RESEARCH PAPER

Title: Effects of temperature and water deficit on cambial activity and woody ring features in *Picea mariana* saplings

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2.1 ABSTRACT

Increase in temperature under the projected future climate change would affect tree growth, including the physiological mechanisms related to sapling responses, which has been examined recently. The study investigated the plant water relations, cambial activity and wood formation in black spruce saplings [*Picea mariana* (Mill.) B.S.P.] subjected to water deficit and warming. Four-year-old saplings growing in three greenhouses were submitted to different thermal conditions: T0, with a temperature equal to the external air temperature; and T +2 and T +5, with temperatures set at 2 and 5 K higher than T0, respectively. We also submitted saplings to two irrigation regimes and studied the effects of a water deficit of 32 days in May–June. We evaluated plant water relations, cambial activity, wood formation and anatomical characteristics from May to October 2010. Lower needle physiology rates were observed during water deficit, with 20-day suspension of irrigation, but after re-watering, non-irrigated saplings attained the same values as irrigated ones in all thermal conditions. Significant differences between irrigation regimes were detected in cambial activity at the end of the water deficit and after resumption of irrigation. Under warmer conditions, the recovery of non-irrigated saplings was slower than T0 and they needed from 2 to 4 weeks to completely restore cambial activity. No significant differences in wood anatomy were observed between irrigation regimes, but there was a sporadic effect on wood density under warming. During wood formation, the warmer conditions combined with water deficit increased sapling mortality by 3.2 and 7.8% for T +2 and T +5, respectively. The black spruce saplings that survived were more sensitive to water availability, and the restoration of cambial activity was slower at temperatures higher than T0. Our results suggest that black spruce showed a plastic response to intense water deficit under warming, but this would compromise their survival.

***Keywords**:* cambial activity, needle water relations, saplings, thermal conditions, water deficit, wood formation.

Abbreviations: Pre-dawn leaf water potential, Ψ_{pd}; midday leaf water potential, Ψ_{md}; stomatal conductance, g_s; maximum photosynthesis rate, A_{max}; T0, greenhouse with a temperature equal to the external air temperature; T+2, greenhouse with temperature of 2 K higher than T0; T+5, greenhouse with temperature of 5 K higher T0.

2.2 INTRODUCTION

In the boreal forest, sapling banks form a reserve of individuals to regenerate the stands following major biotic or abiotic disturbances (Rossi and Morin 2011). Saplings reflect the dynamic evolution of the boreal forest and also constitute a management strategy in the Canadian boreal forests (Lamhamedi and Gagnon 2003, MRNF 2009). Because of climate change, a temperature increase in the boreal forest of ~2–4°C by the year 2060 (Plummer et al. 2006, Logan et al. 2011), combined with a significant increase in the frequency and severity of droughts (IPCC 2007, Seager et al. 2007, Sterl et al. 2008), represents a key challenge for regeneration and survival of forest stands. A demographic change in young forests can reflect phenomena such as self-thinning or shade competition (Lutz and Halpern 2006), but in recent decades, the regional warming has doubled the mortality rate of seedlings in natural stands in the USA (van Mantgem et al. 2009). Peng etal. (2011) found that regional drought increased the adult tree mortality rate in Canada’s boreal forests from 1963 to 2008.

In boreal ecosystems, temperature is the most important factor for tree growth (Körner 2003*a*, 2003*b*). Cambial activity and cell differentiation are determined by temperature (Oribe et al. 2001, Begum et al. 2007, Rossi et al. 2007, 2008*b*). Recent studies have estimated temperature thresholds regulating different phases of xylem phenology in mature black spruce [*Picea mariana* (Mill.) B.S.P.], linking the passage between thermally favorable and unfavorable periods (Rossi et al. 2011). Other research confirmed the influence of cambial age or tree size on radial growth (Rossi et al. 2008*a*, Rathgeber et al.

2011). It is also documented in different species that the climatic sensitivity of radial growth changes with tree age (Rozas et al. 2009, Vieira et al. 2009). However, information is lacking about climatic influence on cambial activity in young trees of the boreal forest. Water availability is another important influencing factor linked to cambial activity and wood formation (Giovannelli et al.2007, Camarero et al. 2010). In the stem, cambium cell division and expansion of newly formed tracheids are processes highly sensitive to the plant's water status (Abe and Nakai 1999, Savidge 2000, Rossi et al. 2009). Past research in 1-year-old seedlings of black spruce evidenced that drought tolerance was mostly through an acclimation of the stomatal conductance and photosynthetic rate (Zine el Abidine et al. 1994), which are strictly linked to an increase in temperature (Sage et al. 2008). Several studies exist on the relation between water conditions and xylem growth (Larson 1963, Shepherd 1964). Saplings can be vulnerable to drought due to the decrease in their ability to uptake soil resources, as observed in past research that evidenced the water-deficit effect on root growth in young plantations of black spruce (Burdett et al. 1984, Bernier 1993). Nevertheless, an evaluation of the combined effects of warming and water deficit on cambial activity in conifer saplings has recently received great interest (de Luis et al. 2011), even if a clear picture is far from being reached, especially in the boreal environment.

Radial growth depends on the link between tree–water relations and carbon balance. Woody ring features provide more information on water transport; these traits have often been used for the characterization of the climatic condition that influences the wood formation (Fonti et al. 2010). Radial growth requires the maintenance of high cell turgor pressure, which has an irreversible influence on cell extension and wall polymer deposition (Proseus and Boyer 2005). The carbohydrate pool also represents a source of energy and carbon skeletons for cambium activity, and could be linked to secondary wall formation (Giovannelli et al. 2011). Wood density is a key parameter for determining carbon investment (Chave et al. 2009), and it depends on tracheid characteristics (Rathgeber et al. 2006). Drought affects several growth features such as xylem anatomy and radial increment (Abe et al. 2003, Corcuera et al. 2004, Arend and Fromm 2007).

Numerous studies have also shown how the wood density of conifers can be strictly correlated to environmental conditions (Chave et al. 2006), in particular, temperature (Gindl and Grabner 2000, Gindl et al. 2000). Recent studies were based on manipulations of the growing conditions of mature black spruce in the field (Lupi et al. 2011, 2012, Belien et al. 2012), which could not control all environmental parameters. In comparison, an artificial control of environmental conditions in a greenhouse provides a localized effect on the whole plant. This can allow xylem development and wood formation of black spruce to be studied in saplings, an age category that has been largely overlooked.

The aim of this paper was to evaluate the effects of three thermal conditions and irrigation regimes on (i) plant water status, gas exchange and CO2 assimilation, (ii) cambial activity and (iii) wood anatomy in black spruce saplings growing in greenhouses. Three thermal conditions were chosen according to the possible future scenarios drawn by recent climate models (Zhang et al. 2000, Rossi et al. 2011). We tested the following hypotheses: (i) the imposition of severe water deficit could induce a reduction in xylem growth as a response to change in leaf water potential, gas exchange and CO2 assimilation; (ii) the cambium could display different sensitivity in terms of decrease in cell division and differentiation rate in response to gradual water-deficit severity according to temperature; and (iii) water deficit under warm conditions could induce the formation of thinner cell walls or smaller cells.

2.3 MATERIAL AND METHODS

2.3.1 Experimental design

The experiment was conducted during the 2010 growing season in Chicoutimi, QC, Canada (48°25′N, 71°04′W, 150 m above sea level) on 4-year-old *P. mariana* (Mill.) B.S.P. saplings growing in plastic reversed-conic pots (volume 4.5 l) filled with peat moss, perlite and vermiculite. In late winter, the saplings were maintained at a temperature close to the external one and sheltered from the snow under a garden tunnel. In April, 1104 saplings of uniform size (height 48.9 ± 4.7 cm and diameter at the collar 8.0 ± 2.0 mm) were selected, fertilized with 1 g l^{-1} of NPK (20-20-20) fertilizer dissolved in 500 ml of water, divided into three groups and transferred to three greenhouses. Three groups were moved to the independent section of three greenhouses where the saplings were subjected to three thermal conditions: T0, with a temperature equal to the external air temperature; and T+2 and T+5, with temperatures of 2 and 5 K higher than T0, respectively. In each section, two different irrigation regimes were applied to the saplings: (i) control (named irrigated saplings), in which soil water content was maintained above 80% of field capacity; and (ii) a dry regime (non-irrigated saplings) in which irrigation was withheld for 32 days during May–June, at the beginning of xylem growth, when plants are supposed to be more susceptible to drought (Rossi et al. 2006*a*). The thermal conditions were maintained quite constant in spring, except on the day of the year (DOY) 122 and from 142 to 152, when a technical problem prevented the expected temperatures being maintained in the greenhouses and the difference in temperature between treatments and control was reduced to +1 and +2° C, respectively. After the restoration of irrigation on DOY 174, the differences in temperature between T0 and T+2 and T+5 were maintained constant at 2 and 5 K higher, respectively.

2.3.2 Sapling mortality

Sapling mortality was monitored from May to October 2010. Three weeks after re-watering, the percentage of mortality was calculated on the total number of saplings that died naturally for each irrigation regime and thermal condition, excluding the number of saplings randomly selected every week for xylem development, wood anatomy and density.

2.3.3 Water relations, gas exchange and CO_2 assimilation

Pre-dawn leaf water potential [Ψ_{pd}] and midday leaf water potential [Ψ_{md}] were measured from May to August on branches of the first whorl of three saplings per treatment (three thermal conditions × two irrigation regimes) with a pressure chamber (PMS Instruments, Corvalis, OR, USA). Similarly, gas exchange and CO_2 assimilation (stomatal conductance gs, mol m^{-2} s^{-1}, and maximum photosynthesis rate, Amax, μmol m^{-2} s^{-1}) were measured from 10:00 to 13:00 under saturating irradiance conditions (1000 μmol m^{-2} s^{-1}) using a portable photosynthesis system (Figure 2.1) (Li-6400, LI-COR, Inc., Lincoln, NB, USA). Measurements were expressed according to the specific needle surface area computed as the ratio of needle dry mass per unit of needle surface area. Needle dry mass was weighed after drying at 65°C for 48 h and the surface area was calculated by scanning projection of sub-samples of needles and using a regression according to Bernier et al. (2001).

2.3.4 Xylem development

Destructive sampling lasted from May to October and consisted of six saplings randomly selected every week from each treatment (three thermal conditions × two irrigation regimes), for a total of 36 saplings per week. Stem disks were collected 2 cm above the root collar of each selected seedling. The samples were dehydrated with successive immersions in ethanol and d-limonene, embedded in paraffin, and transverse sections of 8–10 µm thickness were cut with a rotary microtome (Rossi et al. 2006*a*). The wood sections were stained with cresyl violet acetate (0.16% in water) and examined within 10–25 min with visible and polarized light at ×400–500 magnification to distinguish the differentiation of xylem according to four distinct phases. For each section, the radial numbers of (i) cambial, (ii) enlarging, (iii) wall thickening and lignification, and (iv) mature cells were counted along three radial files. The total number of xylem cells was calculated as the sum of differentiating and mature cells. In the cambial zone, the cells were characterized by thin cell walls and small radial diameters (Rossi et al.2006*b*). During the enlargement phase, the tracheids still showed thin primary walls, but had a radial diameter twice that of the cambial cells and primary cell walls that were not birefringent under polarized light (Kutscha et al. 1975, Antonova and Shebeko 1981). Criteria for discriminating secondary wall formation in cells were the birefringence under polarized light and the coloration due to the reaction of cresyl violet acetate with the lignin, which produced a color change from violet to blue when lignification was complete (Rossi et al. 2006*b*). Thus, a homogeneous blue color over the whole cell wall revealed the end of lignification and the attainment of maturity (Gričar et al. 2005, Rossi et al. 2006*b*).

2.3.5 Wood anatomy and density

Wood sections from the saplings collected during the two last sampling days in October, six saplings randomly selected (three thermal conditions × two irrigation regime for 36 sapling in total per week), were stained with aqueous 1% safranine and fixed on slides with Eukitt® histological mounting medium. A camera mounted on a microscope was used to record numerical images and to measure xylem features with an image analysis system specifically designed for wood cells (WinCell™, Regent Instruments, Inc., Canada). Lumen area, radial diameter and wall thickness of cells were measured at ×400 magnification along a band of 12–18 rows of tracheids, for a total of ~250 µm in thickness. For each anatomical section, earlywood and latewood were identified according to Mork's formula, which classified all cells with lumen smaller than twice a double cell wall as latewood (Denne 1988).

Stem disks from the same saplings were air-dried to a 12% moisture-content state and X-rayed together with a calibration wedge following standard techniques (Polge 1978). Radiographs were digitalized using a scanner, and the acquired grey scaledigital images were treated using semiautomatic procedures (Mothe et al. 1998). Density values were assigned to each pixel of the calibration wedge. Each tree ring was divided into 20 segments of equal width, and the tree-ring density profiles were produced by averaging the values of the pixels inside each segment. For each wood section, the mean density determined by X-ray analysis was compared with the density directly determined by measuring the mass per volume unit to correct the microdensity profiles.

2.3.6 Statistical analysis

The number of cells in the different phases was compared between irrigation regimes with the t-test. Analyses were conducted using GLM procedure in SAS (SAS Institute, Cary, NC, USA).

The dynamics of cell production were assessed by fitting the total number of cells counted on each sampling date with a Gompertz function, using the non-linear regression (NLIN) procedure in SAS. The Gompertz function (1) was defined as:

$$y = A \exp[-e\beta - \kappa\tau] \qquad (1)$$

where y is the number of cells, τ is time computed as DOY, A is the upper asymptote of the total number of cells, β is the x-axis placement parameter and κ is the rate of change parameter (Rossi et al. 2003). The asymptote represented the number of radial cells produced by the saplings during the growing season. Group comparisons were performed between thermal conditions and irrigation regimes by using the fitted curves (Potvin et al. 1990, Giovannelli et al. 2007).

Xylem anatomy and density were analyzed using analysis of variance and the means were performed using Tukey’s test ($P < 0.05$), comparisons of the means were obtained using PDIFF option (Quinn and Keough 2002).

2.4 RESULTS

2.4.1 Growth conditions and saplings mortality

At the beginning of the experiment on DOY 118, the mean daily air temperature in T0 was 8°C (Figure 2.1). During May, T+2 and T+5 were 1.7 and 3.1 K warmer on average than T0. The dry period lasted 32 days, during which the temperature in T0 varied between 15 and 29°C. At the last sampling on DOY 293, temperature in T0 was 6°C. Overall, T+2 and T+5 experienced temperatures of 2.0 and 4.7 K higher than T0, respectively.

None of the irrigated saplings died in the three thermal conditions (Table 2.1). On the contrary, after 3 weeks, re-watering mortality ranged from 2.1 to 12.2% in non-irrigated saplings, with proportionally more dead saplings observed at the higher temperatures.

2.4.2 Water relations, gas exchange and CO2assimilation

After the withholding of irrigation on DOY 142, it was possible to define two stages of water-deficit intensity on the bases of water potential, gas exchange and CO_2 assimilation. From DOY 142 to DOY 158 (mild water deficit), all saplings showed optimal water conditions. Both irrigated and non-irrigated saplings exhibited Ψ_{pd} ranging between −0.4 and −0.7 MPa and Ψ_{md} ranging from −0.5 to −1.4 MPa (Figure 2.2). No marked difference in leaf water potential was observed among the thermal regimes. In these conditions, Amax ranged between 2 and 7 μmol CO_2 m^{-2} s^{-1}, although higher average values of 9 and 14 μmol CO_2 m^{-2} s^{-1} were measured in T+5 and T+2 on DOY 158, respectively (Figure 2.2).

From DOY 159 to 173 (severe water deficit), the Ψ_{pd} and Ψ_{md} values dropped dramatically in non-irrigated saplings, reaching higher values on DOY 173 (−2.7 MPa Ψ_{pd} and −2.9 MPa Ψ_{md}) without evident differences between thermal regimes. Accordingly, in non-irrigated saplings, A_{max} ranged between 0.1 and 0.4 μmol CO_2 m^{-2} s^{-1} and g_s was <0.1 mol m^{-2} s^{-1} in T0, T+2 and T+5 (DOY 161) (Figure 2.2). On DOY 179, 1 week after re-watering, all values of non-irrigated saplings were similar to those observed in irrigated ones, showing that the saplings were able to recover an optimal water status. These conditions persisted for the rest of the summer (Figure 2.2).

2.4.3 Xylem development

Cell division was active from the first sampling date, as shown by the six to nine cells observed in the cambial zone on DOY 124 (Figure 2.3). The number of cells in the cambium decreased until the beginning of June, and then increased with a peak at the beginning of July, on DOY 187. This peak was observed only in irrigated saplings (Figure 2.3). A progressive reduction of cambial activity was observed in the irrigated and non-irrigated saplings at the end of August (DOY 237). The number of cells in the cambial zone decreased synchronously in irrigated and non-irrigated saplings and attained the corresponding number of three to four cells at the beginning of September. During water deficit, the patterns of the number of cambial cells in division were similar among the three thermal conditions, while differences were observed between irrigation regimes ($P < 0.05$), especially after the rehydration (DOY 174) for 2 weeks in T0, and for ~4 weeks in T+2 and

+5. At the end of August, the number of cells in the cambial zone decreased to four, which indicated the end of cell production (Figure 2.3).

The patterns of variation in the number of cells in enlargement, wall thickening and lignification were similar among thermal conditions (Figure 2.3). As for cambial cells, significant differences were detected between irrigation regimes, mostly at the end of the water deficit and after resumption of irrigation. In non-irrigated saplings, a significant decrease in the number of enlarging cells was observed during severe water deficit in T0 and T+2 on DOY 166 ($P<0.001$ and $P<0.01$, respectively) and in T+5 on DOY 173 ($P<0.01$). At the end of water deficit, the number of enlarging cells rapidly decreased to one to two cells in non-irrigated saplings. For about 10–20 days after the resumption of irrigation, significant differences between irrigation regimes were observed in the number of enlarging cells at all thermal conditions ($P<0.05$). At the end of July, the number of enlarging cells of non-irrigated saplings gradually increased in all thermal conditions, but fewer cells in this differentiation phase were detected in T+2 and T+5 (three to four cells) with respect to T0 (four to five cells). For the phase of cell-wall thickening and lignification, significant differences were observed in the irrigation regimes at the end of the water deficit (Figure 2.3). For about 15–20 days after resumption of irrigation, significant differences were detected between irrigated and non-irrigated saplings for cells in both wall thickening and enlargement ($P<0.05$) (Figure 2.3).

The Gompertz function adequately fitted the data through- out the growing period, and was always able to reach an asymptote by the end. Statistical tests detected significant differences in the total number of cells among groups of treatments ($F=7.30$, $P<0.0001$)

(Table 2.2). The highest total numbers of cells were observed in irrigated saplings, with valuesof123.4, 109.9and 109.7 tracheids in T0, T+2 and T+5, respectively (Table 2.2, Figure 2.3). Fewer were produced in non-irrigated saplings of T+2 and T+5, showing an average of 90.7 and 87.7 cells along the tree rings, respectively. Another comparison of the total number of cells between the irrigation regimes revealed a significant difference in the saplings ($F = 32.73$, $P < 0.0001$). However, no significant difference was detected in the comparison among total cells between the thermal conditions ($F = 0.82$, $P = 0.59$) (Table 2.2).

2.4.4 Wood anatomy and density

The anatomical traits of tracheids were compared between irrigated and non-irrigated saplings; no statistical difference was observed (Figure 2.4). For the thermal conditions, sporadic effects were found at high temperature, but these were not consistent during and after water deficit. The cell lumen area generally declined from earlywood to latewood (Figure 2.4). The average lumen area of earlywood was 95.90 mm^2 for T0, and 85.99 mm^2 and 87.24 mm^2 for T+2 and T+5, respectively (Figure 2.4). From earlywood to latewood, no significant differences were observed in all treatments.

From the initial tracheids in earlywood to the last ones in latewood, there was a decrease in the cell length between irrigation regimes in all thermal conditions. However, no significant difference was found (Figure 2.4). Only in the 40–60% portions of the tree ring, the cell length of earlywood was lower on average in non-irrigated saplings (Figure 2.4). From earlywood to latewood, the cell-wall thickness exhibited an overall increase in irrigated and non-irrigated saplings, from 2.1 to 2.7 μm in T0, and at the higher temperatures, from 2.1 to 3.2 μm (T+2 and T+5), respectively. But again, no effect of irrigation regime was observed at all thermal conditions. The cell-wall thickness of earlywood showed a trend in the 40–65% portion of the annual ring at the high temperature, decreasing to 1.6 μm. Wood density increased along the annual tree ring, particularly in latewood. The average values of wood density in the different thermal conditions were 585 kg m^{-3} at T0, 572.19 and 537.48 kg m^{-3} at T+2 and T+5, respectively. No significant difference was observed for wood density between irrigation regimes ($P > 0.05$) (Figure 2.4). However, at the 20% portion of the tree ring, significant differences were observed at T+2 ($P < 0.0001$). The wood density value at T+5 (480.31 kg m^{-3}) was slightly lower than those at T0 and T +2, 562.46 and 562.33 kg m^{-3}, respectively.

2.5 DISCUSSION

Wood formation in black spruce saplings was more affected by 32 days of water deficit than by different thermal conditions during the growing season. As expected, drought induced a transient reduction in cambial activity and cell differentiation, especially at the end of the water-deficit period (severe water deficit) and, in some cases, for several weeks after the recovery of the optimal plant water status. Even if the application of thermal conditions (T+2 and T+5) did not seem to cause a significant effect alone on wood anatomy, the negative effect of water stress (in duration and intensity) was recorded mostly on cambium activity, cell enlargement, cell-wall thickening and lignification. Past studies showed that black spruce saplings had high growth plasticity, i.e., the capacity to maintain growth under drought, to different water regimes during their first growing season (Lamhamedi et al. 2003, Bergeron and Lamhamedi 2004). Similarly, Mayor and Johnsen (1999) affirmed that the drought tolerance traits such as osmotic potential at saturation, modulus of elasticity, turgor and net photosynthetic strongly influenced the growth performances of black spruce plants under reduced soil water availability.

2.5.1 Saplings mortality

The air temperature increase of 2 and 5° C, in conjunction with severe water deficit during wood formation, increased sapling mortality by 5 and 12.2%, respectively, in agreement with Way and Sage (2008) who observed that seedling mortality increased at high temperature. In harvested stands, Ruel et al. (1995) showed that the survival of 3-year-old black spruce correlated on seedling height and seedling health and growth. The mortality of black spruce seedlings reached 21%, but decreased to <10% when stem height exceeded 30 cm (Ruel et al. 1995). However, the observed mortality rate could not be considered as the effective survival of saplings because we had excluded the number of saplings randomly selected every week for anatomical analysis. So, our findings could be an underestimation of the effective survival rate of the plants.

The death of saplings could be due to an alteration in the plant water–carbon balance due to irreversible damages of different primary metabolic pathways that could have gradually taken place during the water deficit (Anderegg et al. 2012). Under mild-to- moderate stress, photosynthesis has been considered the primary physiological process affected by stomatal limitation (Galmés et al. 2011). The limiting process under severe drought still remains unclear, but photosynthesis might be limited by mesophyll conductance. A close relationship between leaf and xylem vulnerability to water stress has been observed (Brodribb and Cochard 2009), and it is accepted that leaves are more prone to cavitations than the stem (Johnson et al. 2011); so, the saplings would be more vulnerable to cavitations than mature trees due to their size. Several studies have affirmed that the reliance on water transport, transpiration and carbon sequestration within trees varies with tree size and is much more negative in small than in adult trees (Domec and Gartner 2002, Phillips et al. 2003). These effects could be related to carbon limitation (Sala et al. 2010). A dysfunction in the phloem transport and long-distance carbon translocation (McDowell

2011), in particular carbon demand for the cambial activity, could lead to the death of saplings.

2.5.2 Needle water relations under water deficit and warming

In our experiment, water deficit greatly influenced the water relations of saplings. During the first phase of the water deficit (from DOY 142 to 158, mild water stress), the Ψ_{pd} value did not drop below −0.7 MPa, and the non-irrigated saplings were able to maintain gas exchange and the photosynthetic rate similar to those of the irrigated ones under all thermal conditions. Stewart et al. (1994) showed that photosynthetic capacity of black spruce seedlings under drought conditions responded positively at Ψ_{pd}= −1.5 MPa. Tan et al. (1992) and Tan and Blake (1997) showed that in drought-stressed sap- lings (Ψ_{pd} ranging from −1.0 to −1.4 MPa) faster-growing black spruce progeny tolerated, and instead postponed, momentary dehydration. From DOY 159 to 173, when severe water deficit was reached, the Ψ_{pd} value of non-irrigated saplings reached −2.2 MPa. At maximum water deficit (DOY 173), g_s and A_{max} were strongly reduced (<80% with respect to the non-irrigated saplings). These results suggested that the efficiency of water translocation was greatly compromised, probably as a cumulative effect of hydraulic failure through cavitations. Under severe water deficit, the non-irrigated saplings were unable to maintain CO_2 assimilation and stomatal conductance. Our results confirmed that stomatal conductance was strongly reduced at leaf Ψ_{pd} of −1.0 MPa, as observed by Bernier (1993) and Stewart et al. (1994). Also, Stewart et al. (1994) showed that stomatal limitation increased to ~40% during a period of intensive water stress (Ψ_{pd} −1.5 MPa), although photosynthesis capacity remained unaffected by drought treatment. In our case, photosynthesis followed the decrease of stomatal conductance, coupling at Ψ_{pd} -2.2 MPa. A week after the resumption of irrigation, non-irrigated saplings had completely recovered their plant water balance. As observed by Stewart et al. (1994), after re-watering, stomatal conductance and photosynthesis rate recovered rapidly even after three cycles of drought. After severe water deficit, the recovery follows two processes: a first stage of leaf re-watering and stomata re-opening (Kirschbaum 1987, 1988), and a second stage, after 10 days of re-watering with the partial recovery (40–60%) of maximum photosynthesis (Bogeat-Triboulot et al. 2007). The different thermal conditions did not influence the recovery of the water status of the needle, while the reduction in root allocation induced by the high temperature could expose black spruce in extreme soil drying events (Way and Sage 2008).

2.5.3 Rate of xylem growth: a matter of water?

The imposition of severe water deficit decreased cell production in non-irrigated saplings. Our findings showed that under limited water availability, non-irrigated saplings exhibited a significant decrease in the number of cells within the newly formed ring in all thermal conditions. The decrease in the annual ring width was attributed to a lower expansion rate of the cambial cell derivatives during the enlarging phase under water deficit. In the early stage of water deficit (mild water stress), cell enlargement is first inhibited, while in a later stage, when the water deficit becomes more severe, cell division is also affected, as observed in past studies (Abe and Nakai1999, Abe et al. 2003, Jyske et al. 2010, de Luis et al. 2011). During severe water stress, the cambium could reduce cell division and save energy for maintaining minimum metabolism and defense (McDowell 2011). In adverse environmental conditions, the control of photosynthesis is reserved for structural growth (carbon demand, sink activity), and the non-structural carbohydrates in tissues indicate the degree to which growth is carbon- limited (Körner 2003*a*, 2003*b*). However, carbon demand and carbon supply cannot be synchronous (Hoch et al. 2003, Sala et al. 2012), and the storage of carbon may be required to maintain hydraulic transport during a severe drought (Sala et al. 2012). The negative effects of drought on the supply and transport of photoassimilates during water deficit and on their accessibility during the re-watering could decelerate and/or stop cell division. Non-irrigated saplings showed fewer cells in division, enlargement and wall thickening than irrigated saplings at all thermal conditions. But after resumption of irrigation, non-irrigated saplings gradually recovered cambial activity in terms of cell enlargement of the cambial derivatives, to different extents according to the thermal conditions. Two weeks after the resumption of irrigation, the number of cells produced by non-irrigated saplings was similar to those by irrigated ones in T0, while this condition was reached after 4 weeks in T+2 and T+5. This finding indicates that cambium and other stages of cell differentiation could display different sensitivity to increasing water-deficit intensity. In addition, the recovery of cambial growth after water deficit could be strongly affected by air temperature.

After the resumption of irrigation, non-irrigated saplings were able to slowly restore hydrostatic pressure within the cambial region at all thermal conditions. A high hydrostatic pressure is required within the cambial region for the enlarging of cambial cell derivatives

(Abe et al. 2003). However, the higher temperature clearly affected xylem formation after water resumption. The cambium activity of non-irrigated saplings needed 2 weeks to be completely restored in T0, but 4 weeks were necessary at higher temperature. This suggests a post-dated effect of water deficit at thermal conditions when the saplings were in water status imbalance. Similar responses in cell enlargement, wall thickening and lignification can manifest in reduced wood production (Arend and Fromm 2007) and growth processes could be completed early (Begum et al. 2007). The restoration of growth may undergo a physiological adjustment to allow the maintenance of water uptake and cell turgor and to accumulate soluble carbohydrates and amino acids for a sufficient carbon gain that supports growth under water deficit (Tan et al. 1992, Chaves et al. 2009).

2.5.4 Effects of water deficit and warming on xylem anatomy and density

Our results showed that xylem anatomy was not affected by water deficit at all thermal conditions, while wood density presented only sporadic changes. The third hypothesis was therefore rejected. The lumen area of earlywood tracheids of non-irrigated saplings was not affected by water deficit at all thermal conditions, even if the withholding of irrigation sporadically affected intra-annual variation in density, as a resilience effect of cells to water deficit. In contrast, the cell size of balsam fir saplings was shown to be quickly affected by a dry period (Rossi et al. 2009), so this could demonstrate the resistance of black spruce saplings. The absence of any effect of irrigation regime on cell length at all thermal conditions could be due to the recovery from water deficit of cell expansion. Cell-wall thickening was also not influenced by the irrigation regime at all thermal conditions;

the presence of trends only in the 40–65% portion of the tree ring at the higher temperature may indicate a possible influence on the apposition of secondary wall and lignification in response to environmental conditions (De Micco et al. 2007). This could reflect an indirect adjustment of wood anatomy (Fonti et al. 2010) and would confirm the high plasticity of black spruce saplings.

The absence of an effect of water deficit on wood density could be related to the observed gradual restoration of radial growth after rehydration. Wood density of Norway spruce earlywood is rather stable under drought (Bouriaud et al. 2005), so the sporadic effect at T+5 may suggest that the wood density of black spruce could be more susceptible to temperature than water deficit, as observed by Gindl et al. (2000) and Gindl and Grabner (2000).

2.6 CONCLUSION

Research on the effects of climate change and increased tree mortality linked to drought are renewing attention to the survival of natural forest regeneration and physiological mechanisms related to saplings responses. Our study indicates that the imposition of severe water deficit affected leaf water potential, gas exchange and CO_2 assimilation in black spruce saplings. During plant-water imbalance, the radial growth and cambium activity were highly sensitive to decrease in soil water. After the resumption of irrigation, stressed saplings were able to resume radial growth and cambium activity according to the thermal conditions, showing great resilience to water deficit. In stressed plants, the recovery of stem growth and cambium activity was slower under warmer condition than in T0. The anatomical properties and wood density of saplings also showed a great resistance to water deficit; however, wood density was slightly susceptible to the thermal conditions. The higher air temperatures in conjunction with water deficit during wood formation increased sapling mortality by 5 and 12.2% for T+2 and T+5, respectively. Our results suggest that black spruce saplings showed a plastic response to intense water deficit under warming, but this would compromise their survival. Nevertheless, an increase in mean temperature coupled with recurrent drought events could exacerbate the water deficit effects on wood formation via an alteration of the plant carbon– water budget.

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Table 2.1 Percentage of dead black spruce saplings during the experiment from May to October at three thermal conditions and two irrigation regimes.

% sapling mortality	**T0**	**T+2**	**T+5**
Irrigated	0	0	0
Non-irrigated	2.1	5.0	12.2

Table 2.2 Comparison between growth response curves, fitted to the cumulative sum of number of radial cells averaged by period (DOY, day of the year) among thermal conditions and between irrigation regimes. *A* is the upper asymptote of the total number of cells, *β* is the *x*-axis placement parameter and *k* is the rate of change parameter, *F*-values were calculated among all groups. Groups represent six single groups of the number of radial cells per irrigation regime and thermal condition. Grey background indicates the water deficit period at three thermal conditions.

Source of variation		***A***	**β**	**κ (10^{-2})**	***F*-value**	***P***
T0	Non-irrigated	98.50	4.06	2.30	573.97	<0.0001
	Irrigated	123.40	3.80	2.13	613.34	<0.0001
T+2	Non-irrigated	90.70	4.22	2.44	518.97	<0.0001
	Irrigated	109.90	4.60	2.73	638.95	<0.0001
T+5	Non-irrigated	87.70	3.85	2.19	516.91	<0.0001
	Irrigated	109.70	4.50	2.62	751.30	<0.0001
Among groups					7.30	<0.0001
Among thermal conditions					0.82	0.59
Between irrigation regimes					32.73	<0.0001

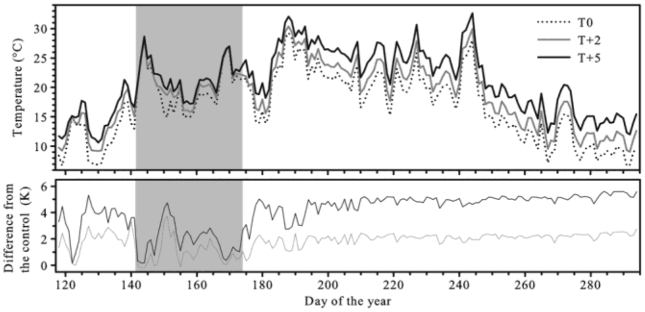

Figure 2.1 Daily temperatures experienced by black spruce saplings of the three thermal conditions during the experiment in the greenhouse.

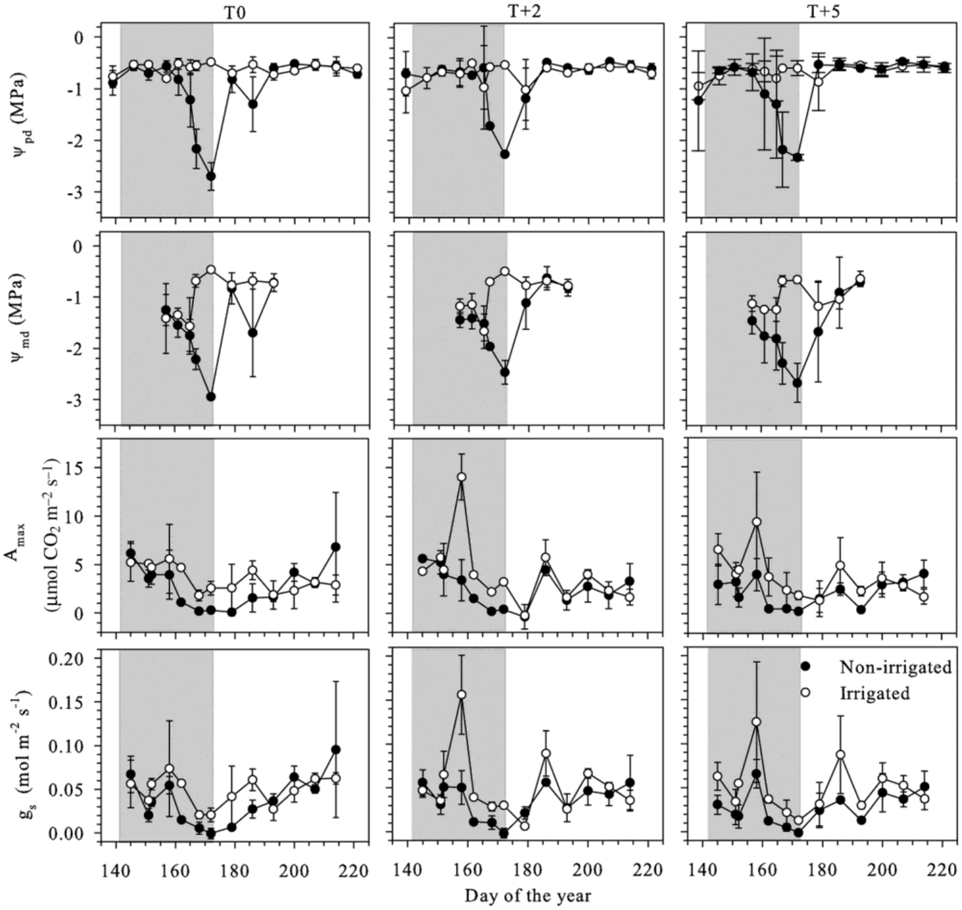

Figure 2.2 Water relations, gas exchange and CO_2 assimilation of black spruce saplings before, during and after the water-deficit period (grey background) at three thermal conditions.

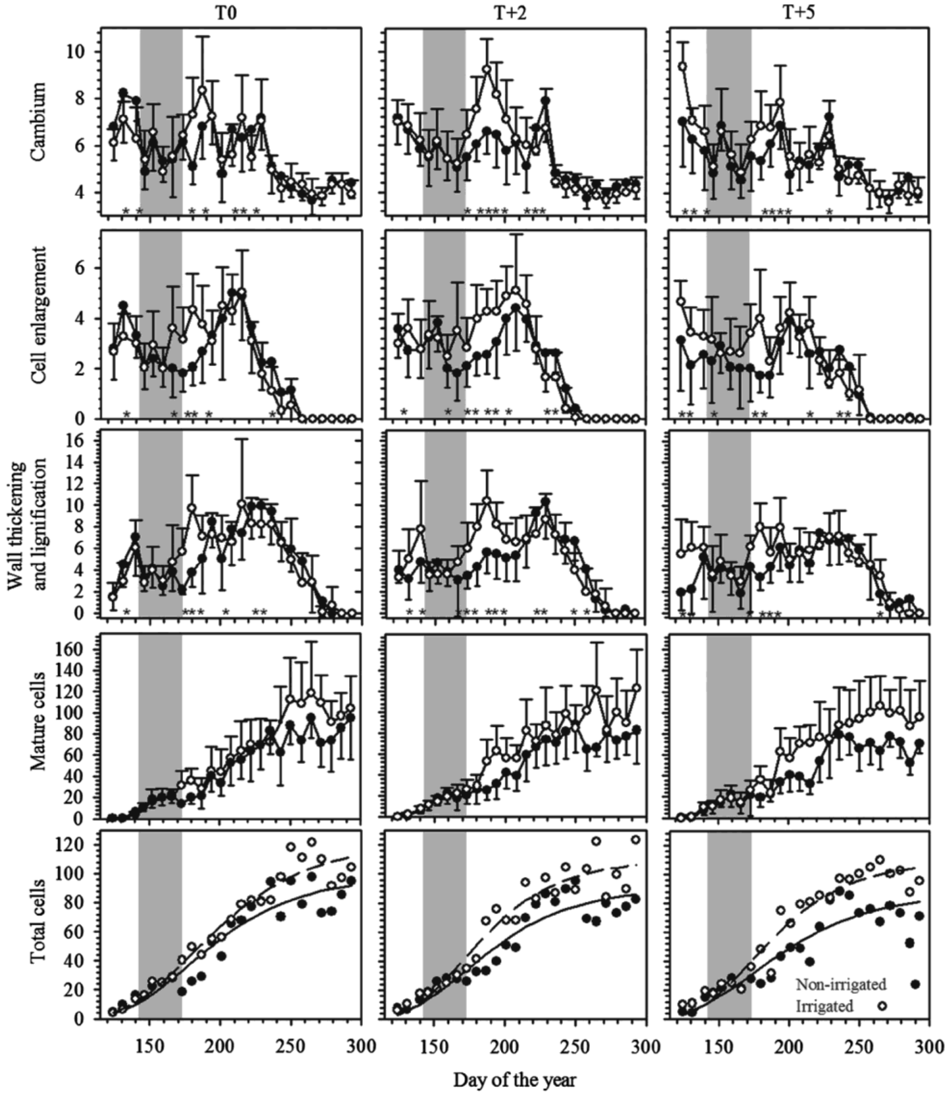

Figure 2.3 Radial number of cambial, enlarging, wall thickening and lignification, and mature cells counted in black spruce saplings before, during and after the water-deficit period (grey background) at three thermal conditions. Vertical bars correspond to the standard deviation. Black and grey curves indicate the total number of cells between the two irrigation regimes (Gompertz). Asterisks indicate significant differences between irrigation regimes.

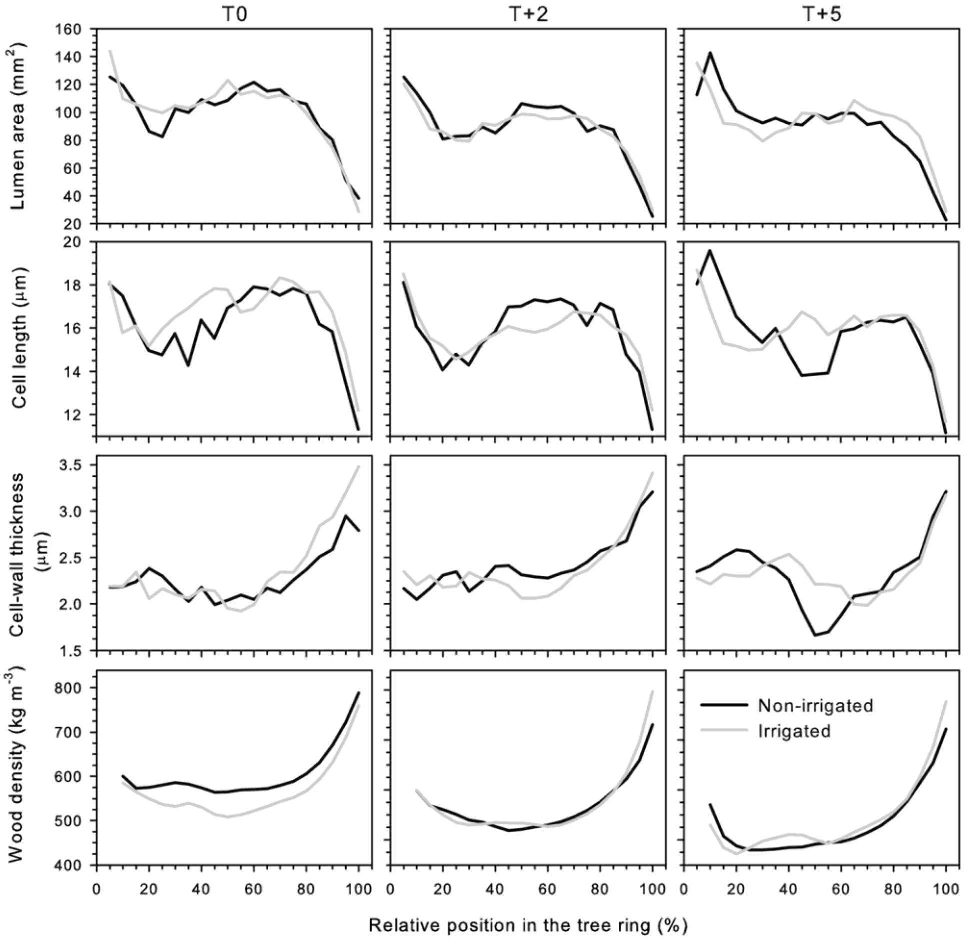

Figure 2.4 Cell features and wood density of the tracheids produced by irrigated and non-irrigated black spruce saplings at three thermal conditions (T0, T+2, T+5) along portions of an annual tree ring. Black and grey curves indicate two irrigation regimes.

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