

Genetic Variation of the Bud and Leaf Phenology of Seventeen Poplar Clones in a Short Rotation Coppice Culture

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Abstract: Leaf phenology of 17 poplar (*Populus* spp.) clones, encompassing spring phenology, length of growth period and end-of-year phenology, was examined over several years of different rotations. The 17 poplar clones differed in their latitude of origin (45°30'N to 51°N) and were studied on a short rotation experimental field plantation, situated in Boom (province of Antwerpen, Belgium; 51°05'N, 04°22'E). A similar, clear pattern of bud burst was observed during the different years of study for all clones. Clones Columbia River, Fritz Pauley, Trichobel (*Populus trichocarpa*) and Balsam Spire (*Populus trichocarpa* × *Populus balsamifera*) from 45°30'N to 49°N reached bud burst (expressed as day of the year or degree day sums) almost every year earlier than clones Wolterson (*Populus nigra*), Gaver, Gibecq and Primo (*Populus deltoides* × *Populus nigra*) (50°N to 51°N). This observation could not be generalised to end-of-season phenology, for which a yearly returning pattern for all clones was lacking. Late bud burst and early leaf fall of some clones (Beaupré, Boelare, IBW1, IBW2, IBW3) was brought about by increasing rust incidence during the years of observation. For these clones, the variability in leaf phenology was reflected in high coefficients of variation among years. The patterns of genetic variation in leaf phenology have implications for short rotation intensive culture forestry and management of natural populations. Moreover, the variation in phenology reported here is relevant with regard to the genetic mapping of poplar.

Key words: *Populus* spp., spring phenology, end-of-season phenology, growing degree day concept, length of growth period.

Introduction

Phenology can be defined as the study of annually recurring biological phenomena, such as bud burst and leaf fall, flowering, fruiting, etc., in the life cycle of plants (Lieth, 1974). Phenology is an important adaptive trait since it determines the duration and timing of the growing season, as well as the period of reproduction (Hänninen, 1990; Reich et al., 1992). In cool and temperate zones, it is a prerequisite for the survival and growth of a tree that its annual development (growth phase

and the frost-tolerant dormant phase) is synchronised each year with the annual course of air temperature (Sakai and Larcher, 1987). The dormant period can be considered as a strategy to avoid unfavorable circumstances (Levins, 1969). It is advantageous for deciduous trees, for example, to shed their leaves before winter and construct new leaves in spring, rather than pay the maintenance costs of keeping them throughout the winter (Kikuzawa, 1995).

For many plant species, phenology is known to be a variable character with a high degree of heritability (El-Kassaby and Park, 1993; Farmer, 1993); in this case it can be modified by selection. Given the large size of most tree populations, genetic variation is unlikely to be explained by drift, but rather by local adaptation. Local adaptation results from a balance between natural selection and gene flow, and will occur if selection is stronger than gene flow (Chaine et al., 2000). Genotypes from northern areas and high elevations, which generally have shorter growing seasons, tend to stop growing earlier in autumn (Pauley and Perry, 1954; Skråpka and Magnussen, 1993). Genotypes that set bud too early tend to be less performing, which could put them at a competitive disadvantage (Li and Adams, 1993; Li et al., 1993; Riemenschneider et al., 1994). Therefore, the timing of bud set is tied to the local climatic cycle.

The timing of leaf unfolding of trees is mainly controlled by temperature (Menzel, 2002). It is generally accepted that a rest break occurs when buds are exposed to temperatures of -5 to +10°C, for several weeks (Flint, 1974; Cannell and Smith, 1983). Subsequently, growth onset will take place after long-term exposure to temperatures above a given threshold (Hänninen, 1995). The degree day-plant phenology interaction is one of the oldest and most studied of ecological concepts (Idso et al., 1978). This concept is based on the idea that certain developmental phases of plant life cycles are correlated with daily accumulations of air temperatures above a certain base value below which physiological activity is assumed to be inhibited (Cannell and Smith, 1986; Murray et al., 1989).

Because *Populus* species are fast growing, easy to replicate via root or stem cuttings, economically important, and because the species contain ecotypes with very different phenological characteristics, they are good models for studying bud and leaf phenology characteristics (Pauley and Perry, 1954; Howe et al., 1995). In addition, interspecific hybridization can be

Table 1 Seventeen poplar clones belonging to six parentage groups, with their gender, clone code number, parents, parent code number, place of origin, latitude and longitude of places of origin

Name	Gender	Clone code number	Parentage	Parentage (abbr.)	Parent code number	Place of origin	Latitude/Longitude
Beaupré	F	S.910-2	<i>P. trichocarpa</i> × <i>P. deltoides</i>	T×D	V.235 × S.1-173	Washington × (Iowa × Missouri)	ca. 49°N, 122°30'W × (42°15'N, 90°45'W) × (38°45'N, 91°15'W)
Boelare	F	S.910-8	<i>P. trichocarpa</i> × <i>P. deltoides</i>	T×D	V.235 × S.1-173	Washington × (Iowa × Missouri)	ca. 49°N, 122°30'W × (42°15'N, 90°45'W) × (38°45'N, 91°15'W)
Hazendans	F	69.039-4	<i>P. trichocarpa</i> × <i>P. deltoides</i>	T×D	V.235 × S.620-225	Washington × Michigan	ca. 49°N, 122°30'W × ca. 43°N, ca. 85°W
Hoogvorst	F	69.038-6	<i>P. trichocarpa</i> × <i>P. deltoides</i>	T×D	V.235 × S.620-225	Washington × Michigan	ca. 49°N, 122°30'W × ca. 43°N, ca. 85°W
Raspalje	F	S.910-10	<i>P. trichocarpa</i> × <i>P. deltoides</i>	T×D	V.235 × S.1-173	Washington × (Iowa × Missouri)	ca. 49°N, 122°30'W × (42°15'N, 90°45'W) × (38°45'N, 91°15'W)
Unal	M	S.910-1	<i>P. trichocarpa</i> × <i>P. deltoides</i>	T×D	V.235 × S.1-173	Washington × (Iowa × Missouri)	ca. 49°N, 122°30'W × (42°15'N, 90°45'W) × (38°45'N, 91°15'W)
Columbia River	M	V.24	<i>P. trichocarpa</i>	T	–	Oregon	45°30'N, 122°40'W
Fritzi Pauley	F	V.235	<i>P. trichocarpa</i>	T	–	Washington	ca. 49°N, 122°30'W
Trichobel	M	S.724-101	<i>P. trichocarpa</i> × <i>P. trichocarpa</i>	T	V.235 × V.24	Washington × Oregon	ca. 49°N, 122°30'W × 45°30'N, 122°40'W
Gaver	M	S.688-22	<i>P. deltoides</i> × <i>P. nigra</i>	D×N	S.71-3 × Gibecq	Illinois × Belgium (Flanders)	40°70'N, 89°60'W × 50°38'N, 3°53'W
Gibecq	M	S.688-30	<i>P. deltoides</i> × <i>P. nigra</i>	D×N	S.71-3 × Gibecq	Illinois × Belgium (Flanders)	40°70'N, 89°60'W × 50°38'N, 3°53'W
Primo	M	S.682-59	<i>P. deltoides</i> × <i>P. nigra</i>	D×N	S.9-2 × Ghoy3	(Iowa × Ontario) × Belgium (Flanders)	(42°15'N, 90°45'W) × (42°40'N, 80°10'W) × 50°44'N, 3°49'W
Balsam Spire	F	TT32	<i>P. trichocarpa</i> × <i>P. balsamifera</i>	T×B	Hastata × Michauxii		
IBW1 D×T	F	71.009-1	<i>P. deltoides</i> × <i>P. trichocarpa</i>	D×T	S.333-44 × S3 ¹	Michigan × (Washington × Idaho)	ca. 43°N, ca. 85°W × (45°70'N, 121°50'W) × (48°15'N, 116°30'W)
IBW2 D×T	F	71.009-2	<i>P. deltoides</i> × <i>P. trichocarpa</i>	D×T	S.333-44 × S3 ¹	Michigan × (Washington × Idaho)	ca. 43°N, ca. 85°W × (45°70'N, 121°50'W) × (48°15'N, 116°30'W)
IBW3 D×T	F	71.015-1	<i>P. deltoides</i> × <i>P. trichocarpa</i>	D×T	S.333-44 × S3 ¹	Michigan × (Washington × Idaho)	ca. 43°N, ca. 85°W × (45°70'N, 121°50'W) × (48°15'N, 116°30'W)
Woltersen	F	1026	<i>P. nigra</i>	N	–	Doesburg, The Netherlands (Ijssel river)	51°59'N, 6°06'E

used to produce pedigrees that are desirable for mapping quantitative trait loci (Newcombe and Bradshaw, 1996; Bradshaw and Stettler, 1999). The field performance of poplar clones is closely tied to the time of bud set and bud burst (Pauley and Perry, 1954). In *Populus*, the date of spring bud burst is one of the most readily mapped quantitative traits, and 98% of the phenotypic variance is under genetic control. As for bud set and leaf fall, bud burst is also an adaptive trait that shows latitudinal and elevational trends within species (Bradshaw, 1996).

The aim of the present study was to examine clonal differences in year-to-year variation in phenology, in particular, differences in spring and end-of-season phenology patterns and length of growth period of 17 poplar clones grown under a

short rotation coppice regime, over several years of three rotation cycles.

Materials and Methods

Experimental plantation and management regime

In April 1996, 17 different poplar (*Populus*) clones were planted in an experimental field plantation in the industrial zone of Boom province of Antwerp (Belgium, 51°05'N, 4°22'E, 5 m above sea level). The plantation is situated on an old waste disposal site, covered with a 2 m thick layer of sand, clay and rubble. The 17 clones, belonging to six parentage groups, i.e. *P. nigra* L. (N) clone Woltersen; *P. trichocarpa* T. and G. (T) clones Columbia River, Fritzi Pauley and Trichobel; *P. trichocarpa* × *P.*

deltoides Marshall (T×D) clones Beaupré, Boelare, Hazendans, Hoogvorst, Raspalje and Unal; *P. deltoides* × *P. trichocarpa* (D×T) clones IBW1, IBW2 and IBW3; *P. deltoides* × *P. nigra* (D×N) clones Gaver, Gibecq and Primo; and *P. trichocarpa* × *P. balsamifera* L. (T×B) clone Balsam Spire, are presented in Table 1 with their gender, parentage, place, latitude and longitude of origin. All clones were planted as 25-cm long dormant, unrooted hardwood cuttings, after being soaked in water for 24 h. They were planted manually to a depth of 22 cm in a double row design. Within the rows, the plant distance was 0.9 m, with alternating inter-row distances of 0.75 m and 1.5 m, resulting in a total plant density of 10 000 trees per ha. A randomized block design was used with 17 clones × 3 replicates (except clone Hoogvorst with 6 replicates, and clones IBW1 and Raspalje with 2 replicates) according to a protocol prescribed by the British Forestry Commission (Armstrong, 1997). Each monoclonal plot (n = 100 trees) was considered as having a double border row (Zavitkovski, 1981). To promote optimal establishment, limited irrigation and weed control were applied. Irrigation was applied only in the first growing season; mechanical weed control on the other hand was carried out at frequent intervals. When mechanical weeding became insufficient, limited chemical weed control techniques were applied. On three occasions, in June 1996, June 1997 and May 2001, herbicides (mixture of glyphosate at 3.2 kg ha⁻¹ and oxadiazon at 9.0 kg ha⁻¹) were used. These herbicides were applied using a spraying device with a hood-covered nozzle to minimize impact on the trees. The cuttings that did not establish in 1996 were replaced in the spring of 1997 with new 25-cm long hardwood cuttings (40 cm for the clones with a mortality rate higher than 10%). At the end of the first growing season (1996), all trees were cut back to a height of 5 cm to create a coppice system with an average number of two to ten stems per stool. So, in the spring of 1997, the trees started their first 4-year rotation cycle (1997–2001). The second 3-year rotation (2001–2003) started in the spring of 2001, after coppicing in January 2001, and ended by cutting the trees back again in the winter of 2003. No fertilisation or irrigation was applied after the establishment of the experiment. Further details on the plantation (layout, management, rotation cycles) can be found in Deraedt and Ceulemans (1998).

Phenology measurements

Spring and autumn phenology, characterised by bud burst and leaf fall, respectively, were monitored at frequent intervals between 1996 and 2003, i.e., during 6 years of two rotation cycles. A subjective scoring system was developed to identify the early to advanced developmental phenology stages on the 17 different poplar clones. The gradual process of leaf unfolding and senescence was quantified by allocating each entire clonal plot (n = 100 trees) to one of six discrete classes, fully described in Table 2. For a detailed description of the bud burst classes, the reader is referred to Turok et al. (1996). Phenological data were collected for all 17 clones in each of the three replicates (n = 3 plots) from autumn 1996 till spring 2003, with the exception of 1997 (only average leaf fall data for 8 clones), 1998 (leaf fall data for 11 clones in all three replicates), 1999 (no data available). In the present study bud burst is defined as the day of the year or the degree-day sum (°C d), at which a clone reaches class 4 of the spring bud phenology classes, while leaf fall is defined as the day of the year at which a clone reaches class 4 of the end-of-season phenology classes. Leaf fall and

Table 2 Description of the six discrete spring (bud burst) and end-of-season (leaf fall) phenology classes. Bud burst classes are according to Turok et al. (1996)

Spring phenology classes	Bud burst
0	Dormant bud; there was no sign at all of any activity. Completely dry outlook and brown colour
1	Buds were slightly swollen and red colour appeared on the bud scales
2	Buds were fully swollen and turned towards a rounded shape. However, there was no sign of breakage of the buds
3	Buds started breaking, wet and sticky. Tip of reddish shoots appeared
4	Bud burst and reddish shoots turned towards a green colour. Very young leaves could be observed
5	Green leaves started growing and venation of leaf could be observed
End-of-season phenology classes	Leaf fall
0	No sign at all of leaf senescence
1	Onset of discoloration with only a few leaves (10%) having changed colour
2	50% of all leaves have changed colour, some leaves have wilted and a few leaves have dropped
3	Almost all leaves (90%) are discoloured with advanced leaf drop
4	Almost all leaves have been dropped
5	End of leaf drop, bare stems remain

bud burst data could be compared among clones and among parentage groups using the day of the year or the degree-day sum, in the case of bud burst (as explained under temperature measurements below) at which phenology class 4 was reached. In addition, the differences in reaching bud burst and leaf fall were compared among years and for all clones.

Only in 1998 were bud burst and leaf fall data available for 11 clones. For 1998, the growth period, defined in this study as the total number of growing days between spring phenology (class 5) and end-of-season phenology (class 5), was calculated. Because some bud burst and leaf fall data sets for 1998 were incomplete, extrapolation and interpolation of the observed data were necessary. The data sets were completed by using exponential (larger gaps > 2 classes) or linear (smaller gaps < 2 classes) relationships between phenology classes and day of the year, and extrapolated or interpolated to the missing data points.

Temperature data sets

Above-canopy temperature measurements were obtained for the 7 years of this study (1996–2003) from a meteorological station in Brasschaat (51°18'N, 4°31'E), in the Belgian Campine region (Carrara et al., 2003), 30 km from the experimental field plantation in Boom. Daily average temperatures were used in

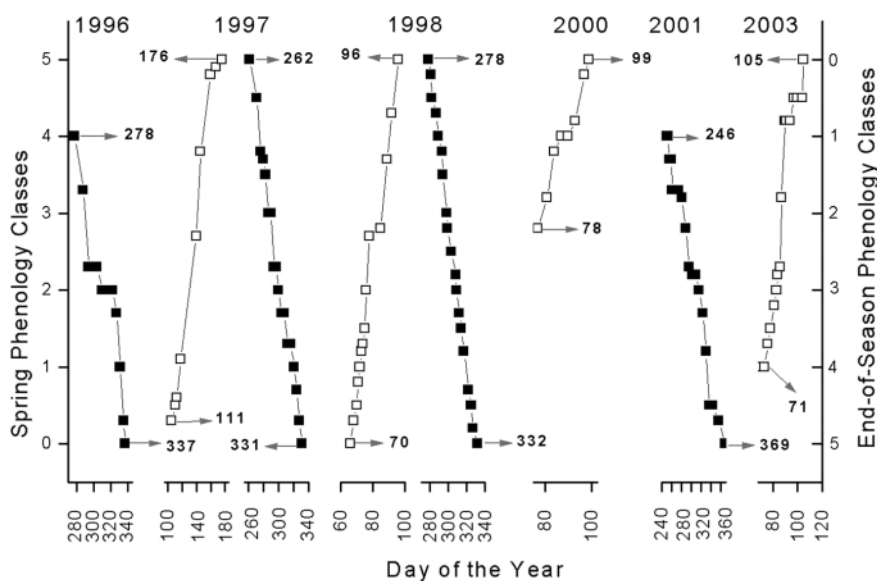


Fig. 1 Course of spring leaf phenology (bud burst) and end-of-season phenology (leaf fall) of *Populus trichocarpa* clone Fritz Pauley in 1996, 1997, 1998, 2000, 2001 and 2003, expressed in terms of six discrete classes reached on specific days of the year. The day of the year at which phenology (spring and end-of-season) started and ended is indicated by the numbers. Closed symbols represent end-of-season classes and open symbols represent spring phenology classes.

the calculation of the degree-day sums ($^{\circ}\text{C d}$) of spring phenology. The growing degree-day (GDD) concept is based on a linear relationship between temperature and developmental rate of the plant (Baker et al., 1984). According to this concept, every stage in the life cycle of a plant can be presented by a temperature sum (degree-day sum), calculated according to the following formula:

$$\text{GDD} = \Sigma(T_m - T_b)$$

where T_m represents the mean daily temperature and T_b the base or threshold temperature. In the present study, the starting date of the degree-day summation and the T_b were chosen arbitrarily, namely 1 January and 0°C , respectively.

Statistical data analysis

Differences in attaining bud burst and leaf fall classes among clones and parentage groups were statistically examined using analysis of variance (ANOVA). The analysis was performed with the SAS statistical software package (SA System 6.12, SAS Institute Inc., Cary, NC), using the mixed procedure (Littell et al., 1996). A randomized block design was applied, with clones as a fixed factor and replicate plot as a random factor. Least square means were pairwise compared for clones and parentage groups. The Tukey–Kramer adjustment was used to control the maximum experimental error rate under any complete or partial null hypothesis. Two means were considered significantly different when the adjusted p value of the ANOVA t -test was <0.05 . The normal distribution of the data was verified with the Shapiro–Wilk statistic (proc univariate in SAS). As bud burst and leaf fall of all clones were measured repeatedly, the repeated measurement statement was used in the mixed procedure to examine differences in attaining bud burst and leaf fall among different years. Moreover, the variation in phenological traits among clones as well as among years was expressed as the coefficient of variation (CV).

Results

An all-embracing impression of spring phenology and end-of-season phenology of one specific clone, Fritz Pauley, over 6 years (1996, 1997, 1998, 2000, 2001 and 2003) is given in Fig. 1. The differences among the years in attaining the different spring phenology and end-of-season phenology classes, and the differences in the rate with which they reached these classes can be seen in Fig. 1. It should be mentioned that the observations for spring phenology in 1997 were made at the beginning of the second growing season, after stems had been coppiced during the previous winter. Therefore, visual observations were sometimes difficult because all buds were only 5 cm above the ground, resulting in a different pattern of spring phenology in 1997 in comparison to other years. In 1997, Fritz Pauley started spring phenology only on 21 April, while in 1998, 2000 and 2003 spring phenology already started around 10–11 March. Also, the rate of phenological bud development of Fritz Pauley differed among the years; in 1997 it took 65 days to reach phenology class 5, while in 1998, 2000 and 2003 it took only about 30 days (Fig. 1). End-of-season phenology was similar (start, end and rate) in 1996, 1997 and 1998, but different in 2001 in comparison with the other years. In 2001, leaf fall started early (before 3 November) and ended late (4 January, 2002).

Spring phenology (bud burst)

The day of the year on which the 17 clones belonging to the six parentage groups reached full bud burst (class 4) in the different years is presented in Table 3. In addition, bud burst data for 1998, 2000 and 2003 were also expressed in degree-day sums (Table 3), according to the concept that spring phenology is strongly controlled by air temperature and that every clone needs a certain temperature sum before breaking bud (Kramer, 1995). The average air temperatures measured from 1 January till bud burst was reached in the last clone, were 9°C (in 1997), 8°C (in 1998), 7°C (in 2000) and 7°C (in 2003). In general, significant differences among clones and among parentage groups within the different years were observed, except

Table 3 Time of bud burst (phenology class 4) of 17 poplar clones belonging to six parentage groups, expressed as day of the year and degree-day sum (DDS: °C d) with standard error (SE) of three replicates in 1997, 1998, 2000 and 2003. T: *P. trichocarpa*, B: *P. balsamifera*, D: *P. deltoides*, N: *P. nigra*, CV = coefficient of variation

Group	Clone	1997		1998		2000		2003		CV (%)	
		class 4 (day of year)	DDS (°C d)	class 4 (day of year)	DDS (°C d)	class 4 (day of year)	DDS (°C d)	class 4 (day of year)	DDS (°C d)	class 4 (day of year)	DDS (°C d)
T × D	Beaupré	137 (16)	1186 (26)	100 (1)	639 (4)	99 (1)	604 (10)	114 (0)	789 (0)	8	15
	Boelare	151 (3)	1160 (23)	99 (10)	634 (2)	99 (1)	604 (10)	113 (1)	750 (9)	8	12
	Hazendans	144 (1)	1060 (9)	104 (2)	663 (11)	108 (1)	670 (7)	113 (1)	740 (9)	4	6
	Hoogvorst	153 (5)	1198 (83)	101 (5)	644 (6)	104 (1)	636 (4)	115 (1)	767 (9)	7	11
	Raspalje	146 (1)	1080 (6)	92 (0)	575 (0)	96 (1)	583 (5)	106 (2)	646 (5)	7	6
	Unal	146 (1)	1090 (16)	96 (0)	613 (0)	102 (3)	624 (20)	107 (0)	676 (4)	5	5
T	Columbia River	155 (6)	1225 (90)	89 (0)	541 (0)	94 (1)	563 (8)	89 (0)	516 (0)	3	4
	Fritzi Pauley	148 (3)	1118 (38)	91 (1)	545 (2)	85 (1)	502 (7)	90 (0)	521 (3)	4	4
	Trichobel	153 (4)	1197 (70)	88 (1)	531 (10)	87 (0)	516 (0)	89 (1)	510 (10)	1	2
D × N	Gaver	151 (4)	1167 (67)	103 (1)	657 (4)	106 (1)	657 (6)	108 (1)	682 (11)	2	2
	Gibecq	155 (2)	1210 (33)	110 (1)	707 (11)	111 (0)	709 (0)	113 (0)	740 (4)	1	3
	Primo	148 (2)	1107 (28)	111 (1)	711 (10)	106 (1)	657 (6)	111 (1)	723 (9)	3	5
T × B	Balsam Spire	152 (6)	1184 (93)	82 (0)	473 (0)	90 (3)	535 (20)	84 (0)	456 (4)	5	9
D × T	IBW1	158 (4)	1261 (66)	105 (1)	664 (5)	101 (2)	618 (15)	115 (1)	779 (30)	7	12
	IBW2	156 (1)	1239 (18)	99 (1)	634 (4)	100 (2)	612 (17)	113 (1)	746 (14)	8	11
	IBW3	155 (5)	1222 (88)	100 (0)	643 (2)	97 (0)	587 (0)	113 (1)	741 (9)	8	12
N	Woltersen	145 (2)	1071 (19)	113 (3)	725 (28)	111 (0)	709 (0)	110 (0)	704 (0)	1	2
CV (%)		4	5	9	11	8	10	10	16		

for bud burst in 1997, when clones and parentage groups were not significantly different in reaching bud burst or in the degree-day sums needed. Clones IBW1, IBW2, IBW3, Beaupré, Boelare, Hazendans and Hoogvorst were severely affected by rust in the summers of 2001 and 2002, resulting in a lower amount of buds and late bud burst in 2003. Moreover, observations on these clones were difficult because not all buds actually reached the first class of spring phenology. Almost every year, the same clones and parentage groups had a significantly earlier or later bud burst in relation to other clones.

Balsam Spire reached bud burst in 1998, 2000 and 2003, respectively on 23 March, 30 March and 25 March. This clone thus needed significantly fewer warm (>0°C) days to reach bud burst (473°C d, 535°C d and 456°C d, respectively) than all other clones, except for the three T clones (Table 3). The latter were also not very demanding; they needed an average degree-day sum of 527°C d (1998, 2000, 2003) and the average day of the year on which the T clones reached bud burst was day 89 (1998, 2000, 2003), resulting in an almost three-week earlier bud burst than clones Woltersen, Gibecq, Gaver, Primo and Hazendans. The local clone (N) Woltersen was, in 1998 and 2000, the last one to reach bud burst (23 April and 20 April, respectively), significantly later than all other clones except the clones above-mentioned. This was reflected in the higher degree-day sums of Woltersen (725°C d and 709°C d in 1998 and 2000, respectively), closely followed by Gaver, Gibecq and Primo, with average degree-day sums of 692°C d

and 674°C d and average bud burst days of 107 and 108, respectively, in 1998 and 2000. For 2003, however, no particular clone had a significantly later bud burst or a significant higher degree-day sum (Table 3). In 1998, 2000 and 2003 the coefficient of variation (CV) was higher for bud burst as well as for degree-day sums in comparison with the CV in 1997, indicating larger differences in bud burst and degree-day sums among clones in those three years (Table 3). The coefficient of variation (CV) among years of bud burst (1998, 2000 and 2003), expressed as day of the year and as degree-day sum, ranged from 1% to 8% (day of the year) and from 2% to 15% (degree-day sum). Clones with higher CVs for bud burst (Beaupré, Boelare, Hoogvorst, IBW1, IBW2 and IBW3) expressed as day of the year as well as degree-day sum (≥7% and ≥11%, respectively) were characterized by an increasing rust infection during those years (Table 3).

The significant clonal differences were also reflected in differences among parentage groups to which they belong, within each year. The T and T × B parentage groups had, in all three years (1998, 2000 and 2003), significantly earlier bud burst dates (27 March, 20 March and 28 March, respectively) than the other parentage groups and thus needed only a few warmer days (>0°C) to reach bud burst (average degree-day sums: 506°C d in 1998, 549°C d in 2000 and 486°C d in 2003). The N and the D × N parentage groups, on the other hand (average degree-day sums: 708°C d in 1998, 692°C d in 2000 and 710°C d in 2003), were more demanding, resulting in a significantly

Table 4 Time of leaf fall (phenology class 4) of 17 clones belonging to six parentage groups, expressed as day of the year with standard error (SE) of three replicates in 1996, 1997, 1998 and 2001. T: *P. trichocarpa*, B: *P. balsamifera*, D: *P. deltoides*, N: *P. nigra*, CV = coefficient of variation

Group	Clone	1996	1997	1998	2001	CV (%)
T×D	Beaupré	323 (6)	296	284 (2)	276 (14)	7
	Boelare	312 (2)	296		288 (7)	5
	Hazendans	330 (2)		318 (4)	309 (4)	3
	Hoogvorst	328 (1)		317 (0)	301 (2)	5
	Raspalje	327 (0)			296 (7)	5
	Unal	313 (3)			281 (0)	5
T	Columbia River	338 (2)	333	335 (5)	328 (2)	5
	Fritzi Pauley	330 (1)	321	319 (2)	323 (4)	4
	Trichobel	325 (0)	328	311 (3)	326 (2)	5
D×N	Gaver	310 (0)		303 (2)	307 (6)	2
	Gibecq	314 (2)		303 (2)	323 (4)	3
	Primo	310 (0)		299 (0)	296 (0)	3
T×B	Balsam Spire	310 (0)		297 (0)	307 (4)	1
D×T	IBW1	327 (0)	308		284 (3)	10
	IBW2	320 (4)	319		277 (4)	10
	IBW3	325 (2)	316		277 (2)	11
N	Wolterson	327 (3)		307 (0)	305 (6)	5
CV (%)		3	4	4	6	

later bud burst (21 April, 19 April and 21 April, respectively) (Table 3).

A significant effect of the year was observed, indicating that day and degree-day sum of bud burst differed significantly with year of observation. Also, a significant clone × year interaction was observed for bud burst expressed as degree-day sums. The clone × year interaction was significant for clones Beaupré, Boelare, Hoogvorst, IBW1, IBW2 and IBW3 between the years 1998 and 2003, and 2000 and 2003. For bud burst expressed as day of the year, the clone × year interaction was not significant.

End-of-season phenology (leaf fall)

Leaf fall was studied and compared among all 17 clones and among all parentage groups, for the years 1996 and 2001 (Table 4). While examining these data sets, it should be kept in mind that Beaupré and Boelare (in 1997) and Beaupré, Boelare, IBW1, IBW2 and IBW3 (in 2001) were severely attacked by rust, namely *Melampsora larici-populina*. For these clones, leaf discoloration and wilting of a major part of the canopy were already observed in mid-September 1997 and near the end of August 2001, resulting in canopies where the majority of the leaves were dead (except for a few leaves near the top).

Although there was no clear yearly returning pattern as for bud burst, clones and parentage groups differed significantly and a distinction could be made between clones and parentage

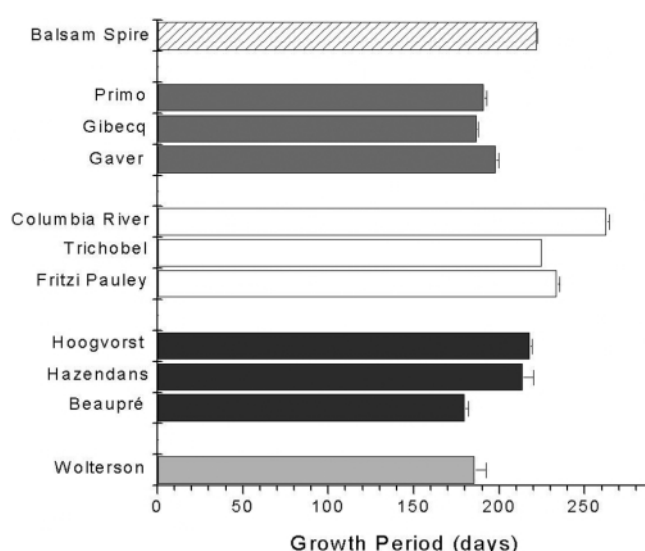


Fig. 2 Length of growth period of 11 different poplar clones in the second growing season of the first coppice rotation (1998). Mean values of three replicates and standard error bars are presented.

groups with earlier and later leaf fall. Only the leaf fall data sets of 1996 and 2001 could be tested for statistic differences. In both years, several clones could be identified with an early leaf fall, namely Beaupré, Boelare, IBW2, Primo and Unal. These clones showed an average leaf fall on 12 November and 10 October, respectively, in 1996 and 2001. This made these clones significantly earlier than the T clones Columbia River, Fritzi Pauley and Trichobel, that showed a significantly later leaf fall (27 November in 1996 and 22 November in 2001) (Table 4). Every year, the low CV value (ranging from 3% in 1996 to 6% in 2001) indicated only small differences in leaf fall among clones. The CV among years of leaf fall ranged from 1% to 11%. High CV values among years ($\geq 7\%$) of some clones (Beaupré, IBW1, IBW2, IBW3) could be related to a higher rust incidence on these clones during the years of observation (Table 4).

The parentage groups differed significantly in leaf fall in 1996, 1998 and 2000, i.e. the D×N parentage group reached leaf fall significantly earlier (7 November) and the T parentage group reached leaf fall significantly later (18 November 1998) than the other parentage groups. Besides the significant effect of year, also a significant clone × year interaction could be observed for leaf fall in the three different years. The interaction was significant for Gibecq and Trichobel between 1996 and 2001, as well as between 1998 and 2001, and for Beaupré, IBW1, IBW2 and IBW3 between 1996 and 1998.

Length of growth period

The growth period was calculated for 11 clones in 1998 and results are represented in Fig. 2. Columbia River (T) had the longest growth period of 263 days, which was significantly longer than the growth period of all other clones. The two other clones of the T parentage group, Fritzi Pauley and Trichobel, were also characterized by a significantly longer growing period, 234 and 225 days, respectively, than the other clones, closely followed by Balsam Spire (222 days), belonging to the T×B parentage group (Fig. 2). The growth period of Beaupré

(180 days) was significantly shorter than the growth period of all other clones except Primo, Gaver, Gibecq and Wolterson (191 days on average; Fig. 2). So Columbia River (T), with the longest growth period, kept its leaves 83 days longer than clone Beaupré (T×D), with the shortest growth period.

Discussion

Genetic variation in tree phenology has primarily been studied using data on vegetative or reproductive phenology derived from experiments involving transplantation of seedlings from natural populations. Differences in phenological traits among populations have been shown for some species (Van Niejenhuis and Parker, 1996; Li et al., 1997), but not for others (Farmer, 1993; Li et al., 1993). In the present study, phenological traits (bud burst and leaf fall) differed significantly among clones and years. Every year (except for 1997) the T clones Columbia River, Fritzi Pauley and Trichobel (latitude of origin 45°30'N to ca. 49°N) as well as the T×B clone Balsam Spire reached bud burst earlier than clones Wolterson (N), Gibecq, Gaver and Primo (D×N) (latitude of origin 50°N to 51°59'N). Differences in timing of bud burst between the first growing season and the following growing seasons, suggested that first-year growth is often not representative of growth in later seasons (Lambeth, 1980). In the present study, a different pattern of bud burst could also be observed in 1997 between the first growing season after coppicing (in December 1996) and the following growing seasons. In the summer of 2002, seven clones (Beaupré, Boelare, Hazendans, Hoogvorst, IBW1, IBW2 and IBW3) were strongly infected with the leaf rust *Melampsora larici-populina*, leaving these clones susceptible to secondary pathogens, early frost damage in autumn and late frost damage in spring. The latter happened in the spring of 2003, explaining why buds of earlier infected clones reached class 1 of spring phenology very late or not at all. This probably resulted in slightly different bud burst data for these infected clones in 2003 in comparison with 1998 and 2000.

Experiments on the spring phenology of poplar indicated that temperature rather than photoperiod is the major factor controlling the initiation of new growth after winter dormancy (Pauley and Perry, 1954). Following this principle, bud burst in the present study was also expressed in degree-day sums, which yielded the same relations among clones, when expressed as day of the year. When studying degree-day relations, the choice of base temperature is of significant importance. Because base temperature is species-dependent, no universally "correct" temperature can be defined. However, because of its convenience and since 0°C proved to be as appropriate as, or even better than, higher base temperatures (Hänninen, 1990; Heide, 1993), a base temperature of 0°C was preferred in the present study. In the study of Sigurdsson (2001), this base temperature gave better results than a threshold of 4°C used in a previous phenological study of *P. trichocarpa* in Iceland (Aradottir et al., 1997).

End-of-season phenology shows important genetic differences between geographically dispersed plantations with different climatic conditions (Weber et al., 1985; Dunlap et al., 1992). The results presented here with regard to the relation of end-of-season phenology and place of origin of the 17 clones have to be interpreted with care as almost all clones were selected hybrids (rather difficult to identify the "place of origin" of a

hybrid) and latitudinal variation was rather limited. In their study, Pauley and Perry (1954) concluded that hybrids between clones of northern and southern origin gave a photoperiodic response (growth cessation) intermediate to the responses of the parentages. Columbia River is the clone of the T parentage group which originated from the lowest (or most southern) latitude (45°30'N) and which reached leaf fall last, as compared to Fritzi Pauley, originating from the highest latitude (ca. 49°N), which reached leaf fall first, from the T parentage group (except in 1998). Clone Trichobel, as a hybrid of Columbia River and Fritzi Pauley, represented in 1997 and 2001 an intermediate pattern of leaf fall within the T parentage group. Although the end-of-season phenology of *P. trichocarpa* was, in the present study, very similar every year (except in 1997), a clear annually returning pattern for the different clones, as found for bud burst, was lacking.

In general, leaves have a finite, genotype-specific life expectancy. Life expectancy (leaf emergence–leaf retention), as well as number and size of leaves, might be increased when trees are grown under intensive culture practices (short rotation coppice culture) rather than under natural conditions (Isebrands et al., 1977). Growth period, defined as the period from emergence to leaf fall, ranged from 180 days for Beaupré to 263 for Columbia River. However, as from 1997, the onset of leaf fall of Beaupré was earlier than that of other clones due to severe rust infection in this clone. Rust infections (*Melampsora larici-populina*) were observed in our plantation on several poplar clones, from two clones in 1997 (Beaupré and Boelare) up to seven clones in 2003 (Beaupré, Boelare, Hazendans, Hoogvorst, IBW1, IBW2 and IBW3).

Since *Populus* has a small genome, it is an attractive model forest tree for gene mapping. Studies aimed at the ideotype concept for poplar also include detailed measures of leaves and branches, and measures of phenological characteristics (Riemenschneider et al., 1996). Moreover, phenological responses of plants to environmental variables have an underlying genetic basis (Dunlap et al., 1992). But these responses can also be influenced by biotic factors, such as leaf rust or insect attacks. Therefore, Quantitative Trait Loci mapping of traits such as phenology and rust resistance should play a prominent part in the identification of the patterns of genetic variation within and among species, and in the design of multispecies hybrids with unique trait combinations (Bradshaw, 1996).

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References

- Aradottir, A. L., Thorgeirsson, H., McCaughey, J. H., Strachan, I. B., and Robertson, A. (1997) Establishment of a black cottonwood plantation on an exposed site in Iceland: plant growth and site energy balance. *Agricultural and Forest Meteorology* 84, 1–9.
- Armstrong, A. (1997) The United Kingdom network of experiments on site/yield relationships for short rotation coppice. Edinburgh: Forestry Commission.
- Baker, D. G., Sharratt, B. S., Chiang, H. C., Zandlo, J. A., and Ruschy, D. L. (1984) Base temperature selection for the prediction of European corn borer instars by the growing degree day method. *Agricultural and Forest Meteorology* 32, 55–60.
- Bradshaw, H. D. Jr. (1996) Molecular genetics of *Populus*. In *Biology of Populus and Its Implications for Management and Conservation* (Stettler, R. F., Bradshaw, H. D. Jr., Heilman, P. E., and Hinckley, T. M., eds.), Ottawa, ON: Research Press, National Research Council of Canada, pp.183–199.
- Bradshaw, H. D. Jr. and Stettler, R. F. (1999) Molecular genetics of growth and development in *Populus*. IV. Mapping QTLs with large effects on growth, form, and phenology traits in a forest tree. *Genetics* 139, 963–973.
- Cannell, M. G. R. and Smith, R. I. (1983) Thermal time, chill days and prediction of bud burst in *Picea sitchensis*. *Journal of Applied Ecology* 20, 951–963.
- Cannell, M. G. R. and Smith, R. I. (1986) Climatic warming, spring bud burst and frost damage on trees. *Journal of Applied Ecology* 23, 177–191.
- Carrara, A., Kowalski, A. S., Neiryneck, J., Janssens, I. A., Curiel Yuste, J., and Ceulemans, R. (2003) Net ecosystem CO₂ exchange of mixed forest in Belgium over 5 years. *Agricultural and Forest Meteorology* 119, 209–227.
- Chaine, I., Belmonte, J., and Mignot, A. (2000) A modelling analysis of the genetic variation of phenology between tree populations. *Journal of Ecology* 88, 561–570.
- Deraedt, W. and Ceulemans, R. (1998) Clonal variability in biomass production and conversion efficiency of poplar during the establishment year of a short rotation coppice plantation. *Biomass and Bioenergy* 15, 391–398.
- Dunlap, J. M., Heilman, P. E., and Stettler, R. F. (1992) Genetic variation and productivity of *Populus trichocarpa* and its hybrids. V. The influence of ramet position on 3-year growth variables. *Canadian Journal of Forest Research* 22, 849–857.
- El-Kassaby, Y. A. and Park, Y. S. (1993) Genetic variation and correlation in growth, biomass and phenology of Douglas-fir diallel progeny at different spacings. *Silvae Genetica* 42, 289–297.
- Farmer, R. E. (1993) Latitudinal variation in height and phenology of Balsam poplar. *Silvae Genetica* 42, 148–152.
- Flint, H. L. (1974) Phenology and genecology of woody plants. In *Phenology and Seasonality Modelling* (Lieth, H., ed.), Berlin: Springer-Verlag, pp.83–97.
- Hänninen, H. (1990) Modelling bud dormancy release in trees from cool and temperate regions. *Acta Forestalia Fennica* 213, 1–47.
- Hänninen, H. (1995) Effects of climatic change on trees from cool and temperate regions: an ecophysiological approach to modelling of bud burst phenology. *Canadian Journal of Botany* 73, 183–199.
- Heide, O. (1993) Day length and thermal time responses of bud burst during dormancy release in some northern deciduous trees. *Physiologia Plantarum* 88, 531–540.
- Howe, G. T., Hackett, W. P., Furnier, G. R., and Klevorn, R. E. (1995) Photoperiodic responses of a northern and southern ecotype of black cottonwood. *Physiologia Plantarum* 93, 695–708.
- Idso, S. B., Jackson, R. D., and Reginato, R. J. (1978) Extending the “Degree Day” concept of plant phenological development to include water stress effects. *Ecology* 59, 431–433.
- Isebrands, J. G., Promnitz, L. C., and Dawson, D. H. (1977) Leaf Area Development in Short Rotation Intensive Cultured *Populus* Plots. TAPPI Forest Biology Wood Chemical Conference, 201–210.
- Kikuzawa, K. (1995) The basis for variation in leaf longevity of plants. *Vegetatio* 121, 89–100.
- Kramer, K. (1995) Phenotypic plasticity of the phenology of seven European tree species in relation to climatic warming. *Plant, Cell and Environment* 18, 93–104.
- Lambeth, C. C. (1980) Juvenile-mature correlations in Pinaceae and implications for early selection. *Forest Science* 26, 571–580.
- Levins, R. (1969) Dormancy as an adaptive strategy. In *Dormancy and Survival*, No. XXIII, Symposia of the Society for Experimental Biology (Woolhouse, H. W., ed.), Cambridge: Cambridge University Press, pp.1–10.
- Li, P. and Adams, W. T. (1993) Genetic control of bud burst and seedlings of coastal Douglas-fir. *Canadian Journal of Forest Research* 23, 1043–1051.
- Li, P., Beaulieu, J., and Bousquet, J. (1997) Genetic structure and patterns of genetic variation among populations in eastern white spruce (*Picea glauca*). *Canadian Journal of Forest Research* 27, 189–198.
- Li, P., Beaulieu, J., Corriveau, A., and Bousquet, J. L. (1993) Genetic variation in juvenile growth and phenology in a white spruce provenance-progeny test. *Silvae Genetica* 42, 52–60.
- Lieth, H. (1974) *Phenology and Seasonality Modelling*. New York: Springer Verlag.
- Littell, R. C., Milliken, G. A., Stroup, W. W., and Wolfinger, R. D. (1996) *SAS System for Mixed Models*. Cary, NC, USA: SAS Institute Inc., 633 pp.
- Menzel, A. (2002) Phenology: Its importance to the global change community. *Climatic Change* 54, 379–385.
- Murray, M. B., Cannell, M. G. R., and Smith, R. I. (1989) Date of bud burst of fifteen tree species in Britain following climatic warming. *Journal of Applied Ecology* 26, 693–700.
- Newcombe, G. and Bradshaw, H. D. (1996) Quantitative trait loci conferring resistance in hybrid poplar to *Septoria populicola*, the cause of leaf spot. *Canadian Journal of Forest Research* 26, 1943–1950.
- Pauley, S. S. and Perry, T. O. (1954) Ecotypic variation of the photoperiodic response in *Populus*. *Journal of the Arnold Arboretum* 35, 167–188.
- Reich, P. B., Walters, M. B., and Ellsworth, D. S. (1992) Leaf life-span in relation to leaf, plant, and stand characteristics among diverse ecosystems. *Ecological Monographs* 62, 365–392.
- Riemenschneider, D. E., Mc Mahon, B. G., and Ostry, M. E. (1994) Population dependent selection strategies needed for 2-year-old black cottonwood clones. *Canadian Journal of Forest Research* 24, 1704–1710.
- Riemenschneider, D. E., Stelzer, H. E., and Foster, G. S. (1996) Quantitative genetics of poplar hybrids. In *Biology of Populus and Its Implications for Management and Conservation* (Stettler, R. F., Bradshaw, H. D. Jr., Heilman, P. E., and Hinckley, T. M., eds.), Ottawa: NRC Research Press, National Research Council of Canada, pp.159–181.
- Sakai, A. and Larcher, W. (1987) *Frost Survival of Plants. Responses and Adaptation to Freezing Stress*. Berlin: Springer-Verlag, 321 pp.
- Sigurdsson, B. D. (2001) Elevated [CO₂] and nutrient status modified leaf phenology and growth rhythm of young *Populus trichocarpa* trees in a 3-year field study. *Trees – Structure and Function* 15, 403–413.
- Skrøppa, T. and Magnussen, S. (1993) Provenance variation in shoot growth components of Norway spruce. *Silvae Genetica* 42, 111–120.
- Turok, J., Lefèvre, F., Cagelli, L., and de Vries, S., compilers (1996) *Populus nigra* Network. Report of the second meeting, 10–12 September 1995, Casale Monferrato, Italy. Rome, Italy: International Plant Genetic Resources Institute, 26 pp.

- Van Niejenhuis, A. and Parker, W. H. (1996) Adaptive variation in jack pine from north central determined by short-term common garden tests. *Canadian Journal of Forest Research* 26, 2006–2014.
- Weber, J. C., Stettler, R. F., and Heilman, P. E. (1985) Genetic variation and productivity of *Populus trichocarpa* and its hybrids. I. Morphology and phenology of 50 native clones. *Canadian Journal of Forest Research* 15, 376–383.
- Zavitkovski, J. (1981) Small plots with unplanted plot border can distort data in biomass production studies. *Canadian Journal of Forest Research* 11, 9–12.

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