EARLY EFFECTS OF LONG NIGHTS ON BUDSET, BUD DORMANCY AND ABSCISIC ACID CONTENT IN TWO POPULATIONS OF *PICEA ABIES*

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ABSTRACT

The daily variation of free and conjugated abscisic acid (ABA) following long night treatments and the number of long nights needed to initiate budset and the build-up of bud dormancy were investigated in seedlings of a northern (north Sweden, estimated critical night length of 2 h) and a southern (Romanian, estimated critical night length of 7.5 h) population of *Picea abies* cultivated in a phytotron. After continuous growth the seedlings from both populations were exposed to a regime of 16 h nights for one or more up to 9 days and in a second experiment the northern seedlings were given 5 h nights for one or more up to 12 days. Only one night with 16 h was needed to induce almost 100% budset about 14 days later in the northern population whereas 4 nights were needed for the southern population. When the northern population was given 5 h nights, the number of nights required for 100% budset increased to 5. Dormancy built up more rapidly in the northern than in the southern population. The northern population reached the same degree of dormancy after 3 nights of 16 h as the southern population after 8 nights of 16 h. The northern population built up dormancy more slowly, but to a higher final level, when exposed to 5 h nights than when exposed to 16 h nights. A transient peak in the content of free ABA in the needles of the northern population was observed at day 4 with 16 h nights. The peak was not attributable to changes in water status of the shoots. The peak in the levels of free ABA in the needles of the southern population occurred after 8 days with 16 h nights. Following exposure to 5 h nights – a less abrupt change – the peak of ABA was smoothed out for the northern population.

Keywords: Abscisic acids, budset, bud dormancy, high pressure liquid chromatography, *Picea abies* populations

INTRODUCTION

The inwintering of *Picea abies*, like that of other forest trees, is a complex process. The role of abscisic acid (ABA) in regulating one or more of the steps in this process has been discussed for 30 years. Inwintering of seedlings begins with a photoperiodic response to long nights which is under strong genetic control, and shows a clinal variation with latitude, longitude and altitude (*e.g.*, DORMLING 1973; HEIDE 1974; DORMLING 1979). This indirect adaptation to the prevailing temperature conditions is an outstanding example of evolution by natural selection, taking place in the limited time available since the most recent glaciation. Physiological and molecular investigations are now needed for further understanding of this evolutionary process. Earlier studies indicate that the response to long nights is rapid. DORMLING *et al.* (1968) reported that 6 but not 3 nights with 16 h darkness were sufficient for inducing growth cessation and budset (*i.e.*, first appearance of bud scales) in a southern population (lat. 51° 47'). A northern population (lat. 66° 50') responded with growth cessation and budset after 6 nights with 6–8 h darkness. At a 4 h night, the treatment had to last for 9 nights to induce budset in the northern population. In a similar study HEIDE (1974) observed a temporary growth cessation (budset was not examined) after 4 nights with a night length of 14 h in *Picea abies* seedlings from lat. 58° 30'.

The environmental signal is probably perceived in the needles (for studies of deciduous trees see WAREING & SAUNDERS 1971). If so, a signal, presently unknown, is transmitted from needles to shoot apex, where the subapical meristems are inactivated leading to inhibition of internode extension. As the apical meristem is still active, bud scales and needle primordia are initiated resulting in the formation of a terminal bud. JOHANSEN *et al.* (1986) and BARROS & NEILL (1986) discussed evidence against ABA being the messenger transmitted from leaf to apex in *Salix* species (which, unlike *Picea*, show apical abortion). On the other hand, for various *Picea* species, exogenous ABA inhibits shoot extension

under long days (HEIDE 1986) and hastens budset under short days (BLAKE *et al.* 1990). ABA could, however, act more indirectly in growth cessation through its well established interactions with the changing water relations of plant cells; for example, decreases in turgor pressure promote the synthesis of ABA (BRAY 1991), which in turn might affect water potential. Correlations between cold acclimation and water potential have been reported for woody plants (MCKENZIE *et al.* 1974; D'AOUST & HUBAC 1986; KACPERSKA 1992) and for maize (CAPELL & DÖRFFLING 1993).

Apart from ABA's putative role in growth cessation and budset, it has further been suggested that it regulates the entry into deep dormancy. Exogenously supplied ABA induced dormancy in *Betula*, though only at nonphysiological concentrations, and endogenous ABA concentrations were correlated with dormancy (RINNE *et al.* 1994). Lateral buds of *Salix viminalis* became more sensitive to exogenously supplied ABA in late autumn as they entered dormancy (BARROS & NEILL 1986). Finally, there is good evidence, particularly from mutants (NORDIN *et al.* 1993) that ABA is involved in the regulation of cold acclimation in *Arabidopsis* and other herbaceous systems that acclimate without growth cessation, budset or bud dormancy.

In short, ABA has putative roles, with varying degrees of experimental support, as a messenger from needles to shoot apex, and in regulating growth cessation, budset, entry into bud dormancy and cold acclimation. These processes are partly independent in *Picea* and other woody plants; for example, buds of *Picea abies* in various degrees of dormancy can have a wide range of frost tolerance according to the history of growth (*e.g.*, DORMLING 1993; QAMARUDDIN *et al.* 1993). It is not currently known whether buds of *Picea* have to enter into and be released from deep dormancy, in order to attain maximum frost tolerance (DORMLING 1993; QAMARUDDIN *et al.* 1993).

WEILER (1980) by using immunoassays showed that the seasonal variation in the contents of free ABA and abscisyl-β-D- glycopyranoside (ABA-GE) in the leaves and buds of *Betula* and *Acer* were significantly species-dependent. The content of both free ABA and ABA-GE was higher in shoots of pine seedlings in response to long nights (ODÉN & DUNBERG 1984). RYU and LI (1994a) have recently investigated the relationship between free ABA and ABA-GE during potato cold acclimation and reported that the ABA-GE was not the source of the increase in free ABA.

In a previous study, a transient peak of free ABA and a trough in the mRNA fraction of the total RNA was recorded 3 days after the start of the long night treatment in a Romanian population of *Picea abies* (QAMARUDDIN *et al.* 1993; CLAPHAM *et al.* 1994). Neither the peak in ABA nor the trough in the mRNA were seen for the northern Swedish population. Since the northern population responded more rapidly to long nights, the peak is thought to have occurred earlier and been missed (QAMARUDDIN *et al.* 1993).

The purpose of the present investigation was to relate the daily changes in free and conjugated ABA during the first 9 or 12 days of long nights to the simultaneous induction of budset and gradual build-up of bud dormancy in seedlings of a northern and a southern population of *Picea abies*. To our knowledge this is the first time such early joint responses to long night treatments have been studied in two contrasting populations of *Picea abies*. In addition water status was measured daily for the northern population during the first 9 long nights.

Terminology

Inwintering refers to the changes in morphology and physiology beginning in late summer that prepare a tree for winter. **Growth cessation** refers to the slowing down and termination of elongation growth. In seedlings of *Picea abies* it is normally accompanied by terminal budset, which is defined by the first appearance of bud scales which occurs 2–3 weeks after induction (DORMLING *et al.* 1968; DORMLING 1973; QAMARUDDIN *et al.* 1993; this study). **Induction of budset** refers here to the response to a limited number of long nights which is followed by budset even if the seedlings are subsequently moved to non-bud-inducing conditions that normally favour elongation growth. **Dormancy** is the suspension of visible growth (*i.e.* elongation growth) of any plant structure containing a meristem (after LANG 1987). Dormant buds normally require a treatment, natural or artificial, before they resume elongation growth in favorable conditions. In seedlings of *Picea abies*, exposure to low, positive temperatures has frequently been observed to release buds from dormancy. But also long nights or long photoperiods have a dormancy-releasing effect (NIENSTAEDT 1967; DORMLING 1993 and unpublished; QAMARUDDIN 1993; this study). Dormancy is a quantitative trait, not an all-or-nothing state (VEGIS 1965). The **build-up** of dormancy can be followed as a gradual process, as is shown in this investigation. We have chosen to measure the degree of dormancy by the number of days required for the resumption of elongation growth in a favorable environment *e.g.* long photoperiod and mild temperature (DORMLING 1993; QAMARUDDIN 1993; this study). A bud with minimum dormancy is sometimes called quiescent.

MATERIALS AND METHODS

Plant materials and cultivation conditions

Two populations of *Picea abies* (L.) Karst., originating from northern Sweden (lat. 66°45'N, 301 m altitude; estimated critical night length of 2 h) and from Romania (lat. 46°28'N, 980 –1,230 m altitude; estimated critical night length of 7.5 h) were cultivated from seed under controlled conditions in growth chambers. Seeds were sown in pots with a mixture of sand and perlite. After a germination period of 2 weeks the seedlings were planted in pots with mineral wool as growth substrate. Two experiments (I, II) with different night-length regimes were carried out. In the first experiment the plants were grown for 5 weeks in continuous light and for 9 weeks in 1h nights for the northern population and 5 h nights for the southern population. Both populations were then exposed to 16 h nights for 1,...,9 days. In the second experiment the northern plants were grown for 15 weeks in 1 h nights and then exposed to 5 h nights for 1,...,12 days. Control plants continued in 1 h nights. The light intensity at the seedling level was 340 μmol $m^{-2} \cdot s^{-1}$ (Osram HQI lamps). The temperature was 20 °C throughout the experiments. The air humidity was 75% RH. A complete nutrient solution of low concentration, 100 mg $N \cdot l^{-1}$ (INGESTAD 1979), was given at each watering occasion, i.e. up to twice a day during continuous growth and once a day after growth cessation.

Experimental design

In both experiment I and II, a randomized complete block design was used for the assessment of budset induction, the degree of bud dormancy attained and the determination of free ABA and ABA-GE. Water status was determined only for the northern population in experiment I (3 blocks) and the number of plants per block and test occasion varied between 2 and 4. In experiment I, the northern population, the number of blocks was 4 for assessments of budset, bud dormancy and free ABA and 3–4 for ABA-GE. For the southern population the number of blocks was 3 for assessments of budset and bud dormancy, 2–3 for free ABA and 1–3 for ABA-GE. The number of plants per block and test occasion varied between 2 and 16. In experiment II the number of blocks was 4 for assessments of budset and bud dormancy, 2 for free ABA and 1–2 for ABA-GE. The number of plants per block and test occasion varied between 3 and 10.

The northern and southern plants were not randomized within a truck to avoid shading of the northern plants by the more rapidly growing southern plants. In both experiments a truck contained 32 pots, each pot with a top diameter of 8 cm and a depth of 7 cm. Four seedlings were grown in each pot. In some pots less than four seedlings were included in the analysis owing to seedling death or abnormal development of the seedlings. The pots used at each test occasion were randomly selected within each block.

Cultivation technique for assessment of terminal budset and build-up of bud dormancy

After each day of the long night treatments (1,...,9 days in expt I and 1,...,12 days in expt II), the plants were transferred back to growth conditions i.e. 1 h night and 20 °C for the northern population and 5 h night, 20 °C for the southern population. The number of long nights needed to induce terminal budset was assessed by recording the number of plants with terminal buds 3 times a week. The subsequent build-up of bud dormancy was determined by recording the number of plants with flushing buds. The median number of days between budset and budburst measured the build-up of bud dormancy. The median was used as some plants never flushed.

Water status measurements in experiment I

Measurements for estimations of daily changes in water potential and water content (% FW) were made every day from one day before the beginning of the 16 h treatment and for the next 9 days. The water potential of the main shoots was measured by the pressure-bomb technique as described in SCHOLANDER *et al.* (1965). Five measurements on each shoot were made.

Free ABA (cis-ABA) and conjugated ABA (abscisyl-β-D-glucopyranoside, ABA-GE) determinations

The methods of sampling, extraction, purification and quantification were used as described in QAMARUDDIN *et al.* (1993) with some modifications. Samples for hormone analysis were taken at a fixed time of the day, i.e. immediately after the end of the night period, just before the plants were transferred to the light period. This was to avoid the influence of possible diurnal variations in hormone levels. Samples from the three last days of the continuous growth period gave a reference level for the ABA content in the two populations. The purification was slightly modified from the previous method (QAMARUDDIN *et al.* 1993). After extraction for 5 min in boiling phosphate-buffered saline (PBS) at pH 7.0, centrifugation and filtration, extracts were applied on the immunoaffinity columns (QAMARUDDIN *et al.* 1993). Guard columns packed with Affigel-10 without polyclonal antibodies to ABA were used on top of the immunoaffinity columns to remove pigments. After washing with 5 ml of PBS and

then 5 ml of water, the guard columns were removed and immunoaffinity columns were further washed with 1 ml of 100 % methanol and eluted with 7 ml of 100 % methanol. After evaporation, the residue was redissolved in 2 % acetonitrile and analyzed on reverse-phase HPLC. The fractions corresponding to free ABA and ABA-GE were identified against authentic ABA. In some cases different gradient and isocratic systems were used to recheck the retention of abscisic acids. ABA-calibration curves were calculated from the peak area obtained with UV-absorption at 265 nm. The identification of the ABA peak from HPLC was confirmed by gas chromatography and mass spectrometry (method in TILLBERG & BJÖRKMAN 1993).

The procedure of WEILER (1980) was used for coupling the carboxyl group of ABA to bovine serum albumin. The polyclonal antibodies to this compound thus cross-reacted with free as well as with ABA-GE. This made it possible to analyze directly in the same sample both free and ABA-GE on HPLC. The peaks corresponding to ABA-GE in few cases were hydrolyzed (30 min, 60 °C, pH 11) and conversion of ABA-GE to free ABA were confirmed on HPLC after purification on immunoaffinity columns.

Statistical calculations

Preliminary analyses indicated, except for the ABA-GE values in experiment I, that the logarithm-transformed values fitted the normal distribution better than the original values. The comparisons in experiment II with respect to controls were based on the logarithms of the ratios free ABA, 5 h/free ABA, 1 h (control) and ABA-GE, 5 h/ ABA-GE, 1 h (control). The model used was

$$y_{ijk} = m + t_j + b_j + e_{ij} + w_{ijk}$$

where: y_{ijk}= original or logarithm-transformed value, m = overall mean, t_i = effect of day i, i = 1, ..., d, d = 9 (expt I), d =12 (expt II), b_j = effect of block j, j = 1, 2, 3, 4 (expt I, northern), j = 1, 2, 3 (expt I, southern) or j = 1, 2 (expt II), $e_{ij} \sim N(0,\sigma^2_e)$, random error between bags, and $w_{ijk} \sim N(0,\sigma^2_w)$, random error within bags, k = 1 or k = 1, 2.

The w_{ijk} are included in the model for the free ABA values from the northern population, since 5 of the 36 combinations of day and block were analysed twice (= 2 bags).. Otherwise, the combinations of day and block were not replicated and some of them were also missing. This implies that only the sum $\sigma^2_e + \sigma^2_w$ could be estimated.

The REML (restricted maximum likelihood) method (*e.g.*, SEARLE *et al.* 1992) was used for the estimation of the variance components σ^2_e, σ^2_w or $\sigma^2_e + \sigma^2_w$. Using those estimates, the parameters m, t_i and b_j were estimated by generalized least squares. Block effects very far from being significant were removed from the model. The obtained estimates were ordered as $\hat{t}_{[1]} < \hat{t}_{[2]}$ and the day corresponding to $\hat{t}_{[9]}$ or $\hat{t}_{[12]}$ was chosen as the peak day. The probability of a correct decision for the peak day was estimated by modifications of the methods in GIBBONS *et al.* (1977).

The above statistical model was also used for the analysis of water potential, water content and days to budset. As the number of observations is not the same for all days and blocks, the least square means were calculated for each day instead of the usual means. The standard errors of the least square means were affixed. For the budburst percentages a model including only day effects was used as there were no block effects. The standard errors of the percentage estimates based $\hat{p}_i$ on n_i plants from day i were calculated by $\sqrt{\hat{p}_i(1-\hat{p}_i)/n_i}$. For the comparative study of days the percentages were transformed according to the arcsin function before the statistical model was applied. In a joint analysis, the model given above was extended by also including the effect of population or the effect of long night treatment. The SAS procedures GLM, IML and VARCOMP were used for the numerical computations.

RESULTS

The induction of budset as well as the build-up of bud dormancy were affected differently in the two populations. Only one 16 h night was needed for the northern population and four 16 h nights for the southern population to induce buds visible about 14 days after the beginning of the treatment in almost all seedlings (Fig. 1 A). However some southern population seedlings formed buds also after two or three 16 h nights (Fig. 1 A; Table 1). With the 5 h night treatment the northern population needed 5 nights to induce buds visible after about 14 days in 100% of the seedlings (Fig. 1 A; Table 1). The build-up of bud dormancy was initiated already after 1 night with 16 h in the northern population (13 days to budburst) whereas a corresponding bud dormancy (12 days to budburst) was built up after 4 nights in the southern population (Fig. 1 B). The attainment in 16 h nights of a maximum bud dormancy of 35 days to budburst in test conditions was observed as early as after 3 long nights in the northern population (Fig. 1 B). In the southern population there was a much slower increase of bud dormancy and not until day 8 was the same degree of bud dormancy attained as in the northern population (Fig. 1 B). With the 5 h night treatment (northern population), the bud dormancy was built up at a much slower rate and not until after day 9 did the 5 h

bud dormancy curve reach the same level as the 16 h curve. Even at day 12, the last sample occasion, no levelling off of the bud dormancy curve was noted (Fig. 1 B). Hence, a higher degree of bud dormancy was obtained after 12 days with 5 h nights than after 9 days with 16 h nights.

For the mean number of days to budset, estimated by least squares (Table 1), the variation between days with 16 h nights for the northern population was significant (p = 0.013) whereas no significant variation was obtained for the southern population (p = 0.52) nor for the northern population exposed to 5 h nights (p = 0.26). In a joint analysis including both populations exposed to 16 h nights, a significant variation between the two populations was observed (p = 0.006). In a corresponding joint analysis, no variation existed between 16 h nights and 5 h nights for the northern population (p = 0.17).

The percentage of plants that flushed within 42 days decreased in both populations, the longer the night exposure lasted (Table 1). This decrease was most pronounced for the northern population exposed to 5 h nights for 1 up to 12 days (p = 0.00025), whereas exposure to 16 h nights for 1 up to 9 days gave a less significant p value of 0.027. The result was not significant for the southern population (p = 0.15). In a joint analysis including both populations, the variation between populations was insignificant (p = 0.36) as well as the variation between the 16 h nights and 5 h nights for the northern population (p = 0.26) in a corresponding analysis.

The result of the statistical analyses of free ABA

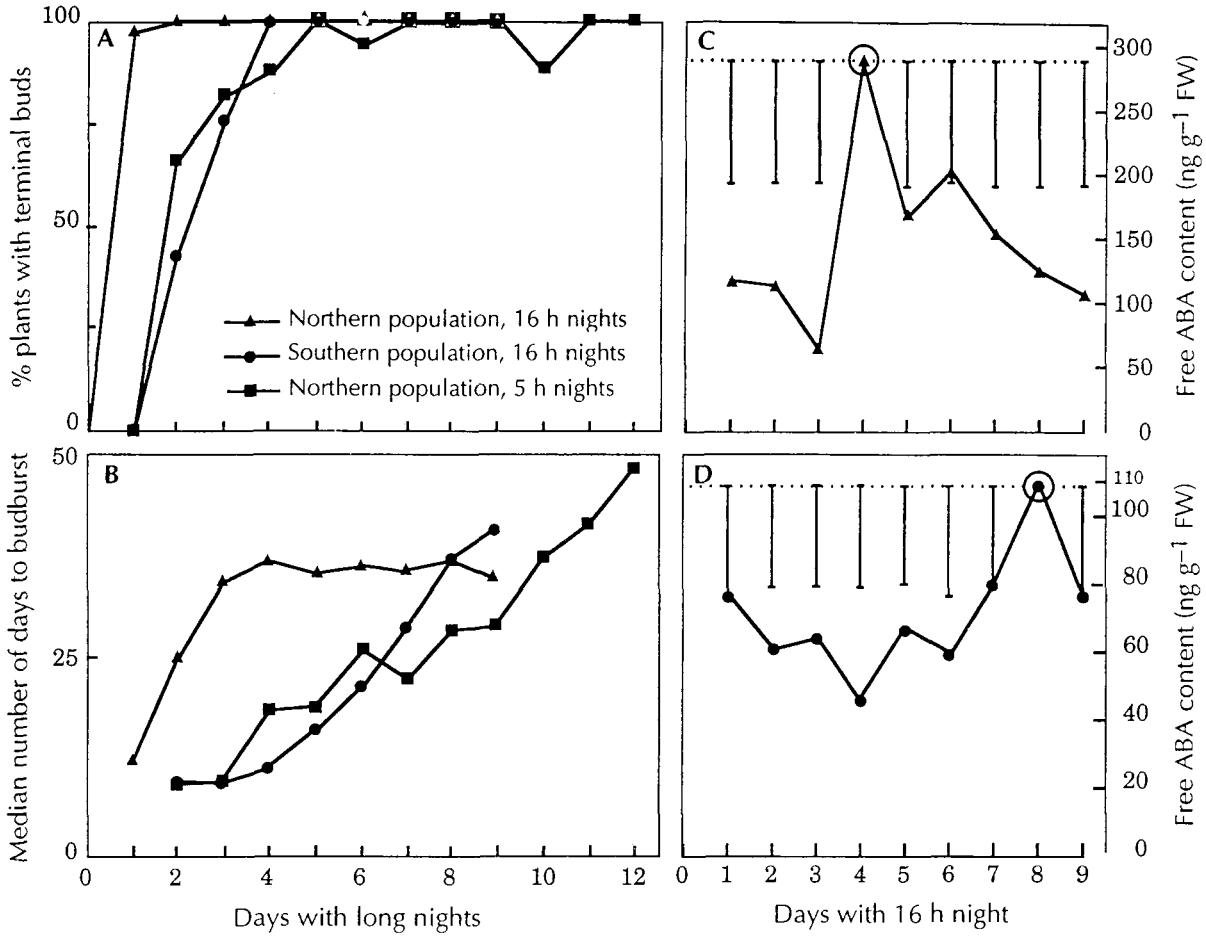

Figure 1 A. Percent plants forming terminal buds as a response to 1,. . . ,9 days with 16 h nights, for a northern and a southern population and as a response to 1,. . . ,12 days with 5 h nights, for the northern population. **B.** The build-up of bud dormancy as a response to long nights. The degree of bud dormancy attained was estimated as the median number of days between budset and budburst when the seedlings were transferred back to growth conditions. **C and D.** The free ABA content of needle extracts was sampled daily during the 16 h night treatment. The mean and standard error of the ABA contents during the last 3 days under continuous growth were 121±20 ng/g fresh weight for the northern population and 125±12 ng/g fresh weight for the southern population. The generalized least-square means for the 9 days have been retransformed to the original free ABA content. For all days except the one with the greates, (encircled) vertical bars are drawn from the level of the peak day showing the standard errors of the comparisons with the peak day. These bars are used to illustrate the confidence in the choice of the peak day.

Table 1 Least square estimates ± standard errors of days to budset and estimated percentages ± standard errors of plants that flushed within 42 days after 1, ..., 9 days in 16 h nights for the northern and the southern population and after 1, ..., 12 days in 5 h night for the northern population

Days	Days to budset			Budburst (%)		
	16h night		5 h night	16 h night		5 h night
	Northern	Southern	Northern	Northern	Southern	Northern
1	13.8 ± 0.4	–	–	96.4 ± 3.5	–	–
2	13.0 ± 0.4	14.6 ± 0.9	14.1 ± 0.6	88.9 ± 6.0	80.0 ± 17.9	81.8 ± 12.9
3	12.2 ± 0.4	15.6 ± 0.6	13.7 ± 0.6	71.9 ± 7.9	100.0 ± 0.0	91.7 ± 8.0
4	12.2 ± 0.4	15.5 ± 0.6	13.5 ± 0.5	70.0 ± 8.4	91.7 ± 8.0	92.9 ± 6.9
5	13.0 ± 0.4	15.0 ± 0.6	13.9 ± 0.5	81.3 ± 6.9	91.7 ± 8.0	87.5 ± 8.3
6	13.1 ± 0.4	14.4 ± 0.6	13.8 ± 0.5	80.0 ± 8.0	83.3 ± 10.8	80.0 ± 10.3
7	13.1 ± 0.4	14.4 ± 0.6	15.4 ± 0.5	77.4 ± 7.5	66.7 ± 13.6	81.3 ± 9.8
8	13.4 ± 0.4	15.1 ± 0.6	13.9 ± 0.5	58.6 ±11.9	66.7 ± 13.6	75.0 ± 10.8
9	13.9 ± 0.4	15.8 ± 0.6	13.3 ± 0.5	75.0 ± 7.7	58.3 ± 14.2	62.5 ± 12.1
10	–	–	13.3 ± 0.5	–	–	57.1 ± 13.2
11	–	–	13.5 ± 0.5	–	–	43.8 ± 12.4
12	–	–	13.7 ± 0.5	–	–	20.0 ± 10.3

Table 2 Estimated probabilities of a correct decision (CD) for a peak day for the content of free ABA and ABA-GE during the first 1, ..., 9 days (Expt I, 16 h night) and 1,....., 12 days (Expt II, 5 h night) of long night treatment in a northern (N) and southern (S) population. The data column gives the number of days x number of blocks and the actual number of observations. The estimated probability should be larger than 0.5 (1+1/9) = 0.55 for 9 days and 0.5 (1 + 1/12) = 0.54 for 12 days (GIBBONS *et al.* 1977)

Population	Variable	Data	Estimated variance components	Peak day	Est. P (CD)
Experiment I (16 h night)					
N	ln (free ABA)	9 × 4 (41)	$\hat{\sigma}_e^2 = 0.107$, $\hat{\sigma}_w^2 = 0.255$	4	0.74
S	ln (free ABA)	9 × 3 (24)	$\hat{\sigma}_e^2 + \hat{\sigma}_w^2 = 0.145$	8	0.63
N	ABA–GE	9 × 4 (35)	$\hat{\sigma}_e^2 + \hat{\sigma}_w^2 = 1795.7$	9	0.32
S	ABA–GA	9 × 3 (22)	$\hat{\sigma}_e^2 + \hat{\sigma}_w^2 = 249.14$	8	0.35
Experiment II (5 h night; 1 h night : control)					
N	ln (free ABA)	12 × 2 (24)	$\hat{\sigma}_e^2 + \hat{\sigma}_w^2 = 0.163$	7	0.42
N	ln (free ABA–GE)	12 × 2 (21)	$\hat{\sigma}_e^2 + \hat{\sigma}_w^2 = 0.389$	1	0.27
N	$\ln\left[\frac{\text{free ABA, 5h}}{\text{free ABA, 1h}}\right]$	12 × 2 (24)	$\hat{\sigma}_e^2 + \hat{\sigma}_w^2 = 0.429$	12	0.27
N	$\ln\left[\frac{\text{ABA-GE, 5h}}{\text{ABA-GE, 5h}}\right]$	12 × 2 (21)	$\hat{\sigma}_e^2 + \hat{\sigma}_w^2 = 0.305$	5	0.37

Table 3 Changes in water status of the northern population 1, ... 9 days in 16 h night. Each value is the least square mean ± standard error using the statistical model. The number of seedlings per day varied from 8 to 12.

Days with 16 h night (No.)	Water potential (Bar)	Water content (% FW)
Last night with 1 h night	−11.9 ± 0.5	78.4 ± 1.0
1	−12.4 ± 0.5	75.7 ± 1.0
2	−10.5 ± 0.5	77.5 ± 1.0
3	−12.7 ± 0.6	75.0 ± 1.1
4	−11.6 ± 0.5	80.5 ± 1.0
5	− 9.7 ± 0.5	80.9 ± 1.2
6	− 9.7 ± 0.5	79.7 ± 1.1
7	− 9.0 ± 0.5	79.4 ± 1.1
8	−10.3 ± 0.5	77.7 ± 1.0
9	− 8.6 ± 0.5	79.0 ± 1.1

and ABA-GE are summarized in Table 2 and in Fig. 1 C & 1 D. In the figure vertical bars are drawn from the level of the peak day showing the standard errors of the comparisons with the peak day. Owing to the imbalance, the standard errors differ slightly between days. These bars are used to illustrate the confidence in the choice of the peak day.

A highest value of free ABA in the seedlings of the northern population was observed after 4 days when the eedlings were exposed to the 16 h night treatment (Fig. 1 C; Table 2). In the southern population there was a peak in free ABA at day 8 (Fig. 1 D; Table 2). The highest value in the northern population exposed to 5 h nights was at day 7 (Table 2). However, the estimated probability of making a correct decision was low for that day therefore no corresponding figures were drawn for this experiment. The identification of free ABA was further confirmed by gas chromatography combined with mass spectrometry. The mass spectrum of purified and methylated needle extract was consistent with the mass spectrum of the methyl ester of standard ABA. Characteristic ion peaks were 190, 162, 134 and 91 (figure not shown here). The overall contents of free ABA were higher than the levels of ABA-GE at almost all sampling occasions.

The day effects differed significantly for both water content (based on fresh weight) and water potential (measured by pressure-bomb) on the shoots (Table 3). The estimated probabilities of correct selection of peak days for water content and water potential were respectively, 0.48 and 0.61. The decrease in water potential at day 3 with 16 h treatment of the northern seedlings is just statistically significant but very small.

DISCUSSION

It is remarkable that only one 16 h night is sufficient to induce almost 100 % budset in the northern population (Fig. 1 A). In agreement with earlier observations (QAMARUDDIN *et al.* 1993) the southern population responded more slowly and required 4 nights of 16 h for 100% budset (Fig. 1 A). This variation in response could be related to the difference in the critical night length between the two populations. Thus, exposure to 16 h nights must be regarded as a more drastic treatment for the northern population than for the southern. This interpretation implies that above a certain night length the night-length response is quantitative, i.e. the response increases with increasing night length within certain limits. Support for a quantitative response is the observation that budset in the northern population was initiated at a much higher rate after 16 h than after 5 h night treatment (Fig. 1 A) as documented earlier (DORMLING *et al.* 1968; HEIDE 1974). DORMLING *et al.* (1968) also observed that the number of days with long nights had to be increased when shorter nights were applied to attain the same budset response. Below a certain night length, however, the response could be regarded as qualitative since seedlings of *Picea abies* can be maintained in continuous growth.

The build-up of bud dormancy was dependent on the night length in such a way that a long night (compared to the critical) induced a rapid build-up of bud dormancy but at a lower level than if a shorter night was applied (Fig. 1 B). This is in agreement with the observations by DORMLING *et al.* (1968) for a southern population of *Picea abies* where more than 50% of the buds induced after 6 long nights (16 h) flushed within 3 weeks after budset (when brought back to 8 h nights) whereas less than 50% of the buds induced after 12 – 18 long nights flushed and after 21 long nights no bud flushing occurred.

In the present investigation the seedlings were cultivated with a night length less than the critical for budset before the long night treatment, a condition more akin to nature, and sampling of ABA was done daily from the onset of night treatment. It was found that the content of free ABA showed a peak 4 days after the initiation of 16 h night in the needles of the northern population (Fig. 1 C; Table 2), and a peak at day 8 for the southern population (Fig. 1 D; Table 2). Considering the large within-population genetic variation in budset of *Picea abies* populations repeatedly reported (ERIKSSON *et al.* 1978; EKBERG *et al.* 1985, 1991; SKRØPPA 1982) the obtained probabilities of correct peak days are strongly indicative of a peak at those days for our probably highly heterogeneous materials. There was a small increase of free ABA at day 7 in the north-

ern population exposed to 5 h nights (the probability of a correct peak day was low, Table 2). Thus, when the seedlings were given short nights during continuous growth before long night treatments the peak of ABA appeared later. This may be a parallel to the longer critical night length observed when the plants were exposed to short nights during the conditions allowing continuous growth before the onset of the long night treatments (DORMLING 1973, 1979). With the 5 h night treatment the response in the population is more protracted, in this way reducing the possibilities of detecting a peak in such a provenance material as ours.

The results for ABA-GE variable differ in the sense that no day is extreme as can be seen from the estimated probabilities in Table 2. The increase in free ABA could not be due to release from the ABA-GE, since the overall levels of ABA-GE (only peak days shown in Table 2) were generally lower than the contents of free ABA in the needles of both populations at all sampling occasions. Whether the increase in free ABA during long night treatment in *Picea abies* could be a result of an increase in the rate of synthesis, a decrease in the rate of destruction, or a decrease in the rate of movement out of the needles remains to be established. It has been suggested that the rate of synthesis is the controlling factor in ABA accumulation (see WALTON 1980). Our results are in accordance with those of RYU and LI (1994a,b) who found that the transitory increase in free ABA in *Solanum commersonii* during cold acclimation was not a result of the conversion of ABA-GE and they discussed the possibility of increased biosynthesis of free ABA.

The water status of the northern population was studied for 9 days after 16 h long night treatment (Table 3). Although the decrease in water potential at day 3 was statistically significant, it was too small to regard as biologically significant; the corresponding decrease in water content was not statistically significant. Therefore, the increase in free ABA in the needle of these seedlings after 4 nights treatment under long nights did not seem to be due to water stress of the shoots. It is commonly thought that reduction in turgor potential and in some cases small changes in cell volume induce ABA accumulation in plants (see TREWAVAS & JONES 1991). However, there are examples where flooding, stem girdling, long photoperiod, mineral deprivation, chilling and drought all induce ABA accumulation without any apparent loss of turgor (see CREELMAN & MULLET 1991). FAN & BLAKE (1994) also found that electrolyte leakage in droughted plants of woody species was the result of ABA accumulation, and leakage appeared to more closely follow ABA accumulation than xylem pressure potential changes.

Gibberellins have long been implicated in the onset of dormancy. The cessation of shoot growth in *Salix* under short days was correlated with reduced content of endogenous gibberellin-like substances (JUNTTILA 1982). The participation of ABA and gibberellins during dormancy acquisition is particularly interesting. Using mutants of tomato it has been shown that the effects of ABA and gibberellins were separated in time and space (KARSSEN & LACKA 1986; KARSSEN *et al.* 1989). The authors suggested that gibberellins were essential for growth, whereas ABA was essential for the imposition of dormancy in the mutants of tomato, although the exact roles of these hormones in dormancy imposition and growth are as yet unclear.

In conclusion, long-night treatments influence ABA metabolism in *Picea abies*. The increase in ABA content precedes budset and build-up of bud dormancy and is a possible trigger for one or more of these processes. This confirms and extends our earlier results (QAMARUDDIN *et al.* 1993; CLAPHAM *et al.* 1994) where we found a strong transient peak in needle ABA content on day 3 after the change to long nights in the southern population but not in the northern population. We presumed that the corresponding peak in the northern population had been missed owing to insufficiently frequent sampling. In the present study, by sampling every day during the dark period, we duly found a strong transient peak in the northern population at day 4 in 16 h nights. Furthermore, the peak of free ABA is smoothed out if the photoperiod is changed less abruptly, corresponding to the slowing down of the other inwintering reactions such as budset and build-up of bud dormancy.

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