nm) is absorbed by the plants, but far-red light (720-740nm) is mostly reflected. There is a diurnal change in R:FR ratio with higher ratio during the day and a short period with a decreasing ratio during twilight when sun elevation is low (Nilsen 1985, Fig. 3). During summer solstice the diurnal variation in R:FR ratio is reduced at higher latitudes since the twilight/darkness period is eliminated. On the other hand, the annual variation in day, night and twilight periods is substantially higher at higher latitudes, causing increased seasonal variation in R:FR ratio. It has been suggested that the change in light quality expressed as R:FR ratio may be of similar significance for growth cessation in the arctic as the photoperiod at lower latitudes (Nilsen 1985). The change in R:FR ratio is known to cause cessation of internode elongation in aspen (*Populus tremula*) (Olsen and Junttila 2002) and initiation of cold acclimation in dogwood (*Cornus stolonifera*). (McKenzie et al. 1974) at the end of the growing season. The effect of red and far-red light on growth cessation of herbaceous species like grass and clover has been less studied.

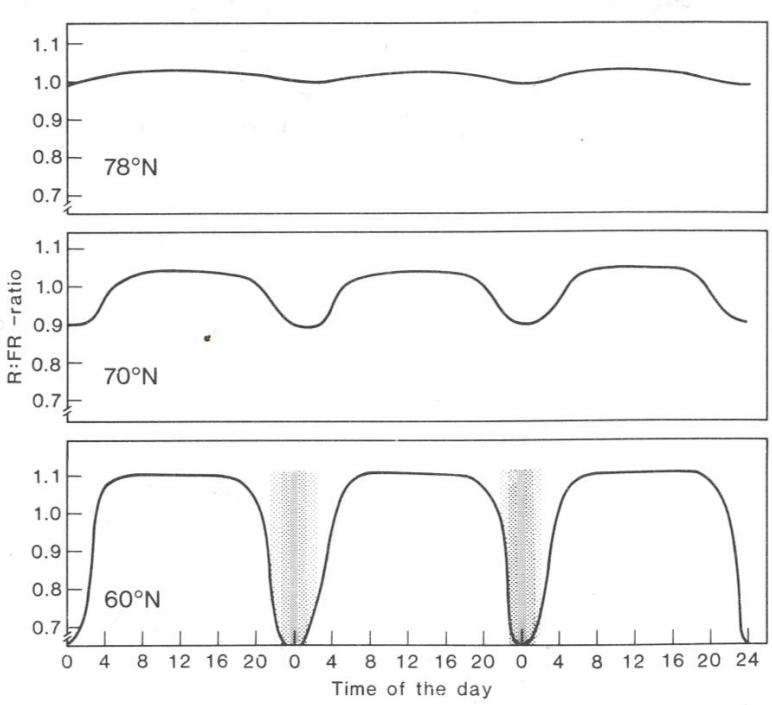

Figure 3. Diurnal alteration in the ratio between red and far-red light at different latitudes. From Nilsen (1985) with premission.

2.3 Agriculture and climate change in the north

Future agriculture is facing enormous challenges. In times of a rapid climate change, increasing population and consumption are placing a demand on increased food production, security and sustainability of agriculture. At the same time, agriculture must also address tremendous environmental concerns. These challenges are outlined by the Food and Agricultural Organisation of the United Nations (FAO) (FAO 2010), and are of concern and discussed globally. The overall effect of climate change is thought to be negative for agriculture globally, but countries in the southern part of the world may be more negatively affected than countries at northern latitudes (Lotze-Campen 2011).

The work of the Intergovernmental Panel on Climate Change (IPCC) points out that the average global temperature is projected to increase by between 1.4° and 5.8°C above 1990 levels by 2100 (Jansen et al. 2007, IPPC 2013). In addition to a temperature rise, the future climate change at northern latitudes involves increased precipitation, increased CO_2 concentration in the air and increased frequency of certain types of extreme weather events,

e.g. floods, heavy storms and droughts (Jansen et al. 2007). The amount and/or optical thickness of clouds in arctic areas are also predicted to increase (IPPC 2013), especially in coastal areas. There are, however, strong seasonal differences in the model scenarios. The highest temperature rise is predicted for winter, autumn and spring for northern latitudes within (ACIA 2005, Hansen-Bauer et al. 2009) resulting in less snow and more unstable snow cover.

Agronomy at higher latitudes like Northern Scandinavia, Iceland, Northern Canada and Greenland is milk and meat production based on perennial forage crops, species and varieties, which are more or less adapted to the short growing season and harsh winters. The possible rise in temperature at higher latitudes will imply lengthening of the growth season. This will be important for agriculture in these regions, particularly in the northern part of Norway, which is at the northern limit of agricultural production today. For this region, the predicted increase in summer temperature can substantially extend the short growing season and thereby increase the potential biomass production. Local projections for Northern Norway indicate that the growing season will be extended be 1-4 weeks in 2021-2050 compared to the reference period 1961-1990 (Uleberg et al. 2014). The largest increase is predicted inland and in the northernmost regions, which today have the shortest growth seasons. Higher mean temperature and longer growing season may enable increased use of legumes and more productive perennial forage grasses.

Even though agriculture in northern areas is often portrayed as a winner in the context of rapid global warming, there are also challenges, in particular related to increased precipitation and milder winters. Climate change may therefore have both positive and negative effects on agricultural production in the north and winter survival will most likely continue to be the main challenge for perennial crop production.

Milder and prolonged autumn followed by milder and more unstable winter with fluctuating temperatures may seriously affect acclimation of plants. Bélanger et al. (2002) predicted 17% shorter hardening period in eastern Canada by 2040-2069 compared to the reference period (1961-1990) and Thorsen and Höglind (2010) predicted that the hardening period would be shortened by up to 21 days in Norway in the period 2071-2100 because of warmer autumns. Warmer autumn may trigger continued growth at low light intensity, which can result in reduced winter hardiness. Increased temperature at the end of the growing season may delay growth cessation and reduce cold acclimation capacity, including photosynthetic acclimation to cold, which is considered the primary target for cold acclimation (Hüner et al. 1993, Crosatti et al. 2013). Northern-adapted plant material stops growth relatively early in

the autumn due to their adaptation to long critical daylength for leaf growth, but they will continue to respire and reduce the stored energy if the temperature in the autumn is high. Southern-adapted material, on the other hand, is adapted to a shorter critical daylength and will continue leaf growth throughout the autumn and have less time to acclimate. It is therefore of high agronomical importance to define the interaction of temperature, daylength and light and how it affects the acclimation of plants. These findings have to be incorporated into future breeding programs for perennial crops.

2.4 Winter hardiness

The winter climate sets limits for growth of many perennial crops in Norway and northern high latitude areas. Therefore, forage production in these areas is dependent on perennial forage species with good winter survival. Being poikilothermic organisms, plants have to survive in the particular environment at site. To do this, they develop mechanisms for either avoiding or tolerating the environmental stresses (Levitt 1980). The different environmental stresses are either of climatic character (abiotic stress) e.g. frost, ice cover, frost heave, drought and flood, or caused by living organism (biotic stress) like low temperature fungi and bacteria (Larsen 1994, Bertrand and Castonguay 2003). Management practices such as late autumn cutting/grazing (Andrews 1987) or soil compaction caused by heavy machinery can also reduce winter hardiness and winter survival of forage grasses (Baadshaug 1973).

Winterkill can be caused by a mixture of the different winter stresses, but low temperature is a direct or indirect cause of all winter stresses caused by the environment. The relative importance of the different types of stresses and damages vary with plant species and differences in climatic conditions between locations and years. The coastal areas, where winter weather is unstable, abiotic stress factors are most prevalent. In these areas, long duration of ice cover (ice encasement) is the major cause of winter damages. Warm spells in winter can cause snow melting which forms non-permeable ice layers when the temperature returns to below zero, causing anoxic conditions for plants (Fridrikson 1954, Andersen 1963, Larsen 1994). The level of tolerance to the anoxic conditions caused by ice encasement is dependent on species and varieties. Some species tolerate only few days, but the most hardy grasses tolerate up to three months (Gudleifsson 2010, Höglind et al 2010). Bertand et al. (2001) and Gudleifsson (2010) tested the ice encasement tolerance of herbaceous crops in the laboratory and ranked the species according to the most tolerant; tufted hairgrass>timothy>perennial ryegrass>white clover> red clover>winter rye>winter barley. Soil type also affects the damage caused by ice encasement and flooding. Sandy soils more easily drain the water

wheras clay soils are less drainable and magnify the stress (Andersen 1963). Former research and registrations showed that the most frequent and severe damage are in Northern Norway, where the winters are longest and most unstable (Flovik 1969, Andersen 1971). Therefore, only winter hardy species like timothy, meadow fescue, smooth meadow grass, orchard grass, and white clover are used in these areas.

In areas with long lasting snow cover, biotic stresses such as low temperature fungi are the most common stresses (Opsahl 1984). *Microdochium nivale* is the most common low temperature fungi in the Nordic countries (Årsvoll 1975) but grasses in Northern Norway are less infected by this species compared to the southern part of the country. In Northern Norway, the most important fungi causing winter injury in grasses have been *Myriosclerotinia borealis* and *Typhula ishikariensis*. They require snow cover for about 150 days or more, and have mostly been found in inland regions of Finnmark and Troms County (Andersen 1992). Ongoing and predicted future climate change indicates highest increase in temperature during winter which will result in fewer days with snow cover. This may reduce infection of *M. borealis* and *T. ishikariensis* in Northern Norway, but increase occurances of other fungi like *M. nivale* and Fusarium and Sclerotinia species which do not require snow cover for attacking plants (Rapacz et al. 2014, Gaudet et al. 2012).

The ability to tolerate freezing is generally considered the most important plant character for winter survival and the level of tolerance is highly correlated with mean winter survival (Pulli et al. 1996, Larsen 1994). The degree of winter hardiness against both biotic and abiotic stresses is dependent on the ability of the plant to cold acclimate during late summer/early autumn (Levitt 1980, Tronsmo 1986). Well-acclimated plants gain more cold hardiness, which makes them more tolerant for the different winter stresses.

However, the correlation between tolerance to different winter stresses varies. Strong relationship has been found between freezing tolerance and ice-encasement tolerance in timothy and perennial ryegrass (Sjøseth 1971, Höglind et al. 2010), but little or no relationship was found between these stresses in several other grass species and white clover (Gudleifsson et al. 1986, Dalmannsdottir et al. 2001).

Inland areas generally have lower temperatures during winter, therefore species/cultivars may reach a higher maximum freezing tolerance than in coastal areas (Larsen 1994). These areas usually have more stable snow cover which protects the plants. Only about 50 cm snow cover is enough to keep 0 to -5 °C underneath the snow (Taulavuori 2013). There are already indications that ongoing climate change is reducing ice cover damage in the lowland in costal Iceland (Gudleifsson 2010), but inland and alpine areas may be more exposed because of

more unstable snow cover. The same applies for Norway. As the climate gets milder, freezing might take over where ice encasement dominates today, whereas ice encasement might take over where snow molds dominate now (Gudleifsson 2009).

2.5 Adaptation and acclimation

Darwin pointed out that individual evolution is influenced by the environment (Darwin 1875). He stated that surviving individuals are the ones who most accurately sense the environment and successfully adapt to it. Plants have to adapt to long term climate change, yearly differences in climate, seasonal differences within years and diurnal differences in both light and temperature. They respond to the environmental contidions both by adaptation and acclimation. Adaptation is a heritable change in structures or functions, which increases the probability of survival of a genotype in a particular habitat. Acclimation involves non-genetic morphological or physiological plasticity to environmental variations (Junttila 1996). Acclimation is also genetically based and linked to adaptation. Phenotypic plasticity is an important character of plants in context with climate change and is often not included in climate models of future distribution of plants. High plasticity and a high capacity for beneficial acclimation may be of major adaptive significance (Junttila 1996). Phenotypic change can be the result of either genetic change or phenotypic plasticity, but the relative contribution of one or the other is still unknown (Merilä and Hendry 2014). Epigenetic modification is another source of 'non-genetic' acclimation, involving environmentally induced epigenetic marking, which can cause changes in phenotypes and contribute to evolutionary changes (Schlichting and Wund 2014).

While adaptation refers to the genetic responses of a population, phenotypic plasticity refers to the differences in physiology, morphology and development caused by exposure of an organism to a changing environment, e.g. acclimation to short-term environmental fluctuations such as changes in temperature, photoperiod and humidity (Jump and Peñuelas 2005). Certain plant characteristics, such as flower structures, seed and fruit anatomy, have low plasticity, but the basic characteristics that are important for plant growth and development, show high plasticity (Junttila 1996). The ultimate capacity of phenotypic plasticity is not fully understood but, since plastic genotypes buffer individuals against short-term environmental fluctuation, these plastic responses might also have important consequences for longer-term effect of climate change (Crawford 1989, Nicotra et al. 2010). Plasticity is evolutionarily favoured when the environment is heterogeneous in time and space, when selection favours different phenotypes in different environments and when no

single phenotype has greatest fitness across all environments (Reed et al. 2011). Plastic and genetic variation is not necessarily dependent on each other, but phenotypic plasticity seems to be able to evolve when the genetic variation is sufficient (Gratani 2014). One might speculate if phenotypic plasticity could somehow compensate for the loss of genetic diversity in the context of performance in a changing climate.

2.5.1 *Cellular cold acclimation and freezing tolerance*

Cold acclimation, i.e. the ability to increase freezing tolerance in response to low non-freezing temperatures, is essential for plants to overwinter in temperate habitats (Levitt 1980, Pollock & Eagles 1988). Cold acclimation is a quantitative trait driven by cold-regulated genes (Hughes and Dunn 1996, Chinnusamy et al. 2007) and controlled by a complex interaction between light and temperature, which is still not fully understood (Gray et al. 1997, Kurepin et al. 2013). The ability to cold acclimate is dependent on the species, cultivar and individual genotype involved (Junttila 1996, Hüner et al. 2012).

Cold acclimation is a rather rapid process and plants can gain a substantial increase in freezing tolerance after one-day exposure to low temperatures, but full acclimation requires more than a week (Palva et al. 2002).

Cold acclimation is a complex process and involves numerous changes in the physiology and biochemistry of the cell. Increased membrane fluidity, accumulation of compatible osmolytes and photosynthetic acclimation are among the biochemical and physiological changes taking place during cold acclimation (Xin and Browse 2000). It is not a result of one biochemical reaction but many different processes. Cold regulated (COR) genes are a part of a highly complex program that regulates changes in the physiological and biochemical processes of the plant. Certain COR genes coding for chloroplast-localized proteins are controlled by the cumulative effect of cold and light (Crosatti et al. 1999). The C-repeat binding factor (CBF) pathway is known as the cold responsive pathway (Thomashow 2010). Overexpression of the CBF genes induces expression of the COR genes and increases the freezing tolerance of the plant (Thomashow et al. 2010). The complete process of the CBF pathway is still not fully understood (Thomashow 2010), nor have the primary sensors involved in sensing low temperature been fully identified (Miura and Furumoto 2013). One of the challenges has been to separate the processes that are important for freezing tolerance (cold regulated) from those who purely respond to low temperature (cold responsive) (Xin and Browse, 2000). However, certain cold stress regulated genes have been identified in several perennial crop species including perennial ryegrass (Zang et al. 2009).

The total effect of photoperiod on cold acclimation and freezing tolerance in herbaceous plants is not clear, but cold acclimation at shorter photoperiod increases freezing tolerance in northern-adapted grasses (Eagles 1994, Lawrence et al. 1973). The level of freezing tolerance is generally expressed as LT_{50} value (lethal temperature) as defined by Levitt (1956). This is the temperature which injures 50% of the plants' tissue or which kills 50% of plants within a population.

The process of cold acclimation

Despite of cold acclimation being a progressive process responding to lowering temperature and shorter days in the autumn, gradually increasing the hardening of herbaceous plants, it can be described quantitatively as different stages of hardening (Fig. 4) (Kacperska-Palacz, 1978, Sakai and Larcher 1987). The different stages were originally defined for winter rape related to the temperature conditions at a site. The first stages are happening when there are relatively high irradiance during late summer/early autumn. Rapacz (1998) defined the first stage as pre-acclimation, occurring when winter rape seedlings are at an early growth stage, with temperatures around +10 to +15°C. At this stage the growth rate decreases and the photosynthetic apparatus acclimates to cold (photoacclimation). As the temperature gradually decreases, the next stage takes place at low positive temperatures (chilling temperatures) above +2°C and below +5° or +10°C. This stage is characterised by accumulation and hydrolysis of carbohydrates and lowering the critical phase transition temperature of the biomembranes. Thereafter, at subzero temperatures (0 to -3°C), adjustment of the osmotic value of the cell sap takes place and poly-unsaturated fatty acids are incorporated into the membrane lipids to maintain the membrane fluidity. The last stage of the acclimation process is induced by prolonged freezing during winter, and maximum freezing tolerance is reached only after exposure to subfreezing temperatures (Kacperska-Palacz 1978, Levitt 1980). At this stage, frost-induced dehydration occurs. It is important to bear in mind that the different stages may overlap. When temperature increases and days get longer in the spring plants are dehardened, losing their freezing tolerance and preparing for spring growth. The unique light conditions in the high north may further affect the succession of the different stages of cold acclimation. Local climate conditions and year-to-year differences in autumn and winter climate together with the adaptive capacity of the plant affect the degree of hardening. Larsen (1994) studied the degree of cold hardiness in the field from autumn to spring in Bodø (north) and Ås (south) in a northern-adapted ('Engmo') and a southern-adapted ('Grindstad') cultivar/ecotype of timothy, and showed that the degree of hardening was generally lower in

the northern coastal climate in Bodø compared to the southern continental climate at Ås. The greatest difference in freezing tolerance between the cultivars did not occur at the stage of maximum tolerance in mid-winter, but at the stage of dehardening in late winter with 'Engmo' maintaining higher tolerance longer than 'Grindstad'. Longer maintenance of tolerance and less sensitivity against warm spells during winter in northern-adapted grass cultivars compared to southern-adapted is documented in other studies (Eagles & Williams, 1992, Jørgensen et al. 2010).

It is still not clear which role light plays in the acclimation to low temperature. Although the induction of freezing tolerance is dependent on exposure to low temperature, the attainment of maximal freezing tolerance is light dependent (Gray et al., 1997). Szalai et al. (2009) showed that the frost hardening of wheat plants at low temperature under low light conditions is much less effective than under normal light conditions. Furthermore, Crosatti et al. (1999) found that the expression of a cold regulated gene (COR14b) in barley is controlled by the cumulative effects of cold and light.

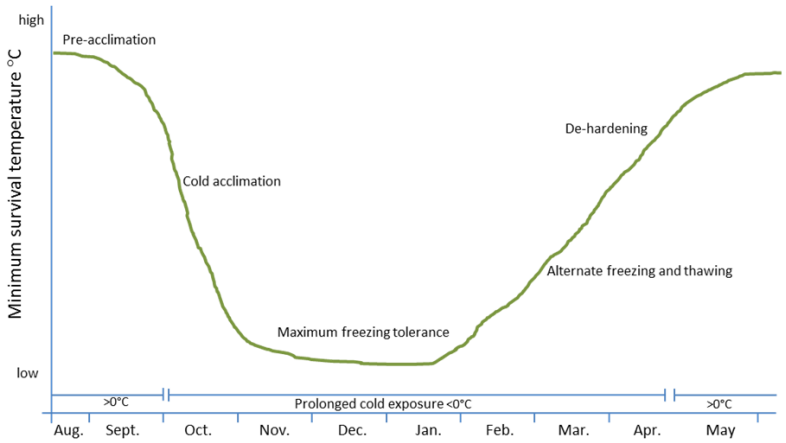

Figure 4. Cold acclimation process. Based on Kacperska-Palacz (1978) and Fowler et al (1983).

Resistance to intracellular freezing

When the plant is exposed to freezing temperatures ice crystals form in the extracellular space between the cell walls (extracellular freezing) (Fig. 5), but this ice formation is not lethal.

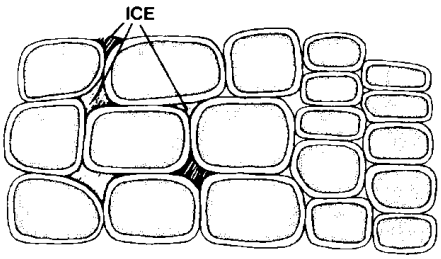

Figure 5. Formation of extracellular ice crystals between cells.

When plants are exposed to freezing temperature for an extended period, the crystals in the cell wall continue to grow and extend into through the plasma membrane into the protoplasm, causing lethal damage (Fig. 6). Freezing resistant species limit the growth of crystals to the cell walls and intercellular space as water within the cells diffuses out and condenses on the growing ice masses. The cell acts as an osmotic system, with the osmotic concentration inside increasing as water diffuses out through the plasma membrane, dehydrating the cell. When ice crystals melt, water returns into the cell and metabolism is resumed. The most common strategy to resist freezing stress is to tolerate dehydration (Gusta 1985, Chen 1994).

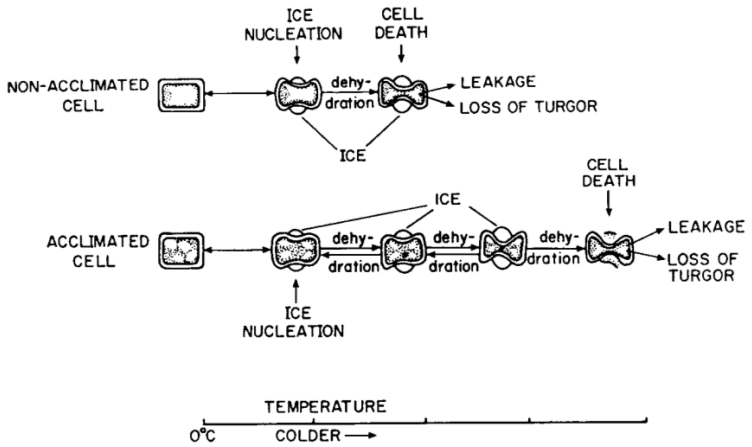

Figure 6. Model illustrating the freezing process in acclimated and non-acclimated plants. In non-acclimated tissue, the ice formation is lethal. In acclimated tissue, dehydration is fully reversible to a characteristic threshold. From Mckerzie and Leshem (1994) with permission.

Stabilization of membranes

One of the important processes involved in the acclimation is the molecular change in membrane lipids. Metabolic change in the membrane lipids can lessen or prevent injury to

plants exposed to low temperature (Lyons 1973, Steponkus 1984). During cold acclimation the amount of unsaturated fatty acids in the membrane lipids increases, this increases the membrane fluidity and lowers the transition temperature (Thompson 1989). The synthesis of the different lipids is a complex process that involves many different enzymes. During acclimation, cold resistant species activate desaturase enzymes which insert 2-3 double bonds into the carbon chain. Higher ratio of unsaturated fatty acid in biomembranes correlates with increased freezing tolerance in e.g. *Arabidopsis* (Uemura et al. 1995) and white clover (Dalmannsdottir et al. 2001) but did not show a direct relationship in rye seedlings (Uemura and Yoshida 1984). Desaturation of fatty acids in plasma membranes during cold acclimation most likely depends on plant species and the level of cold hardiness.

Osmoprotectants

In order to resist osmotic stress, the cell acquires a high concentration of osmoprotectants in the cytoplasm. Osmoprotectants are highly soluble organic compounds, e.g. proline, betain, and polyols, of low molecular weight, which exist in a stable form even in high concentrations (Iba 2002). They protect various cellular components like essential enzymes from denaturation, and are important in increasing the freezing tolerance of the plant by balancing the osmotic difference between the cell's surroundings and the cytosol, preventing excessive dehydration of the cell (Xin & Browse 2000).

Carbohydrate metabolism

Soluble sugars are important osmoprotectants and carbohydrate metabolism plays a crucial role during cold acclimation. However, carbohydrates are also the main source of energy in overwintering plants. When growth declines in late summer, energy is not needed to produce new leaves; instead, photosynthates are transferred to roots or stolons as stored energy for the winter. Accumulation of sugars during cold acclimation in autumn is due to excess of photosynthesis over respiration and growth, and starch-sugar conversion (Wingler 2015, Hüner et al. 2013). Hüner et al. (2013) suggest that sucrose accumulation during cold acclimation is merely a photosynthetic end-product as a response to increased excitation pressure within the photosystem accelerating CO_2 assimilation in cold acclimated plants at low temperature, known as photochemical quenching (see paragraph about photoacclimation). This response of the photosystem both maximizes the carbon storage energy of the plant and increases the osmoprotection of cells during autumn and winter.

Most perennial plants, also legumes like lucerne and clover, store carbohydrate as starch whereas sucrose is the main transport sugar. The storage energy of C3 grasses, like timothy, orchard grass, meadow fescue and perennial ryegrass, is mainly in the form of fructans. Accumulation of carbohydrates as fructans seems to increase the freezing tolerance of grasses (Castonguay et al. 2009; Li et al. 2012) with higher levels within cold hardy variety (Abeynayake et al. 2015). Sucrose phosphate synthase (SPS) is overexpressed at low temperature resulting in increased synthesis of sucrose from products of the Calvin cycle, triose phosphate, which is exported from the chloroplasts into the cytosol where sucrose synthesis takes place (Guy et al. 1992). Rudi et al. (2011) identified a triose phosphate/phosphate translocator (TPT) gene as one of the most differentially expressed genes during cold acclimation in meadow fescue. TPT proteins are located in the chloroplast membrane and are responsible for export of sucrose to the cytosol. Starch, on the other hand, is synthesised within the chloroplasts. Several studies on high latitude forage crops demonstrate higher total water soluble carbohydrates (WSC) such as sucrose, glucose, fructose, stachyose and raffinose in northern-adapted winter hardy cultivars compared to southern-adapted (Frankow-Lindberg 2001, Hanslin and Höglind 2009, Østrem et al. 2011) Starch breakdown during early stage of cold acclimation has been known for a long time. A newly fixed carbon can also be incorporated into WSC rather than starch in the autumn, such a shift being under translational control (Strand et al. 2003) (see Fig. 7).

Hormonal control

Abscisic acid (ABA) and gibberellins (GAs) are important in regulating freezing tolerance. ABA regulates the water balance of the plant and induces gene expression and protein synthesis required for cold acclimation. It also serves as a secondary signal to transduce cold signals (Chinnusamy et al. 2006) and regulates many of the genes associated with increase in freezing tolerance (Gusta et al. 2005). Several studies have demonstrated that treatment with exogenous ABA greatly improves the tolerance to freezing but the extent of freezing tolerance induced in this way has been variable (Gusta et al. 2005). ABA has been suggested to mediate the induction of gene expression and protein synthesis required for cold acclimation (Chen and Gusta 1983, Mantyla et al. 1995).

Gibberellins, which are known to control the elongation growth in plants, are involved in regulating expression of CBFs through production of DELLA proteins (Hüner et al. 2014). Normal levels of biologically active GAs stimulate the degradation of DELLA proteins, but at low levels of active GAs, DELLA proteins accumulate and cause overexpression of CBF and

repressed stem elongation (Achard et al. 2008, Kurepin et al. 2013). It seems that increased excitation pressure within the photosystem II, through gene expression, reduces the levels of active GA in cold acclimated plants, resulting in a dwarf genotype at low temperatures or under excessive irradiance (Hüner et al. 2014).

2.5.2 *Photoacclimation*

The acclimation of the photosynthetic apparatus to cold (photoacclimation) is now considered the primary target for cold acclimation (Hüner et al.1993, Crosatti et al. 2013). The photosynthesis itself serves as an environmental sensor of changes in temperature and irradiance and needs to balance the light energy absorbed by the photosystem in the light reaction with the chemical energy assimilated by the dark reaction (Calvin cycle). The balance between energy input by phytochemistry and energy utilization through metabolism is called photostasis (Öquist and Hüner 2003, Ensminger et al. 2006). Low temperature or high light conditions imbalance the redox state of the photosystem II causing increased excitation pressure, which can result in photoinhibition and reduced photosynthetic activity (Hüner et al. 1993, Ensminger et al. 2006). Photoacclimation is a protective mechanism against photoinhibition; it causes either increased light energy utilization by photochemical quenching (Pq) or improved light energy dissipation as heat (NPQ) through chlorophyll fluorescence (Hüner et al. 2012). Photoacclimation is thus dependent on the plasticity of several physiological and morphological traits in order to develop photostasis and maintain high photosynthetic activity (Hüner et al. 2013).

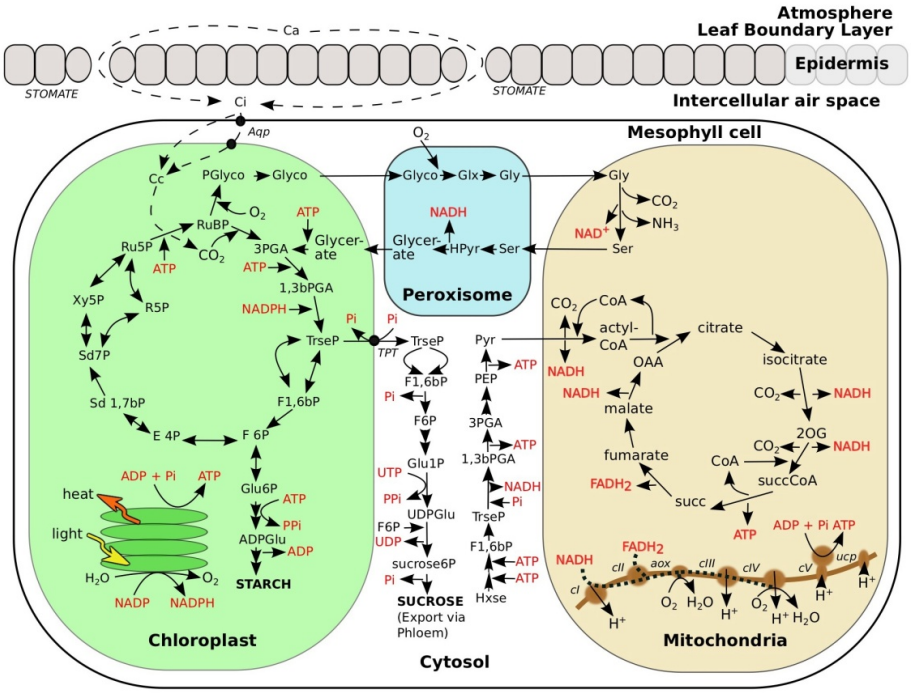

Figure 7. Primary carbon metabolism in a photosynthetic C3 leaf. An abbreviated depiction of foliar CO_2 uptake, chloroplastic light-reactions, chloroplastic carbon fixation (Calvin cycle), chloroplastic starch synthesis, cytosolic sucrose synthesis, cytosolic glycolysis, mitochondrial citric acid cycle, and mitochondrial electron transport. The photorespiration cycle spans reactions localized in the chloroplast, the peroxisome, and the mitochondria. Stacked green ovals (chloroplast) represent thylakoid membranes. Dashed arrows near figure top represent the CO2 diffusion path from the atmosphere (Ca), into the leaf intercellular airspace (Ci), and into the stroma of the chloroplast (Cc). Solid black arrows represent biochemical reactions. From Skillmann et al. (2011) with permission, see reference for more detailed description of the figure.

Rubisco incorporates CO_2 into RuBP (ribulose biphosphate) to form 3PGA within the Calvin cycle (dark reaction). Rubisco is highly temperature sensitive, and its activity is reduced at low temperatures decreasing the demand for ATP and NADH from the light reaction of the photosynthesis, and increasing the excitation pressure. Such a cold-induced increase in the excitation pressure is considered a mechanism for protecting PSII against overexcitation pressure, and has been shown to induce cold acclimation and influence expression of specific COR genes (Crosatti et al. 2013). Photoacclimation to cold-induced photoinhibition seems to be closely related to freezing tolerance (Hüner et al. 1993, Rapacz et al. 2004). Several "repair" mechanisms operating to avoid photoinhibition and maintain photostasis have been identified (Ensminger et al. 2006; Tikkanen et al. 2014). One fast responding mechanism is to divert energy from PSII (which is preferentially excited) to PSI through state transition by connecting the light harvesting complexes (LHC) of PSII and I via the PSI-H subunit (Lunde

et al. 2000), a beautiful collaboration between the two photosystems ☺ (See Fig.8). A more slowly responding mechanism is to reduce the physical size of the LHCII modulated by genetic translation (Walters and Horton 1994). Another important mechanism is the post-translational activation and increased expression of enzymes of the sucrose synthesis pathway (Stitt and Hurry 2002). Overexpression of sucrose phosphate synthase increases phosphor (Pi) release and removal of carbon from the Calvin cycle. This increases substrate availability for CO_2 uptake (ribulose-5-phosphate) and demand for light energy, which, together with increased activity of several enzymes in the Calvin cycle, like Rubisco, accelerates carbon fixation and maintains high photosynthetic activity.

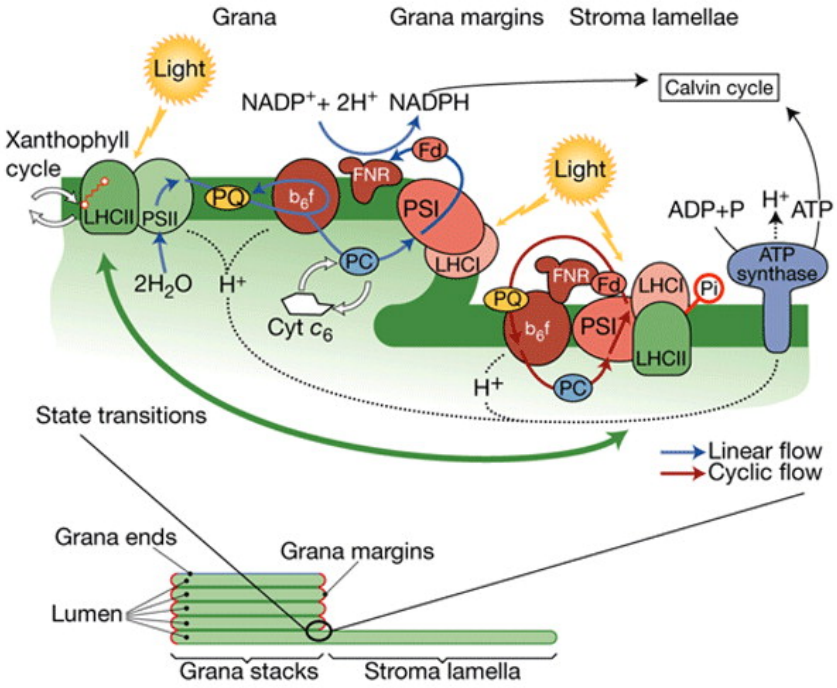

Figure 8. The thylakoid membranes and their domains and the dynamics of the photosynthetic electron-flow machinery (Image drawn by Nicolas Roggli, University of Geneva, Switzerland). From Finazzi et al. (2003) with permission, se references for more detailed description of the figure.

2.6 Growth cessation

Growth cessation is a normal seasonal response of the plant during late summer when uptake of water and nutrients is inhibited and chlorophyll is degraded causing yellowing of leaves. Growth cessation is controlled by temperature and light, however, the physiological and biochemical mechanisms behind growth cessation in herbaceous plant species is still unclear

(Rapacz et al. 2014). In woody species, however, this mechanism has been widely studied with cessation of apical growth being a response to a shorter photoperiod and altered light quality (Olsen 2010). Phytochromes, the light-absorbing molecules, are important sensors of seasonal changes in photoperiod. Hence, the initiation of growth cessation is the first signal for cold acclimation in trees and it has been demonstrated that a short photoperiod significantly enhances cold acclimation in woody species (Weiser 1970, Junttila 1990).

In woody plants, hardening is characterised by two clearly defined steps; firstly growth cessation controlled by photoperiod, and secondly temperature-controlled cold acclimation. Such clearly defined steps of winter hardening are not found in herbaceous species, but growth and cessation of growth in perennial forage crops are strongly affected by photoperiod, especially in ecotypes adapted to northern conditions (Foss 1968, Skjelvåg 1998).

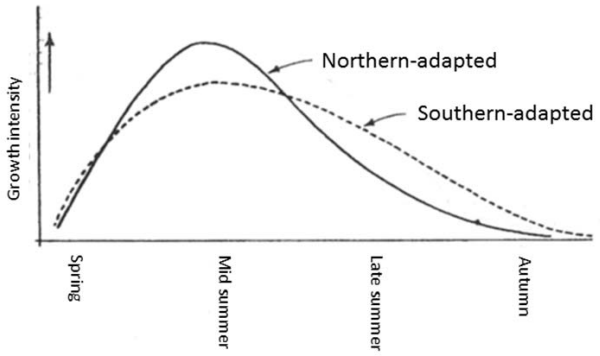

Figure 9. Growth rhythm for northern and southern-adapted populations.

Temperature requirement for plant development generally decreases with increasing photoperiod, so less GDDs are needed to reach a given growth at higher than at lower latitiudes (Bootsma 1984). During the growing season at higher latitudes, northern-adapted grasses are able compensate for low temperatures by longer days in biomass production (Heide et al. 1985).

At the end of the growing season, northern-adapted grasses stop growth earlier (at a longer critical photoperiod for leaf growth) than southern-adapted grasses (Fig. 9) to accumulate sufficient carbohydrate reserves before photosynthesis becomes negligible (Foss 1968, Larsen 1994, Skjelvåg 1998). Northern-adapted varieties normally assimilate more carbohydrate as winter storage compared to southern-adapted varieties as they need higher

storage reserves to survive longer and colder winters (Solhaug 1991). Therefore, when cultivars bred for Northern Norway are moved to the lowland of southern part of the country, they stop growth earlier than necessary for a sufficient cold hardening. On the other hand, southern-Norwegian cultivars moved to the north may continue growth too long, failing to achieve a proper cold hardening (Opsahl 1984) (Fig. 10). The relative importance of photoperiod/light and temperature for growth cessation of perennial crops is still not clear. The redox state of PSII seems to be important not only for cold acclimation since elevated PSII excitation pressure also triggers cessation of elongation growth (Rapacz 2002).

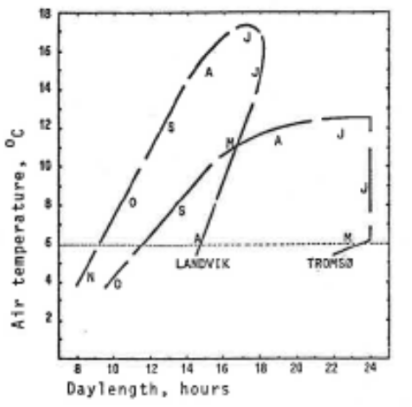

Figure 10. The relation between daylength and air temperature (five-day standard normals) at Landvik (58°20'N) and Tromsø (69°39'N) during each month from April (May) to October (November). From Opsahl (1984) with permission.

2.7 Anoxia

Flooding or water logging of soils may cause stress in plants due to hypoxic or anoxic conditions (Levitt 1980). Due to slow diffusion of oxygen in water, oxygen deprivation in plant roots occur, similar as during ice-encasement. When lack of O_2 limits ATP production from oxidative phosphorylation, a hypoxic situation occurs first. Anoxia follows later when no O_2 is available and ATP is purely produced through fermentative glycolysis (Parent et al. 2008). Plant species adapted to short-term episodes of anoxia may respond by accelerating glycolysis to maintain adequate energy supply under anaerobic conditions (Crawford 2003, Parent et al. 2008). Anaerobic fermentation gradually accumulates anoxic metabolites like ethanol, CO_2 and several carboxylic acids (lactate, citrate, malate, butyrate, propionate). Anaerobic respiration produces less energy than aerobic respiration and this may accelerate the use of carbohydrate reserves (Bertrand et al. 2003, Parent et al. 2008) Tolerance to

anaerobic conditions is clearly species dependent (Gudleifsson 1986, Bertrand et at. 2001). It has earlier been thought that the main reason for cell death under anoxic situation is the accumulation of metabolite and CO_2 (Andrews and Pomeroy 1979). However, the transition from anoxia to air brings a great shock to the plant, which can lead to cell death because of formation of reactive oxygen species (ROS) (McKersie and Leshem 1994). One of the most important adaptive mechanisms of plants to water-logging is development of aerenchyma (gas spaces) within the root cortex in flood tolerant species (Crawford and Braendle 1996, Parent et al. 2008). Such a mechanism may allow cells to avoid anoxia by increasing the available O_2 within the cells and limiting the consumption of both ATP and O_2 (Bailey-Serres and Voesenek 2008).

2.8 Genetic resources and variation

The rapid climate change is reducing the genetic diversity within natural plant species and populations as they migrate northwards to new habitats (Alsos et al. 2012). Especially warmer, shorter winters may disrupt the arctic terrestrial ecosystems (Cooper 2014). If climate change is happening faster than the ability of the species to adapt or migrate into a new habitat, it may gradually lose its fitness and go extinct (Thuiller et al. 2005). On the other hand, climate variation between years may be of greater magnitude than expected temperature changes, thus cultivated plants are constantly exposed to strongly changing environment (Junttila 1996). Maintaining genetic diversity within a species is therefore crucial for its ability adapt both in the short-term and long-term (Jump et al. 2009). Since natural populations and landraces are main genetic resources for new traits in cultivated crops, the loss of genetic variation may involve challenges for future plant breeding. Future breeding for new varieties in a rapid changing climate demands a wide genetic variation available in order to develop varieties able to adapt to new climatic conditions.

2.9 Breeding

The most important factor for traditional selective breeding is the presence of genetic variation for important traits. The basic concept of plant breeding is that genetic gain from selection for a given trait is proportional with the additive genetic variance for that trait (Fehr, 1987). The main goal of plant breeding is to increase yield. At lower latitudes, most breeding programs have focused on breeding for traits like drought resistance and pest resistance in addition to yield (Ceccarelli et al., 2010). In the arctic and subarctic areas, where the

agriculture is mainly based on perennial grasses, a major focus has been breeding for winter survival (Larsen, 1994; Rognli 2013).

Pre-breeding has been defined by Sharma et al. (2013) as follows:

”*Pre-breeding refers to all activities designed to identify desirable characteristics and/or genes from unadapted materials that cannot be used directly in breeding populations and to transfer these traits to an intermediate set of materials that breeders can use further in producing new varieties for farmers. It is a necessary first step in the use of diversity arising from wild relatives and other unimproved materials*.”

2.9.1 *Grass breeding of the past in high latitude areas*

Over the years, the most important trait for selection of crop species and cultivars for northern regions has been the overwintering capacity combined with high biomass production. Timothy has been the most important forage grass species in Northern Norway since around 1920. Cultivars in the past were selected as surviving individuals from populations within farmers’ field, so called landraces. The very winter hardy timothy cv. ‘Engmo’ was based on local seed production of a landrace on the farm Engmo in Troms county, and was available for farmers from 1934 (Schjelderup 1979). ‘Engmo’ has very good freezing and ice encasement tolerance but is susceptible to snow mould fungi (Anderson 1992). Another winter hardy timothy cultivar, ‘Bodin’, was available in 1951 after 25 years of breeding work at Vågønes in Bodø (Schjelderup 1979). Because of unstable seed production in the north due to the short growing season, the commercial seed production was moved stepwise to the southeastern part of Norway, with production only of pre-basic seeds in Troms, basic seeds in Mid-Norway, and commercial seed in Eastern Norway (Schjelderup et al. 1994) This system was implemented to reduce the genetic shift in the timothy cultivars. As it turned out, the commercial seed production in the south reduced the “northern vigour” of the cultivar gradually if several generations were produced in the same region. Andersen (1971) found that six generations of seed propagation in the south reduced the winter survival of timothy with 50%. This emphasises the importance of selective breeding and commercial seed propagation as close to the climate conditions for utilization as possible (Rognli 1987).

In the 1970s, a Nordic collaboration in breeding new adaptive cultivars resulted in an extensive collection of local populations of several species of different geographical origin (Schjelderup et al. 1973). These populations were planted in field tests to determine morphological and physiological changes when subjected to high latitude growth conditions

and winter stress (Foss 1982). This breeding work resulted in new cultivars like 'Noreng' timothy and 'Norild' meadow fescue. Another Nordic grass breeding collaboration initiative was started 1981 (Norgrass) with the aim to breed a new timothy cultivar for the whole of northern region of Scandinavia and Iceland (Helgadóttir & Björnsson 1994). This resulted in the cultivar 'Snorri', finally registered in 2006 (Helgadóttir & Kristjánsdóttir 2006). 'Snorri' was one of the highest yielding in Tromsø in a joint variety testing in the West-Nordic countries and Sweden (Þorvaldsson et al. 2015). However, since the private breeding companies in each country does not receive full royalty for this cultivar, it has not gained commercial interest.

When the professional process of modern plant breeding started early in the twentieth century, there was a development towards uniform high yielding cultivars outcompeting the genetically diverse landraces. Genetic uniformity is even a requirement for the registration of new cultivars today.

Despite development towards homogenous cultivars, some crop species are still based on old landraces with high genetic diversity. Studies have shown that although cultivars of timothy are phenotypically diverse, the genetic similarities among Norwegian timothy genotypes are relatively high (Guo et al. 2003, Fjellheim et al. 2015). Timothy cultivars from Northern Norway are no longer demonstrating the best survival in the north (Larsen and Rognli 2007). The southern cv. 'Grindstad' has performed increasingly better in Northern Norway and this may be explained either by genetic adaptation of 'Grindstad' or reduced fitness in the northern adapted cultivars towards the changing climate (Larsen and Rognli 2007). 'Grindstad' is the most commonly grown cultivar in Norway today and originates from an old Norwegian landrace selected about hundred years ago on a farm in Østfold (Marum and Daugstad 2009). 'Grindstad' has been seed multiplied in a way that allow genetic shifts and adaptation to management and climate, whereas other Norwegian timothy cultivars are multiplied from original and static pre-basic seed (Marum and Daugstad 2009). This might explain the wide adaptation and success of 'Grindstad' over large geographical regions.

2.9.2 *Plant breeding in the future (breeding perspectives)*

Stamp & Visser (2012) have already named the 21st century the century of plant breeding, and state: "we have known for decades that the supply of food will become limited in the not too distant future. Breeding is the only way to slow down this progression". In the context of previously described challenges in relation to future climate change, this is a huge task to

accomplish. The degree of challenges for breeders, geneticist and physiologists will depend on whether the climate change will be abrupt, with dramatic effects, or if it will be gradual and thereby allowing time for plant breeders to respond (Humphreys et al. 2006). The genetic erosion within modern uniform varieties, as the effect of manmade selection for a preferable trait, poses risks since the genetically uniform cultivar is less robust for new environmental or biotic threats. Therefore, new plant genetic resources (PGR) are needed to add new variability into the existing breeding programs. These can for example be found within old landraces or in gene bank accessions. Access to this genetic diversity is critical for the success of breeding programs. It has now become clear that the best strategy for conservation of genetic diversity is a combination of "on the ground" production systems by farmers (*in situ*) and conservation of important genetic resources within gene banks (*ex situ*) (FAO 2010). Gene banks might be increasingly important as provider of new PGR in the future instead of mainly playing a role in conservation. It is therefore important that the PGR within gene banks are well characterised and that the strategies of the gene banks are well defined. New biotechnological methods have shortened the breeding process by many years (Parry et al. 2012) and will be even more efficient in the future with the implementation of genomic selection (Heslot et al. 2015). Further approaches in breeding towards increased efficiency is to develop novel and integrated or holistic approaches, that will both increase production per unit area and simultaneously improve the resource use efficiency of crops (Parry and Hawkesford 2012) and also confer measurable benefits in terms of environmental quality (Humphreys et al. 2006). Because the future climate change is rather unpredictable, breeders must be flexible and have a range of well-characterised germplasm available for new applications (Humpheys et al. 2006). Future adaptation of agriculture to climate change is also very much dependent on the management practices on the farm. The modern farming systems of homogeneous monoculture pastures may benefit by introducing more species rich and multifunctional grasslands, since increased biodiversity, especially legume-grass based, gives yield benefits, and contributes to weed suppression and persistency (Kirwan et al. 2007). Introducing legumes into grassland swards will also reduce the fertilizer requirements and improve N-use efficiency. Good agronomical practices regarding establishment of swards, selection of soils, fertilizing plans, cutting regimes and other management practices are also of great importance as a tool of adaptation for future forage production in a changing climate.

Plant breeding in Norway is a more challenging task than in many other European countries due to the wide latitudinal gradient with highly variable climates and soils from the south to the north and from coastal areas to the inland. This has to be taken into account when

multiplying seed for the market, since the capacity of winter hardiness of perennial forage crops, like timothy, can be significantly reduced after a few generations of seed production in a southern location (Andersen, 1971). In addition, the market for cultivars bred for the marginal environmental areas is small, which makes it economically non-profitable for private seed companies. Breeding for persistency will continue to be of greatest importance in northern Europe. With warmer winters, grass genotypes may need to be designed for greater dependency on photoperiod response rather than low temperatures to stimulate the onset of flowering (Humphreys et al., 2006). Photoperiod control will also be an important factor in determining growth cessation of plants, especially in the arctic, in order to obtain adequate cold acclimation for winter survival in a more unstable winter climate with less snow cover. The grasses within Lolium-Festuca species contain an exceptionally wide range of variation, including adaptation to contrasting temperatures, daylength, rainfall and soils, and can therefore be a feasible PGR for further breeding in arctic and sub-arctic environment (Humphreys et al, 2006)

Several papers are discussing breeding strategies for forage crops in northern areas and ways to improve crops for future climate (e.g. Helgadóttir 1989, Larsen 1994, Tester and Bacic 2005, Gusta and Wisniewski 2013, Rognli 2013). The challenge in breeding for freezing tolerance and winter hardiness in general is that we are dealing with highly complex adaptive mechanisms including genotype x environment interactions. Isolated effects of single stress factors can be addressed in the laboratory, but in order to get a holistic picture of winter survival, a true systems biology approach will be needed (Gusta and Wisniewski 2013). It is argued that breeding approaches should focus on specific traits rather than yield (Tester and Bacic 2005), and new winter-hardy cultivars should be bred for a wide adaptation to winter stresses (Larsen 1994). At the same time, the relatively small gene pool of most crop plants is limiting progress (Tester and Bacic 2005). A breeding effort should therefore be based both on locally adapted material of high yield potential (Helgadóttir 1989) and introgression of exotic material in order to increase the genetic variation and improve the adaptation of forage crops in future climate (Rognli 2013). The modern molecular techniques will most likely be the way forward in introducing desired trait and variation into crop cultivars to make them robust enough for future environmental conditions.

3. MATERIALS AND METHODS

3.1 Description of plant material

The populations used in this work are pre-breeding material and cultivars selected for southern or northern adaptiveness and are a part of the material selected for use in the VARCLIM project. The material has either a wide or a narrow genetic background, represented by southern and northern-adapted breeding populations or cultivars. Adaptation relates to Norwegian growing conditions. The populations were developed by Graminor Ltd. and NIBIO (earlier Bioforsk) in Norway. The background of each population is described in Table 1.

3.1.1 *Timothy*

Timothy (*Phleum pratense* L.) is a forage grass species widely grown in cool and humid regions of the world in both Northern Europe, in northwestern and northeastern parts of North America, and in Japan. It has a good cold tolerance, high yields and good forage quality. It is the most important forage grass in the northern parts of the Nordic countries because of its good winter hardiness and growth potential during a short growing season. Timothy is difficult to breed since it is hexaploid and possibly an auto-allopolyploid (genome constitution AAAABB, see Larsen & Marum 2006). On the other hand, timothy has no vernalisation requirement for flowering, only long day induction (Heide 1982). Timothy has a shallow and fibrous root system and is able to form a storage organ called “haplocorm”. It is formed from the lowest stem internode in the spring and gradually becomes larger with the build up of storage energy. Timothy is less resistant to snow-mould than meadow fescue (Larsen & Marum 2006).

3.1.2 *Perennial ryegrass*

Perennial ryegrass (*Lolium perenne* L.) is the most widely used forage grass species in temperate regions due to its combination of high productivity and feed quality, and tolerance to grazing. The main emphasis in breeding for better cultivars has been to increase the yield and persistency (Wilkins 1991). Further improvement in winter hardiness and adaptation to the light regime at high latitudes is needed to extend the geographic range of this species further north. Perennial ryegrass is also widely used for sports fields, lawns and other amenity areas.

3.1.3 *Festulolium and meadow fescue*

Festulolium are hybrids between the *Festuca* genus and *Lolium*, which are closely related species. *Festulolium* was produced in breeding programs to combine the excellent forage quality of *Lolium* and the winter hardiness of *Festuca* species into *Festulolium* hybrids (Humphreys 2003). These hybrids do not have a long history, being developed around 1970, and promising cultivars are only recently available (Ghesquière et al. 2010). *Festulolium* cultivars have higher biomass production than *Lolium* in addition to having high forage quality, and are promising forage grass cultivars for the Nordic region under future climatic conditions (Østrem et al. 2013).

In the present study, we included several *Festulolium* populations (Table 1), and two Norwegian cultivars of meadow fescue (*Festuca pratensis* Huds.) for reference.

3.1.4 *Red Clover*

Red clover *(Trifolium pratense* L.*)* is a short-lived perennial legume species and the main forage legume in Norwegian agriculture. The yield potential is, most often limited by insufficient persistence due to factors like lack of winter hardiness, unfavourable management regimes (e.g. excess N-fertilization), and diseases like clover rot (*Sclerotinia trifoliorum*). Red clover is known for its ability to fix atmospheric nitrogen into ammonia in symbiosis with the *Rhizobia* bacteria. The symbiotic life causes later spring growth compared to grasses and therefore red clover constitutes a lower fraction in mixtures in the first harvest compared to the second. Red clover makes a nutrient-rich fodder with high protein content, and supplies companion crops with nitrogen as well, thereby reducing the need for fertilizer. Red clover is primarily characterised by a tap-root, but older plants are more fibrous rooted with adventitious roots originating from the upper part of the crown (Taylor & Quesenberry 1996).

Table 1 (3 pages long) Cultivars and populations used in the PhD work

Species	Paper	Name[a]	Adaptation[b]	Country of origin	Ploidy	Description[c]
Perennial ryegrass	1,2,3	Fagerlin	Northern	NO	Diploid	Cultivar (listed 2008). Synthetic polycross of genotypes from cvs. Gunne, Svea, Valinge (SE), Riikka (FI), Norlea (CA), Raidi (LA) and local populations WIR20258, WIR35600 (The Vavilov Institute, RU), and RaigD2, 16-60-1 (NO)
	3	Ivar	Northern	NO	Tetraploid	Cultivar (listed 2007) based on half-sib families from colchicine-induced tetraploid populations of local population (Kleppe, NO) and cvs. Barvestra (NL) and Tove (DK). Mainly selected for winter survival.
	1,2,3	FuRa9805	Southern	IR	Diploid	Population originating from the surviving plants of three candivars from Northern Ireland evaluated at Fureneset, Norway (61.2°N, 5.2°E) for three years
	3	Figgjo	Southern	NO	Tetraploid	Cultivar (listed 2006) originating from a seed mixture of selected plants of Polly (Lolium hybrid, DK), WIR40697 (RU), RaigT5 (NO), and cvs. Tove and Taptoe (DK)
	3	Arka	Continental	PO	Diploid	Polish commercial cultivar
Timothy	1,2	MTV0508-3	Northern	NO	Hexaploid	Originates from MTL9701, selected for persistency for one generation at the southern highland location Løken, Valdres, Norway (59.8°N, 11.5°E, 550 m asl) and two generations at the northern costal location Vågønes, Bodø, Norway (67.3°N, 14.6°E). Each selection cycle consisted of an establishing year, two harvest years under local agricultural management practices and seed harvest of surviving plants in the third ley year.
	1,2	MTL9701 +Grindstad	Southern	NO	Hexaploid	A mixture of cultivar Grindstad and the breeding population MTL9701, the latter originating from a cross conducted in the southeast of Norway, between 12 cultivars listed for use in the Nordic countries, United Kingdom, Netherland, Belgium, Germany and Czech Republic
	4	Grindstad	Southern	NO	Hexaploid	Genetically very broad cultivar based on an old landrace from southeast Norway

Species	Paper	Population name	Adaptation	Country of origin	Ploidy	Description
Red clover	1, 4	B1D2+D3 +VåRk0734	Northern	NO	Diploid	Originated from selected northern Fennoscandian populations intercrossed and subjected to two cycles of different management (fertilizer and harvesting regimes) selection at Vågønes, Bodø, as described for the northern-adapted timothy population.
	4	A1TD1+D2	Northern	NO	Tetraploid	Originating from Fennoscandian tetraploid populations from Kolpo, Syn1/88, Tepa, Betty and SvÅ90052 (Project Norclover). Seed source: VåRk0736-4x, VåRk0735-4x, and VåRk0844-4x crossed together and subjected to two cycles of selection at different management (fertilizer and harvesting regimes) at the two Norwegian research stations Vågønes and Løken.
	4	Bjursele	Northern	SW	Tetraploid	Old cultivar from Västerbotten in Sweden
	1, 4	LøRk0393 / 0395	Southern	NO	Tetraploid	A mixture of the two candivars LøRk0393 and LøRk0395 selected at Løken, Norway from Eastern European red clover, mainly from the Plant Breeding Station Hladke Zivotice (DLF-Trifolium), Czech Republic
	4	MRL97	Southern	NO	Diploid	Based on southern adapted populations (LøRk0499-2x)

Species	Paper	Population name	Adaptation	Country of origin	Ploidy	Description
×Festulolium (Lp^{Fp})	3	FuRs0467	Northern	NO	Diploid	Originates from a wide germplasm pool of several initial triploid hybrids from crosses either between the tetraploid L. perenne population WIR40697 (RU) and F. pratensis cv. Fure (NO) or between cv. Bastion (NL) crossed with cv. Salten, a cultivar originating from Bodø, northern Norway (67°N). The initial hybrids were backcrossed twice onto diploid L. perenne cv. Gunne (SE) and cv. Riikka (FI) and for a few crosses to cv. Norlea (CA). Progenies obtained after the second backcross were exposed to natural selection at Kvithamar (63.3°N, 10.5°E).
	3	FuRs0356	Southern	NO/UK	Diploid	Candivar based on plants backcrossed at IGER (now IBERS, UK) from initial hybrids of diploid L. perenne and Festulolium cv. Prior. Following two periods of three winters in a nursery field at Fureneset 35 selected plants with loloid-type panicles were the base for the candivar
	3	FuRs0142	Southern	NO/UK	Tetraploid	Originates from tetraploid (2n = 4x = 28) families (Ba-11356, Ba-11356-sel, Ba-11358 and Ba-11359 from IGER, UK) which were backcrossed to tetraploid L. perenne cv. Napoleon (DK), cv. Baristra (NL) and candivars LøRa9401 (Graminor, NO) and Jo-0307 (Boreal Ltd., FI)
	3	Felopa	Continental	PO	Tetraploid	Polish commercial cultivar
Meadow fescue	3	Norild	Northern	NO	Diploid	Cultivar (listed 2001) selected in Alta, Finnmark (NO) (69.6°N), from a local population collected in Harstad, Troms (NO) (68.5°N)
	3	Fure	Southern	NO	Diploid	Cultivar (listed 1979) based on a local population from Fureneset, western Norway

[a] Denominations according to the parental origin; Lp: *L. perenne*, Lm: *L. multiflorum*, and Fp: *F. pratensis*, with the abbreviation given as a superscript (e.g. Fp) when the species consist of segments within the *Lolium* genome.
[b] Northern and southern is related to genetic pedigree (background) and adaptation level under Nordic climatic conditions.
[c] Candivar is a new promising candidate cultivar.

3.2 Methods

3.2.1 *Freezing tolerance tests*

Freezing tests were used for evaluation of freezing tolerance in all experiments (Pulli et al. 1996, Larsen 1978). This is a standard method for freezing tests of breeding materials in Norway (Höglind 2010). Whole plants were trimmed and crown segments covered with humid sand inside plastic boxes (Fig. 11). These were exposed to predetermined freezing temperature within a programmable freezer. The temperature was lowered until the minimum test temperature for each treatment was reached (see Fig. 12).

Results of the freezing tests are presented as LT_{50} values, the temperature at which 50% of the plants died, and are scored by visual rating of regrowth after 3-4 weeks at room temperature and 24 h light.

Figure 11. Trimmed plants for freezing tolerance test. Before freeze test they will be covered by sand.

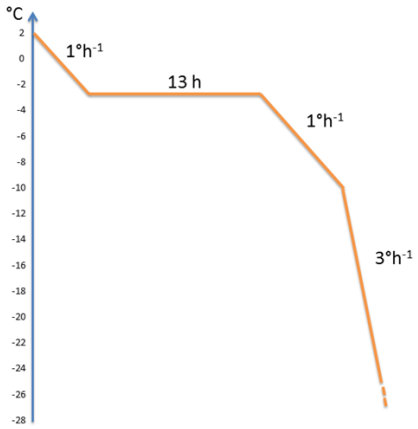

Figure 12. Temperature program for freezing test.

3.2.2 *Fluorescence measurements*

Chlorophyll fluorescence-based techniques were used to study photochemical activity. Fluorimeter (JPI test) was used to study the characteristics of chlorophyll fluorescence based on the energy flow through single reaction centres to estimate photosystem II photochemical activity, the degree of photosynthetic apparatus damage and photoacclimation. In the field studies, Handy Pea fluorimeter (Hansatech Ltd.) was used, while PAM-2500 fluorimeter (Heinz Walz) was used in the laboratory studies. Measurements were made on the mid-section of the youngest fully expanded leaf. Leaves were first dark adapted for 15-30 min, using leaf clips (8 mm diameter) for opening all PSII reaction centres before a short light exposure was given to measure the maximum quantum yield of PSII (*Fv/Fm*), the energy amounts absorbed by the antennas and the energy trapped in PSII reaction. In the laboratory studies plants were in addition treated with far-red light, given to measure F'_0 (minimum chlorophyll fluorescence yield in the dark-adapted/light-adapted leaf) to ensure rapid opening of reaction centres.

3.2.3 *Measurement of leaf elongation rate (LER)*

Leaf elongation (mm $week^{-1}$) was measured on 15 plants per treatment in the laboratory studies. In the field studies, 10 plants were marked in each of three replicate plots during the regrowth after the last cut. The youngest emerging leaf was marked and measured weekly.

3.2.4 *Water-logging treatments*

To test tolerance to water-logging during autumn, pot plants were placed outside on wooden boards with boarders as high as the pots. The inside was covered with plastic to make the space water-tight. The space was filled with water assuring 90 % volumetric water content (VWC) of soil saturation within each pot. Control pots were placed on wooden boards with only a short boarder, just enough to keep soil water concentration at field capacity (25 % VWC).

3.2.5 *Carbohydrate analysis*

Dried crown segments were pulverised and high liquid chromatography (HPLC) was used to analyse the content of different soluble carbohydrates; glucose, fructose, sucrose, maltose and raffinose. Starch was analysed as glucose subunits after enzymatic hydrolysis by alpha-amylase.

3.2.6 *Statistical analyses*

Most statistical analyses were performed using generalised linear model (GLM), mainly ANOVA, in Minitab, SAS, Statistica or R programs. For the freezing test, a logistic model was used. For estimating leaf elongation, a non-linear mixed model was used. Principal component analysis (PCA) was used for grouping data in paper III. In presenting significant differences among mean values, Tukey's *post-hoc* tests were used modestly and presentation of 95% confidence intervals favoured instead. While multiple comparisons of interaction terms may be difficult to interpret without including the main term, the use of adjusted *post hoc* tests are widely discussed as they often are regarded too conservative (Dowling et al., 2003; Garcia, 2004; Kuzon et al., 1996)

4. MAIN RESULTS AND DISCUSSION

The results from the four papers are discussed by looking at the effects of the different climatic factors on the physiological response of the plant during cold acclimation. The three main climatic factors determining crop growth and development are the energy flow by radiation, temperature, and the soil moisture conditions (Skjelvåg 1998). These factors are all tested for in paper I-IV, and how differences within and interaction between those affect the cold acclimation of perennial ryegrass, timothy and red clover in Norway. The results are discussed both in relation to the basic physiological mechanisms *per se* and as evaluations of the pre-breeding material. The research in this PhD work did not include development of new methods, but known methods were used to investigate the physiological and morphological responses, therefore the pros and cons of the methods used are briefly discussed.

4.1 Freezing tolerance of timothy, perennial ryegrass, red clover (paper I, II, IV)

4.1.1 *Effect of acclimation temperature on freezing tolerance*

Paper I and II show that increasing pre-acclimation temperatures, before 1 or 3 weeks of cold acclimation treatment at 2°C, reduced the freezing tolerance in red clover, perennial ryegrass and timothy. Elevated autumn temperatures have also been found to cause reduced freezing tolerance in winter wheat (Hanslin and Mortensen 20010). Cold acclimation at low positive temperature is known to increase the freezing tolerance of plants (Thomashow 1999 and 2010), but even pre-acclimation temperatures as high as 12°C promoted cold acclimation in grasses and clover compared to 15°C (Paper I). A modest increase (1-2°C) in freezing

tolerance can have a dramatic impact on agricultural productivity (Steponkus et al. 1998). Therefore, the ability to start acclimating already at temperatures above 10°C may be increasingly important in future climates. Different night and day temperature did not affect the freezing tolerance in this experiment. Minor diurnal differences in cold tolerance may have existed, but this was not expressed in the overall freezing tolerance. Northern-adapted populations of timothy and perennial ryegrass showed a stronger response to temperature as their freezing tolerance was reduced more at higher pre-acclimation temperatures compared to the southern-adapted populations. This indicates that the predicted increase in autumn temperatures in the future may reduce cold acclimation in northern-adapted cultivars, resulting in lower winter hardiness.

4.1.2. Effect of daylength/irradiance on freezing tolerance

Daylength is a far more stable seasonal environmental cue than temperature in temperate zones. We found that the freezing tolerance of both timothy and perennial ryegrass decreased when acclimation occurred at fast diminishing daylight later in the autumn compared with acclimation earlier in autumn, especially in combination with higher temperature (paper II). As the experiment was performed under natural light conditions, we cannot separate the effect of daylength and light intensity or light quality and circadian regulation. Amount and duration of light during cold acclimation affects the ability of the plant to develop cold tolerance (Crosatti et al. 2013). Increased light intensity at low temperature led to better cold acclimation in genotypes of perennial ryegrass (Harrison et al. 1997, and Höglind et al. 2011). Most likely the irradiance (the amount of light) is even more important *per se* for cold acclimation than the length of the day. Therefore, cloudy days may have reduced the cold acclimation markedly in our study. This might explain the non-significant differences in freezing tolerance between early and intermediate treatments in paper II, whereas a pilot study the year before (2011), showed a significant reduction in freezing tolerance at the intermediate treatment compared to the early treatment (Fig. 12)

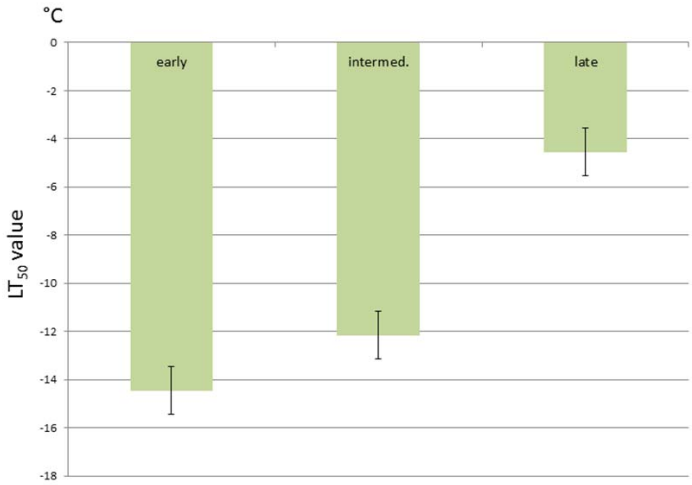

Figure 13. Freezing tolerance expressed as LT50 value within cultivar Grindstad in year 2011. Plants were pre-acclimated for weeks at 6°C and cold acclimated at 2°C for 1 week. This temperature treatment was repeated at three different light periods in the autumn. Early (5 Sept-10 Oct), intermediate (26 Sept-31 Oct) and late (17 Oct-21 Nov autumn light periods.

Northern-adapted populations responded more strongly than southern-adapted to less light at higher temperatures (12°C).The northern-adapted populations had better freezing tolerance than southern-adapted at the early and intermediate light treatments, but the freezing tolerance of both populations was similar at the late light period. This indicates that current adapted breeding materials are not suitable for future climates where acclimation may need to occur under new light × temperature combinations.

4.1.3 *Effect of water-logged soil on freezing tolerance and survival*

The effect of water-logging during autumn on freezing tolerance and survival did not give uniform results (paper IV). The level of freezing tolerance and carbohydrate concentration was more dependent on differences in climate conditions between locations and years, in addition to species and population differences, than the actual water level of soil. Red clover was in general less freezing tolerant compared to timothy, but less sensitive to climatic differences between locations. Species differences in root structure may play a crucial role. In timothy, water-logged soil enhanced the freezing tolerance at the northern site (Holt) with lower autumn temperatures, but reduced the freezing tolerance at the southern site (Fureneset) where autumn temperatures were higher. The harsh climatic conditions at Holt limited survival of red clover, growing plants in pots outside may have stressed the plants further. At

Fureneset, the southern-adapted red clover population was more sensitive to water-logging treatment for two months compared to one month the first year, and survived slightly better at field capacity compared to full water-logging.

4.1.4. *Evaluation of freezing tolerance methods*

Quamme (1978) describes well the criteria for a method when measuring cold hardiness in breeding programs: "*1) the method must allow precise differentiation of hardy cultivars from tender ones, 2) it must be adaptable to measuring large populations and 3) it must be carried out with reasonable expenditure of time and money"*. When the plant tissue is cooled down below 0°C it does usually not freeze immediately, but the tissue remains supercooled (Quamme et al.1982). Therefore, crown segments were covered with humid sand and kept at -3°C for 13 hours for adjustment, to freeze homogenously and avoid supercooling within the tissue, preventing detrimental effects of explosive ice formation within the cells when lowering the temperature further. The rate of cooling, and the rate of thawing, is critical for the survival of the plant (McKersie & Lesheim 1994). In nature the rate of cooling and thawing is usually only a few °C per hour, often not more than 2°C (Palta et al. 1993). This does not disrupt the stability of the plasmamembrane. In the last step of the freezing program (below -10°C) the cooling was accelerated to 3°C per hour. This was mainly because of practical reasons, avoiding having to remove samples from the freezer in the middle of the night, thus minimizing the cost of labour. This acceleration does not give different LT_{50} results for grasses and clover compared with a temperature reduction of 1°C h^{-1} when the temperature is below -10°C (A. Larsen pers. comm. in Höglind et al. 2010).

Though LT_{50} values give a good estimate of short-term freezing tolerance (Larsen 1994, Rapp and Junttila 2000), a full graph showing survival at each test temperature gives a more holistic picture when studying the adaptive responses of populations. Crowns from single plants were used in the freezing tests. Level of freezing tolerance is tissue specific and crown tissue reaches higher level of freezing tolerance compared to leaves and especially roots (McKersie & Lesheim 1994). Survival of the crown is critical for the ability to generate new axillary shoots and roots during regrowth. This method for testing freezing tolerance in the laboratory has been shown to be highly correlated with field testing though plants tested under field conditions commonly express higher tolerance albeit variable (Larsen 1989, Rapp & Junttila 2000). Despite of this, these kinds of laboratory methods have their limits and for several reasons they have mostly been used for basic physiological and genetic studies of cold tolerance. They have not been applied effectively in breeding programmes (Rognli 2013).

These tests are laborious and expensive. Another challenge is choosing the right span of test temperatures within which LT_{50} values lies. When working with new species or testing new plant materials with unknown hardening levels, selecting the adequate test temperatures will only be based on an educated guess. Less distance between test temperatures gives better separation between treatments, but it increases the chance of not reaching the LT_{50} value. As such, this method is not able to discriminate small differences between genotypes (Gusta et al. 1982). In paper I, The LT_{50} values for the northern-adapted perennial ryegrass at 9°C and southern-adapted timothy at 15°C were not reached at the test temperatures used in this study as we underestimated the cold acclimation ability of perennial ryegrass at lower temperatures and overestimated that of timothy at higher pre-acclimation temperature. Larsen (1994) points out that the best stage of differentiation between populations is not at the stage of maximum tolerance in mid-winter, but is obtained at the stage of dehardening in late winter. However, tests which include dehardening can be even more time consuming and expensive. Therefore, winter hardening capacity is probably most conveniently tested during cold acclimation in the autumn.

Estimate of long term freezing tolerance with prolonged freezing tests should also be considered for inducing cell dehydration and achievement of a maximum freezing tolerance (Waalen et al. 2011), but this has not been tested in this study.

4.2. **Photoacclimation in timothy, perennial ryegrass, Festulolium (papers I, II, III)**

4.2.1. *Response to climate*

Pre-acclimation at lower temperatures increased photosynthetic performances in grasses indicating photoacclimation (Hüner 2014) (paper I and II). Red clover was less temperature sensitive than the grasses and did not express low temperature induced photoacclimation (paper I). Fluorescence measurements made both right after pre-acclimation and after cold acclimation at 2°C showed that photoacclimation took place early in the acclimation process, already during pre-acclimation. Both non-photochemical and photochemical mechanisms of photoacclimation were observed in grasses but the photochemical mechanism was predominant, especially at higher irradiance and lower temperature. In paper II, photoinhibition was observed at the early light period (acclimation early in autumn) in a combination with the lowest temperature, and it was probably a response to high excitation pressure (Hüner 1993). Different day and night temperature caused a slightly lower photosynthetic activity compared to a constant temperature (paper II). Among the daylength/irradiance and temperature treatments used in our study, the effect of

daylength/irradiance on photosynthetic activity was stronger than the effect of temperature. Especially the lack of light caused less photosynthetic activity (paper II and III). This was also observed in the field experiments where less photosynthetic activity was recorded at the northern location (Bodø, 67°N) compared to the southern location (Fureneset 61°N). In the south, grasses expressed low temperature induced photoacclimation, by increasing non-photochemical quenching. This correlated with higher winter survival the following spring. In the north, despite of lower photosynthetic activity in northern-adapted grasses during autumn, they survived better than the southern-adapted. The low light conditions in the north may not be sufficient to induce photoacclimation, thus an alternative mechanism for acclimation is suggested.

4.2.2. *Evaluation of methods*

Measurement of chlorophyll fluorescence parameters in cold acclimated plants have been suggested as an indirect measurement of freezing tolerance (Rapacz et al. 2004) and an efficient tool for early detection of temperature stress (Kalaji et al. 2011). Chlorophyll fluorescence measurements made on 40 cultivars of wheat in field trials showed that these measurements could be used as screening method for freezing tolerance (Rapacz & Woźniczka, 2009). When comparing field and laboratory studies the method is shown to be sensitive to environmental changes and is species dependent (Rapacz et al. 2015). Clear relationships observed between photoacclimation and survival in field studies, presented in paper III, in addition to the relationship between decreasing photoacclimation and freezing tolerance with decreasing autumn light and increasing temperature, presented in paper II, indicate that fluorescence measurements might be a valuable tool for the early selection of non-native grasses for high latitude regions.

4.3. **Growth cessation and biomass production (papers I, II, III)**

4.3.2. *Response to climate*

Within the range of the treatments used in our studies, daylength/irradiance had a stronger effect on leaf elongation than temperature, but most importantly, growth was affected by an interaction of autumn light conditions and temperature. Leaf elongation and biomass production increased at higher temperatures, but plants produced less biomass at shorter daylength when leaf elongation was stimulated mainly as etiolated growth response (paper I and II). Etiolated growth is a well-known response to limited light conditions in combination with growth temperatures (Moriyama et al. 2003). Leaf elongation was stimulated gradually

by shorter daylength/less irradiance when day temperature was higher than night temperature (9/3°) compared to a constant temperature 6°C (paper II). There was no general difference between northern-adapted and southern-adapted populations in leaf elongation rate (LER) recorded in the laboratory experiments (paper I and II). The northern-adapted perennial ryegrass cultivar 'Fagerlin' expressed a photochemical form of photoacclimation at 6°C in the early autumn period by increasing biomass production as a response to high excitation pressure. This response is regulated by the redox state of the photosystem, which induces cold acclimation and growth cessation (Hüner 2014). Cv.'Fagerlin' did also reduce LER in the autumn at the northern location (Holt), similar to the northern-adapted *Festuca pratensis* cultivar; this was positively correlated with winter survival in the field (paper III). Perennial ryegrass and *Festulolium* tested in field studies had a gradual reduction in elongation growth during autumn at the southern location (Fureneset), correlating with the non-photochemical quenching of light energy. The *Festulolium* cultivar 'Felopa', adapted to far more southern climate than in Norway, did not show the same temperature and light responses to growth cessation as the more northern-adapted entries, resulting in poor winter survival. The low amount of light at the northern location was not enough to trigger sufficient reduction of the photosystem II (PSII) to trigger growth cessation and decrease in LER. Despite of this, the northern-adapted *Festuca pratensis* cultivar and the northern-adapted perennial ryegrass cultivar 'Fagerlin' and cv. 'Ivar' in particular, effectively reduced growth in the autumn. This indicates that photoperiod or light quality may control growth cessation in grasses adapted to high latitudes involving phytochrome control (Casal et al. 1998). Possibly grasses resemble tree species physiologically regarding growth cessation, more than earlier thought.

3.3.3. *Evaluation of methods*

This method is simple and efficient, but the challenges are related to selection of good leaf candidates. It is important that all leaves are at the same growth stage at the start since their growth rate reduces when they reach maturity. If the marked leaf dies or is destroyed during the experimental period, a new leaf at a similar growth stage has to be selected. I the laboratory, these measurements can easily be tested on genotypic level, but in swards in the field, this must be done by sampling on a population level.

4.3.4. *Breeding material potentials*

Northern adapted populations of timothy and perennial ryegrass suffered more than southern adapted when acclimated at low light levels in combination with higher future temperature in

phytotron studies. Perennial ryegrass did not suffer as much as timothy, and the northern adapted perennial ryegrass cultivar 'Fagerlin', which has showed northern-adapted characters both in field and laboratory studies (papers I, II and III), may thus be a promising germplasm resource for future breeding programs.

In laboratory studies, red clover populations were less temperature sensitive than grasses during acclimation and they expressed low winter survival in semi-field experiments in the north (Holt), but survived well, especially northern-adapted populations, in a milder climate further south (Fureneset). *Festulolium* populations photoacclimated and survived well at Fureneset, but are not adapted to climatic conditions in the north (Bodø) which was reflected in the low winter survival compared to the well adapted *Festuca pratensis*.

5. MAIN CONCLUSIONS AND FUTURE PROSPECTIVES

Main results

Our results indicate that the projected climate change in the north may delay hardening in autumn, reduce photoacclimation and decrease freezing tolerance in grasses. Climate change with warmer autumns could cause inadequate cold acclimation and winter survival at higher latitudes in non-native grass species that have been introduced to Scandinavian grasslands in recent decades. The present species and cultivars may therefore have to be replaced by species and cultivars able to acclimate adequately under new light × temperature combinations, which are unique in the global context for agricultural production.

Our results confirm that the level of freezing tolerance in perennial forage crops is affected by light × temperature interaction during autumn, although temperature had a stronger effect on freezing tolerance under the ranges of temperatures and light conditions tested. However, both the process of photoacclimation and growth cessation seems to depend strongly on light conditions during autumn.

Higher pre-acclimation temperatures and less autumn light reduced the photosynthetic activity and the freezing tolerance in timothy and perennial ryegrass. Higher pre-acclimation temperatures also reduced the freezing tolerance of red clover, although in general, red clover was less sensitive to temperature differences compared to grasses. Northern-adapted populations generally had higher freezing tolerance than the southern-adapted, but not at the combination of shortest daylength/lowest irradiance and highest temperature. Different day and night temperature (9/3°C) compared to a constant temperature (6°C) did not affect the freezing tolerance of grasses. Leaf elongation rate of grasses increased with temperature, and

was higher at shorter daylength/lowest irradiance when this was combined with higher temperatures. Field studies indicated that at high northern latitude (67°N), the amount of light is insufficient to trigger the changes in photosynthetic apparatus that are responsible for growth cessation at more southern latitude (61°C). During growth cessation in the autumn, photosynthetic activity increased at the southern latitude, and it was correlated with increased winter survival. Northern-adapted species and populations started growth cessation earlier at the northern location, but this was not linked with increased activity of the photosystem. This suggests a different pattern of growth cessation controlled more by the photoperiod in the north compared to in the south. Red clover was considerably less freezing tolerant than timothy and perennial ryegrass, but did not show clear effect of water-logging stress on freezing tolerance during acclimation in the autumn. Waterlogged soil (90%) in the autumn enhanced the freezing tolerance of timothy at the northern site Holt (69°N) in combination with the lowest autumn temperature, but reduced it at the southern site Fureneset (61°C) in combination with higher autumn temperature. Prolonged water-logging may therefore negatively affect the cold acclimation at future elevated temperatures, timothy being more affected compared to red clover.

Exactly how the interaction of temperature, light and water-logging affect the processes of growth cessation and hardening of perennial forage in high latitude regions is still not clear.

Future prospective

Although knowledge about the genetic control of the CBF-pathway has increased tremendously in the recent years, there is still a way to go until we fully understand the different mechanisms taking place during plant cold acclimation. Further understanding of the regulatory mechanisms of growth cessation and photoacclimation are important for developing improved, high yielding and persistent forage crops adapted to future climates. Signal transduction of the photosynthesis and sink-source mediated carbon metabolism needs further attention and increased knowledge about the effect of light quality, photoperiod and circadian regulation of growth cessation and freezing tolerance (e.g. as reviewed in Maibam et al. 2013) may increase our understanding of plants adaptation to high latitude conditions. Phytochrome-dependent morphogenesis may be of importance. Studies by Lewandowska-Sabat et al. (2011) in *Arabidopsis* revealed several candidate genes and pathways likely to contribute to adaptation to unique light and temperature regimes at high latitudes. These are worth further comparative studies in perennial forage crops. If future studies could enable us to estimate the span of phenotypic plasticity for cold acclimation capacity within a genotype

and the epigenetic effects, this would be highly valuable in future breeding programs. Our studies show that grass populations/ecotypes locally adapted to high latitude regions cease growth earlier and express an alternative mechanism for growth inhibition during autumn for obtaining adequate winter survival. It is suggested that the adaptation mechanism of northern high latitude grasses may be increasingly required in southern areas of the Nordic region because the light intensity decrease and the temperature increase predicted for these areas will delay cold acclimation. I suggest using natural well-adapted ecotypes from different climate regions to study the basic mechanisms of cold acclimation and growth cessation.

REFERENCE

Abeynayake SW, Etzerodt TP, Jonavičienė K, Byrne S, Asp T, Boelt B (2015) Fructan metabolism and changes in fructan composition during cold acclimation in perennial ryegrass. Front Plant Sci 6:329. doi: 10.3389/fpls.2015.00329

Achard P, Gong F, Cheminant S, Alioua M, Hedden P, Genschik P (2008) The Cold-lnducible CBF1 Factor-Dependent Signaling Pathway Modulates the Accumulation of the Growth-Repressing DELLA Proteins via Its Effect on Gibberellin Metabolism. Plant Cell 20:2117-2129

ACIA (2005) Impacts of a Warming Arctic: Arctic climate Impact Assessment. Cambridge: Cambridge University Press

Alsos IG, Ehrich D, Thuiller W, Eidesen PB, Tribsch A, Schönswetter P, Lagaye C, Taberlet P, Brochmann C (2012) Genetic consequences of climate change for northern plants. Proc R Soc B 279:2042-2051

Andersen IL (1963) Investigation on the wintering of meadow plants in Northern Norway II. Investigations on damages caused by ice and water choking on meadows. Forsk Fors Landb 31: 635-660 (In Norwegian)

Andersen IL (1971) Overvintringsforsøk med ulike grasarter. Forskning og forsøk i landbruket 22:121-134 (In Norwegian)

Andersen IL (1992) Winter injuries in grasslands in northern Norway caused by low temperature fungi. J Agr Sci, supplement No. 7. Disease resistance in agricultural crops, NJF pp 188:13-20

Anderson S (1992) Differences between varieties in resistance to snow mould fungi. J Agr Sci, supplement No. 7.Disease resistance in agricultural crops, NJF pp 188:21-24

Andrews C.J (1987) Low-temperature stress in field and forage crop production – An overview. Can. J. Plant Sci 67:1121-1133

Andrew CJ, Pomeroy MK (1979) Toxicity of anaerobic metaboites accumulating in winter wheat seedlings during ice encasement. Plant Physiol 64:120-125

Baadshaug, OH (1973) Effect of soil type and soil compaction on the wintering of three grass species under different wintering conditions. Acta Agric Scand 23:77-86

Bailey-Serres J, Voesenek LACJ (2008) Flooding stress: acclimations and genetic diversity. Ann Rev Plant Biol 59:313–339

Bélanger G, Rochette P, Castonguay Y, Bootsma A, Mongrain D, Ryan DAJ (2002) Climate change and winter survival of perennial forage crops in Eastern Canada. Agron J 94:1120-1130

Bertrand A, Castonguay Y, Nadeau P, Laberge S, Rochette P, Michaud R, Bélanger G, Benmoussa M, (2001) Molecular and biochemical responses of perennial forage crops to oxygen deprivation at low temperature. Plant Cell Environ 24:1085-1093

Bertrand A &Castonguay Y(2003) Plant adaptations to overwintering stresses and implications of climate change. Can J Bot 81:1145-1152

Bertrand A, Castonguay Y, Nadeau P, Laberge S, Michaud R, Bélanger G, Rochette P, (2003) Oxygen deficiency affects carbohydrate reserves in overwintering forage crops. J Exp Bot 54:1721-1730

Bootsma A (1984) Forage crop maturity zonation in the Atlantic region using growing degree-days. Can J Plant Sci 64:329-338

Casal JJ, Sánchez RA, Botto JF (1998) Modes of action of phytochromes. J Exp Bot 49:127-138
Castonguay Y, Thibault G, Rochette P, Bertrand A, Rochefort S, Dionne J (2009). Physiological Responses of Annual Bluegrass and Creeping Bentgrass to Contrasted Levels of O_2 and CO_2 at Low Temperatures. Crop Sci 49:671
Ceccarelli S, Grando S, Maatougui M, Michael M, Slash M, Haghparast R, Rahmanian M, Taheri A, Al-yassin A, Benbelkacem A, Labdi M, Mimoun H, Nachit M (2010) Plant breeding and climate change. J Agr Sci 149:627-637
Chen THH, Gusta LW (1983) Abscisic acid-induced freezing resistance in cultured plant cells. Plant Physiol 73:71-75
Chen THH (1994) Plant adaptation to low temperature stress. Can J Plant Pathol 16:231-236
Chinnusamy V, Zhu J, Zhu J-K (2006) Gene regulation during cold acclimation in plants. Physiol Plant 126:52-61
Chinnusamy V, Zhu J and Zhu JK (2007) Cold stress regulation of gene expression in plants. Trends Plant Sci 12(10):444-451
Cooper EJ (2014) Warmer Shorter Winters Disrupt Arctic Terrestrial Ecosystems. Annu Rev Ecol Evol Syst 45:271–295
Crawford RMM (1989) Studies in Plant Survival. Blackwell Scientific Publications, Oxford, pp 296
Crawford RMM, Braendle R (1996) Oxygen deprivation stress in a changing environment. J Exp Bot 47:145–159.
Crawford, RMM, (2003) Seasonal differences in plant responses to flooding and anoxia. Can J Bot 81:1224-1246
Crosatti C, Polverino de Laureto P, Bassi R, Cattivelli L (1999) The interaction between cold and light controls the expression of the cold-regulated barley gene *COR 14b* and the accumulation of the corresponding protein. Plant Physiol 119:671-680
Crosatti C, Rizza F,Badeck FW, Mazzucotelli E, and Cattivelli L (2013) Harden the chloroplast to protect the plant. Physiol Plant 147:55-63
Dalmannsdóttir S, Helgadóttir A, Guðleifsson BE (2001) Fatty acid and sugar content in white clover in relation to frost hardiness and ice encasement tolerance. Ann Bot-London 88:753-759
Darwin C (1875) The origin of species by means of natural selection. 6thedn. London: Murray
Dowling A PG, van Veller M G P, Hoberg E P, Brooks D R (2003) A priori and a posteriori methods in comparative evolutionary studies of host-parasite associations. Cladistics19 (3):240–253
Eagles CF, Williams J (1992) Hardening and dehardeningof *Loliumperenne* in response to fluctuating temperatures. Ann Bot 70:333-338
Eagles CF (1994) Temperature, photoperiod and dehardening of forage grasses and legumes. In: Proceedings of COST 814 Workshop in Hamburg, Germany, Brussels, Belgium: European Commission. pp 75–82
Ensminger I, Busch F, Huner NPA (2006) Photostasis and cold acclimation: sensing low temperature through photosynthesis. Pysiol Plant 126:28-44
FAO (2010) The second report on the state of the world`s plant genetic resources for food and agriculture. Commission on Genetic Resources for Food and Agriculture (CGRFA), Food and Agriculture Organization of the United Nations (FAO), Rome. ISBN: 978-92-5-106534-1
Fehr WR (1987) Principles of cultivar develipment Vol. 1 Theory and technique.New York: McGraw-Hill pp 31-33
Finazzi G, Rappaport F, Goldschmidt-Clermon M (2003) From light to life: an interdisciplinary journey into photosynthetic activity. EMBO 4:752-756
Fjellheim S, Tanhuanpää P, Marum P, Manninen O, Rognli OA (2015) Phenotypic or molecular diversity screening for conservation of genetic resources? An Example from a Genebank Collection of the temperate forage grass timothy. Crop Sci 55:1646-1659
Flovik K(1969) Engvekstenes overvintring, norske forsøk og undersøkelser. Nordkalott møtet Sverige 10-16 August 1969. Statens forsøksgård Holt, Tromsø. Stensiltrykk nr.1 pp 11
Foss S (1968) Growth rhythm of timothy varieties. StatensforsøksgårdVoll. Meldingnr. 45:45-518. (In Norwegian)
Foss S (1982) Om vekst hos fleirårige grasplanter (Growth of perennial forage grasses) Landbrukstidende1982 (in Norwegian)

Fowler DB, Limin AE, Gusta LV (1983) Breeding for winter hardiness in wheat. In: Fowler DB, Gusta LV, Slinkard AE, Hobin BA (eds.).New Frontiers in winter wheat production. Proc. Western Canada Winter Wheat Conference, 20-22 June, Saskatoon:Univ. of Saskatchewan pp. 136-184

Frankow-Lindberg BE (2001) Adaptation to winter stress in nine white clover populations: Changes in non-structural carbohydrates during exposure to simulated winter conditions and 'spring' regrowth potential. Ann Bot-London 88:745-751

Fridriksson S (1954) Winter injury of plants in Icelandic hayfields. Univ. Res. Inst. Dept. Agr. Rep. Ser. B-No.7.

Garcia LV(2004) Escaping the Boferroni iron claw in ecological studies. Oikos 105:657-663

Gaudet DA, Tronsmo AM, Laroche A (2012) Climate change and plant diseases. In: Storey KE and Tanino KK (eds.).Temperature adaptation in a changing climate, Nature at risk. CABI Climate change series 3, Groydon: CPI Group Ltd. pp 144-159

Ghesquie`re M, Humphreys MW, Zwierzykovski Z (2010) Festulolium. In: Boller B, Posselt UK, Veronesi F (eds.).Fodder crops and amenity grasses. New York: Springer p 293-316

Gratani L (2014) Plant phenotypic plasticity in response to environmental factors. Adv Bot17 pp http://dx.doi.org/10.1155/2014/208747

Gray GR, Chauvin LP, Sarhan F, Hüner NPA(1997) Cold acclimation and freezing tolerance – a complex interaction of light and temperature. Plant Physiol 114:467-474

Gudleifsson BE, Andrews CJ and Bjornsson H (1986) Cold hardiness and ice tolerance of pasture grasses grown and tested in controlled environments. Can. J. Plant Sci. 66:601-608

Gudleifsson BE (2009) Ice encasement damage on grass crops and alpine plants in Iceland – Impacts of climate change. In: Gusta L, Wisniewski M & Tanino K (eds.).From the laboratory to the field. CAB International pp 163-172

Gudleifsson BE (2010) Ice tolerance and metabolite accumulation of herbage crops in Iceland and impact of climate change. Icel Agric Sci 23:111-122

Guo YD, Yli-Mattila T, Pulli S (2003) Assessment of genetic variation in timothy (*Phleum pratense* L.) using RAPD and UP-PCR. Hereditas 138:101-113

Gusta LV, Fowler DB, Tyler NJ (1982) Factors influencing hardening and survival in winter wheat. In: Sakai A, Li PH (eds.). Plant cold hardiness and freezing stress mechanisms and crop implications. New York: Academic Press

Gusta LV (1985) Physiological aspects of climatic adaptation in plants with special references to high-latitude environments. In: Kaurin Å, Junttila O, Nilsen J (eds.). Plant production in the North.Proceedings from "Plant Adaptation Workshop" Tromsø, Norway. September 4-9, 1983 pp 219-235

Gusta LV and Wisniewski M (2013) Understanding plant cold hardiness: an opinion. Physiol. Plant. 147:4-14

Guy CL, Huber JLA, Huber SC (1992) Sucrose phosphate synthase and sucrose accumulation at low temperature. Plant Physiol 100:502-508

Hansen-Bauer I, Drange H, Førland EJ, Roald LA, Børsheim KY, Hisdal H, Lawrence D, Nesje A Sandven S, Sorteberg A, Sundby S, Vasskog K, Ådlandsvik B (2009*)* Klima i Norge 2100. Bakgrunnsmateriale til NOU Klimatilpassing. Norsk klimasenter, September 2009, Oslo p 148 (in Norwegian)

Hanslin HM, Höglind M (2009) Differences in winter-hardening between phenotypes of *Lolium perenne* with contrasting water-soluble carbohydrate concentrations. Grass Forage Sci 64:187-195

Hanslin HM, Mortensen LM (2010). Autumn growth and cold hardening of winter wheat under simulated climate change. Acta Agric Scand Sect B - Plant Soil Sci 60:437-449.

Harrison J, Tonkinson C, Eagles C, Foyer C (1997) Acclimation to freezing temperatures in perennial ryegrass (*Lolium perenne*). Acta Physiol Plant 19(4):505-515

Heide OM (1982) Effects of photoperiod and temperature on growth and flowering in Norwegian and British timothy cultivars (*Phleum pratense* L.). Acta Agric Scand Sect B - Plant Soil Sci 32:241–252

Heide OM, Hay RKM, Baugerød H(1985) Specific daylength effects on leaf growth and dry matter production in high-latitude grasses. Ann Bot 55:579-586

Helgadóttir Á (1989) Breeding herbage species for northern areas. Acta Agric. Scand. 39:255-268

Helgadóttir Á, Björnsson H (1994) Cooperative breeding for the northern marginal areas. Euphytica 77:221-230

Helgadóttir Á, Kristjánsdóttir TA (2006) SNORRI – A new Nordic timothy variety for areas around the Arctic circle. In: Sveinsson Th. (ed.) Timothy productivity and forage quality possibilities and limitations, NJF seminar 384, Akureyri, Iceland, AUI Report No. 10, pp 43-45

Heslot N, Jannink JL, Sorrells ME (2015) Perspectives for Genomic selection applications and research inplants. Crop Sci 55:1-12

Hughes M A and Dunn MA (1996) The molecular biology of plant acclimation to low temperature. J Exp Bot 47(296):291-305

Höglind M, Bakken AK, Jørgensen M, Østrem L (2010) Tolerance to frost and ice encasement in cultivars of timothy and perennial ryegrass during winter. Grass Forage Sci 65:431-445

Höglind M, Hanslin HM and Mortensen LM (2011) Photosynthesis of *Lolium perenne* L. at low temperature under low irradiance. Environ Exp Bot 70:297-304

Hüner NPA, Öquist G, Hurry VM, Kroll M, Falk S, Griffith M (1993) Photoynthesis, photoinhibition and low temperature acclimation in cold tolerant plants. Photosynth Res 37:19-39

Hüner NPA, Bode R, Dahal K, Hollis L, Rosso D, Krol M, Ivanov AG (2012) Chloroplast redox imbalance governs phenotypic plasticity: the ″grand design of photosynthesis″ revisited. Front Plant Sci 3, doi:10.3389/fpls.2012.00255

Hüner NPA, Bode R, Dahal K, Busch FA, Possmayer M, Szyszka B, Rosso D, Ensminger I, Krol M, Ivanov AG and Maxwell DP (2013) Shedding some light on cold acclimation, cold adaptation and phenotypic plasticity. Botany 91:127-136

Hüner NPA, Dahal K, Kurepin L, Savitch L, Singh J, Ivanov AG, Kane K, Sarhan F (2014) Potential for increased photosynthetic performance and crop productivity in response to climate change: role of CBFs and gibberellic acid. Front Chem 2:18. doi: 10.3389/fchem.2014.00018

Humphreys MW, Canter PJ, Thomas HM (2003) Advances in introgression technologies for precision breeding within the Lolium-Festuca complex. Ann Appl Biol 143:1-10

Humphreys MW, Yadav RS, Cairns AJ, Turner LB, Humphreys J, Skøt L (2006) A changing climate for grassland research. New Phytol 169(1):9-26

Iba K (2002) Acclimation response to temperature stress in higher plants: Approaches of gene engineering for temperature tolerance. Annu Rev Plant Biol 53:225-245

IPCC (2013) *The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (Stocker TF, Qin D, Plattner GK, Tignor M, Allen SK, Boschung J,Nauels A, Xia Y, Bex V, Midgley PM (eds.).Cambridge and New York: Cambridge University Press pp 1535

Jansen E, Overpeck J, Briffa KR, Duplessy JC, Joos F, Masson-Delmotte V, Olago D, Otto-Bliesner B, Peltier WR, Rahmstorf S, Ramesh R, Raynaud D Rind D, Solomina O, Villalba R, Zhang D (2007) Palaeoclimate. In: Solomon S, Qin D, Manning M, ChenZ, Marquis M, Averyt KB, Tignor M, Miller HL (eds.). Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change.Cambridge: Cambridge University Press pp 433-497

Jørgensen M, Østrem L, Höglind M (2010) De-hardening in contrasting cultivars of timothy and perennial ryegrass during winter and spring. Grass Forage Sci 65:38-48

Junttila O (1990) Effects of temperature and photoperiod on frost resistance of white clover (*Trifoliumrepens*) ecotypes. Physiol Plantarum 79:435-438

Junttila O (1996) Plant adaptation to temperature and photoperiod. Agr Food Sci Finland 5: 251-260

Jump AS, Peñuelas J (2005) Running to stand still: adaptation and the response of plants to rapid climate change. Ecol Lett 8:1010-1020

Jump AS, Marchant R, Peñuelas J (2009) Environmental change and the option value of genetic diversity. Trends Plant Sci 14(1):51-58

Kacperska-Palacz A (1978) Herbaceous plant acclimation mechanism, In: Li PH and Sakai A (eds.).Plant cold hardiness and freezing stress.Academic Press pp139-152

Kirwan L, Luscher A, Sebastia MT, Finn JA, Collins RP, Porqueddu C, Helgadottir A, Baadshaug OH, Brophy C, Coran C, Dalmannsdóttir S, Delgado I, Elgersma A, Fothergill A, Frankow-Lindberg BE, Golisnki P, Grieu P, Gustavsson AM, Höglind M, Huguenin-Elie O, Iliadis C, Jørgensen M, Kadziuliene Z, Karyotis T, Lunnan T, Malengier M, Maltoni S, Meyer V, Nyfeler D,

Nykanen-Kurki P, Parente J, Smit HJ, Thumm U, Connolly J (2007) Evenness drives consitent diversity effects in intensive grassland systems across 28 European sites. J Ecol 95:530-539
Kalaji HM, Bosa K, Kośielniak J, Zuk-Gołaszewska K (2011) Effects of salt stress on photosystem II efficiency and CO_2 assimilation of two Syrian barley landraces. Environ Exp Bot 73:64–72
Kurepin LV, Dahal KP, Savitch LV, Singh J, Bode R, Ivanov AG, Hurry V, Hüner NP (2013) Role of CBFs as Integrators of Chloroplast Redox, Phytochrome and Plant Hormone Signaling during Cold Acclimation. Int J Mol Sci 14:12729–63. doi:10.3390/ijms140612729
Kuzon WM, Urbanchek MG, McCabe S (1996) The seven deadly sins of statistical analysis. Ann Plas Sur 37(3):265–272
Larsen A (1978) Freezing tolerance in grasses. Methods for testing in controlled environments. Scientific Reports of the Agricultural University of Norway. Report nr. 195. Vardingstrykkeri, Sarpsborg Vol. 57, nr. 23
Larsen A (1989) Foredling for overvintringsevne hos engvekster (Breeding for winter survival in forage crops) Norsk landbruksforskning (Norwegian Agricultural Research), Supplement no. 5 1989, pp 75-80
Larsen A, (1994) Breeding winter hardy grasses. Euphytica 77:231-237
Larsen A, Rognli OA(2007) Behov for utvikling av tilpasset plantemateriale i Barents-regionen. Bioforsk FOKUS 2(13):9-13 (in Norwegian)
Larsen A and Marum P (2006) Breeding goals and possibilities in future timothy breeding. In: Sveinsson T. Timothy productivity and forage quality –possibilities and limitations -. NJF Seminar 384. Agricultural University of Iceland. Rit Lbhí nr. 10: p 132
Lawrence T, Cooper JP, Breese EL (1973) Cold tolerance and winter hardiness in *Lolium perenne*: II. Influence of light and temperature during growth and hardening. J Agric Sci 80:341-348
Levitt J (1956) The hardiness of plants. New York: Academic Press p 278
Levitt J (1980) Response of plants to environmental stresses, Vol. 1. Chilling, freezing, and high temperature stresses. New York: Academic Press p 497
Lewandowska-Sabat AM, Winge P, Fjellheim S, Dørum G, Bones AM, Rognli OA (2012) Genome wide transcriptional profiling of acclimation to photoperiod in high-latitude accessions of Arabidopsis thaliana. Plant Sci 185-186:143-155
Li C, Rudi H, Stockinger E, Cheng H, Cao M, Fox S, Mockler T, Westereng B, Fjellheim S, Rognli OA, Sandve S (2012) Comparative analyses reveal potential uses of *Brachypodium distachyon* as a model forcold stress responses in temperate grasses. BMC Plant Biol 12:65
Lotze-Campen H (2011) Climate change, population growth, and crop production: An overview, In: Yadav SS, Redden RJ, Hatfield JL, Lotze-Campen H, Hall AE (eds.).Crop Adaptation to Climate Change, John Wiley & Sons Inc., ISBN:9780813820163
Lunde C, Jensen PE, Haldrup A, Knoetzel J, Scheller HV (2000) The PSI-H subunit of photosystem I is essential for state transitions in plant photosynthesis. Nature 408:613-615
Lyons JM (1973) Chilling injury in plants. Annu Rev Plant Physiol 24:445-460
Maibam P, Nawkar GM, Park JH, Sahi VP, Lee SY, Kang CH (2013) The influence of light quality, circadian rhythm, and photoperiod on the CBF-mediated freezing tolerance. Int J Mol Sci 14:11527–43.doi:10.3390/ijms140611527
Mantyla E, Lang V, Palva ET (1995) Role of absisic acid in drought-induced freezing tolerance, cold acclimation, and accumulation of LT178 and RAB18 proteins in Arabidopsis thaliana. Plant Pysiol 107:141-148.
Marum P, Daugstad K (2009) Grindstad timothy: the landrace that became a major commercial variety. Biodiveristy technical bulletin 15:187-190
Mcgregor K, Porteous C (1994) North Sun '94: Solar Energy at High Latitudes. London: James &James Ltd
McKenzie JS, Weiser CJ, Burke MJ (1974) Effects of red and far red light on the initiation of cold acclimation in *Cornus stolonifera* Michx. Plant Physiol 53:783-789
McKerzie BD, Leshem YY (1994) Stress and stress coping in cultivated plants. The Netherlands: Kluwer Academic Publishers p 180
Merilä J, Hendry AP (2014) Climate change, adaptation and phenotypic plasticity: the problem and the evidence. Evolutionary Applications published by John Wiley & Sons Ltd 7 1–14. doi:10.1111/eva.12137

Miura K, Furumoto T (2013) Cold signaling and cold response in plants. Int J MolSci14:5312-5337
Moriyama M, Abe J, Yoshida M (2003) Etiolated growth in relation to energy reserves and winter survival in three temperate grasses. Euphytica 129:351-360
Nelson RJ, Demas GE, Klein SL and Kriegsfeld LJ (2002) Seasonal patterns of stress, immune functions, and disease. Cambridge: Cambridge University Press p 287
Nicotra AB, Atkin OK, Bonser SP, Davidson AM, Finnegan EJ, Mathesius U, Poot P, Purugganan MD, Richards CL, Valladares F, van Kleunen M (2010) Plant phenotypic plasticity in a changing climate. Trends Plant Sci 15(12):684-692
Nilsen J (1985) Light climate in northern areas.In: Junttila O, Kauren Å (eds.) Plant production in the north. Oslo: Norwegian University Press pp 62-72
Olsen and Junttila (2002) Far-red end of day treatment restores WT like length, cell number and GA content in phytochromeA overexpressing hybrid aspen. Physiol Plant 115:448-457
Olsen JE (2010) Light and temperature sensing and signaling in induction of bud dormancy in woody plants. Plant Mol Biol 73:37–47
Opsahl B (1984) Growth climate, agricultural land, soil and grazing resources in Norway. In: Riley H and Skjelvåg (eds.) The impact of climate on grass production and quality. Proceedings of the 10th general meeting of the European grassland federation, Ås Norway 26-20 June 1984 pp 14-23
Öquist G, Huner NPA(2003) Photosynthesis of overwintering evergreen plants. Annu Rev Plant Biol 54:329-355
Østrem L, Rapacz M, Jørgensen M, Höglind M (2011) Effect of developmental stage on carbohydrate accumulation patterns during winter of timothy and perennial ryegrass. Acta Agric Scand Sect B – Plant Soil Sci 61:153-163
Østrem L, Volden B, Larsen A (2013) Morphology, dry matter yield and phenological characters at different maturity stage of ×Festulolium compared with other grass species. Acta Agr Scand Section B- Plant Soil Sci 63:531-541
Palta JP, Weiss LS, Harbage JF, Bamberg JB, Stone JM (1993) Molecular mechanisms of freeze-thaw injury and cold acclimation in herbaceous plants: Merging physiological and genetic approaches. In: Jackson MB, Black CR (eds.). Interacting stresses on plants in a changing climate. NATO ASI Series, Vol I 16 pp 659-679
Palva ET, Thtiharju S, Tamminen I, Puhakainen T, Laitinen R, Svenson J, Helenius E, Heino P (2002) Biological mechanism of low temperature stress response: Cold acclimation and development of freezing tolerance in plants. In: Iwanaga M (compiled by) Genetic Engineering of Crop Plants for Abiotic Stress JIRCAS Working Report 23, 2002 pp 9-15
Parent C, Capelli N, Berger A, Crèvecoeur M, Dat JF (2008) An overview of plant responses to soil waterlogging. Plant Stress 2(1):20-27
Parry MA, Hawkesford M (2012)An integrated approach to crop genetic improvement. J Integr Plant Biol 54(4):250-259
Parry MAJ, Wang J, Araus JL (2012). New technologies, tools and approaches for improving crop breeding. J Integr Plant Biol 54(4):210-214
Pidwirny M (2006)."Earth-Sun Relationships and Insolation". Fundamentals of Physical Geography, 2nd Edition. Date Viewed. http://www.physicalgeography.net/fundamentals/6i.html
Pollock CJ, Eagles CF (1988) Low Temperature and Growth of Plants. In: Long SP & Woodward FI (eds.). Plants and Temperature, Sym, soc. Exp. Biol. Cambridge: The Company of Biologists Limited p 157-181
Pulli S, Hjortsholm K, Larsen A, Gudleifsson B, Larsson S, Kristinsson B, Hömmö L, Tronsmo AM, Ruuth P, Kristensson C (1996) Development and evaluation of laboratory testing methods for winter hardiness breeding. Publications – Nordic Gene Bank 32 p 68
Quamme HA (1978) Selecting temperate fruit crops for cold hardiness In: Li PH and Sakai S (eds.). Plant cold hardiness and freezing stress - Mechanisms and crop implications. New York: Academic Press pp 313-332
Quamme HA, Chen PM and Gusta LV (1982) Relationship of deep supercooling and dehydration resistance to freezing injury in dormant stem tissue of 'Starkrimson Delicious' apple and 'Siberian C' peach. J Am Soc for Hortic Sci 107:299-304
Rapacz M (1998) The effect of day and night temperatures during early growth of winter rape seedlings on their morphology and cold acclimation responses. Acta Physiol Plant 20:67–72

Rapacz M, (2002) Regulation of frost resistance during cold de-acclimation and re-acclimation in oilseed rape: a possible role of PSII redox state. Physiol Plant115:236–243
Rapacz M, Gąsior D, Zwierzykowski Z, Leśniewska-Bocianowska A, Humphreys HW, Gay AP (2004) Changes in cold tolerance and the mechanisms of acclimation of photosystem II to cold hardening generated by anther culture of *Festucapratensis* × *Loliummultiflorum* cultivars. New Phytol 161:105-114
Rapacz M, Woźniczka A (2009)A selection tool for freezing tolerance in common wheat using the fast chlorophyll a fluorescence transient. Plant Breeding 128:227-234
Rapacz M, Ergon Å, Höglind M, Jørgensen M, Jurczyk B, Østrem L, Rognli OA, Tronsmo AM (2014) Overwintering of herbaceous plants in a changing climate. Still more questions than answers. Plant Sci 225:34-44
Rapacz M., Sasal M, Kalaji HM, Kocielniak J (2015) Is the OJIP test a reliable indicator of winter hardiness and freezing tolerance of common wheat and Triticale under variable winter environments? PLOS ONE, DOI:10.1371/journal.pone.0134820
Rapp K and Junttila O (2000) Heritability estimates of winter hardiness in white clover based on field and laboratory experiments. Acta Agric Scand Sect B, Soil and Plant Sci 50:143-148
Reed TE, Schindler DE, Waples RS (2011) Interacting effects of phenotypic plasticity and evolution on population persistence in a changing climate. Conserv Biol 25(1):56-63
Rognli OA (1987) Genetic variation in arctic populations of timothy (*Phleum pratense* L.) – I. Seed production characters. Hereditas 107:27-54
Rognli OA (2013) Breeding for improved winter survival in forage grasses In: Ryozo I, Yoshida M and Matsumoto N (eds.). Plant and microbe adaptations to cold in a changing world: Proceedings of the plant and microbe adaptation to cold conference, 2012 New York: Springer pp 197-208
Rudi H, Sandve SR, Opseth LM, Larsen A, Rognli OA (2011) Identification of candidate genes important for frost tolerance in *Festuca pratensis* Huds. by transcriptional profiling. Plant Sci 180:78-85
Sakai A, Larcher W (1987) Frost survival of plants. Responses and adaptation to freezing stress. Ecol. Stud. 62, Berlin: Springer-Verlag p 321
Schjelderup I (1973) Plan for innsamling av lokale plantepopulasjoner på Nordkalotten. Nord Jordbruksforsk (2) 1973:223-241 (in Norwegian)
Schjelderup I (1979) Lokale populasjoner av grasarter. Norden 6(83):212-213 (In Norwegian)
Schjeldrup I, Aastveit AH, Aastveit K (1994) Winter hardiness in marginal populations of timothy. Euphytica 77:193-198
Schlichting CD, Wund MA (2014) Phenotypic plasticity and epigenetic marking an assessment of evidence for genetic accommodation. Evolution 68:656-672
Schmidhuber J and Tubiello FN (2007) Global food security under climate change.PNAS 104, 19703-19708.
Sharma S, Upadhvava HD, Varshnev RK, Gowda CLL (2013) Pre-breeding for diversification of primary gene pool and genetic enhancement of grain legumes. Front Plant Sci 4:309 doi:10.3389/fpls.2013.00309
Sjöseth H (1971) Winter hardiness in different meadow and pasture plants. Meldinger fra Norges Landbrukshøgskole 50:1-39
Skillmann JB, Griffin KL, Earll S &Kusama M(2011)Photosynthetic productivity: Can plants do better? In: Moreno-Pirján JC (ed.). Thermodynamics-systems in equilibrium and non-equilibrium. In Tech DOI: 10.5772/20192
Skjelvåg AO (1998) Climatic conditions for crop production in Nordic countries.vAgr food sci Finland 7:149-160
Sleper DA, Poehlman JM (1996) Breeding field crops, fourth edition. Iowa State University Press p 494
Solhaug KA (1991) Long day stimulation of dry matter production in Poa alpine along a latitudinal gradient in Norway. Holarctic Ecol 14:161-168
Stamp P, Visser R (2012) The twenty-first century, the century of plant breeding. Euphytica 186:585-591
Steponkus PL (1984) Role of the plasma membrane in freezing injury and cold acclimation. Annu Rev Plant Physiol 35:543-584

Steponkus PL, Uemura M, Joseph RA, Gilmour SJ, Thomashow MF (1998) Mode of action of the *COR15a* gene on the freezing tolerance of *Arabidopsis thaliana*. Proc Natl Acad Sci USA 95:14570-14575

Stitt M, Hurry V(2002) A plant for all seasons: alterations in photosynthetic carbon metabolism during cold acclimation in *Arabidopsis*. CurrOpin Plant Biol 5:199-206

Strand GH and Bekkhus R (2008) Marklagstatistikk, Dyrka og dyrkbart areal. Ressursoversikt fra Skog og landskap 03/2008. (in Norwegian)

Strand Å, Foyer CH, Gustafsson P, Gardeström P (2003) Altering flux through the sucrose biosynthesis pathway in transgenic *Arabidopsis thaliana* modifies photosynthetic acclimation at low temperatures and the development of freezing tolerance. Plant Cell Environ 26:523-535

Stubsjøen M (1994) Norwegian plant production and its challenges. In: Rognli OA, Solberg E and Schjelderup I. (eds .) Breeding fodder crops for marginal conditions. Proceedings of the 18thEucarpia Fodder Crops section Meeting, Loen, Norway 25-28 August 1993. Kluwer Academic Publishers pp 3-10.

Szalai G, Pap M, Janda T (2009) Light-induced frost tolerance differs in winter and spring wheat plants. J Plant Physiol 166 (11):1826-1831

Taulavuori K (2013) Vegetation at northern high latitudes under global warming. In: Dincer I, Colpan CO and Kadioglu F (eds.).Causes, impacts and solutions to global warming. New York: Springer pp 3-16

Tester M and Bacic A (2005) Abiotic stress tolerance in grasses. From model plants to crop plants. Plant Physiol 137:791-793

Thompson GA Jr (1989) Molecular changes in membrane lipids during cold stress. In: Cherry JH (ed.). Environmental stress in plants, biochemical and physiological mechanisms, NATO ASI Series, Berlin, Heidelberg: Springer Verlag pp 249-259

Tikkanen M, Nageswara RM, Aro EM (2014) Photosystem II photoinhibition-repair cycle protects photosystem I from irreversible damage. Biochim Biophys Acta 1837: 210-215

Thomashow MF (1999) Plant cold acclimation: freezing tolerance genes and regulatory mechanisms. Annu Rev Plant Phys 50:571-599

Thomashow MF (2010) Molecular basis of plant cold acclimation: Insights gained from studying the CBF cold response pathway. Plant Physiol 154:571-577

Thorsen SM and Höglind M (2010) Assessing winter survival of forage grasses in Norway under future climate scenarios by simulating potential frost tolerance in combination with simple agroclimatic indices. Agr Forest Meteorol 150:1272-1282

Þorvaldsson G, Østrem L, Öhlund L, Sveinsson Þ, Dalmannsdóttir S, Djurhuus R, Høegh K, Kristjánsdóttir Þ (2015) Climatic adaptation of species and varieties of grass and clover in the West Nordic countries and Sweden. LbhI report. ISSN 1670-5785 pp 43

Taylor NL, Quesenberry KH (1996) Red clover science. Springer Science + Business Media, Dordrecht

Thuiller W, Lavorel S, Araújo MB, Sykes MT, Perntice IC (2005) Climate change threats to plant diversity in Europe. Proc Natl Acad Sci USA 102(23):8245-8250

Tronsmo AM (1986) Winter injury in agricultural crops caused by low temperature fungi. NJF-Seminar no. 84:81-97

Uemura M, Yoshida S (1984) Involvement of plasma membrane alterations in cold acclimation of winter rye seedlings (Secale cereal L. cv Puma). Plant physiol 75:818-826

Uemura M, Joseph RA, Steponkus PL (1995) Cold acclimation of Arabidopsis thaliana – Effect on plasmamembrane lipid composisition and freeze-induced lesions. Plant Physiol 109:15-30

Uleberg E, Hanssen-Bauer I, van Ort B, Dalmannsdottir S (2014) Impact of climate change on agriculture in Northern Norway and potential strategies for adaptation. Climatic Change 122:27-39

Waalen WM, Tanino KK, Olsen JE, Eltun R, Rognli OA and Gusta LV (2011) Freezing tolerance of winter canola cultivars is best revealed by a prolonged freeze test. Crop Sci 51:1988-1996

Walters RG, Horton P (1994) Acclimation of Arabidopsis thaliana to light environment: changes in composition of the photosynthetic apparatus. Planta 195:248-256

Weiser CJ (1970) Cold resistance and injury in woody plants. Science 169:1269-1278

Wilkins PW (1991) Breeding perennial ryegrass for agriculture. Euphytica 52:201-214

Wingler A (2015) Comparison of signaling interactions determining annual and perennial plant growth in response to low temperature. Front Plant Sci 5:794. doi: 10.3389/fpls.2014.00794

Xin Z and Browse J (2000) Cold comfort farm: the acclimation of plants to freezing temperatures. Plant Cell Environ 23:893-902

Zhang C, Fei S, Warnke S, Li L and Hannapel D (2009) Identification of genes associated with cold acclimation in perennial ryegrass. J Plant Physiol 166:1436-1445

Årsvoll K (1975) Fungi causing winter damage on cultivated grasses in Norway. Meld. Norg.Landbr.Høgsk. 54 (9) p 49

Paper I

Hypothesis

I. An increasing pre-acclimation temperature delays growth cessation, disturbs photoacclimation and finally reduces freezing tolerance.

II. The processes are related to the different latitudinal adaptation of the plant material, expecting that northern-adapted populations are more affected by temperature than southern-adapted populations.

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CHILLING/FREEZING STRESS

Temperature Before Cold Acclimation Affects Cold Tolerance and Photoacclimation in Timothy (*Phleum pratense* L.), Perennial Ryegrass (*Lolium perenne* L.) and Red Clover (*Trifolium pratense* L.)

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Keywords
chlorophyll fluorescence; climate change; cold acclimation; freezing tolerance; geographically adapted populations; leaf elongation

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Abstract

The expected temperature rise in late summer/early autumn can change the conditions for acclimation and affect the winter survival of perennial crops. This study examined the effect of the temperature just before the onset of cold acclimation (pre-acclimation) on freezing tolerance of timothy (*Phleum pratense* L.), perennial ryegrass (*Lolium perenne* L.) and red clover (*Trifolium pratense* L.) populations (both cultivars and breeding populations) adapted to either northern or southern parts of Norway. Using phytotron experiments, we studied whether increasing pre-acclimation temperature delays growth cessation, affects photoacclimation and reduces freezing tolerance. Furthermore, we assessed whether these effects were related to the latitudinal adaptation of the plant material. The results showed that a rise in pre-acclimation temperature decreased both cold acclimation capacity and photoacclimation in these species. This affected the freezing tolerance, which was reduced significantly more in northern-adapted population of timothy and perennial ryegrass compared with southern-adapted populations. Red clover was less affected by temperature changes than the grasses.

Introduction

Current climate change models predict that the temperature increase due to global warming will be greatest at higher northern latitudes (IPCC 2013). This may open up new possibilities for forage crop production in these areas, where low temperature and a short growing season are limiting factors for agriculture (Uleberg et al. 2014). However, higher autumn and winter temperatures, combined with the natural short photoperiod in the north, may intensify or pose new types of winter stresses for perennial forage crops.

Herbage plants are exposed to a multitude of winter stresses, for example anoxia caused by ice encasement, snow mould, desiccation and frost. The ability of a plant to withstand the combined effects of these stresses determines its winter hardiness. However, frost is considered the most significant winter stress and freezing tolerance is often used as a measure of winter hardiness (Larsen 1994, Rapp and Junttila 2000, Rognli 2013). Freezing tolerance develops when plants are exposed to low positive temperatures through a process called cold acclimation or hardening (Levitt 1980, Thomashow 1999). Cold acclimation is

induced primarily by non-freezing temperatures below +10 °C (Eagles et al. 1997). Nevertheless, Rapacz (1998a) reported that temperatures in the range of +10 to +15 °C combined with relatively high irradiance during late summer/early autumn increased freezing tolerance and they are referred to as pre-acclimation temperatures. Studies have revealed that the growth rate decreases and the photosynthetic apparatus acclimates to cold (photoacclimation) during pre-acclimation (Rapacz 1998b, Rapacz and Janowiak 1998). Photosynthetic acclimation to cold is considered the primary target for cold acclimation (Hüner et al. 1993, Crosatti et al. 2013). Growth cessation in late summer is considered a prerequisite for cold acclimation in woody species (Kalcsits et al. 2009). Less is known about the control of growth cessation in herbaceous species (Rapacz et al. 2014), but recent studies on acclimation of grasses show that leaf elongation rate and plant height are affected by temperature–photoperiod interactions (Malyshev et al. 2014, Østrem et al. 2014). Dark reactions of photosynthesis are highly temperature-dependent. Photosynthetic activity may thus be inhibited when the relative amount of absorbed photochemical energy is higher than the energy demand for the final products of photochemical reactions (ATP, NADPH). Such an imbalance in photochemical energy can cause damages to PSII, resulting in a cold-induced photoinhibition of photosynthesis. Well-adapted plants are able to acclimate to such conditions (photoacclimation) and maintain high photosynthetic activity (Hüner et al. 2013). During photoacclimation, plants either increase excess energy dissipation as heat (non-photochemical energy quenching) or increase energy utilisation (photochemical quenching) (Hüner et al. 2012). Cold-induced photoacclimation seems to be closely related to freezing tolerance (Rapacz et al. 2004). On this background, it is very likely that higher temperatures at the end of the growing season may delay growth cessation, reduce cold acclimation capacity, and reduce freezing tolerance.

Research on how forage crops respond to the temperature changes at higher latitudes is scarce. Timothy (*Phleum pratense* L.) is the most common forage species at higher latitudes due to its excellent winter survival. However, it seems like the current northern-adapted cultivars are at disadvantage in the current climate with longer growing seasons, and are gradually being replaced by more southern-adapted cultivars (Marum and Daugstad 2009, Østrem et al. 2013). In temperate regions, perennial ryegrass (*Lolium perenne* L.) is the most widely used perennial forage crop due to its high nutritive value and digestibility, but it is of limited use at higher latitudes because of its poor winter survival. Red clover (*Trifolium pratense* L.) cultivars with good persistency in high-latitude conditions are also lacking (Uleberg et al. 2014). Higher temperature and a longer growing season in the north due to climate change may provide possibilities for increased use of perennial ryegrass and red clover. However, simulation modelling indicates a considerable shortening of the acclimation period for perennial grasses during the coming decades (Thorsen and Höglind 2010). Increasing autumn temperature may delay and reduce the cold acclimation of perennial forage species, making them less tolerant to winter stresses.

The aim of this study was to investigate the physiology of climate adaptation as a basis for breeding new cultivars of grasses and clover for future climate conditions at high northern latitudes. We compared photoacclimation, growth rates and freezing tolerance of promising breeding populations of timothy, perennial ryegrass and red clover in different scenarios of increasing temperatures in autumn. Our hypotheses were that: (1) increasing pre-acclimation temperature delays cessation of leaf and petiole elongation, disturbs photoacclimation, and reduces freezing tolerance, and (2) these processes are related to the latitudinal adaptation of the plant material and therefore northern-adapted populations are more affected by increasing autumn temperature than southern-adapted.

Materials and Methods

Plant material and growing conditions

Three forage crop species, timothy, perennial ryegrass and red clover, were studied, each represented by a southern- and a northern-adapted breeding population or cultivar, referred to as populations for the sake of brevity (Table 1). Adaptation relates to Norwegian growing conditions. The perennial ryegrass populations are described by Østrem et al. (2014). The southern-adapted timothy population was a mixture of the south-east Norwegian cultivar Grindstad and the breeding population MTL9701, the latter originating from a cross, conducted in the south-east of Norway, between 12 cultivars listed for use in the Nordic countries, United Kingdom, Netherland, Belgium, Germany and Czech Republic. The northern-adapted timothy population MTV0508-3 originates from MTL9701 and was selected for persistency for one generation at the southern highland location Løken, Valdres, Norway (59.80°N, 11.46°E), and two generations at the northern costal location Vågønes, Bodø, Norway (67.31°N, 14.55°E). Each selection cycle consisted of an establishing year, two harvest years under local agricultural management practices and seed harvest of surviving plants in the third ley year. The southern-adapted red clover population was a mixture of the two tetraploid candivars (i.e. new promising candidate cultivars) LøRk0393 and LøRk0395 selected at Løken, Norway, from Eastern European red clover, mainly from the Plant Breeding Station Hladke Zivotice (DLF-Trifolium,

Table 1 Breeding populations and cultivars adapted to northern or southern regions of Norway used in the experiments

Species	Adaptation	Population name	Ploidy
Perennial ryegrass	Southern	FuRa9805	Diploid
	Northern	Fagerlin	Diploid
Timothy	Southern	MTL9701 + Grindstad	Diploid
	Northern	MTV0508-3	Diploid
Red clover	Southern	LøRk0393/0395	Tetraploid
	Northern	B1D2 + D3 + VåRk0734	Diploid

Czech Republic). The northern-adapted red clover population originated from selected northern Fennoscandian diploid populations intercrossed and subjected to two cycles of different management (fertilizer and harvesting regimes) selection at Vågønes, Bodø, as described for the northern-adapted timothy population. The experiments were conducted in phytotron compartments with the temperature controlled to ±0.5 °C and the air humidity corresponding to a water vapour deficit of 0.5 kPa. Seedlings were planted in tree nursery trays (60 pots in each tray, 1 plant/pot, pot size 40 mm diameter × 85 mm height, 45 mm spacing between plants). The pots were filled with fertilised sphagnum peat and perlite (3 : 1). During establishment, the plants were grown for 4 weeks at 20 °C, 24-h photoperiod. The light source was cool white fluorescent lamps (Philips TLD 58W 840), giving 150 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) at plant level (measured with a quantum sensor, Li-1000, Li-Cor) within the range 400–750 nm. During establishment and throughout the experiment, the plants were watered regularly and fertilised as required with a complete nutrient solution (Hoagland solution, modified from Asher 1978).

Treatments

When grass plants reached the five-leaf stage and red clover the two-leaf stage, they were distributed to three constant pre-acclimation temperatures (9, 12 and 15 °C) and allowed to grow for 5 weeks prior to cold acclimation; 2 weeks at 14 h followed by 3 weeks at 12-h photoperiod. Finally, all plants were cold acclimated at 2 °C and 12-h photoperiod for 3 weeks. Plants were grown under artificial light conditions (PPFD 150 μmol m^{-2} s^{-1}) during the different temperature treatments, and at 2 °C, the PPFD was 70 μmol m^{-2} s^{-1}. The temperature treatments represent the pre-acclimation temperature in northern Norway in August/early September before the actual hardening takes place. The temperature levels correspond to current (9 °C) (normal 1961–1990) and future scenarios until 2050 (12 °C) (Uleberg et al. 2014), while 15 °C represents an extreme temperature increase.

Morphological measurements

Dry weight of aboveground biomass was recorded at the end of the experiment on 15 individual plants/population/treatment (total number of 90 plants per temperature–treatment combination) after drying at 60 °C for 48 h. In grasses, leaf elongation (mm $week^{-1}$) was measured on 15 plants per treatment. One leaf per plant was marked with a thin, plastic-coated wire and measured weekly during the 5 weeks. Similarly, the elongation of the petiole was measured in red clover. The number of leaves was counted on three occasions, when treatments commenced, and before and after cold acclimation at 2 °C. When plants were moved to cold acclimation at 2 °C, new young leaves were marked. The number of leaves was counted on the same individuals as used for measuring for leaf and petiole elongation.

Chlorophyll fluorescence measurements

The photochemical activity of photosystem II (PSII) was studied by measuring chlorophyll fluorescence (PAM-2500 Portable Chlorophyll Fluorometer; Heinz Walz, Effeltrich, Germany) at room temperature on 15 plants at the start of treatments and on 15 plants/population/treatment before and after cold acclimation. These measurements were taken on the same plants used to record leaf and petiole elongation. The measurements were made on the mid-section of the youngest fully expanded leaves, one measurement per leaf constituting a replicate. Before measuring maximum quantum yield of PSII (F_v/F_m), leaves were dark-adapted for 15–60 min in leaf clips (8 mm diameter, Walz) and values of F'_m and F_s were recorded when F_s became stable after re-exposure to light (800 μmol). Within the same leaf clip, F'_0 was measured after far-red light treatment to ensure rapid opening of PSII reaction centres. Current quantum yield of PSII (ϕ_{PSII}) and coefficient of the photochemical (q_p) and non-photochemical (NPQ) quenching of chlorophyll fluorescence were calculated according to Genty et al. (1989), Schreiber et al. (1994) and Bilger and Björkman (1991), respectively.

Freezing tests

Freezing tests were performed at the end of the experiment as described by Pulli et al. (1996) with modifications (Höglind et al. 2010). Plant roots were washed and single plants were trimmed to 3 cm top and 1–2 cm root, with care taken not to destroy the taproot in the case of red clover. The crown segments were placed in plastic boxes covered with fine, humid sand in a programmed freezer with a temperature sensor in each box. The boxes were exposed to predetermined freezing temperatures between −14 °C and

−27 °C for grasses and −6 °C and −16 °C for clover. The temperature was lowered from 2 °C to −3 °C by 1 °C h^{-1} and kept at −3 °C for 13 h to avoid supercooling. Thereafter, the temperature was lowered by 1 °C h^{-1} to −10 °C and by 3 °C h^{-1} until the pre-set temperature was reached for each treatment. Boxes were removed from the freezer at intervals of 2 °C for each of the six temperatures tested. There were two replicate boxes per predetermined test temperature, each containing 10 crown segments per population for each temperature treatment (a total number of 180 plants per box and a total of 720 plants per treatment were used to determine the freezing tolerance). Two boxes per treatment were kept at 2 °C (and in darkness) as a control. The boxes were placed at 2 °C overnight to thaw, and the crown segments were transplanted into fertilised peat with perlite. LT_{50} value, that is the temperature at which 50 % of plants are killed, was estimated based on scoring the regrowth of the plants after 3–4 weeks of growth at 20 °C and 24-h light. Survival of individual plants was rated as dead or alive.

Statistical analysis

A generalised linear model (GLM) approach was used to estimate the effects of pre-acclimation temperature on photochemical activity, biomass production, number of leaves and freezing tolerance. Within species, second- and third-order interaction terms were implemented in the model to evaluate whether northern- and southern-adapted populations responded differently to temperature. As interactions incorporate collinearity in the models, selecting models based on significance of the parameter is not recommended (Kleinbaum et al. 1998). We therefore used model selection based on the Akaike's information criteria corrected for small sample sizes (AICc) to rank models, where the model with lowest AICc value was considered best (Burnham and Anderson 2002). For leaf number and biomass production, a linear model with Gaussian normal distribution and an identity link was assumed. The full model was defined as *Response ~ Treatment*Population*Species,* where Treatment is given as 9, 12 or 15 °C (categorical variable), and Population as northern or southern (categorical) (See Tables S2–S5). For photochemical activity, *series* (start, after pre-acclimation, or after cold acclimation, categorical variable) was included in the full model in addition (See Tables S6–S13). Eleven entries were removed as diagnostic plots indicated them as outliers. Data were log-transformed when residual plots indicated heterogeneity of variance. However, for clarity, we present the untransformed mean values here. For the freezing test, a logistic regression-like model (GLM) assuming binomial distribution (0 = dead, 1 = alive) and a logit link was used. In some of the models, there was no overlap of freezing temperature for the group of dead vs. surviving individuals; hence, a penalised likelihood approach on separate species was used to remove bias (library brglm in R) (Kosmidis and Firth 2009). The full model for each species was defined as *Response ~ Treatment*Population*Pre-determined freezing temperature* (See Tables S14–S17). We used a nonlinear, three-parameter asymptotic mixed model approach for elongation growth (function nlmer in R library lme4). The growth trajectory is described by the function $y \sim Asym + (R_0\text{-}Asym)e^{-e(lrc \times week)}$, where parameters describe the intercept (R_0), the asymptote (*Asym*) and the logistic rate constant (*lrc*) (Crawley 2007) (See Table S1). Individual plant identity was initially included as a random term for all three parameters to avoid pseudoreplication, but was stepwise removed when variance equalled zero or to ensure convergence of the models. To evaluate differences in leaf and petiole elongation rate, overlaps in the 95 % confidence intervals were compared. For nonlinear mixed models, the confidence interval was approximated by mean ± 2 × SE (Gelman and Hill 2008). Predictors were considered significant if their 95 % confidence interval did not include zero. All statistical analyses were performed using R (R version 3.0.1) and Minitab 16 (Minitab Inc., 2010, State College, PA, USA).

Results

Morphology

In general, leaves and petioles elongated most during the first 2 weeks at all pre-acclimation temperatures before growth gradually declined until leaves reached maturity (Fig. 1). However, in grasses, the decline was mainly significant at low temperatures (Table 2). The leaf elongation of perennial ryegrass was on average three-fold higher than that of timothy during the first weeks (Fig. 1). Leaves and petioles of northern-adapted populations elongated more at higher temperatures (12 °C and 15 °C) than at 9 °C during the first 1–2 weeks (Fig. 1, Table 2). However, this difference was significant only between 9 °C and 15 °C in the northern-adapted population of red clover (Table 2). In the southern-adapted populations of red clover and timothy, there were no significant differences between temperatures, but leaf elongation of southern-adapted perennial ryegrass responded to temperature similarly as the northern-adapted population (Fig. 1, Table S1).

In general, plants grown at 9° C produced less biomass than plants grown at higher temperatures. This effect was significant in timothy (both populations) and red clover (northern population) (Table 3). In general, differences in biomass production between the different populations were not related to their latitudinal origin. The only significant difference was between the red clover populations at 9 °C, where southern-adapted had more biomass, and the

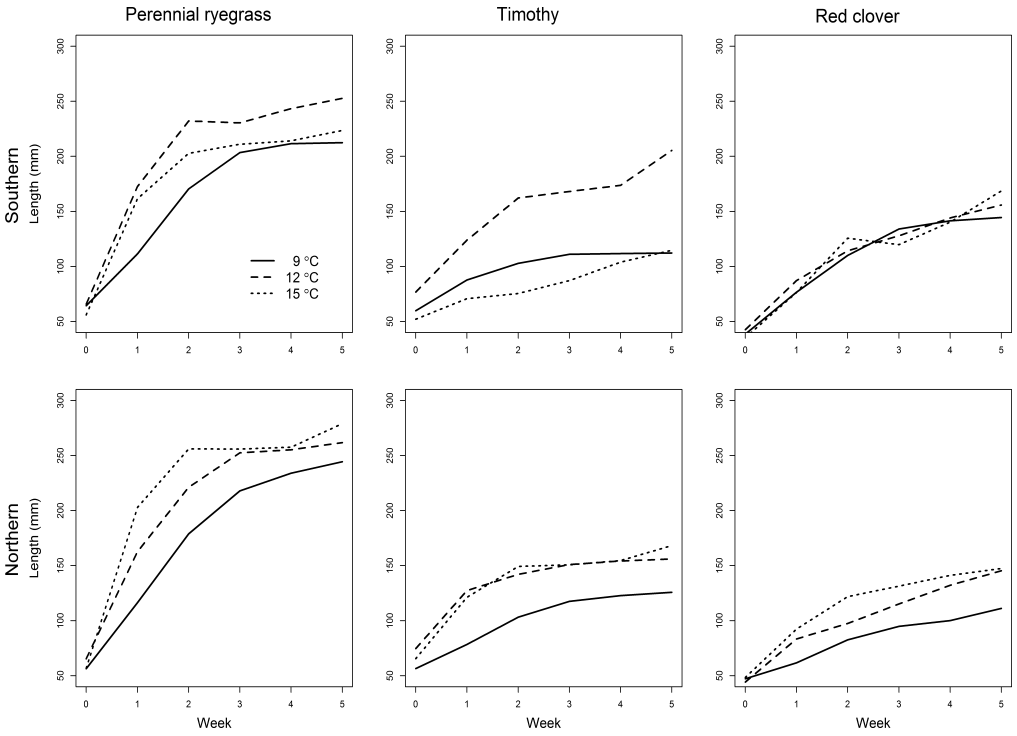

Fig. 1 Elongation growth (mm) of leaves (grasses) and petioles (red clover). Mean of weekly measurements during pre-acclimation treatment at 9 °C, 12 °C and 15 °C.

perennial ryegrass populations at 12 °C where the southern-adapted had less biomass (Table 3, Table S3). Plants produced significantly more leaves at 12 °C and 15 °C than at 9 °C (Table 3), except in the southern-adapted population of red clover where there were no differences. The northern-adapted red clover and perennial ryegrass populations produced more leaves at higher temperatures (12 °C and 15 °C) than the southern-adapted. No new leaves appeared during cold acclimation at 2 °C (data not shown).

Chlorophyll fluorescence

The F_v/F_m values increased during the pre-acclimation treatment in perennial ryegrass, indicating photoacclimation, and this increase was strongest at 9 °C (Tables 4 and S10). The same tendency was observed in timothy, although not statistically significant (Table S10). In both grass species, F_v/F_m values were slightly higher at 9 °C than at 15 °C. During cold acclimation at 2 °C, there were no further changes in this parameter (Table 4). Northern- and southern-adapted populations had similar F_v/F_m values. The only exception was a lower start value of the southern-adapted population of perennial ryegrass compared to the northern. In addition, the southern-adapted population of perennial ryegrass had lower F_v/F_m values at 15 °C than at 9 °C both after the pre-acclimation treatment and after cold acclimation, while this difference was not significant in the northern-adapted population. In contrast with the grasses, the F_v/F_m values did not increase during pre-acclimation in red clover (Table S10). There was a significant increase in non-photochemical light energy quenching coefficient (NPQ) in all populations, except for the northern-adapted population of perennial ryegrass and southern-adapted population of red clover, after pre-acclimation treatments at 9 °C (Table 4). In both populations of perennial ryegrass and the southern-adapted population of red clover, the PSII photochemical activity (ϕ_{PSII}) was lower after pre-acclimation at 15 °C than at 9 °C. In the northern-adapted population of perennial ryegrass and the red clover populations, ϕ_{PSII} was reduced after pre-acclimation at 15 °C compared to the starting values. Differences observed for the photochemical quenching coefficient (q_p) were similar to those observed for ϕ_{PSII}.

Table 2 Influence of pre-acclimation temperature on elongation growth of leaves (grasses) and petioles (clover), estimated by a nonlinear asymptotic model. Elongation growth is expressed as estimated average logistic rate constants (lrc, in bold) with confidence intervals. n = 270 individuals measured at five dates

Species (origin)	Temperature treatment (°C)	lrc estimate	95 % Confidence interval Lower	Upper
Perennial ryegrass (southern)	9	**−0.84**	−1.04	−0.64
	12	**−0.08**	−0.51	0.36
	15	**−0.02**	−0.31	0.26
Perennial ryegrass (northern)	9	**−0.94**	−1.21	−0.67
	12	**−0.16**	−0.30	−0.02
	15	**0.10**	−0.11	0.32
Timothy (southern)	9	**−0.27**	−1.87	1.32
	12	**−0.16**	−0.45	0.12
	15	**−0.22**	−0.67	0.23
Timothy (northern)	9	**−1.01**	−1.34	−0.69
	12	**−0.01**	−0.39	0.38
	15	**−0.15**	−0.48	0.17
Red clover (southern)	9	**−1.37**	−1.78	−0.95
	12	**−1.56**	−2.25	−0.87
	15	**−1.27**	−2.09	−0.45
Red clover (northern)	9	**−1.33**	−1.69	−0.97
	12	**−0.87**	−1.33	−0.41
	15	**−0.43**	−0.65	−0.22

Freezing tolerance

In general, higher pre-acclimation temperatures reduced freezing tolerance in all population compared with lower temperatures (Fig. 2, Table S17). However, the LT_{50} values were not significantly different between the temperature treatments neither in the southern-adapted populations of timothy nor in the northern-adapted population of red clover (Table 5). The northern-adapted populations generally had higher freezing tolerance than the southern-adapted, but freezing tolerance was more strongly reduced by higher pre-acclimation temperature in northern-adapted populations of timothy and perennial ryegrass compared with southern-adapted (Fig. 2, Table S17). In red clover, there was only an effect of pre-acclimation temperature on southern-adapted population, where freezing tolerance was significantly lower after treatment at 15 °C than at 12 °C and 9 °C (Fig. 2, Table S17). The LT_{50} values for the northern-adapted population of perennial ryegrass at 9 °C and southern-adapted timothy at 15 °C were not reached at the test temperatures used in this study.

Discussion

The main findings of this study are that increasing pre-acclimation temperatures increased leaf and petiole elongation rates and reduced freezing tolerance of the populations. Furthermore, photoacclimation occurred in the grass species during pre-acclimation at lower temperature.

The freezing tolerance of plants pre-acclimated at 9 °C was generally higher compared to those at 12 °C and 15 °C, and this confirms the positive effect of low temperature on cold acclimation (Junttila 1996). Northern-adapted populations had higher freezing tolerance than southern-adapted, which confirms the latitudinal adaptation of this breeding material, as plants of northern origin generally reach a higher level of maximum hardiness than the corresponding southern genotypes (Junttila 1996). Under field conditions, northern-adapted populations of timothy harden earlier in autumn and maintain a higher freezing tolerance when de-hardening occurs during late winter than southern-adapted populations (Larsen 1994). A similar response has been shown in perennial ryegrass (Eagles and Williams 1992) and is most likely controlled by temperature–photoperiod interactions (Rapacz et al. 2014).

Nevertheless, the northern-adapted populations of grasses had relatively higher reduction of freezing tolerance than the southern-adapted populations when grown at 12° or 15 °C, compared to the 9 °C treatment. A similar response to temperature change has been reported in a

Table 3 Mean (±standard error) number of new leaves per plant produced during temperature treatment (9 °C, 12 °C, 15 °C) and total biomass of plants at the end of experiment (mg dw plant^{-1}) for northern- and southern-adapted populations of perennial ryegrass (PRG), timothy and red clover. Values within rows with different superscripts are significantly different according to Tukey's test ($P < 0.05$). n = 270

	Number of leaves			Biomass (mg dw plant^{-1})		
	9 °C	12 °C	15 °C	9 °C	12 °C	15 °C
PRG southern	$9.7^{a} \pm 1.0$	$12.7^{ab} \pm 1.6$	$15.3^{b} \pm 1.8$	$470^{a} \pm 33$	$409^{a} \pm 37$	$538^{a} \pm 56$
PRG northern	$13.6^{a} \pm 1.4$	$24.3^{b} \pm 2.3$	$21.0^{b} \pm 2.1$	$494^{a} \pm 48$	$708^{b} \pm 58$	$660^{ab} \pm 49$
Timothy southern	$11.5^{a} \pm 0.7$	$12.9^{a} \pm 1.2$	$18.1^{b} \pm 1.8$	$342^{a} \pm 32$	$561^{b} \pm 38$	$508^{b} \pm 42$
Timothy northern	$9.9^{a} \pm 0.8$	$16.5^{b} \pm 1.6$	$12.5^{ab} \pm 1.7$	$349^{a} \pm 26$	$450^{ab} \pm 38$	$486^{b} \pm 38$
Red clover southern	$4.1^{a} \pm 0.7$	$2.4^{a} \pm 0.7$	$2.1^{a} \pm 0.7$	$441^{ab} \pm 45$	$391^{a} \pm 46$	$583^{b} \pm 56$
Red clover northern	$2.6^{a} \pm 0.4$	$4.1^{a} \pm 0.8$	$9.7^{b} \pm 0.9$	$262^{a} \pm 28$	$484^{b} \pm 64$	$433^{b} \pm 33$

Table 4 Changes in fluorescence parameters in southern- and northern-adapted populations of perennial ryegrass, timothy and red clover measured three times during the experiment; at the start, after pre-acclimation (PA) and after cold acclimation (CA). Estimated mean values (in bold) with 95 % confidence intervals (low–high). n = 799

			Perennial ryegrass		Timothy		Red clover	
		Temp. Treatment	Southern	Northern	Southern	Northern	Southern	Northern
F_v/F_m	Start		**0.643** (0.634–0.652)	**0.703** (0.693–0.713)	**0.722** (0.712–0.731)	**0.711** (0.701–0.720)	**0.779** (0.770–0.789)	**0.780** (0.771–0.790)
	After PA	9 °C	**0.739** (0.730–0.748)	**0.733** (0.723–0.743)	**0.739** (0.730–0.749)	**0.730** (0.720–0.740)	**0.765** (0.756–0.775)	**0.767** (0.757–0.776)
		12 °C	**0.735** (0.725–0.746)	**0.734** (0.724–0.744)	**0.736** (0.726–0.745)	**0.733** (0.724–0.743)	**0.772** (0.763–0.782)	**0.771** (0.762–0.781)
		15 °C	**0.722** (0.712–0.731)	**0.725** (0.716–0.735)	**0.727** (0.716–0.737)	**0.715** (0.705–0.725)	**0.773** (0.764–0.783)	**0.762** (0.752–.0772)
	After CA	9 °C	**0.738** (0.729–0.747)	**0.731** (0.721–0.741)	**0.737** (0.728–0.746)	**0.738** (0.728–0.747)	**0.770** (0.761–0.779)	**0.765** (0.755–0.774)
		12 °C	**0.730** (0.720–0.740)	**0.721** (0.712–0.731)	**0.733** (0.723–0.743)	**0.730** (0.719–0.740)	**0.771** (0.760–0.781)	**0.758** (0.749–0.768)
		15 °C	**0.714** (0.705–0.723)	**0.723** (0.713–0.732)	**0.721** (0.711–0.731)	**0.716** (0.706–0.726)	**0.777** (0.767–0.786)	**0.759** (0.749–0.768)
NPQ	Start		**1.308** (1.181–1.434)	**1.338** (1.207–.1469)	**1.653** (1.522–1.783)	**1.597** (1.466–1.728)	**1.450** (1.324–1.576)	**1.373** (1.247–1.499)
	After PA	9 °C	**1.733** (1.606–1.859)	**1.565** (1.434–1.696)	**1.925** (1.798–2.051)	**1.858** (1.727–1.989)	**1.586** (1.460–1.713)	**1.652** (1.526–1.778)
		12 °C	**1.523** (1.388–1.659)	**1.489** (1.473–1.734)	**1.611** (1.480–1.742)	**1.491** (1.361–1.622)	**1.668** (1.541–1.794)	**1.435** (1.304–1.566)
		15 °C	**1.435** (1.309–1.562)	**1.455** (1.314–1.566)	**1.567** (1.426–1.709)	**1.587** (1.456–1.718)	**1.764** (1.638–1.890)	**1.286** (1.156–1.417)
	After CA	9 °C	**1.541** (1.415–1.668)	**1.508** (1.377–1.639)	**1.958** (1.832–2.085)	**1.848** (1.717–1.978)	**1.758** (1.632–1.884)	**1.548** (1.421–1.674)
		12 °C	**1.617** (1.482–1.753)	**1.402** (1.276–1.529)	**1.815** (1.685–1.946)	**1.759** (1.618–1.900)	**1.635** (1.499–1.770)	**1.661** (1.534–1.787)
		15 °C	**1.613** (1.487–1.740)	**1.535** (1.441–1.662)	**1.704** (1.568–1.840)	**1.959** (1.828–2.089)	**1.708** (1.582–1.834)	**1.491** (1.365–1.618)
ϕ_{PSII}	Start		**0.133** (0.105–0.160)	**0.143** (0.114–0.171)	**0.157** (0.128–0.185)	**0.126** (0.098–0.155)	**0.311** (0.283–0.338)	**0.345** (0.318–.0373)
	After PA	9 °C	**0.142** (0.115–0.170)	**0.154** (0.125–0.183)	**0.103** (0.076–0.131)	**0.093** (0.065–0.122)	**0.298** (0.271–0.326)	**0.266** (0.239–0.294)
		12 °C	**0.129** (0.100–0.159)	**0.124** (0.095–0.152)	**0.139** (0.111–0.168)	**0.116** (0.087–0.144)	**0.226** (0.198–0.253)	**0.320** (0.291–.0348)
		15 °C	**0.088** (0.060–0.115)	**0.084** (0.057–.0112)	**0.103** (0.072–0.134)	**0.072** (0.044–0.101)	**0.204** (0.177–0.232)	**0.268** (0.240–0.297)
	After CA	9 °C	**0.114** (0.087–0.142)	**0.082** (0.053–0.110)	**0.088** (0.061–0.116)	**0.109** (0.080–0.137)	**0.243** (0.215–0.270)	**0.237** (0.210–0.265)
		12 °C	**0.085** (0.056–0.115)	**0.079** (0.052–0.107)	**0.106** (0.078–0.135)	**0.092** (0.061–0.122)	**0.277** (0.248–0.307)	**0.209** (0.182–0.237)
		15 °C	**0.079** (0.052–0.107)	**0.068** (0.041–0.096)	**0.070** (0.040–0.099)	**0.069** (0.040–0.097)	**0.228** (0.201–0.256)	**0.246** (0.218–0.274)
q_p	Start		**0.378** (0.326–0.430)	**0.343** (0.289–0.396)	**0.390** (0.336–0.444)	**0.337** (0.283–.0390)	**0.582** (0.530–0.634)	**0.636** (0.584–0.688)
	After PA	9 °C	**0.341** (0.289–0.393)	**0.365** (0.311–0.419)	**0.275** (0.223–0.327)	**0.240** (0.186–0.294)	**0.594** (0.543–0.646)	**0.545** (0.494–0.597)
		12 °C	**0.296** (0.241–0.352)	**0.287** (0.233–0.341)	**0.320** (0.266–0.374)	**0.267** (0.213–0.320)	**0.437** (0.386–0.489)	**0.595** (0.541–0.649)
		15 °C	**0.207** (0.155–0.259)	**0.200** (0.148–0.251)	**0.249** (0.191–0.307)	**0.185** (0.132–0.239)	**0.424** (0.372–0.476)	**0.504** (0.451–0.558)
	After CA	9 °C	**0.256** (0.204–0.308)	**0.187** (0.134–0.241)	**0.226** (0.175–0.278)	**0.273** (0.219–0.327)	**0.484** (0.432–0.536)	**0.463** (0.411–0.515)
		12 °C	**0.205** (0.150–0.261)	**0.188** (0.137–0.240)	**0.261** (0.207–0.314)	**0.228** (0.170–0.286)	**0.542** (0.486–0.597)	**0.463** (0.371–0.475)
		15 °C	**0.209** (0.158–0.393)	**0.165** (0.113–0.216)	**0.180** (0.124–0.236)	**0.194** (0.140–0.247)	**0.452** (0.401–0.504)	**0.478** (0.427–0.530)

Table 5 Freezing tolerance as LT_{50} value (temperature with 50 % survival, in bold) and 95 % confidence interval for the populations tested

Species (origin)	Temperature treatment (°C)	LT_{50} (°C)	95 % Confidence interval	
			Lower	Upper
Perennial ryegrass (southern)	9	**−15.8**	−15.4	−16.2
	12	**−15.6**	−15.6	−15.7
	15	**−15.0**	−14.5	−15.5
Perennial ryegrass (northern)	9	**−17.9***	−15.2	
	12	**−15.5**	−15.1	−16.0
	15	**−14.0**	−13.2	−14.8
Timothy (southern)	9	**−14.7**	−13.4	−16.1
	12	**−14.7**	−13.9	−15.5
	15	**−12.2***		−14.9
Timothy (northern)	9	**−19.3**	−18.3	−20.3
	12	**−14.8**	−14.1	−15.6
	15	**−14.1**	−13.0	−15.2
Red clover (southern)	9	**−14.1**	−13.3	−14.9
	12	**−14.0**	−13.4	−14.7
	15	**−11.9**	−11.1	−12.8
Red clover (northern)	9	**−15.1**	−13.9	−16.3
	12	**−14.3**	−13.4	−15.1
	15	**−14.6**	−14.0	−15.3

*Estimated values: LT_{50} interval not reached.

study where field-grown timothy plants were exposed to de-hardening conditions in a phytotron during winter, with the northern winter-hardy cultivar 'Engmo' losing freezing tolerance (LT_{50}) more rapidly than the less hardy cultivar 'Grindstad' (Jørgensen et al. 2010). Svenning et al. (1997) observed a similar interaction between cultivar and temperature in white clover (*Trifolium repens*), as the absolute rate of de-hardening was higher in a northern-adapted ecotype (Bodø) than in a southern-adapted cultivar (AberHerald), particularly at higher temperatures. It appears that the northern-adapted populations of grasses are more sensitive to warmer temperatures and that increasing autumn temperatures predicted in the future may therefore reduce the cold acclimation of northern-adapted cultivars, resulting in reduced winter hardiness. Biomass production, number of new leaves and leaf and petiole elongation rate were positively affected by higher temperatures (12 °C and 15 °C) in most populations (Table 3, Fig. 1). In southern-adapted population of timothy leaf, elongation was not significantly different between temperature treatments, but this population produced more biomass by producing more new leaves at 15 °C than at 9 °C. Elongation growth rates increased in northern-adapted populations and southern-adapted population of ryegrass with increasing growth temperature. We examined the effect of increasing pre-acclimation temperature *per se*, under a relatively long photoperiod without including the effect of the declining photoperiod and lower light intensity during late summer and autumn. The long photoperiod used in this study may thus explain the lack of differences in elongation rate between southern- and northern-adapted plants. Growth cessation and photoacclimation in northern-adapted grasses were more affected by short photoperiod during autumn than in southern-adapted in other studies (Østrem et al. 2014), especially at higher autumn temperatures (Malyshev et al. 2014). Biomass production and leaf growth in ecotypes of grass species adapted to higher latitudes are highest when grown in long days at temperatures below 15 °C (Heide et al. 1985). Instead of measuring the leaf elongation of the leaf lamina in red clover, we measured the length of the petiole, a character that is known to be highly plastic (de Kroon et al. 2005). New leaves did not emerge during the treatment at 2 °C, which is probably because this temperature is too low for cell division and growth (Gramig and Stoltenberg 2007) or too slow growing for new leaves to emerge during the experimental period.

The northern-adapted red clover population acclimated at 15 °C in the present study was inadvertently exposed to a short period (4 days) of drought, which could partly explain the similar level of freezing tolerance to the corresponding populations acclimated at 9 °C and 12 °C. Drought increases freezing tolerance in plants in a similar way as low temperature acclimation (Thomas and James 1993).

We recorded photoacclimation in grasses after pre-acclimation at 9 °C, especially in perennial ryegrass. All species showed similar F_v/F_m values before and after cold acclimation at 2 °C, indicating that photoacclimation took place already at pre-acclimation temperatures, as reported previously for oilseed rape (Rapacz 1998b, Rapacz and Janowiak 1998). Grasses, especially perennial ryegrass, had low values of F_v/F_m at the start of this experiment, in contrast with red clover. As a result, plants at higher temperatures were photoinhibited, reflected in the low values of both F_v/F_m and ϕ_{PSII}, especially in southern-adapted perennial ryegrass, indicating that the northern-adapted population photoacclimated better at higher temperature at the particular photoperiod used in this study. Non-photochemical quenching (NPQ) varied between populations and treatments, and it was not directly related to freezing tolerance. Similar results were obtained by Humphreys et al. (2007) who found that although increased NPQ values were observed at highest frequencies in freezing-tolerant *Lolium* × *Festuca* hybrids; this was not a prerequisite for increased freezing tolerance.

The different leaf morphology of grasses and clover, and possibly different species responses to the growing conditions, could explain differences in photochemical activity. In addition, the nitrogen-fixing capacity of the clover makes it less susceptible to undetected nitrogen deficiencies, conditions known to influence the precision of chlorophyll fluorescence measurements. Dicots and monocots have different protein structures within the light-harvesting

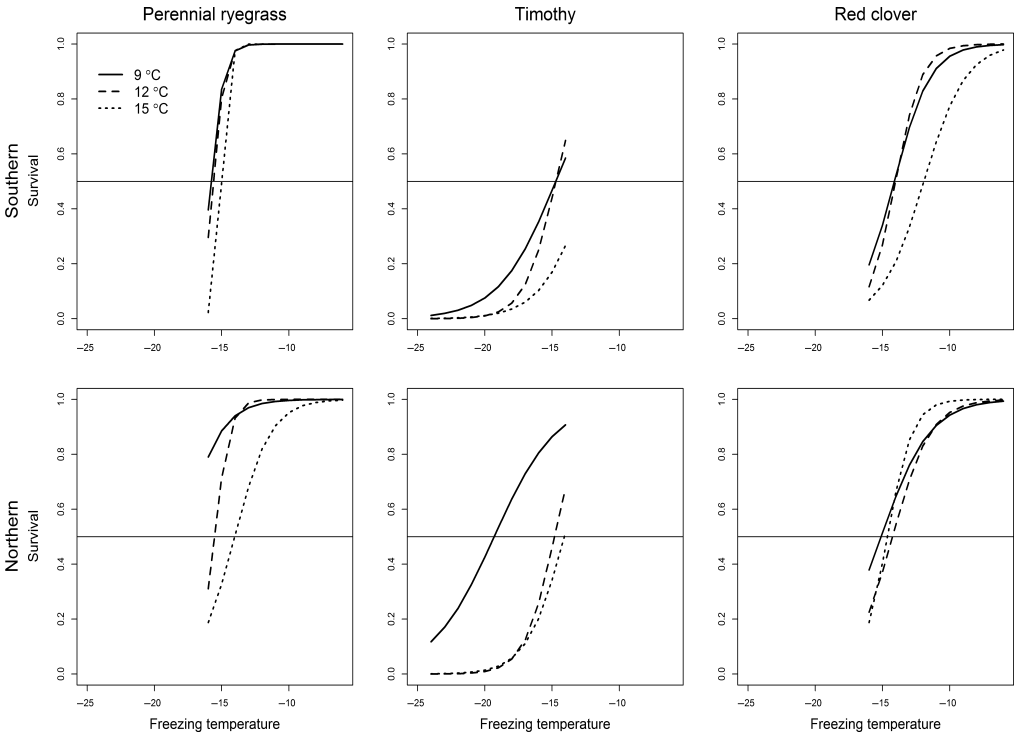

Fig. 2 Survival of plant populations as a function of freezing temperature (°C) in a freezing test at the end of the experiment. The line at 50 % survival indicates the LT_{50} value for the population. Predicted values are presented for each temperature treatment (9 °C, 12 °C, 15 °C).

complex of the photosystems (Huber et al. 2001), which also may affect the flow of photochemical energy under different light and temperature conditions. Red clover is therefore likely to have different mechanisms of photoacclimation than grasses.

In conclusion, higher pre-acclimation temperatures than normal today at higher latitudes reduced the freezing tolerance in red clover, perennial ryegrass and timothy. Furthermore, northern-adapted populations generally expressed higher freezing tolerance than southern-adapted, but the northern-adapted populations of grasses were more sensitive to temperature changes. Red clover was less affected by temperature than the grasses. However, we found no consistent differences between southern-adapted and northern-adapted populations in morphology, biomass production and the utilization of photochemical energy at the photoperiods used in this study. The results obtained confirmed that increasing growth temperature decreases cold acclimation ability and reduces photosynthetic acclimation to cold in grasses. The predicted increase in temperature may therefore reduce the winter hardiness of forage species, which is especially important for alpine and high-latitude regions. The effect of photoperiod × temperature interactions on growth cessation and cold acclimation of these species should be studied further.

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References

Asher, C. J., 1978: Natural and synthetic culture media for spermatophytes. In: M. Jr. Rechcigal, ed. Handbook of Nutrition and Food, Section G: Diets, Culture Media, Food

Supplements. Vol. III. Culture Media for Microorganisms and Plants, pp. 575–609. CRC Press, Cleveland, OH, USA.

Bilger, W., and O. Björkman, 1991: Temperature dependence of violaxanthin de-epoxidation and non-photochemical fluorescence quenching in intact leaves of *Gossypium hirsutum* L. and *Malva perviflora* L. Planta 184, 226–234.

Burnham, K. P., and D. R. Anderson, 2002: Model Selection and Multimodel Inference: A Practical Information-Theoretical Approach, 2nd edn. Springer-Verlag, New York, NY, USA, 488 p.

Crawley, M. J., 2007: The R Book, 1st edn. John Wiley & Sons, Ltd., West Sussex, UK, pp. 1–942.

Crosatti, C., F. Rizza, F. W. Badeck, E. Mazzucotelli, and L. Cattivelli, 2013: Harden the chloroplast to protect the plant. Physiol. Plant. 147, 55–63.

Eagles, C. F., and J. Williams, 1992: Hardening and dehardening of *Lolium perenne* in response to fluctuating temperatures. Ann. Bot. 70, 333–338.

Eagles, C. F., H. Thomas, F. Volaire, and C. J. Howarth, 1997: Stress physiology and crop improvement. In: B. R. Christie, ed. Proceedings of the XVIII International Grassland Congress, Canada 3, pp. 141–150.

Gelman, A., and J. Hill, 2008: Data Analysis Using Regression and Multilevel/Hierarchical Models. – Analytical Methods for Social Research. Cambridge University Press, Cambridge, UK, p. 652.

Genty, B., J.-M. Briantais, and N. R. Baker, 1989: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim. Biophys. Acta 990, 87–92.

Gramig, G. G., and D. E. Stoltenberg, 2007: Leaf appearance base temperature and phyllochron for common grass and broadleaf weed species. Weed Technol. 21, 249–254.

Heide, O. M., R. K. M. Hay, and H. Baugerød, 1985: Specific daylength effects on leaf growth and dry-matter production in high-latitude grasses. Ann. Bot. 55, 579–586.

Höglind, M., A. K. Bakken, M. Jørgensen, and L. Østrem, 2010: Tolerance to frost and ice encasement in cultivars of timothy and perennial ryegrass during winter. Grass Forage Sci. 65, 431–445.

Huber, C. G., A.-M. Timperio, and L. Zolla, 2001: Isoforms of Photosystem II antenna proteins in different plant species revealed by liquid chromatography-electrospray ionization mass spectrometry. J. Biol. Chem. 276, 45755–45761.

Humphreys, M. W., D. Gasior, A. Lesniewska-Bocianowska, Z. Zwierzykowski, and M. Rapacz, 2007: Androgenesis as a means of dissecting complex genetic and physiological controls: selecting useful gene combinations for breeding freezing tolerant grasses. Euphytica 158, 337–345.

Hüner, N. P. A., G. Öquist, V. M. Hurry, M. Kroll, S. Falk, and M. Griffith, 1993: Photosynthesis, photoinhibition and low temperature acclimation in cold tolerant plants. Photosynth. Res. 37, 19–39.

Hüner, N. P. A., R. Bode, K. Dahal, L. Hollis, D. Rosso, M. Krol, and A. G. Ivanov, 2012: Chloroplast redox imbalance governs phenotypic plasticity: the ″grand design of photosynthesis″ revisited. Front. Plant Sci. 3, 255.

Hüner, N. P. A., R. Bode, K. Dahal, F. A. Busch, M. Possmayer, B. Szyszka, D. Rosso, I. Ensminger, M. Krol, A. G. Ivanov, and D. P. Maxwell, 2013: Shedding some light on cold acclimation, cold adaptation and phenotypic plasticity. Botany 91, 127–136.

IPCC, 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 1535 pp.

Jørgensen, M., L. Østrem, and M. Höglind, 2010: De-hardening in contrasting cultivars of timothy and perennial ryegrass during winter and spring. Grass Forage Sci. 65, 38–48.

Junttila, O., 1996: Plant adaptation to temperature and photoperiod. Agr. Food Sci. Finland 5, 251–260.

Kalcsits, L., S. Silim, and K. Tanino, 2009: The influence of temperature on dormancy induction and plant survival in woody plants. In: L. Gusta, K. K. Tanino, and M. E. Wisniewski, eds. Plant Cold Hardiness: From the Laboratory to the Field, pp. 108–118. CAB International, Oxfordshire, UK.

Kleinbaum, D. G., L. L. Kupper, K. E. Muller, and A. Nizam, 1998: Applied Regression Analysis and Other Multivariable Methods, 3rd edn. Duxbury Press, Pacific Grove, CA, USA, pp. 1–798.

Kosmidis, I., and D. Firth, 2009: Bias reduction in exponential family nonlinear models. Biometrika 96, 793–804.

de Kroon, H., H. Huber, J. F. Stuefer, and J. M. van Groenendael, 2005: A modular concept of phenotypic plasticity in plants. New Phytol. 166, 73–82.

Larsen, A., 1994: Breeding winter hardy grasses. Euphytica 77, 231–237.

Levitt, J., 1980: Response of Plants to Environmental Stresses, Vol. 1. Chilling, Freezing, and High Temperature Stresses. Academic Press, New York, NY, USA, 497 p.

Malyshev, A. V., H. A. L. Henry, and J. Kreyling, 2014: Relative effects of temperature vs. photoperiod on growth and cold acclimation of northern and southern ecotypes of the grass *Arrhenatherum elatius*. Environ. Exp. Bot. 106, 189–196.

Marum, P., and K. Daugstad, 2009: Grindstad timothy: the landrace that became a major commercial variety. Biodiv. Tech. Bull. 15, 187–190.

Østrem, L., B. Volden, and A. Larsen, 2013: Morphology, dry matter yield and phenological characters at different maturity stages of X Festulolium compared with other grass species. Acta Agr. Scand. 63, 531–542.

Østrem, L., M. Rapacz, A. Larsen, S. Dalmannsdottir, and M. Jørgensen, 2014: Influences of growth cessation and photoacclimation on winter survival of non-native *Lolium-Festuca* grasses in high-latitude regions. Environ. Exp. Bot. 111, 21–31.

Pulli, S., K. Hjortsholm, A. Larsen, B. Gudleifsson, S. Larsson, B. Kristiansson, L. Hoemmoe, A. M. Tronsmo, P. Ruuth, and C.

Kristensson, 1996: Development and evaluation of laboratory testing methods for winter hardiness breeding. Nordic Gene Bank 32, 68 pp.

Rapacz, M., 1998a: The effect of day and night temperatures during early growth of winter rape seedlings on their morphology and cold acclimation responses. Acta Physiol. Plant 20, 67–72.

Rapacz, M., 1998b: The after-effects of temperature and irradiance during early growth of winter oilseed rape (*Brassica napus* L var oleifera cv Gorczanski) seedlings on the progress of their cold acclimation. Acta Physiol. Plant 20, 73–78.

Rapacz, M., and F. Janowiak, 1998: Physiological effects of winter rape (*Brassica napus* var *oleifera*) prehardening to frost. I. Frost resistance and photosynthesis during cold acclimation. J. Agron. Crop Sci. 181, 13–20.

Rapacz, M., D. Gąsior, Z. Zwierzykowski, A. Leśniewska-Bocianowska, H. W. Humphreys, and A. P. Gay, 2004: Changes in cold tolerance and the mechanisms of acclimation of photosystem II to cold hardening generated by anther culture of *Festuca pratensis* × *Lolium multiflorum* cultivars. New Phytol. 161, 105–114.

Rapacz, M., Å. Ergon, M. Höglind, M. Jørgensen, B. Jurczyk, L. Østrem, O. A. Rognli, and A. M. Tronsmo, 2014: Overwintering of herbaceous plants in a changing climate. Still more questions than answers. Plant Sci. 225, 34–44.

Rapp, K., and O. Junttila, 2000: Heritability estimates of winter hardiness in white clover based on field and laboratory experiments. Acta Agric. Scand. Sect. B Soil Plant Sci. 50, 143–148.

Rognli, O. A., 2013: Breeding for improved winter survival in forage grasses. In: I. Ryozo, M. Yoshida, and N. Matsumoto, eds. Plant and Microbe Adaptations to Cold in a Changing World: Proceedings of the Plant and Microbe Adaptation to Cold Conference, 2012, pp 197–208. Springer, New York, NY, USA.

Schreiber, U., W. Bilger, and C. Neubauer, 1994: Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of in vivo photosynthesis. In: E.-D. Schultze, and M. M. Caldwell, eds. Ecophysiology of Photosynthesis. Springer-Verlag, Berlin, Germany, pp. 49–70.

Svenning, M. M., K. Røsnes, and O. Junttila, 1997: Frost tolerance and biochemical changes during hardening and de-hardening in contrasting white clover populations. Physiol. Plant. 88, 689–695.

Thomas, H., and A. R. James, 1993: Freezing tolerance and solute change in contrasting genotypes of *Lolium perenne* L. acclimated to cold and drought. Ann. Bot. 72, 249–254.

Thomashow, M. F., 1999: Plant cold acclimation: freezing tolerance genes and regulatory mechanisms. Annu. Rev. Plant Physiol. Plant Mol. Biol. 50, 571–599.

Thorsen, S. M., and M. Höglind, 2010: Assessing winter survival of forage grasses in Norway under future climate scenarios by simulating potential frost tolerance in combination with simple agroclimatic indices. Agr. Forest Meteorol. 150, 1272–1282.

Uleberg, E., I. Hanssen-Bauer, B. van Oort, and S. Dalmannsdottir, 2014: Impact of climate change on agriculture in Northern Norway and potential strategies for adaptation. Clim. Change. 122, 27–39.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

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Table S10. Parameter estimates for maximum quantum yield of PSII (F_v/F_m).

Table S11. Parameter estimates for coefficient of the non-photochemical quenching of chlorophyll fluorescence (NPQ).

Table S12. Parameter estimates for coefficient of the Current quantum yield of PSII (ϕ_{PSII}).

Table S13. Parameter estimates for coefficient of the photochemical quenching of chlorophyll fluorescence (q_p).

Table S14. Model comparison (10 best models) for freezing tolerance of timothy.

Table S15. Model comparison (10 best models) for freezing tolerance of perennial ryegrass.

Table S16. Model comparison (10 best models) for freezing tolerance of red clover.

Table S17. Parameter estimates (logit-transformed) of a logistic model for freezing tolerance for the different species tested.

Supplement Paper I

Table S1. Parameter estimates for non-linear model of leaf and petiole elongation. Abbreviations: Asym= asymptote; R_0= intercept; lrc= logistic rate constant.

Species	Population	Temp (°C)	Asym	SE	R_0	SE	lrc	SE
Timothy								
	Southern	9	114.70	±12.43	59.53	±12.96	-0.27	±0.80
	Southern	12	176.25	±27.38	73.64	±6.84	-0.16	±0.14
	Southern	15	87.79	±16.45	50.85	±5.11	-0.22	±0.22
	Northern	9	143.11	±23.31	55.73	±7.92	-1.01	±0.16
	Northern	12	155.50	±10.17	74.82	±5.73	-0.01	±0.19
	Northern	15	161.16	±15.98	67.25	±8.35	-0.15	±0.16
Perennial ryegrass	Southern	9	241.99	±16.05	60.47	±7.06	-0.84	±0.10
	Southern	12	250.44	±10.04	65.19	±12.82	-0.08	±0.22
	Southern	15	223.31	±12.58	56.03	±7.25	-0.02	±0.14
	Northern	9	283.86	±21.09	49.00	±7.56	-0.94	±0.13
	Northern	12	276.42	±0.02	14.79	±0.02	-0.16	±0.02
	Northern	15	272.42	±21.46	56.69	±9.26	0.10.	±0.11
Red clover								
	Southern	9	195.24	±9.87	40.03	±3.64	-1.37	±0.21
	Southern	12	205.79	±8.46	35.00	±9.43	-1.56	±0.34
	Southern	15	198.28	±36.17	37.66	±7.73	-1.27	±0.41
	Northern	9	133.00	±15.25	45.30	±1.32	-1.33	±0.18
	Northern	12	152.11	±17.20	40.83	±5.88	-0.87	±0.23
	Northern	15	148.78	±11.12	47.10	±3.21	-0.43	±0.11

Table S2. Model comparison (10 best models) for biomass. Abbreviations: P=population; Sp=species; T=treatment; df=degrees of freedom; AICc=Akaike's information criterion corrected for small sample size; ΔAICc= difference in AICc-value to best model.

Intercept	T	P	Sp	T×P	TxSp	P×Sp	T×P×Sp	R^2	df	AICc	ΔAICc
5.475	+	+	+	+	+	+	+	0.298	19	273.07	0
5.590	+	+	+	+		+		0.240	11	276.57	3.49
5.565	+	+	+	+	+	+		0.257	15	279.35	6.28
5.666	+	+	+			+		0.217	9	280.20	7.12
5.641	+	+	+		+	+		0.234	13	283.03	9.95
5.687		+	+	+				0.191	9	289.14	16.07
5.763			+					0.168	6	290.26	17.18
5.662	+	+	+	+	+			0.208	13	292.17	19.09
5.764	+	+	+					0.168	7	292.36	19.29
5.738	+		+		+			0.185	10	293.24	20.16

Table S3. Parameter estimates for biomass (log-transformed). Reference value (intercept) represents red clover, northern adapted population and temperature 9°C. Abbreviations: PRG=perennial ryegrass.

	Estimate	SE	t value	P-value
Intercept	5.48	0.10	55.09	<0.000
Species PRG	0.66	0.14	4.69	<0.000
Species timothy	0.33	0.14	2.33	0.020
Population southern	0.52	0.14	3.73	<0.000
Temperature 12°C	0.60	0.14	4.27	<0.000
Temperature 15°C	0.56	0.14	3.98	<0.000
Species PRG × population southern	-0.55	0.20	-2.79	0.006
Species timothy × population southern	-0.57	0.20	-2.85	0.005
Species PRG × temperature 12°C	-0.23	0.20	-1.14	0.257
Species timothy × temperature 12°C	-0.34	0.20	-1.71	0.089
Species PRG × temperature 15°C	-0.25	0.20	-1.24	0.216
Species timothy × temperature 15°C	-0.22	0.20	-1.11	0.267
Population southern × temperature 12°C	-0.73	0.20	-3.67	<0.000
Population southern × temperature 15°C	-0.27	0.20	-1.34	0.181
Species PRG × Population southern × temperature 12°C	0.21	0.28	0.73	0.465
Species timothy × Population southern × temperature 12°C	1.01	0.28	3.58	<0.000
Species PRG × Population southern × temperature 15°C	0.07	0.28	0.23	0.817
Species timothy × population southern × temperature 15°C	0.33	0.28	1.19	0.235

Table S4. Model Comparison (10 best models) for log transformed data of leaf number. See table S2 for abbreviations.

Intercept	T	P	Sp	T×P	T×Sp	P×Sp	T×P×Sp	R^2	df	AICc	ΔAICc
1.458	+	+	+	+	+	+	+	0.635	19	384.07	0
1.570	+	+	+	+		+		0.574	11	407.48	23.41
1.667	+	+	+	+	+	+		0.587	15	408.49	24.42
1.709	+	+	+			+		0.558	9	413.68	29.61
1.805	+	+	+		+	+		0.570	13	414.85	30.78
1.457	+	+	+	+				0.543	9	422.14	38.07
1.858		+	+			+		0.536	7	422.41	38.34
1.554	+	+	+	+	+			0.556	13	423.55	39.48
1.596	+	+	+					0.527	7	427.70	43.63
1.692	+	+	+		+			0.539	11	429.35	45.17

Table S5. Parameter estimates for number of leaves (log + 2 transformed). Reference value (intercept) represents red clover, northern adapted population and temperature 9°C. Abbreviations: PRG=perennial ryegrass.

	Estimate	SE	t value	P-value
Intercept	1.46	0.12	11.94	<0.000
Species PRG	1.22	0.17	7.01	<0.000
Species timothy	0.98	0.17	5.70	<0.000
Population southern	0.22	0.17	1.28	0.202
Temperature 12°C	0.24	0.17	1.41	0.159
Temperature 15°C	0.96	0.17	5.55	<0.000
Species PRG × population southern	-0.49	0.24	-2.02	0.045
Species timothy × population southern	-0.08	0.24	-0.35	0.730
Species PRG × temperature 12°C	-0.30	0.24	1.21	0.226
Species timothy × temperature 12°C	0.17	0.24	0.69	0.489
Species PRG × temperature 15°C	-0.57	0.24	-2.33	0.021
Species timothy × temperature 15°C	-0.91	0.24	-3.71	<0.000
Population southern × temperature 12°C	-0.63	0.24	-2.57	0.011
Population southern × temperature 15°C	-1.46	0.24	-5.97	<0.000
Species PRG × Population southern × temperature 12°C	0.28	0.35	0.813	0.417
Species timothy × Population southern × temperature 12°C	0.30	0.35	0.86	0.393
Species PRG × Population southern × temperature 15°C	1.43	0.35	4.14	<0.000
Species timothy × population southern × temperature 15°C	1.76	0.35	5.09	<0.000

Table S6. Model comparison (10 best models) for Fv/Fm (maximum quantum yield of PSII). Abbreviations: T=treatment; P=population; Se=Serie (start, after PA, after CA); Sp=species; df=degrees of freedom; AICc=Akaike's information criterion corrected for small sample size; ΔAICc= difference in AICc-value to best model.

Intercept	T	P	Se	Sp	T×P	T×Se	T×Sp	P×Se	P×Sp	Se×Sp	T×P×Se	T×P×Sp	T×Se×Sp	P×Se×Sp	T×P×Se×Sp	R^2	df	AICc	ΔAICc
0.763	+	+	+	+		+	+	+	+	+				+		0.770	29	-3955.47	0
0.766	+	+	+	+		+		+	+	+				+		0.767	25	-3952.62	2.85
0.760	+	+	+	+	+		+	+	+	+				+		0.766	25	-3951.51	3.95
0.763	+	+	+	+		+	+	+	+	+				+		0.770	31	-3951.20	4.27
0.763	+	+	+	+	+			+	+	+				+		0.763	21	-3948.73	6.74
0.767	+	+	+	+	+	+		+	+	+				+		0.767	27	-3948.40	7.07
0.765	+	+	+	+	+	+	+	+	+	+		+		+		0.772	35	-3948.21	7.25
0.760	+	+	+	+	+		+	+	+	+				+		0.766	27	-3947.30	8.17
0.763	+	+	+	+				+	+	+				+		0.763	23	-3944.56	9.91
0.763	+	+	+	+		+	+	+	+	+			+	+		0.772	37	-3944.27	11.20

Table S7. Model comparison (10 best models) for NPQ (non-photochemical quenching of chlorophyll fluorescence). For Abbreviations see table S6.

Intercept	T	P	Se	Sp	T×P	T×Se	T×Sp	P×Se	P×Sp	Se×Sp	T×P×Se	T×P×Sp	T×Se×Sp	P×Se×Sp	T×P×Se×Sp	R^2	df	AICc	ΔAICc
1.586	+	+	+	+		+			+	+						0.282	19	93.247	0
1.562	+	+	+	+		+	+		+	+						0.289	23	94.569	1.322
1.596	+	+	+	+	+	+	+		+	+		+				0.300	29	95.036	1.789
1.584	+	+	+	+		+		+	+	+						0.284	21	95.065	1.818
1.559	+	+	+	+		+	+	+	+	+						0.291	25	96.427	3.180
1.593	+	+	+	+	+	+	+	+	+	+		+				0.302	31	96.972	3.725
1.621	+	+	+	+		+				+						0.274	17	97.294	4.047
1.586	+	+	+	+	+	+			+	+						0.282	21	97.465	4.218
1.589	+	+	+	+		+		+	+	+				+		0.289	25	97.771	4.524
1.597	+	+	+	+		+	+			+						0.281	21	98.641	5.394

Table S8. Model comparison (10 best models) for ϕ_{PSII} (Current quantum yield of PSII). For Abbreviations see table S6.

Intercept	T	P	Se	Sp	T×P	T×Se	T×Sp	P×Se	P×Sp	Se×Sp	T×P×Se	T×P×Sp	T×Se×Sp	P×Se×Sp	T×P×Se×Sp	R^2	df	AICc	ΔAICc
0.238	+	+	+	+		+		+	+	+				+		0.710	25	-2268.98	0
0.236	+	+	+	+	+	+		+	+	+				+		0.710	27	-2265.84	3.141
0.238	+	+	+	+		+	+	+	+	+				+		0.712	29	-2264.72	4.264
0.241	+	+	+	+	+	+		+	+	+	+			+		0.713	31	-2264.23	4.754
0.238	+	+	+	+				+	+	+				+		0.704	21	-2261.93	7.053
0.236	+	+	+	+	+	+	+	+	+	+				+		0.712	31	-2261.52	7.468
0.251	+	+	+	+		+		+	+	+						0.703	21	-2260.32	8.661
0.257	+	+	+	+		+			+	+						0.702	19	-2259.94	9.041
0.240	+	+	+	+	+	+	+	+	+	+	+			+		0.715	35	-2259.73	9.259
0.228	+	+	+	+	+	+	+	+	+	+		+		+		0.714	35	-2258.88	10.104

Table S9. Model comparison (10 best models) for q_p (photochemical quenching of chlorophyll fluorescence). For Abbreviations see table S6.

Intercept	T	P	Se	Sp	T×P	Te×Se	T×Sp	P×Se	P×Sp	Se×Sp	T×P×Se	T×P×Sp	T×Se×Sp	P×Se×Sp	T×P×Se×Sp	R^2	df	AICc	ΔAICc
0.469	+	+	+	+		+		+	+	+				+		0.648	25	-1296.24	0
0.466	+	+	+	+	+	+		+	+	+				+		0.649	27	-1292.49	3.751
0.468	+	+	+	+		+	+	+	+	+				+		0.650	29	-1291.92	4.321
0.495	+	+	+	+		+			+							0.637	15	-1291.64	4.602
0.488	+	+	+	+		+		+	+							0.638	17	-1290.32	5.919
0.474	+	+	+	+	+	+		+	+	+	+			+		0.651	31	-1289.78	6.465
0.500	+	+	+	+		+			+	+						0.639	19	-1289.16	7.076
0.465	+	+	+	+	+	+	+	+	+	+				+		0.651	31	-1288.11	8.127
0.493	+	+	+	+	+	+			+							0.637	17	-1287.96	8.280
0.492	+	+	+	+		+		+	+	+						0.641	21	-1287.80	8.437

Table S10. Parameter estimates for maximum quantum yield of PSII (Fv/Fm). Reference value (intercept) represents treatment 9°C, serie after CA, population northern and species red clover. Abbreviations: PRG=perennial ryegrass.

	Estimate	SE	t value	P-value
Intercept	0.763	0.003	221.61	<0.000
Treatment 12°C	-0.003	0.004	-0.94	0.347
Treatment 15°C	-0.004	0.004	-1.17	0.242
Serie	0.002	0.005	0.53	0.600
Serie start	0.014	0.005	3.03	0.003
Species PRG	-0.031	0.005	-6.78	<0.000
Species timothy	-0.028	0.005	-6.10	<0.000
Population southern	0.012	0.004	3.04	0.002
Treatment 12°C × serie after PA	0.007	0.004	1.87	0.062
Treatment 15°C × serie after PA	0.003	0.004	0.88	0.381
Treatment 12°C × serie start	0.006	0.004	1.50	0.135
Treatment 15°C × serie start	0.011	0.004	2.90	0.004
Treatment 12°C × species PRG	-0.005	0.004	-1.14	0.256
Treatment 15°C × species PRG	-0.010	0.004	-2.64	0.009
Treatment 12°C × species timothy	-0.003	0.004	-0.80	0.422
Treatment 15°C × species timothy	-0.012	0.004	-2.95	0.003
Serie after PA × population southern	-0.008	0.006	-1.50	0.135
Serie start × population southern	-0.013	0.006	-2.29	0.022
Species PRG × population southern	-0.010	0.006	-1.75	0.081
Species timothy × population southern	-0.009	0.006	-1.67	0.096
Serie after PA × species PRG	-0.000	0.006	-0.02	0.984
Serie start x species PRG	-0.041	0.006	-7.44	<0.000
Serie after PA × species timothy	-0.008	0.006	-1.33	0.183
Serie start × species timothy	-0.035	0.006	-6.25	<0.000
Serie after PA × species PRG × population southern	0.007	0.008	0.93	0.353
Serie start x species PRG × population southern	-0.049	0.008	-6.28	<0.000
Serie after PA × species timothy × population southern	0.013	0.008	1.68	0.094
Serie start × species timothy × population southern	0.019	0.008	2.45	0.015

Table S11. Parameter estimates for coefficient of the non-photochemical quenching of chlorophyll fluorescence (NPQ). Reference value (intercept) represents treatment 9°C, serie after CA, population northern and species red clover. Abbreviations: PRG=perennial ryegrass.

	Estimate	Std error	t value	P-value
Intercept	1.616	0.053	30.25	<0.000
Serie after PA	-0.034	0.065	-0.51	0.608
Serie start	-0.241	0.065	-3.69	<0.000
Treatment 12°C	-0.013	0.076	-0.18	0.860
Treatment 15°C	-0.143	0.076	-1.90	0.058
Species PRG	-0.120	0.077	-1.57	0.117
Species timothy	0.254	0.077	3.32	0.001
Population southern	0.074	0.053	1.38	0.167
Serie after PA × treatment 12°C	-0.062	0.093	-0.67	0.505
Serie start × treatment 12°C	0.003	0.093	0.03	0.973
Serie after PA × treatment 15°C	-0.037	0.093	-0.40	0.689
Serie start × treatment 15°C	0.053	0.093	0.57	0.566
Serie after PA × species PRG	0.160	0.093	1.72	0.086
Serie start × species PRG	0.038	0.093	0.41	0.681
Serie after PA × species timothy	0.021	0.093	0.23	0.821
Serie start × species timothy	-0.022	0.093	-0.23	0,816
Treatment 12°C × species PRG	0.003	0.108	0.03	0.976
Treatment 15°C × species PRG	0.215	0.107	1.99	0.047
Treatment 12°C × species timothy	-0.107	0.109	-0.98	0.330
Treatment 15°C × species timothy	0.142	0.109	1.30	0.193
Species PRG × population southern	-0.017	0.076	-0.22	0.823
Species timothy × population southern	-0.006	0.076	-0.08	0.933
Serie after PA × treatment 12°C × species PRG	-0.002	0.134	-0.02	0.986
Serie start × treatment 12°C × species PRG	0.018	0.133	0.14	0.891
Serie after PA × treatment 15°C × species PRG	-0.226	0.132	-1.72	0.086
Serie start × treatment 15°C × species PRG	-0.103	0.132	-0.78	0.437
Serie after PA × treatment 12°C × species timothy	-0.167	0.133	-1.26	0.209
Serie start x Treatment 12°C × species timothy	0.108	0.133	0.81	0.418
Serie after PA × Treatment 15°C × species timothy	-0.188	0.133	-1.41	0.158
Serie start x treatment 15°C × species timothy	0.013	0.133	0.10	0.920
Treatment 12°C × species red clover × population southern	0.021	0.076	0.27	0.788
Treatment 15°C × species red clover × population southern	0.181	0.076	2.38	0.017
Treatment 12°C × species PRG × population southern	-0.022	0.078	-0.28	0.781
Treatment 15°C × species PRG × population southern	-0.042	0.076	-0.55	0.583
Treatment 12°C × species timothy × population southern	0.019	0.077	0.25	0.805
Treatment 15°C × species timothy × population southern	-0.134	0.078	-1.73	0.085

Table S12. Parameter estimates for coefficient of the Current quantum yield of PSII (ϕ_{PSII}). Reference value (intercept) represents treatment 9°C, serie after CA, population northern and species red clover. Abbreviations: PRG=perennial ryegrass.

	Estimate	Std error	t value	P-value
Intercept	0.238	0.009	25.17	<0.000
Serie after PA	0.060	0.013	4.42	<0.000
Serie start	0.107	0.013	8.00	<0.000
Treatment 12°C	-0.004	0.008	-0.47	0.638
Treatment 15°C	-0.018	0.008	-2.20	0.028
Species PRG	-0.154	0.012	-13.29	<0.000
Species timothy	-0.141	0.012	-11.94	<0.000
Population southern	0.018	0.012	1.58	0.113
Serie after PA × treatment 12°C	0.003	0.012	0.25	0.804
Serie start × treatment 12°C	0.004	0.012	0.33	0.738
Serie after PA × treatment 15°C	-0.022	0.012	-1.87	0.062
Serie start × Treatment 15°C	0.018	0.012	1.56	0.119
Species PRG × population southern	-0.001	0.017	-0.09	0.930
Species timothy × population southern	-0.020	0.017	-1.22	0.223
Serie after PA × population southern	-0.060	0.016	-3.63	<0.000
Serie start × population southern	-0.053	0.016	-3.23	0.001
Serie after PA × species PRG	-0.009	0.017	-0.56	0.575
Serie start × species PRG	-0.049	0.017	-2.93	0.003
Serie after PA × species timothy	-0.049	0.017	-2.93	0.004
Serie start × species timothy	-0.076	0.017	-4.58	<0.000
Serie after PA × species PRG × population southern	0.042	0.023	1.81	0.071
Serie start × species PRG × population southern	0.026	0.023	1.12	0.265
Serie after PA × species timothy × population southern	0.082	0.024	3.47	0.001
Serie start × species timothy × population southern	0.083	0.023	3.54	<0.000

Table S13. Parameter estimates for coefficient of the photochemical quenching of chlorophyll fluorescence (q_p). Reference value (intercept) represents treatment 9°C, serie after CA, population northern and species red clover. Abbreviations: PRG=perennial ryegrass.

	Estimate	Std error	t value	P-value
Intercept	0.469	0.018	26.39	<0.000
Serie after PA	0.121	0.025	4.77	<0.000
Serie start	0.167	0.025	6.67	<0.000
Treatment 12°C	-0.007	0.016	-0.44	0.663
Treatment 15°C	-0.34.	0.016	-2.21	0.027
Species PRG	-0.274	0.022	-12.61	<0.000
Species timothy	-0.224	0.022	-10.10	<0.000
Population southern	0.038	0.022	1.73	0.084
Serie after PA × treatment 12°C	-0.020	0.022	-0.91	0.362
Serie start × treatment 12°C	0.007	0.022	0.31	0.757
Serie after PA × treatment 15°C	-0.065	0.022	-2.95	0.003
Serie start × Treatment 15°C	0.034	0.022	1.57	0.117
Species PRG × population southern	-0.100	0.031	-3.22	0.001
Species timothy × population southern	-0.092	0.031	-2.99	0.003
Serie after PA × population southern	0.007	0.031	0.22	0.827
Serie start × population southern	-0.046	0.031	-1.46	0.144
Serie after PA × species PRG	0.011	0.031	0.35	0.730
Serie start × species PRG	-0.019	0.031	-0.61	0.544
Serie after PA × species timothy	-0.093	0.031	-2.94	0.003
Serie start × species timothy	-0.069	0.031	-2.22	0.027
Serie after PA × species PRG × population southern	0.053	0.044	1.21	0.226
Serie start × species PRG × population southern	0.083	0.044	1.89	0.059
Serie after PA × species timothy × population southern	0.157	0.044	3.55	<0.000
Serie start × species timothy × population southern	0.147	0.044	3.34	<0.000

Table S14. Model comparison (10 best models) for freezing tolerance of timothy. Abbreviations: T=treatment; F= pre-determined freezing temperatures; P=population; df=degrees of freedom; AICc=Akaike's information criterion corrected for small sample size; ΔAICc=difference in AICc-value to best model.

Intercept	T	F	P	T×F	T×P	F×P	T×F×P	R^2	df	AICc	ΔAICc
8.715	+	0.452	+	+	+			0.354	9	426.11	0
8.514	+	0.441	+	+	+	+		0.354	10	428.21	2.10
8.296	+	0.430	+	+	+	+	+	0.354	12	432.50	6.39
11.453	+	0.594	+		+			0.344	7	432.78	6.66
6.582	+	0.351	+	+		+		0.345	8	433.44	7.32
7.423	+	0.400	+	+				0.343	7	433.94	7.83
10.997	+	0.570	+		+	+		0.344	8	434.66	8.55
9.026	+	0.488	+			+		0.329	6	447.33	21.21
10.037	+	0.547	+					0.325	5	448.76	22.64
6.199	+	0.366		+				0.315	6	460.82	34.71

Table S15. Model comparison (10 best models) for freezing tolerance of perennial ryegrass. For abbreviations see table S14.

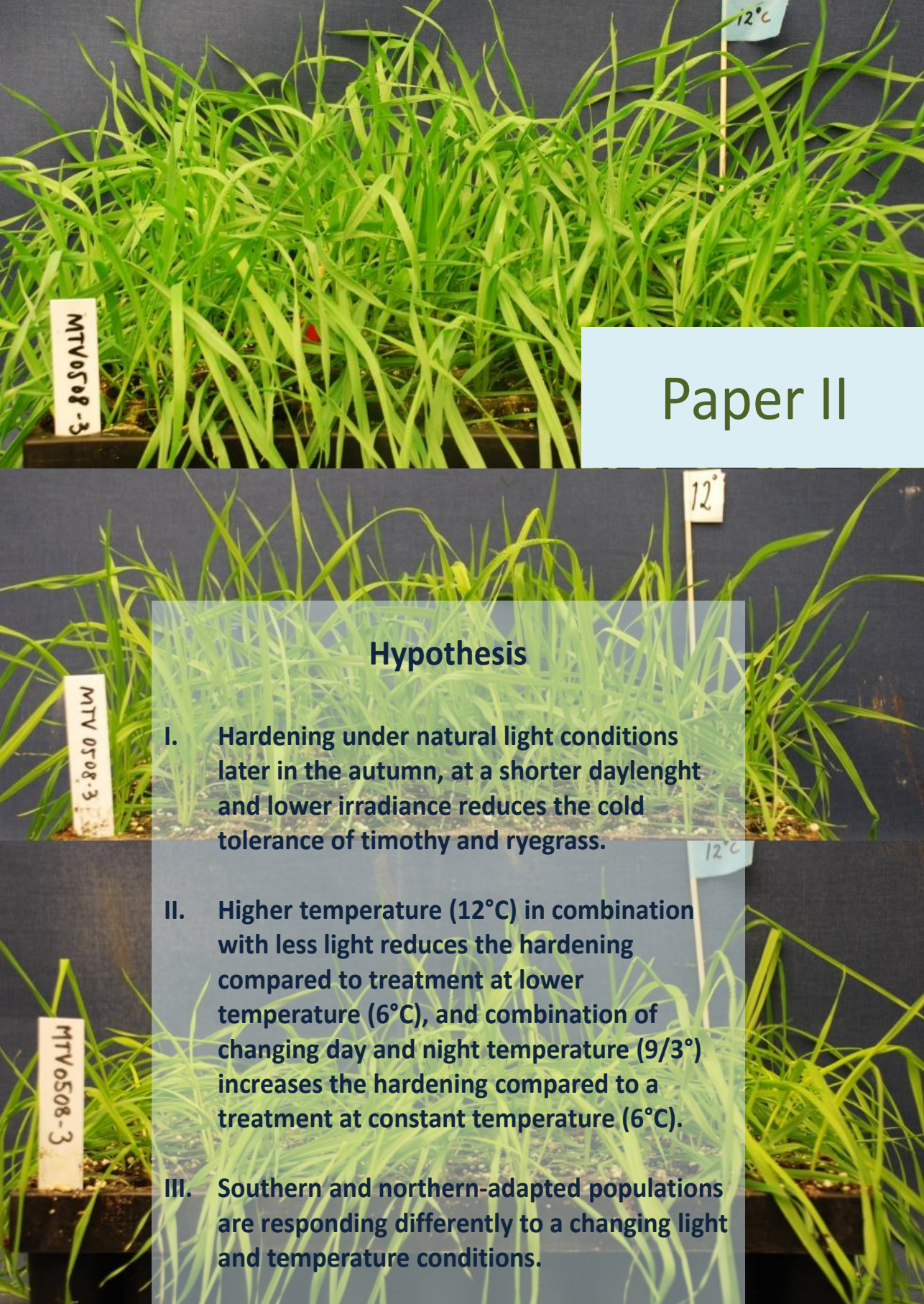
Intercept	T	F	P	T×F	T×P	F×P	T×F×P	R^2	df	AICc	ΔAICc
16.434	+	0.961	+			+		0.408	6	205.52	0
12.687	+	0.710	+	+	+	+		0.415	10	205.98	0.46
14.584	+	0.849	+	+		+		0.411	8	206.47	0.96
16.435	+	0.948	+		+	+		0.410	8	207.63	2.12
12.762	+	0.715	+	+	+	+	+	0.413	12	212.05	6.53
25.400	+	1.520	+	+	+			0.389	9	234.29	28.78
24.567	+	1.466	+		+			0.385	7	235.30	29.79
23.476	+	1.441		+				0.379	6	239.78	34.27
23.277	+	1.436	+	+				0.379	7	241.28	35.77
22.743	+	1.392						0.373	4	242.40	36.88

Table S16. Model comparison (10 best models) for freezing tolerance of red clover. For abbreviations see table S14.

Intercept	T	F	P	T×F	T×P	F×P	T×F×P	R^2	df	AICc	ΔAICc
11.404	+	0.770	+		+			0.421	7	403.88	0
1.071	+	0.746	+		+	+		0.421	8	405.94	2.060
9.856	+	0.660	+	+	+			0.422	9	406.60	2.724
8.310	+	0.550	+	+	+	+	+	0.427	12	407.07	3.187
9.487	+	0.634	+	+	+	+		0.422	10	408.69	4.811
11.255	+	0.744	+					0.409	5	413.19	9.313
10.398	+	0.682	+	+				0.411	7	414.89	11.006
11.575	+	0.767	+			+		0.409	6	415.10	11.221
10.701	+	0.704	+	+		+		0.411	8	416.87	12.991
10.664		0.729	+					0.400	3	418.52	14.645

Table S17. Parameter estimates (logit-transformed) of a logistic model for freezing tolerance for the different species tested. Reference value (intercept) represents northern-adapted population at 9°C.

Species	Predictor	Estimate	SE	z-value	p-value
Timothy	Intercept	8.72	1.27	6.88	0.000
	Treatment 12°C	4.69	2.70	1.74	0.082
	Treatment 15°C	1.11	2.68	0.42	0.678
	Freezing	0.45	0.06	6.97	0.000
	Population southern	-2.12	0.39	-5.41	0.000
	Treatment 12°C × freezing	0.45	0.17	2.72	0.006
	Treatment 15°C × freezing	0.25	0.17	1.50	0.134
	Treatment 12°C × population southern	2.04	0.61	3.36	0.001
	Treatment 15°C × population southern	1.16	0.66	1.77	0.077
Perennial ryegrass	Intercept	16.43	2.25	7.30	0.000
	Treatment 12°C	-1.10	0.45	-2.46	0.014
	Treatment 15°C	-3.09	0.53	-5.86	0.000
	Freezing	0.96	0.15	6.62	0.000
	Population southern	40.58	12.01	3.38	0.001
	Freezing × population southern	2.62	0.76	3.45	0.001
Red clover	Intercept	11.40	0.98	11.63	0.000
	Freezing	0.77	0.07	11.77	0.000
	Treatment 12°C	-0.46	0.44	-1.03	0.303
	Treatment 15°C	-0.03	0.46.	-0.06	0.950
	Population southern	-0.54	0.45	-1.22	0.224
	Treatment 12°C × population southern	0.46	0.62	0.74	0.462
	Treatment 15°C × population southern	-1.67	0.63	-2.64	0.008



Paper II

Hypothesis

I. Hardening under natural light conditions later in the autumn, at a shorter daylenght and lower irradiance reduces the cold tolerance of timothy and ryegrass.

II. Higher temperature (12°C) in combination with less light reduces the hardening compared to treatment at lower temperature (6°C), and combination of changing day and night temperature (9/3°) increases the hardening compared to a treatment at constant temperature (6°C).

III. Southern and northern-adapted populations are responding differently to a changing light and temperature conditions.

Paper II

Interaction of temperature and autumn light conditions affecting cold tolerance of timothy (*Phleum pratense* L.) and perennial ryegrass (*Lolium perenne* L.)

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Abstract

The effect of variable autumn temperatures in combination with decreasing irradiance and daylength on photosynthesis, growth cessation and freezing tolerance in populations of timothy and perennial ryegrass intended for use in northern high latitude regions was investigated. Plants were treated with three different acclimation temperatures; 12˚, 6˚ and 9/3˚C (day/night) for four weeks, followed by one week of cold acclimation at 2˚C under natural light conditions. This procedure was repeated at three different periods in autumn with decreasing sums of irradiance and daylengths. Photoacclimation, leaf elongation and freezing tolerance were studied. The results showed that plants cold acclimated during the lowest irradiance and shortest daylength had lowest freezing tolerance, lowest photosynthetic activity, longest leaves and least biomass production. Higher acclimation temperature (12˚C) resulted in lower freezing tolerance, lower photosynthetic activity, faster leaf elongation and more biomass production. Photochemical mechanisms were predominant in photoacclimation. The northern-adapted populations had a better freezing tolerance than the southern-adapted except when grown during the late autumn period and at the highest temperature, where there were no differences between the populations. Our results indicate that the projected climate change in the north may reduce freezing tolerance in grasses as acclimation will take place at higher temperatures and shorter daylengths with lower irradiance.

Key words: Cold acclimation, chlorophyll fluorescence, freezing tolerance, leaf elongation, geographically adapted populations, climate change

Abbreviations: Fm (F'm) = maximal chlorophyll fluorescence yield in the dark-adapted (light-adapted) leaf; Fo (F'o) = minimum chlorophyll fluorescence yield in the dark-adapted (light-adapted) leaf; Fs = steady-state chlorophyll fluorescence yield in the light-adapted leaf; Fv=Fm-Fo; ϕ_{PSII} = current quantum yield of PSII; q_p = coefficient of the photochemical quenching of chlorophyll fluorescence; NPQ = non-photochemical quenching of chlorophyll fluorescence.

Introduction

The considerable increase in autumn temperature predicted at higher northern latitudes the coming decades (ICPP 2013) in combination with low irradiance and short daylength at these latitudes may intensify or pose new winter stresses for perennial forage crops. Higher future winter temperatures with unstable snow cover and fluctuating weather conditions may increase winter stresses of plants (Bertrand and Castonguay 2003; Uleberg et al. 2014, Cooper 2014). The question is whether perennial plants will be able to cold acclimate during late summer and autumn in the future climate.

Cold acclimation of herbaceous species has been widely studied (reviewed by e.g. Thomashow 1999, Cinnusamy et al. 2006, Sandve et al. 2011, Quellet and Charron 2013, Wingler 2015) and is a process whereby climatically adaptive plants can increase their freezing tolerance in response to low non-freezing temperatures. Freezing tolerance is a dynamic character affected by environmental factors such as temperature and light (Gray et al. 1997) and is both seasonally (Yoshida et al. 1997, Palva et al. 2002) and diurnally (Keily et al. 2013) controlled. A decrease in temperature during late summer/early autumn triggers changes in the gene expression, resulting in increased freezing tolerance of the plant (Cinnusamy et al. 2006). In the plant cell, the chloroplast may act as one of the primary cold sensors of ambient temperatures in addition to the plasma membrane (Miura and Furumoto 2013). As reviewed by Hüner et al. (2014) the formation of an excitation pressure within photosystem II (PS II) in photosynthetic active tissue is the sensor for cold regulated mechanisms; not the low temperature *per se*. Excitation pressure develops as a response to over reduction of PS II because electron transfer through the electron transport chain is too slow (Hüner et al. 2013). This situation occurs either as a response to low temperature, which reduces the rate of assimilation from the carbon cycle and the need for photochemical energy or as a response to high light conditions (Ensminger et al. 2006). This redox sensing signalling through excitation pressure is both species and cultivar dependent (Hüner et al. 2013). Adaptive genotypes can avoid photoinhibition and start a process of photoacclimation either by increasing the rate of energy dissipation by non-photochemical quenching mechanisms (NPQ) or by enhancing the rate of carbon assimilation and photosynthetic performance through a process of photochemical quenching (q_p) (Hüner et al. 2012). As a result, photoacclimated plants exhibit a higher maximum photochemical efficiency (Fv/Fm) and increased photosynthetic activity (ϕ_{PSII}) compared to non-acclimated plants. The capacity of the plant to photoacclimate correlates with

freezing tolerance (Hüner et al. 1993, Rapacz et al. 2004) and tolerance to high light intensities (Rapacz et al. 2008).

In woody species, cold acclimation is a two-step process controlled by a combination of short photoperiod and low temperature, where growth cessation is followed by cold acclimation (Junttila 1996). In grasses and herbaceous species, data on impacts of photoperiod on cold hardening are still scarce. Although temperature seems to be the main factor, cold acclimation of grasses is also triggered by photoperiod, especially at higher temperatures (Malyshev et al. 2014). Likewise, hardening of white clover is enhanced by short photoperiod (Junttila et al. 1990). Recent studies show that the C-repeat binding factor (CBF) cold acclimation pathway in Arabidopsis is regulated by photoperiod (Lee and Thomashow 2012). At higher temperatures, long days caused repression of the CBF pathway, while short days relieved the repression resulting in increased freezing tolerance. Cold acclimation of grasses is also affected by the light intensity (Pollock et al. 1988, Harrison et al. 1997, Höglind et al. 2010), light quality and length of the hardening period (Sjøseth 1964). Winter-hardy cultivars of grasses start hardening earlier and achieve a higher freezing tolerance than southern-adapted cultivars (Larsen 1994). Longer days stimulate dry matter production in perennial grasses (Hay 1990), and the growth of cultivars adapted to higher northern latitudes are most sensitive to photoperiod (Heide 1982, Solhaug 1991). The mechanism behind growth cessation of grasses is still poorly understood (Rapacz et al. 2014) but northern-adapted forage grasses seem to have a specific mechanism for growth inhibition during autumn (Østrem et al. 2014).

The light regime at northern high latitudes is profoundly different from light regimes at temperate and tropical latitudes (Nilsen 1985). In autumn, the daylength and the global irradiance decrease rapidly with modifying effects of clouds (Fig. 1). The light quality is unique at higher latitudes, with diurnal alterations in the ratio of red and far red light different from at lower latitudes (Nilsen 1985). According to future climate projections, the onset of low positive temperatures required for cold acclimation (<10°C) will occur later in the autumn and under considerably shorter daylength and lower irradiance than today (IPCC 2013). The question arises how higher temperature in combination with reduced irradiance and shorter daylength will affect cold acclimation and freezing tolerance of plants in future climate.

Timothy is the most common forage grass species in Northern Norway because of its superior winter survival and good growth at low temperatures and long days. However, with growth seasons extending into late autumn due to climate changes, perennial ryegrass may be better adapted in the north than currently. The purpose of this study was to understand better the

physiological responses of these forage grass species to climate changes in order to breed new improved cultivars for future climatic conditions at higher northern latitudes.

We compared photoacclimation, growth rates and freezing tolerance of promising breeding populations of timothy (*Phleum pratense* L.) and perennial ryegrass (*Lolium perenne* L.) under different combinations of autumn light period and temperature in a phytotron at Holt, Tromsø, Norway (69.68°N, 18.94°E). In the current experiment we exposed plants to natural light during autumn, as successive autumn light periods i.e. lowered irradiance and daylength (Fig. 1). Our hypotheses were that 1) hardening under natural light conditions later in the autumn, at shorter daylength and lower irradiance, reduces the freezing tolerance of timothy and perennial ryegrass; 2) high temperature (12°C) reduces hardening compared to low temperature (6°C), and variable day and night temperatures (9/3°C), compared with a constant temperature (6°C) affect hardening differently because of diurnal effects; 3) cold tolerance is determined by an interaction between temperature and irradiance/daylength, and 4) northern-adapted populations are more sensitive than southern-adapted populations to changing light and temperature conditions.

Material and Methods

Plant material and growth conditions

Two forage grass species, timothy and perennial ryegrass, were studied. Two populations of each species were included, one selected for the northern regions of Norway (northern-adapted) and the other for the southern regions of Norway (southern-adapted). The perennial ryegrass populations were FuRa9805 (southern-adapted) and Fagerlin (northern-adapted), and timothy populations MTL9701+Grindstad (southern-adapted) and MTV0508-3 (northern-adapted). For detailed description of populations see Dalmannsdottir et al. (in press). This plant material was composed with sufficient genetic variation for future breeding in a changing climate and was a part of a broader material used in a Norwegian research project on the genetic and physiological basis for adaptation of Norwegian perennial forage crops to future climates (VARCLIM). The experiment was performed with seedlings to obtain information on the breeding material.

The experiments were conducted in autumn 2012 at Holt, Tromsø (69.68°N, 18.94°E) in phytotron compartments with the temperature controlled to ±0.5°C and the air humidity corresponding to a water vapour deficit of 0.5 kPa. Seedlings were planted in tree nursery trays (60 pots in each tray, 1 plant/pot, pot size 40 mm diameter x 85 mm height, 45 mm spacing

between plants). The pots were filled with fertilised sphagnum peat and perlite (3:1). During establishment, the plants were grown for 4 weeks at 20°C, 24 h photoperiod. The light source was cool white fluorescent lamps (Philips TLD 58W 840), giving 150 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) at plant level (measured with a quantum sensor, Li-1000, Li-Cor) within the range 400-750 nm. During establishment and throughout the experiment, the plants were watered regularly and fertilised as required with a complete nutrient solution (Hoagland solution, modified from Asher 1978). After establishment, similar sized plants were selected for the experiment. Each population was planted in separate trays, which were placed randomly on trolleys within the phytotron compartments.

Experimental design

The whole experiment was conducted in a phytotron during three separate periods in autumn (year 2012) under natural light conditions; 5 Sept-10 Oct (early period), 26 Sept -31 Oct (intermediate period) and from 17 Oct-21 Nov (late period), resulting in three irradiance/daylength treatments here referred to as early, intermediate and late autumn period (Fig. 1). The daylengths decreased approximately from 14 to 9 h (early period), 11 to 6 h (intermediate period) and 8 to 2 h (late period) in the three periods.

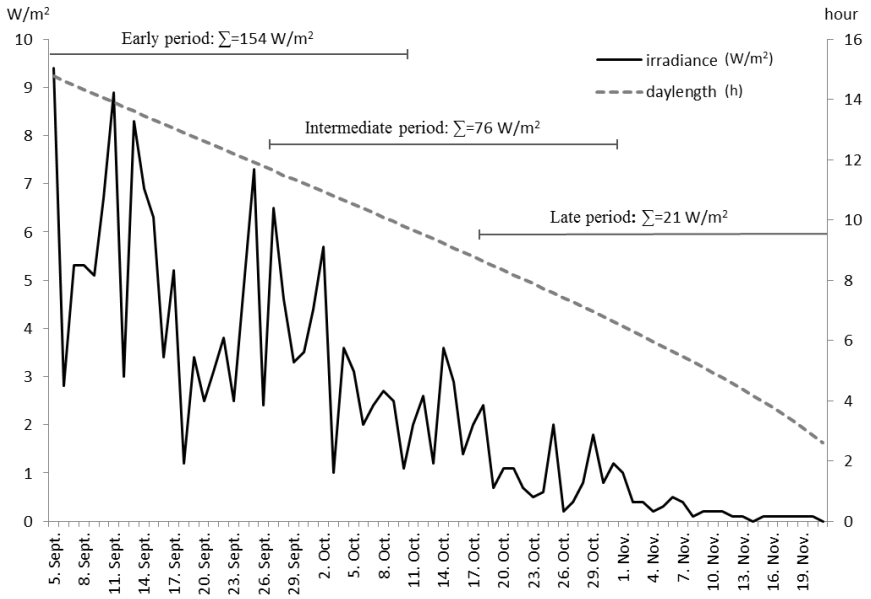

Figure 1. Experimental setup including global irradiation (W/m^2) (whole line) during experimental period in year 2012. A sum of radiation for each light period during autumn (early, intermediate, late) is presented. Daylength in Tromsø (hours) (dotted line).

The sum of global irradiance for each period decreased from 154, 76 to 21 W/m^2, respectively. Apart from the establishment phase, light conditions were natural light during the whole experiment.

After establishment, plants were subjected to three pre-acclimation temperature treatments, 6°, 9/3° (12 h day/12 h night) and 12°C, in combination with the three autumn periods (early, intermediate, late) (Fig. 1). The duration of the pre-acclimation temperature treatments was 4 weeks, and thereafter all populations were cold acclimated at 2°C for 1 week before freezing tests were conducted. The 6°C treatment resembles the current temperature in Sept-Oct in Northern Norway and 12°C an extreme temperature increase in the autumn based on future scenarios until 2050 (Uleberg et al. 2014). We used 6°C and 9/3° day/night (12h/12h) treatments to study the influence of changing day and night temperatures on pre-acclimation efficiency and subsequent freezing tolerance levels. Temperature loggers within chambers certified the temperature accuracy, but water vapour deficit was more unstable for the low temperatures (2 and 6°C), often 30-40% higher than programmed.

Morphological measurements

Dry weight of aboveground biomass of 15 plants per population per treatment was recorded at the start of the temperature treatment and at the end of the experiment, after drying at 60°C for 48 h. Leaf elongation (mm week^{-1}) was measured on 15 plants per treatment (in total 180 individuals for each photoperiod treatment). The youngest emerging leaf on each plant was marked with a thin rubber band and measured weekly during the 5 weeks of temperature treatment.

Chlorophyll fluorescence measurements

The photochemical activity of photosystem II (PSII) was studied by measuring chlorophyll fluorescence (PAM-2500 Portable Chlorophyll Fluorometer; Heinz Walz, Effeltrich, Germany) at room temperature on 15 plants per treatment at the start of treatment, before and after cold acclimation, on the same plants as were used to record leaf elongation. The measurements were made on the mid-section of the youngest fully expanded leaves. Before measuring maximum quantum yield of PSII (F_v/F_m) (indication of photoinhibition), leaves were dark-adapted for 15-60 min in leaf clips (8 mm diameter, Walz) and values of F'_m and F_s were recorded when F_s became stable after re-exposure to actinic red light (800 µmol). Within the same leaf clip, F'_0 was measured after far-red light treatment to ensure rapid opening of PSII reaction centres. Current quantum yield of PSII (ϕ_{PSII}) (photosynthetic activity indicator) and coefficients of the

photochemical (q_p) and non-photochemical (NPQ) quenching of chlorophyll fluorescence were calculated according to Genty et al. (1989), Schreiber et al. (1994) and Bilger and Björkman (1991), respectively.

Freezing test

At the end of the experiment freezing tests were performed as described by Pulli et al. (1996) with modifications (Höglind et al. 2010). Plant roots were washed and single plants were trimmed to 3 cm top and 1-2 cm root. The crown segments were placed in plastic boxes and covered with fine, humid sand in a programmable freezer with a temperature sensor in each box. Before freezing treatments commenced, the temperature was lowered from 2°C to -3°C by 1°C h^{-1} and kept at -3°C for 13 hours to avoid super-cooling of the plants. The boxes were then frozen to pre-determined temperatures between -3°C and -20°C with 2°C intervals, depending on species and treatment. The freezing occurred at a cooling rate of -1°C h^{-1} until -10°C was reached; from then at -3°C h^{-1} until the predetermined temperature was reached for each treatment. There were two replicate boxes per predetermined test temperature; each containing 10 crown segments per population per treatment, i.e. 480 plants per temperature treatment and autumn period summing up to a total number of 4320 plants. Two boxes per population per treatment were kept at 2°C in darkness as a control. After freezing, the boxes were placed at 2°C in the dark overnight to thaw, and the crown segments were transplanted into fertilised peat mixed with perlite. Survival of individual plants was rated as dead or alive and the LT_{50} value, i.e. the temperature at which 50% of plants are killed, was estimated after 3-4 weeks at 20°C and 24 h light (ca. 150µmol m^{-2} s^{-1}).

Statistical analysis

A generalised linear model approach was used to estimate the effects of pre-acclimation temperature on photosynthetic activity, biomass production and freezing tolerance. Model selection was based on the Akaike's Information Criteria corrected for small sample sizes (AICc) (Burnham and Anderson, 2002). For biomass production and photosynthetic activity, a linear model with Gaussian normal distribution and an identity link was assumed. The full model was defined as *Response ~ Treatment*Population*Species*autumn period,* where temperature treatment, population and autumn period are treated as categorical variables. Four entries were removed as diagnostic plots indicated them as outliers. Data was log-transformed when variance was heterogeneous, however, untransformed mean values are presented here for clarity. For the freezing test, a logistic model with logit link function was used. In some of the

models there was no overlap of freezing temperature for the group of dead and surviving individuals, hence penalized likelihood was used to remove bias (library brglm in R) (Kosmidis and Firth 2009). The full model for each species was defined as *Response ~ Treatment*Population*Pre-determined freezing temperature*. We used a non-linear, three-parameter asymptotic mixed model for estimating leaf elongation (function nlmer in the R library lme4). The growth trajectory is described by the function $Leaf\ length \sim Asym + (R_0\text{-}Asym)e^{-e(lrc \times week)}$, where parameters describe the intercept (R_0), the asymptote (*Asym*) and the logistic rate constant (*lrc*) (Crawley 2007). Individual plant identity was included as a random term to avoid pseudoreplication. To evaluate differences in leaf elongation, 95% confidence intervals were compared. For non-linear mixed models, the confidence interval was approximated by mean ± 2 x SE (Gelman and Hill 2008). Predictors were considered significant if their 95% confidence interval did not include zero. All statistical analyses were performed using R (R version 3.0.1) and Minitab 16 (Minitab Inc. 2010, State College, PA, USA). Model comparisons and population statistics are presented in supplementary table S1-14.

Results

Biomass production

Total biomass production decreased gradually from the early autumn period to the late period. However, the decrease was also dependent on temperature (Table 1). During the early autumn period markedly more biomass was produced at 12°C compared to 6 and 9/3°C (Table 1). During the intermediate autumn period, all plants except the northern-adapted population of perennial ryegrass at 6°C produced about 40% less biomass than plants at the two other temperatures (Table 1). During the late autumn period there was no difference in biomass production between temperature treatments. We found no consistent differences in biomass production between northern and southern-adapted populations. However, perennial ryegrass generally produced more biomass than timothy, especially the northern-adapted population, which produced more biomass at 6°C and during the early autumn period than the southern-adapted.

Leaf elongation

The effect of autumn period on leaf elongation was dependent on the temperature treatment and population. Leaf elongation rate was always higher at 12°C than at 6°C and 9/3°C for all

Table 1. Mean values of total biomass of plants (mg dw^{-1}) produced during the experimental treatment for perennial ryegrass (PRG) and timothy, both northern and southern-adapted populations. Confidence intervals (95%) are presented in brackets (lower, upper).

Populations	Temp. °C	autumn period					
		Early		Intermediate		Late	
PRG southern	6	93	(75, 111)	53	(39, 67)	20	(8, 32)
	9/3*	145	(114, 176)	94	(78, 110)	17	(5, 29)
	12	307	(262, 352)	80	(60, 100)	30	(16, 44)
PRG northern	6	166	(137, 195)	55	(37, 73)	16	(4, 28)
	9/3*	147	(125, 169)	91	(64, 118)	32	(14, 50)
	12	259	(216, 302)	129	(104, 154)	33	(13, 53)
Timothy southern	6	55	(43, 67)	49	(37, 61)	16	(8, 24)
	9/3*	108	(90, 126)	77	(65, 89)	14	(6, 22)
	12	172	(135, 209)	88	(70, 106)	21	(11, 31)
Timothy northern	6	76	(56, 96)	41	(29, 53)	17	(7, 27)
	9/3*	84	(70, 98)	67	(57, 77)	19	(11, 21)
	12	237	(188, 296)	87	(71, 103)	22	(18, 26)

*day/night

autumn periods and populations (Table 2, Fig. 2). The effect of temperature on leaf elongation was more pronounced at the late autumn period (Table 2, Fig. 2). Leaf elongation of plants at 12°C was faster at later autumn periods (reflected in higher lrc value, Table 2), though not significantly different for the southern-adapted population of perennial ryegrass at the early and intermediate autumn periods. In the late autumn period, leaves ceased growth earlier at 6°C than at 12 or 9/3°C resulting in longer leaves at 12 and 9/3°C (reflected in lower asymptote values, Table 2). Only the southern-adapted population of perennial ryegrass showed no difference in elongation rate between treatments at 6 and 9/3°C in the early period. During the intermediate autumn period, all populations grew significantly faster at 9/3°C compared to 6°C. In the late autumn period, the same effect was seen for perennial ryegrass, but was not significant in the timothy populations. There was no general difference between southern and northern-adapted populations regarding leaf elongation rate.

Chlorophyll fluorescence

The F_v/F_m values were significantly higher in the intermediate autumn period than in the early and late autumn periods, and similar for all temperature treatments (Fig. 3). In the early autumn period, plants at 12°C generally had higher F_v/F_m, except for plants of the southern-adapted timothy population (Fig. 3). In the late autumn period, plants at 6°C generally displayed the highest F_v/F_m values. The NPQ values were highest in the intermediate autumn period for both species, as observed with F_v/F_m. In timothy, plants at 12°C had the lowest and

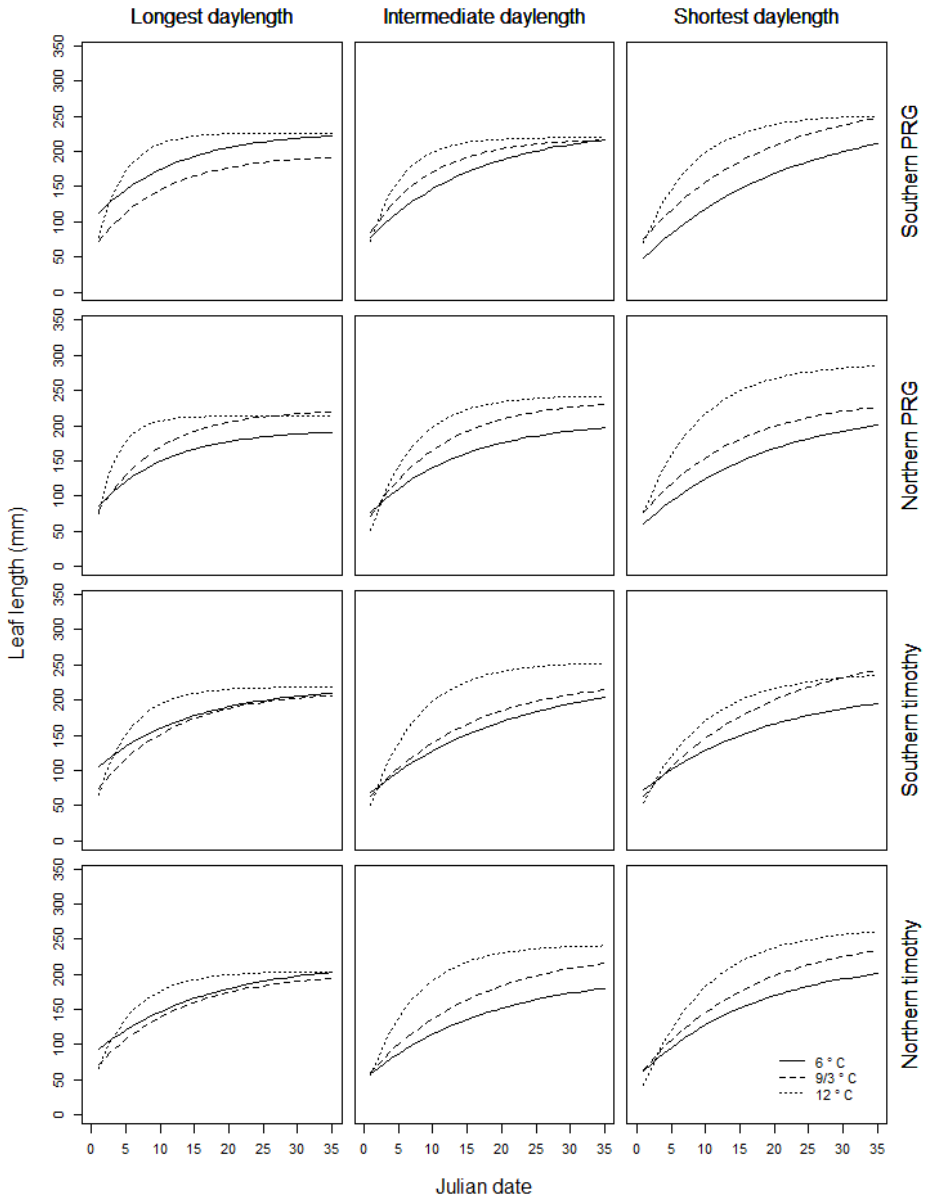

Figure 2. Estimated values of leaf elongation (mm week^{-1}) for timothy and perennial ryegrass (PRG) measured every week during 4 weeks of treatment with pre-acclimation temperatures 6°C, 9/3°C (day/night) and 12°C, and 1 week of treatment with cold acclimation at 2°C for early, intermediate and late autumn period.

Table 2. Influence of temperature on elongation growth of leaves, estimated by a non-linear asymptotic model. Elongation growth is expressed as estimated average logistic rate constants (lrc) and estimated asymptote (asym). Confidence intervals (95%) are presented in brackets (lower, upper).

	Temp.	Early period				Intermediate period				Late periode			
Population	°C	lrc		Asym		lrc		Asym		lrc		Asym	
PRG	6	-2.47	(-2.49, -2.46)	229	(229, 231)	-2.75	(-2.92, -2.58)	234	(217, 254)	-3.08	(-3.09, -3.06)	253	(253, 255)
southern	9/3*	-2.30	(-2.39, -2.21)	196	(183, 210)	-2.19	(-2.21, -2.17)	219	(219, 221)	-2.87	(-2.88, -2.85)	275	(275, 277)
	12	-1.39	(-1.54, -1.24)	227	(205, 250)	-1.53	(-1.61, -1.45)	220	(207, 234)	-2.00	(-2.03, -1.98)	252	(252, 254)
PRG	6	-2.33	(-2.55, -2.12)	195	(174, 218)	-2.62	(-2.64, -2.60)	208	(208, 210)	-2.93	(-3.04, -2.82)	228	(207, 253)
northern	9/3*	-2.20	(-2.37, -2.04)	223	(196, 252)	-2.39	(-2.56, -2.21)	238	(218, 260)	-2.65	(-2.68, -2.62)	241	(242, 244)
	12	-1.09	(-1.10, -1.08)	213	(213, 215)	-1.82	(-1.84, -1.81)	243	(243, 245)	-2.11	(-2.21, -2.01)	288	(271, 308)
Timothy	6	-2.63	(-2.78, -2.48)	220	(205, 237)	-3.02	(-3.25, -2.80)	235	(216, 257)	-2.94	(-2.97, -2.91)	219	(219, 221)
southern	9/3*	-2.40	(-2.56, -2.24)	213	(198, 230)	-2.71	(-2.91, -2.51)	232	(205, 262)	-2.91	(-3.13, -2.69)	276	(250, 303)
	12	-1.58	(-1.61, -1.56)	219	(218, 220)	-1.94	(-1.96, -1.92)	254	(254, 256)	-2.21	(-2.23, -2.18)	240	(240, 242)
Timothy	6	-2.77	(-2.98, -2.56)	218	(205, 233)	-2.88	(-3.10, -2.67)	201	(176, 229)	-2.84	(-2.85, -2.82)	222	(222, 224)
northern	9/3*	-2.52	(-2.66, -2.38)	203	(189, 219)	-2.76	(-2.87, -2.64)	236	(220, 253)	-2.82	(-2.92, -2.72)	260	(242, 281)
	12	-1.73	(-1.82, -1.64)	204	(186, 225)	-1.94	(-2.01, -1.86)	242	(229, 258)	-2.21	(-2.32, -2.11)	266	(250, 284)

*day/night

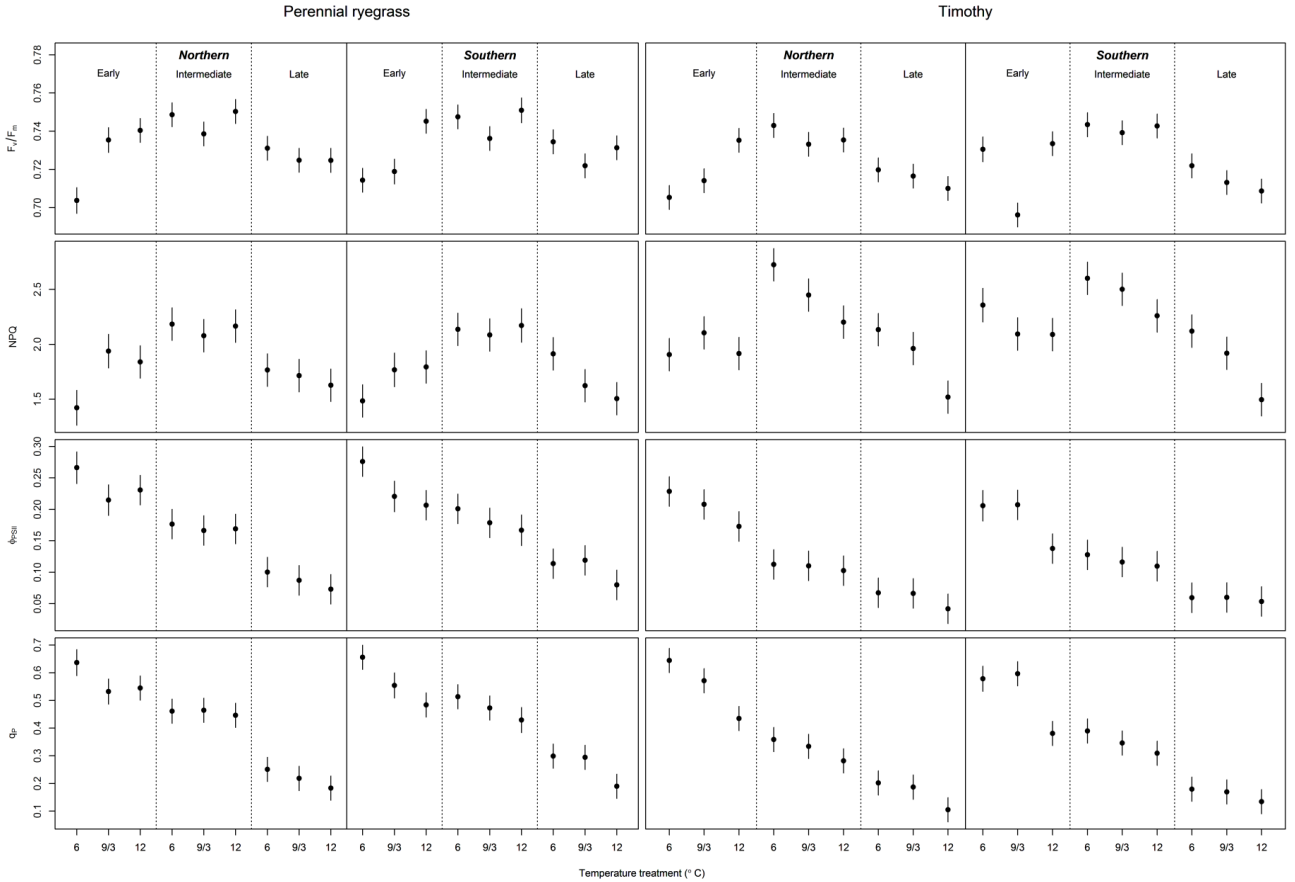

Figure 3. Changes in fluorescence parameters in southern and northern-adapted populations of perennial ryegrass and timothy measured after the three different pre-acclimation temperature treatments. Estimated mean values with 95% confidence intervals for full parametric modell are presented. Abbrevations: E=Early period, I=intermediate period, L=Late period

plants at 6°C the highest NPQ values at both late and intermediate autumn periods (Fig. 3), whereas at the early autumn period there were no significant differences between temperature treatments. This temperature effect was not seen in the northern-adapted and only in the late autumn period in the southern-adapted perennial ryegrass.

Photosynthetic activity (ϕ_{PSII}) was affected more by autumn period than temperature; it decreased with later autumn periods and increasing temperature, and was higher or similar at 9/3°C compared to 6°C (Fig. 3). Photochemical quenching (q_p) showed similar trends as ϕ_{PSII}. Starting values of each population for each temperature and autumn period treatment were, with few exceptions, not significantly different. F_v/F_m varied more at the starting point than NPQ, q_p and ϕ_{PSII}. Since these parameters showed no consistent differences, and their starting points varied much less than after the treatments, they are not presented.

No significant differences were found between northern- and southern-adapted populations regarding photochemical activity.

Freezing tolerance

Plants pre-acclimated in the late autumn period and at the highest temperature displayed lowest freezing tolerance irrespective of species and population (Table 3, Fig. 4). Plants pre-acclimated at 12ºC were less freezing tolerant than plants acclimated at 6º and 9/3ºC. There were no significant differences in freezing tolerance between the 6º and 9/3ºC treatments. There was no significant differences between plants pre-acclimated in the early and the intermediate autumn period. Northern-adapted populations had higher freezing tolerance,

Table 3. Freezing tolerance as LT_{50} value (temperature with 50% plant survival) of perennial ryegrass (PRG) and timothy after different temperature and autumn period treatments. Confidence intervals (95%) are presented in brackets (lower, upper).

		Autumn period					
Population	Temp. °C	Early LT_{50}		Intermediate LT_{50}		Late LT_{50}	
PRG southern	6	-14.0	(-14.4, -13.6)	-14.6	(-15.1, -14.1)	-11.6	(-12.2, -11.0)
	9/3*	-14.2	(-14.6, -13.8)	-13.8	(-14.3, -13.3)	-10.7	(-11.2, -10.2)
	12	-12.2	(-12.6, -11.8)	-10.3	(-10.8, -9.8)	-7.0	(-7.6, -6.3)
PRG northern	6	-15.8	(-15.2, -16.4)	-17.3	(-18.0, -16.5)	-13.6	(-14.2, -13.0)
	9/3*	-15.4	(-16.0, -14.9)	-15.8	(-16.3, -15.4)	-12.9	(-13.5, -12.3)
	12	-13.9	(-14.4, -13.5)	-11.7	(-12.3, -11.1)	-7.0	(-7.6, -6.4)
Timothy southern	6	-15.1	(-15.7, -14.4)	-14.9	(-15.8, -14.1)	-10.5	(-11.1, -9.8)
	9/3*	-14.2	(-14.9, -13.6)	-16.8	(-17.7, -16.0)	-9.9	(-9.2, -10.5)
	12	-11.1	(-11.7, -10.6)	-11.4	(-11.9, -10.9)	-7.7	(-8.3, -7.1)
Timothy northern	6	-17.7	(-18.5, -16.9)	-17.4	(-18.3, -16.5)	-11.8	(-12.5, -11.1)
	9/3*	-16.0	(-16.6, -15.3)	-17.1	(-17.6, -16.6)	-12.2	(-12.9, -11.5)
	12	-12.7	(-13.3, -12.1)	-12.3	(-13.0, -11.7)	-8.2	(-8.9, -7.6)

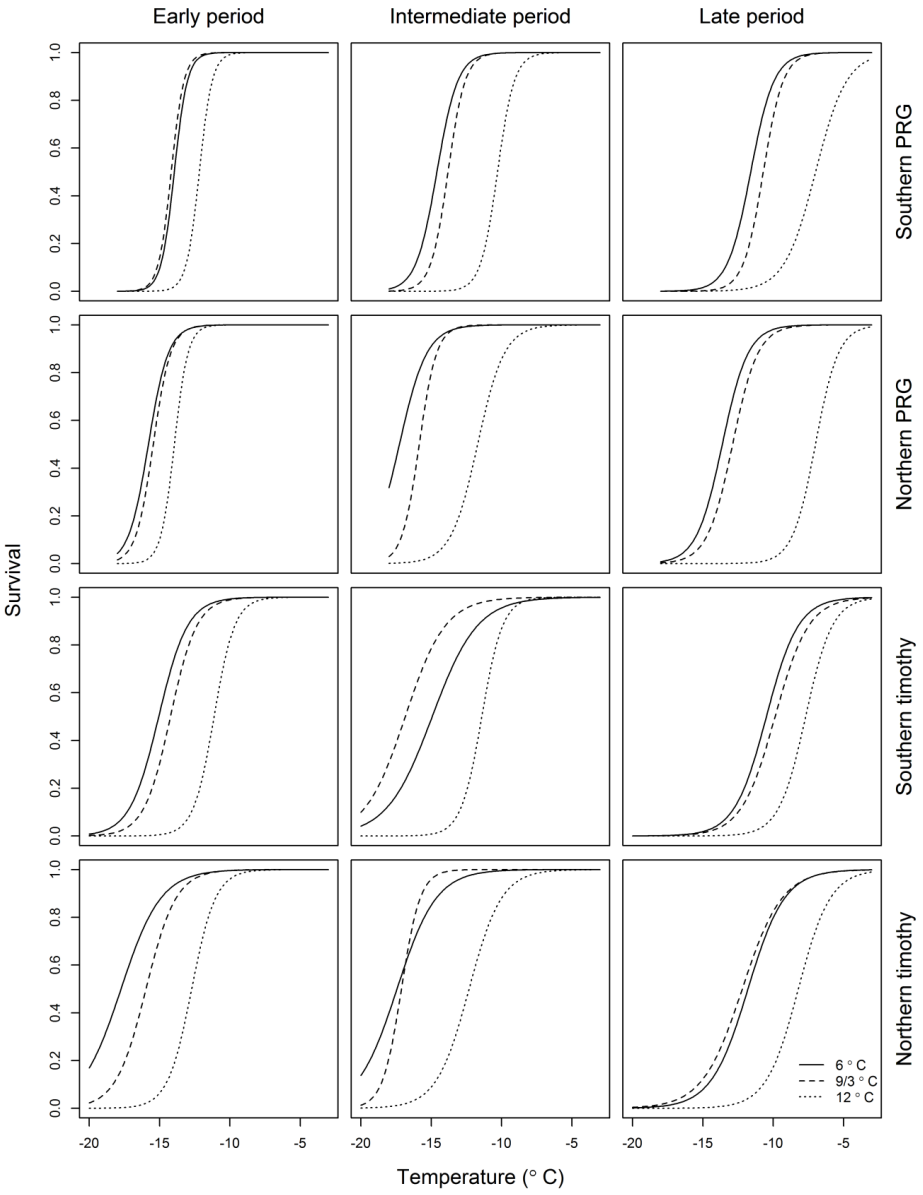

Figure 4. Survival of plant populations as a function of freezing temperature (°C) in a freezing test at the end of the experiment. The line at 50% survival indicates the LT_{50} value for the population. Predicted values are presented for each temperature treatment (6°C, 9/3°C, 12°C) and each light period during autumn (early, intermediate, late).

except in the late autumn period and at the highest acclimation temperature where there were no significant difference between northern and southern-adapted populations. In the intermediate and the late autumn period freezing tolerance was more strongly reduced by the 12°C than the 6°C temperature treatment in northern-adapted populations compared to southern-adapted.

Discussion

We found that interactions between temperature and daylength/irradiance had strong effects on growth, cold acclimation and freezing tolerance of timothy and perennial ryegrass populations with diverse adaptations. A combination of low irradiance/short daylength and higher than normal temperatures, a scenario expected with global warming at higher latitudes, reduced freezing tolerance and photosynthetic activity substantially in all populations. The northern-adapted populations generally had higher freezing tolerance than the southern-adapted, but not at the combination of shortest daylength and highest temperature. This indicates that populations adapted at higher latitudes are vulnerable to the predicted climate changes, which will be most pronounced in these regions.

Temperature and daylength/irradiance effects on growth

All populations responded to a lower irradiance and shorter daylength with reduced dry-matter production. This is in accordance with previous studies of timothy and other high latitude grass species (Heide et al. 1985, Solhaug 1991, Wu et al. 2004). In our study, leaf elongation rate was similar (at 6°C) or increased (9/3°C and 12°C) at later autumn periods. At later autumn light periods, the plants (especially timothy) were suffering from low turgor pressure despite normal soil humidity (data not shown). Leaves of timothy were thin and etiolated, while leaves of perennial ryegrass were narrow but with more turgor than timothy, especially plants of northern-adapted perennial ryegrass. Etiolated growth is a well-known response to limited light conditions and has been shown to increase in timothy and perennial ryegrass during autumn (Moriyama et al. 2003). Peri et al. (2007) also found that etiolated pastures with cocksfoot produced less dry matter. Schnyder and Nelson (1988) found that leaf elongation in tall fescue was up to 65% faster during the dark period during the diurnal cycle, depending on the light intensity and temperature shift. Leaf elongation in grasses is known to increase at higher temperatures and this was also the case in our study and in a previous study where the same populations were tested for temperature response (Dalmannsdottir et al. 2015). Different

day/night temperature, compared to corresponding constant temperature, did affect both dry-matter production and leaf elongation in interaction with autumn period. Higher day temperature (9/3°C) compared to a constant temperature (6°C) stimulated biomass production, but only at the intermediate autumn period. Leaf elongation was stimulated at 9/3°C, but not at 6°C, as a respond to lower irradiance and shorter daylength in our study. Junttila (1985) found that shoot elongation of timothy cultivars was stimulated by alternating temperatures compared to corresponding constant temperatures. The same effect has been shown in pea (*Pisum sativum*) (Grindal et al. 1998) and oilseed rape (*Brassica napus* L. *var. oleifera*) (Rapacz 1998). Stimulation of dry matter production by long days and low temperature is generally stronger during the growing season in winter hardy grasses adapted to high latitude areas (Solhaug 1991, Østgård and Eagles 1971). Our results did not show significant differences in biomass production between northern and southern-adapted populations, except for the northern-adapted population of perennial ryegrass, which produced more dry-matter at 6°C and during the early autumn period compared to the southern-adapted. Furthermore, at the late autumn period, we observed that the northern-adapted populations of both species had a denser growth than the southern-adapted. The northern adapted population of perennial ryegrass, cv. 'Fagerlin', showed relatively high photosynthetic activity and reduced leaf elongation growth in the autumn in a field study in Norway together with high coverage in the following spring (Østrem et al. 2014). These results together with our findings indicate that cv. 'Fagerlin' may be able to utilize a prolonged growth season without sacrificing the level of freezing tolerance. This cultivar may thus be a promising germplasm resource for future breeding programs.

Temperature and daylength effects on photoacclimation

Cold acclimation is known to increase photosynthetic performances (Yamasaki et al. 2002; Hüner et al. 2014) which results in higher PSII photosynthetic activity at lower temperatures (Dalmannsdottir et al. in press). This is supported by our study especially under early autumn light conditions. Photosynthetic activity was more affected by autumn period than temperature, and light conditions during later autumn periods reduced the photosynthetic activity in all populations. Increasing q_p with increasing irradiance/daylength and decreasing temperature shows that the photochemical acclimation mechanism was more predominant than the non-photochemical mechanism, as demonstrated before in a response to temperature in studies with winter rye (Huner 1985) and oilseed rape (Rapacz and Janowiak 1998). Our results did not indicate active NPQ mechanism, but the NPQ values observed in timothy at the two later autumn periods may be caused by etiolation of leaves rather than temperature. In etiolated

leaves and leaves at low light intensities, the amount of active PSII reaction centres is reduced (Miyata et al. 2012) as an adaptive response to protect the photosystem (Tikkanen et al. 2014), resulting in lower NPQ values. The higher sensitivity of timothy compared to perennial ryegrass in relation to leaf etiolation is reflected in the fluorescence measurements. NPQ mechanisms have been found to dissipate excess light during cold acclimation in winter hardy grass species (Humphreys et al. 2007) and northern-adapted cultivars (Rapacz et al. 2004). In our study, there were no differences between southern and northern-adapted populations in respect to q_p mechanisms. A slightly higher ϕ_{PSII} at 6°C compared to 9/3°C indicates lower excitation pressure in plants at 6°C because of lower temperature during the daylight period. Photoinhibition was observed at the early autumn period in combination with low temperature. A shift from the early autumn period (5 Sept -10 Oct) to the intermediate (26 Sept - 31 Oct) reduced the damages of PSII, expressed as higher F_v/F_m values. However, reduction of the autumn light conditions, as within the late autumn period, decreased the Fv/Fm values again probably because the irradiance was below a critical limit for the induction of photoacclimation. After cold acclimation at 2°C the only change in photochemical activity was an increase in F_v/F_m values at the early autumn period, especially at lower temperature (result not presented). Therefore the more active photochemical mechanism of photoacclimation induced during pre-acclimation at the early autumn period was observed as an increasing tolerance to cold-induced photoinhibition of photosynthesis during cold acclimation.

Temperature and daylength effect on freezing tolerance

The treatment with the early autumn period and highest acclimation temperature (12°C) resulted in the lowest freezing tolerance for both species and populations. We have shown that a rise in pre-acclimation temperature (9, 12, 15°C) under controlled light conditions decreased both cold acclimation capacity and photoacclimation in the same populations (Dalmannsdottir et al. in press). Malyshev et al. (2014) found temperature to be a stronger trigger of cold acclimation than photoperiod in an experiment with the grass species *Arrhenatherum elatius*. In the current study, northern-adapted populations had higher freezing tolerances than southern adapted except for the plants growing at the shortest photoperiod and highest temperature were there were no difference between northern and southern-adapted populations. This indicates that today's northern-adapted breeding material may lose its advantages over southern-adapted in the future climate.

Freezing tolerance was reduced in plants at the late autumn period compared to the early and the intermediate period. Treatment at later autumn light conditions includes reduction in

the total irradiation energy, and higher light intensity or irradiance is known to increase cold acclimation in perennial ryegrass (Pollock et al. 1988, Harrison et al. 1997). Light intensity is even more important for cold acclimation than photoperiod (Lawrence et al. 1973). In a pilot study in autumn 2011, the timothy cv. 'Grindstad' expressed lowest freezing tolerance when acclimated at later autumn light conditions (data not shown), significantly different for all three autumn period treatments. In the current study there were no differences between the early and the intermediate autumn period regarding freezing tolerance. Yearly differences with cloudy sky cause differences in irradiance and affect the cold acclimation process. Since we tested effects of natural light conditions during autumn, the effects of irradiance and daylength are confounded and cannot be separated in this study. It is likely that the reduction in irradiance is even more important than shorter daylength in relation to reduced performances of the populations.

We did not register any significant difference in freezing tolerance between 6 and 9/3°C. Studies of Sjøseth (1971) support these finding. On the other hand, Eagles and Williams (1992) found high day and low night temperatures (10/2°C) to give a positive effect on freezing tolerance of perennial ryegrass compared to a constant temperature (10°C). The effect of diurnal temperature differences on cold acclimation seems to be a complicated interaction between daylength, light quality and intensity.

Timothy is known to be considerably more winter hardy than perennial ryegrass (Sjøseth 1971, Jørgensen et al. 2010), but our study did not reveal this huge difference in freezing tolerance, possibly because plants did not reach maximal seasonal hardening after only five weeks of acclimation treatments. On the other hand, the freezing tolerance capacity *per se* does not seem to be the limiting factor for poor survival of perennial ryegrass in high latitude areas, other factors involved in seasonal adaptation like inadequate growth cessation (Østrem et al. 2014), low non-structural carbohydrate accumulation during winter (Østrem et al. 2011), low resistance to ice encasement (Höglind et al. 2010) and susceptibility to fungal diseases (Hofgaard et al. 2003) that contribute more to the low winter survival.

Photoacclimation processes responded more strongly to photoperiod than to temperature whereas freezing tolerance responded more to temperature than photoperiod. Both photoacclimation (photochemical quenching) and freezing tolerance was reduced with decreasing autumn light conditions and increasing temperature. In studies by Rapacz et al. (2004) winter survival of Festulolium grasses correlated with increased energy dissipation and lower photosynthetic activity of PSII before winter.

Our results indicate that the projected climate change in the north may reduce freezing tolerance in grasses because plants will be pre-acclimated at higher temperatures and shorter daylength. Current adapted breeding populations may have unacceptable freezing tolerance in future climate. The present species and cultivars may therefore have to be replaced by species and cultivars which are able to acclimate adequately under new daylength × temperature combinations, combinations which are unique in the global context. Future breeding programs for northern high-latitude areas will need adapted germplasm together with introgression of southern-adapted material in order to produce high yielding and persistent grass cultivars.

Author contributions

Dalmannsdottir, Jørgensen, Rapacz and Rognli designed, guided or participated in performing the experiment. Dalmannsdottir wrote the first draft and corrected the manuscript. Østrem and Larsen provided the plant material. Rødven did most statistical analysis and wrote the chapter on statistical analysis. All co-authors discussed results, reviewed and corrected the manuscript.

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References

Asher CJ. 1978. Natural and synthetic culture media for spermatophytes. In: Rechcigal M Jr., (Ed.), Handbook of Nutrition and Food, Section G: Diets, culture media, food supplements. Vol. III. Culture media for microorganisms and plants, CRC Press, Cleveland, Ohio, pp 575-609

Bertrand A, Castonguay Y (2003) Plant adaptations to overwintering stresses and implications of climate change. Can J Bot 81: 1145-1152

Bilger W, Björkman O (1991) Temperature dependence of violaxanthin de-epoxidation and non-photochemical fluorescence quenching in intact leaves of *Gossypium hirsutum* L. and *Malva perviflora* L. Planta 184: 226-234

Burnham KP, Anderson DR (2002) Model selection and multimodel inference: A practical information-theoretical approach, 2nd edn. Springer-Verlag, New York, 488 p

Chinnusamy V, Zhu J, Zhu J-K (2006) Gene regulation during cold acclimation in plants. Physiol Plant 126: 52-61

Cooper EJ (2014) Warmer shorter winters disrupt arctic terrestrial ecosystems. Annu Rev Ecol Evol Syst 45: 271-295

Crawley MJ (2007) The R book, 1st. edn. John Wiley & Sons, Ltd., West Sussex, pp 1-942

Dalmannsdottir S, Rapacz M, Jørgensen M, Østrem L, Larsen A, Rødven R, Rognli OA (2015) Temperature before cold acclimation affects cold tolerance and photoacclimation in timothy (*Phleum pretense* L.), perennial ryegrass (*Lolium perenne* L.) and red clover (*Trifolium pratense* L.). J Agron Crop Sci doi/10.1111/jac.12149/epdf

Eagles CF, Williams J (1992) Hardening and dehardening of *Lolium perenne* in response to fluctuating temperatures. Ann Bot 70: 333-338

Ensminger I, Busch F, Huner NPA (2006) Photostasis and cold acclimation: sensing low temperature through photosynthesis. Pysiol Plant 126: 28-44

Gelman A, Hill J (2008) Data analysis using regression and multilevel/hierarchical models. – Analytical methods for social research. Cambridge University Press, Cambridge, 652 p

Genty B, Briantais J-M, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim Biophys Acta 990: 87-92

Gray GR, Chauvin LP, Sarhan F, Huner NPA (1997) Cold acclimation and freezing tolerance (A complex interaction of light and temperature). Plant Physiol 114: 467-474

Grindal G, Junttila O, Reid JB, Moe R (1998) The response to gibberellin in *Pisum sativum* grown under alternating day and night temperature. J Plant Growth Regul 17: 161-167

Harrison J, Tonkinson C, Eagles C, Foyer C (1997) Acclimation to freezing temperatures in perennial ryegrass (*Lolium perenne*). Acta Physiol Plant 19: 505-151

Hay RKM (1990) The influence of photoperiod on the dry-matter production of grasses and cereals. New Phytol 116: 233-254

Heide OM (1982) Effects of photoperiod and temperature on growth and flowering in Norwegian and British timothy cultivars (*Phleum pratense* L.). Acta Agric Scand Sect B - Plant Soil Sci 32: 241–252

Heide OM, Hay RKM, Bauergröd H (1985) Specific daylength effects on leaf growth and dry-matter production in high-latitude grasses. Ann Bot 55: 579-586

Hofgaard IS, Vollsnes AV, Marum P, Larsen A, Tronsmo AM (2003) Variation in resistance to different winter stress factors within full-sib family of perennial ryegrass. Euphytica 134: 61-75

Höglind M, Bakken AK, Jørgensen M, Østrem L (2010) Tolerance to frost and ice encasement in cultivars of timothy and perennial ryegrass during winter. Grass Forage Sci 65: 431-445

Humphreys MW, Gasior D, Lesniewska-Bocianowska A, Rapacz M (2007) Androgenesis as a means of dissecting complex genetic and physiological controls: selecting useful gene combinations for breeding freezing tolerant grasses. Euphytica 158: 337-345

Huner NPA (1985) Acclimation of winter rye to cold-hardening temperatures results in an increased capacity for photosynthetic electron transport. Can J Bot 63: 506-511

Hüner NPA, Öquist G, Hurry VM, Kroll M, Falk S, Griffith M (1993) Photoynthesis, photoinhibition and low temperature acclimation in cold tolerant plants. Photosynth Res 37: 19-39

Hüner NPA, Bode R, Dahal K, Hollis L, Rosso D, Krol M, Ivanov AG (2012) Chloroplast redox imbalance governs phenotypic plasticity: the ″grand design of photosynthesis″ revisited. Front Plant Sci 3: 255. doi: 10.3389/fpls.2012.00255

Hüner NPA, Bode R, Dahal K, Busch FA, Possmayer M, Szyszka B, Rosso D, Ensminger I, Krol M, Ivanov AG, Maxwell DP (2013) Shedding some light on cold acclimation, cold adaptation, and phenotypic plasticity. Botany 91: 127-136

Hüner NPA, Dahal K, Kurepin L, Savitch L, Singh J, Ivanov AG, Kane K, Sarhan F (2014) Potential for increased photosynthetic performance and crop productivity in response to climate change: role of CBFs and gibberellic acid. Front Chem 2: 18. doi: 10.3389/fchem.2014.00018

IPCC (2013) Climate Change 2013. The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, pp 1535. doi:10.1017/CBO9781107415324

Jørgensen M, Østrem L, Höglind M (2010) De-hardening in contrasting cultivars of timothy and perennial ryegrass during winter and spring. Grass Forage Sci 65: 38-48

Junttila O (1985) Experimental control of flowering and vivipary in timothy (*Phleum pratense*). Pysiol Plant 63: 35-42

Junttila, O (1996) Plant adaptation to temperature and photoperiod. Agr Food Sci Finland 5: 251-260

Junttila O, Svenning MM, Solheim B (1990) Effects of temperature and photoperiod on frost resistance of white clover (*Trifolium repens*) ecotypes. Physiol Plant 79: 427-438

Keily J, MacGregor DR, Smith RW, Millar AJ, Hallliday KJ, Penfield S (2013) Model selection reveals control of cold signalling by evening-phased components of the plant circadian clock. Plant J 76: 247-257

Kosmidis I, Firth D (2009) Bias reduction in exponential family nonlinear models. Biometrika 96: 793–804

Larsen A (1994) Breeding winter hardy grasses. Euphytica 77: 231-237

Lawrence T, Cooper JP, Breese EL (1973) Cold tolerance and winter hardiness in *Lolium perenne*: II. Influence of light and temperature during growth and hardening. J Agric Sci 80: 341-348

Lee C-M, Thomashow MF (2012) Photoperiodic regulation of the C-repeat binding factor (CBF) cold acclimation pathway and freezing tolerance in Arabidopsis thaliana. PNAS 109: 15054-15059

Malyshev AV, Henry HAL, Kreyling J (2014) Relative effects of temperature vs. photoperiod on growth and cold acclimation of northern and southern ecotypes of the grass *Arrhenatherum elatius*, Environ Exp Bot 106: 189-196

Miyata K, Noguchi K, Terashima I (2012) Cost and benefit of the repair of photodamaged photosystem II in spinach leaves: roles of acclimation to growth light. Photosynth Res 113: 165-180

Moriyama M, Abe J, Yoshida M (2003) Etiolated growth in relation to energy reserves and winter survival in three temperate grasses. Euphytica 129: 351-360

Miura K, Furumoto T (2013) Cold signaling and cold response in plants. Int J Mol Sci 14: 5312-5337.

Nilsen J (1985) Light climate in northern areas. In: Junttila O, Kauren Å (eds) Plant production in the north. Norwegian University Press, Oslo pp 62-72

Østgård O, Eagles CF (1971) Variation in growth and development in natural populations of Dactylis glomerata from Norway and Portugal. II. Leaf Development and Tillering. J Appl Ecol 8: 383-391

Østrem L, Rapacz M, Jørgensen M, Höglind M (2011) Effect of developmental stage on carbohydrate accumulation patterns during winter of timothy and perennial ryegrass. Acta Agric Scand Sect B – Plant Soil Sci 61: 153-163

Østrem L, Rapacz M, Larsen A, Dalmannsdottir S, Jørgensen M (2014) Influences of growth cessation and photoacclimation on winter survival of non-native *Lolium-Festuca* grasses in high-latitude regions. Environ Exp Bot 111: 21-31

Palva ET, Thtiharju S, Tamminen I, Puhakainen T, Laitinen R, Svenson J, Helenius E, Heino P (2002) Biological mechanism of low temperature stress response: Cold acclimation and development of freezing tolerance in plants. In: Iwanaga M (compiled by) Genetic Engineering of Crop Plants for Abiotic Stress JIRCAS Working Report 23, 2002, pp 9-15

Peri PL, Lucas RJ, Moot DJ (2007) Dry matter production, morphology and nutritive value of *Dactylis glomerata* growing under different light regimes. Agroforest Syst 70: 63-79

Pollock CJ, Eagles CF, Sims IM (1988) Effect of photoperiod and irradiance changes upon development of freezing tolerance and accumulation of soluble carbohydrate in seedlings of *Lolium perenne* grown at 2°C. Ann Bot 62: 95-100

Pulli S, Hjortsholm K, Larsen A, Gudleifsson B, Larsson S, Kristiansson B, Hoemmoe L, Tronsmo AM, Ruuth P, Kristiansson C (1996) Development and evaluation of laboratory testing methods for winter hardiness breeding. Nordic Gene Bank publication 32, Alnarp Sweden, 1996, 68 p

Quellet F, Charron J-B (2013) Cold acclimation and freezing tolerance in plants. In: eLS. John Wiley & Sons, Ltd: Chichester. DOI: 10.1002/9780470015902.a0020093.pub2

Rapacz M (1998) The after-effects of temperature and irradiance during early growth of winter oilseed rape (*Brassica napus* L. *var. oleifera, cv.* Góczański) seedlings on the progress of their cold acclimation. Acta Physiol Plant 20: 73-78

Rapacz M, Janowiak F (1998) Physiological effects of winter rape (*Brassica napus* var *oleifera*) prehardening to frost. I. Frost resistence and photosynthesis during cold acclimation. J Agron Crop Sci 181: 13-20

Rapacz M, Gąsior D, Zwierzykowski Z, Leśniewska-Bocianowska A, Humphreys HW, Gay AP (2004) Changes in cold tolerance and the mechanisms of acclimation of photosystem II to cold hardening generated by anther culture of *Festuca pratensis* × *Lolium multiflorum* cultivars. New Phytol 161: 105-114

Rapacz M, Wolanin B, Hura K, Tyrka M (2008) The effects of cold acclimation on photosynthetic apparatus and the expression of COR14b in four genotypes of barley (Hordeum vulgare) contrasting in their tolerance to freezing and high-light treatment in cold conditions. Ann Bot 101: 689-699

Rapacz M, Ergon Å, Höglind M, Jørgensen M, Jurczyk B, Østrem L, Rognli OA, Tronsmo AM (2014) Overwintering of herbaceous plants in a changing climate. Still more questions than answers. Plant Sci 225: 34-44

Sandve SR, Kosmala A, Rudi H, Fjellheim S, Rapacz M, Yamada T, Rognli OA (2011) Molecular mechanisms underlying frost tolerance in perennial grasses adapted to cold climtates. Plant Sci 180: 69-77

Schreiber U, Bilger W. and Neubauer C (1994) Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of in vivo photosynthesis. In: Schultze E-D, Caldwell MM (eds) Ecophysiology of photosynthesis. Germany Springer-Verlag, Berlin, pp 49-70

Schnyder H, Nelson CJ (1988) Diurnal growth of tall fescue leaf blades. Plant Physiol 86: 1070-1076

Sjöseth H (1964) Studies on frost hardening in plants. Acta Agr Scand XIV:178-192

Sjöseth H (1971) Winter hardiness in different meadow and pasture plants. Meldinger fra Norges Landbrukshøgskole 50: 1-39

Solhaug KA (1991) Influence of photoperiod and temperature on dry matter production and chlorophyll content in temperate grasses. Norw J Agr Sci 5: 365-384

Tomashow MF (1999) Plant cold acclimation: freezing tolerance genes and regulatory mechanisms. Annu Rev Plant Phys 50: 571-599

Tikkanen M, Mekala NR, Aro E-M (2014) Photosystem II photoinhibition-repair cycle protects photosystem I from irreversible damage. Biochim Biophys Acta 1837: 210-215

Uleberg E, Hanssen-Bauer I, van Oort B, Dalmannsdottir S (2014) Impact of climate change on agriculture in Northern Norway and potential strategies for adaptation. Clim Change 122: 27-39

Wingler A (2015) Comparison of signaling interactions determining annual and perennial plant growth in response to low temperature. Front Plant Sci 5: 794. doi: 10.3389/fpls.2014.00794

Wu Z, Skjelvåg AO, Baadshaug OH (2004) Quantification of Photoperiodic effects on growth of *Phleum pratense.* Ann Bot 94: 535-543

Yamasaki T, Yamakawa T, Yamane Y, Koike H, Satoh K, Katoh S (2002) Temperature acclimation of photosynthesis and related changes in photosystem II electron transport in winter wheat. Plant Physiol 128: 1087-1097

Yoshida M, Abe J, Moriyama M, Shimokawa S, Nakamura Y (1997) Seasonal changes in the physical state of crown water associated with freezing tolerance in winter wheat. Physiol Plant 99: 363-370

Supplement Paper II

Table S2. Parameter estimates for biomass production. Abbreviations: SD=shortest daylength, MD=intermediate daylength, LD=longest daylength. Reference value (intercept) represents treatment LD, treatment 12°C, population northern and species perennial ryegrass.

	Estimate	SE	t value	P-value
Intercept	5.880	0.096	60.980	<0.000
Treatment MD	-0.944	0.124	-7.583	<0.000
Treatment SD	-1.773	0.124	-14.243	<0.000
Treatment 6°C	-0.912	0.111	-8.191	<0.000
Treatment 9/3°C	-0.678	0.111	-6.088	<0.000
Species timothy	-0.637	0.091	-7.006	<0.000
Population southern	-0.290	0.111	-2.602	0.010
Treatment MD × treatment 6°C	0.313	0.136	2.297	0.022
Treatment SD × treatment 6°C	0.513	0.136	3.761	<0.000
Treatment MD × treatment 9/3°C	0.523	0.136	3.834	<0.000
Treatment SD × treatment 9/3°C	0.418	0.136	3.064	0.002
Treatment 6°C × population southern	0.040	0.111	0.364	0.716
Treatment 9/3°C × population southern	0.072	0.111	0.646	0.518
Treatment MD × population southern	-0.026	0.111	-0.229	0.819
Treatment SD × population southern	-0.129	0.111	-1.156	0.248
Treatment MD × species timothy	0.188	0.111	1.690	0.092
Treatment SD × species timothy	-0.189	0.111	-1.696	0.091
Species timothy× population southern	0.272	0.091	2.987	0.003

Table S1. Model comparison (10 best models) for biomass production. Abbreviations: D=Daylength, T=temperature treatment; Po=population; Sp=species; df=degrees of freedom; AICc=Akaike's information criterion corrected for small sample size; ΔAICc= difference in AICc-value to best model.

Intercept	T	D	Po	Sp	T×D	T×Po	T×Sp	D×Po	D×Sp	Po×Sp	T×D×Po	T×D×Sp	T×Po×Sp	D×Po×Sp	T×D×Po×Sp	R^2	df	AICc	ΔAICc
5.844	+	+	+	+	+	+		+	+	+	+					0.784	23	430.88	0
5.887	+	+	+	+	+				+	+						0.773	15	430.98	0.106
5.784	+	+	+	+	+	+		+	+	+	+			+		0.787	25	431.49	0.609
5.798	+	+	+	+	+	+	+	+	+	+	+					0.787	25	431.86	0.985
5.841	+	+	+	+	+		+		+	+						0.775	17	431.93	1.056
5.738	+	+	+	+	+	+	+	+	+	+	+			+		0.789	27	432.49	1.609
5.861	+	+	+	+	+			+	+	+						0.774	17	433.80	2.927
5.754	+	+	+	+	+	+	+	+	+	+	+		+			0.788	27	434.32	3.437
5.802	+	+	+	+	+			+	+	+				+		0.777	19	434.43	3.554
5.816	+	+	+	+	+		+	+	+	+						0.776	19	434.79	3.913

Table S3. Model comparison (10 best models) for F_v/F_m (maximum quantum yield of PSII) after pre-acclimation treatment. For Abbreviations see table S1.

Intercept	Pp	Po	Sp	T	D×Po	D×Sp	D×T	Po×Sp	Po×T	Sp×T	D×Po×Sp	D×Po×T	D×Sp×T	Po×Sp×T	D×Po×Sp×T	R^2	df	AICc	ΔAICc
0.742	+	+	+	+	+	+	+		+	+		+	+			0.575	28	-3136.74	0
0.742	+	+	+	+	+	+	+	+	+	+		+	+			0.575	29	-3134.97	1.768
0.742	+	+	+	+	+	+	+	+	+	+	+	+	+			0.577	31	-3133.43	3.308
0.741	+	+	+	+	+	+	+	+	+	+		+	+	+		0.577	31	-3132.60	4.140
0.742	+	+	+	+	+	+	+	+	+	+	+	+	+	+		0.579	33	-3131.02	5.712
0.740	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0.583	37	-3127.28	9.459
0.741	+	+	+	+		+	+		+	+			+			0.544	22	-3112.47	24.270
0.741	+	+	+	+		+	+	+	+	+			+			0.544	23	-3110.70	26.037
0.742	+	+	+	+	+	+	+		+	+			+			0.544	24	-3108.32	28.413
0.741	+	+	+	+		+	+	+	+	+			+	+		0.546	25	-3108.32	28.414

Table S4. Model comparison (10 best models) for NPQ (non-photochemical quenching of chlorophyll fluorescence) after pre-acclimation treatment. For Abbreviations see table S1.

Intercept	D	Po	Sp	T	D×Po	D×Sp	D×T	Po×Sp	Po×T	Sp×T	D×Po×Sp	D×Po×T	D×Sp×T	Po×Sp×T	D×Po×Sp×T	R^2	df	AICc	ΔAICc
1.849	+	+	+	+	+	+	+	+	+	+	+	+	+			0.555	31	235.98	0
1.837	+		+	+		+	+			+						0.575	15	236.00	0.011
1.818	+		+	+		+	+			+			+			0.577	19	236.07	0.084
1.868	+	+	+	+	+	+	+	+	+	+	+	+				0.577	27	236.34	0.352
1.852	+	+	+	+		+	+	+		+						0.579	17	237.15	1.165
1.832	+	+	+	+		+	+	+		+			+			0.583	21	237.25	1.263
1.807	+	+	+	+	+	+	+	+	+	+		+	+			0.544	29	237.27	1.289
1.864	+	+	+	+	+	+	+	+		+	+					0.544	21	237.32	1.334
1.843	+	+	+	+	+	+	+	+		+	+		+			0.544	25	237.36	1.370
1.826	+	+	+	+	+	+	+	+	+	+		+				0.546	25	237.65	1.664

Table S5. Model comparison (10 best models) for ϕ_{PSII} (Current quantum yield of PSII) after pre-acclimation treatment. For Abbreviations see table S1.

Intercept	D	Po	Sp	T	D×Po	D×Sp	D×T	Po×Sp	Po×T	Sp×T	D×Po×Sp	D×Po×T	D×Sp×T	Po×Sp×T	D×Po×Sp×T	R^2	df	AICc	ΔAICc
-1.591	+	+	+	+	+	+	+			+						0.721	18	315.17	0
-1.611	+	+	+	+	+	+	+	+		+						0.722	19	315.19	0.025
-1.625	+	+	+	+	+	+	+									0.718	16	315.27	0.101
-1.646	+	+	+	+	+	+	+	+								0.720	17	315.31	0.142
-1.614	+	+	+	+	+	+	+	+		+	+					0.724	21	315.99	0.824
-1.649	+	+	+	+	+	+	+	+			+					0.721	19	316.08	0.912
-1.580	+	+	+	+	+	+				+						0.716	14	316.09	0.926
-1.615	+	+	+	+	+	+										0.714	12	316.11	0.939
-1.601	+	+	+	+	+	+		+		+						0.717	15	316.11	0.946
-1.635	+	+	+	+	+	+		+								0.715	13	316.14	0.974

Table S6. Model comparison (10 best models) for q_p (photochemical quenching of chlorophyll fluorescence) after pre-acclimation treatment. For Abbreviations see table S1.

Intercept	D	Po	Sp	T	D×Po	D×Sp	D×T	Po×Sp	Po×T	Sp×T	D×Po×Sp	D×Po×T	D×Sp×T	Po×Sp×T	D×Po×Sp×T	R^2	df	AICc	ΔAICc
0.524	+	+	+	+	+	+	+			+			+			0.774	22	-1063.91	0
0.519	+	+	+	+	+	+	+	+		+			+			0.774	23	-1063.66	0.257
0.539	+	+	+	+	+	+	+	+	+	+			+	+		0.778	27	-1063.60	0.315
0.514	+		+	+		+	+			+			+			0.771	19	-1063.44	0.480
0.533	+	+	+	+	+	+	+		+	+			+			0.775	24	-1062.79	1.126
0.528	+	+	+	+	+	+	+	+	+	+			+			0.776	25	-1062.54	1.378
0.511	+	+	+	+		+	+			+			+			0.771	20	-1062.11	1.805
0.526	+	+	+	+		+	+	+	+	+			+	+		0.776	25	-1061.97	1.948
0.506	+	+	+	+		+	+	+		+			+			0.772	21	-1061.90	2.019
0.518	+	+	+	+	+	+	+	+		+	+		+			0.775	25	-1061.66	2.253

Table S7. Parameter estimates for maximum quantum yield of PSII (F_v/F_m) after pre-acclimation treatment. Abbreviations: SD=shortest daylength, MD=intermediate daylength, LD=longest daylength. Reference value (intercept) represents treatment LD, treatment 12°C, population northern and species perennial ryegrass.

	Estimate	SE	t value	P-value
Intercept	0.742	0.003	266.39	<0.000
Treatment MD	0.007	0.004	1.68	0.093
Treatment SD	-0.015	0.004	-3.89	<0.000
Treatment 6°C	-0.042	0.004	-10.44	<0.000
Treatment 9/3°C	-0.006	0.004	-1.58	0.115
Species timothy	-0.008	0.003	-2.63	0.009
Population southern	0.002	0.003	0.48	0.634
Treatment MD × population southern	0.002	0.005	0.55	0.586
Treatment SD × population southern	0.001	0.005	0.24	0.809
Treatment MD × species timothy	-0.003	0.005	-0.69	0.490
Treatment SD × species timothy	-0.010	0.005	-2.25	0.025
Treatment MD × treatment 6°C	0.042	0.006	7.40	<0.000
Treatment SD × treatment 6°C	0.047	0.006	8.31	<0.000
Treatment MD × treatment 9/3°C	-0.006	0.006	-1.05	0.295
Treatment SD × treatment 9/3°C	0.004	0.006	0.80	0.424
Treatment 6°C × population southern	0.017	0.005	3.58	<0.000
Treatment 9/3°C × population southern	-0.019	0.005	-4.09	<0.000
Treatment 6°C × species timothy	0.018	0.005	3.80	<0.000
Treatment 9/3°C × species timothy	-0.014	0.005	-2.95	0.003
Treatment MD × treatment 6°C × population southern	-0.021	0.006	-3.22	0.001
Treatment SD × treatment 6°C × population southern	-0.016	0.006	-2.53	0.012
Treatment MD × treatment 9/3°C × population southern	0.017	0.006	2.56	0.011
Treatment SD × treatment 9/3°C × population southern	0.013	0.006	2.01	0.045
Treatment MD × treatment 6°C × species timothy	-0.011	0.006	-1.66	0.098
Treatment SD × treatment 6°C × species timothy	-0.011	0.006	-1.66	0.097
Treatment MD × treatment 9/3°C × species timothy	0.024	0.006	3.70	<0.000
Treatment SD × treatment 9/3°C × species timothy	0.024	0.006	3.70	<0.000

Table S8. Parameter estimates for coefficient of the non-photochemical quenching of chlorophyll fluorescence (NPQ) after pre-acclimation. Abbreviations: SD=shortest daylength, MD=intermediate daylength, LD=longest daylength. Reference value (intercept) represents treatment LD, treatment 12°C, population northern and species perennial ryegrass.

	Estimate	Std error	t value	P-value
Intercept	1.786	0.066	27..21	<0.000
Treatment MD	0.368	0.093	3.96	<0.000
Treatment SD	-0.183	0.093	-1.97	0.050
Treatment 6°C	-0.469	0.096	-4.61	<0.000
Treatment 9/3°C	0.111	0.094	1.18	0.238
Species timothy	0.185	0.076	2.45	0.015
Population southern	0.063	0.076	0.84	0.403
Treatment MD × population southern	-0.032	0.108	-0.29	0.769
Treatment SD × population southern	-0.136	0.108	-1.27	0.207
Treatment MD × species timothy	-0.124	0.108	-1.15	0.249
Treatment SD × species timothy	-0.244	0.108	-2.28	0.023
Treatment MD × treatment 6°C	0.518	0.133	3.89	<0.000
Treatment SD × treatment 6°C	0.672	0.133	5.05	<0.000
Treatment MD × treatment 9/3°C	-0.198	0.132	-1.49	0.136
Treatment SD × treatment 9/3°C	-0.011	0.132	-0.08	0.936
Treatment 6°C × population southern	0.197	0.109	1.81	0.071
Treatment 9/3°C × population southern	-0.151	0.109	-1.40	0.163
Treatment 6°C × species timothy	0.496	0.109	4.57	<0.000
Treatment 9/3°C × species timothy	0.061	0.109	0.56	0.574
Treatment MD × treatment 6°C × population southern	-0.314	0.153	-2.06	0.040
Treatment SD × treatment 6°C × population southern	-0.057	0.153	-0.37	0.708
Treatment MD × treatment 9/3°C × population southern	0.149	0.153	0.98	0.330
Treatment SD × treatment 9/3°C × population southern	0.155	0.153	1.02	0.308
Treatment MD × treatment 6°C × species timothy	-0.055	0.153	-0.36	0.718
Treatment SD × treatment 6°C × species timothy	-0.150	0.1553	-0.99	0.325
Treatment MD × treatment 9/3°C × species timothy	0.271	0.153	1.77	0.077
Treatment SD × treatment 9/3°C × species timothy	0.269	0.152	1.76	0.078

Table S9. Parameter estimates for coefficient of the Current quantum yield of PSII (ϕ_{PSII}) after pre-acclimation. Abbreviations: SD=shortest daylength, MD=intermediate daylength, LD=longest daylength. Reference value (intercept) represents treatment LD, treatment 12°C, population northern and species perennial ryegrass.

	Estimate	Std error	t value	P-value
Intercept	-1.591	0.057	-27.94	<0.000
Treatment MD	-0.266	0.076	-3.52	<0.000
Treatment SD	-1.055	0.075	-13.97	<0.000
Treatment 6°C	0.276	0.068	4.05	< 0.000
Treatment 9/3°C	0.101	0.068	1.49	0.138
Species timothy	-0.265	0.062	-4.30	<0.000
Population southern	-0.056	0.048	-1.15	0.249
Treatment MD × population southern	0.132	0.068	1.94	0.052
Treatment SD × population southern	0.163	0.068	2.41	0.016
Treatment MD × species timothy	-0.268	0.068	-3.95	<0.000
Treatment SD × species timothy	-0.305	0.068	-4.50	<0.000
Treatment MD × treatment 6°C	-0.150	0.083	-1.81	0.071
Treatment SD × treatment 6°C	0.021	0.083	0.25	0.802
Treatment MD × treatment 9/3°C	-0.097	0.083	-1.17	0.243
Treatment SD × treatment 9/3°C	0.124	0.083	1.50	0.133
Treatment 6°C × species timothy	0.072	0.068	1.06	0.290
Treatment 9/3°C × species timothy	0.139	0.068	2.06	0.040

Table S10. Parameter estimates for coefficient of the photochemical quenching of chlorophyll fluorescence (q_p) after pre-acclimation. Abbreviations: SD=shortest daylength, MD=intermediate daylength, LD=longest daylength. Reference value (intercept) represents treatment LD, treatment 12°C, population northern and species perennial ryegrass.

	Estimate	Std error	t value	P-value
Intercept	0.524	0.017	30.37	<0.000
Treatment MD	-0.095	0.025	-3.89	<0.000
Treatment SD	-0.348	0.024	-14.26	<0.000
Treatment 6°C	0.134	0.023	5.82	<0.000
Treatment 9/3°C	0.029	0.023	1.26	0.207
Species timothy	-0.107	0.023	-4.73	<0.000
Population southern	-0.020	0.013	-1.48	0.139
Treatment MD × population southern	0.039	0.019	2.10	0.037
Treatment SD × population southern	0.040	0.019	2.13	0.033
Treatment MD × species timothy	-0.037	0.032	-1.14	0.254
Treatment SD × species timothy	0.040	0.032	1.25	0.213
Treatment MD× treatment 6°C	-0.085	0.032	-2.62	0.009
Treatment SD × treatment 6°C	-0.045	0.032	-1.41	0.160
Treatment MD × treatment 9/3°C	0.001	0.032	0.04	0.969
Treatment SD × treatment 9/3°C	0.041	0.032	1.27	0.205
Treatment 6°C × species timothy	0.071	0.032	2.20	0.028
Treatment 9/3°C × species timothy	0.147	0.032	4.57	<0.000
Treatment MD × treatment 6°C × species timothy	-0.041	0.045	-0.90	0.367
Treatment SD × treatment 6°C × species timothy	-0.088	0.045	-9.94	0.053
Treatment MD× treatment 9/3°C × species timothy	-0.133	0.045	-2.92	0.004
Treatment SD × treatment 9/3°C × species timothy	-0.158	0.045	-3.50	<0.000

Table S11. Model comparison (10 best models) for freezing tolerance of perennial ryegrass. Abbreviations: D=daylength; F= pre-determined freezing temperatures; Po=population; T=Temerature treatment; df=degrees of freedom; AICc=Akaike's information criterion corrected for small sample size; ΔAICc=difference in AICc-value to best model.

Intercept	D	F	Po	T	D×F	D×Po	D×T	F×Po	F×T	Po×T	D×F×Po	D×F×T	D×Po×T	F×Po×T	D×F×Po×T	R^2	df	AICc	ΔAICc
25.50	+	1.862	+	+	+		+	+								0.610	14	791.38	0
24.49	+	1.782	+	+	+		+	+	+							0.611	16	792.71	1.333
28.50	+	2.069	+	+	+	+	+	+								0.610	16	794.04	2.664
39.04	+	2.838	+	+	+	+		+								0.609	12	795.01	3.629
25.49	+	1.858	+	+	+		+	+		+						0.610	16	795.12	3.745
27.29	+	1.977	+	+	+	+	+	+	+							0.611	18	795.68	4.296
24.15	+	1.761	+	+	+		+	+	+	+						0.611	18	796.60	5.220
35.46	+	2.575	+	+	+	+		+	+							0.609	14	796.81	5.429
29.72	+	2.146	+	+	+	+	+	+			+					0.610	18	797.73	6.354
28.54	+	2.075	+	+	+	+	+	+		+						0.610	18	797.79	6.410

Table S12. Table S. Model comparison (10 best models) for freezing tolerance of timothy. For abbreviations see table S11.

Intercept	D	F	Po	T	D×F	D×Po	D×T	F×Po	F×T	Po×T	D×F×Po	D×F×T	D×Po×T	F×Po×T	D×F×Po×T	R^2	df	AICc	ΔAICc
12.88	+	1.024	+	+			+	+	+	+				+		0.559	18	1175.2	0
12.95	+	1.021	+	+		+	+	+	+	+				+		0.560	20	1177.2	1.995
14.72	+	1.159	+	+	+	+	+	+	+	+				+		0.560	22	1177.4	2.194
13.83	+	1.103	+	+	+		+	+	+	+				+		0.560	20	1177.4	2.224
13.79	+	1.101	+	+			+	+	+							0.557	14	1178.6	3.422
14.38	+	1.131	+	+		+	+		+	+			+			0.560	21	1179.0	3.823
14.50	+	1.155	+	+			+		+							0.556	13	1179.1	3.932
15.58	+	1.232	+	+	+	+	+	+	+							0.558	18	1179.5	4.295
16.03	+	1.261	+	+	+	+	+		+	+			+			0.560	23	1179.6	4.413
13.79	+	1.091	+	+		+	+	+	+							0.557	16	1179.6	4.418

Table S13. Parameter estimates (logit-transformed) of a logistic model for freezing tolerance for perennial ryegrass. Abbreviations: SD=shortest daylength, MD=intermediate daylength, LD=longest daylength. Reference value (intercept) represents northern-adapted population at 12°C and LD.

Predictor	Estimate	SE	z-value	p-value
Intercept	26.22	2.56	10.25	<0.000
Population southern	2.06	0.84	2.45	0.014
Treatment 6°C	3.82	0.59	6.52	<0.000
Treatment 9/3°C	3.79	0.58	6.49	<0.000
Treatment MD	-10.84	2.60	-4.17	<0.000
Treatment SD	-18.62	2.59	-7.19	<0.000
Freezing	1.91	0.19	10.02	<0.000
Treatment MD × freezing	-0.60	0.20	-2.95	0.003
Treatment SD × freezing	-0.86	0.20	-4.22	<0.000
Treatment 6°C × MD	3.43	0.84	4.10	<0.000
Treatment 9/3°C × MD	1.90	0.78	2.44	0.015
Treatment 6°C × SD	2.96	0.81	3.64	<0.000
Treatment 9/3°C × SD	2.01	0.76	2.59	0.010
Populations southern × freezing	0.37	0.07	5.33	<0.000

Table S14. Parameter estimates (logit-transformed) of a logistic model for freezing tolerance for timothy. Abbreviations: SD=shortest daylength, MD=intermediate daylength, LD=longest daylength. Reference value (intercept) represents northern-adapted population at 12°C and LD.

Predictor	Estimate	SE	z-value	p-value
Intercept	12.88	1.19	10.80	<0.000
Population southern	1.52	1.27	1.19	0.233
Treatment 6°C	0.03	1.68	0.02	0.983
Treatment 9/3°C	2.99	1.92	1.56	0.120
Treatment MD	-0.09	0.35	-0.25	0.800
Treatment SD	-4.52	0.51	-8.94	<0.000
Freezing	1.02	0.09	10.79	<0.000
Population southern × freezing	0.25	0.12	2.09	0.037
Treatment 6°C × freezing	-0.29	0.12	-2.48	0.013
Treatment 9/3°C × freezing	-0.02	0.14	-0.14	0.886
Treatment 6°C × MD	-0.10	0.47	-0.22	0.828
Treatment 9/3°C × MD	1.83	0.49	3.73	<0.000
Treatment 6°C × SD	0.32	0.66	0.49	0.626
Treatment 9/3°C × SD	0.82	0.66	1.23	0.217
Populations southern × Treatment 6°C	-1.13	1.73	-0.65	0.513
Populations southern × Treatment 9/3°C	-5.41	1.85	-2.92	0.004
Populations southern × Treatment 6°C × freezing	-0.10	0.15	-0.72	0.475
Populations southern × Treatment 9/3°C × freezing	-0.42	0.15	-2.80	0.005

Paper III

Hypothesis

I. The shortage of light energy available for photosynthesis at the northern location during autumn changes the relationship between photoacclimation, growth cessation and winter survival.

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Influences of growth cessation and photoacclimation on winter survival of non-native *Lolium–Festuca* grasses in high-latitude regions

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ABSTRACT

Autumnal growth cessation in non-native grass species is inadequate for sufficient cold acclimation. Cold acclimation is influenced to various degrees by growth cessation and photoacclimation. The effects of both factors on winter survival were investigated by measuring photosynthetic activity with Handy Pea fluorimeter and leaf elongation growth rate (LER) and their effects on winter survival. Triplicate field trials were established at two locations in Norway: Fureneset (61 °N) and Vågønes (67 °N). In total, ten entries of perennial ryegrass (*Lolium perenne*), *Festulolium* hybrids and introgression lines, and meadow fescue (*Festuca pratensis*) were investigated weekly by measuring selected leaves ($n = 3 \times 10$) of the regrowth after the third harvest taken in late August in two successive years.

The results showed that the relationship between photosynthetic performance in autumn, LER and winter survival differed between the locations. In the south (Fureneset), there was a positive correlation between photosynthetic activity before winter and winter survival. In the north (Vågønes), there was no correlation or even a negative correlation between photosynthetic activity before winter and winter survival. Low photosynthetic activity in autumn was associated with a higher level of winter survival in the north for *F. pratensis* cv. Norild and two northern-adapted cultivars of *L. perenne*. Northern-adapted forage grasses can be assumed to have an alternative mechanism for growth inhibition, since in the north the amount of light seems to be insufficient to trigger the changes in photosynthetic apparatus that are responsible for growth cessation in the south. Moreover, with progressing climate change, this adaptation pattern will increasingly be required in more southerly areas of the Nordic region because the light intensity decrease and temperature increase predicted for these areas will delay cold acclimation.

1. Introduction

The prolonged growing season and milder winters predicted in climate change scenarios (ACIA, 2005) may permit wider use of *Lolium perenne* L. (perennial ryegrass) and *Festulolium* in high latitude regions in future. The main challenge for these non-native species, i.e. grasses not naturally growing at these latitudes, is that they do not cease growth in autumn early enough for successful acclimation to the cold (Guy, 1990; Thomashow, 1999; Bocian et al., 2011). Growth cessation, i.e. reduction in leaf elongation growth rate and biomass production, is considered to be a precondition for proper cold acclimation, as has been characterised on a physiological level in *Brassica napus* (Rapacz, 1999) and on a molecular level in *Arabidopsis* mutant lines (Achard et al., 2008). In grasses, growth is reduced earlier in populations adapted to higher-latitude conditions with lowering temperature during autumn (Hay and Heide, 1984).

The initial stage of cold acclimation is dependent on light, so chloroplasts may play a central role as environmental sensors of temperature changes during cold acclimation (Ensminger et al., 2006; Crosatti et al., 2013). When the temperature decreases, a relative over-reduction in PSII is observed. This may trigger cold acclimation-related gene expression (Ndong et al., 2001), down-regulation of growth rate (Rapacz, 1998) and acclimation of the photosynthetic apparatus to cold (Sandve et al., 2011). Photoacclimation prevents or reduces the low temperature-induced

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photoinhibition that occurs when light energy absorption exceeds the capacity for light utilisation (Huner et al., 1993), causing increased excitation pressure within the PSII (Huner et al., 1998). The main mechanism of photoacclimation used by plants is to balance the energy flow within the PSII. At the same time, the excitation pressure is regulated by increasing the rate of energy utilisation or dissipating excess energy as heat, a mechanism known as non-photochemical quenching (Hüner et al., 2013). Thus, freezing tolerance, avoidance of cold-induced photoinhibition and growth cessation seem to be closely interrelated. The ability to avoid low temperature-induced photoinhibition has also been identified as a trait closely related to increased freezing tolerance (Huner et al., 1998; Pocock et al., 2001; Rapacz et al., 2007).

Perennial plants adapted to high-latitude areas grow rapidly during long days and at moderate summer temperatures, and cease growth with a longer photoperiod than plants from southern regions (Heide, 1974; Junttila, 1980). Studies with *L. perenne* have shown that cultivars adapted to northern Europe can acclimate better under shorter light conditions and lower growing temperatures than cultivars adapted to southern Europe (Lawrence et al., 1973). Furthermore, northern ecotypes of *Medicago sativa* (alfalfa) are reported to show increased winter hardiness when acclimated at low temperatures and short photoperiods, while southern ecotypes do not respond to the photoperiod (Hodgson, 1964). The lesser sensitivity of southern ecotypes to cold acclimation cues (light and temperature) is also confirmed in a recent paper (Malyshev et al., 2014). Heide (1985) concludes that many perennial grasses adapted to high latitude environments express specific photoperiodic effects on growth, where long days can compensate for considerable differences in temperature.

Cultivars of *L. perenne* and *Festulolium*, i.e. natural hybrids or hybrid-derivatives involving any *Lolium* and *Festuca* species' combination (Ghesquière et al., 2010) grow well even in high-latitude areas such as the Nordic region (Østrem et al., 2013), and their high yielding and regrowth capacity are appreciated in a prolonged growing season. Improved winter hardiness is the main breeding objective to increase the use of these species. In *Festulolium* the transfer of winter hardiness from *Festuca pratensis* Huds (meadow fescue) into *L. perenne*, and to a lesser degree *L. multiflorum* (Italian ryegrass), has been the main breeding objective either in allotetraploid hybrids or by introgression (Østrem et al., 2007; Ghesquière et al., 2010). The introduction of such non-native species to high-latitude regions necessitates a wide genotypic variability to identify the most suitable genotypes for local climatic adaptation (Rapacz et al., 2014).

Current plant breeding programmes require a more precise definition of all the characteristics contributing to winter damage in perennial grasses. The mechanism behind winter survival cannot be fully understood until there is understanding of the regulation of growth cessation (Bielenberg, 2011). The genetic variation for winter survival in *L. perenne* and *Festulolium* under different climatic conditions has been described, and a correlation ($r = 0.7$) between photosynthetic acclimation to cold and genotypic differences in winter survival in *Lolium* × *Festuca* hybrids has been demonstrated (Rapacz et al., 2004). The temperature rise in late summer and early autumn predicted for the coming decades may decrease cold acclimation ability, growth cessation and photoacclimation to cold (Rapacz, 2002). In high-latitude regions, these processes will be exacerbated by decreased PSII reduction, as the low autumn temperatures needed for cold acclimation are expected to occur at shorter photoperiods and lower light radiation.

The aim of the present study was to examine possible relationships between leaf elongation growth rate and photosynthetic apparatus characteristics during autumn and their effects on winter survival at two latitudinally distinct locations in Norway. It was hypothesised that the shortage of light energy available for photosynthesis at the northern location during autumn would alter the relationship between photosynthetic acclimation to cold, growth cessation and winter survival found in more southern regions. Cultivars and breeding materials of *L. perenne* and *Festulolium* bred for different environments and *Festulolium* of different parental origin and breeding methods were compared with well-adapted cultivars of *F. pratensis*.

2. Materials and methods

2.1. Plant materials

The plant material presented in Table 1 was composed for the study of non-native grasses of the *Festuca–Lolium* complex that are of interest for future breeding in a changing climate. A diverse plant material was needed for sufficient genetic variation and increased reliability of the results. The plant material comprised commercial cultivars (cv.), candivars (i.e. promising new candidate cultivars) and breeding materials of *L. perenne*,*Festulolium* and *F. pratensis*. Described below as *Festulolium*, these are synthetic populations originating from two breeding approaches: tetraploid hybrids and diploid introgression materials from different parent species. The cultivars/candivars are described below with entry numbers as in Table 1. Cv. Fagerlin (entry 1) is a synthetic population containing genotypes from cvs. Gunne, Svea, Valinge (SE), Riikka (FI), Norlea (CA), Raidi (LA) and local populations WIR20258, WIR35600 (The Vavilov Institute, RU), and RaigD2, 16-60-1 (NO). Candivar FuRa9805 (entry 2) originates from the surviving plants of three candivars from Northern Ireland

Table 1
Species origin and basic characteristics of entries used in the experiment.

Entry	ID	Species[a]	Ploidy level[b]	Adapt. type[c]	Country of origin
1	Fagerlin	*Lolium perenne*	D	Northern	NO
2	FuRa9805	*Lolium perenne*	D	Southern	NO
3	Arka	*Lolium perenne*	D	Continental	PO
4	Ivar	*Lolium perenne*	T	Northern	NO
5	Figgjo	*Lolium perenne*	T	Southern	NO
6	FuRs0356	×*Festulolium* (Lp^{Fp})	D	Southern	NO/UK
7	FuRs0467	×*Festulolium* (Lp^{Fp})	D	Northern	NO
8	Felopa	×*Festulolium* (Lm^{Fp})	T	Continental	PO
9	FuRs0142	×*Festulolium* (Lp^{Fp})	T	Southern	NO/UK
10	Fure	*Festuca pratensis*		Southern	NO
10	Norild	*Festuca pratensis*		Northern	NO

[a] Denominations according to the parental origin; Lp: *L. perenne*, Lm: *L. multiflorum*, and Fp: *F. pratensis*, with the abbreviation given as a superscript (e.g. Fp) when the species consists of segments within the *Lolium* genome.
[b] D: diploid, T: tetraploid
[c] Northern and southern is related to genetic pedigree (background) and adaptational level at Nordic climatic conditions.

evaluated at Fureneset, Norway (61°18′N) for three years. Arka (entry 3) is a Polish commercial cultivar. Cv. Ivar (entry 4) was mainly selected for winter survival, and was made as a synthetic population based on half-sib families from colchicine-induced tetraploid populations of local material (pop. Kleppe, NO) and cvs. Barvestra (NL) and Tove (DK). Cv. Figgjo (entry 5) originates from a seed mixture of selected plants of Polly (*Lolium* hybrid, DK), WIR40697 (RU), RaigT5 (NO), and cvs. Tove and Taptoe (DK). The following *Festulolium* candivars from Graminor Ltd. (NO) (entries 6, 7 and 9), were all exposed to two generations of seed propagation before being tested. Candivar FuRs0356 (entry 6) is a synthetic population based on plants backcrossed at IGER (now IBERS, UK) from initial hybrids of diploid *L. perenne* and *Festulolium* cv. Prior, described initially by Lewis et al. (1972). Following two periods of three winters in a nursery field at Fureneset 35 selected plants with loloid-type panicles were the base for the candivar. Candivar FuRs0467 (entry 7) originates from a wide germplasm pool of several initial triploid hybrids from crosses either between the tetraploid *L. perenne* population WIR40697 (RU) and *F. pratensis* cv. Fure (NO) or between cv. Bastion (NL) crossed with cv. Salten, a synthetic population originating from Bodø, northern Norway (67°N). The initial hybrids were backcrossed twice onto diploid *L. perenne* cv. Gunne (SE) and cv. Riikka (FI) and for a few crosses to cv. Norlea (CA). Progenies obtained after the second backcross were exposed to natural selection at Kvithamar (63°28′N). Candivar FuRs0142 (entry 9) originates from allotetraploid ($2n = 4x = 28$) families (Ba-11356, Ba-11356-sel, Ba-11358 and Ba-11359 from IGER, UK) which were backcrossed to tetraploid *L. perenne* cv. Napoleon (DK), cv. Baristra (NL) and candivars LøRa9401 (Graminor, NO) and Jo-0307 (Boreal Ltd. FI). The Polish Festulolium cv. Felopa (entry 8) is described by Zwierzykowski (2004). The *F. pratensis* cvs. Norild and Fure (entry 10) were used as controls, being the main cultivars in the regions in which the two experimental sites were located. Cv. Norild is a synthetic population selected in Alta, Finnmark (NO) (69°55′N), from a local population collected in Harstad, Troms (NO) (68°47′N), and was used at the northern location (Vågønes). Cv. Fure (described above) was used at the southern location (Fureneset).

2.2. Field location and weather data

The study locations were two coastal sites in Norway: Fureneset, Fjaler (61°18′N, 5°21′E, 10 m a.s.l.), and Vågønes, Bodø (67°17′N, 14°27′E, 35 m a.s.l.). Day length during the year varies by several hours between the sites. On 1 August the day length is 19 h 19 min at Vågønes and 17 h 16 min at Fureneset, while on 1 December the corresponding values are 3 h 20 min and 6 h 10 min. Temperature (°C) and radiation (W m^{-2}) for the experimental period (mid-August to early December in 2011 and in 2012) at the two sites and the sampling dates are shown in Fig. 1. There were no major differences in weather conditions between the years, but autumn 2011 was slightly warmer than autumn 2012 at both sites. At Vågønes the solar radiation was lower in 2011 than in 2012, but in autumn 2012 conditions cooled down earlier and an early snowfall stopped growth and disturbed the leaves.

2.3. Field design and measurements

The field trials (randomized plots within three replicates, plot size 6 m^2 per entry) were established in 2010, on 9 July at Vågønes and 31 May at Fureneset. In 2011 and 2012 the trials were fertilised according to local norms, receiving in total 234 and 290 kg nitrogen ha^{-1} year^{-1} at Vågønes and Fureneset, respectively. The third and final cut was taken on 6 September 2011 and 30 August 2012 at Vågønes and 29 August 2011 and 27 August 2012 at Fureneset in accordance with the normal management of these species and to obtain plant stands of the same developmental stage prior to measurements. During the regrowth after the third cut, 10 plants in each of the three replicate plots were individually marked and leaf elongation growth measured weekly on predetermined leaves to

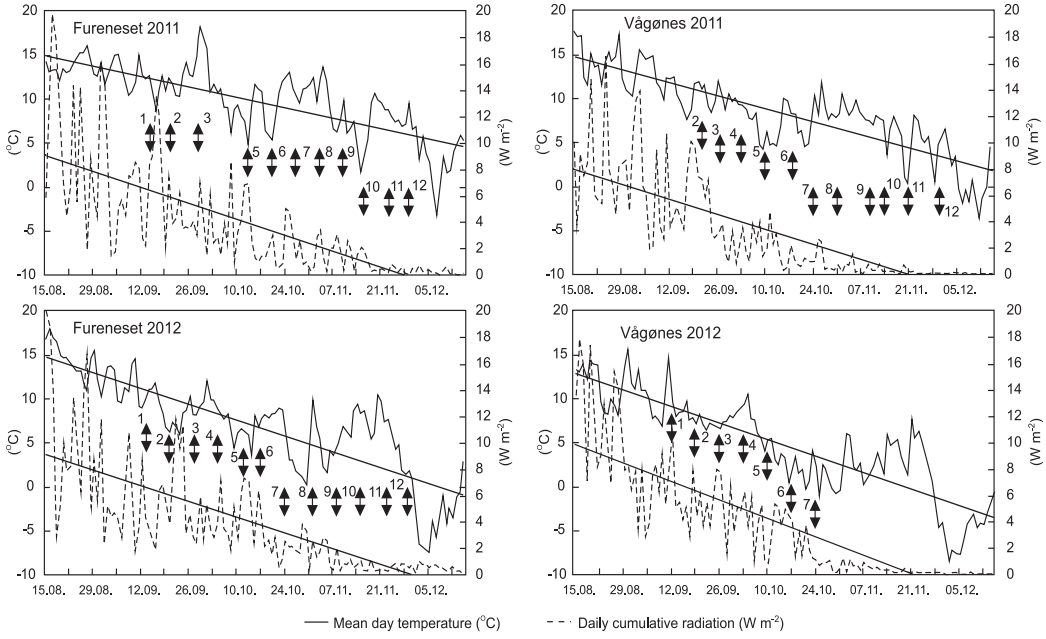

Fig. 1. Temperature (°C) and radiation (W m^{-2}) during the experimental period mid-August to early December in 2011 and 2012 at the southern (Fureneset) and northern (Vågønes) sites. Sampling dates are numbered and indicated by arrows.

determine cumulative leaf growth during the period. Close to the marked plants, fluorescence parameters were measured on 10 plants per plot for each of three replicates. The measurements continued until the days became short in early December 2011, or until grass growth was inhibited by snow or cold in 2012. Winter damage was measured as the cover (%) of live plants in spring when grass growth had started, on 25 May 2012 and 28 May 2013 at Vågønes and 19 April 2012 and 14 May 2013 at Fureneset. Fureneset and Vågønes are hereafter referred to as the southern location (in the south) and the northern location (in the north) respectively.

2.4. Chlorophyll fluorescence measurements

Chlorophyll *a* fluorescence characteristics were studied using a Handy Pea fluorimeter (Hansatech Ltd. Kings Lynn, UK). Chlorophyll fluorescence transient measurements followed by JIP test analysis are a sensitive and complex method for analysing the photosynthetic performance of plants (Strasser and Tsimilli-Michael, 2001). By studying the energy flows through single reaction centres and leaf fragments, it is possible to estimate PSII photochemical activity, the degree of photosynthetic apparatus damage and photosynthetic acclimation (Maxwell and Johnson, 2000).

Light pulse intensity was set to 3000 $\mu mol\, m^{-2}\, s^{-1}$, pulse duration 1.0 s and fixed gain (1×), as described by Rapacz (2007). On the basis of the measurements, parameters for the JIP test (Strasser and Tsimilli-Michael, 2001) were calculated as described by Rapacz et al. (2011). In the present paper the parameters indicating energy distribution in photosynthetic light reactions are presented. Parameters ABS/CS and ABS/RC indicate energy amounts absorbed by PSII antennas. TR_o/CS and TR_o/RC describe the amounts of energy trapped in PSII reaction centres. The energy loss between absorption and trapping indicate photoinhibitory damage in the PSII complex or some adaptive changes leading to increased energy dissipation in antennas (e.g. xantophyll cycle). The measure of this energy loss (F_v/F_m) is also called the maximum quantum yield of PSII because when no other limitations of photosynthetic activity exist, the part of absorbed light energy equal to F_v/F_m will be available for photoassimilate biosynthesis. The parameter F_v/F_m was used to characterise photoinhibitory damage in the plants studied (Krause et al., 1990). The trapped light energy can actually be used for electron transport (ET_o/CS, ET_o/RC) or dissipated from PSII reaction centres (DI_o/CS, DI_o/RC). Thus ET_o-s can be interpreted as parameters describing photosynthetic activity. All the energy fluxes calculated in the present paper were calculated for a single active reaction centre (RC), when "active reaction centre" means the reaction centre from PSII in which quinone A can be reduced when it is excited after dark adaptation (not damaged and coupled with either oxygen evolving complex or PQ pool). All the fluxes may also be expressed for leaf cross-section (CS). The difference between RC and CS-calculated parameters is that in the case of CS the presence of inactive PSII complexes in the measurement area are taken into consideration.

2.5. Statistical analysis

The statistical significance of the effects was analysed using ANOVA multiple general linear model (GLM) with year, location and entry as variables (StatSoft Inc., 2011). Sampling dates (time of measurement) were either analysed separately or averaged for different periods due to the variable course of the weather in both years and at both locations. Prior to analysis, the normality of the distribution was tested using the Shapiro–Wilk *W*-test. Data on spring field coverage were arcsine-transformed prior to analysis due to their non-normal distribution. The statistical significance between means was tested using Tukey's HSD test and the correlation coefficients were calculated in Pearson's model. Principal component analysis (PCA) was used for data grouping. Among all the parameters of chlorophyll fluorescence measured, only those affected by year, location or entry were used for component analysis.

3. Results

3.1. Leaf elongation growth rate (LER, mm day^{-1})

LER decreased over time in both years (Table 2, Fig. 2). LER was significantly higher in the south than in the north ($P < 0.05$) in 2011, especially in the early and late period of the experiment. LER was higher in the north than in the south in 2012 for the first six sampling dates (sd2–7 period). LER responded quickly to temperature changes, and in 2011 warm spells in late October in the south and in early November in the north gave increased leaf growth and increased photoinhibition. Similar responses were found in early autumn in 2012, when a drop in temperature led to a considerable decline in LER.

When analysing LER across locations, years and sampling dates, the entries formed four groups (A, B, C and D) (Fig. 3). Three significantly different groups were found; group A (entry 8-Felopa (3.00 mm day^{-1})) > group B (5-Figgjo, 2-FuRa9805, 9-FuRs0142 (2.26 mm day^{-1})) > D: (1-Fagerlin, 10-Fure/Norild (1.98 mm day^{-1})). Group C (3-Arka, 4-Ivar, 7-FuRs0467, 6-FuRs0356 (2.07 mm day^{-1})) was not significantly different from group B or D. The results presented are based on these four groups. There were no consistent differences between entries of *L. perenne* and *Festulolium*, and the only entry with similar accumulated leaf growth at the two sites in 2011 was *Festulolium* cv. Felopa. In the north, there were no differences between *L. perenne* cv. Fagerlin and *F. pratensis* cv. Norild, while in the south, LER was considerably lower in *L. perenne* cv. Fagerlin than in *F. pratensis* cv. Fure.

There was a significant interaction between entries and location for the first six sampling dates across years ($P < 0.0001$), even when the entries of *F. pratensis* were omitted from the statistical

Table 2
Leaf elongation growth rates (LER) observed for periods of sampling dates (sd) during the experiments (means ± SE), and temperature (°C) (Temp) and radiation ($W\, m^{-2}$) (Rad) for the sampling periods.

Location	Year	All period	Sd 2–7			Sd 8–10			Sd 11–12		
			LER	Temp	Rad	LER	Temp	Rad	LER	Temp	Rad
North (Vågønes)	2011	2.10 ± 0.030 b	2.90 ± 0.054 b	8.41	3.19	1.93 ± 0.045 a	8.17	0.66	0.79 ± 0.026 b	5.02	0.14
	2012	2.73 ± 0.101 a	2.73 ± 0.101 b	7.88	5.12	–			–		
South (Fureneset)	2011	2.85 ± 0.045a	4.08 ± 0.064 a	10.94	4.33	1.86 ± 0.038 a	8.63	1.61	2.01 ± 0.061 a	8.26	0.35
	2012	1.51 ± 0.036 c	2.35 ± 0.047 c	8.58	4.66	0.74 ± 0.020 b	4.75	1.59	0.57 ± 0.027 c	5.72	0.58

Values of the single parameter marked with the same letter did not differ statistically according to Tukey's HSD-test, $P = 0.05$.

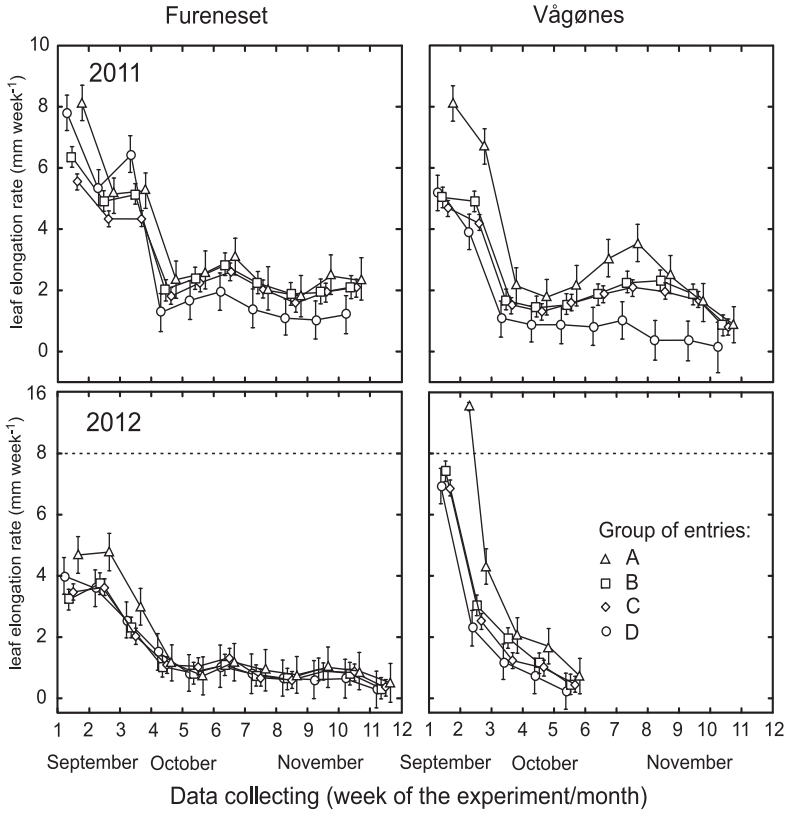

Fig. 2. Changes in leaf elongation growth rate (LER) observed for four groups of plants (A–D) grouped according to their LER ranking (see Fig. 3) at the southern (Fureneset) and northern (Vågønes) locations across both years. Means ± confidence intervals for $P = 0.05$.

analysis (Fig. 4). Analyses of LER for the first six sampling dates across the years showed the same relative differences in LER between the entries for both years, but differed between locations.

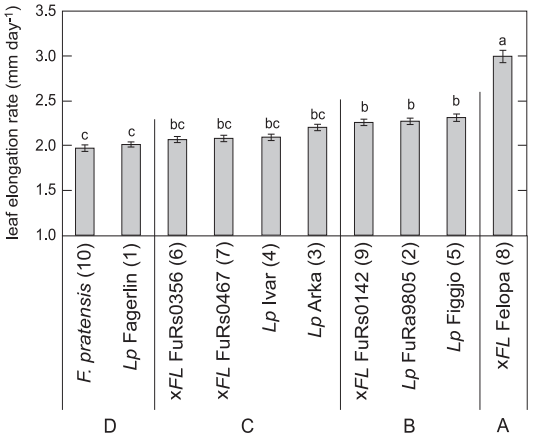

Fig. 3. Grouping of entries (A–D, $N = 959$) based on significant different leaf elongation rates across locations, years and sampling dates. Homogeneity groups (Tukey's HSD-test, $P = 0.05$) are marked with letters over the bars. *Lp* – *Lolium perenne*, ×*FL* – ×*Festulolium*

Neither of the interactions Year × Entry and Year × Sampling Date × Entry were significant (data not shown).

3.2. Photoinhibition

In both locations and years, F_v/F_m was of similar value and decreased during low temperature (especially freezing) episodes in the field (Fig. 5), resulting in an overall large decline in photochemical efficiency in the north in 2012. This effect was highest for group D entries, which in general also had the lowest values of this parameter in the north. This may be an effect of senescence and chlorophyll loss observed at this location (data not shown), in contrast to *Festulolium* cv. Felopa (entry 8, group A), which was characterised by the fastest LER at both locations and the highest F_v/F_m values in the south.

3.3. Photosynthetic activity

Photosynthetic activity before winter differed at the two sites, irrespective of entry (Fig. 6). In the north, photosynthetic activity was much lower than in the south, particularly during the last sampling period. Photosynthetic activity increased from September to November in plants in the south, but not in the north. This may indicate that plants acclimate photosynthetically to cold only in the south. A sudden increase in ET_o/CS was observed in the south during a week in which a temperature drop coincided with an increase in solar radiation at the end of September 2012 (Fig. 1). A

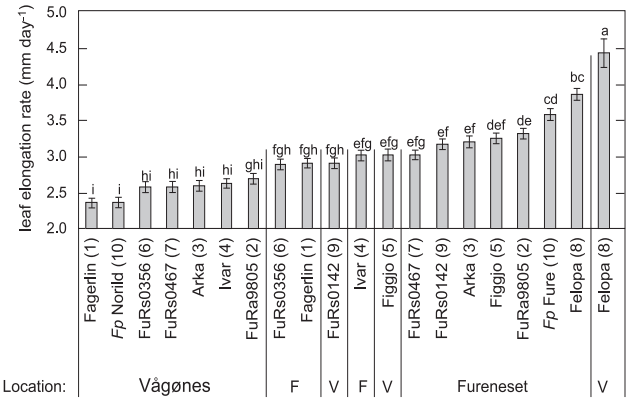

Fig. 4. Leaf elongation rate across northern (Vågønes/V) and southern (Fureneset/F) location and years for sampling dates 2–7. Homogeneity groups (Tukey's HSD-test, $P = 0.05$) are marked with letters over the bars. $N = 411$.

very similar, but reversible and weaker co-occurrence of these events was observed in mid-November 2011. In the north, *F. pratensis* cv. Norild (entry 10) showed the lowest values of photosynthetic activity when the measurements ended because of snow cover in autumn 2012.

3.4. Correlations between growth cessation, chlorophyll-fluorescence characteristics and climatic conditions

No correlation was observed between F_v/F_m and LER. This indicates that there was no direct relationship between LER in autumn and avoidance of photoinhibition. However, there was a clear relationship between LER and selected chlorophyll fluorescence parameters, although there were large differences between locations (Table 3). In the south, almost all electron flow rates in PSII were significantly and negatively correlated with LER. In the north, the correlation between the flow of energy used for electron transport (ET_o/RC) and LER was positive, whereas correlations between LER and other energy flows were very weak and not significant, at least in year one of the study. Linear correlation coefficients between LER and mean daily temperature during sampling dates 2–7 were significant ($P = 0.05$) for all entries in the south and for 1, 2, 4, 7, 9, and 10 in the north. Mean correlation coefficients were significant at both locations. Linear correlation

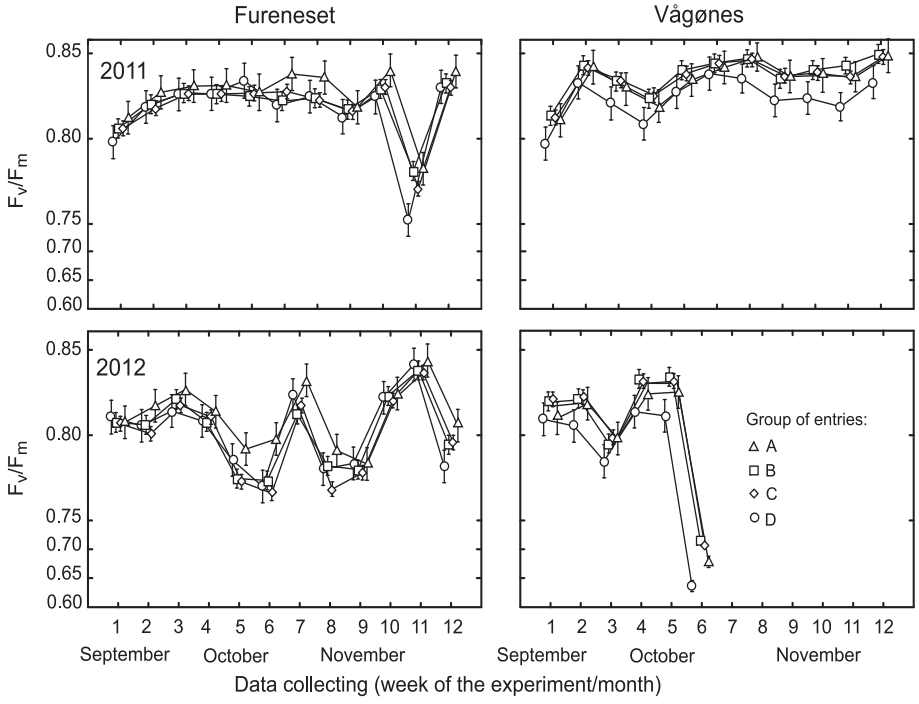

Fig. 5. Changes in the efficiency of light-energy trapping in PSII reaction centres (F_v/F_m) observed at southern (Fureneset) and northern (Vågønes) locations and years for plants grouped according to their leaf elongation rate (see Fig. 3). Means ± confidence intervals for $P = 0.05$. $N = 30$.

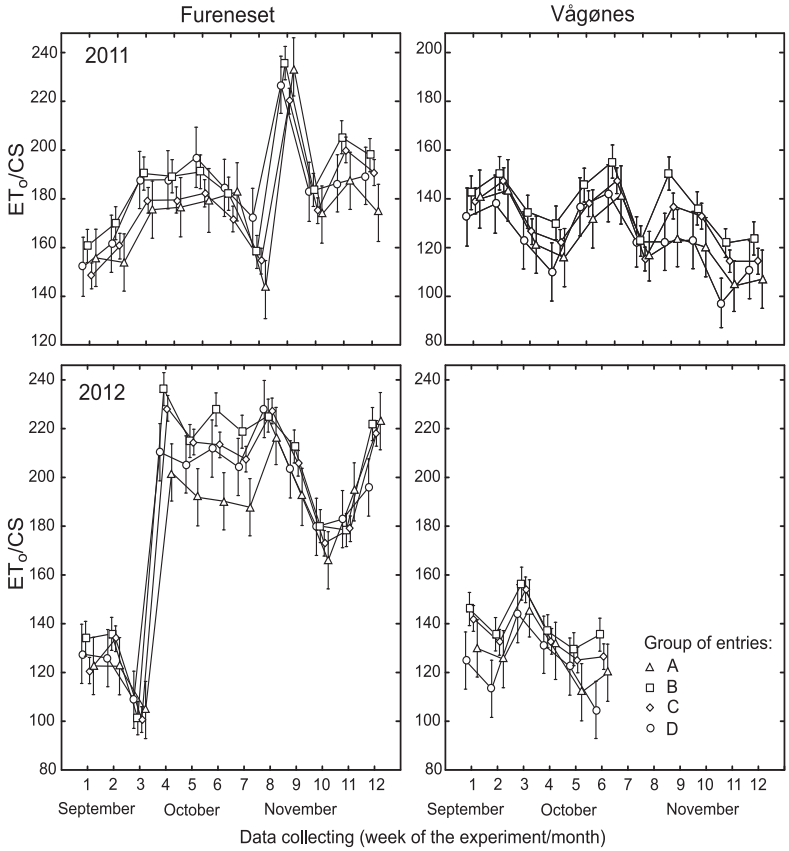

Fig. 6. Changes in phenomenological energy flux for electron transport per leaf cross-section (ET_o/CS) observed at southern (Fureneset) and northern (Vågønes) locations and years for plants grouped according to their leaf elongation rate (see Fig. 3). Means ± confidence intervals for $P = 0.05$. $N = 30$.

coefficients between LER and mean cumulative radiation during the same period were not significant at any location.

3.5. Plant responses during autumn

PCA analysis of three highly independent parameters, i.e. LER, ET_o/CS and F_v/F_m, showed that the results were clearly grouped according to location and sampling date. Thus, temperature differences mainly explained more of the variation than the entry did (Fig. 7). The PCA analysis suggested that LER and properties of

Table 3
Linear correlation coefficients between leaf elongation rate and chlorophyll fluorescence parameters. Values were calculated on the basis of mean values for Entries × Sampling dates (weeks 2–7). $N = 50$. Each mean was calculated from about 300 individual measurements.

Chlorophyll fluorescence parameter[a]	Leaf elongation rate			
	South (Fureneset)		North (Vågønes)	
	2011	2012	2011	2012
F_v/F_m	0.149	0.122	−0.059	0.227
ABS/CS	−0.558*	−0.879*	−0.111	−0.294*
TR_o/CS	−0.580*	−0.874*	−0.115	−0.150
ET_o/CS	−0.343*	−0.807*	0.277*	0.209
DI_o/CS	−0.437*	−0.846*	−0.076	−0.369*
ABS/RC	−0.346*	−0.660*	0.108	−0.112
ET_o/RC	0.149	−0.123	0.677*	0.558*
TR_o/RC	−0.317*	−0.663*	0.099	0.434*
DI_o/RC	−0.300*	−0.602*	0.099	−0.327*

[a] For explanation of the parameters, see Section 2.4.
* Values of the coefficient statistically significant for $P = 0.05$.

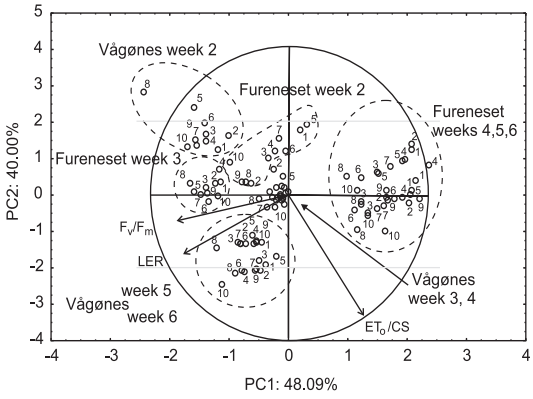

Fig. 7. The result of principal component analysis for leaf elongation rate (LER), ET_o/CS and F_v/F_m based on means for two years for the measurements taken at sampling dates 2–7 (the third week of September and the fourth week of October), which are common for both years, and locations (southern: Fureneset; northern: Vågønes) were considered. Entries are explained in Table 1.

photosynthetic apparatus were affected in a similar way during autumn in all entries investigated at both locations.

3.6. Correlations between autumn parameters and winter survival

At both locations a negative correlation was observed between LER during autumn and overwintering of plants measured as field coverage in spring (Fig. 8). However, this relationship was statistically significant only in the south, probably due to the very small variation in field coverage during spring 2012 and in LER during autumn 2012 observed in the north. A positive correlation between photosynthetic activity before winter (ET_o/CS) and LER was observed in the north, whereas in the south this correlation was negative (Table 3). When photosynthetic activity before winter was correlated with winter survival measured as field coverage in spring the following year, a positive correlation was observed (Fig. 9). This relationship was statistically significant except for the second year of the experiment in the north. When *F. pratensis* (entry 10) was excluded from the analysis, the value of correlation coefficients increased greatly. This response indicates that *F. pratensis* was characterised by different relationships between photosynthetic activity before winter and winter survival compared to other entries, especially in the north. Here *F. pratensis* cv. Norild had the highest field coverage in the spring and very low values of ET_o/CS. This was confirmed by PCA analysis (Fig. 10).

The highest contrasts in chlorophyll fluorescence parameters measured in late autumn were observed between *F. pratensis* (entry 10) and the remaining entries investigated, irrespective of location (supplementary material). *F. pratensis* cv. Norild (entry 10) grown in the north was characterised by higher energy fluxes for energy dissipation (DI_o/RC, DI_o/CS) and a higher amount of energy absorbed by the single, active PSII reaction centre (ABS/RC) and by the leaf cross-section (ABS/CS). In addition, in the south *F. pratensis* cv. Fure (entry 10) had the highest energy flux for energy trapping in PSII (TR_o/CS). In the north, energy flux for electron transport (ET_o/CS), which is an indicator of photosynthetic activity, was highest for FuRs0142 (entry 9), while *F. pratensis* cv. Norild (entry 10) showed some of the lowest values recorded for this parameter. Three parameters with very contrasting data, namely LER, ET_o/CS and ABS/RC, were used for grouping entries according to mean values for sampling dates 2–7 across years (Fig. 10). The PCA analysis clearly demonstrates the difference between locations,

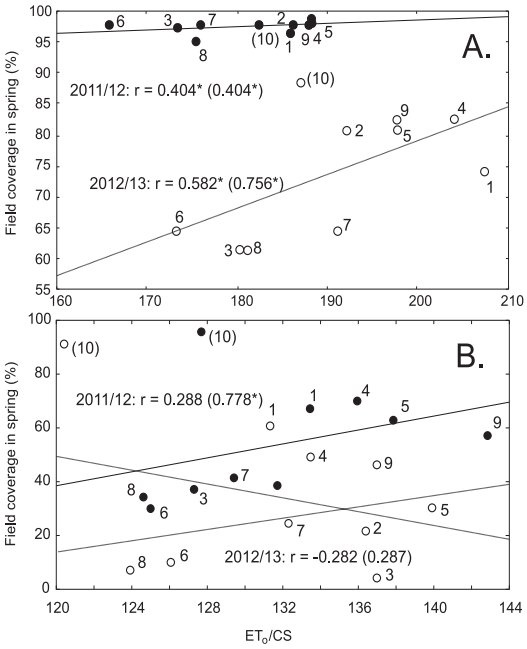

Fig. 9. The relationship between mean energy flow for electron transport during the autumn (ET_o/CS) and field coverage in the spring at southern (A: Fureneset) and northern (B: Vågønes) locations. Data from 2011/12 are indicated with closed symbols and from 2012/13 with open symbols. Correlation coefficients (r) are presented for all the entries with the exception of *F. pratensis* (in parentheses). Values of r statistically significant at $P = 0.05$ are asterisked (based on the means for separate experimental plots). Entries explained in Table 1.

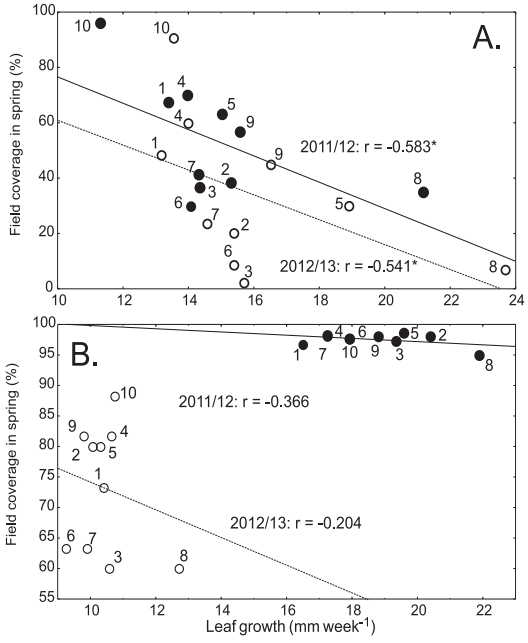

Fig. 8. The relationship between leaf elongation growth in autumn and field coverage in the spring in the south (A) and the north (B). Data from 2011/12 are indicated with closed symbols and from 2012/13 with open symbols. Correlation coefficients (r) values statistically significant at $P = 0.05$ are asterisked (based on the means for separate experimental plots). Entries explained in Table 1.

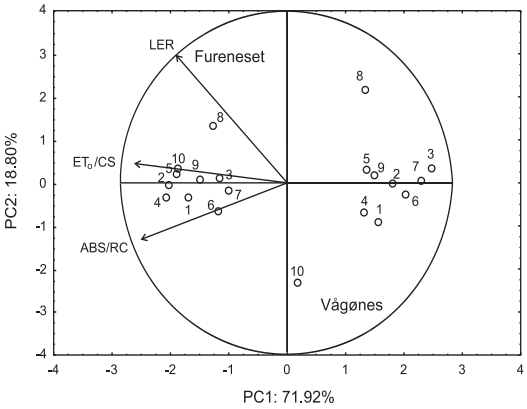

Fig. 10. The result of principal component analysis for leaf elongation growth rate (LER), ET_o/CS and ABS/RC based on means for two years, sampling dates 2–7 (the third week of September and the fourth week of October) and locations (southern: Fureneset; northern: Vågønes). Entries are explained in Table 1.

with a distinct reaction observed in the northern-adapted *F. pratensis* cv. Norild (entry 10) at the northern location as a combined effect of very low LER, low photosynthetic activity and high effectiveness of light energy absorption (ABS/RC) (Supplementary material). A very similar complex of traits was observed in two northern-adapted *L. perenne* cultivars, cv. Fagerlin (entry 1) and cv. Ivar (entry 4). The opposite situation, i.e. similar not only in the north but also in the south, was observed for the Polish *Festulolium* cv. Felopa (entry 8).

4. Discussion

The results obtained suggest that climate change with warmer autumns could cause problems with cold acclimation and winter survival at higher latitudes in non-native grass species that have been introduced into Scandinavian grassland in recent decades. The process of adaptation is slow, and *L. perenne* and *Festulolium* may be regarded as species which are distinct different concerning plant development and persistency compared to the native *Phleum pratense* (timothy), which is the main grassland species and is well adapted to the very variable climatic growing conditions (Østrem et al., 2013). Many reports confirm the close connection between photoacclimation to cold, the increase in freezing tolerance and other winter-hardiness characteristics in forages. However, the effects of low light level and decreased PSII reduction during autumn at higher latitudes were not sufficiently explained.

4.1. Leaf elongation rate (LER)

The consistent similarities in LER between the entries of *L. perenne* and *Festulolium* indicate that there are similar mechanisms behind growth cessation in the two species as demonstrated in the PCA analyses (Fig. 7). *Festulolium* cv. Felopa (entry 8, group A) is adapted to a far more southern climate than that of the current experiment, and it did not show the temperature and light response to growth cessation exhibited by the more northern-adapted entries, resulting in poor winter survival (Hay and Pedersen, 1986). Group D, on the other extreme, consisted of the well-adapted cultivars of *F. pratensis* (cv. Norild and cv. Fure, entry 10) and the northern-adapted *L. perenne* cv. Fagerlin (entry 1). Cultivar Fagerlin responded to changes in light and temperature in the autumn by reducing growth, in contrast to the other entries.

4.2. Lower photosynthetic activity in the north

No major differences between the locations were seen in light energy trapping efficiency, indicating that the plants demonstrated increased efficiency in photoinhibition avoidance (Fig. 5). This photoinhibition avoidance may be the result of increased dissipation of photochemical energy (DI/CS) for example, as was observed in *F. pratensis* cv. Norild and cv. Fure (Supplementary material). Photosynthetic activity (ET_o/CS) (Fig. 6) was lower and also more stable in the north than in the south, where the higher temperature in the south possibly caused the observed variation. This suggests a form of metabolic cessation in the north, which was visible even before the start of measurements in year one.

4.3. Light limitation in the north

In the south, LER seemed to be correlated with the non-photochemical quenching of excess light energy, expressed by the negative correlations with ABS/CS and TR_o/CS (Table 3). This confirms the close relationship between winter survival and photoacclimation, as reviewed by Hüner et al. (2013). In the north, LER was in general limited by the photochemical energy supply, expressed by the high correlation with ET_o/RC, and without over-reduction of PSII. Low amounts of light in the north did not cause a sufficient reduction in PSII to trigger photosynthetic acclimation to cold and a decrease in LER (Ensminger et al., 2006). The clear positive effect of slow LER on winter survival measured as spring coverage, which was also observed in the north (Fig. 8), suggests that there are different mechanisms (not linked to PSII excitation pressure) triggering autumnal growth cessation in the north. A decline in LER as a response to decreasing photoperiod and amount of light, is thus seen in northern-adapted entries only: *L. perenne* cv. Fagerlin (entry 1) and cv. Ivar (entry 4), and especially *F. pratensis* cv. Norild (entry 10). Slow LER was not accompanied by photo-acclimation in the north. Only in the case of *F. pratensis* cv. Norild was slow LER connected with increasing light energy dissipation in PSII (Supplementary material). The distinct differences between *F. pratensis* and the remaining entries (Fig. 10) in relation to photosynthetic activity in the autumn and plant survival in the following spring were probably the effect of a more efficient mechanism of non-photochemical light energy quenching of the excess light energy observed in *F. pratensis* cultivars compared with entries of *L. perenne* and *Festulolium*, as reported by Humphreys et al. (2007), and/or a simple effect of higher winter hardiness. In contrast to *F. pratensis* cv. Norild (entry 10), its counterpart cv. Fure did not show a similar response in the south as cv. Norild did in the north. These results indicate that growth cessation in cv. Fure is dependent on temperature rather than daylength. Such differences between southern and northern-adapted cultivars are generally observed in farmers' fields.

In trees and shrubs, including evergreens, autumnal growth cessation is triggered by decreasing day length (see Heide, 1985). Similar effects may be found in northern-adapted *Lolium–Festuca* grasses, especially those of the allotetraploid type in which northern-adapted *F. pratensis* is included as a parent. Although confirmation of the presence of a similar mechanism in grasses is lacking, some indirect evidence is available. *F. pratensis* exhibits a very strong induction of non-photochemical quenching as a mechanism of photosynthetic acclimation to cold (Rapacz et al., 2004; Humphreys et al., 2007), as was also observed in the present experiment. This mechanism of photosynthetic acclimation is photoperiodically controlled in woody plants (Heide, 1985), with some exceptions in the Rosaceae family, such as apple and pear (Heide and Prestrud, 2005). In conifers too, temperature rather than photoperiod has a major impact on the timing of seasonal changes in photochemical efficiency (Ensminger et al., 2006). In the present study, *F. pratensis* cv. Norild showed a strong correlation between (low) LER and (low) photosynthetic activity (Table 3). Although it cannot be stated categorically that there is a direct relationship between increased dissipation of light energy in autumn and growth cessation, both factors may be regulated by short photoperiod in autumn. As stated previously, a high photosynthetic rate at low temperatures is important for the development of frost tolerance in temperate grasses (Huner et al., 1993, 1998). Harrison et al. (1997) show that a hardy cultivar of *L. perenne* has a higher photosynthetic rate at low temperature than a susceptible cultivar. It can therefore be assumed that maintaining high photosynthetic activity may be more important in the north in the anticipated future climate, as a shortage of photoassimilates may lead to a lower winter survival of plants, which in this study comprised all entries except 1, 4 and 10. This shortage of photoassimilates may become more serious with higher autumn and winter temperatures and a lower light regime, since light is the basic requirement for photoassimilation (see Gray et al., 1997). Maintaining high photosynthetic activity is important to ensure reserve accumulation before rapid photoperiodic reduction as a process occurring simultaneously with photoacclimation.

4.4. What might happen with climate change?

The results indicate two major problems that may occur in plants growing at the latitudes of the Nordic region. With predicted winter climate change, the pattern of adaptation, i.e. with photoperiodically-limited growth rate, combined with photosynthetic activity in autumn, as found in *L. perenne* cv. Fagerlin, will be increasingly required in more southerly regions too, especially with the predicted temperature increase and decrease in light amount (clouds) due to increased precipitation and shorter photoperiods (Hanssen-Bauer et al., 2009). In such conditions, no signal connected to PSII excitation will be sensed in autumn and fast-growing, southern-adapted plants may exhibit decreased winter survival. During the warmer autumns in the north already experienced in recent decades (DNMI, 2014), cold acclimation is taking place at lower levels of light energy than previously, during which the PSII excitation pressure is low and therefore the increased light energy dissipation triggered by short days is less important for photoinhibition avoidance. This mechanism can limit photosynthetic activity while the respiration rate is still high, which may be unfavourable for the carbohydrate status of the plant and overwintering (Østrem et al., 2011).

Climatic adaptation involves increasing tolerance to several stresses on the plants imposed by the environment. The climatic change scenarios seen on a global scale predict an extended growing season in the north in future, narrowing that of UK and maritime Europe today. The major difference between 50, 60 and 70°N is the decreasing radiation and photoperiod in the autumn and the plants' ability to cope with the combination of light and temperature over years. With rapid changes from summer to winter, photoperiodic control may be more reliable than temperature for triggering cold acclimation as opposed to temperature as a stimulus for hardening in more unpredictable weather (Eagles, 1989). Since breeders have to choose one of these adaptational strategies, for the high north a photoperiodical response combined with a moderate or sufficient photosynthetic activity which may exploit a warm autumn without depleting its reserves before winter, would be beneficial.

5. Conclusions

Predicted climate change in the high north may delay cold acclimation, as it will take place when the days are shorter, resulting in reduced winter survival in grassland species. The results indicated that in the north, the amount of light is insufficient to trigger the changes in photosynthetic apparatus that are responsible for growth cessation in the south. This pattern of adaptation implies that future grass breeding programmes for non-native species should pay more attention to selecting genotypes with photoperiodically-controlled growth cessation which also maintain photosynthetic activity in the autumn. With progressive climate change, this pattern of adaptation will also be required in more southerly areas of Scandinavia due to the temperature increase and light amount decrease predicted for these areas. The clear relationship observed here between photoacclimation and leaf elongation growth rate indicates that fluorescence recording might be a valuable tool for the early selection of non-native grasses for high latitude regions.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.envexpbot.2014.10.008.

References

Achard, P., Gong, F., Cheminant, S., Alioua, M., Hedden, P., Genschik, P., 2008. The cold-inducible cbf1 factor-dependent signaling pathway modulates the accumulation of the growth-repressing DELLA proteins via its effect on gibberellin metabolism. Plant Cell 20, 2117–2129.

ACIA, 2005. Arctic Climate Impact Assessment. Cambridge University Press, Cambridge.

Bielenberg, D.G., 2011. Knowing when not to grow. New Phytol. 189, 3–5.

Bocian, A., Kosmala, A., Rapacz, M., Jurczyk, B., Marczak, L., Zwierzykowski, Z., 2011. Differences in leaf proteome response to cold acclimation between *Lolium perenne* plants with distinct levels of frost tolerance. J. Plant Physiol. 168, 1271–1279.

Crosatti, C., Rizza, F., Badeck, F.W., Mazzucotelli, E., Cattivelli, L., 2013. Harden the chloroplast to protect the plant. Physiol. Plant. 147, 55–63.

DNMI. Norwegian Meteorological Institute http://met.no/English/ (accessed 16.09.2014).

Eagles, C.F., 1989. Temperature-induced changes in cold tolerance of *Lolium perenne*. J. Agric. Sci. 113, 339–347.

Ensminger, I., Busch, F., Huner, N.P.A., 2006. Photostasis and cold acclimation: sensing low temperature through photosynthesis. Physiol. Plant. 126, 28–44.

Ghesquière, M., Humphreys, M.W., Zwierzykovski, Z., 2010. *Festulolium*. In: Boller, B., Posselt, U.K., Veronesi, F. (Eds.), Fodder Crops and Amenity Grasses, Handbook of Plant Breeding, 5. Springer Science+Business Media, LLC, pp. 293–316.

Gray, G.R., Chauvin, L.-P., Sarhan, F., Huner, N.P.A., 1997. Cold acclimation and freezing tolerance: a complex interaction of light and temperature. Plant Physiol. 114, 467–474.

Guy, C.L., 1990. Cold acclimation and freezing stress tolerance: role of protein metabolism. Annu. Rev. Plant Physiol. Plant Mol. Biol. 41, 187–223.

Hanssen-Bauer I., Drange H., Førland E.J., Roald L.A., Børsheim K.Y., Hisdal H., Lawrence D., Nesje A., Sandven S., Sorteberg A., Sundby S., Vasskog K., Ådlandsvik B., 2009. Klima i Norge 2100. Bakgrunnsmateriale til NOU Klimatilplassing [Climate in Norway 2100. Background material to NOU Climate Adaptation], Norsk klimasenter, September 2009. Oslo, 148 pp. [In Norwegian]. Available online at: http://met.no/Klima/Fremtidsklima/KlimaiNOrge 2100/filestore/klima-norge-2100.

Harrison, J., Tonkinson, C., Eagles, C., Foyer, C., 1997. Acclimation to freezing temperatures in perennial ryegrass (*Lolium perenne*). Acta Physiol. Plant. 19, 505–515.

Hay, R.K.M., Heide, O.M., 1984. The response of high-latitude Norwegian grass cultivars to long photoperiods and cool temperatures. Proceedings of the 10th General Meeting of the European Grassland Federation, Ås Norway, pp. 46–50.

Hay, R.K.M., Pedersen, K., 1986. Influence of long photoperiods on the growth of timothy (*Phleum pratense* L.) varieties from different latitudes in northern Europe. Grass Forage Sci. 41, 311–317.

Heide, O.M., 1974. Growth and dormancy in Norway spruce ecotypes: II. After-effects of photoperiod and temperature on growth and development in subsequent years. Physiol. Plant. 31, 131–139.

Heide, O.M., 1985. Physiological aspects of climatic adaptation in plants with special reference to high-latitude environments. In: Kaurin Å, Junttila, O., Nilsen, J. (Eds.), Plant Production in the North. Norwegian University Press, Tromsø, Oslo, Bergen, Stavanger, pp. 1–21.

Heide, O.M., Prestrud, A.K., 2005. Low temperature, but not photoperiod, controls growth cessation and dormancy induction and release in apple and pear. Tree Physiol. 25, 109–114.

Hodgson, H.J., 1964. Effect of photoperiod on development of cold resistance in alfalfa. Crop Sci. 4, 302–305.

Humphreys, M.W., Gasior, D., Lesniewska-Bocianowska, A., Zwierzykowski, Z., Rapacz, M., 2007. Androgenesis as a means of dissecting complex genetic and physiological controls: selecting useful gene combinations for breeding freezing tolerant grasses. Euphytica 158, 337–345.

Huner, N.P.A., Öquist, G., Hurry, V.M., Krol, M., Falk, S., Griffith, M., 1993. Photosynthesis, photoinhibition and low temperature acclimation in cold tolerant plants. Photosynth. Res. 37, 19–39.

Huner, N.P.A., Öquist, G., Sarhan, F., 1998. Energy balance and acclimation to light and cold. Trends Plant Sci. 3, 224–230.

Hüner, N.P.A., Bode, R., Dahal, K., Busch, F.A., Possmayer, M., Szyszka, B., Rosso, D., Ensminger, I., Krol, M., Ivanov, A.G., Maxwell, D.P., 2013. Shedding some light on cold acclimation, cold adaptation and phenotypic plasticity. Botany 91, 127–136. doi:http://dx.doi.org/10.1139/cjb-2012-0174.

Junttila, O., 1980. Effect of photoperiod and temperature on apical growth cessation in two ecotypes of Salix and Betula. Physiol. Plant. 48, 347–352.
Krause, G.H., Somersalo, S., Zumbusch, E., Weyers, B., Laasch, H., 1990. On the mechanism of photoinhibition in chloroplasts: relationship between changes in fluorescence and activity of photosystem II. J. Plant Physiol. 136, 472–479.
Lawrence, T., Cooper, J.P., Breese, E.L., 1973. Cold tolerance and winter hardiness in *Lolium perenne* II. Influence of light and temperature during growth and hardening. J. Agric. Sci. 80, 341–348.
Lewis, E.J., Tyler, B.F., Chorlton, K.H., 1972. Annual Report of the Welsh Plant Breeding Station. Aberystwyth, UK, pp. 34–37.
Malyshev, A.V., Henry, H.A.L., Kreyling, J., 2014. Relative effects of temperature vs: photoperiod on growth and cold acclimation of northern and southern ecotypes of the grass *Arrhenatherum elatius*. Env. Exp. Bot 106, 189–196.
Maxwell, K., Johnson, G.N., 2000. Chlorophyll fluorescence–a practical guide. J. Exp. Bot. 51, 659–668.
Ndong, C., Danyluk, J., Huner, N.P.A., Sarhan, F., 2001. Survey of gene expression in winter rye during changes in growth temperature, irradiance or excitation pressure. Plant Mol. Biol. 45, 691–703.
Østrem, L., Larsen, A., Pašakinskienė, I., 2007. Prebreeding of Festulolium (Lolium × Festuca hybrids). Proceedings XXVI Eucarpia Fodder Crops and Amenity Grasses Section, Perugia, Italy, pp. 47–51.
Østrem, L., Rapacz, M., Jørgensen, M., Höglind, M., 2011. Effect of developmental stage on carbohydrate accumulation patterns during winter of timothy and perennial ryegrass. Acta Agric. Scand. B 61, 153–163.
Østrem, L., Volden, B., Larsen, A., 2013. Morphology, dry matter yield and phenological characters at different maturity stages of ×*Festulolium* compared with other grass species. Acta Agric. Scand. B 63, 531–542.
Pocock, T.H., Hurry, V., Savitch, L.V., Hüner, N.P.A., 2001. Susceptibility to low-temperature photoinhibition and the acquisition of freezing tolerance in winter and spring wheat: the role of growth temperature and irradiance. Physiol. Plant. 113, 499–506.
Rapacz, M., 1998. Physiological effects of winter rape (*Brassica napus* var. *oleifera*) prehardening to frost. II. Growth, energy partitioning and water status during cold acclimation. J. Agron. Crop Sci. 181, 81–87.
Rapacz, M., 1999. Frost resistance and cold acclimation abilities of spring-type oilseed rape. Plant Sci. 147, 55–64.
Rapacz, M., 2002. Regulation of frost resistance during cold de-acclimation and re-acclimation in oilseed rape: a possible role of PSII redox state. Physiol. Plant. 115, 236–243.
Rapacz, M., 2007. Chlorophyll *a* fluorescence transient during freezing and recovery in winter wheat. Photosynthetica 45, 409–418.
Rapacz, M., Gąsior, D., Zwierzykowski, Z., Leśniewska-Bocianowska, A., Humphreys, M.W., Gay, A.P., 2004. Changes in cold tolerance and the mechanisms of acclimation of photosystem II to cold hardening generated by anther culture of *Festuca pratensis* × *Lolium multiflorum* cultivars. New Phytol. 162, 105–114.
Rapacz, M., Gąsior, D., Kościelniak, J., Kosmala, A., Zwierzykowski, Z., Humphreys, M. W., 2007. The role of the photosynthetic apparatus in cold acclimation of *Lolium multiflorum*. Characteristics of novel genotypes low-sensitive to PSII over-reduction. Acta Physiol. Plant. 29, 309–316.
Rapacz, M., Sasal, M., Gut, M., 2011. Chlorophyll fluorescence-based studies of frost damage and the tolerance for cold-induced photoinhibition in freezing tolerance analysis of Triticale (×*Triticosecale* Wittmack). J. Agron. Crop Sci. 197, 378–389.
Rapacz, M., Ergon Å, Höglind, M., Jørgensen, M., Jurczyk, B., Østrem, L., Rognli, O.A., Tronsmo, A.M., 2014. Overwintering of herbaceous plants in a changing climate. Still more questions than answers. Plant Sci. 225, 34–44. doi:http://dx.doi.org/10.1016/j.plantsci.2014.05.009.
Sandve, S.R., Kosmala, A., Rudi, H., Fjellheim, S., Rapacz, M., Yamada, T., Rognli, O.A., 2011. Molecular mechanisms underlying frost tolerance in perennial grasses adapted to cold climates. Plant Sci. 180, 69–77.
StatSoft, Inc., 2011. STATISTICA (data analysis software system), version 10. www.statsoft.com.
Strasser, R.J., Tsimilli-Michael, M., 2001. Stress in plants, from daily rhythm to global changes, detected and quantified by the JIP-test. Chim. Nouv. 75, 3321–3326.
Thomashow, M.F., 1999. Plant cold acclimation: freezing tolerance genes and regulatory mechanisms. Annu. Rev. Plant Physiol. Plant Mol. Biol. 50, 571–599.
Zwierzykowski, Z., 2004. Amphiploid and introgression breeding within the *Lolium–Festuca* complex–achievements and perspectives. In: Yamada, T., Takamizo, T. (Eds.), Development of a Novel Grass with Environmental Stress Tolerance and High Forage Quality Through Intergeneric Hybridization Between *Lolium* and *Festuca*. National Agriculture and Bio-oriented Research Organization, NARO, Japan, pp. 17–29.

Supplement Paper III

Chlorophyll fluorescence parameters [1)] measured in entries (see Table 1) at the end of autumn (week 11).

Location	Entry	ABS/RC	TR_o/RC	ET_o/RC	DI_o/RC	ABS/CS	TR_o/CS	ET_o/CS	DI_o/CS
Southern location (Fureneset)	1	2.322^{ab}	1.926^{ab}	0.880^{a}	0.396^{a}	493.6^{bcd}	409.9^{bc}	189.3^{ab}	83.75^{ab}
	2	2.416^{a}	2.018^{a}	0.871^{ab}	0.398^{a}	507.3^{abcd}	423.7^{bc}	184.3^{ab}	83.55^{ab}
	3	2.381^{a}	1.990^{a}	0.826^{bcd}	0.391^{a}	488.8^{cd}	409.2^{bc}	171.2^{b}	79.59^{b}
	4	2.248^{bc}	1.878^{bc}	0.869^{ab}	0.369^{ab}	513.3^{abc}	429.0^{bc}	200.1^{a}	84.35^{ab}
	5	2.299^{ab}	1.918^{abc}	0.843^{abc}	0.381^{a}	498.8^{bcd}	416.4^{abc}	184.2^{ab}	82.35^{ab}
	6	2.329^{ab}	1.941^{ab}	0.858^{ab}	0.389^{a}	480.1^{d}	400.4^{abc}	178.1^{ab}	79.75^{b}
	7	2.318^{ab}	1.932^{ab}	0.842^{abc}	0.386^{a}	494.9^{bcd}	412.6^{ab}	181.5^{ab}	82.29^{ab}
	8	2.158^{c}	1.817^{c}	0.782^{d}	0.341^{b}	505.2^{abcd}	425.2^{ab}	183.9^{ab}	79.99^{b}
	9	2.332^{ab}	1.951^{ab}	0.863^{ab}	0.381^{a}	521.9^{ab}	436.7^{c}	195.9^{a}	85.15^{ab}
	10	2.349^{ab}	1.965^{ab}	0.808^{cd}	0.384^{a}	535.7^{a}	448.4^{a}	185.9^{ab}	87.32^{a}
Northern location (Vågønes*)	1	2.353^{bcd}	1.997^{bcd}	0.814^{ab}	0.356^{c}	358.3^{abc}	303.9^{b}	124.6^{b}	54.38^{bc}
	2	2.427^{b}	2.060^{b}	0.793^{bcd}	0.367^{bc}	352.3^{bcd}	298.9^{bc}	115.3^{bcd}	53.46^{bc}
	3	2.303^{d}	1.955^{d}	0.771^{de}	0.347^{c}	336.3^{cde}	285.5^{cd}	113.1^{cd}	50.84^{c}
	4	2.404^{bc}	2.042^{bc}	0.787^{cde}	0.362^{bc}	358.3^{abc}	304.5^{b}	117.7^{bc}	53.83^{bc}
	5	2.366^{bcd}	2.014^{bcd}	0.806^{abc}	0.352^{c}	361.4^{ab}	307.4^{ab}	123.9^{b}	54.01^{bc}
	6	2.408^{bc}	2.030^{bcd}	0.776^{de}	0.378^{b}	325.0^{e}	273.9^{d}	105.5^{d}	51.03^{c}
	7	2.316^{cd}	1.969^{cd}	0.777^{de}	0.347^{c}	335.5^{cde}	285.0^{cd}	113.4^{cd}	50.52^{c}
	8	2.358^{bcd}	1.998^{bcd}	0.767^{de}	0.360^{bc}	330.8^{de}	280.2^{cd}	107.6^{d}	50.62^{c}
	9	2.356^{bcd}	2.000^{bcd}	0.823^{a}	0.356^{c}	381.4^{a}	323.6^{a}	133.8^{a}	57.72^{b}
	10	2.586^{a}	2.155^{a}	0.763^{d}	0.430^{a}	375.9^{a}	313.4^{ab}	111.5^{cd}	62.53^{a}

Values of the single parameter within the same location marked with the same letter did not differ statistically according to Tuckey's HSD test (P=0.05).

*Data from 2012 unavailable.

[1)] for explanation of the parameters, see section 2.4

Paper IV

Hypothesis

I. Waterlogging of soil reduces cold hardening of clover and timothy, prolonged waterlogging being more severe.

II. Different climate conditions between years and location interacts with waterlogging treatment.

III. The effect of waterlogging is dependent on species and population.

Effect of water-logging on winter hardiness of red clover (*Trifolium pratense* L.) and timothy (*Phleum pratense* L.)

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Abstract

Climate change is predicted to cause increased autumn precipitation in the Nordic region, which may result in higher incidence of waterlogged soils. An outdoor pot experiment was performed during two consecutive growth seasons in 2010 and 2011 at two sites in Norway (Fureneset (69°39'N) and Holt (59°45'N)) to examine whether such water-saturation of soils affects cold hardening of red clover (*Trifolium pratense* L.) and timothy (*Phleum pratense* L.). Populations of red clover, adapted to southern or northern Nordic climates, and a Norwegian cultivar of timothy, were treated with two levels of soil water saturation (25% and 90%) for one or two months during natural cold hardening outdoor in autumn. The temperature at which 50% of plants were killed (LT_{50}) was determined during autumn and winter to estimate the degree of cold hardening of plants expressed as freezing tolerance. Climatic differences between years and locations, as well as species and populations, exerted stronger effects on the freezing tolerance and carbohydrate concentration than the degree of water saturation of the soil. Waterlogged soil (90%) in the autumn enhanced the freezing tolerance of timothy at the northern site Holt (lowest autumn temperature) but reduced it at the southern site Fureneset (highest autumn temperature). The southern-adapted red clover population survived poorly after two months of water-logging with no plants surviving in fully water saturated soil in the spring of 2011, while no clover plants survived at Holt irrespective of duration of water-logging. Timothy was considerably more freezing tolerant than red clover, and the northern-adapted clover was more freezing tolerant than the southern-adapted. Red clover demonstrated a better winter survival at Fureneset compared to Holt in both experimental years. Prolonged water-logging may negatively affect the cold acclimation and freezing tolerance at future elevated temperatures, timothy being more affected compared to red clover.

Key words: water-logging, freezing tolerance, cold hardening, anoxia, carbohydrates, climate change

Introduction

Future climate change scenarios for Norway indicate increased precipitation during autumn (RegClim, 2005), in addition to warmer autumns and winters at high latitude regions (IPCC, 2013). Autumn precipitation is likely to increase most markedly in the coastal areas of Western and Northern Norway (Engen-Skaugen, 2007). This will most likely lead to increased waterlogging of soils or flooding, defined as the presence of water in soil in excess of field capacity (Levitt, 1980). Water-logged soil or flooding cause anaerobic conditions and oxygen deficiency in the soil and plant roots, potentially enhancing the effects of flood-induced stress. A decline in O_2 availability causes energy shortage in the plants due to reduced ATP production from cell metabolism (Parent et al., 2008). When lack of O_2 limits ATP production from oxidative phosphorylation, a hypoxic situation occurs first. Anoxia follows later when no O_2 is available and ATP is purely produced through fermentative glycolysis (Parent et al., 2008). Plant species adapted to short-term episodes of anoxia may respond by accelerating glycolysis to maintain adequate energy supply under anaerobic conditions (Crawford, 2003; Parent et al., 2008).

Most studies have focused on the effects of anaerobic conditions, caused by flooding, on plants during the growing season. Yet, responses to oxygen deprivation in plants could be different when these conditions prevail during winter when metabolic processes are slow due to low temperatures (Crawford, 2003). The effect of flooding on temperate grasses has been shown to be temperature dependent with higher temperature during flooding reducing survival (Beard and Martin, 1970). In addition, if flooding leads to increased consumption of carbohydrate reserves during autumn, this may deplete the stored energy reserves and lead to energy shortage later in the winter. Thus, prolonged energy shortage may reduce winter survival of the plants (Licausi, 2010).

Winter hardening of plants takes place during autumn through cold acclimation at low positive temperatures, as days become shorter. Pomeroy and Andrews (1981) found that flooding combined with low temperature reduced the freezing tolerance of winter wheat and winter barley. Jurczyk et al. (2013) showed that meadow fescue (*Festuca pratensis* Huds.) genotypes increased their freezing tolerance faster when cold acclimated under flooding

compared to non-flooded plants, although the effect on freezing tolerance has been shown to be both species and genotype dependent (Jurczyk et al., 2015). Waterlogged soil during autumn may freeze and form ice layers during winter resulting in ice encasement of plants and anoxic condition. Timothy is known to be quite tolerant to these conditions (Höglind et al., 2010; Andrews, 1997) whereas red clover is very sensitive (Pulli et al., 1996; Gudleifsson, 2010).

Oxygen deprivation increases the accumulation of CO_2, ethanol and other TCA-cycle metabolites within plants, though at a different levels within red clover and timothy (Gudleifsson, 1994; Bertrand et al., 2001). Further, oxygen deficiency accelerates the use of carbohydrate reserves (Bertrand et al., 2003).

Timothy (*Phleum pratense* L.), the most common forage grass species in the northern parts of the Nordic region, has been reported to be more resistant to waterlogged conditions and to maintain higher carbohydrate reserves than red clover (*Trifolium pratense*) under oxygen deficiency (Bertrand et al., 2003). The greater tolerance of timothy to anaerobic conditions at low temperature correlates with a slower glycolytic metabolism in timothy compared to red clover (Bertrand et al., 2001). Red clover is today at its geographical limit for winter survival at high northern latitudes and has low persistency in meadows in Norway. However, because of the climate change, it may become more important as a protein source and nitrogen supplier to the soil and it will be a key component in the development of future sustainable livestock farming systems. Joint Nordic breeding programs for generating red clover cultivars for the northern regions were active at the end of the last decade with the aim to develop populations possessing wide adaptation to both climatic conditions and cultivation practices in the region (Helgadóttir, 1996; Helgadóttir et al., 2000). Nordic breeding companies are still making an extensive effort to breed new red clover cultivars (The Norwegian Official List of Varieties, 2015), but cultivars with sufficient persistence for northern high latitudes are still lacking.

In this experiment, we investigated whether waterlogging of soil in autumn affected the freezing tolerance of two populations of red clover and timothy and winter survival of additional red clover populations. We compared two locations with different climatic condition in order to see if a colder climate would promote different effect on hardening under waterlogged soil compared to a milder climate. Further, we tested the effect of shorter (1 month) and longer (2 months) period with waterlogged soil. Both northern-adapted and

southern-adapted populations of red clover were tested in order to evaluate the adaptive capacity of red clover for anaerobic soil situation during cold acclimation.

Materials and methods

Experimental design

A semi-field experiment was carried out at two sites in Norway; Fureneset, Fjaler (61°18' N) and Holt, Tromsø (69°39' N) during two autumns 2010 (year 1) and 2011 (year 2). Ten (year 1) and 12 (year 2) plants were planted evenly spaced in each pot with a volume of 10 dm^3, height 22.8 cm, and diameter 29 cm. The pots were placed randomly on wooden boards insulated with mineral wool to protect plants against cold air from underneath. The waterlogging treatments were applied by placing the pots randomly in watertight boxes with a water height of either 3 cm (field capacity, intended to maintain approx. 25 % volumetric water content (VWC) of soil saturation) or 20 cm (full waterlogging, approx. 90% VWC of soil saturation). At field capacity, the water and air contents of the soil are considered ideal for crop growth. Water saturation percentage within pots was measured regularly with a moisture meter (HH2, Delta-T devise, Cambridge, England) and during rainy days runoff water was let out through an opening within the 3 cm high boxes for maintaining 25 % VWC. The waterlogging treatments were carried out for either one or two months, with the exception of year 1 at Holt where the water froze within the boxes before the 1 month treatment was finished. Therefore, pots were left within the boxes with frozen water until tested for freezing tolerance in winter or until thawing next spring. Autumn starts earlier at the northern location, thus the treatments started on the 16th and 27th of September at Holt and Fureneset, respectively, in year 1, and on the 1st and 26th of September at Holt and Fureneset, respectively, in year 2. The pots were covered by snow during winter at Holt. Additional pots were used to test overwintering. Two (year 1) and three (year 2) pots per treatment and population/cultivar were left for wintering outside until spring when they were scored for plant survival. Temperature loggers within the pots indicated no differences in temperature between waterlogged pots in boxes and pots at field capacity.

Plant material and establishment

Two red clover populations, a southern-adapted (origin Czech Republic) and a northern-adapted (origin Northern Norway), and one commercial timothy cultivar of south Norwegian origin (cv. Grindstad) were used in the experiment. Details about the origin and composition of the two red clover populations can be found in Dalmannsdottir et al. (2015). In addition, we wanted to obtain information from broader breeding materials, thus additional red clover populations, i.e. Bjursele, LøRk0498, MRL97 and A1TD1+D2, were tested for waterlogging resistance in year 2 by measuring survival and biomass production in spring. Bjursele is an old cultivar from Västerbotten in Sweden (64°20' N) (Åkerberg 1974). LøRk0498 is a breeding population with a broad genetic background based on natural selection at Løken (1999) from a mixture of Nordic gene bank accessions, populations from Russia, North America and Europe. MRL97 is a breeding population based on southern adapted populations (LøRk0499-2x). A1TD1+D2 is a northern adapted tetraploid red clover breeding population originated from Fennoscandian tetraploid populations intercrossed and subjected to two cycles of selection under different management regimes (fertilizer and harvesting) at the two Norwegian research stations Vågønes (67.3° N, 14.6° E) and Løken (59.8° N, 11.5° E). Each selection cycle consisted of an establishing year, two harvest years under local agricultural practice and seed harvest of surviving plants in the third ley year.

Seedlings were established in the greenhouse in early summer and transferred to 10 dm^3 pots 1.5 months prior to the start of the water-logging treatments. The growth medium was 3:2 (vol/vol) peat:fine sand. Plants were fertilized according to demand, or approx. 2 g/pot/week with 19-4-12 (N-K-P). At the start of the experiment, plants at Holt were smaller the first year compared to the second year.

Weather conditions

Daily mean temperature and monthly precipitation is presented in Figure 1. Mean autumn temperature was always higher at Fureneset than at Holt. Mean air temperature was markedly higher in the autumn months (Sept, Oct. Nov.) of 2011 (year 2) (Tromsø:7°C, Fureneset: 10.2°C) compared to 2010 (year 1) (Tromsø: 3.4°C, Fureneset: 6.6°C). Precipitation was, on average, up to four times higher at Fureneset compared to Holt in the beginning of autumn.

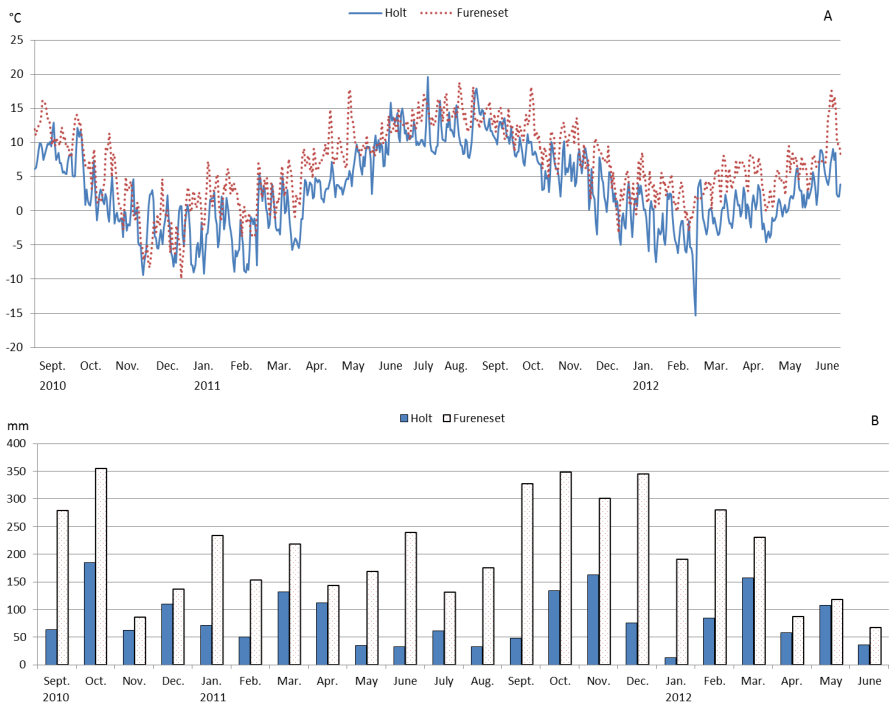

Figure 1. Mean daily temperature at 2 m height (A) and monthly precipitation (B) at the experimental sites obtained from "on site" weather stations.

Survival and biomass production

Plants from two pots per treatment/population/year were scored for survival in the following spring and biomass production was recorded from the same pots. Biomass was also recorded in two other pots at the start of the water-logging treatments. Shoots were cut 2 cm from the soil surface and dried at 60°C for two days before weighing. The timothy cv. 'Grindstad' was tested both years, for red clover in year 1 only two populations were tested for survival, while 6 additional populations were scored for survival in year 2.

Freezing test

The degree of hardening of the plants was estimated as freezing tolerance using LT_{50} tests (temperature at which 50% of plants die) twice per site and year. In year 1 at Holt, LT_{50} tests

were performed on the 29th of October (1 month treatment) and on the 23rd of November (2 month treatment), while at Fureneset, the LT_{50} test were performed on the 27th of October (1 month treatment) and on the 19th of January (2 month treatment). In year 2, freezing tests for both one and two-months waterlogging were performed in mid-January at both locations (Table 2). The LT_{50} tests were performed using programmable freezers according to Pulli et al. (1996). In brief, roots were washed and crown segments prepared with 3 cm shoot length and about 1-2 cm roots in timothy, in red clover the taproot was included. The crown segments were covered with humid sand in plastic boxes and the temperature was lowered from 2°C to -3°C by 1°C h^{-1}, and boxes were kept at -3°C for 13 hours to avoid super-cooling of the plants. The boxes were then gradually frozen at a cooling rate of -1°C h^{-1} until -10°C was reached, and from then at -3°C h^{-1} until the pre-set temperature was reached for each treatment. Unfrozen controls were kept at a constant temperature of 2°C. Ten plants per population were used for each temperature, including two replicates per temperature, all together 20 plants per each of six pre-set temperatures. LT_{50} was estimated by scoring the number of dead plants after 3-4 weeks growth at room temperature and continuous light.

Carbohydrate measurements

Plants were analysed for carbohydrate content in year 2. Crown segments including 1.5 cm shoot and 1.5 cm root were sampled from two pots per treatment at the start of experiment and in spring from all treatments, and dried at 60°C before pulverised in a mixer mill (Retsch MM 400, Germany). There were four parallel samples with four plants in each. Total soluble carbohydrate content was analysed by HPLC as described by Pociecha and Dziurka (2015). In short, ground tissue was mixed with 50 cm^3 de-ionized water and shaken for 1 hour, diluted with acetonitrile 1:1 (v/v), and centrifuged. The supernatant was filtered through a 0.22 µm membrane filter and analysed using Hamilton RCX-10 250 4.01 mm column (Hamilton, Reno, NV, USA) with 1 cm^3 min^{-1} flow rate and ESA Coulochem II Analytical Cell 5040 detector (ESA, Chelmsford, MA, USA) with 100 mm aqueous NaOH solution as the mobile phase.

Starch was estimated as glucose released after enzymatic hydrolysis of pellets kept after soluble sugars analysis as described by Bach et al. (2015). Alpha-amylase in 50 mM potassium phosphate buffer (pH 6.9) with 6.7 mM of NaCl, and amyloglucosidase in 200 mM

sodiumacetate buffer (pH 4.5) were used to break down the starch. After enzymatic treatment samples were centrifuged, supernatant was collected, diluted with acetonitrile 1:1 (v/v) and analyzed by HPLC for glucose content.

Statistical analysis

LT_{50} values were calculated by probit analyses using the logistic distribution in PROC PROBIT in SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). Feducial limits (α=0.05) were used to test any significant differences between treatments. Biomass production and carbohydrate content were analysed using ANOVA general linear model (GLM) in Minitab 16 (Minitab Inc., 2010, State College, PA, USA) with a model that included location, population, degree (per cent %) and duration (months) of waterlogging and their interactions.

Results

Freezing tolerance

Climatic differences between years and locations together with species and population differences had a stronger effect on freezing tolerance and carbohydrate concentration than water levels of the soil. The LT_{50} values for timothy and red clover are presented in Table 1 and 2. Timothy was always more freezing tolerant than red clover. The northern adapted population of red clover was significantly more freezing tolerant than the southern adapted, but both populations responded similarly to water saturated soil. Freezing tolerance was affected by location and differences in natural hardening conditions between years. There was an interaction between location, year and soil waterlogging degree reflected in the LT_{50} values. Especially timothy demonstrated differential responses towards full (90%) waterlogging at the two locations between the two years. Waterlogged soil enhanced the hardening of timothy at Holt in year 1, whereas at Fureneset there was no difference in freezing tolerance between timothy grown at field capacity or in fully waterlogged soil (Table 1). However, in year 2, timothy had better freezing tolerance at field capacity than with waterlogged soil after two months treatment at both locations (Table 2). After 1 month treatment at Holt in year 1 , red clover plants at field capacity were most freezing tolerant,

while after 2 months , the plants in fully waterlogged soil were most freezing tolerant (Table 1). At Fureneset in year 1 (Table 1) northern-adapted red clover at field capacity gained higher freezing tolerance after two months and plants at field capacity expressed a higher freezing tolerance than plants from a fully waterlogged soil after two months treatment. In year 2, there was no difference in freezing tolerance of red clover between one and two months of treatment (Table 2).

Table 1. Freezing tolerance as LT_{50} values and 95% confidence interval for timothy and red clover at two levels of soil water saturation at two sites in Norway (Fureneset and Holt) in year 1 (autumn 2010).

			Fureneset			Holt		
	Degree of saturation (%)	months	LT_{50}	95% Confidence interval		LT_{50}	95% Confidence interval	
				lower	Upper		lower	upper
Timothy (cv. Grindstad)	25	1	-17.0	-17.7	-16.2	-17.3	-18.0	-16.6
	90	1	-16.5	-17.3	-15.8	-19.2	-19.9	-18.5
	25	2	-23.9	-25.0	-22.7	-17.1	-18.3	-16.0
	90	2	-23.2	-24.3	-22.1	-22.6	-23.9	-21.5
Red clover (N-pop; origin Northern Norway)	25	1	-10.6	-11.4	-9.8	-12.5	-13.3	-11.8
	90	1	-11.7	-12.5	-11.0	-10.9	-11.7	-10.2
	25	2	-16.5	-17.6	-15.5	-13.3	-14.5	-12.2
	90	2	-12.6	-13.5	-11.7	-15.4	-16.7	-14.3
Red clover (S-pop, origin Czech Republic)	25	1	-9.5	-10.3	-8.6	-9.1	-9.9	-8.3
	90	1	-10.3	-11.1	-9.5	-7.0	-7.7	-6.2
	25	2	-9.9	-10.9	-9.0	-6.0	-7.3	-4.7
	90	2	-8.7	-9.8	-7.5	-12.0	-13.4	-11.0

Table 2. Frost tolerance as LT_{50} values and 95% confidence interval for timothy and red clover at two levels of soil water saturation at two sites in Norway (Fureneset and Holt) in year 2 (autumn 2011).

			Fureneset			Holt		
	Degree of saturation (%)	Months	LT_{50}	95% Confidence interval		LT_{50}	95% Confidence interval	
				lower	Upper		lower	upper
Timothy (cv. Grindstad)	25	1	-14.2	-15.2	-13.2	-16.9	-17.9	-15.9
	90	1	-13.1	-14.0	-12.0	-17.6	-18.6	-16.7
	25	2	-14.2	-14.9	-13.5	-16.1	-17.0	-15.3
	90	2	-12.9	-13.6	-12.2	-13.9	-14.7	-13.1
Red clover (N-pop; origin Northern Norway)	25	1	-12.7	-13.7	-11.7	-16.7	-17.7	-15.7
	90	1	-11.3	-12.3	-10.3	-17.0	-18.0	-16.0
	25	2	-11.6	-12.2	-11.1	-13.0	-13.8	-12.1
	90	2	-12.4	-13.0	-11.9	-15.0	-15.8	-14.1
Red clover (S-pop, origin Czech Republic)	25	1	-9.7	-10.7	-8.6	-12.5	-13.4	-11.5
	90	1	-10.3	-11.3	-9.3	-11.3	-12.3	-10.4
	25	2	-10.0	-10.5	-9.4	-9.6	-10.5	-8.8
	90	2	-10.6	-11.1	-10.0	-10.0	-10.8	-9.2

Survival

No red clover plants survived until spring at Holt in year 1, while 60-85% of the timothy plants survived (Table 3). At Fureneset in year 1, timothy and northern-adapted red clover survived well regardless of treatment. In contrast, the southern-adapted red clover population survived poorly after two months of soil water treatment, with no surviving plants in fully waterlogged soil. In year 2, with additional red clover populations tested (Table 4), all survived well at Fureneset, except for the southern-adapted population, which showed poorest survival. At Holt, red clover populations survived poorly, except for the northern populations A1TD1+D2 and Bjursele, which had around 50% or more survival at the field capacity treatment. Similar fractions (60-90%) of timothy plants survived until spring in year 2, at both Holt and Fureneset. The surviving plants were smaller and produced less biomass at Holt compared to Fureneset at harvest in the spring. Snow mould was detected in dead plant materials, especially on clover, in the spring at Holt.

Table 3. Number of surviving plants and biomass in spring 2011 (year 1). Mean values and standard error of mean presented for two replicating pots, each containing 10 plants.

			Fureneset		Holt	
	Degree of saturation (%)	Month with treatm.	% survival	dm g/plant	% survival	dm g/plant
Timothy (cv. Grindstad)	25	1	100	4.0±0.2	73	1.0±0.3
	90	1	95	4.3±0.7	60	1.7±0.3
	25	2	95	2.8±0.5	85	1.4±0.1
	90	2	100	2.9±0.1	85	1.5±0.1
Red clover (N-pop; origin Northern Norway)	25	1	90	6.3±1.2	0	-
	90	1	80	5.8±0.3	0	-
	25	2	75	2.0±0.5	0	-
	90	2	80	1.7±0.3	0	-
Red clover (S-pop, origin Czech Republic)	25	1	90	2.3±0.7	0	-
	90	1	95	2.4±1.0	0	-
	25	2	20	1.2±1.2	0	-
	90	2	0	-	0	-

Table 4. Number of surviving plants and biomass in spring 2012 (year 2). Mean values and standard error of mean presented for three replicating pots, each containing 12 plants. Standard errors are absent for treatments having only one pot with surviving plants.

			Fureneset		Holt	
	Degree of saturation (%)	Month with treatm.	% survival	dm g/plant	% survival	dm g/plant
Timothy (cv. Grindstad)	25	1	67	8.1±0.8	61	11.0±1.00
	90	1	75	6.9±1.5	86	0.79±0.34
	25	2	89	7.3±0.5	89	10.7±1.33
	90	2	86	6.9±0.9	72	0.44±0.15
Red clover (N-pop; origin Northern Norway)	25	1	86	7.0±1.4	36	0.38±0.13
	90	1	89	6.1±0.9	0	-
	25	2	97	6.6±0.3	33	0.56±0.14
	90	2	97	6.9±1.0	47	0.88±0.03
Red clover (S-pop, origin Czech Republic, H-Ziv)	25	1	69	10.7±1.1	0	-
	90	1	47	10.5±2.8	17	0.38±0.14
	25	2	72	7.6±1.3	0	-
	90	2	64	6.6±1.6	19	0.24±0.13
Red clover (Bjursele)	25	1	83	8.2±0.7	53	0.82±0.25
	90	1	89	8,1±0.9	17	0.41±0.02
	25	2	86	6.5±0.9	42	0.85±0.06
	90	2	92	6.2±0.3	0	-
Red clover (LøRk0498)	25	1	89	8.7±0.8	19	0.44±0.17
	90	1	89	8.2±0.6	0	-
	25	2	94	6.9±0.4	11	0.33±0.18
	90	2	89	6.7±0.5	6	0.26±0.02
Red clover (MRL97)	25	1	89	6.4±0.1	19	0.39±0.09
	90	1	83	6.0±0.2	8.3	0.26
	25	2	89	7.6±0.5	6	0.36
	90	2	92	8.2±1.8	8.3	0.38
Red clover (A1TD1+D2)	25	1	97	6.5±0.1	50	1.21±0.13
	90	1	94	6.0±0.6	3	0.40
	25	2	93	6.1±0.4	86	0.65±0.06
	90	2	97	6.8±0.5	22	1.00±0.05

Carbohydrate content

In general, concentration of carbohydrates was not affected by waterlogging. Location had the strongest effect on total carbohydrate content, with higher concentrations of all carbohydrates at Holt compared to Fureneset, except for the concentration of raffinose, which was higher at Fureneset, and starch in timothy, which was very low and not different between locations. All carbohydrate fractions measured are presented in Table 5 except for maltose, for which the content changed similarly as the starch content, and raffinose, which was found only low concentrations. Occasionally, the red clover populations contained significantly higher

concentrations of carbohydrates when waterlogged (90%), especially the northern-adapted population. Apart from that, the only differences in carbohydrate content were found between northern and southern-adapted red clover populations at Holt where glucose content was slightly higher and starch content slightly lower in the northern-adapted population. Timothy contained significantly less carbohydrates compared to the red clover populations, except for fructose, which was at higher concentrations in timothy. The starch content declined during the experiment at both locations and for both species. At the same time, soluble carbohydrates increased in red clover and timothy mainly because of increase in the sucrose concentration. The carbohydrate content was not affected the duration of waterlogging.

No correlation was found between freezing tolerance (LT_{50} values) and total soluble carbohydrates (corr. -0.025, P=0.908) or starch (corr. 0.100, P=0.643).

Table 5. Content (mg/g dw) of glucose, fructose, sucrose and starch in northern-adapted and southern adapted red clover, and timothy in year 2. Values at start (0 months), after 1 month and after 2 months of soil water-logging are presented. Mean values are presented ± standard error of means.

			Glucose			Fructose			Sucrose			Starch		
Location	Treatm. (months)	Saturation (%)	Red clover N	Red clover S	Timothy Grindstad	Red clover N	Red clover S	Timothy Grindstad	Red clover N	Red clover S	Timothy Grindstad	Red clover N	Red clover S	Timothy Grindstad
Fureneset	0	start	2.6 ±0.3	2.3 ±0.3	1.0 ±0.0	1.4 ±0.1	1.7 ±0.1	3.2 ±1.2	12 ±1	16 ±2	3.4 ±0.2	107 ±13	53 ±7	2.3 ±0.3
	1	25	7.4 ±1.2	2.3 ±0.4	1.5 ±0.2	2.0 ±0.1	1.0 ±0.0	3.5 ±0.5	85 ±11	74 ±11	23 ±2	37 ±1	16 ±2	2.5 ±0.4
	1	90	6.4 ±1.1	4.7 ±1.3	1.8 ±0.3	2.0 ±0.2	1.9 ±0.7	4.6 ±1.0	76 ±5	90 ±9	26 ±3	24 ±3	18 ±4	1.7 ±0.2
	2	25	5.7 ±1.0	5.1 ±1.3	1.8 ±0.1	1.9 ±0.2	1.8 ±0.3	5.6 ±0.8	83 ±6	64 ±5	24 ±2	35 ±4	28 ±5	1.4 ±0.3
	2	90	11.5 ±1.4	5.9 ±1.0	1.6 ±0.2	4.8 ±0.6	1.9 ±0.5	4.7 ±0.6	81 ±13	80 ±6	24±1	43 ±7	29 ±7	1.7 ±0.5
Holt	0	Start	9.4 ±1.4	23.7 ±1.5	18.2 ±3.2	2.9 ±0.6	9.3 ±0.7	12.1 ±0.6	83 ±5	102 ±6	37 ±3	125 ±29	97 ±13	3.1 ±0.6
	1	25	5.1 ±1.0	7.9 ±0.8	2.8 ±0.4	2.5 ±0.4	7.2 ±2.1	13.1 ±2.8	169 ±10	162 ±7	62 ±4	78 ±8	59 ±4	1.8 ±0.1
	1	90	8.9 ±1.5	13.0 ±1.6	4.3 ±0.8	7.1 ±0.6	11.8 ±1.6	16.8 ±1.7	190 ±16	206 ±23	100 ±30	81 ±4	88 ±9	1.3 ±0.2
	2	25	3.6 ±0.3	7.9 ±0.8	4.0 ±0.4	2.6 ±0.3	5.3 ±0.7	17.0 ±1.6	166 ±12	157 ±8	57 ±5	81 ±6	56 ±15	2.0 ±0.3
	2	90	6.2 ±2.0	8.3 ±0.7	4.5 ±0.8	5.0 ±2.5	7.2 ±0.7	17.0 ±1.4	238 ±12	211 ±15	62 ±7	79 ±5	69 ±7	1.5 ±0.3

Discussion

Waterlogging of soil during autumn had no consistent effect on cold acclimation of red clover expressed as freezing tolerance. However, location and year-to-year differences in climate interacted with the waterlogging treatment. In year 2, the mean air temperature during autumn was 3.6°C higher than in year 1 at both locations. We have earlier shown that low pre-acclimation temperatures per se have a positive effect on freezing tolerance of the same red clover populations and timothy (Dalmannsdottir et al., 2015). The higher autumn temperature in year 2 is reflected in a much lower freezing tolerance this year compared to year 1 of both timothy and the northern-adapted red clover at Fureneset where freezing tests were performed at the same time in mid-winter both years. The combined year to year effect of climate and water-logging treatment led to enhanced cold hardening of timothy in waterlogged soil at lower temperature (year 1 at Holt) whereas at higher temperature (year 2 at Holt and Fureneset) waterlogged soil reduced cold hardening of timothy. Studies from Poland also reported increased hardening of meadow fescue acclimated under flooding (Jurczyk et al., 2013). Further studies with meadow fescue and perennial ryegrass showed a temperature dependent effect of flooding treatment, and the presence of species and genotype differences (Jurczyk et al., 2015). Andrews and Pomery (1989) also found exposure of winter cereals to low-temperature flooding prior to ice encasement to increase the ice encasement tolerance. An earlier Norwegian study of full soil water saturation of timothy showed no reduction in cold hardening when the temperature during autumn was high, i.e. water was needed for plant growth, whereas when autumn temperatures were lower, LT_{50} values increased for the most frost-sensitive timothy cultivar (Østrem et al., 2009). There was no clear effect of waterlogging *per se* during cold acclimation in autumn on the freezing tolerance. Waterlogging stress induces several metabolic changes within the plant cell in addition to other related abiotic and biotic stress factors. Therefore the effects of waterlogging can be complex and include several interactions, for instance it seems that temperature interactions are important. Despite non-significant differences in freezing tolerance between 1 and 2 months of water-logging treatments of the southern-adapted red clover population at Fureneset in year 1, prolonged waterlogging effectively reduced the survival of this population in the spring. This may be due to an interaction with frost periods in December and in late winter. The lack of surviving red clover plants at Holt in the spring of year 1 is most probably an effect of ice-encased soil during the whole winter period.

Differences in carbohydrate concentrations in our study seem to be merely an effect of decreasing temperature during autumn. Breakdown of starch and storage carbohydrate into fructose and sucrose are well-known responses of plants to colder temperature during the acclimation process in addition to accumulation of carbohydrates due to excess photosynthesis over respiration and growth (Levitt, 1980; Thorsteinsson et al., 2002). Red clover stores carbohydrates in the form of starch instead of fructans and has a different energy storage mechanism than timothy. Studies in Canada showed that total non-structural carbohydrates (TNC) increased in red clover during autumn, reached a maximum in November and then decreased during the overwintering period, with a faster rate of decrease under oxygen deficiency (Bertrand et al., 2001). In timothy, on the other hand, TNC increased progressively from autumn to February, with a higher increase under oxygen deficiency (Bertrand et al., 2003). This can partly explain the different results obtained for red clover and timothy in our study. The slightly different content of TNC in the northern and southern-adapted red clover is in accordance with findings in white clover, where northern-adapted populations had slower rates of carbon reserve utilisation at low positive temperature compared to southern-adapted populations (Frankow-Lindberg, 2001). Norwegian studies have shown an increase in TNC in timothy from October to March with highest concentrations in the most winter-hardy cultivars (Østrem et al., 2011). Further, carbohydrate concentrations during winter were affected by plant-developmental stage and age (Østrem et al., 2009).

In addition to climate differences in our study, differences observed between Holt and Fureneset may have been caused by growth stage of the plants, with generally larger plants at Fureneset, and larger plants at Holt in year 2 compared to year 1. The morphological differences in root structure between species could also explain the different responses to waterlogged soil. When emptying the boxes after one or two months of waterlogged soil, a strong odour appeared indicating anaerobic fermentation within the boxes. When removing the pots, the root system of timothy showed a strong effect of soil water treatment. Roots in pots with fully waterlogged soil were all present in the upper layer of the pot, while those with field capacity were spread around in the pot and particularly dense at the bottom of the pot. No such effect on the root system was observed in red clover.

The fibrous root system of timothy might be advantageous in waterlogged soil in comparison with the taproot structure of red clover, which does not produce adventitious roots as prolific as timothy. The formation of adventitious roots may be an important morphological adaptation to increase the interface between water saturated soil and atmospheric surface as these roots are commonly formed near the base of the stem with a lateral growth parallel to the water/soil surface (Parent et al., 2008).

Conclusions

Climatic differences between years and locations together with species and population differences had a stronger effect on cold hardening, expressed as freezing tolerance and carbohydrate concentration, than water treatment of the soil. Full water-saturation of soil in autumn enhanced the freezing tolerance of timothy at colder temperatures (Holt), but reduced it at higher temperatures (Fureneset). Red clover adapted to the northern Norwegian climate (northern-adapted) generally showed higher frost tolerance than red clover population adapted to the southern Norwegian climate (southern-adapted). Increased abiotic stress caused by higher levels of water saturation in soil together with increased autumn temperature may reduce cold hardening of perennial forage crops like timothy.

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References

Andrews, C.J., Pomeroy, M.K., 1981. The effect of flooding pretreatment on cold hardiness and survival of winter cereals in ice encasement. Can. J. Plant Sci. 61, 507-513.

Andrews, C.J., Pomeroy, M.K., 1989. Metabolic acclimation to hypoxia in winter cereals.

Plant Physiol. 91, 1063-1068.

Andrews, C.J., 1997. A comparison of glycolytic activity in winter wheat and two forage grasses in relation to their tolerance to ice encasement. Ann. Bot. 79 (Supplement A), 87-91.

Bach, A., Kapczyńska, A., Dziurka, K., Dziurka, M., 2015. Phenolic compounds and carbohydrates in relation to bulb formation in Lachenalia 'Ronina'and 'Rupert'in vitro cultures under different lighting environments. Sci. Hortic. 188, 23-29.

Beard, J.B., Martin, D.B., 1970. Influence of water temperature on submersion tolerance of four grasses, Agron. J. 62, 257-259.

Bertrand, A., Castonguay, Y., Nadeau, P., Laberge, S., Rochette, P., Michaud, R., Bélanger, G., Benmoussa, M., 2001. Molecular and biochemical responses of perennial forage crops to oxygen deprivation at low temperature. Plant Cell Environ. 24, 1085-1093.

Bertrand, A., Castonguay, Y., Nadeau, P., Laberge, S., Michaud, R., Bélanger, G., Rochette, P., 2003. Oxygen deficiency affects carbohydrate reserves in overwintering forage crops. J. Exp. Bot. 54, 1721-1730.

Crawford, R.M.M., 2003. Seasonal differences in plant responses to flooding and anoxia. Can. J. Bot. 81, 1224-1246.

Dalmannsdottir, S., Rapacz, M., Jørgensen, M., Østrem, L., Larsen, A., Rødven, R., Rognli, O.A., 2015. Temperature before cold acclimation affects cold tolerance and photoacclimation in timothy (*Phleum pretense* L.), perennial ryegrass (*Lolium perenne* L.) and red clover (*Trifolium pratense* L.). J. Agron. Crop Sci. doi:10.1111/jac.12149.

Engen-Skaugen, T., 2007. Refinement of dynamically downscaled precipitation and temperature scenarios. Climate change 84, 365-382.

Frankow-Lindberg B.E., 2001. Adaptation to winter stress in nine white clover populations: Changes in non-structural carbohydrates during exposure to simulated winter conditions and 'spring' regrowth potential. Annals of Botany 88, 745-751.

Gudleifsson, B.E., 1997. Survival and metabolite accumulation by seedlings and mature plants of timothy grass during ice encasement. Annals of Botany 79, 93-96.

Gudleifsson, B.E., 2010. Ice tolerance and metabolite accumulation of herbage crops in Iceland and impact of climate change. Icel. Agric. Sci. 23, 111-122.

Helgadóttir, Á., 1996. Red clover (*Trifolium pratense* L.) varieties for northern regions. Acta Agric. Scand., Sect. B, Soil and Plant Sci. 46, 218-223.

Helgadóttir, Á., Larsen, A., Marum, P., Fritsen, H., Lindvall, E., Miettinen, E., 2000. Prebreeding of red clover (*Trifolium pratense* L.) of northern areas. Acta Agric. Scand., Sect. B, Soil and Plant Sci. 50, 187-190.

Höglind, M., Bakken, A.K., Jørgensen, M., Østrem, L., 2010. Tolerance to frost and ice encasement in cultivars of timothy and perennial ryegrass during winter. Grass Forage Sci. 65, 431-445.

IPCC, 2013. Climate Change 2013. The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge, United Kingdom and New York: Cambridge University Press, 1535 pp. doi:10.1017/CBO9781107415324.

Jurczyk, B., Kręosju, T., Kosmala, A., Rapacz, M., 2013. Different mechanisms trigger an increase in freezing tolerance in *Festuca pratensis* exposed to flooding stress. Environ. Exp. Bot. 93, 45-54.

Jurczyk, B., Rapacz, M., Krępski, T., 2015. Photosynthetic apparatus responses to short-term low-temperature flooding may contribute to freezing tolerance changes in forage grasses. J. Agron. Crop Sci. 201, 49-56.

Levitt, J., 1980: Response of plants to environmental stresses, Vol. 1. Chilling, freezing, and high temperature stresses, Academic Press, New York 497 pp.

Licausi, F., 2010. Regulation of the molecular response to oxygen limitations in plants (Minireview). New Phytol. 190, 550-555.

Østrem, L., Höglind, M., Jørgensen, M., 2009. Vinterbiologi i endra klima – vassoverskot (in Norwegian). Bioforsk conference, Oslo, 2009.

Østrem, L., Rapacz, M., Jørgensen, M., Höglind, M., 2009. Impact of frost and plant age on compensatory growth in timothy and perennial ryegrass during winter. Grass Forage Sci. 65, 15-22.

Østrem, L., Rapacz, M., Jørgensen, M., Höglind, M., 2011. Effect of developmental stage on carbohydrate accumulation patterns during winter of timothy and perennial ryegrass. Acta Agric. Scand., Sect. B, Soil and Plant Sci. 61, 153-163.

Parent, C., Capelli, N., Berger, A., Crèvecoeur, M., Dat J.F., 2008. An overview of plant responses to soil waterlogging. Plant Stress 2(1), 20-27.

Pociecha, E., Dziurka, M., 2015. *Trichoderma* interferes with cold acclimation by lowering soluble sugars accumulation resulting in reduced pink snowmould (*Microdochium nivale*) resistance of winter rye. Environ. Exp. Bot. 109, 193–200.

Pulli, S., Hjortsholm, K., Larsen, A., Gudleifsson, B., Larsson, S., Kristinsson, B., Hömmö, L., Tronsmo, A.M., Ruuth, P., Kristensson, C., 1996. Development and evaluation of laboratory testing methods for winter hardiness breeding. Publications – Nordic Gene Bank 32, 68 pp.

RegClim, 2005. Norges klima om 100 år. Usikkerhet og økt risiko. (In Norwegian). http://regclim.met.no**.**

The Norwegian Official List of Varieties, 2015. The Plant Variety Board. Norwegian Food Safety Authority, Norway 18. March 2015, 22 p. http://www.plantesortsnemnda.no.

Thorsteinsson, B., Harrison, P.A., Chatterton, N.J., 2002. Fructan and total carbohydrate accumulation in leaves of two cultivars of timothy (*Phleum pratense* Vega and Climax) as affected by temperature. J. Plant Physiol. 159, 999-1003.

Åkerberg, E., 1974. Red clover – an interesting species for genecological studies. Hereditas 77, 177-182.

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