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Growth Potential and Course of Frost Tolerance in Winter Wheat (*Triticum aestivum* L.) as Influenced by Variable Temperature and Snow Cover Conditions

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ABSTRACT

The anticipated future changes in temperature, precipitation and snow cover caused by global warming may affect winter survival of autumn sown wheat. More variable weather conditions may cause an increased frequency of periods with alternating freezing and thawing and less stable snow covers. In the present study, the course of plant frost tolerance and growth potential was studied by exposing cold acclimated plants of winter wheat to conditions with alternating periods of freezing and thawing (either –1 or +5°C), and differing durations of snow cover. Tests of frost tolerance and determination of growth potential were performed each time the temperature or snow cover conditions were changed. Periods without snow cover and +5°C caused dehardening, with loss of frost tolerance being more pronounced during the first dehardening period than in the second one. The ability to reharden after a dehardening period decreased towards the end of the experimental period. Mild periods during winter also seemed to exhaust plant growth potential, possibly by increasing respiration rate while photosynthesis was still restricted. The results indicate some of the challenges we may face regarding overwintering of winter wheat in a future climate.

Keywords: climate change, dehardening, hardening, phenological development, plant vigour

INTRODUCTION

Plants' tolerance to freezing temperatures varies during the winter as a consequence of complex interactions between the plant and various environmental factors. The anticipated future changes in temperature, precipitation and snow cover duration caused by global warming (Christensen *et al.* 2001) may hence affect the ability of winter wheat to survive winter in different ways. An increase in temperature, giving milder winters, may reduce the risk of frost related damages. However, combined with increased weather variability, the consequences are not easily predictable. Semenov and Porter (1995) found, when modelling winter wheat yield in response to future weather scenarios, that changes in the variability of either temperature or precipitation during summer had a larger impact on yield than changes in average conditions. It is reasonable to assume that the same situation could apply also for winter conditions and plant survival.

Increased weather variability may cause repeated cycles of freezing and thawing to occur during winter, potentially making the plants more vulnerable to frost damages. Mild periods will reduce the plants' frost tolerance by dehardening, and although they may reharden to a certain extent when the temperature is again lowered, earlier experiments have shown that winter wheat plants lose most of their ability to reharden at some point during winter. Fowler *et al*. (1996b) and Prasil *et al*. (2004) studied the course of frost tolerance during periods of different lengths (0-98 days) with 2 or 4°C. They found that the plants' frost tolerance started declining, and the ability to cold acclimate and reharden was reduced, at the time when their vernalization requirement was fulfilled and a transition from vegetative to generative stage of development occurred. In a study by Mahfoozi *et al*. (2001), a short photoperiod was found to delay the transition from vegetative to generative development after vernalization saturation, and as a consequence the plants retained their ability to reharden for a longer time than plants grown at long day conditions. Studies of reciprocal near-isogenic lines for alleles determining vernalization requirement (*Vrn-A1* and *vrn-A1* representing spring and winter habit, respectively) (Limin and Fowler 2006), and studies of the expression-level and -duration of genes determining degree of frost tolerance (Fowler *et al.* 1996a; Danyluk *et al.* 2003; Dhillon *et al.* 2010), further confirm this close relationship between attainable level of frost tolerance and phenological development, as influenced by vernalization and photoperiod.

Experiments with hardening and dehardening periods have often been performed by exposing plants for temperatures in the range 10-20°C during dehardening (e.g. Pomeroy *et al.* 1975; Fowler *et al.* 1996b). Temperatures this high are unlikely to occur during winter in most of Northern Europe. Hence, in the present experiment the aim has been to simulate periods of hardening and dehardening more similar to the conditions we may expect in the field.

Winter survival is most commonly recorded by rating dead or living plants. Surviving plants may however differ extensively in vigour when spring arrives, depending on the weather and growing conditions during the preceding autumn and winter. Weak plants with diminished reserves at the end of winter may lag behind in spring growth and hence not be able to exploit the growing season to the same degree as plants of better conditions. Weakened plants will also be less tolerant to stress, as for instance conditions inducing desiccation in late winter/early spring. At present, there is still a lack of knowledge regarding the impact of a changed winter climate on plant growth potential. The objective of the present work has been to study the influence of cycles with freezing and thawing periods and differing

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Week number	1	2	3	4	5	6	7	8	9	10	11
A	Snow, -1 °C										
B	Snow, -1 °C			No snow, +5°C		Snow, -1 °C		No snow, +5°C		Snow, -1 °C	
C	Snow, -1 °C							No snow, +5°C		No snow, -5°C	

Fig. 1 Temperature and snow cover conditions given during the 11 weeks long experimental period for treatments A, B and C.

lengths of snow cover on plant frost tolerance and growth potential.

MATERIALS AND METHODS

Plant material and experimental design

Winter wheat (*Triticum aestivum* L. cv 'Magnifik') was sown in perforated and well-drained polythene boxes filled with soil on October 17th 2006 at the Norwegian Institute for Agricultural and Environmental Research in Stjørdal. Each box (37 × 27 × 15 (depth) cm) contained 40 plants, giving a plant density similar to 400 plants m^{-2}. The soil was a silty loam with a layer of sand (1 cm) on the top to prevent clodding. Mineral fertilizer was added at sowing at rates of 3.6 g N, 1.0 g P, and 2.7 g K m^{-2}.

The boxes were placed in a greenhouse for germination and growth for four weeks after sowing. They were given long day conditions (16 h) by a mixture of HQI® metal halide lamps (approx. 140 µmol m^{-2} s^{-1}) and natural light conditions, and a temperature decreasing from 16/12°C (day/night) at the beginning of the period, to 10/6°C at the end of the period.

After four weeks, the plants were moved to a growth chamber for hardening at 2.5°C for a month. During hardening, light was given by HQI® metal halide lamps at a fluence rate of approximately 200 µmol m^{-2} s^{-1} (8 h day).

The hardened plants were divided into three groups of different treatments (**Fig. 1**). Group A, the control plants, were covered with an artificial snow cover and kept at –1°C throughout the experimental period of 11 weeks. Treatment B simulated an unstable winter where the conditions changed every second or third week between snow cover and –1°C, and no snow with +5°C. The plants in group C got seven weeks of stable snow cover and then thawing conditions (+5°C) without snow for two weeks. Thereafter they were given a simulated spring frost period with –5°C and no snow cover. Artificial snow was made by covering the plant boxes with moist felt (approx. 7 mm) overlaid with opaque plastic to inhibit evaporation of moisture. In order to ensure gas exchange for these "snow" covered plants, all boxes were placed on pallets. To avoid fungal infections, all plants were sprayed with a suspo-emulsion of azoksystrobin (4 mg m^{-2}) and fenpropimorf (11.2 mg m^{-2}) (Amistar Pro produced by Syngenta Crop Protection, Basel, Switzerland) prior to covering the boxes with the artificial snow.

Determination of frost tolerance

Tests of frost tolerance were performed according to the method described by Limin and Fowler (1988) each time the simulated winter conditions were changed throughout the experimental period. Plants were washed free from soil, cut to 2 cm root length and 3 cm top, and placed in moist sand in aluminium trays in a programmable freezer. The temperature was lowered from 2 to –3°C by 1°C h^{-1} and kept at this level for 12 hrs. Thereafter temperature was lowered by 1°C h^{-1} until the set minimum temperature of each test was reached. During this period, two samples of ten plants per cultivar, originating from two different boxes, were removed from the freezer at intervals of 2-3°C for each of five test temperatures within the range –10 to –20°C, or –12 to –22°C. The sampled plants were placed at 2°C over night for thawing and then transplanted into pots with fertilized peat. Individual plants were rated dead or alive after three weeks of regrowth at 18°C and long day (18 h) conditions. An LT_{50} (the temperature at which 50% of the plants were killed) was thereafter estimated as a measure of plant frost tolerance for each replicate, and a standard deviation was calculated.

Each test also included two control samples of plants. They were washed, trimmed and placed in moist sand as described above, and thereafter kept at 2°C before they were planted in peat and placed at 18°C at the same time as the frozen plants.

Determination of growth potential

Plants were sampled for determination of growth potential each time the simulated winter conditions were changed throughout the experimental period. Their immediate growth potential was regarded as a measure of plant vigour. At each sampling, 20 plants from each of two replicated boxes were cut to 4 cm height above soil surface. Ten of these plants were thereafter cut at 1 cm height, and fresh and dry weights were recorded for the cut plant material. The plants which were cut at 4 cm height only, were left to grow for three weeks at 18°C and long day (18 h) conditions whereupon they were cut at 1 cm height, as well. Growth potential was expressed as yield (g dry weight), recorded as the difference between harvested plant material cut at 1 cm height after three weeks of growth and initial plant material between 1 and 4 cm height at sampling.

At each sampling, a relative growth potential (yield/initial plant material between 1 and 4 cm height at sampling) was recorded for both replicates and a standard deviation calculated.

Statistical analyses

Statistical evaluation of the results was done by an analysis of variance using SAS general linear model procedure. LSD tests at the 5% level of significance were calculated to determine differences in plant frost tolerance and growth potential between treatments and sampling times.

RESULTS

Frost tolerance

After four weeks of hardening at +2.5°C, plant frost tolerance (LT_{50}) was recorded as –16°C. Tests of frost tolerance performed during the experimental period showed that the plants continued hardening also after having been transferred to their different treatments and simulated winter conditions. For the control plants (treatment A), which were kept under an artificial snow cover at –1°C, mean LT_{50} was estimated to –19°C at the end of the experimental period (**Fig. 2A**). However, for unknown reasons, there was a large difference in recorded LT_{50} between the two replicates of this test, and hence the apparent increase in frost tolerance during treatment A was not statistically significant.

Treatment B was initiated by giving the plants three weeks with –1°C and an artificial snow cover after hardening. These conditions lowered the plants' LT_{50} with several degrees, and at –20°C, the set minimum temperature in this test of frost tolerance, 70% of the plants were still alive (**Fig. 2B**).

As expected, the periods without snow cover and + 5°C caused dehardening and loss of frost tolerance. Treatment B

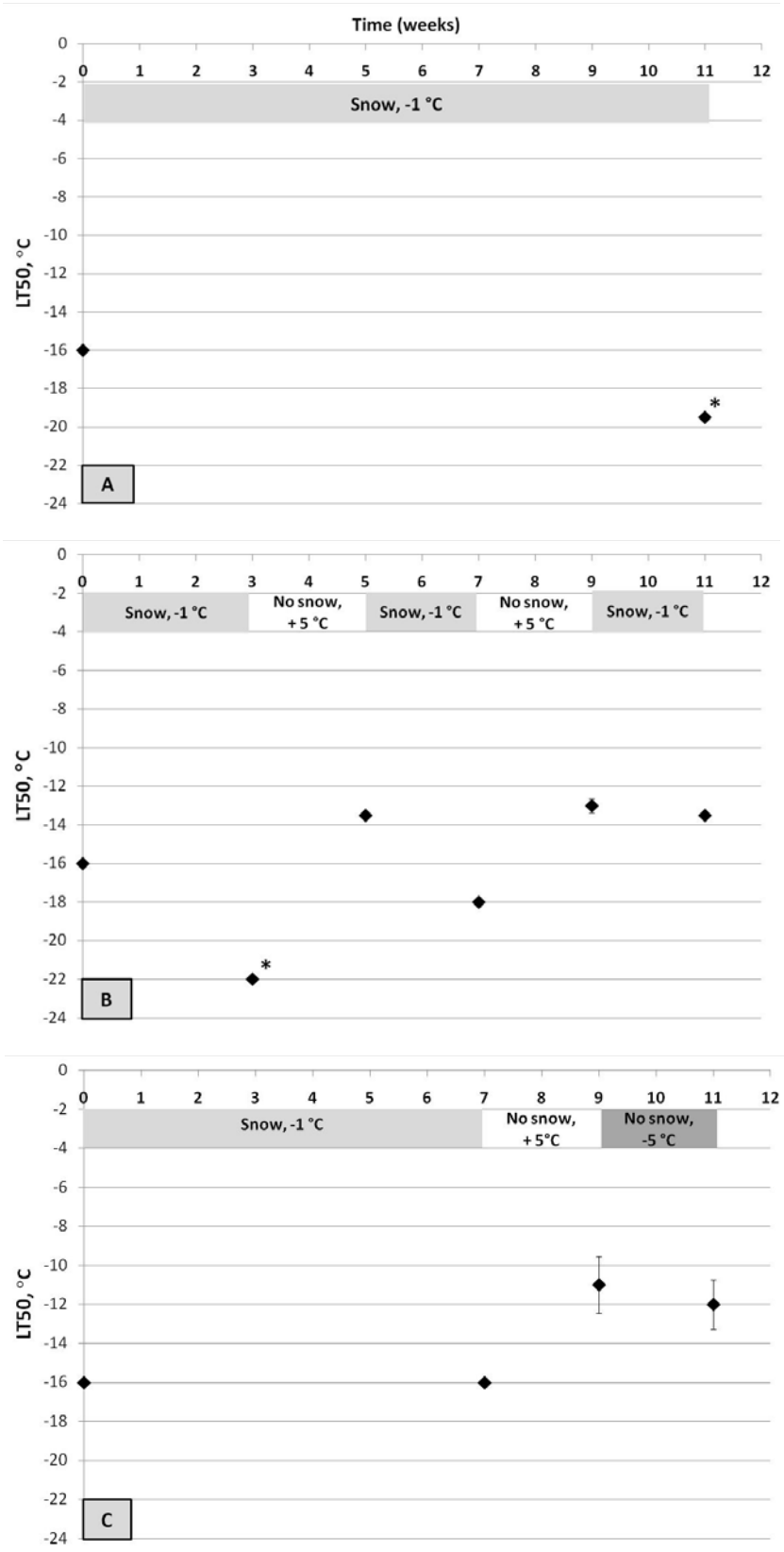

Fig. 2 Frost tolerance (LT_{50}) recorded at each change of temperature and snow cover conditions in treatment A, B, and C. Error bars indicate standard deviations. *70% of the plants survived at –20°C, the lowest test temperature, in one (A) or both (B) replicates.

included two such dehardening periods. Loss of frost tolerance was larger during the first dehardening period, between week three and five, than during the second one, between week seven and nine. Recorded LT_{50} changed from < –20 to –13.5°C during the first period, and from –18 to –13°C during the second period.

By moving the plants in treatment B back to an artificial snow cover and –1°C after the first dehardening period, plant frost tolerance was again increased, although not to the same low level as recorded before the dehardening period. However, after the second period of dehardening, two weeks of –1°C did not induce rehardening and change of LT_{50}.

The plants in treatment C only received one period without snow cover and + 5°C, between week seven and nine. As in treatment B, the plants started dehardening and lost some of their acquired frost tolerance during this period ($p = 0.08$). Estimated mean LT_{50} before and after dehar-

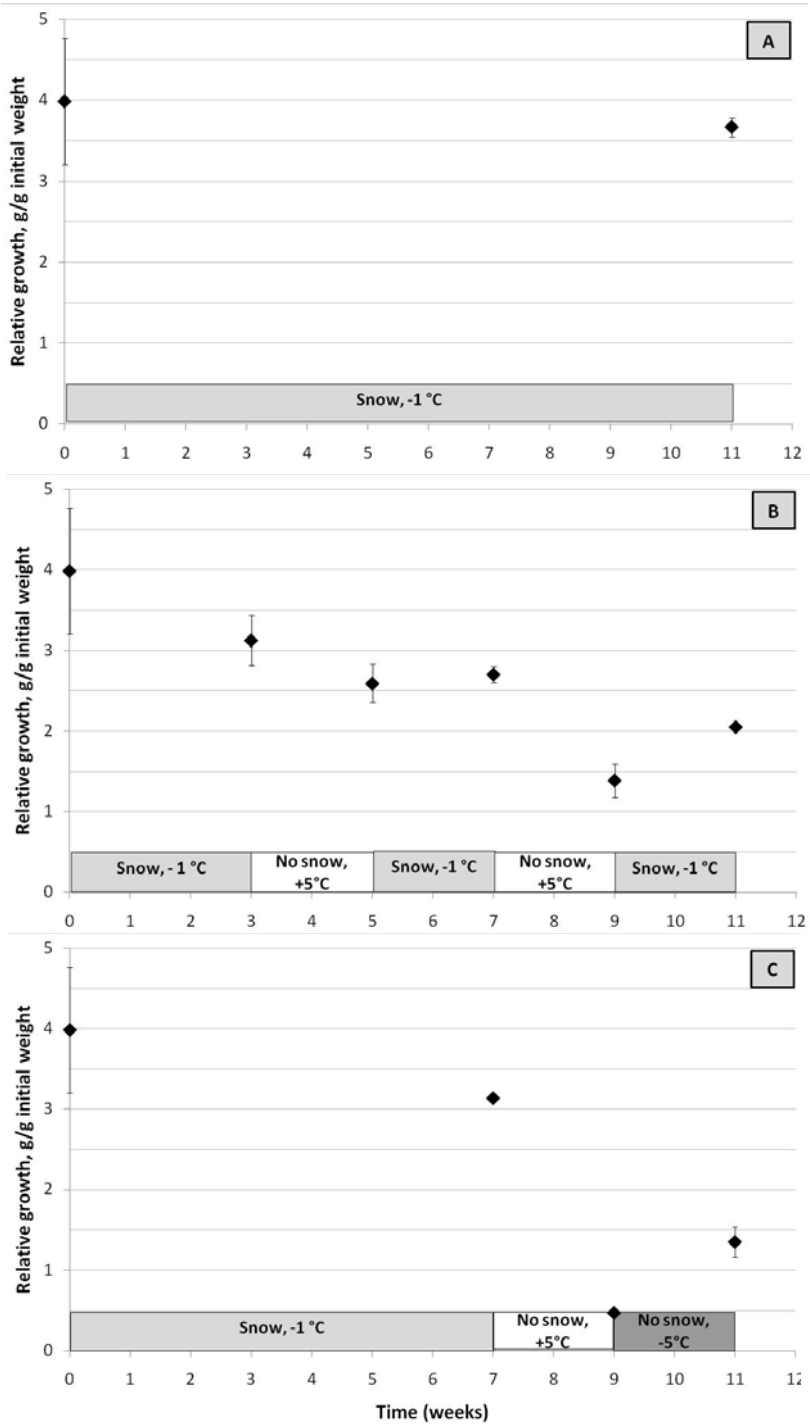

Fig. 3 Relative growth potential as determined by three weeks of growth at 18°C and long day conditions after each change of temperature and snow cover conditions in treatment A, B, and C. All recordings are given as means of two replicates of ten plants each. Error bars indicate standard deviations.

dening was –16 and –11°C, respectively (**Fig. 2C**). After the two weeks of dehardening, these plants were given conditions simulating a spring frost period with –5°C and no snow. Estimated mean LT_{50} recorded after two weeks with spring frost indicated that no rehardening occurred during this period.

Growth potential

There was no significant difference in relative growth potential between the start and the end of the 11 week long experimental period in treatment A, where the plants were kept under a constant, artificial snow cover at –1°C (**Fig. 3**). Treatment B did not cause any statistically significant differences in growth potential, either. However, results from this treatment indicated that the second period with 5°C and no snow cover (week 7-9) reduced the plants' vigour ($p = 0.1$). In treatment C, there was a significant reduction in growth potential during the period with 5°C and no snow between week seven and nine. The plants' growth potential stayed at this reduced level during the final weeks of both treatment B, with an artificial snow cover and –1°C, and treatment C, with no snow and –5°C.

DISCUSSION

Tests of frost tolerance performed during the experimental period showed that the plants had not obtained their maximum attainable level of frost tolerance during the preceding four weeks of hardening. According to an equation that calculates rate of winter wheat hardening in the model FROSTOL (Bergjord *et al.* 2008), an LT_{50} of about –21°C should

be expected for the winter wheat cultivar used in this experiment after four weeks at 2.5°C. Recorded LT_{50} after hardening was, however, only –16°C. Poorer light conditions inside the growth chamber as compared to outdoor field conditions probably slowed down the rate of hardening by reducing photosynthesis. Earlier experiments have shown that attainable level of frost tolerance is influenced by light conditions through the photosynthetic production of energy necessary for cold acclimation (Griffith and McIntyre 1993; Huner *et al.* 1998). The first days of the experimental period, where the temperature was lowered to –1°C, may also have acted as a 'second phase hardening'. A 'second phase hardening' at subzero temperatures is well known to increase plants' frost tolerance somewhat further beyond the level achieved by hardening at above zero temperatures (e.g. Herman *et al.* 2006).

During recent years, several genes associated with an increase in plant frost tolerance have been identified (Båga *et al.* 2007), genes which are induced as the temperature is lowered and hardening initiated. Initiation of cold hardening occurs when the temperature gets lower than 10°C (Limin and Fowler 1985). Proteins found to be highly correlated with the ability to tolerate cold, as for instance the *WCS120*-family, have been shown to accumulate to high levels in frost tolerant cultivars as hardening proceeds (e.g. Fowler *et al.* 1996a; Vitamvas and Prasil 2008). Through an interaction between genotype and environment, a number of physical and biochemical changes are induced in order to protect the plant cells from lethal freezing (Alden and Hermann 1971). Amongst these changes is a reduction of the plants' crown water content (Fowler and Carles 1979), which depresses the cells' freezing point, restricts intracellular ice formation, and down regulates metabolic activity and energy consumption (Kalberer *et al.* 2006).

Periods of dehardening on the other hand, reduce the expression of genes associated with frost tolerance (Vitamvas and Prasil 2008) and increase the crown water content (Gusta and Fowler 1976). As demonstrated during the periods with 5°C and no snow in the present study, dehardening may occur within the same temperature range as hardening. Whether certain temperatures cause hardening or dehardening to occur depends on the environmental history of the plants. Gay and Eagles (1991) have demonstrated how equal temperatures can induce both hardening and dehardening depending on plant history. Annual ryegrass which was grown at 15°C, acclimated at 2°C, and thereafter dehardened at 6, 8, or 10°C had almost the same frost tolerance as plants grown at 15°C and thereafter hardened at 6, 8, or 10°C.

The rate of dehardening in treatment B seemed slightly faster during the first period with 5°C between week three and five, as compared to the corresponding period between week 7 and 9 (**Fig. 2B**). During the first and the second dehardening period, the plants' LT_{50} changed from < –20 to –13.5°C, and from –18 to –13°C, respectively. Considering that recorded LT_{50} was lower before the first dehardening period than before the second one, this seems to be in accordance with Gusta and Fowler (1976) who found that rate of dehardening was higher during the first three days, when the plants' frost tolerance was high, than later in the dehardening period, when the frost tolerance was lower. In treatment C, loss of frost tolerance during dehardening followed the same rate as that of the last dehardening period in treatment B, with a change in LT_{50} from –16 to –11°C (**Fig. 2C**).

The plants were able to reharden when they were returned to –1°C after the first dehardening period in treatment B, although they did not regain the same level of frost tolerance as before dehardening (**Fig. 2B**). No rehardening was, however, seen after the last period with 5°C between week seven and nine. Nor did treatment C, where the plants were given –5°C after a corresponding, late dehardening period (weeks 7-9), seem to induce rehardening, although this could not be verified statistically due to rather large differences between replicates of the test (**Fig. 2C**). Reduced abilities of rehardening in late winter is in accordance with several earlier experiments which have revealed a close relationship between phenological development and attainable level of frost tolerance, and a down regulation of genes inducing frost tolerance once the plants were fully induced to generative development (Fowler *et al.* 1996a, 1996b; Mahfoozi *et al.* 2001; Danyluk *et al.* 2003; Prasil *et al.* 2004; Limin and Fowler 2006; Dhillon *et al.* 2010). The induction of generative development in winter wheat is regulated through the plants' requirement of vernalization and long day conditions. Vernalization occurs within the temperature range –1 to 15°C, with optimum temperatures around 5°C (Porter and Gawith 1999). Considering that 50 days with optimum vernalization temperature are assumed sufficient to saturate the vernalization requirement of winter wheat (Ritchie 1991), it is reasonable to believe that, in the present experiment, the vernalization requirement was fulfilled when the plants in treatment B were moved back to conditions with –1°C and snow cover after the first period of dehardening (week 3-5) (Slafer and Rawson 1996; Mahfoozi *et al.* 2000, 2001; Danyluk *et al.* 2003). However, as the plants were given short day conditions from hardening onwards, they probably remained at a vegetative stage of development right after vernalization saturation, and hence they were still able to reharden after the first dehardening period.

In an experiment by Bergjord *et al.* (2009), short day conditions delayed the induction of generative development by about one month after vernalization saturation, which in the present experiment would be around week nine. Hence when the plants in treatment B and C were given hardening conditions (–1°C and snow) after the latest dehardening period (weeks 7-9), they were most likely fully induced to generative development, and thus the genes inducing frost tolerance would be down regulated at this time. A total loss of the ability to reharden does, however, not correspond to results of Andrews *et al.* (1974), who found that field sampled winter wheat plants were able to increase their frost tolerance after snow thaw and exposure to lower temperatures in spring. The present experiment was conducted under artificial conditions with simulated mild and freezing periods, and earlier studies of plant frost tolerance have shown that plant response to temperature may be somewhat different in field than under controlled experimental conditions (Gusta and Fowler 1976). In addition, rehardening is an energy requiring process, and the observed reduction in growth potential after the latest dehardening period could indicate that the plants' storage of carbohydrates was depleted, rendering less energy available for rehardening. Still, although rates of hardening and dehardening may be slightly different in field as compared to the present results, the study gives important knowledge about plant responses to cycles with freezing and thawing periods and differing lengths of snow cover.

As the results indicate, the occurrence of mild periods during winter may exhaust both the plants tolerance to cold, and their growth potential. A mild period will increase plant respiration and perhaps also induce plant growth. Photosynthesis will, however, be restricted by the short photoperiod prevailing during winter in Northern Europe, giving a negative carbon balance. The increased demand for metabolites caused by higher respiration and possible growth must hence be covered by depletion of earlier accumulated carbohydrate reserves. In this view it is not surprising that the occurrence of a mild period in late winter, when the plants have already been depending on and depleted their storages of carbohydrates for several weeks, had a more dramatic consequence for plant growth potential than a similar incident during early winter (**Fig. 3B, 3C**).

The present results indicate some of the challenges we may face regarding overwintering of winter wheat in a future climate. Milder winter seasons and increased weather variability will make scenarios with repeated cycles of freezing and thawing plausible. The plants' rehardening capacity may hence constitute an important factor regarding the

ability to survive winter. Mahfoozi *et al*. (2006) have earlier concluded that mechanisms which can extend the plants' vegetative phase and hence also their ability to retain a high level of frost tolerance, are of great importance for winter wheat survival in regions with long, mild winters. However, sometimes temperature shifts happen too fast for any rehardening to occur. A mild period followed by a cold spell could be detrimental, especially if thawing has left the plants without an insulating snow cover to protect them against such low temperature incidents. Midwinter thaws followed by moderately cold freezing periods have earlier been known to be a common cause of winter injury in the eastern USA (Olien 1967). A more rapid depletion of the plants' carbohydrate reserves in a milder winter climate will also weaken the plants and make them more vulnerable for such damages. Repeated cycles of thawing and freezing will most probably increase the depletion of carbohydrate reserves even further as both the dehardening and rehardening processes require energy. In view of the present results, it seems reasonable to expect that the anticipated future climatic changes will make winter wheat in Northern Europe more vulnerable for different kinds of winter damage.

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REFERENCES

Alden J, Hermann RK (1971) Aspects of the cold-hardiness mechanism in plants. *The Botanical Review* **37**, 37-142

Andrews CJ, Pomeroy MK, Delaroch IA (1974) Changes in cold hardiness of overwintering winter-wheat. *Canadian Journal of Plant Science* **54**, 9-15

Båga M, Chodaparambil SV, Limin AE, Pecar M, Fowler DB, Chibbar RN (2007) Identification of quantitative trait loci and associated candidate genes for low-temperature tolerance in cold-hardy winter wheat. *Functional and Integrative Genomics* **7**, 53-68

Bergjord AK, Bakken AK, Skjelvåg AO (2009) The relationship between frost tolerance and generative induction in winter wheat (*Triticum aestivum* L.) under field conditions. *Canadian Journal of Plant Science* **89**, 1031-1039

Bergjord AK, Bonesmo H, Skjelvåg AO (2008) Modelling the course of frost tolerance in winter wheat I: Model development. *European Journal of Agronomy* **28**, 321-330

Christensen JH, Raisanen J, Iversen T, Bjorge D, Christensen OB, Rummukainen M (2001) A synthesis of regional climate change simulations - A Scandinavian perspective. *Geophysical Research Letters* **28**, 1003-1006

Danyluk J, Kane NA, Breton G, Limin AE, Fowler DB, Sarhan F (2003) TaVRT-1, a putative transcription factor associated with vegetative to reproductive transition in cereals. *Plant Physiology* **132**, 1849-1860

Dhillon T, Pearce SP, Stockinger EJ, Distelfeld A, Li C, Knox AK, Vashegyi I, Vagujfalvi A, Galiba G, Dubcovsky J (2010) Regulation of freezing tolerance and flowering in temperate cereals: The VRN-1 connection. *Plant Physiology* **153**, 1846-1858

Fowler DB, Carles RJ (1979) Growth, development, and cold tolerance of fall-acclimated cereal-grains. *Crop Science* **19**, 915-922

Fowler DB, Chauvin LP, Limin AE, Sarhan F (1996a) The regulatory role of vernalization in the expression of low-temperature-induced genes in wheat and rye. *Theoretical and Applied Genetics* **93**, 554-559

Fowler DB, Limin AE, Wang SY, Ward RW (1996b) Relationship between low-temperature tolerance and vernalization response in wheat and rye. *Canadian Journal of Plant Science* **76**, 37-42

Gay AP, Eagles CF (1991) Quantitative analysis of cold hardening and dehardening in *Lolium*. *Annals of Botany* **67**, 339-345

Griffith M, McIntyre HCH (1993) The interrelationship of growth and frost tolerance in winter rye. *Physiologia Plantarum* **87**, 335-344

Gusta LV, Fowler DB (1976) Effects of temperature on dehardening and rehardening of winter cereals. *Canadian Journal of Plant Science* **56**, 673-678

Herman EM, Rotter K, Premakumar R, Elwinger G, Bae R, Ehler-King L, Chen S, Livingston DP (2006) Additional freeze hardiness in wheat acquired by exposure to -3°C is associated with extensive physiological, morphological, and molecular changes. *Journal of Experimental Botany* **57**, 3601-3618

Huner NPA, Oquist G, Sarhan F (1998) Energy balance and acclimation to light and cold. *Trends in Plant Science* **3**, 224-230

Kalberer SR, Wisniewski M, Arora R (2006) Deacclimation and reacclimation of cold-hardy plants: Current understanding and emerging concepts. *Plant Science* **171**, 3-16

Limin AE, Fowler DB (1985) Cold-hardiness response of sequential winter-wheat tissue segments to differing temperature regimes. *Crop Science* **25**, 838-843

Limin AE, Fowler DB (1988) Cold hardiness expression in interspecific hybrids and amphiploids of the Triticeae. *Genome* **30**, 361-365

Limin AE, Fowler DB (2006) Low-temperature tolerance and genetic potential in wheat (*Triticum aestivum* L.): Response to photoperiod, vernalization, and plant development. *Planta* **224**, 360-366

Mahfoozi S, Limin AE, Ahakpaz F, Fowler DB (2006) Phenological development and expression of freezing resistance in spring and winter wheat under field conditions in north-west Iran. *Field Crops Research* **97**, 182-187

Mahfoozi S, Limin AE, Fowler DB (2001) Developmental regulation of low-temperature tolerance in winter wheat. *Annals of Botany* **87**, 751-757

Mahfoozi S, Limin AE, Hayes PM, Hucl P, Fowler DB (2000) Influence of photoperiod response on the expression of cold hardiness in wheat and barley. *Canadian Journal of Plant Science* **80**, 721-724

Olien CR (1967) Freezing stresses and survival. *Annual Review of Plant Physiology* **18**, 387-408

Pomeroy MK, Andrews CJ, Fedak G (1975) Cold hardening and dehardening responses in winter-wheat and winter barley. *Canadian Journal of Plant Science* **55**, 529-535

Porter JR, Gawith M (1999) Temperatures and the growth and development of wheat: A review. *European Journal of Agronomy* **10**, 23-36

Prasil IT, Prasilova P, Pankova K (2004) Relationships among vernalization, shoot apex development and frost tolerance in wheat. *Annals of Botany* **94**, 413-418

Ritchie JT (1991) Wheat phasic development. In: Hanks RJ, Ritchie JT (Eds) *Modeling Plant and Soil Systems*, Agronomy Monograph 31. ASA-CSSA-SSSA, Madison, WI, pp 31-54

Semenov MA, Porter JR (1995) Climatic variability and the modeling of crop yields. *Agricultural and Forest Meteorology* **73**, 265-283

Slafer GA, Rawson HM (1996) Responses to photoperiod change with phenophase and temperature during wheat development. *Field Crops Research* **46**, 1-13

Vitamvas P, Prasil IT (2008) WCS120 protein family and frost tolerance during cold acclimation, deacclimation and reacclimation of winter wheat. *Plant Physiology and Biochemistry* **46**, 970-976