Reproductive development and seed ripening in *Betula papyrifera* along an altitudinal thermal gradient in eastern Appalachia (Canada)

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Abstract: In several gymnosperm tree species of the circumboreal forest, reproductive development is closely associated with the accumulation of degree-days during the growing season. We wanted to verify whether this pattern holds for a widespread angiosperm species such as paper birch (*Betula papyrifera* Marsh), a broadleaf tree distributed throughout the North American boreal forest. Stations to simultaneously measure the thermal sum (degree-days > 5 °C, DD) and the reproductive development (anatomical development and seed germination) were installed in four sites along an altitudinal thermal gradient totalling 541 m a.s.l. in the eastern Appalachian Mountains (Canada). In 2004, sites 1, 2, 3 and 4, received 973, 1099, 1266 and 1359 DD, respectively. A strong relationship was found between thermal sum and reproductive development (anatomical development: $r^2 > 0.90$; seed germination $r^2 > 0.72$). This relationship seems to be based on the absolute value of heat-sum received because (*i*) rates of reproductive development are twice as high in colder sites (1 and 2) than in warmer sites (3 and 4), and (*ii*) total seed germination did not differ among the sites. Along with this, we noted a lesser production of full seeds in colder sites, coupled with a higher success of germination in the coldest site (site 1), which appears to be a mechanism of phenotypic adaptation to cold.

Key words: reproductive development, thermal sum, *Betula papyrifera*, germination, variety.

Résumé : Chez plusieurs espèces d'arbres gymnospermes de la forêt circumpolaire, le développement reproductif est étroitement associé à la somme des dégrés-jours au cours de la saison de croissance. Les auteurs ont voulu vérifier si ce patron vaut également pour des espèces d'angiospermes largement distribuées comme le bouleau à papier (*Betula papyrifera* Marsh.), une espèce à feuilles larges distribuée dans l'ensemble de la forêt boréale Nord Américaine. Les auteurs ont installé des stations pour mesurer simultanément la somme thermique (degrés-jours > 5 °C, DD) et le développement reproductif (développement anatomique et germination des graines); ils ont installé ces stations sur quatre sites le long d'un gradient thermique altitudinal sur 541 mètres dans l'est des montagnes Appalaches (Canada). En 2004, les sites 1, 2, 3 et 4, ont reçu respectivement 973, 1099, 1266 et 1359 DD. On observe une forte corrélation entre la somme thermique et le développement reproductif (développement anatomique : $r^2 > 0{,}90$; germination des graines $r^2 > 0{,}72$). Cette relation semble basée sur la valeur absolue de la somme thermique reçue parce que (*i*) les taux de développement reproductif sont deux fois plus grands sur les sites les plus froids (1 et 2) que sur les sites les plus chauds (3 et 4) et, (*ii*) la germination totale des graines ne diffère pas entre les sites. Les auteurs ont également noté une plus faible production de graines pleines sur les sites les plus froids, couplée avec un degré plus élevé du succès de germination sur le site le plus froid (site 1), ce qui semble constituer un mécanisme d'adaptation phénotypique au froid.

Mots-clés : développement reproductif, somme thermique, *Betula papyrifera*, germination, variété.

[Traduit par la Rédaction]

Introduction

The developmental sequence of the reproductive structures and of seed maturation appears to be closely related to the progression of the thermal period (thermal sum) in plants in general (Trudgill et al. 2005), and more specifically in arborescent species (Sarvas 1967). For instance, the flowering season begins when the proportion (percentage) of the annual thermal sum (degree-days > 5 °C, DD) locally reaches 1% in *Alnus glutinosa* L., 4%–5% in *Betula verrucosa* Ehrh., 11%–12% in *Quercus robur* L, 9%–10% in *Picea abies* L., and 15%–17% in *Pinus sylvestris* L. (Sarvas 1967). In *Betula pendula* Roth, blossoming (flowering) induction occurs at ca. 2000 degree-hours > 2 °C (DH), and ovary primordia are visible at ca. 3000 DH (Dahl and Fredrikson 1996). The degree of anatomical development is also related to the attainment of a minimal heat sum, as the sequential stages of gametophyte development and seed ripening follow a sigmoid function of the thermal sum in *Picea mariana* Mill. (Meunier et al. 2007), in *P. abies*, and *Pinus sylvestris* (Almqvist et al. 1998), while a linear function of heat sum fits best in *B. pendula* (Dahl and Fredrikson 1996). Embryo maturity is also generally associated with

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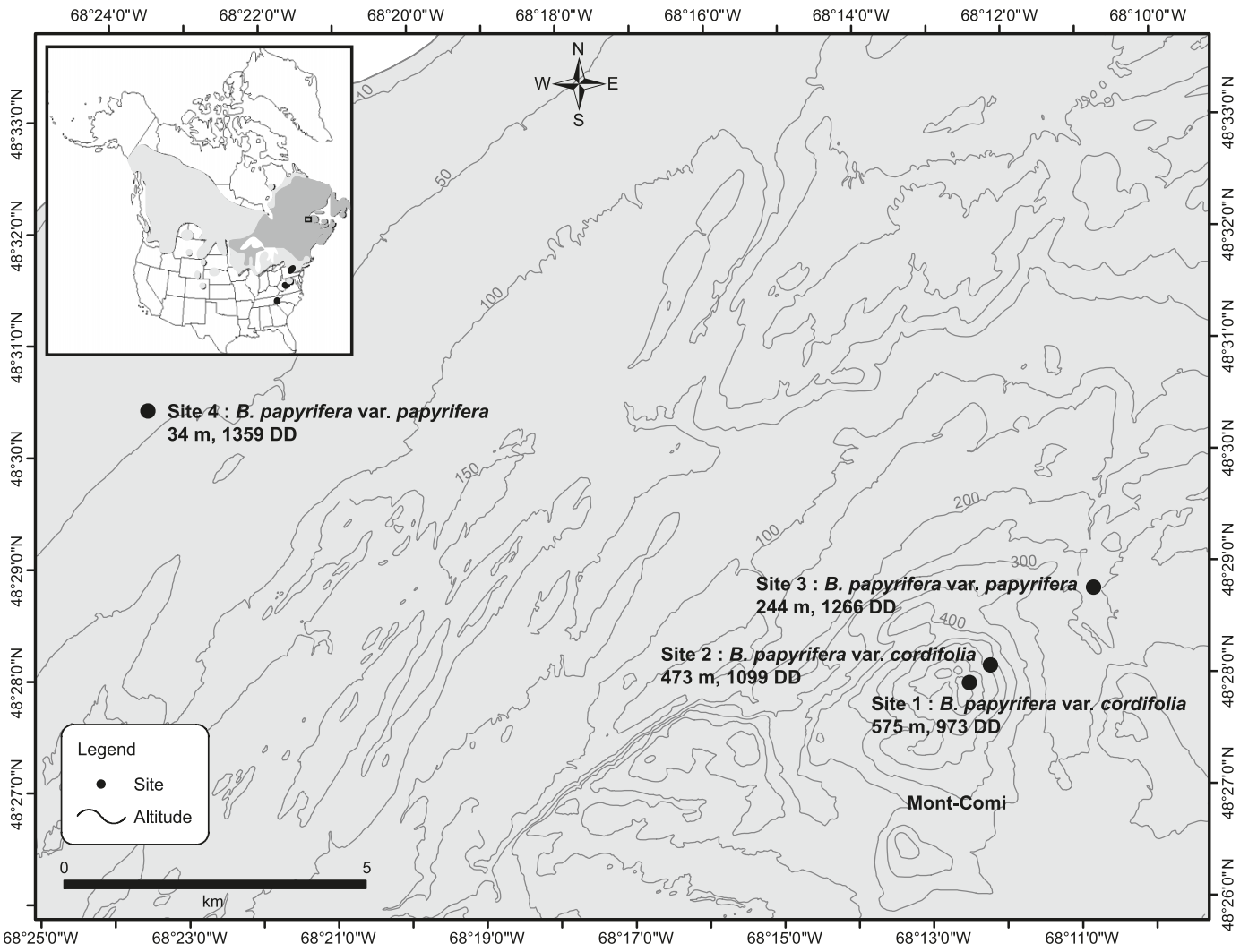
Fig. 1. Geographical location of the four sites chosen along an altitudinal thermal gradient in the eastern Appalachian mountains (Canada). Insert: Geographical distribution of *Betula papyrifera* modified from eFloras (2006*a*, 2006*b*), dots represent disjointed stations of the distribution area. Areas filled in pale grey, black, and medium grey, represent the distribution of var. *papyrifera*, var. *cordifolia*, and areas where both are present, respectively.



reaching a minimal thermal threshold in *Pinus sylvestris* (Henttonen et al. 1986), in *Picea glauca* (Moench) Voss (Zasada 1988), and *Picea mariana* (Mill.) BSP (Sirois et al. 1999). Heat deficit in the final phase of seed ripening appears to be associated with lower germination percentage in *Acer rubrum* L. (Tremblay et al. 1996), *P. abies*, *Pinus sylvestris* (Almqvist et al. 1998), and *P. mariana* (Sirois et al. 1999) in northern sections of their natural geographical range.

In *Betula papyrifera* Marsh., the results obtained are more variable. In the Appalachian mountains, germination in this species is positively correlated with heat sum (Ruel and Ayres 1996) although it is negatively correlated in all other parts of this species' natural range (Bevington 1986; Benowicz et al. 2001). These results could reflect phenological plasticity in *Betula*, which is known for its considerable morphological variability and its frequent hybridization. For example, two varieties of *B. papyrifera*: var. *cordifolia* and var. *papyrifera*, occur in northeastern America (Flora of North America Editorial Committee 1997). These previous studies assumed temperature variations by differences in altitude or latitude without any temperature measurements taken in situ to explain the effect of heat on germination.

In this study, we explain the reproductive development (anatomical development and seed germination) of *B. papyrifera* in relation to the accumulation of thermal sum (DD). Possible encounters with species varieties in the area's study were controlled a posteriori. With a setting taking simultaneous measurements of temperature and reproductive development in sites distributed along an altitudinal thermal gradient, we (*i*) describe the sequence of female anatomical development, and (*ii*) study the relation between DD and reproductive development, as assessed by anatomical analyses and seed germination tests.

Materials and methods

Study sites and study species

The study area is located in the southern boreal forest in the Appalachian mountains (48°33′0″N–48°27′30″N,

Fig. 2. Female reproductive stages of *Betula papyrifera*. (*a*) Stage 1: B, bract; O, ovary. (*b*) Stage 2: TO, ovary tissue; TPO, pre-ovule tissue. (*c*) Stage 3: TO, ovary tissue; LO, ovule primordium. (*d*) Stage 4: LO, ovulary lobe; TO, ovary tissue. (*e*) Stage 5: T, integuments; CMM, megaspore mother cell. (*f*) Stage 6: T, integuments; M, haploid megaspore; N, nucellus.

68°10′30″W–68°24′00″W) in Canada (Fig. 1). The low, hilly landscape is formed by Appalachian sedimentary rocks (Robitaille and Saucier 1998). Growing season (mean daily temperature > 5 °C) lasts 170 d from May to mid-October, and corresponds to 1402 DD (1971–2000) (Environment Canada 2006). Four sites, representing areas of maximum elevation differences, were selected following these criteria: a difference of 100 to 200 m of altitude between them, easy accessibility, and possessing a sexually mature population of *B. papyrifera*.

Betula papyrifera is a monoecious tree whose male and female catkins begin to form during the previous growing season, rest during winter, and finish ripening in the following growing season. In female catkins, flowers are grouped three per scale and expand simultaneously with leaves in spring. In eastern North America, *B. papyrifera* var. *cordifolia* occurs mostly in humid areas, on rocky slopes, in cooler habitats, and at elevations higher than 800 m a.s.l. (Flora of North America Editorial Committee 1997). *Betula papyrifera* var. *papyrifera* and *B. papyrifera* var. *cordifolia* are frequently confused, since they look alike and the form of their leaves is too variable to serve as an unique identification criterion (Brittain and Grant 1965). For these reasons, we proceeded to characterize populations in each site following these criteria: leaf morphology, bract shape, and wing forms on seeds (Brittain and Grant 1965; Farrar 1996; Flora of North America Editorial Committee 1997).

Thermal sum

From mid-April until the end of December 2004, a thermal probe (Hobo® H8 Pro Series) was installed on a tree trunk at a height of 2 m in all sites; the probes were protected from direct sunlight by an opaque white plastic shield and the forest canopy. Their hourly records allow the calculation of the mean daily temperature (T_{md}, °C) that was used to calculate the thermal sum (DD) using the following equation:

$$DD = \sum_{i=1}^{365} (T_{md} - 5)$$

where i represents each day where mean daily temperature (T_{md}) was > 5 °C.

Anatomical development

In each site, one tree was chosen to monitor female anatomical development, and this was done following two criteria: easy access to the tree's branches and a large number of reproductive catkins. In early May 2004, we began harvesting at least three female catkins three times a week from one tree per site. At the beginning of June we increased the lapse of time between harvests to 7 d, because similar studies lead us to believe in a slowing down in embryo development (Sirois et al. 1999). When flowering was completed in all sites (early July) we spaced out harvesting to intervals of 14 d. At this time, pollination was over at all sites.

Each sample was fixed in formaldehyde-acetic acid-alcohol (FAA) for at least 48 h. They were then transferred and kept in 70% alcohol for later use. According to their developmental stage, either entire catkins or seeds were dehydrated and then embedded in paraffin following a modified method by Johansen (1940). Buds were opened while viewing with a binocular microscope at a magnification of 25× to extract the female catkin or the developing fruits, prior to their embedding in paraffin. Sections of 7 µm were prepared with an electronic microtome Shandon Finesse®E+ (Thermo Scientific). Slides were stained with hematoxylin and eosin (Ruzin 1999) and residual paraffin was dissolved with Citrisolv™ (Fisherbrand) clearing agent in a carousel-type automatic slide stainer Shandon Varistain® 24–4 (Thermo Scientific). Covers were permanently mounted with a Shandon Consul®, (Thermo Scientific) cover slipper.

The identification of gametophyte developmental stages was based on previous studies of Maheshwari (1950*a*, 1950*b*), Owens and Blake (1986), and Reiser and Fischer (1993). For each harvest date, at least three catkins or fruits per tree were examined to determine their latest developmental stage. We studied the relation between thermal sum and anatomical developmental stage with a covariance analysis (ANCOVA) of one factor (DD) and one covariate (site). We transformed DD and development stage in neperian log to follow statistical test conditions.

Seed germination

Germination was determined with seeds harvested from five trees per site, including trees monitored for female anatomical development. A minimum of three female catkins per tree were harvested on each site following a 2 week interval between early August until mid-October (when seed dispersal is almost completed), for a total of six harvest dates per site. Catkins were air-dried until the seeds were released and then kept at 4 °C until the germination test. In each site, seeds were mixed together to obtain an estimate of population seed germination. To simulate a natural process of germination, each harvest was divided into two treatments: one with a cold treatment (21 d at 3–4 °C on humidified VersaPak™ cotton) and one without cold treatment. Each treatment was subdivided into 3 replicates of 50 seeds randomly chosen for a total of 144 lots (4 sites × 6 harvests × 2 treatments × 3 replicates). To test germination for all seeds only once, the treatments were placed in an environmental chamber (Conviron® CMP 3244) in the following design: 8 lots per tray (2 harvests × 4 sites) for a total of 18 trays randomly placed in the environmental chamber. The germination test was run for 28 d at 30 °C for 8 h in the light and 20 °C for 16 h in the dark, following a modified method from the International Seed Testing Association (1999). The number of germinated seeds (radicle > 1 mm) was noted, and these were removed each day (Farmer 1997). When this test was over, each nongerminated seed

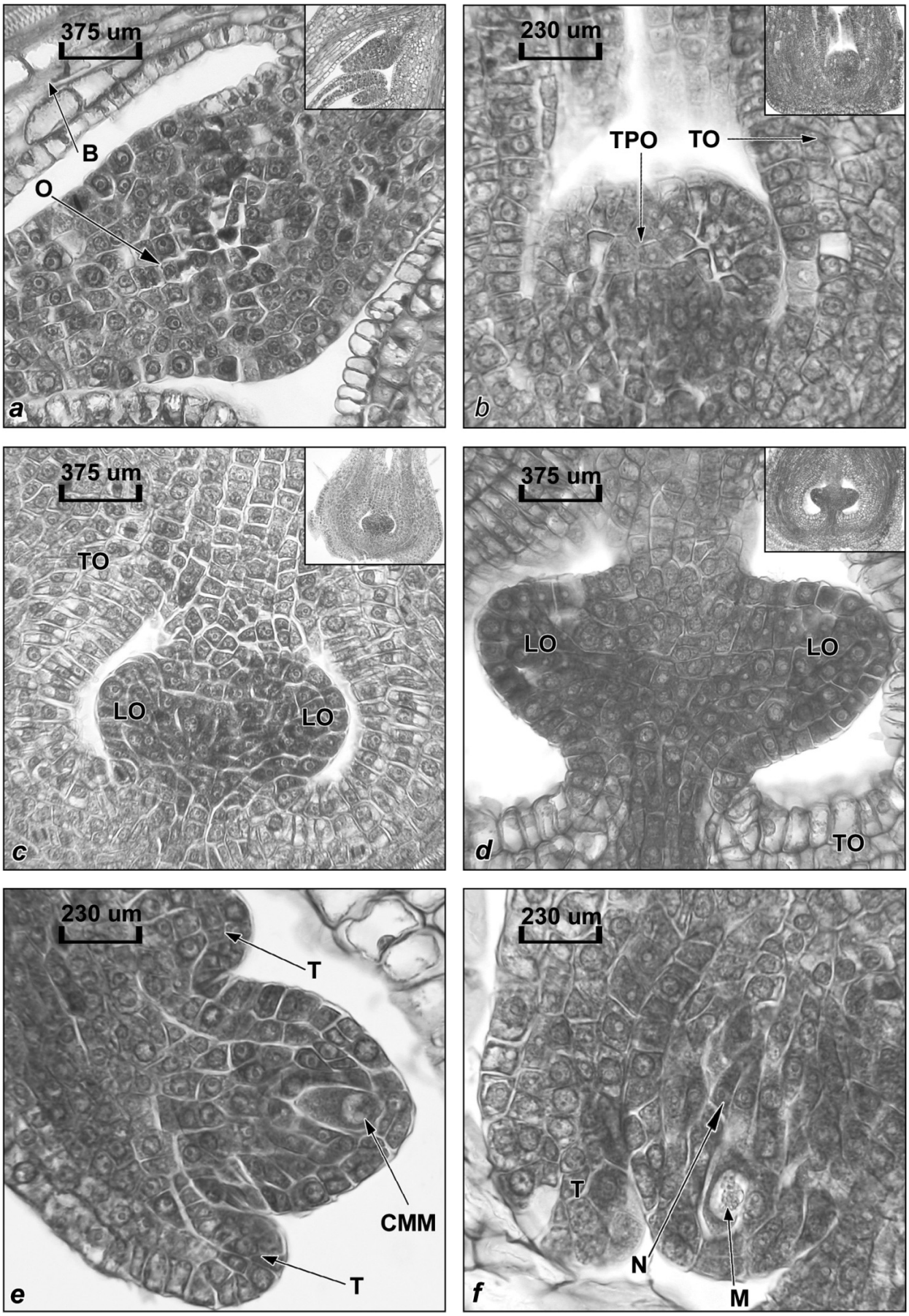

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Fig. 3. Female reproductive stages of *Betula papyrifera*. (*g*) Stage 7: MN, two-nucleate megaspore; N, nucellus; T, integuments. (*h*) Stage 8, megagametophyte: CAP, antipodal cells; NP, polar nuclei; OO, egg cell; N, nucellus; T, integuments. (*i*) OO, egg cells; N, nucellus; T, integuments. (*j*) Stage 9, two-cell proembryo: CA, apical cell; CB, basal cell; E, acellular endosperm. (*k*) Stage 10, four-cell embryo: PE, proembryo; CB, basal cell. (*l*) Stage 11, globular embryo: EG, Globular embryo; S, suspensor; CB, basal cell.

was examined to determine whether they were full, half-full, or empty following a procedure established by Patterson and Bunce (1931). The percentage of seeds that germinated was calculated on full seeds only, and transformed in arcsine, as recommended by Bevington (1986). The number of full and empty seeds were compared between sites with a Kruskal–Wallis test followed by a Tukey's test. We compared total germination between sites for each harvest with a covariance analysis (ANCOVA) of two factors (DD and treatment) and one covariate (site). For this test, we excluded results from site 2 because the germination percentage was dramatically low (0.22%). Then we used the Weibull function (Plait 1962; Brown 1987; Brown and Mayer 1988) to describe and compare seed germination profiles from the six harvests in sites 1, 3, and 4. The Weibull function was applied to the germination percentage of each lot with the nonlinear procedure of Gauss-Newton, using SAS 9.1.3 (SAS Institute Inc., Cary, N.C.). To describe and compare germination profile, we used the Weibull function described as follows:

$$y = M(1 - \exp\{-[k(t - l)]^c\})$$

where M represents total germination, l is first day where germination is > 0, k is the germination rate when 63% of total germination is reached, and c is the shape of the curve (Plait 1962; Brown 1987; Brown and Mayer 1988).

Results

Study sites and study species

Altitude differences between the four sites (541 m a.s.l.) induced a thermal gradient of 386 DD and reflect a regional adiabatic gradient of –3.25 °C. Morphological analysis of *B. papyrifera* revealed that the entire *Betula* population at sites 1 and 2 is composed of var. *papyrifera*, while at sites 3 and 4 the *Betula* population is composed of var. *cordifolia*.

Anatomical development and thermal sum

The first stage of female anatomical development identified was the formation of bracts and ovaries without any internal anatomical features (stage 1, Fig. 2*a*). Later on, a spherical area of tissue (the pre-ovule tissue) forms inside the ovary (stage 2, Fig. 2*b*). Then, this pre-ovule tissue begins to separate to form two ovule primordia (stage 3, Fig. 2*c*). Ovule primordia then expand laterally toward the ovary wall (stage 4, Fig. 2*d*). Following nucellus formation, the megaspore mother cell, characterized by cell enlargement, is established. This cell is located internal to the epidermis of the nucellus, which is made up of two layers of cells. By this stage, integuments have begun to form (stage 5, Fig. 2*e*). When the integuments cover over half the nucellus, megasporogenesis appears to be completed as the remaining haploid megaspore is present (stage 6, Fig. 2*f*). By the time the integuments are fully formed, the megaspore has divided into a two-nucleate megagametophyte (stage 7, Fig. 3*g*). Later on, the mature megagametophyte (stage 8) consisted of two polar nuclei, three antipodal cells (Fig. 3*h*), and an egg (Fig. 3*i*); we did not observe synergids. Following double fertilization, the zygote undergoes mitosis to form a basal and an apical cell (stage 9, Fig. 3*j*). Subsequent mitotic divisions in the apical cell establish a structure with four cells (stage 10, Fig. 3*k*), which then forms a globular embryo. Successive divisions of the basal cell forms a very short suspensor (stage 11, Fig. 3*l*).

The sequence of developmental stages is positively correlated with thermal sum in each site ($\text{Pearson}_{\alpha = 0.05}$; $r^2 > 0.90$). The covariance analysis shows an interaction between sites and thermal sum ($p < 0.001$). Slope comparison shows similarity in developmental seed rate between sites 1 and 2 (Tukey $q = 0.75 < q_{0.05(28,4)}$), and between site 3 and 4 (Tukey $q = 1.57 < q_{0.05(28,4)}$). The slope of site 1 is different from those of site 3 (Tukey $q = 5.71 > q_{0.05(28,4)}$) and site 4 (Tukey $q = 8.03 > q_{0.05(28,4)}$). The slope of site 2 is also different from those of site 3 (Tukey $q = 4.93 > q_{0.05(28,4)}$) and site 4 (Tukey $q = 7.12 > q_{0.05(28,4)}$). Values of the slopes representing the developmental rate of seeds are almost twice as high in sites 1 and 2 (m_1: 1.14; m_2: 1.07) compared with those of sites 3 and 4 (m_3: 0.63; m_4: 0.50) (Fig. 4).

Seed germination and thermal sum

Although there is no difference between the quantity of half-full seeds between sites (Kruskal–Wallis $p = 0.052$), we noted a higher number of empty seeds in sites 1 and 2 (Kruskal–Wallis $p < 0.001$) and higher number of full seeds in sites 3 and 4 (Kruskal–Wallis $p < 0.001$) (not shown). Germination percentage is positively correlated to the thermal sum reached at harvest date for all sites with or without cold stratification (r^2 between 0.72–0.82) (Fig. 5). Cold treatment had no effect on germination ($p = 0.719$). There was a significant interaction between sites and thermal sum ($p = 0.007$). The slope, which expresses seed maturation rate, is two times higher in site 1 (0.11 and 0.11) compared with site 3 (0.07 and 0.05) and 4 (0.05 and 0.04), with or without cold stratification (Fig. 5). The progression of germination among seeds harvested on the 30 July, the 12 August, 28 August, and 11 September did not fit the Weibull model. Only the two last harvests (24 September and 8 October) for sites 1, 3, and 4, fitted the Weibull model. Average germination rate (k) at those harvest dates varied between 0.03 and 0.49 (all sites and harvest dates combined). We observed significant differences between the beginning of germination (l) and total germination (M) only in seeds collected on 8 October. Site 1 displays the lowest germination rate (k) for each harvest date (Table 1).

Discussion

Anatomical development

In this study, we took samples before the onset of bud burst (beginning of May) until the near completion of seed dispersal from catkins (mid-October). This gave us the op-

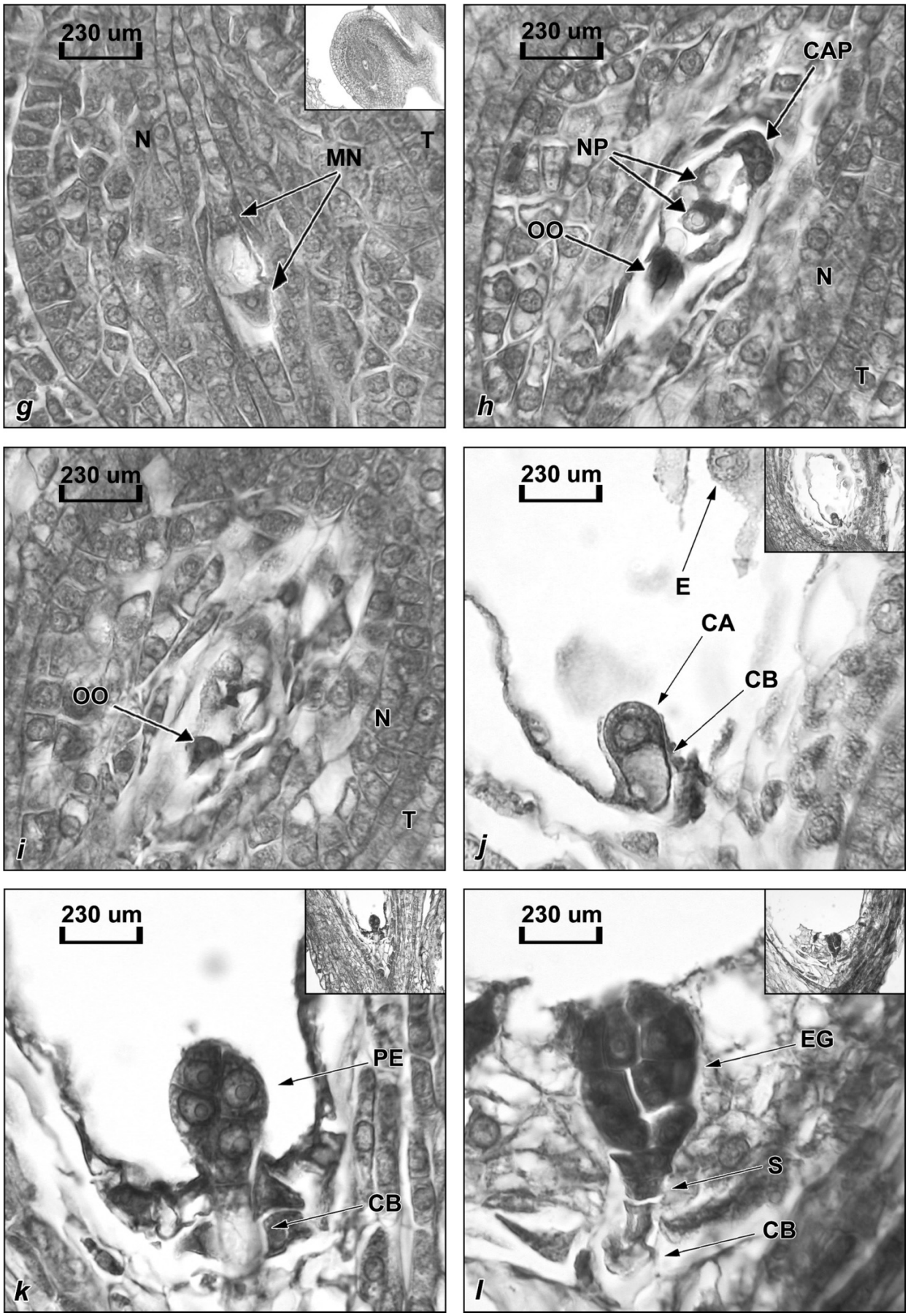
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Fig. 4. Anatomical development stage of *Betula papyrifera* in relation to thermal sum (DD > 5 °C) in four sites (1, 2, 3, 4) along an altitudinal thermal gradient in 2004 (n = 1). Apparent variation in the number of points is a consequence of the repetition of stages for the same thermal sum.

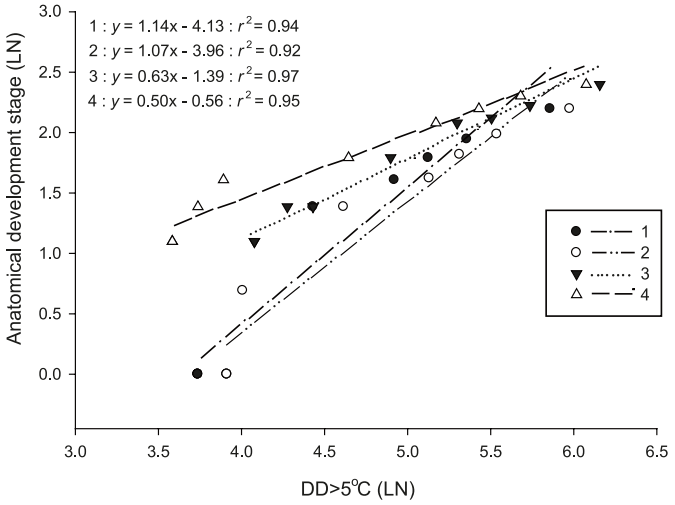
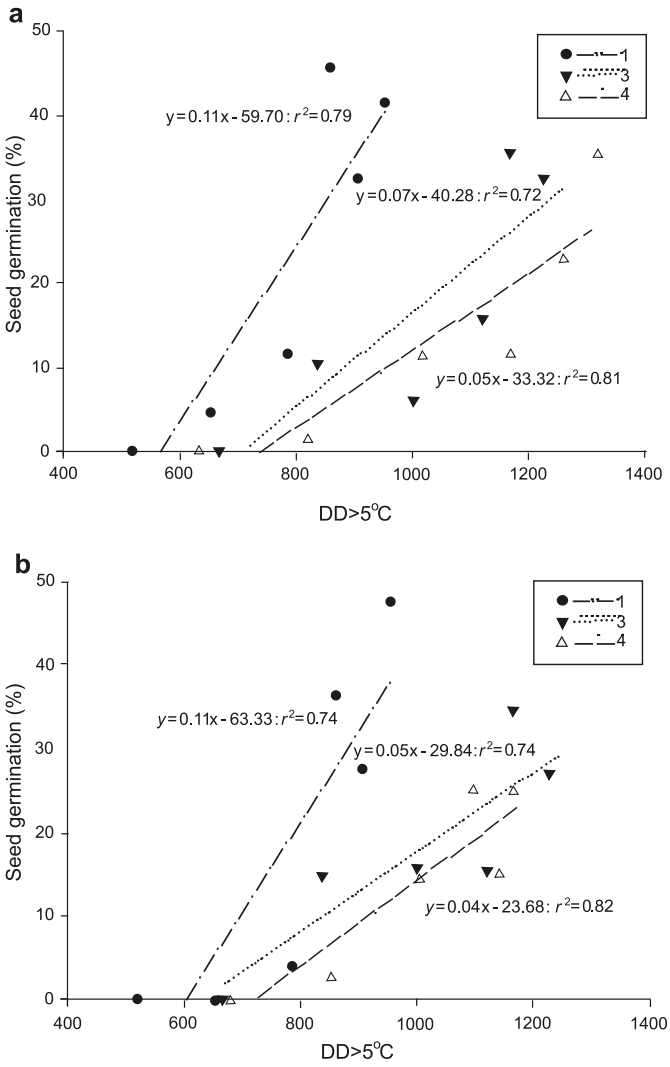


Fig. 5. Seed germination of *Betula papyrifera* in relation to thermal sum (DD > 5 °C) for six harvests (*a*) without cold stratification treatment or (*b*) with cold stratification treatment in three sites (1, 3, and 4) along an altitudinal thermal gradient (n = 3).



portunity to observe floral development and seed ripening of *B. papyrifera* in sites distributed between 34 and 575 m (a.s.l.) and corresponding to a thermal gradient of 386 degree-days. During the first day of harvest, female catkins were still buds, and anatomical analysis of the floral bracts and gynoecium showed that they were in a stage of development attained at end of the preceding growing season (Owens and Blake 1986). After the bud scales open, the peduncles of female catkins elongate and development of the reproductive structures resumes. Our anatomical analysis covers the developmental progression occurring from the fourth stage before the formation of the megaspore mother cell up to the first stages of embryo formation. Observations past those stages were not possible owing to the hardening of the ovary wall forming the pericarp, which resulted in tissue tearing. Overall, development of the megaspore mother cell described here corresponds to that in the *Betula* genus model (Benson 1894), whereas the megagametophyte development is of the monosporic *Polygonum* type (Maheshwari 1950*a*) as found in *B. pendula* (Dahl and Fredrikson 1996). We observed a delay between pollination and the complete formation of the megagametophyte, as is frequently the case in the order Fagales (Benson 1894; Dahl and Fredrikson 1996; Sogo and Tobe 2005, 2006).

Reproductive development and thermal sum

Despite the limitation created by the single year and tree per site sampled in this study, the relationship shown between reproductive development and thermal sum remains robust. The anatomical development begins later but at a higher rate in colder sites (sites 1 and 2) compared with warmer sites (sites 3 and 4). Indeed, until the third harvest date (3 June), female reproductive structures had barely reached the pre-ovule stage (stage 2) in colder sites (1 and 2) while at the same time, megaspore mother cells (stage 6) were already formed in several flowers in the warmer sites (3 and 4). This phenological lag is likely associated with delayed bud burst in trees in sites 1 and 2 compared with sites 3 and 4. Differences in reproductive development tend to decrease later on, owing to the developmental rate being twice as fast in colder sites compared with warmer sites, as shown by the slope values from the relationship between development and thermal sum (Fig. 4). By the end of the growing season, this delay in the reproductive development observed in colder sites does not seem to affect germination capacity. The covariance analysis on total germination and thermal sum does not show any significant differences between sites. Also, the values of the M parameter of the Weibull function show no difference in total germination percentage between sites for 24 September harvest (just before complete seed dispersion). This similarity in germination percentage among full seeds despite a delayed initiation of reproductive development at colder sites (1 and 2) is made possible by the seed developmental rate being twice as high in colder sites than in warmer sites. The exclusion of germination data from site 2 in the covariance analysis limits the analogy one can make between our results and those of Bevington (1986) and Benowicz et al. (2001),

Table 1. Value of parameters, *l*, *k*, *c*, *M* and indices of adjustment (r^2_{adj} and SE_{res}) of the Weibull function adjusted to the averaged cumulative germination ($n = 6$) of *Betula papyrifera* seeds harvested on 9 and 24 September and 8 October 2004, in three sites (1, 3, and 4), along an altitudinal thermal gradient.

Harvest date and site no.	*l*	*k*	*c*	*M*	r^2_{adj}	SE_{res}
11 Sept.	**%DD**					
1	88	n.c.	n.c.	n.c.	n.c.	n.c.
3	87	6.98 (6.94–7.02)	0.25 (0.21–0.29)	0.66 (0.57–0.75)	17.28 (16.38–18.18)	0.995	0.322
4	86	n.c.	n.c.	n.c.	n.c.	n.c.
24 Sept.	**%DD**					
1	93	8.36a (7.20–9.49)	0.03a (–0.10–0.17)	0.61a (0.19–1.03)	56.70a (–33.61–147.00)	0.986	0.956
3	92	6.53b (6.26–6.80)	0.30b (0.27–0.33)	0.84a (0.71–0.97)	36.25a (35.45–37.05)	0.995	0.603
4	93	8.42a (7.67–9.18)	0.40b (0.26–0.54)	0.68a (0.34–1.03)	20.91a (19.44–22.38)	0.970	0.717
8 Oct.	**%DD**					
1	98	10.02a (8.96–11.09)	0.22a (0.08–0.35)	0.53a (0.22–0.84)	50.05a (37.78–62.33)	0.990	0.822
3	97	6.64b (6.55–6.72)	0.49b (0.46–0.52)	0.77a (0.72–0.83)	30.21b (30.02–30.40)	0.999	0.206
4	97	5.59c (4.85–6.33)	0.22a (0.18–0.25)	1.64b (1.27–2.01)	31.69c (31.23–32.14)	0.995	0.714

Note: %DD, the proportion (%) of annual thermal sum (degree-day > 5 °C) cumulated at harvest date. The Weibull function is described as follows: $y = M(1 - \exp\{-[k(t-l)]^c\})$, where *M* represents total germination, *l* is the first day where germination is > 0, *k* is the germination rate when 63% of total germination is achieved, and *c* is the curve shape. Identical letters right of parameter value's indicate nonsignificant difference ($\alpha = 0{,}05$) between sites for a given harvest date, based on the 95% confidence interval values between parentheses. First fourth harvest dates were not convergent (n.c.) with the Weibull function and convergence occurs only at site 3 at the fifth harvest (11 Sept.)- accordingly, the value of the parameters of the Weibull function could not be assessed for sites 1 and 4.

in which the total and the rate of germination were higher in trees growing in colder sites. We cannot provide a satisfactory explanation for the uniquely low (0.22%) germination of seeds from site 2. The birch trees sampled there were producing a normal amount of male and female catkins and their reproductive structures seem to develop normally into variously filled seeds. It seems therefore that the seeds from site 2 were in some physiological state, unrelated to climate, which prevents their germination. Bevington and Hoyle (1981) mentions that viable seed of *B. papyrifera* is not only influenced by climate, but also by site and mother tree.

Our results also suggest that available thermal sum at colder sites (1 and 2) is associated with the decreased proportion of full seeds in *B. papyrifera*. Indeed, this species seems able to modulate mature-seed production according to the available thermal sum, as is the case in black spruce (Sirois 2000). This modulation of mature-seed production under a regional climatic gradient was recently suggested as one of the most important mechanism of phenotypic adaptation in plants towards climatic changes (Trudgill et al. 2005) and was also demonstrated in *P. mariana* (Meunier et al. 2007). It remains to be demonstrated whether these phonological traits are associated with the varieties *papyrifera* and *cordifolia* found in the study area. Nevertheless, this tradeoff made between seed quantity and seed quality could be seen as an adaptive mechanism at reduced thermal sum in several plants.

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