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Environmental Regulation of Dormancy and Frost Hardiness in Norwegian Populations of Wood Strawberry (*Fragaria vesca* L.)

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ABSTRACT

Effects of temperature and photoperiod on plant dormancy development and cold hardiness were examined in six latitudinal and altitudinal populations of wood strawberry (*Fragaria vesca* L.). Following exposure to natural seasonal changes and/or artificial manipulation of the plant environment, the degree of dormancy was essayed by forcing plants at 20°C and 20 h photoperiod, while cold hardiness was essayed by artificial freezing. Dormancy development in plants grown outdoors under natural autumn temperature and daylength conditions at 61°N differed greatly in two successive years with contrasting temperature conditions. When temperature was relatively high during late October and the first half of November, the plants attained the deepest state of dormancy by late November, while low temperature during the same period resulted in a gradual loss of dormancy throughout autumn. The responses of plants grown at controlled temperatures, likewise confirmed that relatively warm conditions are required for SD dormancy induction in wood strawberry. The capacity for runner formation and petiole elongation were identified as the most sensitive criteria of the state of dormancy. Growth vigour and flowering generally decreased with increasing latitude of population origin. Development of frost hardiness required exposure to short days (SD) and near-freezing temperatures for several weeks. Non-hardened plants grown at 18°C and 18-h photoperiod, were all killed at -12°C (all surviving -6°C), regardless of population origin. However, hardened plants exposed to natural out-door conditions for 10 weeks from September 27 to December 5 all survived freezing at -15°C, many at -18°C, and some even at -21 and -24°C. In such hardened plants there was a clinal increase in frost hardiness with increasing latitude of population origin. The results are discussed in relation to results with cultivated strawberry.

Keywords: artificial freezing, frost tolerance, latitude, photoperiod, rest, temperature
Abbreviations: LD, long day(s); **SD**, short day(s)

INTRODUCTION

Seasonal regulation of growth and dormancy is vital for winter survival of plants living in temperate and cold climates. This requires a reliable environmental signal that can be sensed and processed by the plant well in advance of the cold season. The role of short photoperiods in autumn as the principal dormancy-inducing signal was first demonstrated by Garner and Allard (1923) and has later been documented for a wide range of temperate trees and shrubs (references in Thomas and Vince-Prue 1997). Photoperiodic control of dormancy induction is well known also in many temperate perennial herbs. In many of these plants dormancy is accompanied by the formation of resting storage organs such as bulbs, corms and tubers and other perennating organs (Thomas and Vince-Prue 1997; Heide 2001).

In the genus *Fragaria* vegetative growth and stolon formation are stimulated by long days (LD) and warm temperatures, while flowering and dormancy induction are controlled by a pronounced interaction of photoperiod and temerature (Heide 1977; Guttridge 1985). In the cultivated octoploid strawberry (*F.* x *ananassa*) prolonged exposure to short day (SD) conditions results in gradually decreasing growth and declining leaf size; stolon formation is stopped while flower primordia are initiated, and the plants attain a dormant state. However, this dormant state is only quantitative and can be considered a state of semi-dormancy. Thus, so-called dormant plants, maintained under natural autumn conditions, retain the capability to resume growth, albeit at a reduced rate, if transferred to a heated greenhouse even in November when the deepest dormancy is attained (Jonkers 1965; Jahn and Dana 1966). Yet, restoration of the full growth potential requires chilling for several weeks at temperatures ranging from -2 to +6°C, the effect being enhanced by LD (Guttridge 1985; Lieten 1997; Sønsteby and Heide 2006). In cv. 'Korona' and 'Elsanta' marked growth reduction is visible after 5 weeks of SD exposure, while 10 or more weeks of SD are required for attainment of the dormant state that requires chilling to be released (Sønsteby and Heide 2006). Whereas extended SD exposure induced the typical dormant state at 15°C, no such effect was seen at 6°C. Since 6°C is within the range of temperatures that are fully effective in breaking of dormancy in strawberry (Guttridge 1985; Lieten 1997), it appears that the dormancy-inducing effect of SD is continuously nullified at this temperature (Sønsteby and Heide 2006).

Because of the complex genetic constitution of the octoploid cultivated strawberry, there is increasing interest in using diploid species such as the wood strawberry (*Fragaria vesca*) as a model plant for genetic analyses in strawberry (Battey *et al*. 1998), and a genetic linkage map of important genetic markers has been constructed (Sargent *et al*. 2004). A long-term goal of the Norwegian strawberry breeding is to increase winter hardiness. Due to the complexity involved in the regulation and enhancing freezing tolerance, the progress in the improvement of cultivars using traditional screening methods have had limited success. Thus, the development of molecular markers for freezing hardiness would facilitate the selection work for this trait. Diversity studies of diploid *F. vesca*, are considered to lead to the identification of molecular and biochemical marker compounds (M. Alsheikh, pers. comm.). However, the

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Table 1 Geographic origin of the six Norwegian populations of *Fragaria vesca* used in the investigations.

Population	Latitude ºN	Longitude ºE	Altitude m a.s.l.
Ås	59º40′	10º45′	70
Namsos	64º30′	11º35′	5
Grytøy	68º50′	13º25′	15
Alta	69º55′	23º0′	40
Haugastøl	60º35′	07º50′	1080
Hardanger	60º10′	06º35′	15

full utilization of such genomic information must build on an integration of genetic and physiological approaches for an understanding of the environmental control mechanisms involved. Therefore, we have previously studied the environmental control of flowering and its variation in a range of Norwegian populations of *F. vesca* (Heide and Sønsteby 2007). Here, we report of related studies on the environmental control of dormancy and cold hardiness in the same populations.

MATERIALS AND METHODS

Plant material and cultivation

The experiments were carried out in the Ås phytotron and in the field at the Kise Experimental Station (Nes Hedmark, 60° 40'N, 10° 11'E). Six Norwegian populations of *Fragaria vesca* L. of different latitudinal and altitudinal origin were used in the experiments (**Table 1**). Runner plants were collected in May 2004 and planted in the field at Kise as described by Heide and Sønsteby (2007), each population being represented by three separate clones. For experimental use, runners of the collected plants were sampled in August-September and rooted and established in 10 cm plastic pots in a greenhouse maintained at 18 ± 2°C and 18-h photoperiod. Plants used for the freezing experiments were transplanted into 12 cm pots in order to provide a larger soil volume with larger temperature buffering capacity. Throughout the experiments, the plants were grown in a peat-based potting compost (90% peat, 10% clay), with the addition of 1:5 v/v of granulated perlite. The soil mixture was fertilized with 300 g 80 l^{-1} of Osmocote controlled release fertilizer (14% N, 4.2% P, 11.6% K + micronutrients, release rate 3-4 months) (Scotts UK Ltd., Nottingham, UK). The plants were regularly watered with tap water as required.

Four experiments were conducted. In Expt. 1, rooted and well established plants of all six populations were moved out-doors and exposed to natural temperature and day-length conditions from September 15, 2005. Samples of plants were then moved into a heated greenhouse maintained at 20 ± 2°C and 24-h photoperiod after 5, 8, 11, 14 and 17 weeks (on Oct. 18, Nov. 8, Nov. 29, Dec. 20 and Jan. 10, respectively), for testing the dormancy state. The experiment was repeated in 2006 with identical timing of all operations and treatments. The temperature conditions in the two years are shown in **Fig. 1**. Expt. 2 was conducted in a phytotron and included only populations 'Ås', 'Haugastøl' and 'Alta'. These populations were chosen because they were representing latitudinal and altitudinal extremes of Norwegian wild strawberry populations. On September 25, 2007, well established plants were placed in two day-light phytotron compartments maintained at 6 ± 1°C and 15 ± 1°C respectively, and natural (SD) photoperiod. Supplemental lighting was provided by Philips HPTI 400 W lamps at a photosynthetic photon flux density (PPFD) of 150 µmol quanta m^{-2} s^{-1} throughout the light period (0800 – 1800h). After 3, 7, 11 and 15 weeks of exposure to these conditions, samples of plants were moved into a growth room for forcing at 21°C and 24-h photoperiod. During the forcing period the plants received a 12-h basic illumination with Philips HPI-T 400 W lamps (270 µmol quanta m^{-2} s^{-1}, 0800-2000 h), and daylength extension to 24 h was provided by 75 W incandescent lamps at a PPFD of about 7 µmol quanta m^{-2} s^{-1}. Expts. 3 and 4 were freezing tests with non-hardened, respective hardened plants of all six populations. In Expt. 3, the plants were grown in a heated greenhouse maintained at 18 ± 2°C and 18-h photoperiod (0600 – 2400 h) for 5 weeks starting on October 26, 2004. Supplemental lighting was provided by high pressure sodium lamps (SON-T) at a PPFD of about 200 µmol quanta m^{-2} s^{-1} throughout the photoperiod (0600 – 2400 h). After conditioning for an additional week during which temperature and daylength were gradually reduced to 6°C and 12-h photoperiod, the plants were exposed to freezing at temperatures ranging from -6 to -30°C. In Expt 4 the plants were grown out-doors under natural temperature and daylength conditions for 10 weeks (Sep. 27 to Dec. 6, 2005) and then exposed to freezing at temperatures ranging from -9 to -48°C. During freezing the plants were placed on moist felt pads in perforated plastic trays. The freezing was performed in darkness in freezing cabinets at a freeze and thaw rate of 2°C h^{-1} and exposure to the respective freezing temperatures for 2 h. Control plants were exposed to 0°C in darkness for 48 h for comparison. Since in Expt. 4 the plants were frozen before intake, they were left to thaw and condition in darkness at 2°C for three days before the freezing treatments were started. After completion of the freeze and thaw cycle, the plants were left to thaw at 2°C for 24 h, whereupon the plants were moved into a greenhouse maintained at 18 ± 2°C and 20 h photoperiod for recording of plant survival and growth performance.

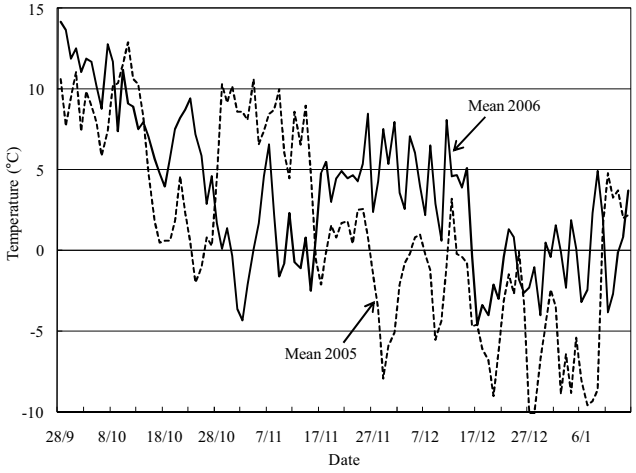

Fig. 1 Daily mean temperature records during the experimental period at Kise Experimental Station, Nes Hedmark in the years 2005 and 2006. Data from the Norwegian Meteorological Institute's weather station located at Kise.

Experimental design, data observation and analysis

The experiments were fully factorial of the split-plot design. All experiments were replicated with three randomized blocks with 5 plants of each of the three clones of each population (see above), giving a total of 15 plants of each population in each treatment. In the dormancy induction experiments (Expts. 1 and 2), all stolons were removed and the number of leaves and their petiole length were recorded at the end of the conditioning treatments. During the following forcing period, growth and flowering were monitored by weekly observations and, at termination of the experiments after 5 weeks of forcing, the following parameters were recorded on each plant: number and length of inflorescences, total number of flowers, number of runners and leaves, and the petiole length of three fully developed leaves. In the freezing tests the following parameters were recorded after 5 weeks at 18°C and 20-h photoperiod: number of surviving plants, number of new leaves, number of dead leaves, and number of runners. In addition, the degree of injury of surviving plants were estimated by observation of the extent (scale 1-5) and intensity (scale 1-3) of discolouration (browning) of longitudinal crown sections as described by Marini and Boyce (1977). ANOVA analyses were performed by standard procedures using a MiniTab® Statistical Software programme package (Release 14; Minitab Inc., State College, PA, USA). Since primary analyses revealed non-significant differences among the three clones within each population, the clones were treated as replications in the final statistical analyses.

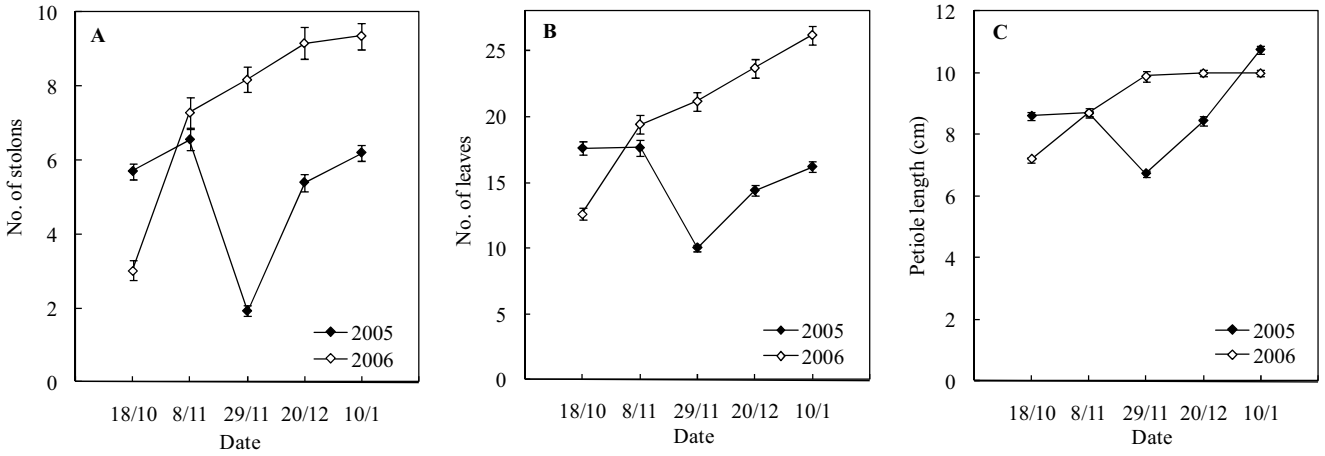

Fig. 2 Dormancy state of wood strawberry plants. This state was expressed by the capacity for stolon (**A**) and leaf formation (**B**), as well as petiole elongation of new leaves (**C**) after forcing for 5 weeks at various stages of the autumn in the years 2005 and 2006. The data are means ± SE of six populations, each represented with 15 plants per treatment.

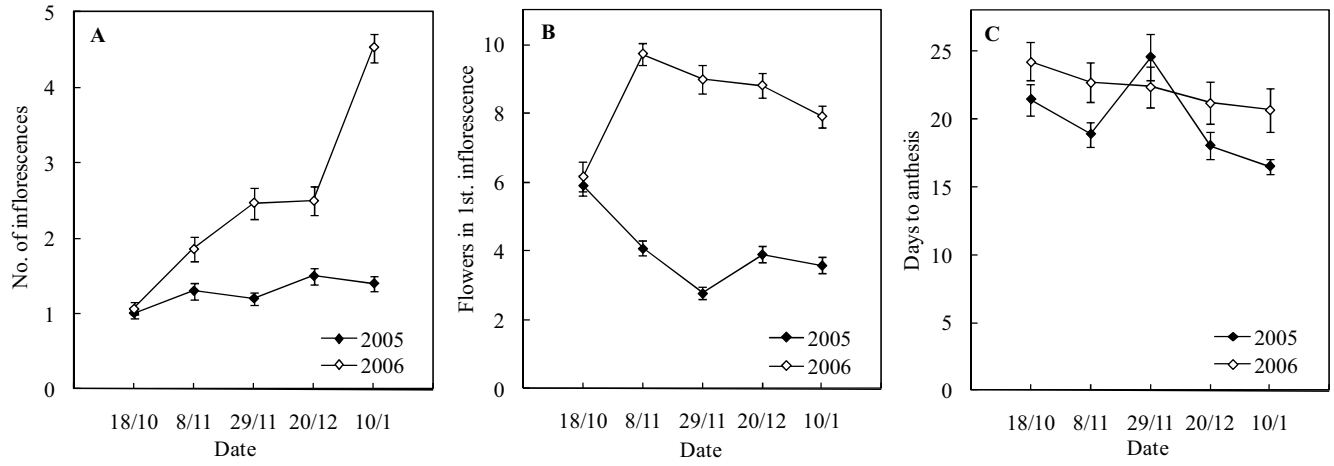

Fig. 3 Flowering capacity (A, B) and days to anthesis (C) of wood strawberry plants as essayed by forcing for 5 weeks in LD (24 h) at 20°C at various stages during autumn in 2005 and 2006. The data are means ± SE of five populations, each represented by 15 plants per treatment (Alta population did not flower).

RESULTS

Dormancy regulation

In Expt. 1 both temperature conditions and plant responses differed markedly in the two years of experimentation. While the temperature during a three-week period starting on October 28 was relatively high with an average mean of about 9°C in 2005, the average was close to zero during the same period in 2006 (**Fig. 1**). During the next four weeks, however, the relations were reversed, although with lower temperatures during both years. This was associated with principally different dormancy responses of the plants in the two years (**Fig. 2**). Whereas dormancy increased during the early part of autumn and reached the deepest state by the end of November in year 2005, there was a gradual loss of dormancy throughout the same period in 2006. Thus, while the capacity for runner and leaf formation and petiole elongation all decreased from Oct. 18 through Nov. 8 to Nov. 29, and then increased again by Dec, 20 and Jan. 10 in the 2005 season, there was a gradual increase in the capacities for these processes over the entire period in 2006. Growth vigour and runnering generally decreased among the populations with increasing latitude and altitude of population origin (data not shown). The extent and earliness of flowering also varied markedly in the two years (**Fig. 3**). Flowering increased with extended exposure to natural SD and autumn temperature in both years, but more so in year 2006 than in 2005. Also, the seasonal trends varied markedly between the two years. As for vegetative growth, the dormancy state of initiated flower buds (as expressed by days to anthesis) was deepest by the end of November in year 2005, while there was a slow and gradual loss of dormancy throughout the autumn in 2006. The results also confirmed

Table 2 Probability levels of significance of ANOVA for the various dormancy indicators studied in Experiment 1.

Source of variation	No. of inflorescences*	Flowers in 1st inflorescence*	Days to anthesis*	No. of stolons	No. of leaves	Petiole length (cm)
Year (A)	0.01	0.003	n.s.	0.04	0.02	<0.001
Date (B)	<0.001	0.001	0.02	<0.001	<0.001	<0.001
A x B	<0.001	<0.001	n.s.	<0.001	<0.001	<0.001
Population (C)	<0.001	<0.001	0.002	<0.001	<0.001	<0.001
A x C	<0.001	<0.001	n.s.	<0.001	<0.001	<0.001
B x C	<0.001	n.s.	n.s.	0.03	n.s.	0.02
A x B x C	n.s.	n.s.	n.s.	0.02	n.s.	0.04

*Population Alta excluded in flowering parameters

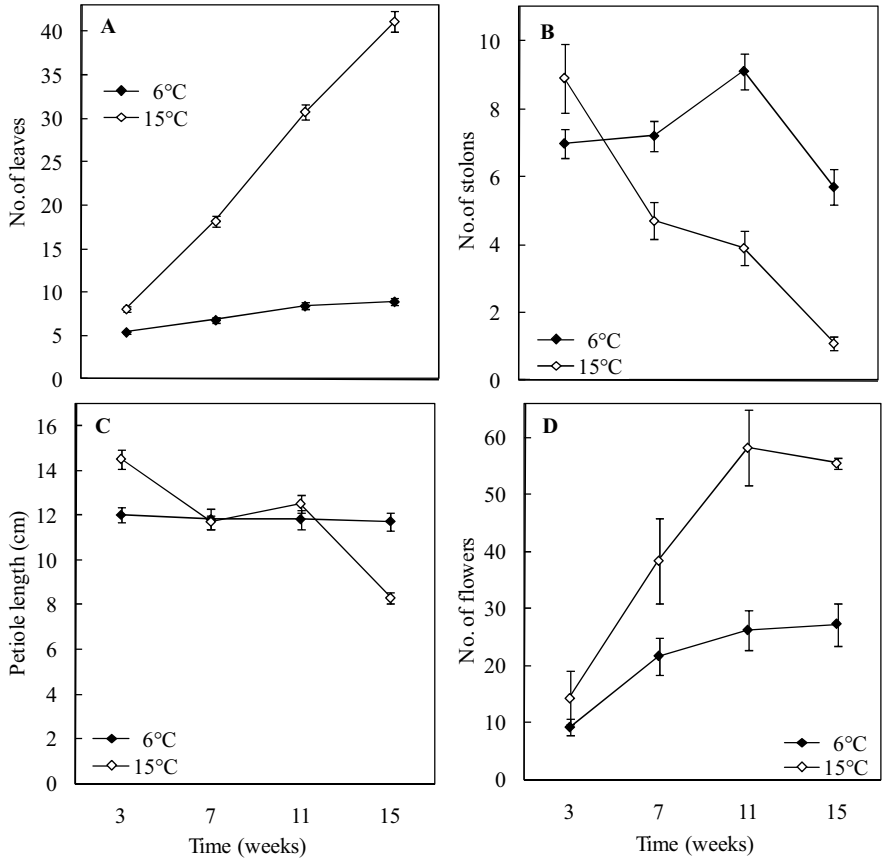

Fig. 4 Growth and flowering performance of three distant populations of wood strawberry grown at constant temperatures of 6 and 15°C and 12 h photoperiod for varying lengths of time. Panel (**A**) denotes the cumulative number of leaves produced during the cultivation period, while the other panels denote number of stolons (**B**), petiole length (**C**) and number of flowers (**D**) as recorded after 5 weeks of forcing at 21°C and 24 h photoperiod. The data are means ± SE of the populations Ås, Haugastøl and Alta, each represented by 15 plants per treatment (except panel (**D**) in which the non-flowering Alta population is excluded).

our earlier finding (Heide and Sønsteby 2007) that the Alta population from 70°N has an extreme floral induction requirement and did not flower even after exposure to out-door conditions until January 10 in either year. The probability levels of significance for these various responses are shown in **Table 2**.

In an attempt to verify these temperature effects, the dormancy-inducing effect of natural autumn SD was examined at two controlled temperature conditions in the phytotron (Expt. 2). Starting on September 25, plants of populations 'Ås', 'Haugastøl' and 'Alta' were exposed to natural autumn SD conditions at 6 and 15°C for 3, 7, 11 and 15 weeks, followed by forcing at 20°C and 24-h daylength. As shown in **Fig. 4A**, the plants continued to grow and produce new leaves during SD at both temperatures but at a much higher rate at the higher temperature. However, the capacity for stolon formation under subsequent forcing was steadily reduced in plants grown at 15°C and, after 15 weeks, it was almost completely blocked, while at 6°C the capacity for stolon formation remained more or less constant (**Fig. 4B**). The ANOVA revealed a significant main effect of temperature ($P = 0.03$) on the runnering capacity, and a highly significant ($P < 0.001$) interaction of temperature and length of exposure, the latter also having a highly significant main effect ($P < 0.001$). A similar but less pronounced temperature effect was observed on petiole elongation, the capacity for elongation gradually decreasing with time at 15°C, but remaining more or less constant at 6°C (**Fig. 4C**). For this parameter, however, the main effect of temperature was non-significant, due to a highly significant ($P < 0.001$) interaction of temperature and length of exposure, the main effect of which also being highly significant.

In concurrence with our previous findings (Heide and Sønsteby 2007), flower initiation took place in SD at both temperatures, the number of flowers always being higher at 15 than at 6°C (**Fig. 4D**). At both temperatures the number of initiated flowers increased with extended exposure for up to 11 weeks, whereupon it levelled off. As in the previous experiments no flowering took place in the 'Alta' population, whereas the 'Ås' population had particularly rich flowering. Inflorescence height at anthesis was not significantly affected by temperature, but decreased with time from about 16 cm after 3 weeks of exposure to about 8 cm after 15 weeks (data not shown).

Frost hardiness

Non-hardened plants were all killed by freezing to -12°C and lower temperatures regardless of population origin, whereas all survived -6°C (**Table 3**). However, the plants surviving -6°C showed various degrees of injury on leaves and inflorescences and in the extent and degree of crown tissue browning, indicating that the lethal temperature was not much below -6°C. The degree of injury after exposure to -6°C also varied significantly ($P < 0.05$) among the populations, the high-latitude populations 'Grytøy' and 'Alta' being less injured than the other populations (**Table 4**). Whereas all other populations flowered with one or two inflorescences, plants of the 'Alta' population did not flower at all, confirming our earlier observation (Heide and Sønsteby 2007) that this population has an unusual floral induction requirement (data not shown).

On the other hand, hardened plants exposed to natural out-door conditions for 10 weeks during late autumn all survived freezing at -15°C, many at -18°C, and some even at -21 and -24°C (**Table 5**, **Fig. 5**). Both plant survival and the degree of sub-lethal leaf injury (**Table 5**) and crown tissue browning of surviving plants (**Table 6**) varied significantly among the populations, frost hardiness generally increasing with increasing latitude of population origin. On

Table 3 Number of surviving plants (out of 15) of six Norwegian populations of *Fragaria vesca* L. as recorded five weeks after freezing at different temperatures (Experiment 3).

Freezing temperature (ºC)	Population					
	Ås	Namsos	Grytøy	Alta	Haugastøl	Hardanger
0	15	15	15	15	15	15
-6	15	15	15	15	15	15
-12, -18, -24, -30	0	0	0	0	0	0

Table 4 Freezing injuries in non-dormant plants of six populations of *Fragaria vesca* exposed to 0 and 6°C. Data for surviving plants only (Experiment 3).

Population	Number of green leaves		Number of dead leaves		Tissue browning (1-5)		Intensity of discoloration (1-3)	
	Freezing temperature (°C)		Freezing temperature (°C)		Freezing temperature (°C)		Freezing temperature (°C)	
	0	-6	0	-6	0	-6	0	-6
Ås	14.3 b*	7.9 c	0.5 c	5.5 ab	1.0	2.9 ab	1.0	2.0 ab
Namsos	16.4 ab	9.2 bc	1.5 ab	5.5 ab	1.0	2.5 bc	1.0	1.9 ab
Grytøy	13.1 b	13.5 ab	0.7 abc	1.1 c	1.0	1.8 c	1.0	1.6 b
Alta	18.4 a	14.7 a	1.6 a	3.9 b	1.0	2.1 bc	1.0	1.6 b
Haugastøl	14.9 b	6.3 c	1.5 ab	6.8 a	1.0	3.5 a	1.0	2.3 a
Hardanger	16.4 ab	9.7 bc	.6 bc	5.7 ab	1.0	2.7 abc	1.0	2.3 a
Mean	15.6 a	10.2 b	1.1 a	4.7 b	1.0 a	2.6 b	1.0 a	2.0 b
Probability levels of significance (ANOVA) foundation of variation								
Population (A)		0.02		0.005		0.04		n.s.
Temperature (B)		< 0.001		< 0.001		< 0.001		< 0.001
A x B		< 0.001		< 0.001		< 0.001		< 0.001

Table 5 Number of surviving plants (and injured plants) of six Norwegian populations of *Fragaria vesca* as recorded five weeks after freezing at different temperatures (n = 15). (Experiment 4).

Freezing temperature (ºC)	Population					
	Ås	Namsos	Grytøy	Alta	Haugastøl	Hardanger
0	15	15	15	15	15	15
-9	15	15	15	15	15	15
-12	15 (1)	15	15	15	15 (1)	15
-15	15 (10)	15 (6)	15 (0)	15 (2)	15 (2)	15 (4)
-18	8 (8)	11 (6)	10 (4)	14 (2)	5 (5)	6 (5)
-21	0	4 (4)	3 (3)	8 (8)	6 (6)	5 (5)
-24	0	3 (3)	2 (2)	9 (9)	0	0
-30, -48	0	0	0	0	0	0

Table 6 Freezing injury estimated as extent of crown tissue browning on a scale from 1 (no discoloration) to 5 (discoloration extending the entire length of the crown) for six Norwegian populations of *Fragaria vesca*. (Expt. 4).

Freezing temperature (ºC)	Population					
	Ås	Namsos	Grytøy	Alta	Haugastøl	Hardanger
0	1.0	1.0	1.0	1.0	1.0	1.0
-9	1.0	1.0	1.0	1.0	1.0	1.0
-12	1.1	1.0	1.0	1.0	1.1	1.0
-15	1.8	1.7	1.0	1.3	1.1	1.7
-18	4.0	2.5	2.7	1.4	4.2	4.0
-21	5.0	4.5	4.7	4.0	4.4	4.4
-24	5.0	4.7	4.8	3.9	5.0	4.8
-30, -48	5.0	5.0	5.0	5.0	5.0	5.0

the other hand, the high-altitude 'Haugastøl' population was as susceptible to frost injury as the low-altitude 'Ås' and 'Hardanger' populations from the same latitude. Also root survival and regrowth varied in a similar way among the populations (data not shown). In some cases plants survived by formation of new roots from the vascular tissue at the base of the crowns after all old roots had been killed by freezing (see e. g. -18°C plant in **Fig. 5**).

DISCUSSION

The pattern and extent of dormancy development varied significantly between the two years of experimentation (**Fig. 2**). In 2005 when the plants experienced relatively mild temperatures up to mid November, the dormancy state increased during the early part of autumn and reached its deepest state by late November. This is in full agreement with the results of similar experiments with cultivated strawberry in the Netherlands (Jonkers 1965) and Great Britain (Guttridge 1985; Battey *et al*. 1998) where autumns

Fig. 5 Appearance of *Fragaria vesca* plants, population Ås, after freezing at -12, -15, -18 and -21°C and subsequent growth for 5 weeks at 18°C and 20 h photoperiod. Note severe root injury and formation of adventitious roots after freezing at -18°C. The plants frozen at -21°C died.

are relatively mild. In contrast, in 2006 when the temperature in periods was close to zero, there was an early and continuing loss of dormancy during the entire autumn (**Fig. 2**). Also the phytotron experiment with constant temperatures indicated that dormancy did not develop at 6°C, while the normal semi-dormant state was attained at 15°C (**Fig. 4**). This concurs with the responses of 'Korona' and 'Elsanta' strawberry which likewise developed the usual semi-dormant state at 15°C but not at 6°C (Sønsteby and Heide 2006). A large number of experiments (see review by Guttridge 1985) have shown that temperatures ranging from -2 to 6°C are fully effective in breaking of dormancy in cultivated strawberry, whereas 9.5 and 10°C are almost ineffective (*cf*. Lieten 1997). The results suggest that within this range of temperatures, the dormancy inducing effect of SD is continuously nullified by low temperature (*cf*. Sønsteby and Heide 2006). This interpretation is fully compatible with the dynamic changes in the response of strawberry plants to seasonal changes in the natural environment. Thus, in the early part of autumn when temperatures are still favourable, SD causes dormancy induction, while later in the season when temperature is approaching freezing, dormancy is gradually released despite even shorter photoperiods (Jonkers 1965; Guttridge 1985; Battey *et al*. 1998). Although the results of the present experiments were not always clear cut for all dormancy parameters, they tend to support this hypothesis, and it is likely that autumn temperature fluctuations between 0 and 10°C in the two years of experimentation is the basis for the contrasting results. As reported for cultivated strawberry (Jahn and Dana 1966; Sønsteby and Heide 2007), initiation and immergence of new leaves did not cease in SD but continued at a constant rate at both 15 and 6°C (**Fig. 4A**), although leaf size steadily declined in SD. On the other hand, reduced capacity for leaf formation under subsequent forcing at high temperature and LD conditions was one of the symptoms of the semi-dormant state (**Fig. 2**).

Development of deep cold hardiness in wood strawberry required exposure to near-freezing temperatures for an extended period. This type of response is well known in woody species where cold hardening commonly takes place in two steps: First, growth has to be stopped by SD, and secondly, deep hardening takes place at temperatures slightly above or close to 0°C (Junttila and Robberecht 1998; Larcher 2003). Similar responses are known in perennial grasses and winter cereals (see Junttila and Kaurin 1987), while the responses of other herbaceous plants may vary (Heide 2001). In white clover, which has no marked winter dormancy (Junttila and Robberecht 1998), stolon elongation is reduced while cold hardening is enhanced by SD. However, development of deep cold resistance (-19°C) required exposure to near-freezing temperatures for several weeks.

In the present experiments with wood strawberry the temperature at which about 50% of the plants were killed (T_{50}) changed from slightly below -6°C in non-hardened plants (**Table 2**) to -18 to -24°C, depending on plant geographic origin (**Table 4**), in well hardened plants. Also, the data for hardened plants clearly indicate a clinal trend of increasing frost hardiness with increasing latitude of population origin, the T_{50} decreasing from -18°C in population 'Ås' with origin about 60°N to -24°C in the 'Alta' population from 70°N (**Table 4**). Similar differences in cold hardiness were found in white clover populations of varying latitudinal origin (Svenning *et al*. 2000). However, there was no parallel increase in frost hardiness of wood strawberry of alpine origin. Thus, the 'Haugastøl' population with origin 1080 m a. s. l. was as susceptible to low freezing temperatures as the low-altitude populations from the same latitude (**Tables 3-5**). It seems likely that early snowfall in autumn and stable snow cover throughout winter and early spring, that prevail at such high elevation, provides a protected winter environment that have prevented hard selection for low temperature tolerance.

Although examples of extreme cold hardiness in strawberry are mentioned by Darrow (1966), with some cultivars surviving -40°C or even lower temperatures, there is no experimental evidence to support this. Using a freezing rate of 1°C h^{-1} Marini and Boyce (1977) found that hardened 'Catskill' plants all survived -4°C, while all were killed at -20°C, the T_{50} being about -12.5°C. Sub-lethal temperatures of -8, -12 and -16°C caused various degrees of injury on leaf and inflorescence primordia and browning of crown tissues. Using the same freezing method as the present experiments, Nestby and Bjørgum (1999), likewise found that the strawberry cultivars 'Bounty', 'Senga Segana' and 'Korona' were all killed at -20°C, with various degrees of injury at temperatures of -8, -12 and -16°C. Based on these comparisons it can be concluded that high-latitude wood strawberry populations are hardy and can resist 3-6°C lower temperature than most cultivated varieties. They should therefore, be scrutinized for the presence of useful genetic hardiness markers that can be utilized in breeding of hardy strawberries.

The experiments also demonstrated that there is no direct relationship between dormancy state and cold hardiness of strawberry plants. Thus, by December 6 when the hardened plants were exposed to freezing, the plants had passed the deep state of dormancy but were still very frost tolerant. This is in agreement with the results of Stushnoff and Junttila (1986) who found that buds of a range of boreal trees in North Norway could resist freezing at -40°C in April after dormancy had been fully released. On the other hand, it has also been found that the dormant condition will retard loss of hardiness (Irving and Lanphear 1967). Such experiments demonstrate that frost resistance can be maintained also after alleviation of dormancy as long as the plants are maintained at low temperature. It is when growth is re-initiated during prolonged thaw periods in late winter that serious hardiness problems arise. With the predicted and ongoing global varming (Serreze *et al*. 2000), the frequency of such incidences are increasing and will result in greater risk of frost injury during late winter and early spring (cf. Myking and Heide 1995). On the other hand, our present and earlier results (Sønsteby and Heide 2006), indicate that milder autumn conditions, which is also associated with global warming, would be positive for dormancy induction and stabilization of cold hardiness in both wild and cultivated strawberry plants.

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