

Photoperiodic responses of a northern and southern ecotype of black cottonwood

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Howe, G. T., Hackett, W. P., Fournier, G. R. and Klevorn, R. E. 1995. Photoperiodic responses of a northern and southern ecotype of black cottonwood. – *Physiol. Plant.* 93: 695–708.

Photoperiod is an important signal controlling the onset of dormancy in perennial plants. Short days typically induce growth cessation, the initiation of cold acclimation, the formation of a terminal bud, bud dormancy and other adaptive responses. Photoperiodic ecotypes have evolved in many species with large latitudinal distributions. The photoperiodic responses of two northern ($53^{\circ}35'$ and $53^{\circ}50'N$) and two southern ($34^{\circ}10'$ and $40^{\circ}32'N$) genotypes of black cottonwood (*Populus trichocarpa* Torr. & Gray) were characterized by growing trees under a range of photoperiods in the greenhouse and growth chamber. Short days induced bud set in both ecotypes, resulting in trees with fewer leaves and less height growth than trees grown under long days. Short days also enhanced anthocyanin accumulation in the northern ecotype and decreased branching of the southernmost genotype. Two aspects of the photoperiodic response were evaluated for each trait: critical photoperiod, which was defined as the longest photoperiod that elicited a short-day response, and photoperiodic sensitivity, which was defined as the change in response per unit change in photoperiod. For each of the traits analyzed, the northern ecotype had a longer critical photoperiod and greater photoperiodic sensitivity than did the southern ecotype. The short critical photoperiod and reduced photoperiodic sensitivity of the southern ecotype resulted in a significant delay in bud set compared to that of the northern ecotype, even under a 9-h photoperiod. Typically, photoperiodic ecotypes have been characterized as having different critical photoperiods. Ecotypic differences in photoperiodic sensitivity, however, indicate that differences in the photoperiodic response curves cannot be completely described by the critical photoperiod alone. These results also suggest that the critical photoperiod, photoperiodic sensitivity and speed of bud set have a common physiological basis. Bud set occurred earlier in the northern ecotype primarily because bud scale leaves were initiated earlier. For one of the northern genotypes, leaf primordia that were initiated under long days subsequently differentiated into bud scale leaves after the trees were transferred to a 9-h photoperiod. This demonstrates that primordia initiated under long days are not necessarily committed to becoming foliage leaves. The response to photoperiod did not differ appreciably between the greenhouse and growth chamber conditions that were tested.

Key words – Anthocyanin, black cottonwood, branching, bud scale, bud set, height growth, long day, photoperiod, *Populus trichocarpa*, short day.

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Introduction

Photoperiod is one of the most important environmental cues controlling the onset of dormancy in perennial plants. In many temperate-zone species, short days (SDs)

induce growth cessation, the initiation of cold acclimation, the formation of a terminal bud and bud dormancy (Vince-Prue 1975, 1985, Nooden and Weber 1978, Junttila and Nilsen 1993). By responding to SDs, plants are able to synchronize cold acclimation and dormancy in-

Received 2 September, 1994; revised 29 December, 1994

duction with the end of the growing season and the onset of low temperatures in the fall. Because the length of the growing season varies latitudinally, photoperiodic responses often differ between northern and southern populations of the same species. In the northern hemisphere, for example, trees from southern locations typically require shorter days to induce bud set than do northern trees (Pauley and Perry 1954, Vaartaja 1959, Heide 1974, Häbjørg 1978, Juntila 1980). Consequently, northern populations initiate dormancy earlier in the year and are, therefore, adapted to the shorter growing seasons found at higher latitudes. Although the term "ecotype" is frequently used to refer to populations from latitudinal extremes (Pauley and Perry 1954, Vaartaja 1959, Heide 1974, Häbjørg 1978, Juntila 1980, Oleksyn et al. 1992), the response to photoperiod often shows a clinal pattern of genetic variation that is associated with both latitudinal and elevational gradients (Pauley and Perry 1954, Häbjørg 1972, 1978, Heide 1974, Farmer 1993). In black cottonwood (*Populus trichocarpa* Torr. & Gray), large ecotypic differences are exhibited when northern and southern trees are grown in common-garden tests under the same photoperiodic regime, demonstrating that photoperiodism is under strong genetic control (Pauley and Perry 1954). SDs may also contribute to other morphological and metabolic changes associated with dormancy induction including anthocyanin accumulation, leaf abscission, accumulation of bark storage proteins and changes in biomass partitioning (Garner and Allard 1923, Nitsch 1957, Coleman et al. 1991, Oleksyn et al. 1992).

One of the most dramatic changes that occurs in response to SDs is the formation of a terminal bud (or abscission of the shoot apex in some species; Critchfield 1960, Juntila 1976, Goffinet and Larson 1981). This response has been widely studied because it is an important early event in dormancy induction, involves dramatic changes in plant development and serves as a useful marker for other adaptive responses. Tree growth, for example, is often positively correlated with delayed bud set (Rehfeldt 1989, 1992a,b, Riemenschneider et al. 1992, Farmer 1993, Li and Adams 1993, Li et al. 1993). Late bud set, however, is also associated with an increased risk of damage from fall frost and winter cold (Campbell and Sorensen 1973, Kuser and Ching 1980, Juntila and Kaurin 1985), as well as other stresses (Dietrichson 1964). Because of its adaptive value, the timing of bud set can be used as a predictor of climatic adaptation for developing seed-transfer guidelines in tree improvement programs (Mikola 1982, Campbell 1986, Campbell and Sugano 1987).

Bud set is a complex process that involves at least two fundamentally different processes: bud scale formation and the cessation of internode elongation and leaf development. In *Populus*, bud scale formation involves a major shift in the developmental pathway of the leaf. Under long days (LDs), foliage leaves are produced consisting of a lamina, petiole and two small stipules. Under SDs, however, the developmental pathway of the leaf is al-

tered, resulting in the production of bud scale leaves. The stipules of the bud scale leaves become greatly enlarged and form the protective scales that eventually enclose the shoot apex (Critchfield 1960, Goffinet and Larson 1981). During this process, the petiole of the bud scale leaf forms an abscission zone and the lamina eventually aborts (Goffinet and Larson 1982). Proceeding from the base of the bud to the shoot apex, the mature resting bud of black cottonwood consists of rudimentary bud scales formed from the stipules of the last foliage leaves, 1–3 abortive bud scale leaves, plus the embryonic leaves and leaf primordia that will develop into mature foliage leaves during the following growing season (Critchfield 1960).

We compared the photoperiodic responses of a northern and southern ecotype of black cottonwood by growing trees under a range of photoperiods in the greenhouse and growth chamber. Photoperiod extensions were used to minimize differences in the amount of photosynthetically active radiation (PAR) between the SD and LD treatments. We show that SDs induce bud set, promote anthocyanin accumulation and inhibit branching. In general, the northern ecotype had a longer critical photoperiod and a greater photoperiodic sensitivity, where sensitivity is defined as the change in response per unit change in photoperiod. Ecotypic differences in photoperiodic sensitivity suggest that differences in the photoperiodic response curves cannot be completely described by the critical photoperiod alone.

Abbreviations – ANTHO, anthocyanin score; BSI, bud scale initiation; DTB, days to bud set; FR, far-red light; HTG, height growth; NL, number of newly emerged leaves; NWB, nodes without branches; R, red light; S, photoperiodic sensitivity.

Materials and methods

Plant material

Clonal material from five black cottonwood trees originating from British Columbia and California were chosen to represent contrasting photoperiodic ecotypes (Tab. 1). Greenwood stem cuttings were collected from trees growing in arboreta and rooted in the greenhouse in St. Paul, MN. Stem segments approximately 11 cm long were dipped in Hormex 3 rooting powder (0.3% indole-3-butyric acid; Brooker Chemical Co., North Hollywood, CA, USA) and rooted in a 1:1 (v:v) mixture of Perlite and vermiculite under mist. After 4–6 weeks, the rooted cuttings were transplanted to soil (Baceto Pro Plant Mix, Michigan Peat Co., Houston, TX, USA) and subsequently maintained in the greenhouse under either a 21- or 24-h photoperiod by extending the natural photoperiod with incandescent light. Stock plants were fertilized weekly with 20-10-20 fertilizer and treated with insecticides as needed. For each experimental replication, shoot tips ca 11 cm long were rooted as described above and transplanted to soil using one tree per pot. The treatments were initiated approximately 2 weeks later when the

Tab. 1. Origin of black cottonwood clones.

Clone	Origin	N. latitude	Source of material
<i>Northern ecotype</i>			
BC-1	Prince George, BC	53°50'	Accession PGE-2, BC Forest Service, Vernon, BC
BC-2	Prince George, BC	53°35'	Accession 20-4, BC Forest Service, Vernon, BC
<i>Middle-latitude clone</i>			
BC-3	Chilliwack, BC	49°10'	Accession 1-13, Univ. of Washington, Seattle, WA and Washington State Univ., Puyallup, WA
<i>Southern ecotype</i>			
CA-1	Hydesville, CA	40°32'	Univ. of California Arboretum, Davis, CA
CA-2	Claremont, CA	34°10'	Accession 15696, Rancho Santa Ana Botanic Garden, Claremont, CA

rooted cuttings had an average height of 20 cm and 14 visible leaves or nodes.

Greenhouse experiment

Rooted cuttings were randomly assigned to eight photoperiodic regimes (9, 11, 13, 15, 17, 19, 21 and 24 h), each consisting of 8 h of natural light plus a photoperiod extension at the end of the night. Shade cloth was used to control the duration of the main light period and the photoperiod was extended with light from a 100-W clear incandescent bulb (Philips Lighting Co., Somerset, NJ, USA) which provided a photosynthetic photon flux density (PPFD) of 25–50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ midway between the bottom and top of the crown. The ratio of red to far-red light (R:FR ratio, 660/740 nm) was approximately 1.0 during the main light period and 0.7 during the extended photoperiod. PPFD was measured using a LI-85 light meter with a quantum sensor and R:FR ratios were measured using a LI-1800 spectroradiometer with a LI-1800-11 remote cosine sensor (LI-COR, Inc., Lincoln, NB, USA). The daily minimum and maximum temperatures averaged 22 and 27°C during the day, and 19 and 27°C during the night. The first replication was conducted from 21 November to 13 January with one tree per clone in each treatment. The second replication was conducted from 6 March to 28 April with two trees per clone in each treatment. Height, number of leaves, branching and anthocyanin accumulation were recorded periodically for 53 days. The average number of trees per experimental unit was 1.3, rather than 1.5, because trees damaged by insects (thrips) were not included in the analyses. We compared the average performance of the two northernmost clones (BC-1 and BC-2) with that of the two southernmost clones (CA-1 and CA-2) to judge latitudinal differences in the response to photoperiod. BC-1 and BC-2 are collectively referred to as the "northern ecotype," whereas CA-1 and CA-2 are referred to as the "southern ecotype" (Tab. 1). A single middle-latitude clone (BC-3) was included to provide additional information on latitudinal variation in the photoperiodic response.

Greenhouse vs growth chamber comparison

To determine whether the photoperiodic effects observed in the greenhouse were exhibited in the growth chamber, we also grew trees under a 13- or 21-h photoperiod in a Conviron E-15 or PGW-36 growth chamber (Controlled Environments, Inc., Pembina, ND, USA). Trees were grown at a constant temperature of 18–20°C under a combination of cool-white fluorescent (Philips F72T12/CW/VHO, 160 W) and incandescent (Philips, 60 W, clear) lamps which provided a PPFD of 250–300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and a R:FR ratio of 1.3 to 1.4. Both treatments consisted of a 13-h main light period. In the 21-h treatment, the 13-h main light period was extended with 8 h of low intensity incandescent light as described above. The 13-h photoperiod was replicated three times and the 21-h photoperiod was replicated twice with an average of 3.1 trees per clone in each replication. Height, number of leaves, branching and anthocyanin accumulation were recorded periodically for 45 days.

Bud scale initiation

Trees were transferred from a 21-h photoperiod in the greenhouse to a 9-h photoperiod in the growth chamber to induce bud set. Following bud set, we determined when the first bud scale leaf was initiated relative to the onset of the SD treatments by using the procedure of Goffinet and Larson (1981). This was done by comparing how many leaves and primordia were present when the SD treatments were initiated (initial measurements) with how many leaves were present after the trees had set bud (final measurements). "Leaves" are defined as foliage leaves that are visible to the unaided eye and "primordia" are defined as embryonic leaves and leaf primordia that cannot be seen without dissecting the shoot apex. Because destructive sampling is required to determine the number of primordia, the initial and final measurements were made on separate, but comparable, groups of trees (Goffinet and Larson 1981). Trees growing in the greenhouse under a 21-h photoperiod were randomly assigned to two groups. Shoot apices were harvested from one group to count the number of primordia and the second group was

transferred to a 9-h photoperiod in the growth chamber. Each group consisted of 5–6 trees per clone. Shoot apices were stored in FAA (5% formalin, 5% acetic acid, 90% ethanol) until primordia were counted under a dissecting microscope at 60 \times magnification. Leaves were counted on the trees that were not destructively sampled at the beginning of the SD treatment and 66 days later. This experiment consisted of two replications and the growth chamber conditions during the 9-h main light period were as described above.

The relative timing of bud scale initiation (BSI) was measured in plastochnrons by using the last primordium initiated under LDs as a reference point. Because the reference primordium was assigned a plastochnron of zero, a BSI that is negative or equal to zero indicates that the first bud scale leaf was initiated under LDs. Conversely, a BSI that is positive indicates that the first bud scale leaf was initiated after the trees were transferred to SDs. Following bud set, BSI was calculated by subtraction ($BSI = [final\ number\ of\ leaves + 1\ bud\ scale\ leaf] - [initial\ number\ of\ leaves\ and\ primordia]$). For example, if 10 leaves and primordia were present at the beginning of the 9-h treatment and 10 leaves were present at the end of the experiment, we deduced that the first bud scale leaf differentiated from the first primordium initiated under SDs ($BSI = [10 + 1] - [10] = 1$).

Measurements

For each experiment, we recorded total height, number of leaves, number of sylleptic branches, anthocyanin accumulation and the presence or absence of a terminal bud. Height growth (HTG) and the number of new leaves (NL) were calculated by subtracting the initial from the final measurements. Plastochnron (days/NL) was calculated for the first 12 days of the experiment. This period was chosen because none of the trees had set a terminal bud. For each node, a sylleptic branch was counted if any leaves could be seen protruding from the axillary bud scales. These data were used to calculate the number of nodes without branches (NWB) for each tree. NWB was used to evaluate branching because this measure was relatively unaffected by tree size (see Results). In contrast, both the total number of branches per tree and the percentage of nodes with branches were positively correlated with the number of nodes per tree. Relative anthocyanin accumulation was evaluated by recording the average color of the top four petioles using a 1 to 10 score. A score of 1 indicates that no red color was visible and 10 denotes maximum anthocyanin accumulation (based on anthocyanin levels previously observed in trees grown under SDs). An anthocyanin score (ANTHO) was calculated for each tree by subtracting the initial anthocyanin accumulation from the largest value recorded during the experiment for each tree. The date of bud set was determined by visually examining the shoot apex each day. Bud set was recorded when the stipules of the foliage leaves covered the shoot apex and the youngest foliage

leaf was offset from the central axis of the shoot apex. Following bud set, the bud scale leaves continued to develop and eventually replaced the stipules of the foliage leaves as the main structures that enclosed the shoot apex. For trees that formed a temporary bud (but subsequently resumed growth) we calculated the number of days to bud set (DTB) by averaging the values for each bud set date for that tree. If these trees set a temporary bud, but were actively growing on the last day of the experiment, a value of 58 days was used for the second bud set date. Fifty-eight days represents a minimum estimate for DTB because the last observations were made on day 53 and the average time between the appearance of the last leaf and the date of bud set was 5 days. Only one of the southern trees failed to set a terminal bud under the 11-h photoperiod. A value of 58 days was used for this individual to calculate the mean DTB for this treatment.

Statistical analyses

Data from the greenhouse experiment were analyzed as a randomized complete block design with a factorial arrangement of treatments (Tab. 2A). Effects for photoperiod and ecotype were considered fixed, whereas block and clone-within-ecotype were treated as random effects. Photoperiod \times clone \times block means were calculated for each variable and used in a weighted analysis of variance to compute Type IV sums-of-squares using the SAS GLM procedure (SAS 1985). Because the interaction of photoperiod and clone-within-ecotype was nonsignificant for each variable ($\alpha > 0.10$), the corresponding sum-of-squares was included in the error term. For the northern and southern ecotypes, single degree of freedom contrasts were used to test differences between pooled SD and LD treatments for each trait. SD treatments were defined as those treatments that induced bud set. Because the critical photoperiod for bud set varied by ecotype, the pooled SD and LD treatments were composed of different photoperiodic treatments for the northern and southern ecotypes (see Results). Photoperiod \times ecotype least-square means and standard errors were calculated and used for presenting the data. In general, treatment differences were considered nonsignificant if the probability level (α) exceeded 0.10.

The critical photoperiod for bud set was defined as the longest photoperiod that induced bud set in any of the trees. For traits other than bud set, objective estimates of the critical photoperiods were obtained by determining the longest photoperiod that produced a response significantly different from the 21-h treatment (if all of the shorter photoperiods were also significantly different from the 21-h treatment). Based on visual inspection of the photoperiodic response curves and results from the statistical analyses, we chose to use an intermediate level of probability for calculating critical photoperiods ($\alpha < 0.05$).

The photoperiodic sensitivity (S) of each trait was

Tab. 2. Analyses of variance. The northern and southern ecotypes were each represented by two clones of black cottonwood, whereas the middle-latitude ecotype was represented by a single clone (A, C, and D). Only the northern and southern ecotypes were used in the analysis of photoperiodic sensitivity (B).

A. Greenhouse experiment

Source of variation	df
Block	1
Photoperiod	7
Ecotype	2
Photoperiod × ecotype	14
Clone-within-ecotype	2
Error	49

B. Analysis of photoperiod sensitivity

Source of variation	df
Block	1
Trait	3
Ecotype	1
Trait × ecotype	3
Clone-within-ecotype	2
Error	19

C. Greenhouse vs growth chamber environment

Source of variation	df
Environment	1
Block-within-environment	3
Photoperiod	1
Photoperiod × environment	1
Ecotype	2
Ecotype × environment	2
Ecotype × photoperiod	2
Ecotype × photoperiod × environment	2
Clone-within-ecotype	2
Error	26

D. Analysis of bud scale initiation

Source of variation	df
Block	1
Ecotype	2
Clone-within-ecotype	2
Error	4

evaluated by determining the slope of the photoperiodic response curve at the critical photoperiod. To facilitate comparisons among the traits, the sensitivities were standardized by dividing the trait values by the square-roots of the corresponding error-mean-squares from the analyses of variance. $S = |(Y_{CP+2}/\text{ems}) - (Y_{CP-2}/\text{ems})|/4$, where Y_{CP+2} and Y_{CP-2} are the means for the treatments 2 h longer and 2 h shorter than the critical photoperiod, respectively; ems is the error-mean-square from the analysis of variance; and the denominator "4" is the difference in photoperiod between the Y_{CP+2} and Y_{CP-2} treatments. This general procedure was modified for certain traits. Sensitivities for DTB were calculated using Y_{CP}

and Y_{CP-2} because no values for Y_{CP+2} are possible. For the southern ecotype, the sensitivities for HTG were calculated using Y_{CP+2} and Y_{CP} because Y_{CP+2} was not tested, and for both ecotypes, the sensitivities for ANTHO were calculated by using the steepest parts of the response curves because no critical photoperiods could be determined. Sensitivities were calculated for each clone and block separately, resulting in a total of four values for the northern ecotype and four values for the southern ecotype (2 clones/ecotype × 2 blocks). These values were used in an analysis of variance to test overall differences among the traits and differences between the ecotypes for each trait (Tab. 2B). Sensitivity least-square means were used for presenting the data.

To compare photoperiodic responses in the greenhouse and growth chamber, we conducted a combined analysis by using the data from the 13- and 21-h treatments from the greenhouse and growth chamber experiments. These data were analyzed essentially as described for the greenhouse experiment, except that blocks were considered nested within the greenhouse and growth chamber "environments" (Tab. 2C), and "environment" was considered a fixed effect. Expected mean squares were calculated using the RANDOM option of the SAS GLM procedure (SAS 1985) and the main effect of environment was tested for each trait using a synthetic error term (Snedecor and Cochran 1967, p. 368–369). Within each ecotype and environment, the SAS PDIF option was used to test differences between the 13- and 21-h treatments (i.e. preplanned comparisons were made between selected environment × photoperiod × ecotype means).

The same general procedures were used to analyze bud scale initiation (Tab. 2D). Differences in BSI and the number of primordia between the northern and southern ecotypes were tested by using the clone-within-ecotype mean square as an error term. For BSI, individual clone means were also tested to see if they were significantly less than 1.0.

Results

Short days induce bud set, promote anthocyanin accumulation and inhibit branching

Days to bud set. – SDs induced the formation of a terminal bud in both the northern and southern ecotypes (Fig. 1A). Although the percentages in Fig. 1A include all trees that formed a bud during the experiment, bud set was temporary for some of the trees and growth resumed a few days later. Under the 11-h photoperiod, for example, 75% of the southern trees formed a bud during the experiment (Fig. 1A), but only 40% of the trees had a terminal bud on the last day of the experiment. The formation of a temporary bud occurred primarily in trees of the southern ecotype. The timing of bud set was roughly correlated with the latitude of origin of the trees. Under the 9-h photoperiod, for example, the northern-, middle- and southern-latitude clones formed buds after 16.8, 28.0 and

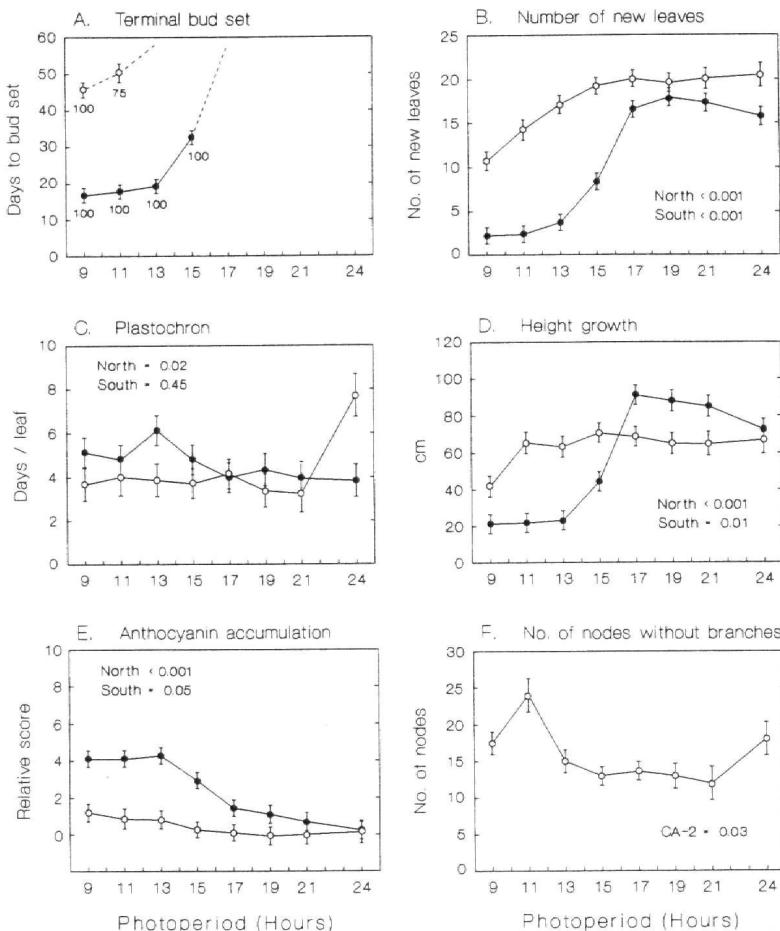


Fig. 1. Photoperiodic responses of a northern (●) and southern ecotype (○) of black cottonwood growing in the greenhouse. Each point is the mean of 3 to 6 trees (\pm SE), except for branching, which is the mean of 1 to 3 trees from a single southern clone (CA-2). For terminal bud set, the numerical values are the percentages of trees that formed a permanent or temporary bud during the experiment. Treatments in which all of the trees formed a terminal bud are connected by solid lines and treatments in which some (or all) of the trees were actively growing on the last day of the experiment are connected by dashed lines. For the latter treatments, minimum estimates for DTB were calculated assuming that all trees set bud immediately after the experiment ended. For each ecotype, differences between the pooled SD and LD treatments were tested and the level of significance (α) is presented in either the upper left-hand corner (Panels C and E), or lower right-hand corner (Panels B, D and F) of the figure. The pooled SD treatments included all treatments that induced bud set (see panel A). Because the northern and southern ecotypes have different critical photoperiods, the pooled SD and LD treatments were defined separately for each ecotype.

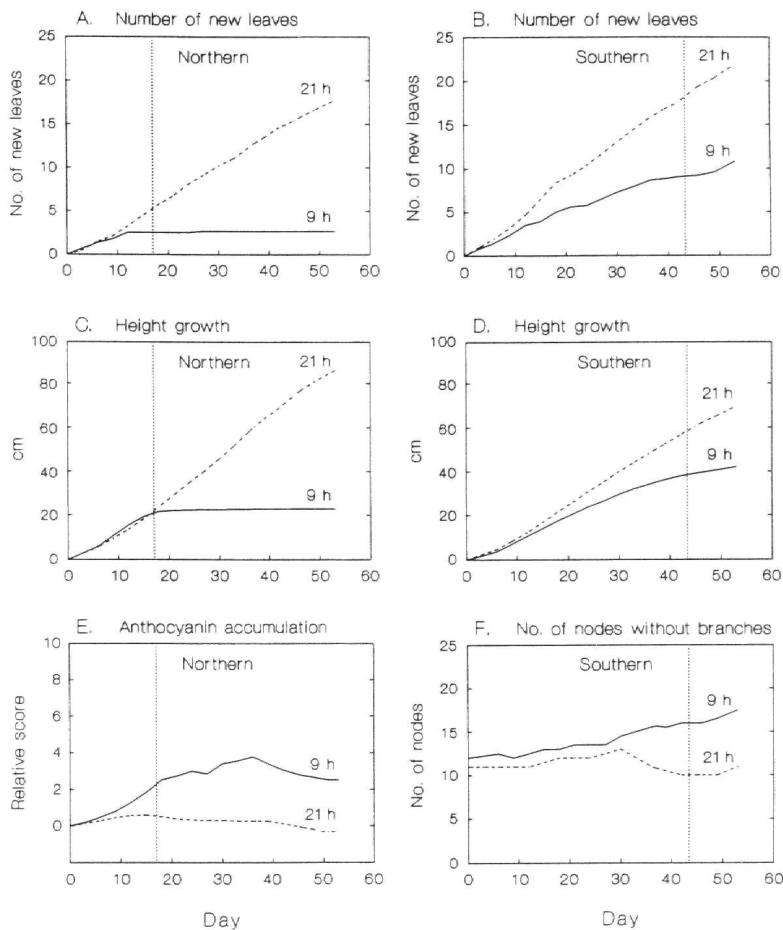
45.7 days, respectively (data for the middle-latitude clone are not shown).

Newly emerged leaves. — Although both ecotypes produced significantly fewer leaves under SDs ($\alpha < 0.001$; Fig. 1B), the rate of leaf emergence during the first 12 days was not dramatically affected by photoperiod (Fig. 1C). For the northern ecotype, a statistically significant increase in plastochron was observed under SDs (i.e. slower rate of leaf emergence; $\alpha = 0.02$; Fig. 1C), but this increase was relatively small compared to the large reduction in NL (Fig. 1B). For the southern ecotype, the difference in plastochron between the SD and LD treatments was nonsignificant ($\alpha = 0.45$). NL was roughly correlated with the latitude of origin of the genotypes. Under the 9-h photoperiod, the northern-, middle- and southern-latitude clones produced 2.2, 3.9 and 10.7 new leaves, respectively (data for the middle-latitude clone are not shown). In the northern ecotype, the 9-h photoper-

iod appeared to have a minor effect on the rate of leaf emergence prior to the date of bud set (Fig. 2A). Bud set was recorded when the stipules of the foliage leaves covered the shoot apex. On average, this occurred 5 days after the emergence of the last foliage leaf. For the southern ecotype, differences in the rate of leaf emergence between the 9- and 21-h treatments were apparent well before the average date of bud set (Fig. 2B). Under the 9-h photoperiod, bud set was first observed in trees of the southern ecotype on day 36, but some of the trees were still growing on the last day of the experiment (day 53). The large variation in the timing of bud set explains why NL did not plateau during the experiment for the southern ecotype (Fig. 2B).

Bud scale initiation. — Based on the mean BSI of the northern ecotype, the first bud scales were formed from primordia initiated under LDs ($\bar{x} = -0.16$, Fig. 3). A BSI that is negative or equal to zero indicates that the first bud

Fig. 2. Time-courses for photoperiodic responses in a northern and southern ecotype of black cottonwood growing in the greenhouse. Trees were grown under either a 9-h (—) or 21-h (----) photoperiod. Each point is the mean of 3 to 6 trees, except for branching, which is the mean of 1 to 3 trees from a single southern clone (CA-2). The dotted vertical line indicates the average date of bud set under the 9-h photoperiod.



scale leaf was initiated under LDs, and a BSI that is positive indicates that the first bud scale leaf was initiated after the trees were transferred to SDs. Only one of the northern clones, however, had a BSI that was significantly less than 1.0 (BC-2: $\bar{x} = -0.31$; $\alpha = 0.04$). A BSI of 1.0 corresponds to the first primordium initiated under SDs. In contrast, the bud scales of the middle-latitude and southern clones were formed from primordia that developed under SDs ($\bar{x} = 0.98$ and 5.7, respectively). The difference in BSI between the northern and southern ecotypes was statistically significant ($\alpha = 0.04$). Dissection of the shoot apex also allowed us to determine the average number of primordia (embryonic leaves and leaf primordia distal to the youngest visible leaf). The number of primordia averaged 3.4, 2.7 and 3.9 for the northern-, middle- and southern-latitude clones, respectively. Although the difference in the number of primordia between the northern and southern ecotypes was statistically non-significant ($\alpha = 0.24$), the shoot apex of the southern

ecotype was more compact. The southern ecotype had an average of eight leaves and primordia less than 2 cm in length, but the northern ecotype had only five (i.e. the youngest primordium occurred at LPI -8 for the southern ecotype and LPI -5 for the northern ecotype; Larson and Isebrands 1971). The number of primordia did not differ between the ecotypes, however, because the number of visible leaves distal to the 2-cm index leaf was also greater for the southern ecotype.

Height growth. — Both the northern and southern ecotypes had significantly less HTG under SDs ($\alpha < 0.001$ and $\alpha = 0.01$, respectively; Fig. 1D). In addition, HTG of the genotypes was roughly correlated with their latitude of origin. Under the 9-h photoperiod, the northern-, middle- and southern-latitude clones grew 21.3, 26.0 and 41.8 cm, respectively (data for the middle-latitude clone are not shown). In the northern ecotype, SDs had no detectable effect on HTG prior to the date of bud set (Fig.

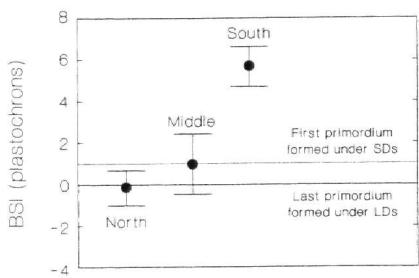


Fig. 3. Initiation of bud scale leaves in northern-, middle- and southern-latitude clones of black cottonwood. Trees were transferred from a 21- to a 9-h photoperiod to induce bud set. The relative timing of bud scale initiation was measured in plastochrons by using the last primordium initiated under LDs as a reference. A BSI that is negative or equal to zero indicates that the first bud scale leaf was initiated under LDs and a BSI that is positive indicates that the first bud scale leaf was initiated after the trees were transferred to SDs. Each point is the mean of 8 to 24 trees (\pm SE). The difference between the northern and southern ecotypes was significant at the 0.04 level of probability.

2C). In contrast, differences in HTG between the SD and LD treatments were apparent well before bud set occurred in the southern ecotype (Fig. 2D).

Anthocyanin accumulation. – When the northern ecotype was grown under SDs, a substantial increase in anthocyanin was observed in the petioles of the uppermost leaves (Fig. 1E), and to a lesser degree in the laminae and stem. The difference in ANTHO between the SD and LD treatments was statistically significant for both the northern and southern ecotypes ($\alpha < 0.001$ and $\alpha = 0.05$, respectively; Fig. 1E). The increase in ANTHO became apparent in the northern ecotype about 10 to 15 days after the onset of the 9-h photoperiod (Fig. 2E). ANTHO continued to increase slowly until it peaked around day 35, then it gradually declined until the end of the experiment. The time-course for ANTHO is not presented for the southern ecotype because the increase in ANTHO under SDs was barely detectable (Fig. 1E).

Branching. – Because axillary bud burst proceeded acropetally, branching appeared to be inhibited by the shoot apex. We characterized the branching of the trees by measuring the number of nodes without branches

(NWB). Unlike the number of branches per tree or the percentage of nodes with branches, NWB was relatively unaffected by tree size (number of nodes) once the trees were large enough to produce branches. With few exceptions, the nodes that lacked branches occurred adjacent to one another at the top of the tree. In general, NWB was lowest for the southern ecotype (i.e. the southern ecotype was more branched). Under the 21-h photoperiod, for example, NWB of the southern clones ranged from 11 to 25 nodes (for CA-2 and CA-1, respectively). The northern clones, however, averaged 27 nodes without branches (this is a minimum estimate for NWB because 80% of the northern trees had no branches at all). Unfortunately, NWB is not a useful measure unless the trees are large enough to produce branches. If the average NWB of a genotype is 25 nodes, for example, then many of the trees that are shorter than 26 nodes will have no branches and NWB will primarily reflect tree size, rather than branching habit. This was true for most of the trees grown under SDs. Using the southernmost clone (CA-2), however, we were able to measure NWB for all of the treatments. Based on the performance of CA-2, SDs seemed to inhibit branching. Although the variation among the SD treatments was large, the trees in the 9- and 11-h photoperiods had a significantly greater NWB than trees grown under LDs ($\alpha = 0.03$; Fig. 1F). This effect was subsequently confirmed in the growth chamber: trees grown under a 9-h photoperiod had a mean NWB of 17.1 (CA-2; data from the bud scale initiation experiment), compared to 11.2 for trees grown under a 13-h photoperiod (13 h is greater than the critical photoperiod for CA-2; data from the greenhouse vs growth chamber comparison).

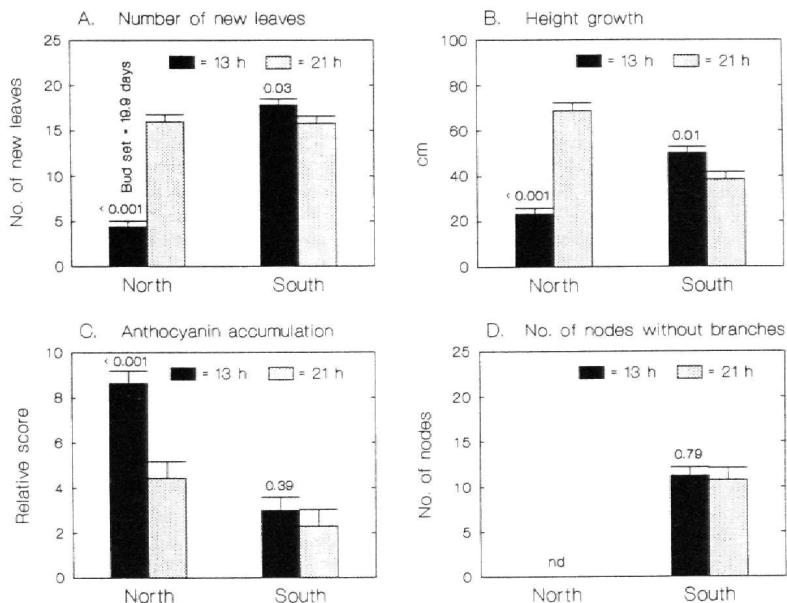
Critical photoperiods differ between the northern and southern ecotypes

Estimation of critical photoperiods in the greenhouse. – The critical photoperiods for the northern ecotype were 15 h for DTB, NL, HTG and ANTHO (Tab. 3). The critical photoperiods for the southern ecotype, however, were 11 h for DTB and NL and 9 h for HTG. The critical photoperiod for ANTHO could not be determined for the southern ecotype and the critical photoperiods for plastochron and NWB could not be determined for either ecotype. Based on the visual assessment of bud set (DTB),

Tab. 3. Critical photoperiods and photoperiodic sensitivities (S) for two ecotypes of black cottonwood growing in the greenhouse. Probability levels (α) are presented for judging differences in sensitivity between the northern and southern ecotypes. Each ecotype was represented by two clones of black cottonwood. Dashes indicate that a critical photoperiod could not be determined.

Trait	Northern ecotype		Southern ecotype		Probability level
	Critical photoperiod	Photoperiodic sensitivity (S)	Critical photoperiod	Photoperiodic sensitivity (S)	
Terminal bud set (DTB)	15	1.42	11	0.71	0.08
Number of new leaves (NL)	15	1.39	11	0.74	0.08
Height growth (HTG)	15	1.34	9	0.95	0.32
Anthocyanin score (ANTHO)	15	0.67	–	0.18	0.16

Fig. 4. Photoperiodic responses of a northern and southern ecotype of black cottonwood growing in the growth chamber. Northern and southern ecotypes were grown under a 13- and 21-h photoperiod for 45 days. Each bar is the mean of 11 to 21 trees (+SE), except for branching, which is based on a mean of 5 to 9 trees from a single southern clone (CA-2). For each ecotype, differences between the 13- and 21-h photoperiods were tested and the level of significance (α) is presented above the bar for the 13-h treatment.



the critical photoperiod for the middle-latitude clone was 13 h (data not shown).

Examination of critical photoperiods in the growth chamber. – We also grew trees in the growth chamber under two contrasting photoperiods to determine whether our estimates of the critical photoperiods were reasonable. We chose to use a 13-h photoperiod because it was expected to elicit a SD response in the northern ecotype, but a LD response in the southern ecotype. A 21-h photoperiod was chosen as the best LD treatment for both ecotypes.

Terminal bud set. – The northern trees set bud under the 13-h photoperiod in the growth chamber, but the southern trees did not (Fig. 4A). These results confirm that the critical photoperiod for bud set was greater than 13 h for the northern ecotype, but less than 13 h for the southern ecotype. Comparable results were obtained for both NL and HTG (Fig. 4A,B). Overall, the differences between the greenhouse and growth chamber environments were nonsignificant for DTB, NL and HTG (data not shown).

Anthocyanin accumulation. – The results in the growth chamber confirm that the critical photoperiod for ANTHO was greater than 13 h for the northern ecotype, but less than 13 h for the southern ecotype (Fig. 4C). A substantial increase in ANTHO occurred shortly after the trees were transferred from the greenhouse to the growth chamber and this increase persisted for at least 2 weeks after the SD treatments were begun. Although ANTHO tended to be greater in the growth chamber at the end of

the experiment (day 45), this difference was nonsignificant ($\alpha = 0.20$).

Nodes without branches. – The branching of CA-2 did not differ between the 13- and 21-h photoperiods in either environment (Figs 1F and 4D). Branching tended to be greater in the growth chamber than in the greenhouse (i.e. lower NWB), but this difference was statistically nonsignificant ($\alpha = 0.31$).

Sensitivity to photoperiod

Photoperiodic sensitivity was evaluated for each trait by determining the slope of the photoperiodic response curve at the critical photoperiod (Tab. 3). Compared to the southern ecotype, the northern ecotype was more sensitive to photoperiod for each trait except branching (the sensitivity for branching could not be determined for either ecotype). Differences in sensitivity between the ecotypes were statistically significant for DTB and NL ($\alpha = 0.08$), but nonsignificant for HTG and ANTHO ($\alpha = 0.32$ and 0.16, respectively).

Discussion

Short days induce bud set, promote anthocyanin accumulation and inhibit branching

We characterized the photoperiodic responses of a northern and southern ecotype of black cottonwood by growing trees under a range of photoperiods in the greenhouse and growth chamber. SDs induced the formation of a

terminal bud in both ecotypes, resulting in trees that were shorter and had fewer leaves than trees grown under LDs (Fig. 1). SDs also promoted anthocyanin accumulation in both ecotypes and inhibited branching of the southernmost clone (Fig. 1).

Terminal bud set. — Although we evaluated terminal bud set by measuring DTB, NL, BSI and HTG, we focus our discussion on NL and BSI because they are generally the most informative measures of SD-induced bud set. The main difference between NL and DTB is that the former is measured on a developmental scale (newly emerged leaves), whereas the latter is measured on a time scale (days). NL and DTB would be perfectly correlated if the rate of leaf emergence was the same for all photoperiods and genotypes. This was not the case, however. SDs increased the plastochnon of the northern ecotype (i.e. the northern trees had a significantly slower rate of leaf emergence under SDs; Fig. 1C). In addition, the plastochnon of the northern ecotype was generally greater than that of the southern ecotype (Fig. 1C). NL is more informative than DTB because it can be measured on treatments that do not induce bud set, thereby potentially detecting subtle effects on growth that do not lead to bud formation. HTG, in contrast, is the least reliable measure of bud set because it is particularly sensitive to extraneous environmental factors such as temperature, nutrient availability and light quality (Downs and Borthwick 1956, Vince-Prue 1984).

Although all of the trees formed a terminal bud under the shortest photoperiod (9 h), the northern ecotype set bud after producing 2.2 new leaves (16.8 days) compared to 10.7 new leaves for the southern ecotype (45.7 days; Fig. 1A,B). What accounts for this difference? First, it is important to note that NL is dependent on two factors; (1) the number of primordia present when the trees are transferred from LDs to SDs, and (2) the timing of initiation of the first bud scale leaf. When seedlings of eastern cottonwood (*Populus deltoides*) were transferred from LDs to SDs, the first primordium initiated under SDs differentiated into the first bud scale leaf (Goffinet and Larson 1981). If this were true for both ecotypes of black cottonwood, then NL would be identical to the number of primordia, and ecotypic differences in NL must result from corresponding differences in the number of primordia. Alternatively, if the ecotypes have the same number of primordia, then the smaller NL of the northern ecotype indicates that initiation of the first bud scale leaf must have occurred earlier in the northern ecotype. We determined when bud scale leaves were initiated in each ecotype to distinguish between these two possibilities.

By dissecting shoot apices of trees grown under LDs, we found that the numbers of primordia were the same for both ecotypes. We also determined when the first bud scale leaf was initiated relative to the onset of the SD treatment. In the northern ecotype, the first bud scale leaf differentiated from the last primordium initiated under LDs ($BSI = -0.16$; Fig. 3). In the southern ecotype,

however, the first bud scale leaf usually developed from the sixth primordium initiated under SDs; the first five primordia usually differentiated into foliage leaves ($BSI = 5.7$). These results demonstrate that bud set occurred earlier in the northern ecotype because bud scale leaves differentiated earlier, not because fewer primordia were present at the shoot apex. In *Populus deltoides* only primordia initiated under SDs differentiated into bud scale leaves (Goffinet and Larson 1981). This suggested that primordia initiated under LDs might be committed to becoming foliage leaves. For black cottonwood, however, the mean BSI for the northern ecotype was negative (-0.16), and for one of the northern clones (BC-2), the BSI was significantly less than 1 ($BSI = -0.31$). This indicates that primordia initiated under LDs can, indeed, differentiate into bud scale leaves in response to SDs, at least in some northern clones of black cottonwood.

The contrast between our results and those of Goffinet and Larson (1981) is probably due to differences in the geographic origin of the trees, although species differences could play a role. Goffinet and Larson studied a single seed source from LaCrosse, WI (44°N), but we tested genotypes originating from British Columbia ($53^\circ35'$ and $53^\circ50'\text{N}$) to southern California ($34^\circ10'$ and $40^\circ32'\text{N}$). Interestingly, our middle-latitude clone of *P. trichocarpa* ($49^\circ10'\text{N}$) responded like the WI *P. deltoides* seed source; the first bud scale leaf was initiated immediately after the trees were transferred to SDs ($BSI = 0.98$).

It was somewhat surprising that bud set occurred so late in the southern ecotype under the 9-h photoperiod (Fig. 1A). Although bud set might have occurred earlier if we had tested shorter photoperiods, the southern ecotype never experiences photoperiods as short as 9 h in nature. The shortest day experienced by the southernmost clone, for example, is about 10 h. Therefore, within the range of naturally occurring photoperiods, bud set occurred much more slowly in the southern ecotype than in the northern ecotype (i.e. NL and DTB were relatively large for the southern ecotype). As discussed above, the slow response of the southern ecotype was mainly due to delayed initiation of bud scale leaves. Why does the southern ecotype respond relatively slowly to SDs compared to the northern ecotype? One explanation is that the critical photoperiod and speed of bud set are controlled by the same physiological process. According to this hypothesis, the southern ecotype has evolved a short critical photoperiod and short critical photoperiods are invariably associated with the inability to respond quickly to inductive photoperiods. A shorter critical photoperiod is also associated with a slower response to SDs in photoperiodic ecotypes of *Salix pentandra* (Junttila 1980, 1982). Compared to the southern trees, the northern trees of *Salix* stopped growing much sooner after they were transferred to a 12-h photoperiod (Junttila 1980). The results for *Salix* are particularly interesting because both ecotypes originated from northern locations ($59^\circ40'$ and $69^\circ39'\text{N}$) and apical growth cessation was measured under 12-h days, well

below the critical photoperiod for the southern ecotype (16.5 h).

It has been suggested, however, that the speed of bud set and critical photoperiod are regulated by separate mechanisms (Junttila 1982). If this were true, the speed of bud set might be influenced by natural selection separately from the critical photoperiod. Because the southern ecotype experiences a very gradual drop in temperatures in the fall, the slow response of the southern ecotype could be adaptive. The average daily minimum temperatures experienced by CA-1, for example, decline steadily from about 8–11°C in October to about 4–5°C in January, with only an 80% probability of freezing temperatures by 1 January. Therefore, the slow response of the southern ecotype may delay the cessation of height growth and allow greater use of favorable growing temperatures in the fall. Alternatively, the slow response of the southern ecotype may result from a relaxation of natural selection. In black cottonwood, the response to photoperiod is believed to be under polygenic control (Pauley and Perry 1954) and rapid bud set may require fixation of favorable alleles at a number of loci. If selection is relaxed in moderate climates, unfavorable alleles may accumulate at some of these loci, resulting in trees that retain the ability to respond to SDs, but that exhibit a reduction in the speed of bud set.

In contrast to the southern ecotype, the northern trees of black cottonwood responded relatively quickly to the 9-h photoperiod. Bud set occurred in 16.8 days and only 2.2 new leaves were produced. Prior to bud set, however, SDs had only a minor effect on the rate of leaf emergence and no detectable effect on HTG (Fig. 2A,C). What accounts for the abrupt cessation of growth and why does it occur 2 to 3 weeks after the onset of the SD treatment? The delay in growth cessation does not appear to result from a delay in the perception of SDs because other responses are observed in cottonwood trees prior to bud set. These responses include changes in leaf development (Goffinet and Larson 1981), accumulation of bark storage proteins (Coleman et al. 1991) and changes in anthocyanin accumulation (discussed below). Instead, the 2- to 3-week delay in height growth cessation appears to correspond to the time needed for bud formation to occur. Bud formation is a protracted process that requires the differentiation and development of bud scale leaves. During this period, leaf primordia that are already committed to becoming foliage leaves continue to emerge and expand. Maintenance of rapid growth under SDs should reduce the number of days to bud set by increasing the rate of leaf emergence and bud scale development.

Internode elongation stops abruptly, however, about 5 days after the emergence of the last foliage leaf (at least in the northern ecotype). What controls the timing of this event? Because the bud scale leaves are undergoing dramatic developmental changes during this period (Goffinet and Larson 1981), we speculate that the developmental stage of the bud scale leaves is communicated to the rest of the shoot apex, ultimately leading to the

cessation of internode elongation. This model accounts for the long delay in apical growth cessation after the trees are transferred to SDs and the close coordination between apical growth cessation and bud scale development. Although the timing of apical growth cessation may be controlled by the developing bud scale leaves, the newly expanded foliage leaves are believed to be the primary sites of photoperiodic perception in most tree species (reviewed in Vince-Prue 1975) and responses observed in other parts of the plant appear to be initiated via a signal translocated from these leaves (Fuchigami et al. 1971a,b).

In contrast to that of the northern ecotype, the rate of leaf emergence and HTG of the southern ecotype declined substantially prior to bud set (Fig. 2B,D). The apparent difference between the northern and southern ecotypes may be misleading, however, because the 9-h photoperiod is 6 h below the critical photoperiod for the northern ecotype, but only 2 h below the critical photoperiod for the southern ecotype. When the northern ecotype was grown under a much longer photoperiod (15 h), the response was similar to that of the southern ecotype; leaf emergence and HTG declined slowly before bud set occurred on day 32 (data not shown).

Unlike black cottonwood, trees that do not form a terminal bud may be capable of stopping height growth soon after experiencing SDs. In species of *Salix* and *Betula*, for example, SDs cause the shoot apex to abscise, rather than form a terminal bud (Junttila 1976). The terminal bud for the next growing season (pseudoterminal) is formed from an axillary bud formed under LDs. This could be an evolutionary adaptation to environments that require a particularly fast response to SDs.

Anthocyanin accumulation. – Although SDs enhanced anthocyanin accumulation in both ecotypes, the difference in ANTHO between the SD and LD treatments was small for the southern ecotype (Fig. 1E). Because anthocyanin accumulation is promoted by soluble carbohydrates (Ishikura 1976, Kramer and Kozlowski 1979, Murray and Hackett 1991, Tsukaya et al. 1991), the larger ANTHO values for the trees grown under SDs may indicate that carbohydrate metabolism was altered by SDs. Anthocyanins increase dramatically in the leaves of many tree species in the fall. The leaves of black cottonwood turn yellow, however, rather than the reds and purples typical of anthocyanins. Nonetheless, our results suggest that SD-induced anthocyanin accumulation may contribute to the fall coloration observed in many species. It is generally agreed, however, that low temperatures play a significant role in enhancing fall coloration (Kramer and Kozlowski 1979), perhaps by inducing the accumulation of soluble carbohydrates. In the northern ecotype, ANTHO values were noticeably larger 10 to 15 days after the onset of the 9-h photoperiod (Fig. 2E), but HTG did not plateau until day 16 or 17 (Fig. 2C). Therefore, it appears that anthocyanin accumulation and internode elongation are regulated somewhat independently.

Branching. – Sylleptic branches were particularly common on trees of the southern ecotype. Sylleptic branches are those branches that elongate from axillary buds that have not experienced winter dormancy or a prolonged period of rest (Richards and Larson 1981). Because most of the clones produced few branches, only one of the southern clones could be used to evaluate the effect of photoperiod on branching. For this clone (CA-2), branching was significantly less under SDs than under LDs (i.e. NWB was greater; Fig. 1F). Although these results are based on relatively few trees, the same effect was observed in the growth chamber (see Results). These results suggest that axillary bud burst is inhibited prior to bud set, resulting in changes in branching habit under SDs. Under the 9-h photoperiod, for example, changes in branching were apparent 30 to 35 days after the onset of SDs, but the average DTB was 43 (Fig. 2F). Although there is an apparent increase in apical dominance under SDs, the reduction in branching may not result from changes in the activity of the shoot apex, but may involve physiological changes in the axillary buds themselves, or in some other organ such as the leaves.

Differences between the SD and LD treatments were largely due to differences in photoperiod

Four lines of evidence suggest that the effects of the treatments resulted primarily from differences in photoperiod per se. First, because low levels of light were used to extend the photoperiod, differences in PAR between the photoperiodic treatments were small. Second, the photoperiod extensions were given at the beginning rather than the end of the day to minimize nonphotoperiodic effects of the treatments (Downs and Hellmers 1975). Third, we observed a typical LD response for each trait when the northern trees were grown under SDs, but were given a 30 min R night break in the middle of the night (data not shown). Finally, when the southern ecotype was grown under two contrasting photoperiods that were not extended with incandescent light (i.e. the 9-h photoperiod used in the bud scale initiation experiment and the 13-h photoperiod used for the greenhouse vs growth chamber comparison), the trees exhibited contrasting SD and LD responses (13 h is greater than the critical photoperiod for the southern ecotype). These last two observations demonstrate that differences in the duration of the photoperiod extension itself (i.e. differences in the duration of incandescent light) are not responsible for the contrasting SD and LD responses that we observed.

The response to photoperiod differs between the northern and southern ecotypes

Based on the data from the greenhouse experiment, the northern and southern ecotypes appear to have different photoperiodic response curves (Fig. 1). In general, photoperiodic response curves could differ in at least three

basic parameters: critical photoperiod, photoperiodic sensitivity and magnitude of the photoperiodic response. Critical photoperiod is defined as the longest photoperiod that elicits a SD response (Downs and Borthwick 1956), and ecotypic differences in critical photoperiod for either bud set or height growth have been well documented (Downs and Borthwick 1956, Vaartaja 1959, Junttila 1980, Downs and Bevington 1981). We found similar differences in critical photoperiod between the northern and southern ecotypes of black cottonwood. In addition, the northern and southern ecotypes appear to have different photoperiodic sensitivities, where sensitivity is defined as the change in response per unit change in photoperiod. The term "photoperiodic sensitivity" has been used previously (Downs and Borthwick 1956, Vaartaja 1959), but has rarely been defined (Vaartaja 1959). Ecotypes may also differ in the magnitude of their photoperiodic response, where magnitude is defined as the difference between the maximum and minimum responses observed over a full range of photoperiodic treatments. Vaartaja's use of the term "photoperiodic sensitivity" refers essentially to this parameter (Vaartaja 1959). We did not estimate the magnitude of the photoperiodic response for black cottonwood because it is unlikely that we tested sufficiently short photoperiods for the southern ecotype (Fig. 1B).

In general, the northern ecotype had a longer critical photoperiod and greater photoperiodic sensitivity than did the southern ecotype (Tab. 3). Ecotypic differences in photoperiodic sensitivity indicate that differences in the response curves cannot be completely described by the critical photoperiod alone (i.e. the response curves for the southern ecotype did not resemble "northern" curves shifted to shorter critical photoperiods). By comparing the photoperiodic response curves of the northern and southern ecotypes, it is clear that the relatively late bud set of the southern ecotype (discussed above) and the low photoperiodic sensitivity are two manifestations of the same phenomenon.

The performance of the middle-latitude clone was consistent with the hypothesis that the differences between the northern and southern clones were related to latitude of origin. For the middle-latitude clone, the critical photoperiod for bud set was 13 h (data not shown), compared to 11 h for the southern ecotype and 15 h for the northern ecotype. The middle-latitude clone also exhibited an intermediate response for DTB, NL, HTG and ANTHO when the responses of the three ecotypes were compared under the 9-h photoperiod.

The photoperiodic response was the same in the greenhouse and growth chamber

The response to photoperiod may not be the same in trees grown in different environments. Critical photoperiod and bud morphology, for example, differed between trees grown in the greenhouse and growth chamber (Junttila 1980, Goffinet and Larson 1981). In our experiments,

however, the response to photoperiod was essentially the same when the trees were tested in the greenhouse and growth chamber environments (cf. Figs 1A,B vs 4A; 1D vs 4B; 1E vs 4C; 1F vs 4D).

Acknowledgments. — We thank Michael Carlson, British Columbia Forest Service, Vernon, BC; Thomas Hayduk, Rancho Santa Ana Botanic Garden, Claremont, CA; Paul Heilman, Washington State Univ., Puyallup, WA; and Ellen Zagory, Univ. of California Arboretum, Davis, CA for providing plant material.

References

- Campbell, R. K. 1986. Mapped genetic variation of Douglas-fir to guide seed transfer in southwest Oregon. — *Silvae Genet.* 35: 85–96.
- & Sorensen, F. C. 1973. Cold-acclimation in seedling Douglas-fir related to phenology and provenance. — *Ecology* 54: 1148–1151.
- & Sugano, A. I. 1987. Seed zones and breeding zones for sugar pine in southwestern Oregon. — *Res. Pap. PNW-RP-383*, 18 pp. US Dept of Agric., Forest Serv., Pacific Northwest Research Station, Portland, OR.
- Coleman, G. D., Chen, T. H. H., Ernst, S. G. & Fuchigami, L. 1991. Photoperiod control of poplar bark storage protein accumulation. — *Plant Physiol.* 96: 686–692.
- Crutchfield, W. B. 1960. Leaf dimorphism in *Populus trichocarpa*. — *Am. J. Bot.* 47: 699–711.
- Dietrichson, J. 1964. The selection problem and growth-rhythm. — *Silvae Genet.* 13: 178–184.
- Downs, R. J. & Bevington, J. M. 1981. Effect of temperature and photoperiod on growth and dormancy of *Betula papyrifera*. — *Am. J. Bot.* 68: 795–800.
- & Borthwick, H. A. 1956. Effects of photoperiod on growth of trees. — *Bot. Gaz.* 117: 310–326.
- & Hellmers, H. 1975. Environment and the Experimental Control of Plant Growth. — Academic Press, New York, NY, pp. 31–82. ISBN 0-12-221450-1.
- Farmer, R. E. Jr. 1993. Latitudinal variation in height and phenology of balsam poplar. — *Silvae Genet.* 42: 148–153.
- Fuchigami, L. H., Evert, D. R. & Weiser, C. J. 1971a. A translocatable cold hardiness promoter. — *Plant Physiol.* 47: 164–167.
- , Weiser, C. J. & Evert, D. R. 1971b. Induction of cold acclimation in *Cornus stolonifera* Michx. — *Plant Physiol.* 47: 98–103.
- Garner, W. W. & Allard, H. A. 1923. Further studies in photoperiodism, the response of the plant to relative length of day and night. — *J. Agric. Res.* 28: 871–920.
- Goffinet, M. C. & Larson, P. R. 1981. Structural changes in *Populus deltoides* terminal buds and in the vascular transition zone of the stems during dormancy induction. — *Am. J. Bot.* 68: 118–129.
- & Larson, P. R. 1982. Lamina abortion in terminal bud-scale leaves of *Populus deltoides* during dormancy induction. — *Bot. Gaz.* 143: 331–340.
- Häbjörn, A. 1972. Effects of photoperiod and temperature on growth and development of three latitudinal and three altitudinal populations of *Betula pubescens* Ehrh. — *Meld Nor. Landbruksforsk.* 51: 1–27.
- 1978. Photoperiodic ecotypes in scandinavian trees and shrubs. — *Meld. Nor. Landbruksforsk.* 57: 1–20.
- Heide, O. M. 1974. Growth and dormancy in Norway spruce ecotypes (*Picea abies*). I. Interaction of photoperiod and temperature. — *Physiol. Plant.* 30: 1–12.
- Ishikura, N. 1976. Seasonal changes in contents of phenolic compounds and sugar in *Rhus*, *Euonymus* and *Acer* leaves with special reference to anthocyanin formation in autumn. — *Bot. Mag. Tokyo* 89: 251–257.
- Junttila, O. 1976. Apical growth cessation and shoot tip abscission in *Salix*. — *Physiol. Plant.* 38: 278–286.
- 1980. Effect of photoperiod and temperature on apical growth cessation in two ecotypes of *Salix* and *Betula*. — *Physiol. Plant.* 48: 347–352.
- 1982. The cessation of apical growth in latitudinal ecotypes and ecotype crosses of *Salix pentandra* L. — *J. Exp. Bot.* 33: 1021–1029.
- & Kaurin, Å. 1985. Climatic control of apical growth cessation in latitudinal ecotypes of *Salix pentandra* L. — In *Plant Production in the North* (A. Kaurin, O. Junttila and J. Nilsen, eds), pp. 83–91. Norwegian University Press, Tromsø. ISBN 82-00-07395-8.
- & Nilsen, J. 1993. Growth and development of northern forest trees as affected by temperature and light. — In *Forest Development in Cold Climates* (J. Alden, J. L. Mastrandio and S. Odum, eds), pp. 43–55. Plenum Press, New York, NY. ISBN 0-306-44480-1.
- Kramer, P. J. & Kozlowski, T. T. 1979. Physiology of Woody Plants. — Academic Press, New York, NY, pp. 277–280. ISBN 0-12-425050-5.
- Kuser, J. E. & Ching, K. K. 1980. Provenance variation in phenology and cold hardiness of western hemlock seedlings. — *For. Sci.* 26: 463–470.
- Larson, P. R. & Isebrands, J. G. 1971. The plastochron index as applied to developmental studies of cottonwood. — *Can. J. For. Res.* 1: 1–11.
- Li, P. & Adams, W. T. 1993. Genetic control of bud phenology in pole-size trees and seedlings of coastal Douglas-fir. — *Can. J. For. Res.* 23: 1043–1051.
- , Beaulieu, J., Corriveau, A. & Bousquet, J. 1993. Genetic variation in juvenile growth and phenology in a white spruce provenance-progeny test. — *Silvae Genet.* 42: 52–60.
- Mikola, J. 1982. Bud-set phenology as an indicator of climatic adaptation of Scots pine in Finland. — *Silva Fenn.* 16: 178–184.
- Murray, J. R. & Hackett, W. P. 1991. Dihydroflavonol reductase activity in relation to differential anthocyanin accumulation in juvenile and mature phase *Hedera helix* L. — *Plant Physiol.* 97: 343–351.
- Nitsch, J. P. 1957. Photoperiodism in woody plants. — *Proc. Am. Soc. Hortic. Sci.* 70: 526–544.
- Noorden, L. D. & Weber, J. A. 1978. Environmental and hormonal control of dormancy in terminal buds of plants. — In *Dormancy and Developmental Arrest* (M. E. Clutter, ed.), pp. 221–268. Academic Press, New York, NY. ISBN 0-12-177050-8.
- Oleksyn, J., Tjoelker, M. G. & Reich, P. B. 1992. Growth and biomass partitioning of populations of European *Pinus sylvestris* L. under simulated 50° and 60°N daylengths: evidence for photoperiodic ecotypes. — *New Phytol.* 120: 561–574.
- Pauley, S. S. & Perry, T. O. 1954. Ecotypic variation of the photoperiodic response in *Populus*. — *J. Arnold Arbor.* 35: 167–188.
- Rehfeldt, G. E. 1989. Genetic variances and covariances in freezing tolerance of lodgepole pine during early acclimation. — *Silvae Genet.* 38: 133–137.
- 1992a. Breeding strategies for *Larix occidentalis*: adaptations to the biotic and abiotic environment in relation to improving growth. — *Can. J. For. Res.* 22: 5–13.
- 1992b. Early selection in *Pinus ponderosa*: compromises between growth potential and growth rhythm in developing breeding strategies. — *For. Sci.* 38: 661–677.
- Richards, J. H. & Larson, P. R. 1981. Morphology and development of *Populus deltoides* branches in different environments. — *Bot. Gaz.* 142: 382–393.
- Riemenschneider, D. E., McMahon, B. G. & Ostry, M. E. 1992. Use of selection indices to increase tree height and to control damaging agents in 2-year-old balsam poplar. — *Can. J. For. Res.* 22: 561–567.
- SAS Institute, Inc. 1985. SAS User's Guide: Statistics, Version 5 Ed. — SAS Institute, Inc., Cary, NC. 956 pp. ISBN 0-917382-66-8.

- Tsukaya, H., Ohshima, O., Naito, S., Chino, M. & Komeda, Y. 1991. Sugar-dependent expression of the CHS-A gene for chalcone synthase from petunia in transgenic *Arabidopsis*. – Plant Physiol. 97: 1414–1421.
- Vaartaja, O. 1959. Evidence of photoperiodic ecotypes in trees. – Ecol. Monogr. 29: 91–111.
- Vince-Prue, D. 1975. Photoperiodism in Plants. – McGraw-Hill, London, pp. 333–383, ISBN 0-07-084048-2.
- 1984. Contrasting types of photoperiodic response in the control of dormancy. – Plant Cell Environ. 7: 507–513.
- 1985. Photoperiod and hormones. – In Encyclopedia of Plant Physiology, New series, Vol. 11. Hormonal Regulation of Development: Role of Environmental Factors (R. P. Pharis, D. M. Reid and F. D. Beall, eds), pp. 308–364. Springer-Verlag, New York, NY. ISBN 0-387-10197-7.

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