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Quantitative genetics of bud phenology, frost damage, and winter survival in an F_2 family of hybrid poplars

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Abstract We studied the quantitative genetics of bud phenology, fall frost damage, and winter survival in an F_2 family (no. 822) of *Populus* hybrids derived from a cross between two full-sub F_1 hybrids (*P. trichocarpa* (Torr. & Gray×*P. deltoides* Bartr.). Field traits studied included the timing of bud set (BS_F) in Minnesota and Oregon, the timing of bud flush (BF_F) in Oregon, as well as fall frost damage (FD_F) and winter survival (WS_F) in Minnesota. We conclude that Family 822 has substantial genetic variability for all field traits, BS_F and BF_F are under moderate to strong genetic control (H^2_i=0.48–0.80), FD_F and WS_F are under low to moderate genetic control (H^2_i=0.27–0.40), and late bud set is associated with increased frost damage and decreased winter survival. In a warm greenhouse, we measured the timing of bud set and the number of new leaves on trees growing under either an 8-h photoperiod (BS_{SD} and NL_{SD}) or a natural photoperiod (NP) from August to December (BS_{NP} and NL_{NP}). We found that BS_{SD}, NL_{SD}, and NL_{NP} are under moderate genetic control (H^2_i=0.53–0.70), but the heritability of BS_{NP} could not be determined because few trees set bud in the warm greenhouse under the NP. By comparing results from the greenhouse experiments with results from the field, we conclude that the genetic correlation between BS_{SD} and BS_F (0.53–0.60) is relatively modest and that NPs in the fall are relatively ineffective at promoting bud set under warm greenhouse temperatures, although bud set readily occurred in the field. Although, low levels of light pollution in the greenhouse might have affected BS_{NP}, results from both greenhouse and field experiments suggest that genetic differences in photoperiodic responses play a modest role in explaining genetic differences in the timing of bud set under natural field conditions. Therefore, genetic differences in responses to other environmental factors, such as temperature, deserve greater attention.

Key words Bud flush · Bud set · Frost hardiness · Photoperiod · Short day

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Introduction

Dormancy, in general, is defined as the temporary suspension of visible growth of any plant structure containing a meristem (Lang 1987). Endodormancy, which develops in the fall, is characterized by a requirement for sustained exposure to low, near-freezing temperatures before active shoot growth can resume in the spring. Because plants are more resistant to cold and other stresses when they are endodormant (Bigras 1996), endodormancy is an important adaptive strategy for perennial plants native to areas with cold winter temperatures. The cessation of shoot elongation is a prerequisite for the development of both bud endodormancy and substantial cold hardiness (Junttila 1989; Bigras 1996). Because bud set is temporally associated with the cessation of shoot elongation in the fall, it may be a useful marker for the impending initiation of cold acclimation and endodormancy. Conversely, because bud flush marks the initiation of active shoot elongation in the spring, it may be a useful indicator that endodormancy release and substantial cold deacclimation have already occurred.

In support of these conclusions, two lines of evidence suggest that bud set and bud flush are important adaptive traits in natural populations. First, there is ample evidence that the timings of bud set and bud flush in common garden tests are associated with the length of the growing

season at the genotype's site of origin. Genotypes from northern areas and high elevations, which generally have shorter growing seasons, tend to stop growing and set bud earlier in the fall (Pauley and Perry 1954; Smithberg and Weiser 1968; Campbell and Sorensen 1973; Kuser and Ching 1980; Ernst and Fechner 1981; Skrøppa and Magnussen 1993). Although the relationships for spring bud flush are more complex, genotypes from colder climates, e.g., northern, high elevation, and continental climates, typically flush earlier in common garden tests (although the opposite may be true in southern test plantations with mild winters) (Kriebel and Wang 1962; Worrall and Mergen 1967; Kuser and Ching 1980; Worrall 1983; Brissette and Barnes 1984). These observations suggest that patterns of genetic variation for bud set and bud flush have been molded by natural selection.

Second, selective forces that can explain these patterns of genetic variation have been demonstrated. Genotypes that stop growing and set bud late in the season are prone to frost damage (Campbell and Sorensen 1973; Kraus and Lines 1976; Kuser and Ching 1980; Junttila and Kaurin 1990), but genotypes that set bud too early tend to be shorter, which could put them at a competitive disadvantage (Rehfeldt 1992a, b; Riemenschneider et al. 1992, 1994; Li and Adams 1993, Li et al. 1993). Therefore, the timing of bud set is tied to the local climatic cycle, presumably because this adaptation limits cold injury and increases growth and fitness.

The timing of bud flush is also important – early flushing genotypes are prone to damage from late spring frosts (Nienstaedt and King 1970; Aitken and Adams 1995; Schermann et al. 1997). The later flushing habit of southern and maritime genotypes, for example, seems to be an adaptation to climates with mild winters and long springs – climates that have warm spells, which would promote bud flush, followed by damaging frosts (Campbell and Sugano 1979). Conversely, early bud flush (i.e., the ability to flush when temperatures are cooler) seems to be an adaptation to colder climates where short growing seasons limit growth and development and where late spring frosts are relatively uncommon (Campbell and Sugano 1979; Worrall 1983; Farmer and Reinholt 1986). More detailed discussions of the complex relationships between spring bud flush and the local environment, as well as the influence of test environments, are available (Campbell and Sugano 1979; Worrall 1983; Farmer and Reinholdt 1986).

In seasonally indeterminate species such as poplars, short days (SDs) are typically viewed as the primary factor influencing the timing of bud set (Junttila 1989; Howe et al. 1995; Jeknić and Chen 1999), although low temperatures are also involved (Håbjørg 1972a, b; Downs and Bevington 1981). Because other factors, such as soil moisture and nutrients, also affect bud set, the timing of bud set under field conditions is influenced by a multiplicity of factors and their interactions. In contrast to bud set, the timing of bud flush is primarily controlled by temperature (Junttila 1989). Although secondary environmental factors such as daylength can influence the timing of bud flush, the relative impacts of these secondary factors seem to be less than they are for bud set. For example, daylength may influence bud flush only in the very unusual cases when winter chilling has been insufficient (Chandler and Thielges 1973; Worrall 1983).

Species of *Populus* are good models for studying bud phenology and cold hardiness in trees because they contain ecotypes with contrasting phenological characteristics (Howe et al. 1995; Pauley and Perry 1954), can be clonally replicated via root or stem cuttings, are fast growing and economically important, and because they have seasonally indeterminate growth. That is, they will grow continuously under long days (LDs) but will stop growing, form a terminal bud, and become endodormant under SDs (Howe et al. 1995; Jian et al. 1997; Jeknić and Chen 1999). In addition, interspecific hybridization can be used to produce pedigrees that are desirable for mapping quantitative trait loci (Bradshaw and Stettler 1995; Newcombe and Bradshaw 1996).

Using an F_2 family of poplar hybrids, we sought to (1) shed light on the genetic and physiological relationships among bud phenology, fall frost damage, and winter survival, (2) determine whether genetic differences in photoperiodic responses in controlled environments are closely tied to genetic differences in bud set under field conditions, and (3) assess the genetic control and variability of bud phenology, fall frost damage, and winter survival as a prelude to analyses of quantitative trait loci (QTLs).

Materials and methods

Pedigree

We studied an F_2 family (no. 822) of *Populus* hybrids derived from a cross between two full-sib F_1 hybrids (*P. trichocarpa* Torr. & Gray ×*P. deltoides* Bartr.). The pedigree's black cottonwood female parent (no. 93–968) originates from western Washington (48°N), whereas the pedigree's eastern cottonwood male parent (no. S7C4) originates from Texas (31°N). F_2 progeny were clonally replicated and evaluated in the field and in controlled environments. The number of F_2 clones analyzed ranged from 301 to 359 (Table 1).

Field experiments

Plantations were established in St. Paul, Minnesota (44°59′N, 93°10′W) in the springs of 1996 and 1997, and in Corvallis, Oregon (44°34′N, 123°16′W), in the spring of 1997. In Minnesota, the trees from the SD experiment (described below) were pruned to a single stem, grown under a 21-h photoperiod in the greenhouse, acclimated outdoors under shade, then transplanted to a cultivated agricultural field on 8–10 June, 1996. A second plantation was established at the same location on 3 May 1997, using 20-cm unrooted cuttings from a clone bank in Corvallis. Each Minnesota plantation was established as a randomized complete block design with single-tree plots and two replications. The spacing was 1.5 m within rows, and either 2.1 or 2.3 m between rows. The trees were periodically watered for 1 month after establishment.

The Oregon plantation was established in May of 1997 on a cultivated agricultural field using 20-cm unrooted cuttings. This plantation was established at a spacing of 1.5×2.6 m using a ran-

Table 1 Statistics for F_2 hybrid poplar clones measured in the field or in the greenhouse. H^2_i and H^2_c are the individual-tree and clone mean heritabilities, respectively

Environment	Trait	Number of clones[a]	Mean	Range of clone means	H^2_i	H^2_c
Field						
St. Paul, Minn.	Bud set (BS_F)	349	284.5[b]	244–324[b]	0.48	0.75
	Frost damage (FD_F)	301	3.0[c]	–0.8–9.5[c]	0.27	0.52
	Winter survival (WS_F)	349	55.3[d]	–13–116[d]	0.40	0.68
Corvallis, Ore.	Bud set (BS_F)	348	267.6[b]	228–329	0.72	0.91
	Bud flush (BF_F)	356	78.1[b]	63–103[b]	0.80	0.94
Minn. and Ore.	Bud set (BS_F)	340	276.3[b]	239–327[b]	0.51	0.81
Greenhouse						
SD photoperiod	Bud set (BS_{SD})	359	32.1[e]	16–67[e]	0.53	0.80
	New leaves (NL_{SD})	359	9.3[f]	3.1–28.6[f]	0.63	0.86
Natural photoperiod	Bud set (BS_{NP})	321	–[g]	24–?[g]	–[g]	–[g]
	New leaves (NL_{NP})	321	49.4[h]	2.9–90.9[h]	0.70	0.83

[a] Number of clones for which least-square means could be estimated
[b] Days from December 31
[c] Fall frost damage score (0–10). Negative scores result from the calculation of least-square clone means
[d] Winter survival (%). Values that do not fall between 0 and 100% result from calculation of least-square clone means
[e] Days to bud set after the imposition of an 8-h photoperiod
[f] Number of new leaves produced within 60 days after the imposition of an 8-h photoperiod
[g] These values could not be determined because too few trees set bud
[h] Number of new leaves produced from the middle of August until the middle of December

domized complete block design with single-tree plots and four replications. The field was fertilized once with 50 kg·ha^{-1} of N (urea) and irrigated by overhead sprinklers every 2 weeks for 4 h. In all plantations, weeds were controlled by either pre- or post-emergent herbicides, mowing, or hand weeding.

Bud set was measured every 3 to 11 days beginning in the first week of August. Measurements ceased on 9 November in St. Paul and on 25 November in Oregon. 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. Bud set was recorded if all healthy apices on the tree had a terminal bud, and the number of days to bud set from 31 December (BS_F) was determined. A relatively small number of trees set bud, then resumed growth. For these trees, the first date of bud set was used in statistical analyses. In the Minnesota plantations, some trees were damaged or killed by frost near the end of the growing season, before bud set had occurred. For these trees, the date of the killing frost was used as the date of bud set for calculating BS_F. Therefore, the number of days to bud set for trees damaged by frost may be biased slightly downward.

In Minnesota, frost damage was measured for 5 successive weeks at the end of the growing season, and a frost damage score (FD_F) was developed which incorporated information on both the timing and severity of frost damage. A score of 10, 9, 8, 7, or 6 indicates that the aerial part of the tree was apparently killed by frost on measurement week 1, 2, 3, 4, or 5, respectively. A score of 5, 4, 3, 2, or 1 indicates that the stem was initially damaged by frost on week 1, 2, 3, 4, or 5, respectively. A score of 0 indicates that no damage to the stems or buds was observed.

Percent winter survival (WS_F) was measured in the Minnesota plantations the spring after establishment. On an individual-tree basis, values are 0 or 100%.

The timing of bud flush was measured in Oregon the spring after establishment. Bud flush was recorded when the first fully unfolded leaf was observed, and the number of days to bud flush from 31 December (BF_F) was determined.

Short-day (SD) experiment in the greenhouse

Hardwood stem cuttings (20 cm) were collected from trees growing in Corvallis, Oregon, shipped to St. Paul, Minnesota, then stored at 4°C. In late January and early February of 1995 and 1996, the cuttings were dipped briefly in a solution of 1000 ppm indole-3-butyric acid and rooted in a 1:1 (V:V) mixture of perlite and vermiculite under mist. Except for the SD treatment described below, the plants were grown under a 21-h photoperiod by extending the natural photoperiod (NP) with incandescent light. The rooted cuttings were potted in Strong-Lite Universal Mix in 13.3×13.3×15.9-cm plastic pots, then fertilized weekly with 20-10-20 fertilizer and treated with insecticides as needed.

To achieve uniform plant material, we grouped the trees in each block (greenhouse bench) into two size classes. The trees in the large-size class were placed under SDs about 1 week earlier than trees in the short-size class. In 1996, the trees in Block 1 were placed under an 8-h photoperiod on either 25 February or 3 March. The trees in Block 2 were placed under a separate SD treatment on either 13 March or 22 March. In 1997, the SD treatments were initiated on either 5 April or 14 April, for Block 1, and 10 April or 19 April, for Block 2. In addition, the rooted cuttings were pruned to a single leader a few days prior to the initiation of each SD treatment. For each tree, the pruned leader was chosen so that the resulting plants had roughly the same height and number of visible leaves or nodes. When this approach was used, the trees had a relatively uniform height of 13 cm and 17 visible leaves or nodes when the SD treatments were initiated. The trees were covered with shade cloth prior to 16:30, and uncovered after 8:30. Precise control of the photoperiod was achieved using an electronic timer linked to 100-W incandescent lamps located beneath the shade cloth. Average daily minimum and maximum temperatures were 19.7° and 30.3°C.

After 2 weeks under SDs, but set was measured every 1–4 days as described above. For trees that had set bud, we counted the final number of leaves, then removed those trees from the experiment. After 60 SDs, the experiment was terminated, and the numbers of leaves on the remaining plants were counted. We calculated the number of new leaves (NL_{SD}) from the initial and final leaf numbers, and the number of days to bud set from the beginning of the SD treatment (BS_{SD}). For trees that had not set a bud during the experiment, a value of 65 was used for BS_{SD} (Howe et al. 1995).

Natural photoperiod (NP) experiment in the greenhouse

In the summer of 1996, rooted cuttings were prepared, and the resulting trees were pruned to a uniform size as described above.

Initial leaf counts were made between 12 and 13 August. On 13 August, the trees were placed under a NP by discontinuing the daylength extensions, and bud set was recorded every 2 to 8 days until the middle of December. The trees were shielded from sodium vapor lamps located outdoors and in nearby greenhouses by placing black plastic on one side of the greenhouse. At night, light levels in the greenhouse ranged between 0.01 and 0.05 μmol·s^{-1}·m^{-2} using a Li-Cor LI-189 light meter and LI-190SA quantum sensor (LI-COR, Lincoln, Ne.). Average daily minimum and maximum temperatures were 20.9° and 30.4°C. Because final leaf counts could not be made within a single day, the length of the experiment varied by block, ranging from 123 to 127 days. The number of new leaves (NL_{NP}) and number of days to bud set from 31 December (BS_{NP}) were calculated as described above.

Statistical analyses

Analyses of variance based on Type IV sums of squares were conducted using the GLM procedure of SAS (Statistical Analysis System version 6.12, SAS Institute, Cary, N.C.). Residuals were calculated, and the PLOT and NORMAL options of PROC UNIVARIATE were used to judge the normality of errors. Although the residual plots looked fairly normal, statistical tests indicated that the residuals deviated from normality for each trait ($P<0.01$), and data transformations did little to improve the situation. Therefore, untransformed data were used in all analyses, and the probabilities we report should be considered approximate. The COVTEST option of PROC MIXED was used to estimate variance components by restricted maximum likelihood (REML) treating *Clone*, and all interactions with *Clone*, as random effects. The remaining effects were considered fixed. For traits measured in either the Oregon plantation or the NP experiment, the statistical model was:

$$X_{ij}=\mu+B_i+C_j+\varepsilon_{ij} \quad (1)$$

where X_{ij} is the observation for the j^{th} clone in the i^{th} block, μ is the population mean, B_i is the effect of the i^{th} block, C_j is the effect of the j^{th} clone, and ε_{ij} is the experimental error. Within-family individual-tree heritabilities were estimated as:

$$H^2_i=\sigma^2_c/[\sigma^2_\varepsilon+\sigma^2_c] \quad (2)$$

where σ^2_c and σ^2_ε are the *Clone* within family and *Error* variance components, respectively. The clones we analyzed belonged to a single F_2 family produced by crossing two full-sib F_1 hybrids, therefore the genetic variance among clones is less than that among unrelated clones. Clone mean heritabilities were estimated as:

$$H^2_c=\sigma^2_c/[(\sigma^2_\varepsilon/b_c)+\sigma^2_c] \quad (3)$$

where b_c is the coefficient for σ^2_c from the *Clone* expected mean square according to the RANDOM option of PROC GLM.

Initial analyses indicated that the *Year*×*Clone* interactions were non-significant ($P>0.16$) for two of the Minnesota field traits (BS_F and WS_F), as well as for the traits measured in the SD experiment (BS_{SD} and NL_{SD}). Therefore, these interactions were pooled into the error terms, resulting in the following model:

$$X_{ijk}=\mu+Y_i+B_j(Y_i)+C_k+\varepsilon_{ijk} \quad (4)$$

where X_{ijk} is the observation for the k^{th} clone in the j^{th} block in the i^{th} year, μ is the population mean, Y_i is the effect of the i^{th} year, $B_j(Y_i)$ is the effect of the j^{th} block in the i^{th} year, C_k is the effect of the k^{th} clone, and ε_{ijk} is the experimental error. Individual-tree and clone mean heritabilities were estimated according to Eqs. 2 and 3.

Year and *Year*×*Clone* terms were omitted from the combined analysis of BS_F in Minnesota and Oregon because only a single year was included in the Oregon experiment. In addition, these terms were non-significant in the analysis of BS_F in Minnesota ($P>0.35$). Therefore, the following model was used for the analysis of BS_F in Minnesota and Oregon:

$$X_{ijk}=\mu+S_i+B_j(S_i)+C_k+S_iC_k+\varepsilon_{ijk} \quad (5)$$

where X_{ijk} is the observation for the k^{th} clone in the j^{th} block in the i^{th} state, μ is the population mean, S_i is the effect of the i^{th} state, $B_j(S_i)$ is the effect of the j^{th} block in the i^{th} state, C_k is the effect of the k^{th} clone, S_iC_k is the interaction of the i^{th} state and k^{th} clone, and ε_{ijk} is the experimental error. Within-family individual-tree heritabilities were estimated as:

$$H^2_i=\sigma^2_c/[\sigma^2_\varepsilon+\sigma^2_{sc}+\sigma^2_c] \quad (6)$$

where σ^2_{sc} is the *State*×*Clone* variance component, and σ^2_c and σ^2_ε are as described above. Clone mean heritabilities were estimated as:

$$H^2_c=\sigma^2_c/[(\sigma^2_\varepsilon+b_{sc}\sigma^2_{sc})/b_c+\sigma^2_c] \quad (7)$$

where b_c and b_{sc} are the coefficients for σ^2_c and σ^2_{sc} from the *Clone* expected mean square.

Because the *Year*×*Clone* interaction was significant for FD_F in Minnesota ($P<0.0003$), the models and heritability equations for FD_F are the same as those described in Eqs. 5–7 except that the effects of *Year* (Y_i), *Block within Year* ($B_j(Y_i)$), and *Year*×*Clone* (Y_iC_k) replace those of *State* (S_i), *Block within State* ($B_j(S_i)$), and *State*×*Clone* (S_iC_k). BS_{NP} was not analyzed because too few trees set bud.

Genetic correlations (r_g and r^*_g) were calculated using two methods. For correlations between traits measured on different trees (e.g., BS_F in Minnesota vs BS_F in Oregon), genetic correlations were calculated according to Burdon (1977):

$$r_g=r_p/[H_{c(x)}\cdot H_{c(y)}] \quad (8)$$

where r_p is the phenotypic correlation between clone means for trait *x* and trait *y*, and $H_{c(x)}$ and $H_{c(y)}$ are the square-roots of the heritabilities of clone means. For traits measured on the same trees (e.g. BS_F in Minnesota vs FD_F in Minnesota), genetic correlations were estimated as:

$$r^*_g=\sigma_{c(xy)}/[\sigma^2_{c(x)}\cdot\sigma^2_{c(y)}]^{\frac{1}{2}} \quad (9)$$

where $\sigma^2_{c(x)}$ and $\sigma^2_{c(y)}$ are the *Clone* variance components for traits *x* and *y*, and $\sigma_{c(xy)}$ is the corresponding covariance component, calculated according to the formula $[(\sigma^2_{c(x+y)}-\sigma^2_{c(x)}-\sigma^2_{c(y)})/2]$.

Results and discussion

Bud phenology

In the field, bud set (BS_F) and bud flush (BF_F) were under moderate to strong genetic control (Table 1). Based on a combined analysis of trees growing in Minnesota and Oregon, the individual-tree heritability of BS_F was 0.51. This heritability reflects the presence of a modest *Clone*×*State* interaction ($F=2.13$; $P<0.0001$), probably due to the contrasting climatic conditions in Minnesota and Oregon. The corresponding heritabilities for BS_F within Minnesota and Oregon were 0.48 and 0.72, respectively. In Oregon, the individual-tree heritability of BF_F was 0.80, which was higher than the heritability of any other trait measured in either the field or greenhouse. We did not analyze BF_F in Minnesota because many of the trees did not survive the harsh winters (Fig. 1A, B).

The heritabilities we report are individual-tree heritabilities applicable to our F_2 inbred population. Because we analyzed a single F_2 family, the variance component for clone (σ^2_c) estimates only a portion of the genetic variance that is expected to be found within and among F_2 families derived from our two original parents. On the other hand, because our clones were derived from inter-

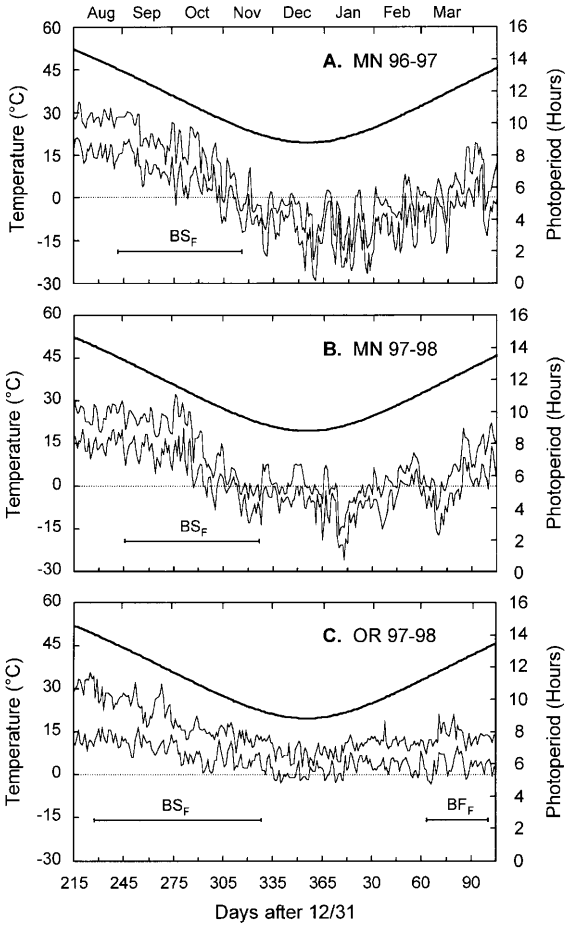

Fig. 1A–C Photoperiod (top line) and daily temperature extremes (bottom two lines) in St. Paul, Minnesota in 1996–1998 (**A**, **B**) and in Corvallis, Oregon in 1997–1998 (**C**) during periods of time when bud set (BS_F) and bud flush (BF_F) were measured on an F_2 family of hybrid poplars growing in the field. *Horizontal bars* indicate the range of F_2 clone means for each trait

specific hybridization, genetic variances and heritabilities should be greater than they would be for non-hybrid material. Despite these differences, our heritabilities are consistent with those reported for trees collected from natural populations. Broad-sense heritabilities for bud set were 0.71 and 0.81, for example, for populations of black cottonwood trees collected from northern Idaho and British Columbia (Riemenschneider et al. 1994). In this experiment, heritabilities for bud set were higher than those of any other trait, except for traits related to the severity and extent of *Melampsora* leaf rust (Riemenschneider et al. 1994). In a population of black cottonwood trees collected from the Pacific Northwest, the heritability of bud set was 0.62 (Weber et al. 1985), and in a population of balsam poplars collected from Minnesota, Wisconsin, and Michigan, the heritability was 0.65, higher than the heritability of any other trait (Riemenschneider et al. 1992). In a number of coniferous tree species, narrow-sense heritabilities for bud set and growth cessation were moderate to high (Rehfeldt 1992a, b; Li and Adams 1993), although low heritabilities were observed for some species and age classes (Li and Adams 1993; Li et al. 1993).

Bradshaw and Stettler (1995) reported that bud flush was under strong genetic control in an F_2 family of hybrid poplars that is closely related to the family we studied. Based on heritabilities of clonal means, bud flush had the highest heritability of any trait. Heritabilities for bud flush have also been reported for trees collected from natural populations. In balsam poplar, for example, broad-sense heritabilities ranged from 0.21 to 0.47 on an individual-tree basis, indicating that bud flush was under moderate genetic control, at best (Farmer 1993). Other reports indicate that bud flush is under strong genetic control in *Populus* (Farmer 1970; Thomas et al. 1997; Dunlap and Stettler 1996) as well as other angiosperm and coniferous tree species (Worrall 1983; Bongarten and Hanover 1986; Mebrahtu and Hanover 1989; Aitken and Adams 1997).

Both BS_F and BF_F varied considerably among F_2 clones (Fig. 2A, B). Nonetheless, the difference in BS_F between the "earliest" and "latest" clones was more than twice the corresponding difference in BF_F (Fig. 1C). In Oregon, for example, mean bud set dates ranged from 16 August to 25 November (101 days), but mean bud flush dates ranged from 4 March to 13 April (40 days). Similar results have been observed in pure species of *Populus* (Ernst and Fechner 1981; Brissette and Barnes 1984; Farmer 1993; Dunlap and Stettler 1996). When trembling aspens were planted in Michigan, for example, growth cessation occurred as much as two months earlier in trees from Alaska than in trees from Michigan (Brissette and Barnes 1984). The Alaskan trees, which are normally exposed to very long days during the growing season (>18 h), set bud by the end of June, apparently in response to the shorter days found in Michigan (<15 h). In contrast, a few of the Michigan trees continued growing into September. Despite the large difference in the timing of bud set, the difference in bud flush between the Alaskan and Michigan trees was only about 2 weeks. Similar results were observed for narrowleaf cottonwood from Colorado – virtually no clonal differences were observed for bud flush, but there were large differences in the timing of bud set (Ernst and Fechner 1981).

Why is the genetic variability for bud set often much larger than it is for bud flush? Compared to bud flush, the environmental control of bud set is more site-specific and, thus, more genetically variable. This is because of the *indirect* relationship between the photoperiodic signals that promote bud set and the timing of the fall frosts. Trees from different latitudes necessarily experience different photoperiodic regimes, even if the temperatures are similar. Conversely, trees from different elevations can experience identical daylengths but dramatically different temperature regimes. The site-specificity of photoperiodic responses is clearly illustrated when trees are moved northward into a colder environment. Although earlier bud set would promote frost hardiness in the new environment, the longer daylengths in the north actually delay bud set, often leading to frost damage. In

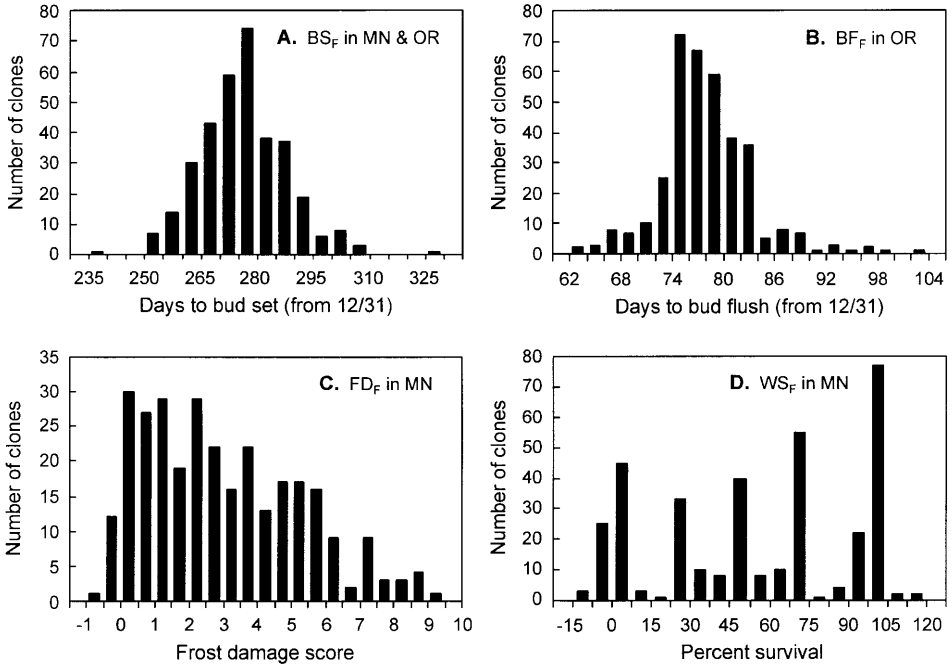

Fig 2A–D Distributions of least-square clone means for bud set (**A**), bud flush (**B**), frost damage (**C**), and winter survival (**D**) measured on an F_2 family of hybrid poplars growing in the field in St. Paul, Minnesota (*MN*) or Corvallis, Oregon (*OR*). Negative scores for frost damage (FD_F) as well as values for winter survival (WS_F) that do not fall between 0 and 100% result from calculation of least-square clone means

nature, strong selective pressures have lead to the evolution of different critical photoperiods in different populations. Although photoperiodic signals are fairly reliable for predicting the onset of freezing temperatures, specific critical photoperiods are adaptive in only a limited range of environments.

The environmental control of bud flush is considerably different. If the chilling requirement has been satisfied, the timing of bud flush is largely dependent on the accumulation of flushing temperatures (Campbell and Sugano 1979; Hänninen 1990). Because of the *direct* relationship between the temperature signals that promote bud flush and the local temperature regime (which determines the timing of spring frosts), a single type of bud flush response may be adaptive in a fairly wide range of environments. For example, if trees are moved into a colder environment where late spring frosts are common, there is a corresponding decrease in flushing temperature accumulation, and a compensating delay in bud flush that contributes to the avoidance of frost damage. Because a single flushing requirement may allow bud flush to coincide with the onset of favorable growing temperatures in a broader range of environments, genetic differences among populations are less. Nonetheless, geographically based patterns of genetic variation have been observed for bud flush. Trees from northern locations and high elevations tend to flush earlier than do southern genotypes in common garden tests, perhaps because they have been exposed to shorter frost-free seasons in their native environments, leading to selection for genotypes that begin growing very early in the spring (Burley 1966; Campbell and Sugano 1979; Worrall 1983; Farmer 1993).

Bud phenological traits are genetically variable and, at least, moderately heritable. In a number of experiments, the heritability of bud phenology was higher than that of any other measured trait (Riemenschneider et al. 1992; Bradshaw and Stettler 1995). The relatively high heritabilities for bud set and bud flush indicate that these traits are relatively unaffected by microenvironmental influences and exhibit little genotype×environmental interaction. High levels of genetic variation are maintained due to diversifying selection for phenological characteristics that promote adaptation to local climatic and photoperiodic regimes. Because the parents of our pedigree originated from widely different latitudes, it is likely that much of the variation in our F_2 population resulted from genetic differentiation of this type. Nonetheless, the presence of substantial within-population genetic variation is the rule for both bud set (Pauley and Perry 1954; Weber et al. 1985; Riemenschneider et al. 1992, 1994) and bud flush (Worrall and Mergen 1967; Bongarten and Hanover 1986; Farmer 1993; Thomas et al. 1997). This has been interpreted as evidence for adaptation to local environments that are extremely heterogeneous in time and space (Campbell 1979). Therefore, within-population genetic variability may have contributed to the phenotypic variability we saw in the F_2. In our experiment, this "within-population" variation would have arisen as heterozygous combinations of alleles within the two parents of the pedigree.

Fall frost damage and winter survival

Because the Minnesota plantations were damaged by fall frosts, we measured frost damage (FD_F) in Minnesota using a 0–10 score that incorporated information on both the timing and severity of damage. Although many trees were severely damaged or killed, other trees exhibited no

Table 2 Genetic correlations for F_2 hybrid poplar clones measured in the field or in the greenhouse

Environment	Trait	Field						Greenhouse	
		St. Paul, Minn.			Corvallis, Ore.		MN/OR	SD	
		BS_F	FD_F	WS_F	BS_F	BF_F	BS_F	BS_{SD}	NL_{SD}
Field									
St. Paul, Minn.	Bud set (BS_F)	–	–	–	–	–	–	–	–
	Frost damage (FD_F)	0.72	–	–	–	–	–	–	–
	Winter survival (WS_F)	–0.30	–0.70	–	–	–	–	–	–
Corvallis, Ore.	Bud set (BS_F)	0.81	0.57	–0.25	–	–	–	–	–
	Bud flush (BF_F)	–0.09	0.11	–0.04	–0.12	–	–	–	–
Greenhouse									
SD photoperiod	Bud set (BS_{SD})	0.59	0.23	–0.23	0.53	–0.21	0.60	–	–
	New leaves (NL_{SD})	0.51	0.26	–0.22	0.45	–0.21	0.51	0.93	–
Natural photoperiod	New leaves (NL_{NP})	0.44	0.20	–0.01	0.33	–0.23	0.37	0.46	0.59

frost damage. On a clone mean basis, FD_F ranged from –0.8 to 9.5 (Fig. 2C; calculation of least-square means resulted in negative numbers for some clones). The heritability of FD_F was moderately low (H^2_i=0.27), apparently due to large microenvironmental effects and a significant *Clone×Year* interaction (P<0.0003). No such *Clone×Year* interaction was observed for the other traits measured in Minnesota. The importance of microenvironmental influences is demonstrated by the large amount of variability observed within the 1997 plantation; the mean FD_F score for Block 2 was more than three times that of Block 1 (FD_F=4.5 vs. 1.2). The reason for this difference is unclear, although it was associated with a modest difference in BS_F. In Block 2, mean BS_F was 83.0 days compared to 68.2 days for Block 1. Moderately low heritabilities for fall cold hardiness have been reported for natural populations of Douglas-fir (Aitken and Adams 1996). Arithmetic means for winter survival (WS_F) ranged from 0 to 100%. Calculation of least-square means, however, resulted in values ranging from –13 to 116% (Fig. 2D).

Relationships among field traits

There was a strong negative genetic correlation between FD_F and WS_F (–0.70), suggesting that fall frost damage contributed substantially to the mortality that occurred during the fall and winter. Although we did not measure survival until the spring, the severe frost damage that we observed on many of the clones supports this conclusion. Nonetheless, frost susceptible genotypes may also be less able to acclimate to low midwinter temperatures. In dogwood, ecotypic differences in cold hardiness are primarily associated with differences in the timing of cold acclimation rather than differences in the ability to acclimate to low temperatures (Fuchigami et al. 1971). Cold acclimation began early in a frost-hardy genotype from North Dakota but was delayed in a less-hardy genotype from Washington, although both genotypes were able to acclimate to temperatures of –196°C. Our results suggest that differences in the timing of cold acclimation may be equally important in our population.

Trees that stop growing and set bud earlier tend to be more resistant to frost damage (Campbell and Sorensen 1973; Ying and Bagley 1976; Kuser and Ching 1980; Junttila and Kaurin 1990). In our experiment, the genetic correlations between BS_F and FD_F were 0.57 and 0.72 (Table 2), indicating that trees that grew later into the fall experienced greater frost damage. The weaker correlation (0.57) is a conservative estimate based on measurements of BS_F in Oregon and FD_F in Minnesota. This value may underestimate the true genetic correlation because BS_F was not measured in Minnesota. The stronger correlation (0.72) was derived from measurements made solely in the Minnesota plantation (i.e., BS_F and FD_F were measured on the same trees).

These genetic correlations support the conclusion that the timing of bud set is associated with adaptability. The weak negative genetic correlations between BS_F and WS_F (r_g=–0.25 and –0.30; Table 2) provide direct evidence that early bud set is associated with trees that are better able to survive the winter. The weaker correlation (–0.25) is a conservative estimate because it was derived from measurements of BS_F in Oregon and WS_F in Minnesota. The stronger correlation (–0.30) was derived from measurements made on the same trees in the Minnesota plantations. Because least-square means for WS_F fell into five fairly discrete classes, we also investigated the relationships among these traits by calculating FD_F and BS_F for each of these five classes (WS_F≤12%; 13–37%; 38–62%; 63–87%, and ≥88%). The clones with the highest survival had a mean FD_F of 1.3 compared to a FD_F of 5.0 for the clones with the lowest survival (P=0.05). The timing of bud set also differed among the classes. Compared to the clones with the greatest mortality, the clones with the highest survival set bud an average of 8.6 days earlier in Minnesota, and 7.6 days earlier in Oregon (P=0.05).

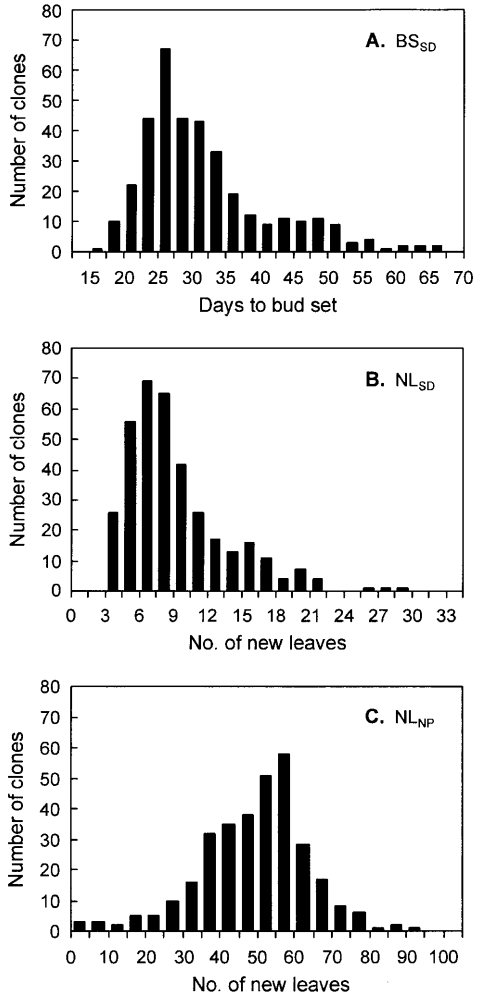

Fig. 3A–C Distributions of least-square clone means for bud set (**A**) and number of new leaves (**B**) for trees growing under an 8-h photoperiod (*SD*), and number of new leaves (**C**) for trees growing under a natural photoperiod (*NP*). All traits were measured on an F_2 family of hybrid poplars growing under warm temperatures in a greenhouse

The timing of bud set is often positively correlated with seasonal height growth. Genotypes that grow later into the fall tend to be taller (Rehfeldt 1992a, b; Riemenschneider et al. 1992, 1994; Li and Adams 1993). Because of the interrelationships discussed above, breeding for increased height growth may result in genotypes that set bud later and are more susceptible to fall frosts. We did not measure seasonal height growth because our trees were pruned during the growing season to remove leaders that were damaged by insects. Nonetheless, because positive genetic correlations between bud set and height grown have been reported for both *P. balsamifera* and *P. trichocarpa* (Riemenschneider et al. 1992, 1994), our results support the conclusion that breeding for increased height growth could have an adverse impact on frost hardiness and winter survival. In addition, our results indicate that bud set could be used as an indirect selection criterion for improving frost hardiness and winter survival even when bud set is measured in an environment that is substantially different from the environment in which frost hardiness is desired. As discussed above, the timing of bud set in Oregon was associated with both frost hardiness and winter survival in Minnesota, although for winter survival, this association was relatively weak.

In the Oregon plantation, there was a weak negative genetic correlation (–0.12) between BS_F and BF_F. Because the correlation between bud set and bud flush was stronger when bud set was measured in the greenhouse (Table 2), this relationship is discussed in greater detail below.

Bud set in the greenhouse

In photoperiodic species, the timing of bud set is regulated mainly by photoperiod and temperature. Low night temperatures, for example, tend to increase the critical daylength and promote SD-induced bud set (Håbjørg 1972a, b; Downs and Bevington 1981). Nonetheless, SDs are often viewed as the primary signal for bud set because SDs typically induce bud set under warm, non-inductive temperatures, while low night temperatures often fail to cause bud set under non-inductive LD photoperiods (but see Håbjørg 1972a). Because photoperiod and temperature probably regulate bud set through different sets of genes, it would be beneficial to study these processes independently. Therefore, we studied the independent effect of photoperiod by measuring SD-induced bud set under warm greenhouse conditions.

SD-induced bud set in the greenhouse (BS_{SD}) was highly variable among F_2 clones and under moderately strong genetic control (H^2_i=0.53). The earliest F_2 clone set bud 16 days after the start of the 8-h photoperiod, whereas other clones were still growing after 2 months (Fig. 3A). The number of new leaves (NL_{SD}) was also measured in the greenhouse (Fig. 3B). Because leaf production stops when bud set occurs, NL_{SD} is an alternative measure of bud set that is based on a developmental scale rather than a chronological scale (Howe et al. 1995). The heritability of NL_{SD} was 0.63 and the genetic correlation between NL_{SD} and BS_{SD} was 0.93, which demonstrates the close relationship between these traits.

Genetic correlations between BS_{SD} and BS_F were used to judge the relative impact of photoperiodic responses on the timing of bud set in the field. Surprisingly, these correlations were relatively modest. The genetic correlations between BS_{SD} and BS_F ranged from 0.53 to 0.60 (Table 2), indicating that BS_{SD} explained only about 28–36% of the genetic variation in BS_F. Slightly lower genetic correlations were observed between NL_{SD} and BS_F. These correlations ranged from 0.45 to 0.51 (Table 2).

What accounts for these modest genetic correlations? First, the trees in the field were much larger than the trees in the greenhouse when they were exposed to SDs. Although this could be a factor, the smaller greenhouse

trees were clearly able to respond rapidly to SDs – some trees set bud after only 16 days. Another difference is that the trees in the field were exposed to a gradually decreasing photoperiod, but the trees in the greenhouse were exposed to a constant 8-h photoperiod. Therefore, if the primary signal for bud set is a *decrease* in daylength, rather than SDs per se (Van Huystee et al. 1967; Greer et al. 1989), the genetic correlations between BS_{SD} and BS_F might be lower than expected. If this were the case, we should obtain stronger genetic correlations if the trees in the greenhouse and in the field are given the same photoperiodic regime. To test this hypothesis, we compared the growth of trees grown in a warm greenhouse under a natural photoperiod (NP) with trees grown in the field.

Although bud set occurred readily in the field, bud set occurred late, or not at all, when the trees were grown in the greenhouse under a NP. In the greenhouse, for example, only 29.5% of the trees had a terminal bud by the middle of December, but in the Minnesota plantations, 86.2% of the trees had a terminal bud when they were last measured in the beginning of November. The trees that had not set bud by this time had already been damaged or killed by frost, so their bud set dates could not be determined. In Oregon, which has a photoperiodic regime similar to that of Minnesota, all of the trees had a terminal bud by 25 November. These results suggest that NPs are relatively ineffective at inducing bud set under warm temperatures, but this needs to be evaluated further. During the night, light pollution from street lamps and other greenhouses was slightly higher in the greenhouse (PPFD=0.02–0.05 $\mu mol \cdot s^{-1} \cdot m^{-2}$) than it was in the field (undetectable). Although these light levels are very low, we cannot exclude the possibility that they inhibited bud set in the greenhouse to some degree.

Although few trees set bud in the greenhouse, the production of new leaves seemed to be inhibited by the NP. At the end of the experiment, NL_{NP} varied from 2.9 to 90.9 among the clones, with an individual-tree heritability of 0.70 (Fig. 3C). In addition, the genetic correlations between NL_{NP} and bud set (BS_F or BS_{SD}) ranged from 0.33 to 0.46 (Table 2), suggesting that NL_{NP} is a reasonable measure of the photoperiodic responses of these clones. Nonetheless, the genetic correlations between the greenhouse and field traits were not improved by growing the trees under a NP rather than under SDs. In fact, the correlations between NL_{NP} and BS_F (r_g=0.33–0.44) were lower than the correlations between NL_{SD} and BS_F (r_g=0.45–0.51). Thus, these results provide no evidence that the trees in the field mainly responded to a decreasing photoperiod rather than to SDs per se.

Considering both greenhouse experiments, factors other than daylength appear to be important for inducing bud set in the field and for eliciting differences among genotypes. What are these factors? One logical candidate is temperature. Low temperatures interact with photoperiod to induce bud set, and genotypes may respond differently to temperature treatments (Downs and Bevington 1981). Alternatively, other environmental differences may have been important instead. Unlike the trees in the field, the trees in the greenhouse were well-watered and fertilized weekly, and these conditions can inhibit bud set. Therefore, we hypothesize that (1) nonphotoperiodic environmental factors are important for inducing bud set under NPs (i.e., in the field), (2) our clones differed in their response to these factors, and (3) these conditions are responsible for the modest genetic correlations we observed between bud set in the greenhouse and bud set in the field. The influence of low temperatures, in particular, would be worth investigating in greater detail.

Genotypes that set bud late in the greenhouse had greater frost damage in the field (r_g=0.20–0.26 for FD_F vs. BS_{SD}, NL_{SD}, NL_{NP}). The relationship between delayed bud set and frost damage was stronger, however, when both bud set and frost damage were measured in the field (r_g=0.57–0.72 for FD_F vs. BS_F). Furthermore, genotypes with delayed bud set in the greenhouse had lower winter survival (r_g=–0.22 to –0.23 for WS_F vs. BS_{SD}, NL_{SD}). These results support the conclusion that late bud set is associated with increased frost damage and decreased winter survival.

Early bud set in the greenhouse was associated with late bud flush in the field, but this relationship was weak (r_g=–0.21 to –0.23 for BF_F vs. BS_{SD}, NL_{SD}, NL_{NP}). A similar trend was observed between bud set and bud flush in the field, but this relationship was even weaker (r_g=–0.09 to –0.12 for BF_F vs. BS_F). This negative correlation was surprising because early bud set is often associated with earlier, rather than later, bud flush. During seedling production, for example, SDs are used to induce early bud set, and these treatments can cause early bud flush the following spring (Odlum and Colombo 1988). The reason for this is unclear, although trees exposed to SDs might become endodormant earlier, fulfill their chilling requirement sooner and, thus, flush earlier in the spring. At the genetic level, the timing of bud set and bud flush are also positively correlated in a number of species (Eriksson et al. 1978; Rehfeldt 1992b; Schermann et al. 1997).

As discussed above, our correlations between bud set and bud flush were strongest when bud set was measured in the greenhouse, suggesting that this association was mostly related to photoperiodic responses rather than to the timing of bud set per se. One explanation for this effect is that our genotypes differed in their sensitivity to LDs. Genotypes that are particularly sensitive to LDs (i.e., those with short critical photoperiods) tend to set bud later in the fall. If these genotypes are also very sensitive to the effects of LDs on bud flush, they may flush earlier in the spring as well. LDs promote bud flush of foliated and defoliated poplar trees shortly after bud set (Goffinet and Larson 1982; Howe et al. 1999). LDs also promote bud flush of foliated and leafless trees during endodormancy release, particularly if the trees are insufficiently chilled (Olmsted 1951; Farmer 1968; Chandler and Thielges 1973; Worrall 1983). If daylength differentially affected the timing of bud flush in our F_2 family, this suggests that early bud flush was constrained by insufficient chilling, at least in some genotypes. This is possible because the winter temperatures in Corvallis are mild.

Practical significance

Our results have implications for designing breeding strategies for hybrid poplars and other trees. First, our results indicate that the genetic variability and heritabilities of bud phenology, fall frost hardiness, and winter survival are sufficiently high that these traits are amenable to genetic improvement. Second, the high genetic correlation between bud set in Minnesota and Oregon (0.81) indicates that it should be possible to breed genotypes that perform reasonably well in a range of environments, at least for this trait. Third, the moderate genetic correlations between bud set in the greenhouse (BS_{SD}, NL_{SD}, NL_{NP}) and bud set in the field (BS_F) indicate that it should be possible to screen many genotypes in controlled environments for appropriate bud set responses, then conduct expensive field tests using a much smaller number of desirable clones. Because the correlations between controlled environments and the field were only moderate, however, nursery tests will probably be more effective for making early selections for bud set. Fourth, because increased height growth is genetically associated with delayed bud set (Rehfeldt 1992a, b; Riemenschneider et al. 1992, 1994; Li and Adams 1993), our results support the conclusion that breeding for increased height growth could result in delayed bud set, increased frost damage, and decreased winter survival. Fifth, our results indicate that advanced generation breeding can be used to incorporate germplasm from mild climates into breeding programs designed for harsher climates. For example, we obtained F_2 genotypes that were cold-hardy in Minnesota from grandparents that originated from much milder climates in Washington and Texas. Therefore, it should be possible to incorporate desirable characteristics, such as disease resistance, from trees native to milder climates, while still maintaining adaptability of advanced generation materials. Finally, our results suggest that it will be possible to map quantitative trait loci for bud phenology, fall frost hardiness, and winter survival using this hybrid poplar pedigree.

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