Xylogenesis in black spruce subjected to a rain exclusion in the field

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Abstract

The climate models for Eastern Canada predict increases in temperature and precipitation in the next 50 years, with the higher latitudes expected to experience the greatest rates of warming. Despite the increase in precipitation, more extreme and frequent drought periods will occur in spring and summer. An experiment was conducted on black spruce [*Picea mariana* (Mill.) BSP] trees excluded from precipitation during June-September by the installation of plastic under-canopy roofs in four sites of the boreal forest of Quebec. Xylem growth and physiological traits of treated and control trees were monitored at short-time resolution to study the effects of summer drought on plants growing in their natural environment. The rain exclusion reduced cell area and increased cell wall thickness of xylem, but no change was observed in timings or amount of cell production. In the southern sites, treated trees showed lower stem radius variations than the control. After removal of the under-canopy roofs, trees quickly recuperated their normal water status. The results demonstrated the physical effects of reduction in water availability on xylem and the resistance of black spruce to one summer of drought, most likely due to the ability to collect water from sources other than the superficial soil horizon.

Keywords: Picea mariana, Xylogenesis, Drought stress, Boreal forest

Introduction

The climatic models for Eastern Canada predict increases in temperature and precipitation in the next 50 years (Plummer et al. 2006). The increase in precipitation will occur mainly in winter in the form of snowfall, while extreme conditions with drought events should be experienced in summer and spring, when the main growth processes of plants take place (Burke et al. 2006; Zhang et al. 2000). Because of the low temperatures characterizing the boreal forest, water evaporation is generally reduced and soils often remain wet throughout the growing season. As a result, boreal species are adjusted to live with high or close-to-optimal moisture conditions. However, increased evapotranspiration due to climate warming as well as more frequent and longer drought events are expected to produce potentially severe water stresses in these high latitudes ecosystems (Easterling et al. 2000).

It is well-known that temperature plays an important role in cambium activity and wood formation in trees of cold climates (Gruber et al. 2010; Rossi et al. 2008). However, the influence of moisture conditions on radial growth has also been demonstrated, with positive effects of precipitations on growth observed in several boreal conifers (Deslauriers et al. 2003b; Duchesne and Houle 2011; Hofgaard et al. 1999). Several metabolic processes, such as photosynthesis and cell differentiation, are a function of the water status of plants (Taiz and Zeiger 2006). In trees, most water is stored in the thin layer of living cells comprised between bark and cambium. Therefore, changes in water status are necessarily reflected in xylem and phloem, and in the resulting radial growth of the stem (Steppe et al. 2006). During the early stages of water deficit, water potential decreases up to -1.9 MPa for Japanes cedar, and the reduced hydrostatic pressure precludes the accomplishment of complete cell expansion (Abe et al. 2003). With stress of higher intensity, or persisting longer, water potential falls below -2 MPa for white spruce, and photosynthesis and cell divisions in cambium also begin to decline (Abe et al. 2003). Thus, if occurring with low intensity, summer droughts are expected to induce only slight modifications to wood anatomy, in particular the production of cells with smaller diameters and thicker cell walls (Arend and Fromm 2007; Gruber et al. 2010). Nevertheless, field drought experiments in Finland were able to markedly reduce secondary growth in mature Norway spruce, which produced tree rings with less tracheids (Jyske et al. 2010).

As growth and water status are both physically and physiologically related, understanding of the effects of drought events on wood formation require that cambial activity is coupled with the physiological traits of trees. Although several previous investigations have explored the effect of drought on tree growth in natural and controlled environments (Eilmann et al. 2009; Rossi et al. 2009a; Swidrak et al. 2011), the impacts of a prolonged summer drought on both the secondary growth and physiology of trees still remain largely unexamined. Despite the latest knowledge on the cellular, molecular, and developmental processes underlying wood formation, the recent literature still strongly recommends more experimental studies to assess how the secondary meristem copes with extreme drought events (Hartmann 2010; Jentsch et al. 2007).

The aim of this paper was to study the effect of summer droughts on xylem phenology and anatomy of mature trees growing in their natural environment. Manipulative experiments of rain exclusion were applied on mature black spruce [*Picea mariana* (Mill.) BSP] trees in four sites at different altitudes and latitudes in the boreal forest of Quebec, Canada. Black spruce is one of the most common boreal tree species (Krause and Morin 2005) and is endemic to Northeastern America. It is a slow growing species that often establishes immediately after forest fires. The root system is shallow, mostly to be found in the upper 20cm of the organic soil.

A multidisciplinary approach including high resolution monitoring of xylogenesis, tree physiology and stem radius variation was used to provide as complete information as possible on the water status of the trees and thus quantify the level of stress that was caused by the treatment. The lower water availability will reduce water potentials, which are expected to produce physical and physiological effects in trees, identified by modifications in (i) xylem cell size, (ii) activity of sources (i.e. photosynthesis), and (iii) activity of sinks (cell production), according to increasing levels of stress.

Material and Methods

Study area and experimental design

The study was carried out in four permanent plots [Simoncouche (abbreviated as SIM), Bernatchez (BER), Mistassibi (MIS) and Camp Daniel (DAN)] located along a latitudinal and altitudinal gradient, in the boreal forest of the Saguenay-Lac-Saint-Jean region, Quebec, Canada (Table 1).

The sites consisted of mature, even-aged black spruce stands characterized by a typical boreal climate, with cold winters and cool summers (Table 1). The mean annual temperature in the years preceding the experiment at the four sites was between -0.9 and 12.0 °C. May-September rainfall ranged between 402 and 532mm and increased with latitude. The soils are podzolic with different depths among sites. The organic layer in SIM ranged between 10 and 20 cm, with the maximum rooting depth limited by a shallow bedrock. In the other sites, the organic layer was deeper and attained 20-40 cm

In each site, ten dominant or codominant trees with upright stems and similar growth rates were chosen, five control and five treated trees. Trees with polycormic stems, partially dead crowns, reaction wood or evident damage due to parasites were avoided. The selection was based on the proximity among the treated trees to allow the installation of the equipment for rain exclusion on the same cluster of individuals. In 2010, plastic transparent under-canopy roofs were installed during late May-early June to exclude the treated trees from precipitation. The majority of the root system of black spruce is localized at a distance of 90-200 cm from the stem collar (Polomski and Kuhn 1998). Accordingly, the plastic roofs extended for at least 3 m from the stem of each tree and drained the rain in sinking points of the soil to avoid water flowing back towards the stem collar. The plastic roofs were removed in September to ensure the survival of trees. The five control trees per site were left untreated as a control.

The volumetric water content (VWC) of the soil was measured weekly in four points at a distance of 1 m from the stem of each tree and at a depth between 12-20 cm with a time domain reflectometry soil moisture meter (TDR). All VWC measurements were divided by the maximum observed value to standardize results and differences in VWC between the treatments were calculated as the percentage difference between treated and control plots. Rainfall data was recorded every 15 minutes on all sites and stored as hourly sums by means of CR10X dataloggers (Campbell Scientific Corporation). Daily sums of precipitation were later calculated with the time series obtained from the 24 measurements per day.

Tree physiology

The physiology of the selected trees was monitored weekly throughout the growing season in SIM and BER, the two sites experiencing the most extreme growth conditions (Rossi et al. 2011; Turcotte et al. 2011). Short canopy branches were collected at 8-10 m on the tree using a telescopic branch pruner and photosynthetic rate was measured using the LI-6400XT Portable Photosynthesis System (Li-Cor Biosciences, Lincoln, Nebraska) with the 6400-22L Lighted Conifer Chamber. Because Li-Cor uses a standard leaf area, the actual leaf area of each sample was adjusted using a regression based on the dry sample weight (Flower-Ellis and Olsson 1993). Mid-day (Ψmd) and pre-dawn (Ψpd) water potentials were measured with a pressure chamber (Model 610 Pressure Chamber, PMS instruments). For logistical reasons, the pre-dawn water potential was simulated in the morning by wrapping the sampled branches in aluminum foil for one hour before taking measurements (Bucci et al. 2004).

Xylem dynamics

Cambium phenology was studied from May to October on control and treated trees at weekly time resolution. Microcores were collected from the stem following a spiral trajectory between 90 and 170 cm above ground using Trephor (Rossi et al. 2006a). The wood samples were embedded in paraffin, cut into sections of 7 µm with a rotary microtome, fixed on slides and stained with cresyl violet acetate (0.15% in water). The phenology of xylem was followed under visible and polarized light at 400-500× by counting the number of cells (i) in the cambium, (ii) in radial enlargement, (iii) in cell wall thickening and lignification, and (iv) the number of mature cells along three radial files (Deslauriers et al. 2003a; Rossi et al. 2006b). Rows with cells with large tangential sizes were selected, to ensure that only cells cut at their middle longitudinal part were considered. Cambial cells were characterized by thin cell walls and small radial diameters. During cell enlargement, the primary cell walls were still thin, but the radial diameter was at least twice that of a cambial cell. Observation under polarized light discriminated between the phases of enlargement and cell wall thickening. Because of the arrangement of cellulose microfibrills, the developing secondary walls glisten when observed under polarized light, whereas no glistening is observed in enlargement zones, where the cells are still composed of just primary wall (Abe et al. 1997). The progress of cell wall lignification was detected with cresyl violet acetate reacting with the lignin (Rossi et al. 2006b). Lignification appeared as a color change from violet to blue. A homogeneous blue cell wall revealed the end of lignification and the tracheid reaching maturity (Gricar et al. 2005). The number of cells in each developmental stage was averaged for each tree.

One sample per tree, collected in October, at the end of the growing season when wood formation was finished, was stained with safranin to measure cell features. A camera fixed on an optical microscope was used to record numerical images at a magnification of 20x. Lumen area and cell wall thickness were measured in the tree rings produced in 2009, the year before the treatment, and 2010 on three radial files per section using Wincell^{Tm} (Deslauriers et al. 2003a) and were averaged for each tree and year. The effect of the treatment on cell number and cell characteristics was tested through General Linear Models (GLM) with repeated measurements using treatment and site as model factors.

Stem radius variations

Ten automatic point dendrometers per site were installed on treated and control trees at a height of 2 m on the stem to monitor radius variations during and after the period of rain exclusion. Dendrometers were based on a precision linear variable differential transducer (LVDT) enclosed in an aluminum housing and fixed to the tree with stainless steel rods having a thermal linear expansion coefficient of 17 µm m^{-1} C^{-1}. With this equipment, the percentage of metal expansion was less than 1% of stem variation. A sensing rod held against the surface of the bark measured the radius variations, which in our monitoring represented the overall variation in size of xylem and phloem together. The sensitivity of dendrometers to temperature and humidity was negligible due to the use of dimensionally stable compounds in their manufacture and the dead bark was partially removed to minimize error due to hygroscopic thickness variations. As the stem changed in size, the core of the LVDT moved and translated the displacement in an electrical signal. Measurements were taken every fifteen minutes and stored in CR-1000 dataloggers (Campbell Scientific Corporation) providing precise and high-resolution data of radius variation during the growing season.

Results

Soil water content and tree physiology

In general, the two northern sites had higher VWC, and control plots showed more fluctuations, mainly during the rainy periods (Fig. 1). When measurements started, between DOY 173 and 179, VWC in treated plots was already slightly lower than that of the control. During the period of treatment, VWC was substantially lower in the treated plots, ranging from 46.8% of reduction in DAN, the northern site, to 64.4% in SIM, the southern site. After removal of the under-canopy roofs, the difference in VWC was maintained for one and three weeks in SIM and BER, respectively. During the last measurements on DOY 285, DAN and MIS still showed a reduction of 25-39% in VWC between treated and control plots (Fig. 1).

Lower Ψpd and Ψmd were observed in the treated trees during the whole period of rain exclusion in SIM, and from DOY 174 in BER (Fig. 2). During the treatment, the difference in water potential between treated and control trees was larger in SIM than in BER, and was 0.16-0.17 MPa for Ψpd and 0.26-0.33 MPa for Ψmd. In both sites, the difference in water potential decreased rapidly after the removal of the under-canopy roofs. The photosynthetic rate of trees ranged between 1.6 and 6.2 µmol $CO_2 m^{-2} s^{-1}$, was highly variable and showed the same fluctuations in treated and control trees throughout the growing season. Higher values were regularly observed from the beginning of August in treated trees in BER. In SIM, the photosynthetic rate in September diverged markedly between treatments, but the difference disappeared with the ending of rain exclusion (Fig. 2).

Xylem dynamics

The radial rows of cells had a clear pattern of variation in the number of tracheids in the different developmental stages during the year, which resulted in a decreasing curve of cambial cells, two delayed bell-shaped curves of radial enlarging and wall thickening and lignifying cells, and a rising curve of mature cells (Fig. 3). All patterns of variation are similar between treatments and among sites. However, the number of cells in control trees in SIM was always markedly higher than that observed in treated trees and in the other sites. Cell enlargement started before the application of the under-canopy roofs, except for control trees in BER, where the first enlarging cells were detected one week after the treatment began. During late summer, the number of enlarging cells decreased to zero between DOY 207 and 243. The earliest and latest endings of cell enlargement were observed in DAN and SIM, respectively (Fig. 3). The first cells in wall thickening and lignification were detected between mid-May and the end of May, at the beginning of rain exclusion. Mature cells were observed from DOY 151 in SIM and DOY 172 in MIS and DAN. In the northern sites, the ending of xylogenesis occurred between DOY 242 and 270, on average 10 days earlier than in the southern sites, where a higher number of cambium cells were produced (Fig. 3).

Xylem characteristics

Fig. 4 shows the number of xylem cells and their size in control and treated trees for 2009, the year before the treatment, and 2010, the year of the treatment. Cell production along a radial row varied between 10 and 47, with significant differences observed among sites (F=7.74, $p<0.001$, Table 3). No significant interaction year×treatment was found ($p>0.05$), indicating that the number of cells produced by the cambium was not affected by the treatment. This was particularly clear in SIM, where both control and treated trees reduced cell production in 2010, but with the same intensity (Fig. 4). Lumen area ranged from 307 μm^2 in DAN to 394 μm^2 in BER and cell wall thickness varied from 2.3 µm to 3.9 µm. For both variables, significant differences were observed between 2009 and 2010 ($p<0.05$ for lumen area and $p<0.001$ for wall thickness) but variations differed between treatments, as shown by the significant interaction year×treatment ($p<0.05$ for lumen area and $p<0.01$ for wall thickness, Table 2). The GLM demonstrated that rain exclusion reduced cell area and increased cell wall thickness of xylem, but did not affect the number of cells produced by the cambium (Fig. 4).

Stem radius variations

Stem diameter varied according to the diurnal rhythms of water storage depletion and replenishment and during precipitation events (Fig. 5). Greater variations were observed at the beginning of the growing season, between mid-May and the end of June. After that, stem increase was markedly reduced, finally attaining a plateau from the beginning of August. The highest and lowest stem radius variations were observed in BER and DAN, respectively. During and after the treatment, no difference in stem radius variation between treated and control trees was observed in DAN, the northern site. In the three other sites, stem radius variations of the control were lower than those of treated trees. In BER and SIM, a difference between the treatments occurred soon after the installation of the experiment, indicating that stem radius of treated trees increased more slowly and to lesser extent than that of the control. The pattern of treated trees was similar to that of the control, as shown by the low variations in the difference between treatments. In MIS, the differences between control and treated trees occurred later, at the beginning of July (DOY 185). The pattern of the treated trees differed from that of the control, this is clear from the higher variations in the difference. After removal of the under-canopy roofs, the difference between both treatments decreased, but treated and control trees only attained similar final values of stem radius variation in BER.

Discussion

This paper presents a manipulative experiment of rain exclusion on mature black spruce growing in four sites of the boreal forest of Quebec, Canada, with the aim of studying the effects of summer drought on xylem phenology and anatomy in trees growing in their natural environment. Drought was expected to cause a decrease in water potential, with modifications of the activity of sources and sinks within the tree. The results showed significant changes in the size of the xylem cells, but no substantial change was observed in photosynthesis and cell production, thus only partly confirming the expectations.

The treatment affected the turgor pressure in the trees by reducing the water potential of xylem during cell development. Cell enlargement is physically sensitive to changes in hydrostatic pressure during the early stages of a water deficit (Abe et al. 2003; Steppe et al. 2006). Changes in the size of cells and cell walls are a well-known physiological response to drought, which plants activate to prevent cavitation and to increase the anatomical strength of their tissues (Hacke et al. 2001). Thicker cell walls and smaller cells result in a higher wood density, rendering tree rings less vulnerable to future drought events. These stronger cells may be important to maintain a minimal conductivity. However, these anatomical modifications require larger supplies of cellulose and lignin for cell wall production, and, in the long run, result in lower growth rates and water storage capacities (Durante et al. 2011; Hacke and Sperry 2001). Cells with reduced lumen area evidently imply that a more-than-proportionally smaller area is available for water conductance from the roots to the canopy (Anfodillo et al. 2011). Thus, trees with a less efficient network of water transport could be more sensitive to subsequent drought events. Also, as tree rings in conifers remain active for several years, repeated drought events could significantly reduce the overall conductivity of the stem, which could affect water availability for all metabolic processes of the needles. In contrast with our findings, Eilmann et al. (2009) found that as the environment gets drier, trees build more effective water conducting systems, with larger tracheids and thinner cell walls. However, that study was conducted on Scots pine, which is a species adapted to low water availabilities and soil moisture, and no extreme drought conditions were applied.

Cell division is a growth process occurring in the cambium, which is strictly related to the source of energy, the process of photosynthesis and its products (Deslauriers et al. 2009; Taiz and Zeiger 2006). Bigras (2005) affirmed that different levels of stress have different impacts on tree physiology. Photosynthesis of white spruce was not affected by drought until a severe stress of -2.0 MPa was reached (Bigras 2005). In our experiment, simulated Ψpd hardly fell below -1.5 MPa, and photosynthesis was only slightly inhibited, which demonstrated that the water stress was not sufficient to cause a physiological reaction of the trees. Consequently, cell production also remained unchanged. Moreover, trees are most susceptible to environmental signals in the first period of cell division, during cambium reactivation (Frankenstein et al. 2005). Accordingly, there is evidence that the timings of the water stress are particularly important for producing a marked effect on the secondary meristem. As shown in Fig. 3, cell division and even cell enlargement had already started at the end of May, when snowmelt was just completed and the sites were accessible to set up the experiment. We assume that the expected increases in winter precipitation supplying abundant water during snowmelt (Easterling et al. 2000), associated with an earlier cambium reactivation due to the warmer air temperatures (Lupi et al. in press; Rossi et al. 2011), may still provide adequate conditions for secondary growth in spring. Since the number of cambium cells is determining for the total number of cells produced, the start under more favorable conditions at the beginning of the growing season may compensate for the consequences of a severe drought during summer. This is supported by the results, which showed that the applied drought during summer had no influence on the total number of cells produced.

In SIM, the number of cambium and mature cells was markedly higher in control. As shown in Fig 4, control trees also produced more cells than treated trees in 2009. This indicated that there was an initial difference in growth between treatments before the experiment, and that the lower cell production in treated trees was not due to drought. Such a conclusion was confirmed by the lack in significant interaction year^{x}treatment found by GLM (Table 2).

The results clearly showed that the treated trees of southern sites (SIM and BER) were less able to rehydrate at night and during rainfall events. Nevertheless, after removing the plastic roofs from the stem, the treated trees, especially in BER, were able to rehydrate within 23 days and finally showed no difference from the control. The trees in MIS and SIM also showed a rehydration, although not complete. In DAN, there was no difference between treated and control trees, which can be explained by the differences in soil characteristics. In the northern sites, soils are deeper, with steady accumulations of organic matter producing thicker organic layers (Rossi et al. 2009b), that need more time to dry out or rehydrate.

Despite the lower variations in stem radius, there was evidence that treated trees shrunk and swelled according to the circadian cycle (Downes et al. 1999). As was shown by Giovannelli et al. (2007) in poplar, a higher stem shrinkage may take place during the early stages of drought, when tree water potential begins to decrease. Our results showed that the trees in SIM and BER were still able to rehydrate, because they follow the same pattern as the control, but with lower amplitudes, being still influenced by rainfall and nocturnal rehydration. This indicated that the sources of water were either still accessible or not completely cut off by the under-canopy roofs. However, the treatment appeared more effective and the treated trees were less able to rehydrate in MIS. These results suggested that site characteristics can play an important role in the responses of trees to drought.

Although root grafts between trees are known to have the capacity to transport water and nutrients efficiently between individuals (Stone and Stone 1975), the functionality of these structures in black spruce has never been demonstrated. Since the under-canopy roofs were installed at a height of about 2 m, the canopy was still directly exposed to rain. A hypothesis is proposed that this species has the capacity to absorb rain, dew or fog water directly from needles, has already previously been shown in other conifers (Boucher et al. 1995; Limm et al. 2009). However, even if foliar absorption could maintain a sufficient water potential and stomatal conductance for accomplishing photosynthesis during the treatment, it seems unlikely that the amount of water absorbed by needles was able to incessantly rehydrate the stems of treated trees. Despite the well-known superficial root system of the genus *Picea* and the evident reduction in soil moisture under the roofs, alternative sources of water drawn by roots in the deeper layers of soil cannot be excluded and should be specifically considered in the next investigations.

This study presents a manipulative experiment of rain exclusion lasting from June to September applied on mature black spruce in four sites of the boreal forest of Quebec, Canada. The water stress produced by the treatment modified the anatomical characteristics of wood but did not affect timings and amount of xylem growth. Trees quickly recuperated their normal water status after the removal of the under-canopy roofs. The results demonstrate the resistance of mature trees to one summer of rain exclusion, most likely due to the ability to collect water from sources other than the superficial soil horizon.

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Tables

Table 1: Location and climatic characteristics of the four study sites, listed in decreasing latitude. Altitude is reported in m above sea level (a.s.l.).

Site	Latitude	Longitude	Altitude (m a.s.l.)	Mean annual temperature	Maximum temperature (°C)	Minimum temperature (°C)	May-September precipitation (mm)
DAN	50° 41'N	72° 11'W	487	-0.9	34.2	-47.1	401.8
MIS	49° 43'N	71° 56'W	342	1.0	35.1	-42.4	425.4
BER	48° 51'N	70° 20'W	611	0.3	33.1	-39.8	500.2
SIM	48° 12'N	71° 14'W	338	2.0	35.7	-39.7	532.2

Table 2: F-Value resulting from GLM with repeated measurements using treatment and site as model factors, for cell number, cell area and cell wall thickness. One and two asterisks indicate differences at p<0.05 and p<0.01, respectively.

	Cell number	Lumen area	Cell wall thickness
Year	3.03	6.38*	7.87**
Treatment	0.60	1.64	4.32*
Site	7.74**	1.74	1.82
YearxTreatment	0.18	4.95*	8.39**
YearxSite	12.85**	0.89	1.68
YearxTreatmentxSite	0.84	0.34	0.73

Figure captions

Figure 1: Volumetric water content of the soil (VWC) in the treated (white dots) and control (black dots) plots during (grey background) and after the rain exclusion treatment. Vertical bars represent the standard deviation.

Figure 2: Ψmd, Ψpd and photosynthetic rate of treated (white dots) and control (black dots) trees during (grey background) and after the rain exclusion treatment. Vertical bars represent the standard deviation.

Figure 3: Radial number of cells, enlarging, wall thickening and mature cells in developing xylem of treated (white dots) and control trees (black dots) before, during (grey background) and after the rain exclusion treatment. Vertical bars represent the standard deviation.

Figure 4: Number of cells, cell area and cell wall thickness in the treated (white dots) and control (black dots) trees in 2009 (the year before treatment) and 2010 (the year of treatment, grey background). Vertical bars represent the standard deviation.

Figure 5: Mean daily stem radius variations (mm) in control (black line) and treated trees (grey line) before, during and after the rain exclusion treatment (grey window). The thicker black line shows the difference between treated and control trees.

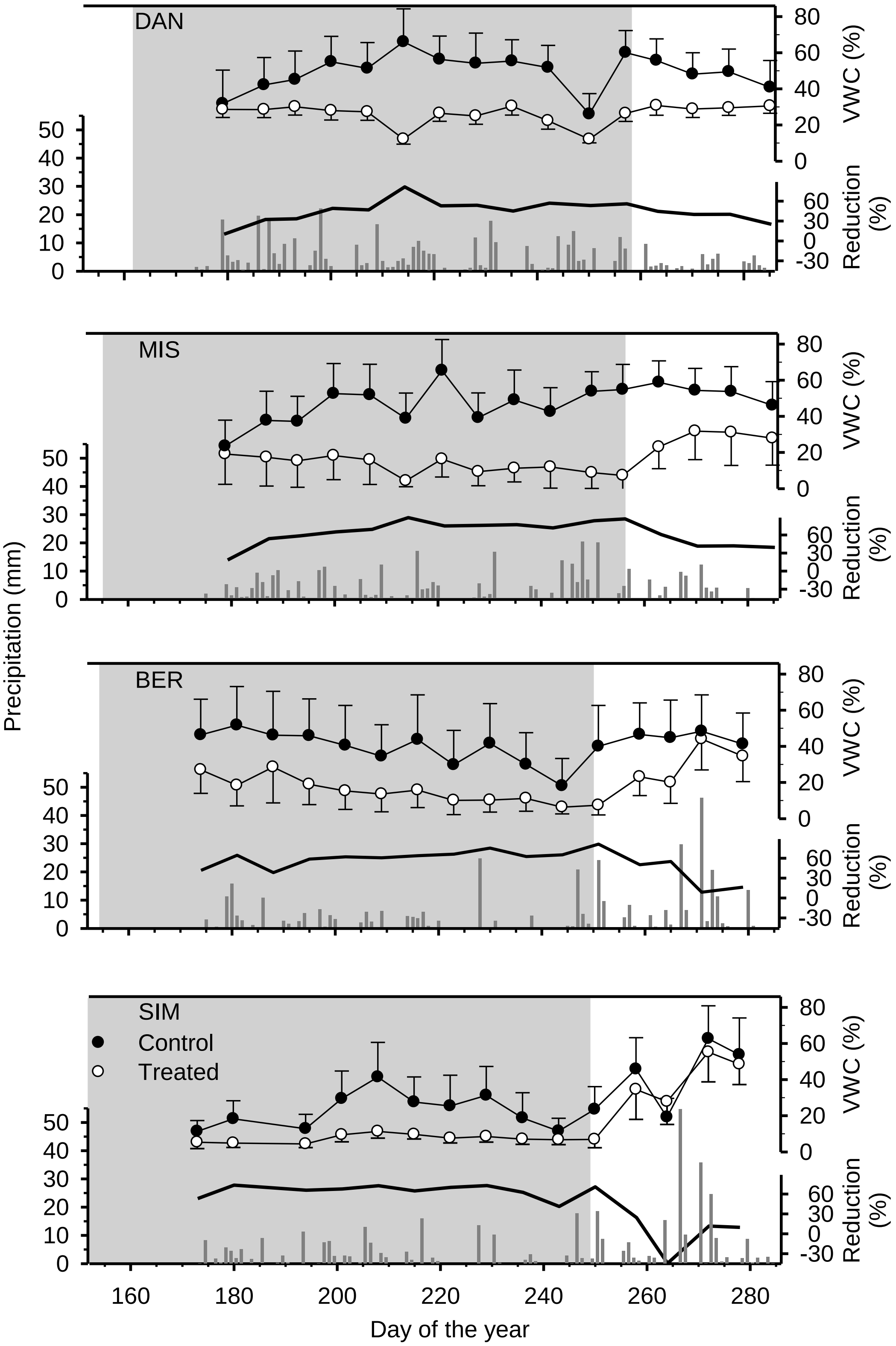

DAN
MIS
BER
SIM
● Control
○ Treated
VWC (%)
80
60
40
20
0
Reduction (%)
60
30
0
-30
Precipitation (mm)
50
40
30
20
10
0
160
180
200
220
240
260
280
Day of the year

Figure 1

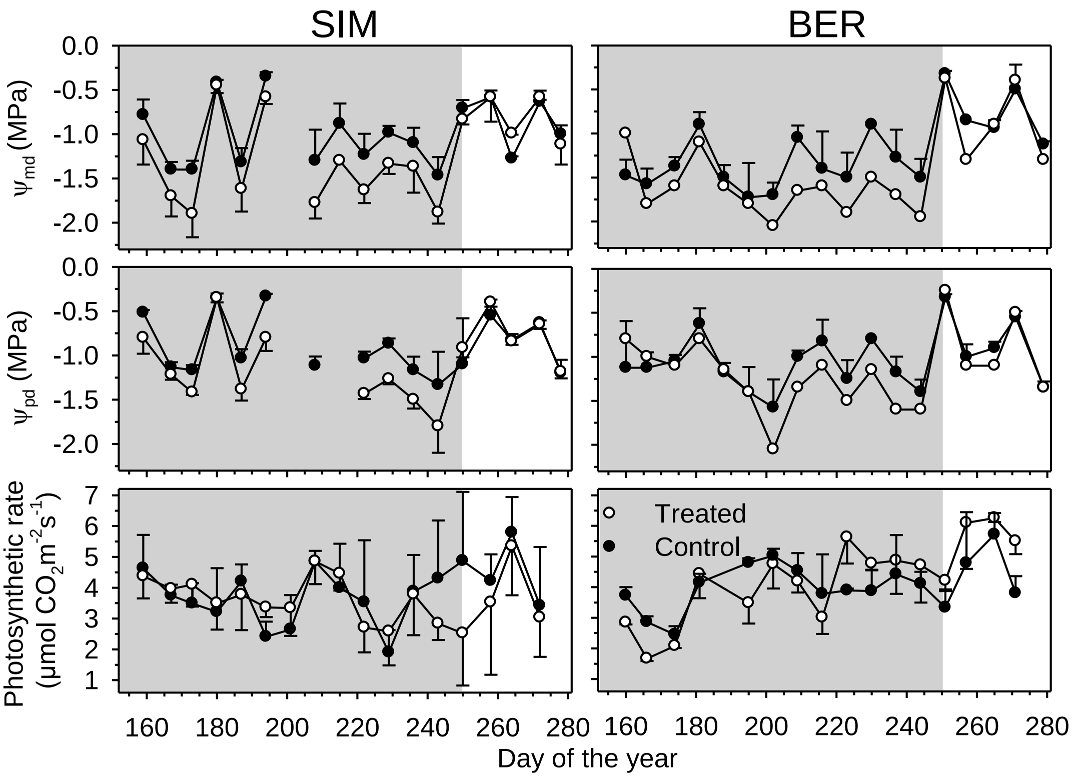

SIM
BER
0.0
-0.5
-1.0
-1.5
-2.0
Ψ_{md} (MPa)
Ψ_{pd} (MPa)
Photosynthetic rate
(µmol $CO_2 m^{-2} s^{-1}$)
7
6
5
4
3
2
1
○ Treated
● Control
160
180
200
220
240
260
280
Day of the year

Figure 2

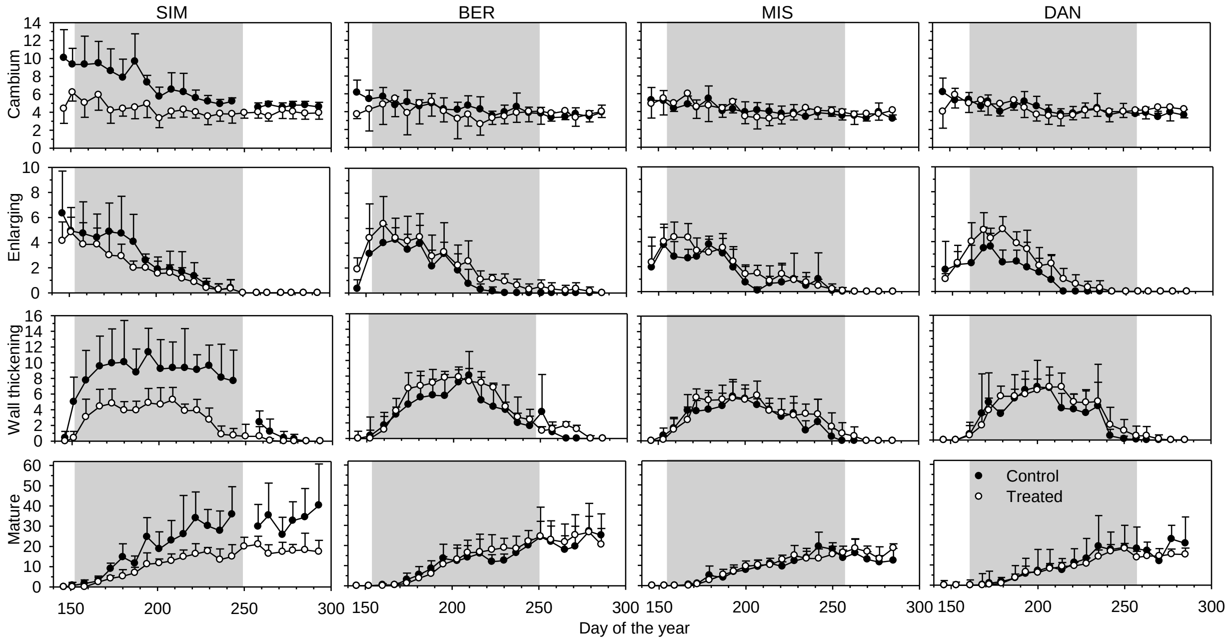


Figure 3

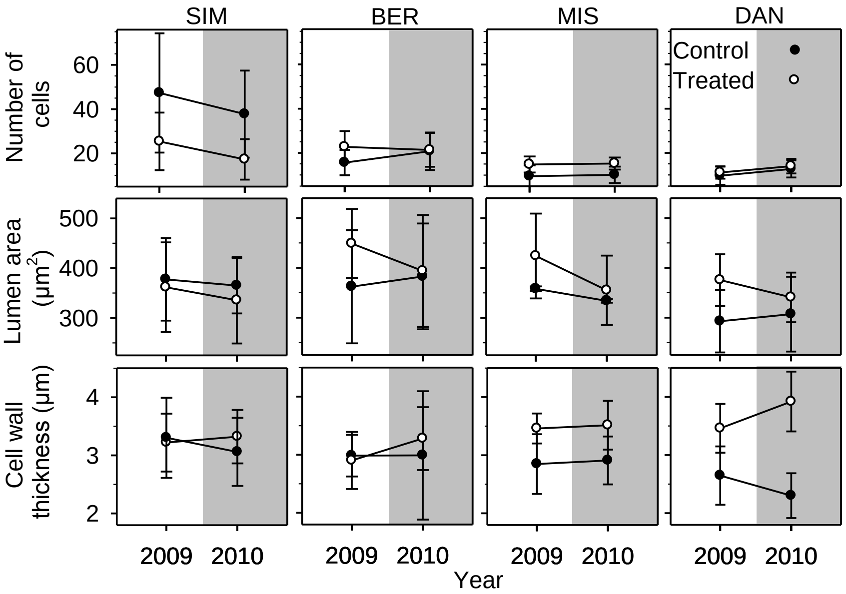
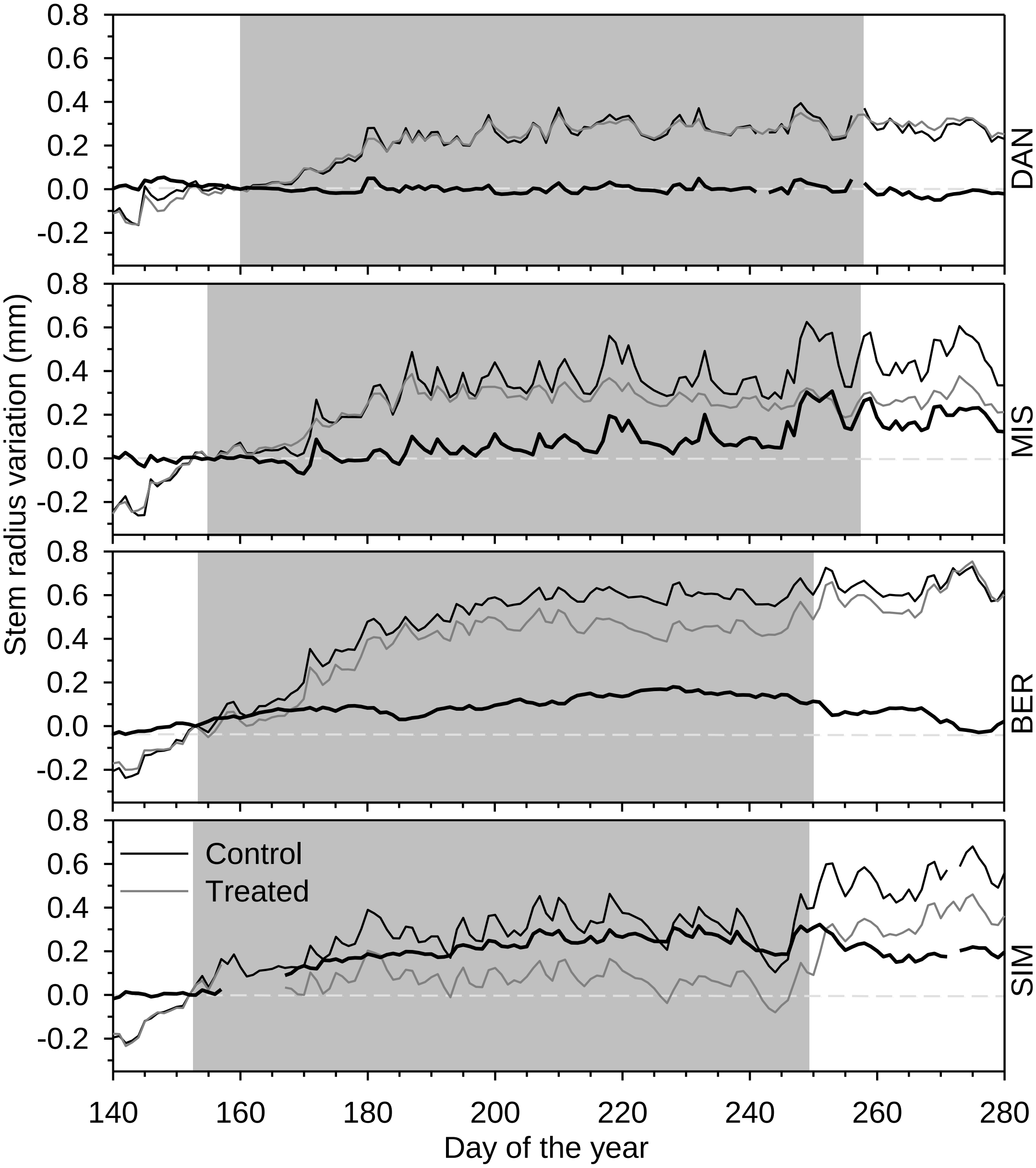


Figure 4



Stem radius variation (mm)
0.8
0.6
0.4
0.2
0.0
-0.2
DAN
MIS
BER
SIM
Control
Treated
140
160
180
200
220
240
260
280
Day of the year

Figure 5