Sixteen years of winter stress: an assessment of cold hardiness, growth performance and survival of hybrid poplar clones at a boreal planting site

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ABSTRACT

In recent years, thousands of hectares of hybrid poplar plantations have been established in Canada for the purpose of carbon sequestration and wood production. However, boreal planting environments pose special challenges that may compromise the long-term survival and productivity of such plantations. In this study, we evaluated the effect of winter stress, that is, frequent freeze-thaw and extreme cold events, on growth and survival of 47 hybrid poplar clones in a long-term field experiment. We further assessed physiological and structural traits that are potentially important for cold tolerance for a selected set of seven clones. We found that trees with narrow xylem vessels showed reduced freezing-induced embolism and showed superior productivity after 16 growing seasons. With respect to cold hardiness of living tissues, we only observed small differences among clones in early autumn, which were nonetheless significantly correlated to growth. Maximum winter cold hardiness and the timing of leaf senescence and budbreak were not related to growth or survival. In conclusion, our data suggest that reduction of freezing-induced embolism due to small vessel diameters is an essential adaptive trait to ensure long-term productivity of hybrid poplar plantations in boreal planting environments.

Key-words: boreal forest; freezing-induced embolism; frost damage; vessel diameter.

INTRODUCTION

Intensive plantation forestry with fast-growing hybrid poplars has been advocated for its CO_2 sequestration potential, and in Canada thousands of hectares of hybrid poplar plantations have been established under the federal 'Forest 2020' afforestation initiative to help meet greenhouse gas reduction targets (Dominy *et al.* 2010). The boreal and sub-boreal planting environments throughout Canada pose special challenges however, and planting stock needs to be well adapted to harsh winter conditions. While

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drought tolerance and productivity of hybrid poplars have been relatively well researched (Monclus *et al.* 2006; Hogg, Brandt & Michaelian 2008; Silim *et al.* 2009; Soolanayakanahally *et al.* 2009; Schreiber *et al.* 2011), studies that investigate winter biological traits of hybrid poplars are limited, particularly linking these traits with growth performance (cf. McCamant & Black 2000; Tsarouhas, Kenney & Zsuffa 2001; Friedman *et al.* 2008). Specifically, extreme minimum temperatures and frequent freeze-thaw events may play an important role in survival and long-term productivity of hybrid poplar plantations in boreal planting environments.

Plants exposed to frequent freeze-thaw events must develop mechanisms to overcome the stress applied to their organs to remain functional. A recent study by Schreiber *et al.* (2011) showed a strong negative relationship between height and branch vessel diameters of hybrid poplars (*Populus* spp.) as well as trembling aspen (*Populus tremuloides* Michx.) at a boreal planting site. This planting site is characterized by frequent freeze-thaw events in spring, and winter minimum temperatures as low as –40 °C. A possible mechanism explaining the unexpected negative relationship between growth and vessel diameter may be the occurrence of frost-induced embolisms. It has previously been shown that wider xylem conduits are more likely to embolize when exposed to frequent freeze-thaw events (e.g. Davis, Sperry & Hacke 1999; Pittermann & Sperry 2003; Mayr, Gruber & Bauer 2003; Cobb, Choat & Holbrook 2007; Choat *et al.* 2011) and thus trees with wider vessels would experience impaired water conduction after budbreak in spring, decreased photosynthetic rates and eventually reduced growth (Wang, Ives & Lechowicz 1992; Castro-Diez *et al.* 1998; Cavender-Bares *et al.* 2005). These conditions may be particularly pronounced in poplars as they do not seem to refill winter embolism by developing root pressure, a behaviour that, for example, is seen in birch or alder species (Sperry *et al.* 1994; Hacke & Sauter 1996).

Freeze-thaw events, besides inducing embolism, may also significantly contribute to frost injury and shoot dieback over winter. Living tissues are mostly affected when water transitions from the liquid into the crystalline state, causing plasma membrane destruction and eventually cell death (Sakai & Larcher 1987). Injury to tissues can also occur by

means of extracellular freezing, resulting in considerable desiccation stress on the protoplasm as a result of water removal from cells (Burke *et al*. 1976). Multiple freeze-thaw events during winter may amplify the extent of frost injury due to mechanical wear of cell components, caused by volume changes of water from the liquid into the crystalline state and vice versa. Frost injury damage often results in cankers, dieback, frost cracked stems and distortion of developing organs (Cayford *et al*. 1959; Zalasky 1976). Late spring frosts can also severely harm a tree in which growth is already initiated and early tissues are not lignified, particularly due to warmer winters and earlier dormancy release (Beaubien & Hamann 2011).

In the present study, we analysed a long-term, repeatedly measured field experiment with 47 hybrid poplar clones in central Alberta, Canada. Height and winter survival were evaluated in autumn and spring for the first four years after planting. Subsequently, height, diameter at breast height (DBH) and survival were measured at the end of an additional 12 growing seasons. Further, we evaluated physiological and structural traits that are considered potentially important for cold adaptation. The timing of leaf senescence and budbreak was quantified for all clones, and in a selected subset of seven clones we measured the amount of native xylem embolism in autumn, winter and spring of 2010 and 2011, as well as frost hardiness of living tissue several times throughout autumn of 2011. The objective of this paper is to identify traits relevant for cold adaptation, ensuring survival and long-term productivity of hybrid poplars in a boreal planting environment. Specifically, we hypothesized that the degree of freezing-induced embolism plays an important role in cold adaptation and growth performance of hybrid poplars based on previous research (Schreiber *et al*. 2011), in which the authors found a strong negative relationship between vessel diameter and tree height at a boreal planting site. We also evaluated repeated field measurements in conjunction with historical climate data to understand genetic differences in growth and survival among clones as a function of environmental stressors. This research may help to guide selection of appropriate hybrid poplar clones and species for future afforestation efforts in boreal environments by identifying key traits in adaptation to winter stress.

MATERIALS AND METHODS

Plant material

We evaluated a hybrid poplar field trial established by Alberta-Pacific Forest Industries Inc. near Boyle, Alberta, Canada (54°49′N, 113°31′W, 570 m a.s.l.). The trial was established with 47 clones in June 1993 (see Supporting Information Table S1 for the list of clones). Over-winter dormant stock was planted in a randomized complete block design with five blocks and five-tree row plots of each clone in each block. Trees were planted on a 2 m within row by 3 m between row spacing. The trial was managed intensively for the first three years using cultivation and hand weeding, after which pulp mat sheets were placed around each tree to control competition. Height and DBH measurements for all clones were taken annually in autumn until 2008, except for the year 1998, and additional height measurements in spring were recorded for the years 1994–1997 to capture winter dieback during the first 4 years after planting. As height and DBH in 2008 were highly correlated ($r = 0.94$, $P < 0.05$), we only discuss one trait, but the complete set of measurements is provided in the Supporting Information Table S1. Other measurements taken on all individuals of the experiment were the timing of leaf senescence and budbreak (see details below).

For the evaluation of physiological and wood structural traits, we sampled a total of seven clones with contrasting growth performances for subsequent testing (Table 1). These clones were labelled as 'High', 'Average' and 'Poor' performing genotypes based on height in 2008 and survival (Fig. 1). The chosen subset included two clones of each performance group plus the 'Walker' clone as a reference, as it is widely used in plantations and shelterbelts in western Canada. Cold hardiness, xylem vessel diameter and the

Table 1. Seven selected hybrid poplar clones with code, parental background, performance grouping, height and DBH data measured in 2008, as well as percent survival for the time period 1993–2008

Clone ID	Code/name	Genus	Female parent (species/hybrid[a])	Male parent (species/hybrid[a])	Performance group	Height 2008 (m)	DBH 2008 (cm)	Survival 1993–2008 (%)
24	Walker	*Populus*	*deltoides*	×*petrowskyana*	Walker	14.0 (0.6)	14.7 (1.1)	96.0
33	P38P38	*Populus*	*balsamifera*	*simonii*	High	14.3 (0.6)	19.8 (1.1)	84.0
36	Brooks #1	*Populus*	*deltoides*	×*petrowskyana*	High	13.7 (0.6)	17.5 (1.1)	87.5
18	4435	*Populus*	*balsamifera*	×*euramericana*	Average	12.3 (0.9)	11.0 (1.7)	35.3
48	TACN 1	*Populus*	*laurifolia*	*nigra*	Average	11.9 (0.7)	13.3 (1.4)	40.0
52	DTAC 24	*Populus*	*angulata*	*trichocarpa*	Poor	8.3 (0.6)	8.3 (1.2)	65.0
32	DTAC 22	*Populus*	*angulata*	*trichocarpa*	Poor	6.2 (0.6)	4.5 (1.1)	60.0

[a]Hybrids are designated by an × in front of the parent.
The standard error of the mean is given in parentheses.
P. × *petrowskyana* is a hybrid of *P. laurifolia* and *P. nigra*.
P. × *euramericana* is a hybrid of *P. deltoides* and *P. nigra*.
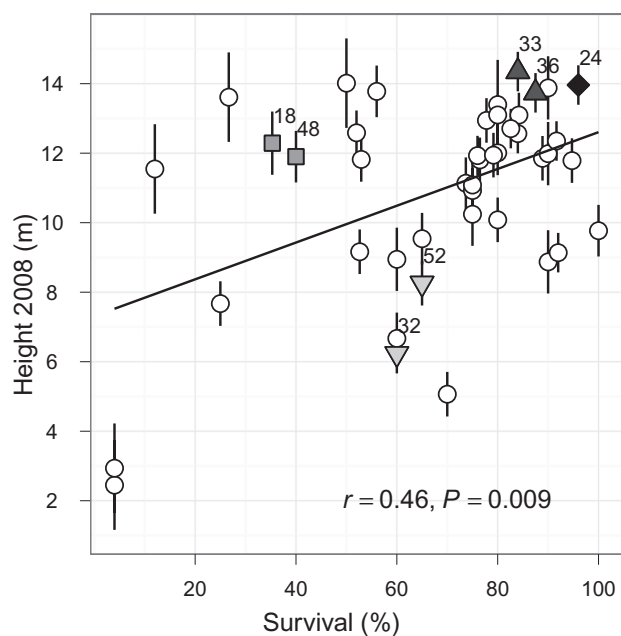DBH, diameter at breast height.

Figure 1. Relationship of average height and survival of each clone in 2008 after 16 growing seasons. Survival, expressed in percent, was calculated as individual tree count per clone at the end of 2008. Symbols represent performance groups: Triangles: high performer; squares: average performer; upside -down triangles: poor performer; diamond: reference clone Walker; open circles: all other clones.

amount of native embolism were measured on these seven clones with eight replicates per clone on multiple dates (see sections below). All samples were taken from 2 to 3-year-old branches from approximately 6 m using a 4 m telescope pruner and were processed within the next 7 d. The material was packed in plastic bags and stored at 4 °C in a walk-in refrigerator at the University of Alberta.

Climate data

Daily minimum and maximum temperatures (*TMIN*, *TMAX*) for the period from 1980 to 2009 were obtained from the *National Climate Data and Information Archive* (http://www.climate.weatheroffice.gc.ca) for the climate station *Athabasca 2* (Station ID 3060321). Furthermore, a 30 year daily average temperature (*TAV*) was calculated to identify seasonal climate anomalies. Winter dieback of the leader was calculated as the difference in height between the spring measurement of a given year and the preceding height measurement in autumn. Freeze-thaw events throughout the winter season were calculated as the difference between *TMAX* and *TMIN* for only those days on which *TMAX* was equal to or greater than 5 °C and *TMIN* was equal to or less than −5 °C (Figs 2 & 4).

Spring and autumn phenology

Timing of leaf senescence and budbreak were measured in autumn 2010 and spring 2011, respectively, on an eight-level

senescence scale based on Fracheboud *et al.* (2009) and a five-level bud development scale (see Supporting Information Table S2). Leaf senescence was measured on 21 September, 23 September, 25 September, 28 September and 2 October, and budbreak on 8 May, 11 May, 13 May, 15 May and 17 May. Budbreak was represented by score 2 (leaves extended but unfolded), and leaf senescence by score 4 (more yellow than green leaves). The dates of budbreak and leaf senescence were calculated for each individual tree. If the required score was recorded multiple times, the date of the phenological event was calculated as an average. If the required score was not directly recorded, the date of the phenology event was inferred by means of linear regression from the bracketing scores.
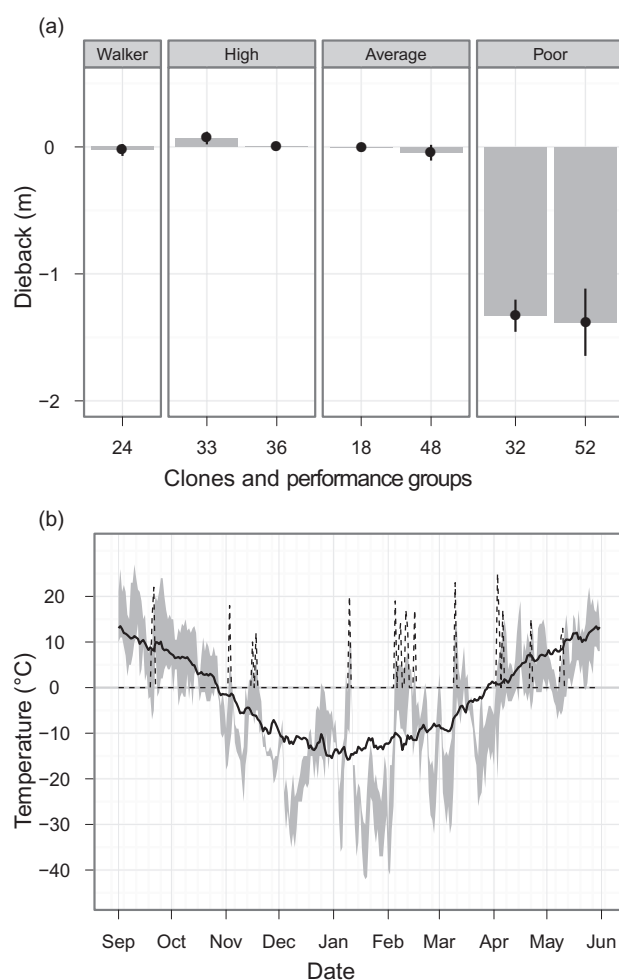

Figure 2. (a) Mean winter dieback for the season 1995/1996 in metres for seven selected clones grouped by performance (Walker, high, average, poor). Error bars represent the standard error of the mean. (b) Daily minimum and maximum temperature for the winter season 1995/1996 (grey-shaded ribbon) and the 30 year daily average temperature (solid black line) for the time period 1980–2009. The dashed grey line represents freeze-thaw events for days when *TMIN* was equal or less than −5 °C and *TMAX* was equal or greater than +5 °C (see text for details).

Cold hardiness

Cold hardiness of living tissue was measured using the electrolyte leakage method (Zhang & Willison 1987; Morin *et al.* 2007), which quantifies the amount of frost damage in living tissue by measuring the electrolyte leakage from the symplast into the apoplast due to damaged plasma membranes. Plant material was collected in 2011 on 22 August, 12 September and 10 October. Current year branches were cut into 5 cm pieces and placed in 30 mL high-density polyethylene bottles (Fisherbrand, Fisher Scientific Company, Ottawa, ON, Canada). To induce ice formation, 5 mL of deionized water was added to the sample before freezing treatments were applied. The freezing treatments were 8 °C (control), −5, −10, −20, −40 °C on 22 August; 8 °C (control), −10, −20, −40, −60 °C on 12 September; and 8 °C (control), −10, −20, −40, −80 °C on 10 October. A programmable freezer (Model 85-3.1A, Scientemp Corp., Adrian, MI, USA) was used to cool samples at a rate of 7 °C per hour, holding the target temperature for 1 h, before re-warming to 8 °C. Subsequently, each segment was cut into 5 mm pieces, 20 mL deionized water was added, and samples were stored for 15–18 h at 8 °C and manually shaken three times during storage. After storage, the amount of electrolyte leakage was measured at room temperature using a conductivity meter (Oakton Acorn CON 6 Meter, Oakton Instruments, Vernon Hills, IL, USA). Conductivity readings were taken before (c1) and after (c2) all samples were boiled at 100 °C for 30 min. The relative amount of electrolyte leakage (REL) was calculated as (c1/c2) × 100 and used to determine the amount of cell lysis (L) in percent, where $\overline{REL}_c$ is the mean value of the control samples:

$$L = \frac{REL - \overline{REL}_c}{100 - \overline{REL}_c} \times 100$$

Native embolism

Percent native embolism (PLC_N) was measured using the flushing method (Sperry, Donnelly & Tyree 1988). Long branches were cut from trees in 2010 and 2011 on 2 October, 8 April and 23 May. In the laboratory, two 14 cm-long segments were cut from these branches under water, and hydraulic conductivity (K_h) was measured as described previously (Hacke & Jansen 2009). Briefly, K_h was calculated as the quotient of flow rate through a segment and the pressure gradient. The tubing apparatus consisted of an elevated water reservoir connected to an electronic balance (CP225D; Sartorius, Göttingen, Germany) via Tygon tubing. The balance was interfaced with a computer using Collect 6 software (Labtronics, Guelph, Canada) and logged K_h every 10 s. An initial measurement represented the native conductivity of the segment. The segment was then flushed with filtered (0.2 μm) measuring solution (20 mM KCl + 1 mM CaCl$_2$) at 40 kPa for 15 min, and the maximum conductivity was determined. PLC_N was calculated as the percentage loss of conductivity relative to the maximum conductivity.

Vessel diameter

Xylem vessel diameters (Dv) were taken from a previous study (Schreiber *et al.* 2011) for analysis in a new context. All other traits in this study were measured on the same individual trees as xylem vessel diameters in Schreiber *et al.* (2011). Briefly, mean hydraulic vessel diameters were calculated based on the Hagen–Poiseuille equation. The vessel diameter that corresponds to the average lumen conductivity was calculated as $Dv = [(\Sigma d^4)/n)^{1/4}]$, where n is the number of vessels measured, and d is the individual vessel lumen diameter.

Statistical analysis

Statistical analyses were carried out using the R programming environment (R Development Core Team 2011). Data exploration and plotting were carried out using the R packages *plyr* (Wickham 2011) and *ggplot2* (Wickham 2009). To take advantage of the blocked experimental design, the data were analysed using a mixed effects model available through the R package *lme4* (Bates, Maechler & Bolker 2011) with the *lmer()* function. The dependent variables were native embolism (PLC_N) and cell lysis (L), and the fixed effects in this model were *group* (Walker, High, Average and Poor) and *sample date*. The random terms were *block, clone* and each clone's unique *ID* to remove temporal pseudoreplication for multiple measurements on the same clone at different dates. In cases where inferences were based on multiple statistics, experiment-wise *P* values were reported using the Holmes adjustment according to Peres-Neto (1999) for multiple correlations, or using the Tukey adjustment for multiple mean comparisons following analysis of variance.

RESULTS

Growth and survival in the field

Survival and height after 16 growing seasons was highly variable among the 47 clones tested in the field experiment (Fig. 1, Supporting Information Table S1). Overall, height and survival were significantly correlated ($r = 0.46$, $P = 0.009$, d.f. = 42 due to three clones that had no surviving individuals by the final measurement). The high-performing clones as well as the Walker clone showed high survival (84, 87.5 and 96%) and were among the tallest trees after 16 growing seasons (14.3, 13.7 and 14 m). The average-performing clones showed low survival (35.3% and 40%) but were among the tallest trees (12.3 and 11.9 m). The poor-performing trees had intermediate survival rates (60 and 65%) but were the shortest of the seven clones (6.2 and 8.3 m).

Putative frost damage, measured as the height of each tree in autumn minus the height of the leader after budbreak in spring, is shown in Figure 2a. Of the first four years, the 1995/1996 winter appears the most extreme with a variety of potential stress conditions (Fig. 2b, and see

Supporting Information Fig. S1 for data from all winter seasons). The 1995/1996 winter season shows very low extreme cold events exceeding −40 °C, which are rare events that were only observed at four other dates between 1980 and 2009 (data not shown). In addition, winter temperatures in 1996 were highly fluctuating with unusual warm periods in January and the first half of February, followed by large temperature drops of 35 to 50 °C. Furthermore, a late spring frost in early May 1996 was recorded with minimum temperatures of −7 °C. The 1995/1996 winter season coincided with an average dieback of approximately 1.35 m of the two clones 32 and 52, selected from the poor-performance group (Fig. 2a). Other clones from the same performance group suffered an average dieback of approximately 30 cm during the 1995/1996 winter season. No significant dieback was observed in the other performance groups.

Cold hardiness

The onset of cold hardiness of living tissue, evaluated as percent cell lysis (%L) occurred relatively early, between mid-August and mid-September on all hybrid poplars tested (Fig. 3a). Late summer measurements showed significant differences between performance groups at −10 and −20 °C on 22 August (Table 2). It should be noted that frost events of −10 or −20 °C in mid-August are extremely unlikely, and were not observed over the course of this field experiment (data not shown). Nevertheless, we note that cell lysis at −10 °C on 22 August significantly correlated with height (Fig. 3b). For cell lysis at both −10 and −20 °C in late summer, significant differences were found between the high- and poor-performing groups (Table 2). In contrast, by mid-September and especially mid-October, all clones appear to be well hardened. By early October, cell lysis was low even when tested under extreme artificial freezing conditions of −40 and −80 °C (Table 2). While we did observe significant differences between average- and poor-performing groups in October, these differences were not correlated with height as in August and remained below 33% (Table 2, Supporting Information Fig. S2, Supporting Information Table S3).

Native embolism

The degree of native embolism was measured three times. The first measurement was taken at the end of the growing season in 2010 and prior to any major frost event representing a baseline or control (Fig. 4); the second measurement was taken during the peak period of freeze-thaw events in early April 2011, when plants were still dormant; and finally, the third measurement was taken well into the subsequent growing season at the end of May 2011. Performance groups differed significantly at the 2 October and 23 May measurements, but not at the 8 April measurement (Fig. 4a, Table 2). Within groups and across dates, only the average- and poor-performing groups differed significantly in the amount of embolism when measured on different dates
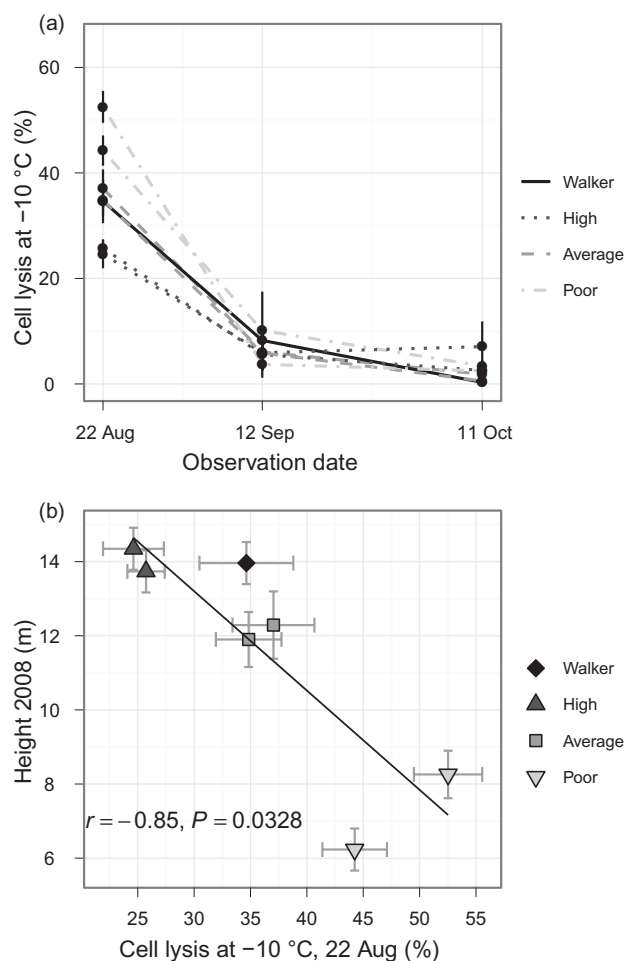

Figure 3. (a) Mean cell lysis at −10 °C measured three times in autumn 2011 (22 August, 12 September and 11 October). A solid line signifies the Walker clone; a dotted line signifies individuals of the high-performing group; a dashed line signifies the average-performing group; and a dot-dashed line signifies the poor-performing group. (b) Correlation between tree height and average cell lysis at −10 °C for 22 August. Error bars represent the standard error of the mean.

(Table 2). No significant differences in native embolism were found for Walker and the high-performing group across different dates. Notably, native embolism in May was positively correlated with vessel diameter (Fig. 5a) and negatively correlated with tree height (Fig. 5b).

Timing of budbreak and leaf senescence

The onset of leaf senescence in autumn and the timing of budbreak in spring appeared to be remarkably uniform among performance groups, which showed virtually identical timing (Table 2). In addition, variance of clones within performance groups was minimal, with all clones breaking bud or showing leaf senescence within a week (Supporting Information Fig. S3). No significant correlations of leaf senescence and budbreak with native embolism, vessel diameter or height were found.

Table 2 Means (cell lysis, PLC_N) and medians (day of year) of physiological and phenological parameters for each performance group

Physiological/phenological parameter	Performance group			
	Walker	High	Average	Poor
Cell lysis (%)				
22 August, −10 °C	34.6 (4.2)[AB]	25.2 (1.5)[A]	35.9 (2.3)[B]	48.4 (2.3)[C]
22 August, −20 °C	73.8 (2.0)[AB]	70.3 (1.5)[A]	69.5 (1.9)[A]	79.1 (1.5)[B]
11 October, −40 °C	4.9 (0.7)[AB]	14.3 (5)[AB]	1.4 (0.5)[B]	18.8 (2.2)[AC]
11 October, −80 °C	17.0 (2.1)[AB]	24.6 (4.4)[AB]	12.7 (2.2)[B]	32.6 (1.6)[AC]
PLC_N (%)				
2 October	30.0 (4.1)[AB,a]	21.7 (2.4)[A,a]	38.3 (3.5)[BC,b]	47.8 (3.6)[C,b]
8 April	19.8 (3.3)[A,a]	19.8 (2.6)[A,a]	19.8 (1.8)[A,a]	28.0 (3.0)[A,a]
23 May	26.8 (3.7)[A,a]	31.7 (3.0)[AB,a]	45.7 (5.1)[BC,b]	57.0 (3.5)[C,b]
Phenology (day of year)				
Leaf abscission	267 (24 September)	267 (24 September)	267 (24 September)	266.5 (23 September)
Bud break	135 (15 May)	135 (15 May)	135 (15 May)	134 (14 May)

The standard error of the mean is given in parentheses. Different uppercase letters indicate significant mean differences after Tukey adjustment for multiple mean comparisons *between* performance groups for each date (to be read horizontally). Different lowercase letters indicate significant differences *within* performance group for different dates (to be read vertically, only for PLC_N). No letters indicate that no statistical significance testing was carried out.

DISCUSSION

Our results allow us to investigate and discuss several alternative mechanisms that may play a role in cold adaptation and potentially impacting growth performance of boreal forest trees. The synchrony of budbreak and leaf senescence with the available growing season, the timely onset of frost hardiness and absolute winter hardiness, and the structural xylem properties are all potentially important traits for cold adaptation. The most notable result appears to be a strong differentiation of performance groups in measurements of native embolism (Fig. 4), a strong positive correlation of native embolism with vessel diameter (Fig. 5a) and a strongly negative correlation of native embolism with height (Fig. 5b). Notably, these correlations are not just a result of a size effect, which has been previously discussed in Schreiber *et al*. (2011). In our study, branches were similar in age and were taken from the same height, regardless of the size of trees. In this situation, vessel diameters may be expected to be wider in larger trees than in smaller ones (Weitz, Ogle & Horn 2006). We observed the opposite, suggesting that the trend in vessel diameter was not a consequence of a size effect.

These results suggest that narrow vessel diameters minimize the extent of freezing-induced embolism. Embolized xylem tissue would result in decreased hydraulic conductivity, which in turn limits photosynthetic rates and eventually growth (Wang *et al*. 1992; Castro-Diez *et al*. 1998; Tyree 2003; Cavender-Bares *et al*. 2005). Similarly to height, survival was negatively correlated with vessel diameter ($r = -0.56$, $P = 0.192$), cell lysis ($r = -0.39$, $P = 0.394$) and native embolism ($r = -0.70$, $P = 0.107$). While none of these correlations were significant, the trends do indicate that under boreal planting environments, there appear to be no fundamental differences in tradeoffs with respect to height versus survival. Adaptive traits that increase the probability

of survival (e.g. small vessel diameters) also result in larger trees after multiple growing seasons. Hence, narrower vessels would explain the observed greater height for trees in an environment that is characterized by frequent freeze-thaw events. A negative correlation between vessel diameter and tree height is likely restricted to boreal or high-elevation environments (e.g. Fisher *et al*. 2007; Schreiber *et al*. 2011), while an opposite correlation has been observed in tropical environments (Poorter *et al*. 2010; Zach *et al*. 2010; Fan *et al*. 2012).

Further, our data showed that the amount of native embolism (PLC_N) decreased over winter and increased again right after budbreak at the start of the growing season (Fig. 4a,b). A decrease in PLC_N from autumn to winter was not expected and may be due to recovery of embolized vessels. Mayr, Schwienbacher & Bauer (2003) observed similar trends for conifers at the alpine timberline and proposed the existence of refilling mechanisms that enable species to recover from embolism in late winter. In diffuse-porous beech trees (*Fagus sylvatica*), Cochard *et al*. (2001) observed similar results and postulated restoration of branch hydraulic conductivity due to a combination of active refilling of embolized vessels through root pressure (although rather weak in beech), and through the formation of new functional xylem after cambial activity was initiated. In the current study, however, we observed a recovery mechanism that must have occurred before growth was initiated (Fig. 4a,b). As poplars are not known for developing root pressure, an alternate hypothesis may be that in late autumn when soils were still unfrozen and leaves were shed, near atmospheric xylem pressures led to bubble dissolution.

Figure 4a,b also shows a significant increase in PLC_N for the average- and poor-performing groups when measured on 8 April and 23 May of 2011 (Table 2), indicating a possible threshold vessel diameter at which freezing-induced
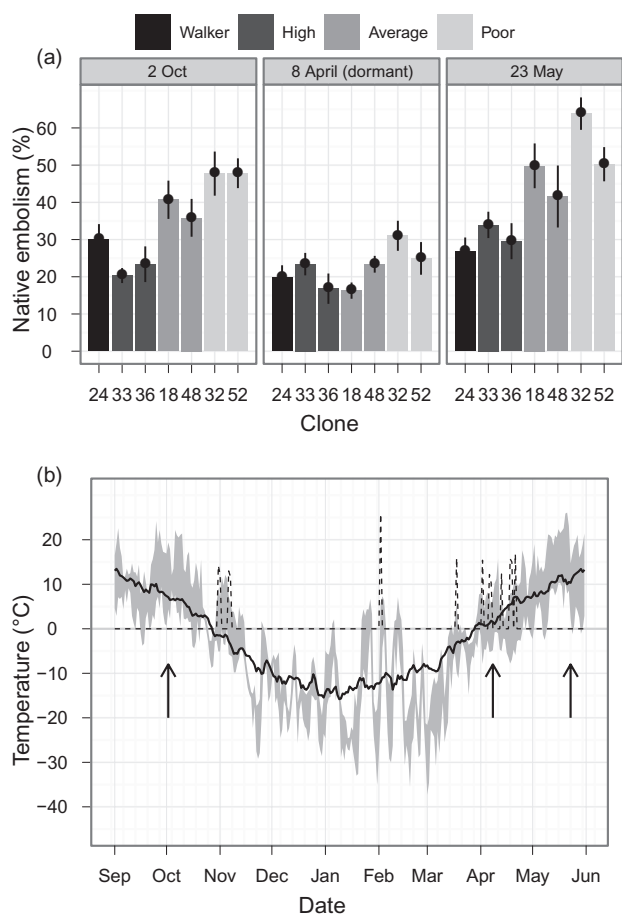
Figure 4. (a) Mean native embolism in percent measured over three dates in 2010/2011 (2 October, 8 April, 23 May) is given for the seven selected clones grouped by performance (Walker, high, average and poor). Error bars represent the standard error of the mean. (b) Daily minimum and maximum temperature for the winter season 2010/2011 (grey-shaded ribbon) and the 30 year daily average temperature (solid black line) for the time period 1980–2009. The dashed grey line represents freeze-thaw events for days when *TMIN* was equal or less than –5 °C and *TMAX* was equal or greater than +5 °C (see text for details). The arrows indicate the sample dates.

embolism increased significantly. The reference clone Walker, as well as the high-performing group, did not show significant differences between the three dates at which PLC_N was measured (Table 2), suggesting an optimal mean vessel diameter of < 28 µm (Fig. 5a) given the local climate conditions. Previous studies (Davis *et al.* 1999; Pittermann & Sperry 2003) demonstrated that plants with a mean conduit diameter below 30 µm experienced little embolism following a single freeze-thaw event at a xylem pressure of –0.5 MPa, while species with conduit diameters greater than 30 µm exhibited considerable embolism. Fisher *et al.* (2007) also observed mean vessel diameters of 27.5 µm for high-elevation populations of *Metrosideros polymorpha* compared with 35.5 and 32.9 µm for populations found at middle and low elevation, respectively. Taken together, these results underpin the adaptive significance of vessel

diameter in influencing tree height and performance in an environment characterized by frequent freeze-thaw events, and that mean vessel diameter may be an important trait to consider for poplar breeding programmes in the Canadian prairies.

As a possible alternative trait that could be important for cold tolerance, we tested the effect of frost injury, measured as %L, on tree height (Fig. 3). These results only show clear differences in clonal performance for %L at –10 °C and only for trees sampled on 22 August, implying a very early date for the onset of frost hardiness. High-performing trees appear to be hardier than average- and poor-performing trees in August and were hardy enough to sustain moderate sub-zero temperatures without major damage. By the end of August, all groups were hardy enough to sustain moderate sub-zero temperatures without major damage. By October, all clones could withstand –40 °C frost events that were extremely rare in the field, even in mid-winter. Once hardy, these clones even withstood extreme experimental treatments of –80 °C in October, which agrees with
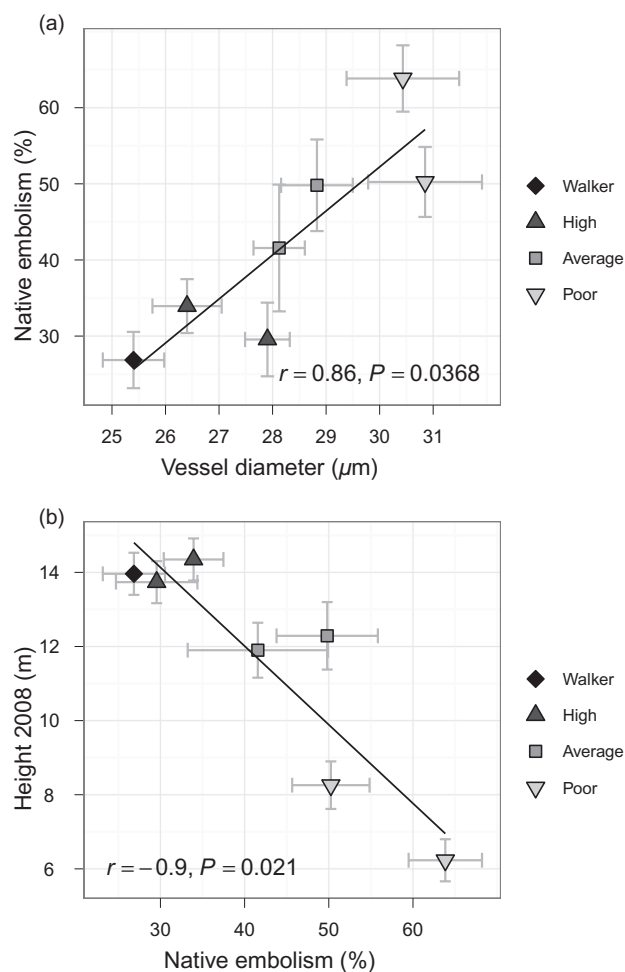

Figure 5. Correlations between mean native embolism measured in May of 2011 and vessel diameter (a) as well as tree height (b) for seven selected clones grouped by performance. Error bars represent the standard error of the mean.

previous research showing that poplar cells can, once hardy, survive extreme freezing through vitrification (Hirsh, Williams & Meryman 1985). We therefore conclude that damage of living tissue due to lack of cold hardiness in hybrid poplar clones is not a plausible explanation for the observed differences in performance. Although we observed a negative relationship of cell lysis in August with performance, the observed correlation does not indicate causation as August temperatures were never near −10 °C at our study site over the entire 16-year growing period.

During the winter of 1995/1996, we observed severe dieback of poor-performing clones (Fig. 2a). Figure 2b demonstrates that highly fluctuating daily temperatures characterized this winter. Temperatures in January varied between +10 and −42 °C; in February between +8 and −41 °C; and in March between +14 and −32 °C. April appeared to be normal relative to the 30 year temperature average. Early May was characterized by a distinctive temperature drop which can be considered a late spring frost. We neither observed an unusually low amount of snow, which could have increased the total degree of frost damage, nor was there an unusual drought event preceding the winter of 1995/1996, which could have weakened the trees (data not shown). Hence, we hypothesize that the observed dieback may be a cumulative effect of a high number of freeze-thaw events in winter as well as a late spring frost in May (Fig. 2b).

Parent type (Table 1) may also play into the vulnerability of these different groups as Walker as well as the high- and average-performing trees share many of the same parent species, including *Populus balsamifera*, *Populus deltoides* and *Populus nigra*. Of these species *P. balsamifera* is the northernmost North American hardwood; *P. deltoides* is native to the continental and eastern United States; and *P. nigra* is native to Europe and central Asia. Hybrids of these three species often share characteristics for superior growth performance and survival in boreal planting environments. *Populus trichocarpa*, on the other hand, a common parent in the poor-performing group, is a species of moist and bottomland sites of the Pacific Northwest which may have contributed to the poor performance of its hybrids in the cold and dry Canadian prairies.

Finally, synchronization of budbreak and the onset of leaf senescence with the available growing season could not serve as a plausible explanation for differences among clones in growth and survival. The timing of budbreak in spring appears to be remarkably uniform among performance groups and among tested clones, even though a wide variety of hybrids from diverse genetic backgrounds was included in this field trial (Supporting Information Table S1).

By excluding several alternate hypotheses, we conclude that the degree of native embolism restricts hydraulic conductivity during the growing season, and ultimately limits tree height and performance in boreal planting environments. Vessel diameter appears to be a key trait responsible for variation in native embolism in environments that experience frequent freeze-thaw events. Interestingly, we did not find significant differences in native embolism over time in the high-performing group. This suggests that small vessel diameters minimize freezing-induced embolism throughout the year, which in turn maximizes xylem conductivity. We should provide a cautionary note, however, as we arrive at our conclusions by exclusion of alternate explanations, and obviously we cannot exhaustively test all conceivable traits that are potentially responsible for cold adaptation, growth and survival. Additional systematic studies in controlled environments and field conditions should strengthen or challenge our conclusions regarding key traits for predicting growth performance of hybrid poplars in boreal environments.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Mean winter dieback and the respective climate for the years 1993/94, 1994/95, 1995/96 and 1996/97. Dieback is given in metres for seven selected clones grouped by performance (Walker, High, Average, Poor). Error bars represent the standard error of the mean. Daily minimum and maximum temperatures are shown as a grey-shaded ribbon and the 30-year average daily temperature for the period 1980–2009 as a solid black line. The dashed grey line represents freeze-thaw events for days when *TMIN* was equal or less than –5 °C and *TMAX* equal or greater than +5 °C (see text for details).

Figure S2. Non-linear model fit (y = a/x + b) to visualize the hardening process of seven selected clones over three sampling dates faceted by performance groups. The model was calculated using the method of non-linear least squares given as the function *nls()* in R.

Figure S3. Boxplots representing the day of year at which leaf senescence score 4 (a) and budbreak score 2 (b) was reached, grouped by performance. Leaf senescence score 4 = more yellow than green leaves. Budbreak score 2 = leaves extending but unfolded. Incomplete boxplots are due to lack of spread in data.

Table S1. Hybrid poplar clones with code, parental background, height and DBH data measured in 2008 as well as percent survival for the time period 1993–2008. Grey-shaded rows indicate the seven-clone subset analysed in this study. Clones are ordered based on height in 2008. Standard error is given in parentheses. DOY = day of year. Missing values are indicated as *n/a*.

Table S2. Phenology score key to assess timing of leaf senescence and budbreak. Boldface indicates scores and codes used for further analyses in this study.

Table S3. Mean cell lysis for seven selected clones by performance groups for all sampling dates and temperatures.