Research paper

The effects of throughfall exclusion on xylogenesis of balsam fir

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A 20–40% reduction in soil moisture is projected for the boreal forest of Eastern Canada for the period 2070–99 relative to 1971–2000. In order to better predict the effects of a reduced water supply on the growth of balsam fir (*Abies balsamea* (L.) Mill.), a dominant tree species of the boreal forest, we simulated 2 consecutive years of summer droughts (starting in July) by means of throughfall exclusion. Four 100-m² plots were established in 2010 with polyethylene sheets maintained 1.3–2 m aboveground and redirecting the water outside the plots. Wood microcores were extracted weekly from mature trees from April to October 2011 to analyse the time dynamics of wood formation in that year. The number of tracheids formed during and before treatment and their anatomical characteristics were determined through microscopic analyses. The growth of lateral and terminal branches and the water potential of balsam fir seedlings were also monitored. Throughfall exclusion significantly reduced soil water content by 5.8% in 2010 and 10.5% in 2011. Xylogenesis was affected significantly by the treatment. Tracheids were 16.1% smaller in diameter and their cell wall was 14.1% thicker during both years. The treatment delayed by more than a week the start of the tracheid differentiation process in the second year with a concomitant decrease (26%) in the number of tracheids produced. The seedlings displayed a 32% reduction in growth and a 40% reduction in leaf water potential. Our results suggest that a future regime of increased frequency and intensity of droughts could have negative effects on the duration of xylogenesis and the production of xylem cells in balsam fir.

Keywords: *Abies balsamea*, climate change, drought, radial/secondary growth, tracheid, water stress.

Introduction

Global aridity and drought frequency have increased significantly in the last 60 years in Africa, southern Europe, East and South Asia, eastern Australia, Alaska and northern Canada (Intergovernmental Panel on Climate Change 2012). For the boreal forest of Québec, Canada, a 20–40% reduction in soil moisture is projected during the growing season for the period 2070–99 compared with 1971–2000 (Houle et al. 2012). Such a decrease in soil moisture is likely to be associated with an increase in the frequency and intensity of droughts (Schar et al. 2004). In comparison with other forest growth drivers expected to undergo significant changes in the coming

decades (e.g., temperature and CO_2), reduced water availability has received little attention (Hartmann 2011, Beier et al. 2012).

Water deficits affect a variety of physiological processes in plants. A reduction in plant turgor pressure triggers trees to close stomata to reduce transpiration (Hsiao 1973). As a result, both photosynthesis and growth can be negatively affected (Hsiao et al. 1976). Our understanding of drought-related impacts on tree growth is, however, complicated by delayed responses resulting in non-linear effect of drought over time (Allen et al. 2010). For instance, the growth of beech (*Fagus sylvatica* L.) was lowest in the year following a severe drought in Europe (Granier et al. 2007). In the study of growth patterns

from dead oak trees (*Quercus* spp. L.), this response lag was shown to persist much longer: a majority of trees exhibited pre-mortality growth declines often correlated with drought events that occurred 2–52 years prior to mortality (Pedersen 1998). The post-drought decline of trembling aspen trees (*Populus tremuloides* Michx.) in western North America was related to the persistence of hydraulic damages that increase the susceptibility to cavitation (Anderegg et al. 2013). Alternatively, the exhaustion of carbon reserves is also forward as a possible explanation for post-drought decline (McDowell et al. 2008).

In this context, the anticipated increase in carbon sequestration rates of boreal forest ecosystems due to global warming (Hyvönen et al. 2007) could be partially offset by growth reductions caused by an increased frequency of drought events. For example, the severe rainfall deficit and summer heat of 2003 in Europe caused a massive reduction of 30% in gross primary productivity, cancelling in a single year the equivalent of 4 years of net ecosystem carbon sequestration (Ciais et al. 2005). Of the 95 recent experiments involving changes to precipitation regimes, only 2 looked at tree growth in response to drought in boreal ecosystems, highlighting the need for additional studies on that matter (see Beier et al. 2012). The formation of wood in conifers, or xylogenesis, involves cambium mitosis, enlargement of the cambial derivatives, wall thickening and lignification, with cell apoptosis marking the completion of the differentiation process (Catesson 1994). The successive phases of cell division and differentiation, or phenophases, can display contrasting temporal patterns as they occur at different times in the growing season (Deslauriers et al. 2003). Therefore, the consequences of a drought event on tree growth will depend on the timing of the event and the affected phenophases. A better understanding of the effects of drought on each phenophase could improve our capacity to predict how trees will be affected by future changes in water availability.

Studies of xylem formation in trees subjected to natural episodes of drought have shown a reduction in the rate of xylem differentiation and the annual amount of xylem produced (Hsiao 1973, Abe et al. 2003, Eilmann et al. 2009, Jyske et al. 2010). Xylem cells produced under drought stress can have different properties, including smaller diameters and thicker cell walls (Abe and Nakai 1999, Abe et al. 2003, Eilmann et al. 2009, Rossi et al. 2009, Gruber et al. 2010, Jyske et al. 2010). This reduction in size is the direct consequence of a reduced turgor pressure during cell enlargement and is thought to limit the risk of cavitation (Sperry et al. 2006). With increasing water stress and negative pressure in the xylem, a thicker cell wall is needed to increase the resistance of the xylem conduits against the risk of implosion (Hacke et al. 2001).

The phenology of xylem formation could also be affected by droughts. In Québec, Canada, balsam fir (*Abies balsamea* (L.)

Mill.) radial growth ended 4 weeks earlier and this was attributed to warm temperatures combined with high relative humidity and low precipitation which caused stomata to close, impairing transpiration during the 2 weeks preceding the end of growth (Duchesne and Houle 2011). There are no reported effects of drought on the beginning of growth, although stem rehydration is a prerequisite for cambium mitosis (Turcotte et al. 2009) perhaps because of the preponderant role of temperature on cambium reactivation (see Begum et al. 2013). To the best of our knowledge, the effects of drought on each of the phenophases of xylogenesis have yet to be demonstrated experimentally. Throughfall exclusion for ~90 days on mature black spruce trees (*Picea mariana* (Mill.) Britton, Sterns & Poggenb.) was limited to changes in the tracheids morphology (Belien et al. 2012). In another study, balsam fir seedlings subjected to a 20 day water exclusion did not, however, alter their xylogenesis phenology (Rossi et al. 2009).

The susceptibility of trees to drought depends on their life stage. For instance, seedlings in the understorey could be more susceptible to drought because of their small and superficial rooting that prevents water uptake from the deeper soil layers (McDowell et al. 2008). Their limited carbon reserves could also make them more vulnerable to carbon starvation following an extended period of reduced photosynthesis (Hartmann 2011). Nevertheless, the effects of experimental drought on the xylogenesis of younger trees appear to be quite similar to those observed in mature trees with effects on xylem morphology or cell division rates (Abe et al. 2003, Rossi et al. 2009, Galle et al. 2010). A good understanding of the differential response of young and mature trees to drought is necessary to better predict the consequences of a drought stress on the age structure of uneven-aged forest stands (Beier et al. 2012).

In this study, a throughfall exclusion experimental design was used to study the effect of 2 consecutive years of summer drought on the xylogenesis of balsam fir, a dominant species of the boreal forest of Eastern Canada. Our hypotheses were that the drought would (i) reduce the size of the differentiating tracheids and (ii) increase tracheid wall thickness during both treatment years, (iii) hasten the ending of xylogenesis and (iv) reduce the number of tracheids produced particularly in the second year of throughfall exclusion as 65–80% of the annual ring will be formed when the treatment will be started in July (Deslauriers et al. 2003). We hypothesized that the growth reduction of seedlings would be more severe than for mature trees.

Materials and methods

Study area

The study area is located in the Laurentian Mountains of Québec, Canada (47°17′N; 71°14′O; 800 m above sea level).

The 60-year-old even-aged balsam fir stand is dominated by balsam fir mixed with paper birch (*Betula papyrifera* Marsh.) and white spruce (*Picea glauca* (Moench) Voss). The stand density is 2024 stems ha^{-1} (diameter at breast height (DBH) ≥9 cm) with a basal area of 18.6 m^2 ha^{-1}. The climate is continental with cold winters and warm summers. Between 1981 and 2006, mean temperature and annual precipitation were −0.3 °C and 1535 mm, respectively. The soil is a sandy till classified as an Orthic Humo-Ferric Podzol. The mean slope is 8% with a western aspect.

Throughfall exclusion

Four plots were established in the spring of 2010 at least 50 m apart from each other. Each plot was centred on a group of three healthy dominant or co-dominant balsam fir trees in order to have at least 3 m from each stem to the edges of the plot. For each plot, a throughfall exclusion shelter was established with a transparent 10 m × 10 m polyethylene film (Harnois Industries Inc., Saint-Thomas de Joliette, QC, Canada) laid down over a tent-like structure made out of ropes and wooden posts with the apex aligned with the slope (Figure 1). By gravity, the intercepted water was redirected outside the plots and perpendicularly to the slope. Openings were made in the polyethylene sheets to surround every tree stem, and the sheet was sealed around each stem with sheathing tape. The polyethylene sheets were maintained 1.3–2 m aboveground to allow proper air flow beneath them. The built-up of heat was limited to 0.3 °C on average, as measured with temperature data loggers (HOBO H8 Pro Series, Onset Computer Corp., Cape Cod, MA, USA). In 2010, the experiment was started in the first week of July and ended on 5 August. In 2011, the experiment was also started in the first week of July, but ended 1 month later, on 8 September.

In addition to the three trees located in the throughfall exclusion shelters, two balsam fir trees were used as a control (Figure 1). They were also healthy dominant or co-dominant trees and were located within a 5 m × 10 m area contiguous to the upper side of each shelter. Control ($N = 8$) and treated trees ($N = 12$) had on average a DBH of 21 ± 3.5 and 22 ± 5.7 cm, respectively, and a height of 16.1 ± 2.2 and 16.7 ± 2.7 m, respectively. No significant differences in DBH or height were detected between treatments.

Weather data

Weather parameters were measured in a clearing located ~500 m from the plots. Temperature sensors (HMP35CF, Campbell Scientific Inc., Logan, UT, USA) recorded air temperature at a height of 3.3 m and rainfall was measured by averaging readings from two different pluviometers: a tipping bucket rain gauge (TE-525, Texas Electronics, Dallas, TX, USA) and a precipitation gauge (35–1558, Fisher and Porter, Albany, NY, USA). Daily volumetric soil water content (SWC) of the mineral
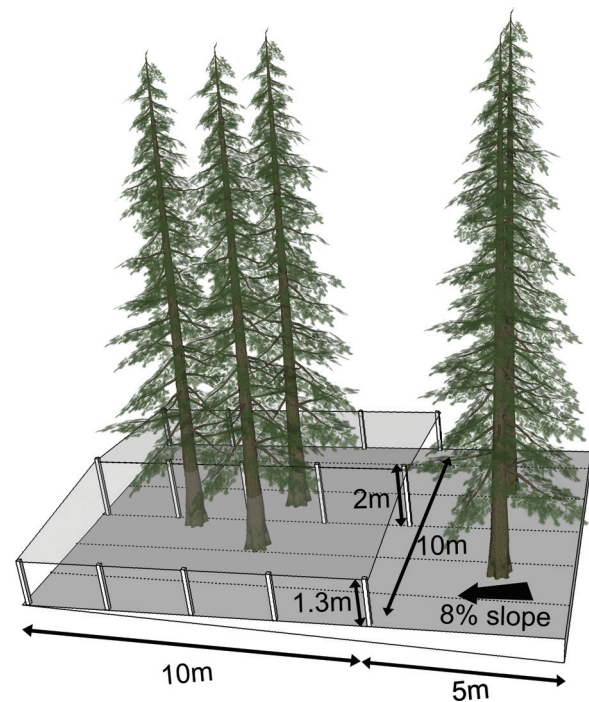

Figure 1. Schematic description of the throughfall exclusion shelter, including the location of the three treated balsam fir trees as well as the two control balsam fir trees. The dashed lines identify the transects used to monitor SWC.

soil (22 cm depth) was measured in a nearby stand using time-domain reflectometry (CS615, Campbell Scientific Inc., Logan, UT, USA). Measurements were made every 15 min and hourly averages were recorded by the data logger (CR-1000, Campbell Scientific Inc., Logan, UT, USA).

Volumetric SWC in the plots was measured weekly during treatment in both years using a FieldScout TDR-300 device with 20-cm rods and standard calibration (accuracy: ±3% vol; Spectrum Technologies Inc., Plainfield, IL, USA). Rods were inserted vertically and measurements were made at 1 m intervals along five 15 m transects parallel to the slope and 2 m apart crossing both control and treatment areas (Figure 1).

Sample collection

Using a Trephor tool (Rossi et al. 2006*a*), wood microcores (2.5 mm in diameter and 20 mm long) were extracted weekly from each tree stem during the second treatment year (2011), from April to October. No wood samples were taken during the first year of treatment (2010) because the experiment was set up in June of that year and the analysis of wood cores (see the 'Model fitting' section) required a sampling period covering the entire snow-free season. The sampling followed a spiralling upward transect centred at breast height and all samples were taken at least 10 cm apart to minimize the formation of resin ducts (Forster et al. 2000). The samples were stored at 4 °C in Eppendorf microtubes filled with a 10% ethanol solution. An

additional series of microcores was collected upon termination of xylogenesis, in mid-October 2011, and used for anatomical measurements of the tracheids formed in the years 2008–11.

Histological analyses

In the laboratory, the water from the microcores was removed through successive immersions in ethanol and Histosol™ and embedded in paraffin according to Rossi et al. (2006a). Transverse sections were cut with a rotary microtome (6–10 μm in thickness), stained with cresyl violet acetate (0.16% in water) after removal of the paraffin and observed under visible and polarized light at a magnification of ×400–500 to differentiate cells in the cambium and the differentiating xylem.

The number of cambial cells, enlarging cells, wall thickening cells and mature cells were counted along three radial files and averaged for every sample of each tree. Cambial cells were characterized by thin cell walls and less than half the radial diameter of enlarging cells. Both cambial and enlarging cells could be differentiated from other cell phases by their absence of secondary cell wall, a structure that shines under polarized light (Kutscha et al. 1975). Cells in the process of lignification and wall thickening showed violet and blue walls, the colour blue revealing the deposition of lignin on the cell walls (Thibeault-Martel et al. 2008). Mature tracheids were characterized by completely blue walls.

For anatomical measurements, parallel sections were stained with safranin (1%) following the standard preparation procedure described above, and numerically photographed with a camera fixed on an optical microscope at a magnification of ×400. Cell and lumen diameter (μm) as well as cell wall thickness (μm) were measured on three radial files per section using Wincell software (Regent Instruments Inc., Quebec City, QC, Canada).

Model fitting

The cumulative amount of cells produced was modelled with a modified Gompertz function (Rossi et al. 2003) for each tree:

$$Y = A \exp[-e^{(\kappa(\beta-t))}]$$

where Y is the cumulative amount of cells produced, A is the annual amount of cells produced, κ is the rate of change, t is the time computed in days of year (DOY) and β is the time at inflection point, i.e., when maximal growth rate is reached. The cumulative amount of cells produced was calculated as the sum of enlarging, wall thickening and mature cells. Three phenological signals were extracted from the models: (i) beginning of cell enlargement, determined as the moment when the daily cell production ($Y_t - Y_{t-1}$) reached 0.1 cell day^{-1}, (ii) maximal growth rate and its corresponding date, determined as the highest value of daily cell production ($Y_t - Y_{t-1}$) and (iii) end of

wall thickening, determined as the moment when daily cell production ($Y_t - Y_{t-1}$) declined <0.1 cell day^{-1}. In addition, the starting date for the wall thickening phase was obtained by applying the Gompertz function to the sum of wall thickening and mature cells (enlarging cells were excluded) and determining the moment when the daily cell production ($Y_t - Y_{t-1}$) reached 0.1 cell day^{-1}. All regression showed a normal distribution of the residuals (Motulsky and Ransnas 1987). The 'nlm' procedure from the R software was used with ordinary least squares method for parameter estimation (R Development Core Team 2012). In addition to the abovementioned phenological signals, the duration of xylogenesis was calculated as the difference in days between the beginning of cell enlargement and the ending of wall thickening.

Separating the tracheids produced before and during treatment

With xylogenesis starting in May, the differentiation of many cells was already in progress or even completed prior to the initiation of the treatment in early July (Figure 5). These cells were probably not affected in their development (i.e., diameter and wall thickness) contrary to differentiating cells produced after the start of the treatment. In order to better detect treatment effects on the differentiation of tracheids, it was thus decided to narrow the study to morphological impacts of the tracheids produced after the beginning of the treatment for the measured years. To do so, the individual Gompertz models previously fitted on the cumulative amount of cells produced in 2011 were used to identify, for each tree, the fraction of differentiating cells produced after 1 July relative to the total number produced that year, on average 44 ± 3%. Assuming similar growth patterns between years and based on the total number of tracheids produced in 2008–10, we identified for each tree and each previous year the tracheids corresponding to the fraction produced after 1 July 2011. The morphological parameters corresponding to each tracheid were then averaged per tree for each year (2008–11).

Seedling growth and water potential

Due their shade tolerance, balsam fir stands generate important banks of seedlings in the understorey (Morin 1994), and this study site was no exception. In each plot, three balsam fir seedlings located under the throughfall exclusion shelters (>2 m from the edges), and two others located outside the treatment (>2 m from the edges) were sampled in late August 2011. Based on the homogeneity of the canopy structure in this even-aged stand, we assumed a similar light regime between treatments. Sampled seedling height ranged between 70 and 110 cm, and their age was visually estimated between 10 and 20 years. Because of their size, seedlings are not suited for weekly extractions of wood cores. In addition, the terminal growth of seedlings is perhaps more ecologically

significant than radial growth in order to assess their capacity to survive and grow in the understorey. For each seedling, the 2011 terminal growth (leader) was measured to the nearest 5 mm, and the 2011 lateral growth of three distinct upper branches was also measured to the nearest 5 mm and averaged. To determine the water status of each seedling, the midday water potential of three distinct lateral branches was measured using a pressure chamber instrument (model 610, PMS Instrument Co., Albany, OR, USA) and their water potential was averaged.

Statistical methods

To test our hypotheses of the effects of drought on tracheid characteristics, the 2 years of treatment were combined in a mixed-model linear analysis of covariance (ANCOVA) with repeated measures, assuming a compound symmetry variance–covariance structure. The pre-treatment value of each morphological parameter (average of 2008 and 2009), limited to tracheids produced since 1 July as for treatment years, was used as a covariate when significant at $P < 0.05$, considering throughfall exclusion treatment and year as fixed factors, and subject and block as random factors. In order to verify the hypothesis that xylem production would not be affected in the first year but perhaps in the second year, each year was tested separately in an ANCOVA with previous growth (average of 2008 and 2009) as a covariate. A similar approach was used to detect a treatment effect on the phenology of xylem formation in 2011. Significant correlations with pre-treatment growth ring width (average of 2008 and 2009) were often observed ($P < 0.05$). For such cases, pre-treatment growth ring width was used as a covariate. Finally, 'treatment' was used in a mixed-model ANOVA as a fixed factor, with random factors 'subject' and 'block', to detect an effect of the treatment on the seedling growth and water potential. Data were tested a priori to meet the assumptions of ANOVA (e.g., normality and homoscedasticity). Statistical analysis, modelling and plots were done using the R software (R Development Core Team 2012).

Results

Climate

From May to September, average temperatures were 12.6 and 12.2 °C in 2010 and 2011, respectively (Figure 2), <1 °C higher than the normal of 11.2 °C (1971–2000). The precipitations recorded for the period of May to September 2010 (596 mm) were slightly lower than the average for 1975–2000 (654 ± 47 mm), mostly because of small amounts of precipitation in August (38 mm compared with an average of 130 ± 46 mm for 1975–2000). Precipitations were higher in 2011 totalling 747 mm for the period of May to September, with 226 mm falling in August (Figure 2). The average volumetric SWC measured in a nearby stand for the period of May to September was also lower in 2010 (30 ± 8.5%) than in 2011 (40 ± 4.5%). Soil water content was highest following snowmelt in May (42 ± 2.7% in 2010 and 46 ± 1.3% in 2011) and decreased somewhat steadily in the following months with large between-year differences in August and September probably due to contrasting levels of precipitation (Figure 2). Soil water content was 23 ± 1.6 and 24 ± 2.8% in August and September 2010, respectively, compared with 34 ± 4.6 and 41 ± 2.5% in August and September 2011, respectively.

Treatment effect on SWC

The SWC measured inside the treated plots was significantly higher in 2011 than in 2010 ($P < 0.01$). During both years, the throughfall exclusion shelter significantly reduced SWC relative to the control ($P < 0.01$; Figure 3). The average SWC in treated and control plots, respectively, was 7.7 ± 0.2 and 13.5 ± 0.3% in 2010, and 11.7 ± 0.3 and 22.2 ± 0.5% in 2011. The contrasting precipitation regime between years affected the efficiency of the treatment, with reductions in SWC of 5.8 and 10.5% being recorded under the throughfall exclusion shelter in 2010 and 2011, respectively (Figure 3). Indeed, lower precipitations caused a reduction in throughfall exclusion in 2010, decreasing the treatment effect over SWC that year. Probably due to the lateral movement of incoming rain as well as the
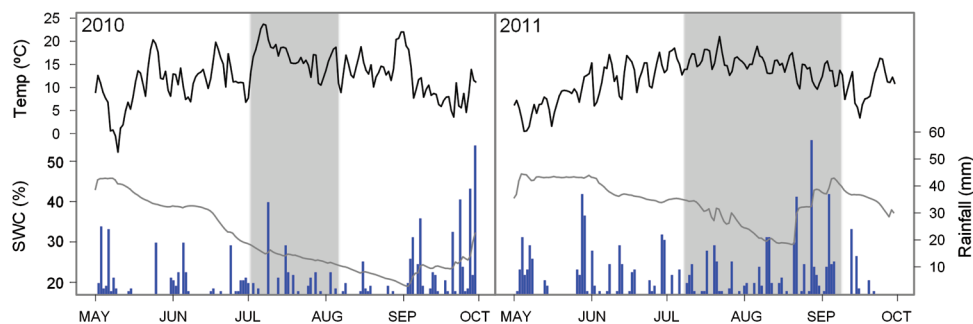
Figure 2. Daily air temperature (black curve), SWC (grey curve) and rainfall (vertical bars) from May to October 2010 and 2011 measured 500 m from the experimental site. The periods of throughfall exclusion are in the shaded areas.

water coming from the shelter itself, SWC was higher near the edges of the throughfall exclusion shelters (10.2 ± 0.5%) than in the centre (7.5 ± 1.0%; Figure 3).

Characteristics of the new tracheids

No differences in tracheid characteristics (wall thickness, cell and lumen diameter) were detected between the 2 years of treatment ($P > 0.34$; Table 1). No initial differences were observed between treatments in 2008–09 ($P > 0.55$). All three characteristics were however significantly different between treatments ($P \leq 0.02$; Table 1). The diameter of tra-
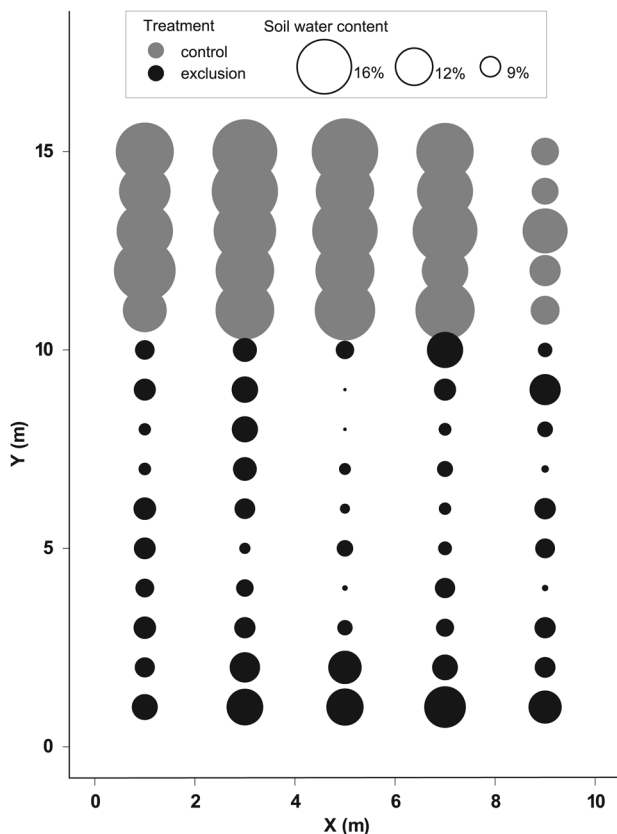

Figure 3. Average SWC in control and throughfall exclusion plots during treatment in 2010 and 2011.

Table 1. Probability values from mixed-model ANOVAs applied to the tracheid morphology data from the treated (12 trees) and control (8 trees) plots, before and during the treatment

		Tracheid diameter	Lumen diameter	Wall thickness
Before treatment	Treatment (T)	0.97	0.86	0.55
During treatment	Covariate	**0.04**	**<0.01**	0.58
	T	**0.02**	**<0.01**	**<0.01**
	Year (Y)	0.34	0.35	0.99
	T × Y	0.99	0.80	0.34

Pre-treatment tracheid characteristics (*covariate*: average of 2008–09) were used as covariate when significant at $P < 0.05$. Significant P values are in bold ($P < 0.05$).

cheids was 16.1% smaller in treated trees with a mean cell diameter of 20.1 ± 1.3 μm compared with 23.9 ± 0.9 μm for the control (Figure 4). A reduction in lumen size was also observed, with a diameter that was 30.8% smaller in treated trees (16.5 ± 0.9 and 11.4 ± 1.4 μm in control and treated trees, respectively). The cell walls of treated trees were 14.1% thicker than those of control trees (4.3 ± 0.1 and 3.7 ± 0.1 μm, respectively; Figure 4).

Phenology of wood formation in 2011

In 2011, the cambium was in general composed of five cells prior to the onset of xylogenesis but reached eight to nine cells at the peak of its mitotic activity (Figure 5). The first enlarging cells were observed on average on 19 May for control trees, but 8 days later in trees under the throughfall exclusion shelters (27 May; $P < 0.01$; Table 2). The number of enlarging cells during the growing season followed a bell-shaped curve, reaching seven cells at its peak. The first cells in the final stage of wall thickening were observed on 31 May in control trees and 9 days later in treated trees (9 June; $P < 0.01$; Table 2). The seasonal amount of cells in that phenophase also followed a bell-shaped curve, with an average maximum of 10 cells (Figure 5). At its peak, the daily growth rate was 0.61 ± 0.05 cells day^{-1}, with no significant differences between treatments (Table 2). The growing season averaged 99 days and ended on 30 August for all trees (Table 2). Xylogenesis was already in progress when the treatment was initiated in early July, with eight cambial cells, five enlarging cells, seven wall thickening cells and approximately five mature tracheids already formed (Figure 5). When the treatment ended in September, there were no more enlarging cells although the process of wall thickening was not completed.

Number of tracheids

In the 2 years preceding the experiment, an average of 32 ± 2 tracheids were produced annually (Figure 6), with no difference in annual xylem production being detected between treatments ($P = 0.64$). An average of 33 ± 3 tracheids were produced in the first year of treatment (2010), also without a significant difference between treatments ($P = 0.80$). In 2011, however, annual xylem production in control trees increased to 39 ± 3 tracheids while treated tree production was reduced by 26% (29 ± 4 tracheids, $P = 0.03$; Figure 6). On an average, 56 ± 3% of the annual amount of tracheids was already differentiating or mature when the treatment was started in 2011.

Seedlings growth and water potential

The growth of the leader and lateral branches of treated seedlings were reduced by an average of 31% ($P = 0.10$) and 32% ($P = 0.01$), respectively, in 2011 (Figure 7), and no seedling mortality was observed. Seedlings subjected to the throughfall exclusion treatment had a 40% reduction in midday water
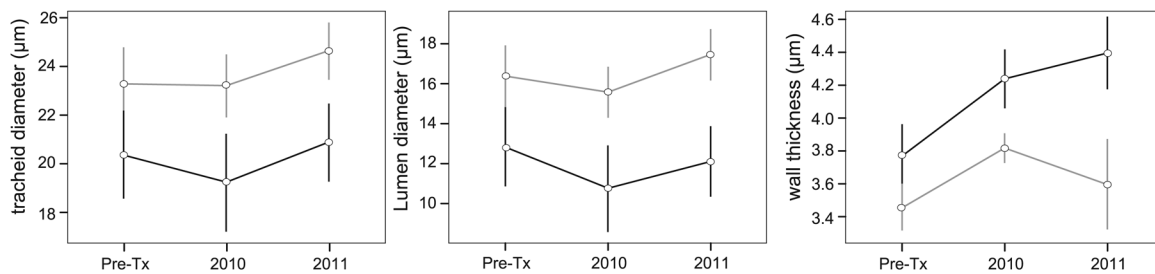
Figure 4. Anatomical characteristics of the tracheids produced after 1 July before treatment (Pre-Tx; average of 2008 and 2009) and during treatment in 2010 and 2011. Control trees are in grey, treated trees in black and vertical bars are the standard error.
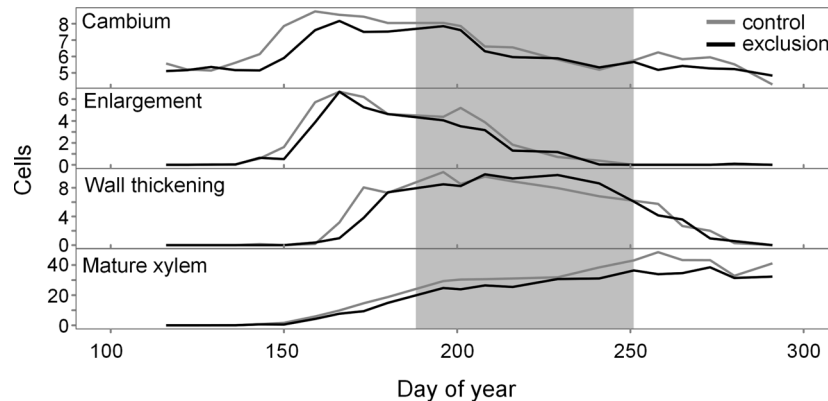

Figure 5. Average number of cambial cells, enlarging cells, wall thickening cells and differentiated tracheids in control and treated balsam fir in 2011. Control trees are in grey and treated trees in black, and the period of throughfall exclusion is in the shaded area.

Table 2. Average values for phenology and productivity parameters (and standard error) of balsam fir in control and treated plots in 2011 ($N = 20$ trees)

	2008–09 ring width (µm)	Onset of enlargement	Onset of wall thickening	End of wall thickening	Duration[a] (days)	Maximum growth rate[b] (cells day^{-1})	Timing of maximum growth
Control	1242 (91)	May 19 (2 days)	May 31 (2 days)	September 5 (7 days)	109 (8)	0.61 (0.05)	June 26 (2 days)
Treated	1033 (159)	May 27 (2 days)	June 9 (2 days)	August 24 (8 days)	89 (9)	0.60 (0.06)	June 27 (2 days)
Statistical analysis							
Covariate	–	**<0.01**	**<0.01**	0.07	**0.03**	0.08	0.97
Treatment	0.42	**<0.01**	**<0.01**	0.46	0.24	0.28	0.64

[a]Duration was calculated as the difference in days between the onset of enlargement and the ending of wall thickening.
[b]Maximum growth rate was calculated as the maximum daily growth.
Probability values from mixed-model ANOVAs with pre-treatment ring width (covariate: average of 2008–09) as covariate when significant at $P < 0.05$. Significant P values are in bold ($P < 0.05$).

potential averaging -1.38 ± 0.05 relative to -0.98 ± 0.04 MPa in control seedlings ($P < 0.01$; Figure 7).

Discussion

Effects on tracheid characteristics

In agreement with our first two hypotheses, throughfall exclusion affected the morphology of the tracheids. The reduction in cell and lumen diameter and the increase in cell wall thickness show that the enlargement and wall thickening stages of xylogenesis were affected by the treatment. The formation of tracheids starts with cell divisions in the cambium producing cambial derivatives. Cell expansion depends exclusively on hydrostatic pressure: as water potential lowers, cell turgor decreases, limiting the expansion of new cells (Beth Kirkham et al. 1972, Deri Tomos et al. 1989). Experimental water limitation has been previously observed to reduce tracheid diameter in balsam fir seedlings (Rossi et al. 2009) as well as in mature trees of *Pinus sylvestris* L. (Sterck et al. 2008, Gruber et al. 2010) and *Cryptomeria japonica* (L.f.) D. Don (Abe and Nakai 1999, Abe et al. 2003). This phenomenon could be considered as an adaptation to ensure sufficient water transport while minimizing the negative pressure in order to avoid xylem injuries due to cavitation (Sperry et al. 2006).

Consistent with the reduction in tracheid diameter, an increase in cell wall thickness with reduced water availability has also been documented (Eilmann et al. 2009, Jyske et al. 2010). A higher negative pressure in the xylem enhances the risk of implosion, and the vessel walls thicken to prevent the cells from collapsing (Hacke and Sperry 2001). Comparing 47 woody species, Hacke et al. (2001) determined that the thickness of the xylem cell wall is proportional to the negative pressure it has to withstand. In our study, we observed a 40% decrease in xylem water potential in seedlings growing in the throughfall exclusion plots. Although the water status of mature trees was not measured per se in this experiment, the changes observed in the tracheids' morphology suggest that the prolonged throughfall exclusion also decreased the trees' water potential. Balsam fir could be relatively sensitive to extended drought periods as it is a shallow rooted species (Schultz 1969 cited by Hix et al. 1987).
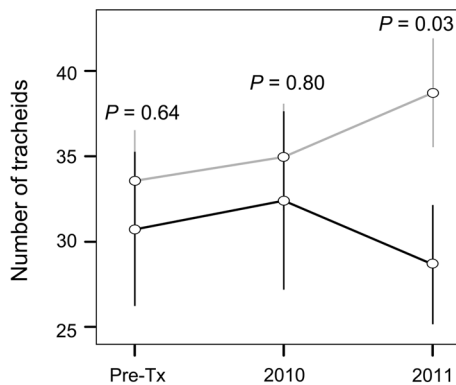

Figure 6. Annual number of tracheids produced before (Pre-Tx; average of years 2008 and 2009) and during throughfall exclusion years. Control trees are in grey, treated trees in black and vertical bars are the standard error. P values refer to the effect of the treatment as measured with mixed-model ANCOVAs.
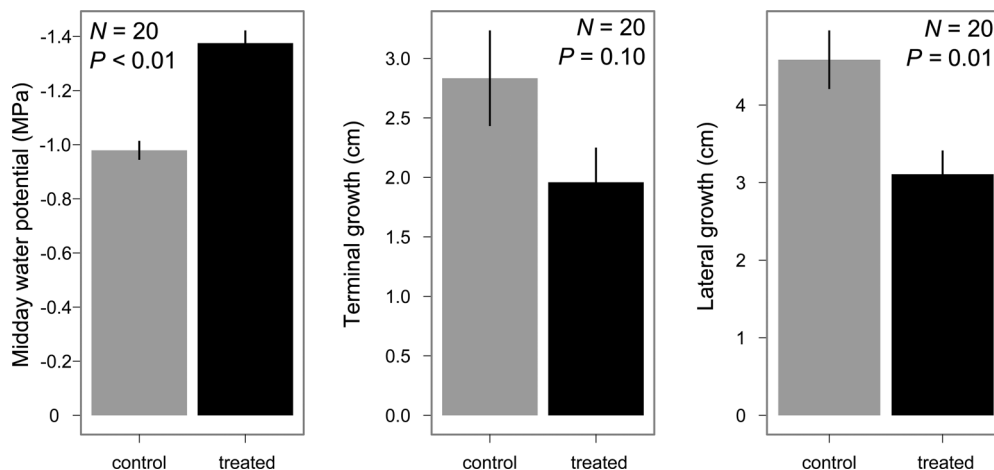
Effects on the phenology of wood formation

The hypothesis stating that xylogenesis would end prematurely in treated plots was not verified. In fact, the 26% reduction in the number of tracheids produced in 2011 by treated trees was not associated with an earlier growth ending. This hypothesis, based on the comparison of trees growing under naturally contrasting water regimes (see Gruber et al. 2010), has not been supported yet by data from experimental drought studies (Arend and Fromm 2007, Rossi et al. 2009, Belien et al. 2012). Our result is consistent with previous findings that the timing of growth cessation is a highly variable parameter poorly correlated with climate variables (Rossi et al. 2006b, Duchesne et al. 2012). While the hypothesis of a premature ending of xylogenesis was rejected, the phenology was nonetheless affected by the treatment. We observed a delay of more than a week in the beginning of cell differentiation in the second year (2011) of treatment. Cell enlargement and wall thickening phases started in late May and early June, respectively, at least 1 month before the treatment was started in July. Therefore, the observed delay in cell differentiation was caused by a reaction to the preceding-year treatment. While drought-induced mortality in trees can be observed many years following a given event (Pedersen 1998, Bigler et al. 2007, Hartmann 2011), only a few examples of non-lethal lagged effects have been documented. Following the exceptional European drought in the summer of 2003, a pronounced growth reduction was observed in the following year (Granier et al. 2007). The same observation was made in a mature boreal aspen stand of Canada following a 3-year-long drought (2001–2003) (Krishnan et al. 2006). However, to our knowledge, our study is the first to report a delayed onset of cell differentiation caused by a drought stress in the preceding year. During the xylogenesis process, the enlargement of a cambial derivative is prior to its wall thickening. Thus, as both phases were delayed by the drought, the delay observed for wall thickening can be

Figure 7. Average growth and water potential in control and treated balsam fir seedlings. P values refer to the effect of the treatment as measured with mixed-model ANOVAs.

accounted for by the delay in cell enlargement. In turn, the start of cell enlargement relies on the start of mitosis in the cambium and the production of cambial derivatives. It is therefore possible that the delayed cell enlargement was due to delayed mitosis in the cambium. The reduced number of tracheids produced that year proves that the cambium activity was affected, although we cannot determine if this effect was specific to the start of cambium mitosis. Both cambium mitosis and cell enlargement rely heavily on available water (Beth Kirkham et al. 1972, Hacke and Sperry 2001), and cambium reactivation has been shown to be preceded by 4–6 weeks of stem rehydration in black spruce (Turcotte et al. 2009). Droughts can cause hydraulic damages in trees which can affect various water-dependent processes for several years following the end of the event (Anderegg et al. 2013). In the current study, a persistent deterioration of the trees' hydraulic capacities could have slowed down the spring rehydration process of the cambium. The cambium water potential serves as a metabolic regulator of mitotic activity and has the capacity to influence later stages of differentiation (Abe and Nakai 1999, Arend and Fromm 2007). Perhaps a delay in reaching sufficient water potential in the cambium could have provoked a later initiation of cambium mitosis and cell differentiation.

Effects on the production of tracheids

As we hypothesized, the effect of drought on xylem production was more pronounced in the second year of treatment. Indeed, the treatment had no effect on the number of tracheids produced in 2010, while a 26% decline was observed in 2011. The lack of effects on xylem production in the first year of treatment was expected given that a majority of the new tracheids forming the growth ring ($56 \pm 3\%$) were already in the differentiation process and/or completed when the treatment was started. Delayed effects from the first year drought cannot be separated from current aggravating effects due to the second-year drought, and several mechanisms related to carbon and water availability can explain this result. Among them, impaired hydraulic conductivity could have reduced the rates of water-dependent processes of wood formation such as cambium mitosis and tracheid differentiation, as cited earlier to explain the delayed onset of xylogenesis. Other water-dependent processes occurring during the growing season could have been impacted, such as photosynthesis or carbon distribution within the tree (Krishnan et al. 2006), reducing the amounts of carbon allocated to xylogenesis (e.g., Hartmann 2011). For instance, the reduced growth of oak saplings (*Quercus pubescens* Willd.) subjected to 2 consecutive years of summer drought (22–38% reduction in ring width) was attributed not only to impaired photosynthesis but also to enhanced protection and repair processes which consumed significant amounts of carbon (Galle et al. 2010). The comparison of balsam fir growth vulnerability to drought relative to other species is difficult to establish due to the lack of published data. Mature black and Norway spruce trees growing in Canada and Denmark, respectively, displayed no reduction in radial growth in response to a single experimental drought (Beier et al. 1995, Belien et al. 2012). However, mature Norway spruce trees subjected to spring droughts of 60–75 days for 4–5 consecutive years in southern Finland reduced their annual ring width by 11–21% every year (Jyske et al. 2010). The rooting plasticity as well as the water reserves of a given species are often cited, but the vulnerability of the water transport system to drought-induced embolism could also play a critical role in the drought tolerance of forest species (Choat et al. 2012).

Effects on seedling growth

A 32% reduction in the terminal growth of balsam fir seedlings growing under the exclusion shelter as compared with control seedlings was observed. In this experiment, seedlings and trees were not subjected to an identical treatment, as the seedlings' canopy was not in contact with rainwater while trees could benefit from the foliar absorption of a fraction of the intercepted rainfall (Breshears et al. 2008). In a greenhouse study with 7-year-old balsam fir seedlings subjected to a 20-day drought in June–July, Rossi et al. (2009) observed a significant reduction in shoot growth during the treatment and in the following weeks that was compensated for before the end of the growing season after the cessation of the treatment. The shorter drought duration in that study could explain the different results. The water potentials observed in the current study were similar to those of 2-year-old Scots pine and Sitka spruce trees (~ -1.5 MPa) after 30 days of water stress under controlled conditions (Jackson et al. 1995). Initial differences in available light between treatments could have affected the growth of seedlings, but the even-aged structure of the stand supposes a homogeneous understorey light regime. In addition, the likelihood of contrasting light environments between treatments was reduced by the random selection of seedlings as well as the proximity of control and treated seedlings within each plot.

Conclusions

In order to study the impact of droughts on balsam fir growth, an in situ experiment consisting in throughfall exclusion during summer was conducted for 2 consecutive years in a typical balsam fir boreal forest of Québec, Canada. The treatment reduced the SWC and had significant impacts on several aspects of xylogenesis. The new tracheids formed were smaller, with thicker cell walls, suggesting a significant response to decreasing tree water potential. The treatment also delayed by more than a week the start of the differentiation process of cambial derivatives in reaction to the preceding-year treatment. Although previous observations have

shown that drought may have a delayed effect on tree growth, this is the first experimental observation of a drought stress affecting the phenology of xylogenesis. The drought also reduced by 26% the number of tracheids produced in the second year of treatment. Balsam fir seedlings were also affected, with a 32% reduction in terminal growth. Our results suggest that a future regime of increased frequency and intensity of droughts will have negative effects on the duration of xylogenesis, the production of xylem cells and the regeneration of balsam fir. Balsam fir could be particularly sensitive to droughts as compared with black spruce trees since a similar protocol applied to this species did not affect its xylem production or phenology (Belien et al. 2012). The delayed effect of the throughfall exclusion treatment observed here underlines the importance of conducting longer term studies on the effect of drought on tree growth.

Conflict of interest

None declared.

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