

# Photoperiodic induction of dormancy and freezing tolerance in *Betula pubescens*. Involvement of ABA and dehydrins

Annikki Welling, Päivi Kaikuranta and Päivi Rinne

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In many woody plants photoperiod signals the initiation of dormancy and cold acclimation. The photoperiod-specific physiological and molecular mechanisms have remained uncharacterised. The role of abscisic acid (ABA) and dehydrins in photoperiod-induced dormancy and freezing tolerance was investigated in birch, *Betula pubescens* Ehrh. The experiments were designed to investigate if development of dormancy and freezing tolerance under long-day (LD) and short-day (SD) conditions could be affected by manipulation of the endogenous ABA content, and if accumulation of dehydrin-like proteins was correlated with SD and/or the water content of the buds. Experimentally, the internal ABA content was increased by ABA application and by water stress treatment under LD, and decreased by blocking the synthesis of ABA with fluridone under SD. Additionally, high humidity (95% RH) was applied to establish if accidental water stress was involved in SD. ABA content was monitored by gas chromatography-mass spectrometry with selective ion monitoring (SIM). Short days induced a transient increase in ABA content, which was absent in 95% RH, whereas fluridone treatment decreased ABA. Short days induced a typical pattern of bud desiccation and growth cessation regardless of the treatment, and improved freezing tolerance except in the fluridone treatment. ABA content of the buds was significantly increased after spraying ABA on leaves and after water stress, treatments that did not induce cessation of growth and dormancy, but improved freezing tolerance. In addition to several constitutively produced dehydrins, two SD-specific proteins of molecular masses 34 and 36 kDa were found. Photoperiod- and experimentally-induced alterations in ABA contents affected freezing tolerance but not cessation of growth and dormancy. Therefore, involvement of ABA in the photoperiodic control of cold acclimation is more direct than in growth cessation and dormancy. As the typical desiccation pattern of the buds was found in all SD plants, and was not directly related to ABA content or to freezing tolerance, this pattern characterises the onset of photoperiod-induced growth cessation and dormancy. The results provide evidence for the existence of various constitutively and two photoperiod-induced dehydrins in buds of birch, and reveal characteristics of dormancy and freezing tolerance that may facilitate further investigations of photoperiodic control of growth in trees.

**Key words** – ABA, abscisic acid, *Betula*, birch, dehydrins, dormancy, fluridone, freezing tolerance, water stress.

A. Welling, P. Kaikuranta and P. Rinne (corresponding author, fax 358-8-5531500), Dept of Biology, Univ. of Oulu, P.O. Box 400, FIN-90570 Oulu, Finland.

## Introduction

Woody plants in temperate zones have evolved both dormancy and freezing tolerance mechanisms that allow survival during unfavourable periods. The phenology of induction, completion and release of bud dormancy and

freezing tolerance have been investigated extensively, but less information is available on their basic biochemical nature (Seeley 1994, Hughes and Dunn 1996). Dormancy and freezing tolerance can be independent (Irving and Lanphear 1967). However, freezing tolerance cannot develop fully without cessation of growth (Fuchigami et

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al. 1971), which also marks the initiation of dormancy, and therefore they may be partially interrelated. In addition, their induction is closely synchronised by the same environmental factors although their termination may be individual. Cold acclimation has been extensively studied in herbaceous plants, which develop freezing tolerance in response to low temperature (Hughes and Dunn 1996). Photoperiodic control of growth is pivotal to the survival of many woody plants, but there still is a lack of information on the underlying processes.

Abscisic acid (ABA), a well-known stress-inducible plant hormone and growth inhibitor, has long been studied as a potential mediator of short day (SD)-induced cessation of growth and initiation of bud dormancy in trees. Its involvement has remained questionable, mainly due to inconsistent changes in endogenous ABA concentrations and weak responses to exogenously applied ABA (Powell 1987). ABA has an important role in the induction and maintenance of seed dormancy, by suppressing precocious germination and inducing the accumulation of protein reserves (Black 1991). Furthermore, seeds accumulate ABA concomitantly with late embryogenesis abundant (LEA) proteins. Dehydrins, a subgroup of these LEA proteins, referred to as the D-11 family, can be induced in vegetative tissues by a variety of environmental stresses (e.g. drought, cold, salt) and by external application of ABA (Dure 1993). Various stresses cause dehydration of plant tissues which respond by producing dehydrins. Dehydrins may protect cells against dehydration damage, although the mechanisms have not yet been established (Close 1996).

The ability to tolerate desiccation is also one of the most important factors in the development of freezing tolerance (Lee and Chen 1993). Cells become highly dehydrated at freezing temperatures, as water moves across the plasma membrane to the cell wall, where it contributes to ice formation (Griffith et al. 1993). In certain species short photoperiod, low temperature and water stress lead to increased freezing tolerance, decreased water content and accumulation of ABA, and applied ABA alone has been shown to enhance freezing tolerance (see Lee and Chen 1993). The role of ABA in the development of freezing tolerance may be connected with the regulation of gene expression, as acquisition of freezing tolerance is mediated by altered gene expression and some of the novel polypeptides are related to dehydrins or to other ABA-responsive proteins (Dhindsa et al. 1993).

We are investigating the physiological and molecular mechanisms involved in the photoperiodic control of vegetative growth, and the interrelationship between dormancy and freezing tolerance. We use birch as an experimental plant because it shows strong photoperiodic responses. In our earlier experiments with birch we showed that dormancy can be induced in the course of a few weeks of SD exposure, during which the ABA concentration increases and water content decreases in the buds (Rinne et al. 1994). In the present experiments, the in-

volvement of dehydrins and ABA in the photoperiodic induction of dormancy and freezing tolerance was investigated. This was done by studying whether manipulations that yield high and low internal ABA contents could initiate or inhibit dormancy and/or freezing tolerance under LD and SD. Dehydrin accumulation was investigated in relation to development of these phenomena.

**Abbreviations** – LEA, late embryogenesis abundant.

## Materials and methods

### Plant material and growth conditions

Seedlings of *Betula pubescens* Ehrh. were grown under long-day (LD) conditions (24-h day, 18°C) similar to those described previously (Rinne et al. 1994) except that the photosynthetic photon flux was constant, 85 µmol m<sup>-2</sup> s<sup>-1</sup>. Only fast-growing coppice shoots were used in the present experiments and only one plant was grown per 12-l pot. The experiment was started when the shoots were 15 cm. Some of the plants were left intact for 21 days (controls), some were given exogenous ABA for 10 days and others underwent water stress for 21 days. The water-stressed plants were given 50 ml water per pot every 4th day to prevent wilting. Internal bud ABA levels were increased by spraying ABA on the leaves with 25 ml of 50 µM (±)ABA (Fluka) in 0.5% (v/v) Tween 20, once a day. In a separate experiment it was established that Tween 20 did not affect the phenomena under study. Quantitative ABA analyses were carried out to monitor ABA uptake by the buds (described later).

Half of the plants were transferred to SD conditions at the beginning of the experiment. Except for a 12-h day the growth conditions were similar to those under LD. Some of the plants were left intact for 21 days (controls) and others were treated with 1-methyl-3-phenyl-5-(3[trifluoromethyl]phenyl)-4-(1H)-pyridinone (fluridone, Riedel-de Haën, Sheelze, Germany) for 10 days. Each day 2.5 mg of fluridone (100 mg l<sup>-1</sup>) in aqueous methanol (0.4%) was given via the roots. A separate experiment showed that 0.4% methanol had no effect. In addition, some of the SD plants were grown at 95% RH for 21 days (corresponding to a water vapour pressure deficit of -6.9 MPa).

Plants for freezing tests and protein analyses were grown in an additional experiment under similar growth conditions to the earlier experiment, except that the concentration of exogenous ABA was 25 µM and fluridone was given at a dose of 0.2 mg once a day for one week. It was concluded that this treatment was sufficiently severe, as symptoms similar to those induced with higher concentrations were observed (i.e. discolouration of the leaves and production of anthocyanin).

### Measurement of dormancy development

Dormancy development was measured after 10 and 21 days of treatment by transferring the plants to LD con-

trol conditions (see above) for visual determination of bud bursting. Buds were classified as burst as soon as at least one leaf had emerged. Bursting was expressed as an  $\Sigma_{11}$  value, which incorporates both the speed of bursting and the number of burst buds. For example, if a bud bursts on day 1 of observation,  $\Sigma_{11} = 11$  and if on day 12,  $\Sigma_{11} = 0$ .

#### Measurement of freezing tolerance

The freezing tolerance of buds of 2 to 4 seedlings was determined after 1 and 3 weeks in each treatment. Pieces of stems (15 cm) with ca 5 nodes were sprayed with water, wrapped in aluminium foil and placed in a 1-cm layer of sand in an aluminium box, which was then filled with sand and left to stand overnight in a cold room (2°C). The boxes were placed in a computer-controlled freezer and the temperature lowered to -4.6, -9 or -13.5°C at a rate of 1.5, 2 and 2.5°C h<sup>-1</sup>, respectively, and the desired temperature maintained for 30 min. The temperature inside the box was measured with a bulb thermometer. Finally, boxes were placed in an ice-bed in a cold room (2°C) until the sand temperature was 0°C. After thawing, the shoots were cut to single node cuttings, placed on a perforated styrofoam plate and cultured in water as described previously (Rinne et al. 1994). Freezing tolerance of the bud meristem was estimated visually after three weeks of culture. The injury level is based on classification according to colour (see Ritchie 1991). Dead or injured meristems were typically brown or tanned compared to the light-green of living meristems. Meristems were chosen because the resistance against freezing of the regenerative tissue is particularly vital to plant survival.

#### Quantitative analysis of ABA

ABA was measured in the three uppermost leaves at the beginning of the experiment and in the corresponding leaves after 1, 4, 10 and 21 days, by which time they were proximally lower. Bud samples were collected from the same trees as the leaf samples but due to their small size buds from stems and branches were pooled. Each sample of four replicates consisted of leaves or buds from 2 or 3 seedlings. The samples were weighed, frozen in liquid nitrogen and freeze-dried, to determine dry weight and water content. They were then homogenised in liquid nitrogen in a mortar with a pestle. The extraction and partitioning steps were carried out in 20-ml liquid-scintillation vials and the solutions were agitated with magnetic stirrers. The samples were extracted for 1 h at 4°C in 5 ml 50 mM sodium phosphate buffer, pH 7.5, with 0.02% sodium diethyldithiocarbamate as antioxidant and [<sup>2</sup>H<sub>4</sub>]ABA as internal standard. They were then filtered and slurried with an equivalent amount (w/w) of insoluble polyvinylpyrrolidone for 20 min, filtered again and partitioned twice against diethyl ether. The aqueous phase was adjusted to pH 2.7 and partitioned

three times with diethyl ether. These diethyl ether fractions were evaporated to dryness with nitrogen gas, dissolved in 400 µl 50% methanol in 30 mM acetic acid and purified by high-performance liquid chromatography. A gradient of methanol and water, both in 30 mM acetic acid, was used as mobile phase, the methanol content being increased from 10 to 90% in 15 min. The retention time for ABA was 11 min at a flow rate of 1 ml min<sup>-1</sup>. ABA was measured by gas chromatography-mass spectrometry with selective ion monitoring (SIM). Ions at 190.1 and 194.1 were monitored and amount of ABA in the sample calculated using a standard curve drawn from the area ratios of known amounts of ABA and [<sup>2</sup>H<sub>4</sub>]ABA. The endogenous ABA level was calculated in µg g<sup>-1</sup> dry weight.

#### Protein extraction, SDS-PAGE and western blotting of proteins

For protein analysis axillary buds of a similar size were collected. One bud per sample was ground in an Eppendorf tube with a glass rod in 30 µl SDS-PAGE sample buffer (62.5 mM Tris-HCl, pH 6.8, 10% (v/v) glycerol, 2% (w/v) SDS, 5% (v/v) 2-β-mercaptoethanol, 0.0125% (w/v) bromophenol blue, 1 mM PMSF) and centrifuged at 20 000 g for 10 min. Protein, 12 µl from each sample, was loaded onto the gel and proteins were separated by discontinuous SDS-PAGE with a Mini-Protean II electrophoresis unit (Bio-Rad) using a 4% stacking gel and a 12% separating gel according to the manufacturer's directions. Polypeptides were detected by staining the gels with Coomassie brilliant blue G-250 according to Neuhoff et al. (1988).

For immunoblotting, proteins from unstained replicate gels were electroblotted onto 0.2-µm nitrocellulose membranes (Bio-Rad) using a Mini Trans-Blot Electrophoretic Transfer Cell (Bio-Rad). Electroblotting was carried out at 4°C for 1 h 15 min at 100 V in a transferring buffer of 25 mM Tris, 192 mM glycine, 20% (v/v) methanol (pH 8.3). The membrane was blocked with 3% (w/v) dry milk in TBS (25 mM Tris and 150 mM NaCl; pH 7.5 with HCl) using 0.01% (v/v) antifoam A (Sigma) and probed at 1:1000 dilution with an antibody directed against a synthetic dehydrin peptide containing the consensus sequence TGEKKGIMDKIKEK-LPGQ (Close et al. 1993). The membrane was then incubated with goat anti-rabbit IgG alkaline phosphatase conjugate. The secondary antibody was detected using an alkaline phosphatase conjugate substrate kit (Bio-Rad).

#### Statistics

The effects of exogenous ABA, water stress, fluridone and 95% RH on ABA and water content were tested against control treatments at the same day length using the Scheirer-Ray-Hare extension of the Kruskal-Wallis test (Sokal and Rohlf 1995).

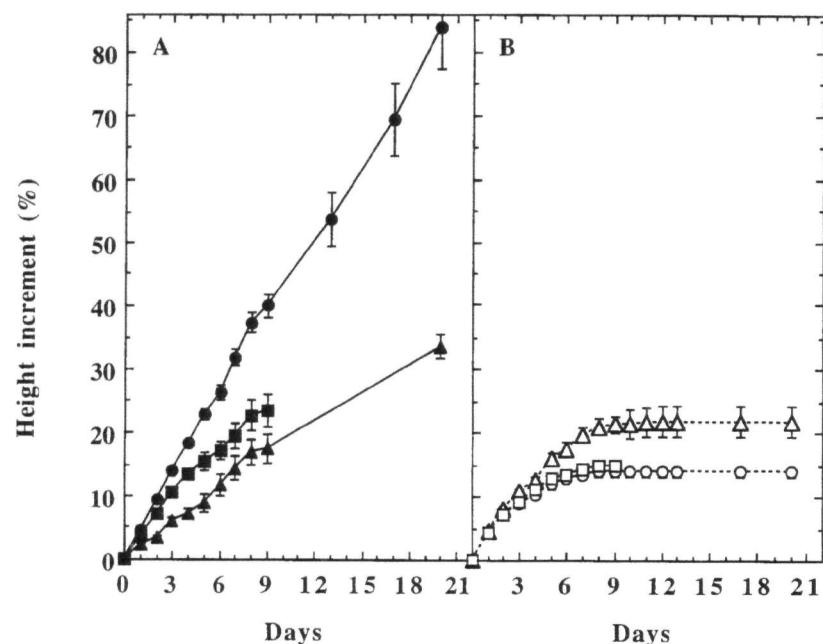


Fig. 1. Shoot elongation in seedlings of *Betula pubescens* grown under (A) LD (24-h day) and (B) SD (12-h day) regimes at 18°C. Means  $\pm$  SE, n = 20–63. ●, LD control; ○, SD control; ■, external ABA treatment (25 ml 50  $\mu$ M ABA day $^{-1}$  sprayed onto the leaves); ▲, water stress in LD; △, 95% RH; □, fluridone treatment (2.5 mg plant $^{-1}$  day $^{-1}$ ).

## Results

### Cessation of growth and induction of dormancy

Growth of the SD control and fluridone-treated plants ceased within one week under SD conditions (Fig. 1B), and although elongation growth was faster under 95% RH, its cessation was postponed only by 4 days in some individuals as compared with the SD controls. Under LD conditions, the ABA and water stress treatments reduced growth but were unable to halt it completely in 9 and 20 days, respectively (Fig. 1A). The growth of the water-stressed plants was reduced to about one-third of that of the LD control plants after 20 days of treatment.

Dormancy development was examined only in the SD plants, as only they had ceased growing (Tab. 1). Ten days under the SD regime reduced bud bursting to one-half and 21 days prevented it almost completely, implying that dormancy had been induced by 21 days under these conditions.

Tab. 1. Induction of dormancy in seedlings of *Betula pubescens* after 10 or 21 days under SD control conditions (12-h day, 18°C, 75% RH) or 95% RH or fluridone (2.5 mg plant $^{-1}$  day $^{-1}$ ) measured in terms of bud bursting capacity. Means of the  $\Sigma_{11}$  values refer to the speed of bursting and the number of burst buds. If a bud bursts on the first day of observation  $\Sigma_{11} = 11$ , and if it bursts on day 12,  $\Sigma_{11} = 0$ ; n = 1–3, number of buds per plant = 7.

Treatment (short days)	Days	$\Sigma_{11}$ Mean	SE
Control	10	6.200	0.788
Fluridone	10	4.143	1.945
Control	21	0.042	0.045
95% RH	21	0.048	0.048

Tab. 2. Effect of 1 and 3 weeks exposure of birch seedlings to LD conditions (24-h day, 18°C, 75% RH), exogenous ABA treatment in LD (25 ml 25  $\mu$ M ABA day $^{-1}$  sprayed onto the leaves) or water stress in LD, or under SD conditions (12-h day, 18°C, 75% RH) or 95% RH or fluridone treatment in SD (0.2 mg plant $^{-1}$  day $^{-1}$ ) on the viability of the bud apical meristem after exposure to -4.6, -9 or -13.5°C, as a percentage of undamaged buds, n = 2–4, ND = not determined.

Time	Treatment	Viability, % undamaged buds		
		-4.6°C	-9°C	-13.5°C
1 week	LD control	8.0	0.0	0.0
	LD ABA	4.2	0.0	0.0
	LD water stress	14.3	29.4	0.0
	SD control	20.0	0.0	ND
	SD fluridone	20.0	0.0	ND
	SD 95% RH	46.1	0.0	ND
3 weeks	LD control	0.0	0.0	0.0
	LD ABA	50.0	45.2	16.2
	LD water stress	39.3	17.9	0.0
	SD control	100.0	96.2	100.0
	SD fluridone	36.8	4.5	0.0
	SD 95% RH	100.0	100.0	100.0

### Freezing tolerance

Only the water-stressed plants expressed increased freezing tolerance after one week (Tab. 2), with no further increase during the next two weeks. By 3 weeks, ABA-treated plants had increased freezing tolerance relative to water-stressed plants, but significantly less than plants under SD conditions. There was no difference in freezing tolerance between 95% RH and SD control plants, but fluridone treatment reduced freezing tolerance of birch buds significantly (Tab. 2).

### Water status

Water content decreased most (12%) in buds of water-stressed plants growing under LD conditions (Fig. 2A), the difference relative to the LD control plants being significant ( $H = 11$ ,  $df = 1$ ,  $P < 0.001$ ). Bud water content

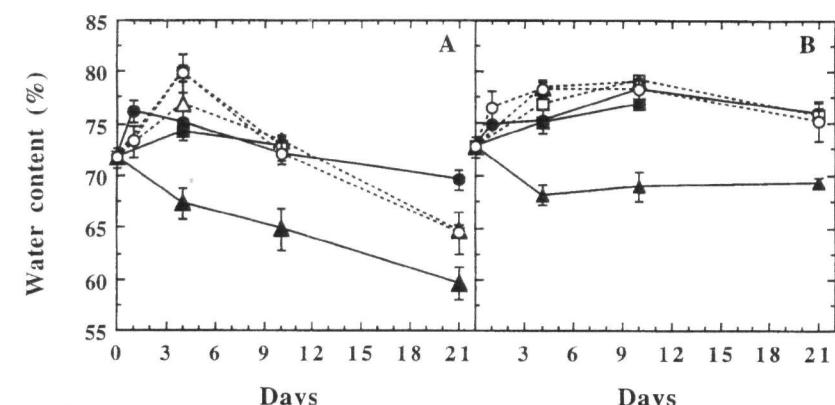


Fig. 2. Bud (A) and leaf (B) water content in seedlings of *Betula pubescens* grown under LD (24-h day, filled symbols) and SD (12-h day, open symbols) at 18°C, 75% RH. Means  $\pm$  SE, n = 2–4. ●, LD control; ○, SD control; ■, external ABA treatment (25 ml 50  $\mu$ M ABA day $^{-1}$  sprayed onto the leaves); ▲, water stress; △, 95% RH; □, fluridone treatment (2.5 mg plant $^{-1}$  day $^{-1}$ ).

remained stable in ABA-treated plants during the 10-day study period. There was an increase in bud water content in all the SD plants on day 4, after which it decreased from ca 80 to 65%. There was no statistical difference in bud water content between the LD and SD control plants, but the trend in the latter was an ascending one after day 4, while in LD it was stable. The water content of the leaves was approximately the same as that of the buds at the beginning of the experiment (Fig. 2B), but in contrast to the buds, water content in the leaves remained stable throughout, except in the water-stressed plants, where it decreased significantly ( $H = 13.2$ ,  $df = 1$ ,  $P < 0.01$ ).

### Endogenous ABA

The ABA content of the buds was almost 10 times higher than that of the leaves (Fig. 3A–D). A transient peak was observed in the SD control plants, but there was no increase under 95% RH (Fig. 3B). Fluridone treatment reduced the bud ABA content significantly ( $H = 7.5$ ,  $df = 1$ ,  $P < 0.01$ ), while ABA treatment (Fig. 3E)

increased it ( $H = 9.5$ ,  $df = 1$ ,  $P < 0.01$ ), as did water stress (Fig. 3A), although the difference was not statistically significant ( $H = 1.7$ ,  $df = 1$ ,  $P > 0.1$ ).

Day length had no effect on the leaf's ABA content, as the levels did not differ between the SD and LD control plants (Fig. 3C and D), and ABA content was also unchanged under 95% RH in SD. Fluridone treatment reduced ABA content significantly ( $H = 6.5$ ,  $df = 1$ ,  $P < 0.05$ ). Under LD conditions, both exogenous ABA (Fig. 3E,  $H = 6.5$ ,  $df = 1$ ,  $P < 0.05$ ) and water stress (Fig. 3C,  $H = 4.4$ ,  $df = 1$ ,  $P < 0.05$ ) resulted in increased ABA content, but the values in the water-stressed plants dropped to close to the pre-stress level in 3 weeks.

### Protein pattern

The protein pattern in one dimensional SDS-PAGE showed the main differences between LD and SD treatments. A 62-kDa protein that was expressed in LD disappeared during the first week in SD (Fig. 4). During the next two weeks 30- and 31-kDa proteins accumulated in all SD treatments, although the 30-kDa protein was less abundant in fluridone treatment. According to the immunoblot these proteins were not dehydrin-like proteins. A number of dehydrins were expressed constitutively in birch buds under all the treatments (Fig. 5), their molecular masses being approximately 19, 26, 44, 60, 71 and 89 kDa, of which 19 and 26 kDa were the most abundant. Two other dehydrin-like proteins, 34 and 36 kDa, accumulated during the first week in SD, similarly in all treatment-groups, although two weeks later they were

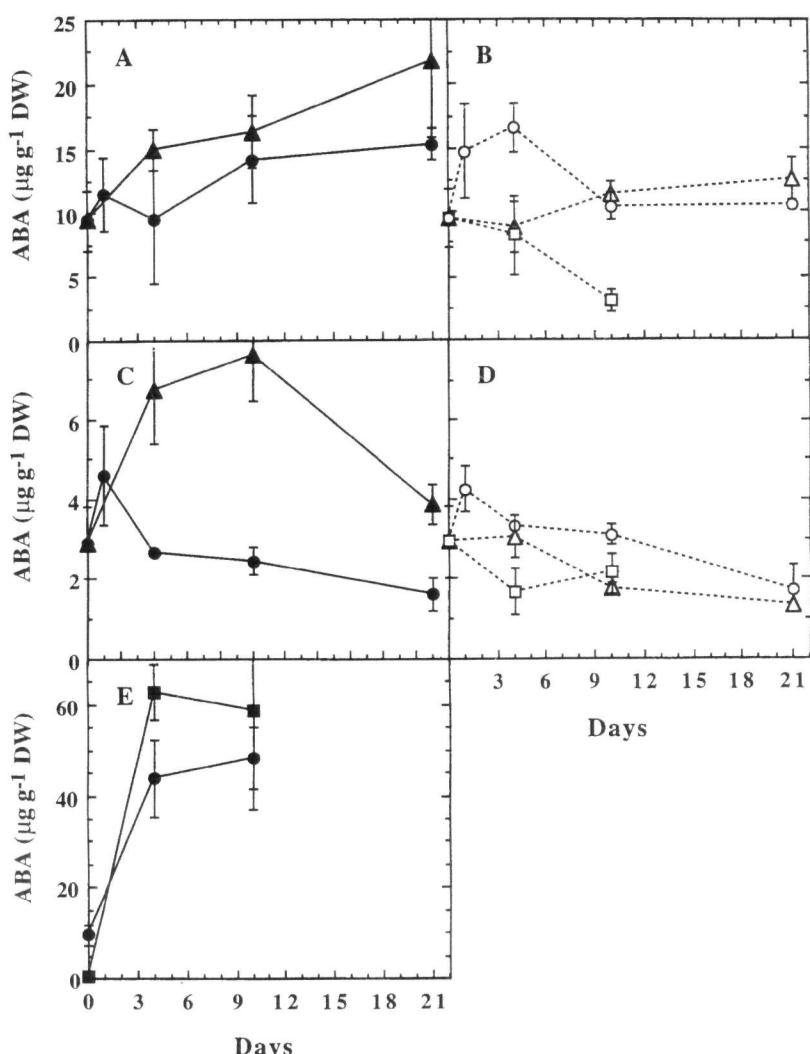


Fig. 3. ABA ( $\mu\text{g g}^{-1}$  dry weight) in the buds (A) and leaves (C) of seedlings of *Betula pubescens* in the course of LD (24-h day, 18°C, 75% RH) and in the buds (B) and leaves (D) in the course of SD (12-h day, 18°C, 75% RH). Means  $\pm$  SE,  $n = 3$  in leaves,  $n = 2$ –4 in buds. ●, LD control; ○, SD control; ▲, water stress; △, 95% RH; □, fluridone treatment ( $2.5 \text{ mg plant}^{-1} \text{ day}^{-1}$ ). ABA level in the leaves (■) and buds (●) (E) of seedlings treated with external ABA in LD (25 ml  $50 \mu\text{M}$  ABA  $\text{day}^{-1}$  sprayed onto the leaves).

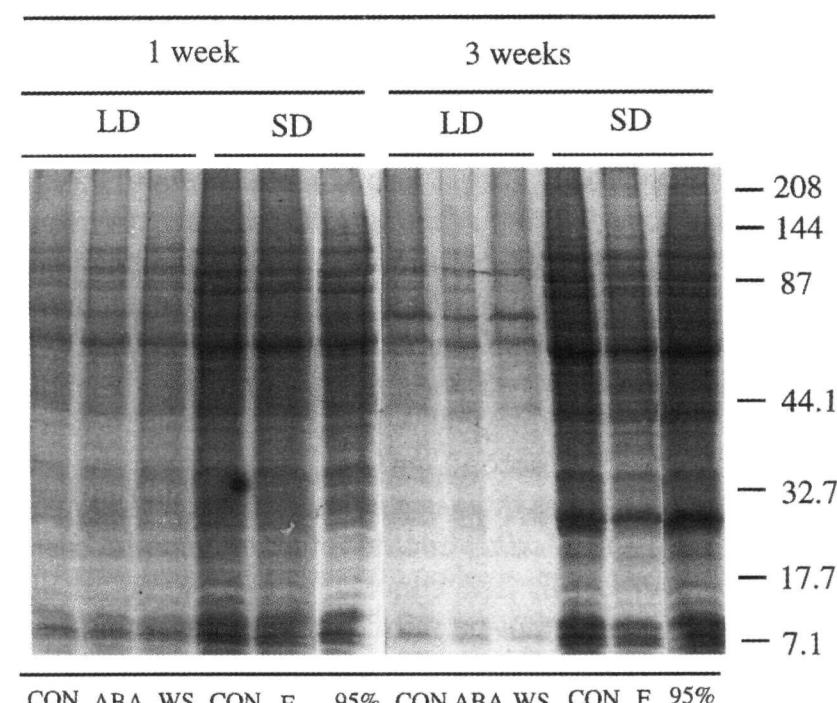


Fig. 4. SDS-PAGE profile of bud proteins extracted from birch grown for 1 or 3 weeks under LD control conditions (CON, 24-h day, 18°C, 75% RH), with external ABA treatment in (ABA, 25 ml  $25 \mu\text{M}$  ABA  $\text{day}^{-1}$  sprayed onto the leaves) and under water stress (WS), or under SD control conditions (CON, 12-h day, 18°C, 75% RH) or with 95% RH (95%) or fluridone treatment (F,  $0.2 \text{ mg plant}^{-1} \text{ day}^{-1}$ ). Molecular masses of the protein standards are indicated on the right.

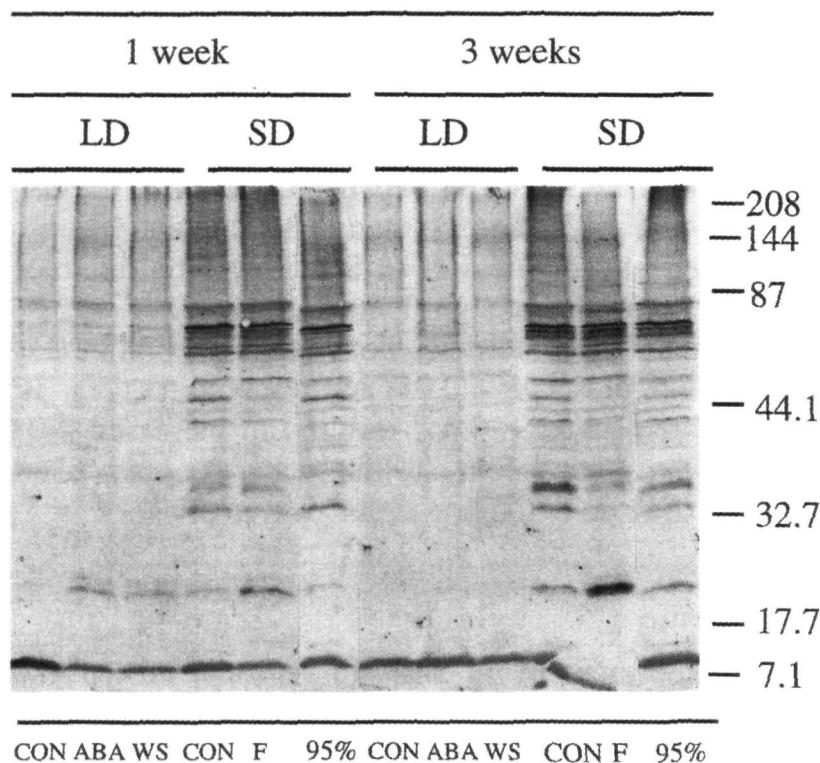


Fig. 5. Western blot analysis of bud proteins extracted from birch grown for 1 or 3 weeks under LD control conditions (CON, 24-h day, 18°C, 75% RH), with external ABA treatment (ABA, 25 ml 25 µM ABA day<sup>-1</sup> sprayed onto the leaves) and under water stress (WS), or under SD control conditions (CON, 12-h day, 18°C, 75% RH) or with 95% RH (95%) or fluridone treatment (F, 0.2 mg plant<sup>-1</sup> day<sup>-1</sup>). Molecular masses of the protein standards are indicated on the right.

less abundant in buds of fluridone-treated plants and 95% RH (Fig. 5).

## Discussion

The results with birch demonstrate that a SD photoperiod is the main environmental factor that synchronises the initiation of the development of both dormancy and freezing tolerance. One week of exposure to SD was sufficient to stop growth (Fig. 1B) and three weeks to induce bud dormancy (Tab. 1). Although SD lead to a transient increase in ABA content, it seems unlikely that the alterations in ABA concentrations are directly involved in the cessation of growth and dormancy: First, growth cessation was induced under SD conditions with basal ABA at 95% RH and with negligible ABA as a result of fluridone treatment (Fig. 3B); second, growth cessation was not induced by water stress or exogenous ABA under LD even though endogenous ABA contents were about the same or higher than in SD. The conclusion that effects of SD are multiple and cannot be mimicked by water stress are supported by our earlier results with birch demonstrating that simultaneous exposure to SD and water stress did not advance dormancy (Rinne et al. 1994). It is possible that environmental factors cause alterations in sensitivity to ABA, as suggested earlier with water-stressed plants (Rinne et al. 1994). Similar changes in bud sensitivity to ABA might be involved in exposure to SD.

The first indications of the involvement of ABA in the development of freezing tolerance in woody plants came

from bioassays (Irving and Lanphear 1968, Irving 1969). Studies with herbaceous plants later confirmed a role for ABA in the regulation of gene expression during cold acclimation (reviewed by Hughes and Dunn 1996). Our results with birch demonstrate that endogenous ABA has a role in freezing tolerance of trees. Increased endogenous ABA contents during both water stress and ABA treatment were correlated with increased freezing tolerance (Fig. 3A,E, Tab. 2), and decreased endogenous ABA content due to blockage of ABA biosynthesis by fluridone (Fig. 3B) was correlated with decreased freezing tolerance under SD (Tab. 2). A similar decrease in chilling tolerance following fluridone treatment of maize has been attributed to ABA, although endogenous ABA levels were not monitored (Anderson et al. 1994). As ABA or water stress could not completely compensate for the SD effect in the development of freezing tolerance in our experiments and as freezing tolerance developed also in the 95% RH with no transient increase in ABA content (Tab. 2), it is possible that SD and water stress have separate pathways for its induction as proposed by Chen and Li (1978), and that there are other metabolic alterations besides accumulation of ABA that contribute to the subsequent development of freezing tolerance in SD.

The observed alterations in bud water content during the early acquisition of dormancy in SD (Fig. 2) suggest significant changes in the water status of the buds. It has been observed that under SD treatment water content is positively correlated with water potential values and negatively with apoplastic water (P. Rinne, A. Welling and P. Kaikuranta, unpublished data), and therefore it is obvious that the decrease in water content is due to cellular dehydration. Water content increased briefly during the first few days under SD conditions and then decreased significantly during the next 5 to 6 days (Fig. 2A). No comparable pattern was observed with any treatment under LD conditions. This typical desiccation was caused by internal factors specific to the buds, as no comparable dehydration occurred in the leaves (Fig. 2B). Furthermore, under water stress treatment both the leaves and buds suffered dehydration, with no initial increase in water content.

Of all the factors tested that of photoperiod was the most important in altering the protein pattern in birch buds. The disappearance of a 62-kDa protein and appearance of 30 and 31 kDa proteins were clearly under photoperiodic control (Fig. 4), but the function of these proteins remains to be elucidated. Two other proteins, 34 and 36 kDa, that accumulated in birch buds under SD were identified as dehydrin-like proteins (Fig. 5). Dehydrin expression has been shown to be related to situations that induce cellular dehydration (reviewed by Close 1996). Similarly, the accumulation of dehydrin-like proteins in birch buds was correlated with the cellular water status (Figs 2 and 5), but only if desiccation was due to photoperiod. Although the exact functions of dehydrins are not yet known (Close 1996), it seems

plausible that the photoperiod-related dehydrins protect from dehydration specific structures that are co-expressed in SD and do not appear under water-stressed conditions. Dehydrin expression has been related to freezing tolerance of various herbaceous (reviewed by Close 1996) and a few woody plants (Arora and Wisniewski 1994, Wisniewski et al. 1996). The present results are important in showing that their expression might be more complex. In fact, the majority of dehydrin-like proteins in birch buds were expressed constitutively in all treatments under LD and SD conditions. Similar results regarding unstressed tissues have been obtained by others (Close et al. 1993, Wisniewski et al. 1996). Schneider et al. (1993) proposed that some of the dehydrins also play a role in normal cellular metabolism, although they are required at higher concentrations at times of water stress.

In conclusion, the results show that in birch photoperiod is the major environmental factor affecting the development of both dormancy and freezing tolerance. The specific physiological alterations leading to cessation of growth and dormancy under SD remain an enigma, but could be characterised as programmed dehydration, that is specific to buds. Accumulation of ABA was a characteristic of the development of freezing tolerance, but not of cessation of growth and dormancy. We document here that many dehydrin-like proteins are expressed constitutively in birch buds and that two other dehydrins, 34 and 36 kDa, are under photoperiodic control. Based on their accumulation levels in different treatments, they may play a role in freezing tolerance. The characterisation of physiological responses in dormancy and freezing tolerance are important for further investigations of the photoperiodic control of vegetative growth.

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