

UNIVERSITE DU QUÉBEC

WOOD FORMATION AND PHYSIOLOGY OF MATURE BLACK SPRUCE (*PICEA MARIANA*
(MILL.) BSP) SUBJECTED TO RAIN EXCLUSION IN THE FIELD

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Mai 2015

THÈSE PRÉSENTÉE À
L'UNIVERSITÉ DU QUÉBEC À CHICOUTIMI
COMME EXIGENCE PARTIELLE
DU DOCTORAT EN BIOLOGIE

*“Suppose we suddenly wake up and see that what we thought to be this and that, ain't this and that
at all?”*

-Jack Kerouac, The Dharma Bums

Summary

Climate change causes more frequent and severe periods of drought in summer and higher overall temperatures in the northern regions of the world. This may produce potentially severe water stresses in the boreal forest. The boreal forest of Québec normally has low temperatures and water evaporation, meaning that the soils remain wet throughout the growing season. Boreal species are adapted to live with optimal moisture conditions, and the harsh climate with long cold winters and short growing seasons result in a slow development of the ecosystem. This makes for an ecosystem that is potentially vulnerable, but its' reaction to climate change is not yet well-known.

The objective of this study was to evaluate the impact of climate change, drought in particular, on wood formation, cambium phenology, water relations and physiology of mature black spruce trees (*Picea mariana* (Mill.) BSP) in their natural environment. Different measurement approaches were used to present a large picture of the effect of rain exclusion on black spruce's water relations. An experiment was installed to exclude precipitation, combined with high-resolution monitoring of stem radius variations, anatomy and physiology of mature black spruce trees. It was hypothesised that drought will cause a clear decrease in water potential; (1) resulting in a decrease in cambium activity and photosynthesis, fewer and smaller tracheids with thicker cell walls and a shorter period of wood formation; (2) this will lead to an increase in stem shrinking and a decrease in stem radius variations; (3) during drought black spruce may resort to alternative ways to absorb water.

The experiment was conducted on four research sites along a latitudinal gradient in the Saguenay Lac Saint-Jean Region, Quebec, Canada. On each site five mature black spruce trees were excluded from precipitation at the root system during the summers of 2010, 2011 and 2012. Plastic roofs were installed in May to cover the entire root system of each tree, and removed in September to ensure the winter survival of the trees. Five trees per site were left untreated and used as a control. In addition a greenhouse experiment was conducted to test for the possibility of foliar absorption as an alternative water acquisition strategy in black spruce.

During the three summers of the experiment different aspects of tree growth and physiology were studied at different temporal resolutions. On the two southern sites water potential and photosynthesis were measured weekly. Micro-cores were collected on all sites and automatic point dendrometers were installed to provide year-round data about the trees' stem radius variations. During the rain exclusion the soil water content quickly decreased on all sites, this shows that rain was successfully excluded from the root system. During winter and spring the soil rehydrated to its normal water status before the next growing season.

Overall the results suggest that black spruce trees do not seem to be susceptible to the short-term negative consequences of reduced water availability to the roots. Phenology of wood formation was not affected. It was expected that the effects of the treatment would soon be visible in a decrease in water potential, followed by a reduction in photosynthesis. But no difference in photosynthesis and water potential was found between the treated and control trees in any of the years.

The stem radius variations were analysed using a sequential analysis technique and the daily patterns of shrinking and swelling were extracted. On three out of four sites the trees subjected

to rain exclusion showed larger stem contractions in summer, and larger winter contractions were found on the northern sites. There was no significant difference in the cumulative stem expansion between control trees and treated trees over the three years. The analysis of stem radius variations showed that in spite of the reduced water availability the trees are still able to maintain their daily cycles of contraction and expansion.

The cell characteristics of control and treated trees were compared after the three years of the rain exclusion. Ring width and the number of cells produced did not differ between control and treated trees. During the first year of the experiment lumen area decreased and cell wall thickness increased, this is an expected reaction to drought because it reinforces the tracheids and helps prevent cavitation. In the second and third year however, lumen area and cell wall thickness returned to the pre-treatment values. In contrast with the expectations the effect of the rain exclusion did not persist or increased when the treatment was repeated.

Since black spruce did not seem to be strongly affected by the rain exclusion, the possibility of foliar absorption was explored in a greenhouse experiment using black spruce saplings. Twenty plants were excluded from irrigation, and twenty plants were irrigated normally. The root system was covered with plastic sheets, and after a period of desiccation the plants were sprayed at night with water with and without colorant. Water potential and photosynthesis were measured the following day. The colorant was not absorbed by the needles and water potential and photosynthesis were not improved by the canopy spraying. The results of this experiment do not show evidence for the existence of foliar absorption in black spruce saplings.

Résumé

Les changements climatiques causeront des périodes de sécheresse plus fréquentes et plus sévères en été, et des températures plus élevées dans les régions nordiques du monde. Ceci produira des stress hydriques potentiellement sévères dans la forêt boréale. Normalement, la forêt boréale du Québec est caractérisée par des basses températures et peu d'évapotranspiration, gardant ainsi les sols humides pendant la saison de croissance. Les espèces boréales sont adaptées pour vivre dans des conditions d'humidité optimales, alors que les longs hivers arides ne font que ralentir le développement de l'écosystème. En ce sens, une augmentation des températures créerait un écosystème potentiellement vulnérable, cependant les réactions aux changements climatiques sont encore peu connues.

L'objectif de cette étude était d'évaluer l'impact des changements climatiques, plus précisément la sécheresse, sur la formation du bois, la phénologie du cambium, les relations hydriques et la physiologie de l'épinette noire (*Picea mariana* (Mill.) BSP) dans son milieu naturel. Différentes mesures ont été employées pour présenter une vue d'ensemble de l'effet d'une exclusion de pluie sur les relations hydriques de l'épinette noire. Un dispositif expérimental a été mené pour exclure la précipitation, combinée avec un suivi à haute résolution des variations radiales du tronc, de l'anatomie et de la physiologie de l'épinette noire mature. Les hypothèses étaient qu'une sécheresse causera une réduction en potentiel hydrique; (1) suivi par une réduction en activité cambial et photosynthétique, moins de trachéïdes, des trachéïdes plus petites ayant des parois plus épais et une période de formation de bois plus courte; (2) ceci mènera à une plus grande contraction du tronc et éventuellement à une réduction en variations radiales; (3) pendant la sécheresse l'épinette noire pourra employer des moyens alternatives pour absorber l'eau.

L'expérience a été menée sur quatre sites d'étude le long d'un gradient latitudinal dans la région du Saguenay-Lac-Saint-Jean, Québec, Canada. À chacun des sites, cinq arbres matures ont été exclus de la précipitation au niveau du système racinaire pendant les étés de 2010, 2011 et 2012. Des toiles en plastique ont été installées en mai afin de couvrir complètement le système racinaire puis ont été retirées en septembre pour assurer la survie des arbres en hiver. Cinq arbres par site ont été laissés sans traitement et ont servi de témoin. De plus, une expérience en serre a été réalisée pour tester la possibilité d'absorption foliaire comme mécanisme d'absorption d'eau alternatif.

Pendant les trois étés de l'expérience, les différents aspects de la croissance et de la physiologie ont été étudiés à différentes résolutions temporelles. Dans les deux sites au sud, le potentiel hydrique et la photosynthèse ont été mesurés à chaque semaine. Des micro-carottes ont été collectées sur chaque site et des dendromètres automatiques étaient installés pour fournir des données sur les variations radiales du tronc tout au long de l'année. Pendant l'exclusion de pluie le contenu en eau du sol diminuait rapidement, ce qui démontre que l'eau a été exclue du sol. Pendant l'hiver et le printemps suivant le sol s'est réhydraté.

En général, les résultats suggèrent que l'épinette noire n'est pas sensible aux conséquences négatives à court terme d'une réduction d'eau au niveau du système racinaire. La phénologie de la formation du bois n'a pas été affectée. Il était attendu que l'effet du traitement aurait mené une réduction du potentiel hydrique et une réduction de la photosynthèse. Par contre, aucune différence n'a été trouvée entre les arbres témoins et traités.

Les variations radiales du tronc ont été analysées avec une technique d'analyse séquentielle et les patrons journaliers de rétrécissement et gonflement ont été extraits. Pour trois des quatre sites les arbres soumis à l'exclusion de pluie ont montrés des contractions plus larges en été et les plus larges contractions hivernales ont quant à elle été observées dans les sites au nord. Il n'y avait pas de différence significative dans l'expansion cumulative entre les arbres témoins et stressés sur les trois années. L'analyse des variations radiales du tronc a démontré que, malgré la réduction de l'eau disponible, les arbres sont tout de même capables de maintenir leurs cycles journaliers de contraction et expansion.

Les caractéristiques cellulaires des arbres traités et témoins ont été comparées après les trois années de l'expérience. Le nombre de cellules produites n'était pas différent entre les deux traitements. Pendant la première année de l'exclusion, l'aire du lumen a diminué et l'épaisseur des parois a augmenté. Ceci est une réaction attendue puisque que les trachéides se sont renforcées afin de prévenir la cavitation. Par contre, dans la deuxième et troisième année l'aire du lumen et l'épaisseur des parois sont retournées aux largeurs prétraitement. Contrairement aux attentes, l'effet de l'exclusion de pluie n'a pas augmenté ni persisté avec la répétition du traitement.

Puisque l'épinette noire ne semblait pas être fortement affectée par l'exclusion de pluie, la possibilité de l'absorption foliaire a été explorée dans une expérience en serre avec des jeunes épinettes. Vingt plants étaient exclus d'irrigation et vingt plants étaient arrosés normalement. Le système racinaire a été couvert de toiles en plastique et suite à une période de dessiccation les plantes ont été arrosées en soirée avec de l'eau avec et sans colorant. Le colorant n'était pas absorbé par les aiguilles et le potentiel hydrique ainsi que la photosynthèse n'ont pas augmenté suite à l'arrosage. Les résultats de cette expérience ne démontrent pas d'évidence pour l'absorption foliaire chez des jeunes épinettes noires.

Remerciements

Ça fait si longtemps, que j'ai hâte à ce moment, écrire les remerciements pour mon doctorat. Premièrement et avant tout il y a Hubert Morin, mon directeur de thèse. Celui avec le bureau magique, où tu peux rentrer avec des problèmes, mais où tu sors toujours un peu plus de bonne humeur qu'avant. Merci Hubert, pour tout pendant ces 4 années. Merci aussi à Annie Deslauriers, ma co-directrice qui a toujours été disponible, et qui me faisait souvent penser aux choses que j'aurais pu oublier. Et merci à Sergio Rossi, qui a eu une patience interminable avec moi lors de l'analyse des données, en particulier des dendromètres, et l'interprétation et présentation des résultats.

Ensuite il y a toute l'équipe du labo d'écologie végétale et animale, avec Germain Savard comme brave capitaine. En plus des nombreuses étudiants qui ont travaillé sur l'analyse des échantillons et sur le terrain il y a Caroline Soucy, qui gère son labo cellulaire, il y a Jean-Guy Girard, sans qui l'installation n'aurait pas été possible. Il y a François Gionest, avec qui j'ai eu le privilège de faire un magnifique voyage à Mirage, qui est aussi le maître des dendromètres, de la météo et de n'importe quel projet que tu veux faire sur le terrain. Les journées en forêt n'auraient pas été pareilles si ce n'était pas pour Danielle Laprise; si moi j'aime faire du terrain, Danielle l'adore, pour moi c'est un exemple! Et que dire de ma Mireille, elle était là lors de ma toute première journée de terrain, et ensuite au labo. Merci Mireille, de toujours avoir eu une oreille pour mes petits et grands problèmes.

Il y a quelques étudiants qui méritent grandement une mention spéciale. Premièrement Vanessa Tremblay et Pascal Lapointe, qui ont réussi à expliquer comment utiliser la sacrée machine à photosynthèse à une fille qui parlait à peine français. Pendant la deuxième année le poids de mon terrain a été largement porté par Maryse Labreque. Et finalement il y a Philippe Cournoyer-Farley,

grâce à qui mon dernière année de terrain a été très agréable. Le troisième chapitre de cette thèse n'aura pas existé si ce n'était pas pour l'enthousiasme de Joseph Gaudard, qui l'a initiée comme projet de fin de baccalauréat. Merci à vous tous.

Je remercie également les amis du labo, tout spécialement Émilie Pamerleau, qui a toujours été à côté de moi et avec qui j'ai pu partager la lourdeur, des fois, du doctorat. C'était nécessaire, merci pour toutes les pauses-pommes, j'espère qu'on en aura encore et toujours.

Au travers le monde il y a Kerstin Treydte et Bastian Ullrich qui m'ont accueilli et aidé pour l'analyse des isotopes. Et il y a Noelia Gonzalez-Munoz, qui m'a toujours fait croire que c'est vraiment possible; finir le doctorat en gardant la folie dans le cœur.

Merci à mes amis de Chicoutimi. Il y a 4 ans je ne vous connaissais pas encore, maintenant vous êtes une partie indispensable de ma vie. Merci mille fois pour toutes les bières, les soupers, les marches, les discussions et les fous-rires. Je n'ai vraiment pas assez de mots, mais vous êtes dans le plus profond de mon cœur. Chacun qui a croisé mon chemin m'a aidé -des fois surement sans s'en rendre compte- de ne pas lâcher, de toujours aller plus loin, et souvent, d'oublier.

Un dernier mot pour ma famille. Malgré la distance, mes brèves visites à la Belgique m'ont toujours rempli de nouvel énergie, leurs visites m'ont fait plus que plaisir. Les conversations sur tout et rien avec ma sœur, et les petits mots de mon frère ont fait que je ne me suis pas senti trop loin

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INTRODUCTION

Problem statement

The boreal forest represents about 33% of the global forested area and is an important source of forest products (Dumais et al., 2007, Tremblay et al., 2007). A large portion is unmanaged and mainly dominated by natural disturbances. This ecosystem is composed of only a few tree species, living under harsh conditions, but still contains a rich diversity in ecosystems and landscapes (Lynch et al., 2003). The boreal forest has an important function in the dynamics of the world's carbon budget and nearly all fresh water delivered by rivers to the Arctic Ocean originates from the boreal zone. Black spruce (*Picea mariana* (Mill. B.S.P) is the most common tree species in the boreal forest of Eastern North America (Mailly and Gaudreau, 2007). The species only occurs in North America and has a high economic value. Because of its good fiber quality the wood is highly valued for paper production and construction wood.

Climate changes in the boreal forest are already happening and are significantly more intense in the Northern regions (Gauthier et al., 2014). Recent climate models for Eastern Canada predict that the mean annual temperature will increase about 3-4°C and that precipitation will increase 5-20% by 2050 (Plummer et al., 2006) The predicted increase in temperature may have a positive impact on forest productivity as long as water is not limiting (Boisvenue and Running, 2006). Despite the general increase in precipitation throughout the year; early spring conditions, when growth is starting, shall be variable with extremes (Zhang et al., 2000, Burke et al., 2006, Walsh et al., 2011). An increase of precipitation in winter, but a decrease of precipitation in the months of June, July and August is expected. Moreover, drought events will be more frequent and of longer duration (Burke et al., 2006). In combination with higher evaporation due to increasing temperature lower water availability will result in more severe water stresses for the trees. Recent literature (Hartmann, 2010) strongly recommends more experimental studies to assess the impact of

drought events on tree vitality in different species and environments. With the increasing awareness of an anthropogenic induced climate change, an increasing need in estimating the impact of environmental changes on intra-annual tree growth is arising.

In spite of the importance of black spruce and the boreal ecosystem, the response of mature trees to climate change is not yet well understood. To our knowledge a thorough experiment, over several years and sites, on mature black spruce trees has never been carried out before. In this work we present an original experiment combining real-time growth measurements with manipulation of precipitation on mature trees in their natural environment along a lati- altitudinal gradient in the boreal forest.

Current knowledge

Future changes in wood formation caused by climate change can be understood by using current observations. Wood formation is based on cambial cell division and expansion and secondary cell wall production. As is demonstrated by numerous studies (Hughes, 2002) tree ring and wood characteristics are influenced by climate and are likely to record deficits, such as lack of water during growth. Temperature and precipitation are known to have an influence on growth processes (Lussier, 1994, Deslauriers and Morin, 2005). Over a large timescale drought can explain the year-to-year tree ring variation of boreal species such as black spruce (Girardin and Tardif, 2005). The majority of tracheids of black spruce is formed between the beginning of June and the end of July (Deslauriers et al., 2003, Deslauriers and Morin, 2005, Rossi et al., 2006b, Rossi et al., 2008). During this period in particular environmental signals are important for trees and cell formation (Frankenstein et al., 2005). This means that the environmental conditions stimulating wood growth should be optimal in exactly this short time frame of cell-formation.

Temperature is an important factor determining tree growth and forest productivity (Rossi et al., 2008), but also water is often a limiting factor. Plants use large amounts of water of which most is lost to transpiration, the remaining amount of water is of vital importance for photosynthesis and cell growth. Plants can continue to absorb water as long as their water potential is lower than that of the soil water. When the soil water potential decreases, plants have two general mechanisms to cope with water stress: Minimising water loss and maximizing water uptake (Chaves et al., 2003). Physiological processes in trees, such as stomatal conductance and cell growth, are a function of water potential, therefore water potential is a common measure used to determine the water status of a plant and the impact of environment on the physiology. For black spruce a water potential between -2.7 and -2.9MPa is considered a severe water deficit (Balducci et al., 2013), but stomatal closure may already occur at -1.3MPa (Grossnickle and Blake (1986), Blake and Sutton (1987)). In general, the permanent wilting point is reached when the water potential reaches values of -1.5MPa, meaning that plants cannot maintain turgor pressure. Turgor pressure is essential for many physiological processes and hydromechanical stability of the plant. Cell expansion is one of the first processes affected by water deficit, followed by cell wall synthesis, stomatal conductance and photosynthesis (Bélanger et al., 1996, Pantin et al., 2012). This is consistent with the findings of Stewart et al. (1995) that drought may cause a large decrease in stomatal conductance of black spruce seedlings, whereas photosynthetic capacity remains largely unaffected. Photosynthesis and stomatal conductance of white spruce remained unaffected by drought up to a water potential of -2MPa (Bradley et al., 2001). High CO₂ levels, also associated with climate change, may compensate for the decreased stomatal conductance. This may result in unchanged rates of photosynthesis even with lower water availability. Black spruce annual growth is known to be positively related with June-August precipitation of the current year and of the 2 years prior to the current year (Dang and Lieffers 1988).

Trees may be able to resort to other water absorption mechanisms during periods of drought. Previous research on conifers (Boucher et al., 1995, Turgeon et al., 2005) showed that trees have the capacity to absorb rain, dew or fog water directly into the leaves. This way dew or fog can increase the water potential of the shoots and the stomatal conductance (Boucher et al., 1995).

According to Abe et al. (2003), cell expansion is restricted by a decline in hydrostatic pressure during the early stage of water deficit. During the later stages of water deficit cambial cell division also declined. A limited water supply and low water potentials may lead to cavitation of the tracheids (Sperry et al 2002). Moreover, plants that experienced severe water stress are particularly susceptible to cavitation (Hacke et al., 2000, McLachlan and Clark, 2004). A way to prevent cavitation and increase mechanical strength is to form smaller tracheids with thicker cell walls (Fortin et al., 2008). Arend and Fromm (2002) showed that drought events in early summer can induce wood anatomical modifications like the formation of smaller tracheids. In a drought experiment in Finland Jyske et al. (2010) found that mature Norway spruce produced fewer tracheids with thicker cell walls, mainly in the latewood. In the boreal forest it was found that mature balsam fir produced smaller tracheids with thicker cell walls during two summers of artificial drought (D'Orangeville et al., 2013). The timing of cambium activity and thus the growing season is mainly influenced by temperature. However, it was found that under influence of drought, the growing season of other conifers, hence the duration of xylem activity, is shorter as compared to irrigated trees (Eilmann et al., 2009, Gruber et al., 2010). Experiments by Rossi et al. (2009) showed that short periods of drought do not have a severe impact on radial growth of young balsam fir but it was not excluded that longer periods of drought may have an indirect effect on plant growth. However, the effect of longer and repeated periods of drought on growth of mature black spruce trees is still largely unknown.

Normally, trees experience a shrinking of the stem during the first part of the day due to transpiration, followed by an expansion phase due to rehydration during the afternoon and the evening. Apart from these diurnal changes, also dry and wet periods of various durations can be recognised in the stem radius variations (Zweifel and Häsler, 2001). Changes in stem radius are proportional to the water content in the bark (Zweifel et al., 2000) and a decrease in stem radius can be caused by a depletion of water in the bark (Pregent and Poliquin, 2006). Automatic dendrometers are commonly used to monitor trees' stem radius variations. However, Gruber et al. (2008) found that diurnal fluctuations, measured by dendrometers in stem radius reflected primarily the bark water content, rather than actual changes in growth. Therefore dendrometers are an appropriate tool to provide information about the water status of the trees and physiology. Following Zweifel et al. (2006) dendrometer data can be used as a direct physiological measure of drought stress. A decline in a plants water status is reflected in an increase in stem shrinking during the early stages of drought (Giovannelli et al., 2007). In addition, stem radius variation is directly influenced by precipitation and sap flow during the night and is negatively correlated with air temperature (Deslauriers et al., 2007).

Objective and Hypotheses

The objective of this study was to evaluate the impact of climate change, drought in particular, on wood formation, cambium phenology, water relations and physiology of mature black spruce trees in their natural environment. A multidisciplinary experimental approach was used to apply climate control, through exclusion of precipitation and along a latitudinal and altitudinal gradient, combined with high-resolution monitoring of growth and physiology of mature black spruce trees.

The specific objective of the experiment was to study the effects of an artificially induced drought on radial increment and radial variation, cambium phenology, physiology and tracheid properties of mature black spruce trees.

It was hypothesised that drought will cause a decrease in water potential; (1) resulting in a decrease in cambium activity and photosynthesis, fewer and smaller tracheids with thicker cell walls and a shorter period of wood formation; (2) this will lead to an increase in stem shrinking and a decrease in stem radius variations; (3) during drought black spruce may resort to alternative ways, other than directly via the root system, to absorb water.

Study sites and methodological approach

Four permanent research sites along a latitudinal and altitudinal gradient in the Saguenay-Lac-Saint Jean Region, Québec, Canada were used in this experiment: Simoncouche (abbreviated as SIM), Bernatchez (BER), Mistassibi (MIS) and Camp Daniel (DAN). The southern site (SIM) is located on the southernmost distribution limit of black spruce stands.

Each site consisted of typical mature, even aged pure black spruce stands. The understorey vegetation on the sites contained *Kalmia angustifolia*, *Ledum groenlandicum*, *Cornus canadensis*, with soil vegetation consisting mainly of *Sphagnum spp.* The sites consist of large areas of bedrock, covered by a thick layer of fine-grained marine sediments. There is a thin organic layer at SIM, and a thicker organic layer at the Northern sites. Climate details for each site are presented in chapter 1.

Ten dominant trees with upright stems and branches that are easily reachable were selected. Damaged trees; trees with partially dead crowns or with reaction wood were avoided. Transparent 4µm thick plastic under-canopy roofs were installed on each site at the beginning of June 2010,

2011 and 2012, excluding the root system of at least five mature 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. Five trees per site were left untreated as a control.

Several aspects of tree growth, physiology and water relations were studied in this project, to provide a large picture of the effects of rain exclusion on black spruce. During the rain exclusion all sites were visited weekly to measure the volumetric soil water content (VWC) and to collect micro-cores (Rossi et al., 2006a). In SIM and BER also water potential and photosynthesis were measured weekly on control and treated trees, to provide an indication of the trees' basic physiological reaction to the rain exclusion. These measurements were only done on the two southern sites because they are more easily accessible and nearby.

For the growth measurements the microcores were treated in the laboratory and 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 was counted (Deslauriers et al. 2003a; Rossi et al. 2006b). At the end of each growing season one sample per tree was prepared for analysis of the wood anatomy. Lumen area and cell wall thickness were measured in the tree rings produced in 2008, 2009, the 2 years before the treatment, and in 2010, 2011 and 2012 on three radial files per section using Wincell (Deslauriers et al. 2003a).

In addition to these measurements of wood anatomy and cell development, dendrometers were used to study the radial variation of the stem at a high 15-minute resolution. Dendrometers

record not only the radial growth of trees, but also the daily water-related expansion and contraction and are thus an appropriate tool to provide information about the water status of a tree.

The possible alternative water sources of black spruce were explored using isotopes of different water sources and compare them to isotopes inside the plant. Secondly a greenhouse experiment was done on forty black spruce saplings, of which half was excluded from irrigation. The crown of the saplings was sprayed with normal water and colored water. Water potential and photosynthesis were measured the following day to evaluate the occurrence of foliar absorption.

Structure of the thesis

This thesis contains 4 main chapters, comprising two published articles, one submitted article and one finished chapter, followed by a general conclusion.

The first chapter gives an overview of the effect of one summer of rain exclusion on the xylem phenology and anatomy of mature trees growing in their natural environment. Also the raw data of stem radius variations is presented in this chapter. This chapter showed that black spruce did not seem to suffer any severe growth reduction or even mortality when the root system was excluded from direct rainfall.

The second chapter deals with the seasonal patterns of shrinking and rehydration throughout the years and with the daily patterns of contraction and expansion. A new method using Cusum charts for the analysis of chronologies is presented in this chapter and the interest of year-round stem radius variation measurements is pointed out. Black spruce seemed to be able to rehydrate, even if the root system was excluded from direct rainfall. It was therefore hypothesized that black spruce might have been able to employ other water acquisition strategies.

Preliminary results (Belien et al 2012) showed that black spruce seemed to be able to rehydrate, even if the root system was excluded from direct rainfall. It was therefore hypothesized that black spruce might have been able to employ other water acquisition strategies. The third chapter explores the possibility of foliar water absorption by black spruce saplings, in particular during a period of drought. It was verified in a greenhouse experiment if water potential or photosynthesis increased after nocturnal needle wetting.

The fourth chapter focuses on wood anatomy of the trees during the three years of the experiment. Tracheid characteristics are studied and complemented with a xylem vulnerability curve for black spruce. This chapter allows to have a better idea about the hydraulic and mechanic characteristics of black spruces xylem.

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**CHAPTER 1. XYLOGENESIS IN BLACK
SPRUCE SUBJECTED TO RAIN
EXCLUSION IN THE FIELD**

Published in : Canadian Journal of Forest. Research. 42: 1306-1315 (2012)

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Xylogenesis in black spruce subjected to rain exclusion in the field

Abstract

The predicted climate warming and more frequent and longer droughts are expected to produce potentially severe water stresses in the boreal forest. The aim of this experiment was to study the effect of a summer drought on xylem phenology and anatomy of mature black spruce [*Picea mariana* (Mill.) BSP] trees in their natural environment. The trees were excluded from rain during June-September 2010 by the installation of under-canopy roofs in four sites of the boreal forest of Quebec. Xylem phenology, stem radius variations and physiological traits of treated and control trees were monitored at short-time resolution. At the end of the growth season cell characteristics were measured. The rain exclusion reduced the cell area of the xylem, but no significant change was observed in cell wall thickness, cell production or phenology. Stem radius variations of the treated trees were lower, but followed the same pattern as the control. After removal of the exclusion, trees and soil quickly recovered their normal water status. One summer of drought lead to the formation of smaller tracheids, but showed that black spruce is resistant to this rain exclusion treatment. This is likely due to the ability to collect water from sources other than the superficial soil horizon.

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

Résumé

L'augmentation de la température et des périodes de sécheresse plus extrêmes et plus fréquentes prévues produiront des stress hydriques potentiellement sévères dans la forêt boréale. Le but de cette expérience était d'étudier l'effet de la sécheresse estivale sur la phénologie et l'anatomie du xylème des épinettes noires matures [*Picea mariana* (Mill.) BSP] en milieu naturel. Les arbres ont été exclus de la précipitation de juin à septembre 2010. Des toiles imperméables ont été installées sous la canopée dans quatre sites de la forêt boréale de Québec. Le développement du xylème, la variation radiale du tronc et les traits physiologiques des arbres traités et témoins ont été surveillés à une haute résolution temporelle. À la fin de la saison de croissance les caractéristiques cellulaires ont été mesurées. L'exclusion de la pluie a diminué la superficie des cellules du xylème, mais aucun changement significatif n'a été observé dans l'épaisseur des parois, la quantité des cellules ou leur phénologie. Les variations radiales du tronc des arbres traités étaient plus basses mais suivaient le même motif que celles des témoins. Après avoir retiré les toiles imperméables, le sol et les arbres ont rapidement récupéré leur statut hydrique normal. Un été de sécheresse a entraîné la formation des trachéides plus petits, mais démontre que l'épinette noire est résistante au traitement d'exclusion de pluie. L'espèce a probablement une capacité de récupérer l'eau provenant de sources autres que le sol superficiel.

Introduction

The climatic models for Eastern Canada predict increases in temperature and precipitation in the next 50 years (Plummer et al., 2006, IPCC, 2007). 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 (Zhang et al., 2000, Burke et al., 2006). 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 latitude ecosystems (Walsh et al., 2011).

It is well-known that temperature plays an important role in cambium activity and wood formation in trees of cold climates (Rossi et al., 2008b, Gruber et al., 2010). 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 (Hofgaard et al., 1999, Deslauriers et al., 2003b, Duchesne and Houle, 2011). Several metabolic processes like photosynthesis, cell expansion and cell division are a function of turgor pressure (Lockhart, 1965, Kirkham et al., 1972). Changes in water status are thus necessarily reflected in the characteristics of xylem and phloem, and in the resulting radial growth of the stem (Bouchard and Pothier, 2011). A reduction in cell expansion has often been ascribed to a decrease in turgor pressure (Eckstein, 1990, Abe et al., 2003, Woodruff et al., 2004) and an increase in cell wall thickness results from reductions in turgor-driven cell expansions (Woodruff et al., 2008). During the early stages of water deficit the water potential decreases and inhibits stomatal conductance, photosynthesis and cell division decline after a

continued stress (Abe et al., 2003, Chaves 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 (Despont et al., 2002, 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 remains 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 (Cahall and Hayes, 2009, Hartmann, 2010).

The aim of this experiment was to study the effect of a summer of rain exclusion 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 (Mailly and Gaudreau, 2007) 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 2.0 °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 co-dominant 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 (Despont et al., août 2007). 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 (Casabon and Pothier, 2007, Cernusak et al., 2009). Short canopy branches were collected at 8-10 m height 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 based on the dry weight of samples collected at the start of each season using a regression according to Flower-Ellis and Olsson (2011). Mid-day (Ψ_{md}) and pre-dawn (Ψ_{pd}) 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 (Farquhar et al., 1989).

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 \times 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 microfibrils, 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 20 \times . 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[™] (Deslauriers et al., 2003a) and were averaged for each tree and year. Since the measurements were repeated on the same experimental unit in 2009 and 2010, a repeated measures analysis of variance was used to compare the results from the two years. A general linear model (GLM) was used with sampling time in the repeated statement and treatment and site as model factors to test for the effect of treatment and year (before and during the treatment) on cell number and cell characteristics. To test if the proportion of latewood was affected by the treatment a generalized linear mixed model (GLIMMIX) was used with year as a repeated measure in the random statement. All analyses were performed using SAS 9.2 (SAS institute Inc., Cary, NC).

Stem radius variations

Ten automatic point dendrometers (Agricultural Electronics Corp., Tucson, AZ,) 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 $17 \mu\text{m m}^{-1} \text{C}^{-1}$. 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 where the sensing rod touched the tree 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 fluctuated according to the rainy periods (Fig. 1). When measurements started, between DOY (day of the year) 173 and 179, VWC in treated plots of BER was already slightly lower than that of the control. The VWC was substantially lower in the treated plots during the period of treatment, the average reduction in VWC ranged from 46.8% of reduction in DAN, to 67.9% in MIS. 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 Ψ_{pd} and Ψ_{md} were observed in the treated trees during the whole period of rain exclusion in SIM, and from DOY 174 until the end of the treatment 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 Ψ_{pd} and 0.26-0.33 MPa for Ψ_{md} . 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 $\mu\text{mol CO}_2\text{m}^{-2}\text{s}^{-1}$, was highly variable on both sites. 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 ($F=7.43$, $p<0.01$, Table 2). No significant interaction year \times 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 . No significant interaction was found for cell wall thickness ($p>0.05$, Table 2), there was a difference in cell wall thickness between the treated and control plots ($F=8.90$, $p<0.01$), but this difference was already present in the year before the experiment (Fig.4). For lumen area, significant differences were observed between 2009 and 2010 ($F= 6.13$, $p<0.05$) but variations differed between treatments, as shown by the significant interaction year \times treatment ($F= 4.27$, $p<0.05$, Table 2). The GLM demonstrated that rain exclusion reduced lumen area of xylem, but did not affect the number of cells produced by the cambium (Fig. 4). The proportion of latewood ranged from 0.21 in BER to 0.37 in DAN, there was a decrease during the second year in the treated trees in SIM and in the control trees of BER and DAN. The proportion of latewood increased in the other plots (Table 2, Fig. 4). The interaction year \times treatment did not influence the proportion of latewood ($F=0.08$, $p>0.05$).

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. 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 treated trees were lower than those of control. 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.

An important consequence of drought is cavitation, which is caused by air-seeding and occurs when air is pulled through the pit membranes and fills the entire conduit (Cruiziat et al., 2002). More and larger pits per conduit increase the susceptibility to cavitation spreading between conduits, following the *rare pit* hypothesis (Christman et al., 2009). In general, smaller conduits are more resistant to cavitation, because they contain fewer pits and have better mechanical strength (Sperry et al., 2006, Fortin et al., 2008). Cell expansion is physically sensitive to changes in hydrostatic pressure during the early stages of a water deficit (Abe et al., 2003, Bouchard and Pothier, 2011). The turgor of cells declines proportionally with xylem water potential (Chertov et al., 2009), resulting in a decrease of cell expansion (Lockhart, 1965). According to the Hagen-Poiseuille law, hydraulic conductivity increases with the number or size of conduits, therefore reduced lumen dimension leads to a smaller area being available for water transport (Cruiziat et al.,

2002, Anfodillo et al., 2006, Anfodillo et al., 2011). For a long time, cavitation was thought to be irreversible, but it has been shown that embolisms can be reversed, even under negative water potentials (Sobrado et al., 1992). Even though the reduced hydraulic conductivity can be recuperated by rebuilding the water conduits, repeated drought may lead to a reduced growth and the formation of smaller vessels in the following year, because the anatomical modifications require larger supplies of cellulose and lignin (Cannell et al., 1976, Pizzolato, 2008, Galle et al., 2010).

Often a higher wood density due to drought can be explained by a higher amount of latewood (Domec and Gartner, 2002). However, in this study the proportion of latewood was not affected by the treatment (Fig. 4, Table 2). Since the proportion of latewood and total number of cells did not change, the decrease in the overall lumen area can be ascribed to the applied drought treatment. Other studies have reported a reduction in lumen diameter under dry conditions for Scots pine [*Pinus sylvestris*] (Sterck et al., 2008, Heijari et al., 2010). However, Eilmann et al. (2009) found that an uncommon drought event caused the formation of larger conduits for Scots pine, a species that is well adapted to a wide range of hydrological conditions (Poyatos et al., 2007).

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 (Bradley et al., 2001). In our experiment, simulated Ψ_{pd} 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. 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 finished and the

sites were accessible to set up the experiment. Trees are most susceptible to environmental signals in the first period of cell division which is during cambium reactivation (Frankenstein et al., 2005).

The expected increase in winter precipitation will provide abundant water during snowmelt (Walsh et al., 2011). Even though there is more snow, the melting will not be delayed, photosynthesis will thus not be inhibited and cambium reactivation is expected to occur earlier due to the increasing temperatures (Casabon and Pothier, 2007, Chang et al., 2009, Frechette et al., 2011). Adequate conditions for secondary growth in spring are thus assumed. Since the number of cambium cells is determining for the total number of cells produced (Rossi et al., 2008a), 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 the control plot. 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^xtreatment 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. In SIM and BER the treated trees followed the same pattern of de- and re-hydration, but with lower amplitudes (Fig. 5). This means that they were still influenced by rainfall events, nocturnal rehydration and changes in sapflow (Sevanto et al., 2008), indicating that 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 suggest that site characteristics play an important role in the responses of trees to drought.

Root grafts between trees are known to have the capacity to transport water and nutrients efficiently between individuals (Pregent and Y., 2001, Tarroux and DesRochers, 2011), but the functionality of these structures in black spruce has never been demonstrated. Previous studies have shown that absorption of water through the foliage can contribute to the tree water balance (Boucher et al., 1995, Turgeon et al., 2005). Even when no precipitation reaches the soil, water that is intercepted by the canopy can play a role in mitigating water stress and improve drought survival (Breshears et al., 2008). Since the under-canopy roofs were installed at a height of about 2 m, the canopy was still directly exposed to rain. Black spruce may thus have the capacity to absorb rain, dew or fog water directly from the needles. 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. It was clear that the treatment caused a reduction in the soil moisture content, but it is possible that the trees were able to cope with the drought by redistributing their water uptake to deeper soil layers (Eggemeyer et al., 2009), even if black spruce, like many other boreal species, is known to have a superficial root system (Strong and Roi, 1983). These alternative sources of water should be considered in further 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 clearly resulted in the formation of smaller tracheids but did not affect timings and amount of xylem growth. Trees quickly recovered 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.

Acknowledgments

This work was funded by the Natural Sciences and Engineering Research Council of Canada and the Consortium Ouranos. The authors thank F. Gionest, C. Soucy, S. Pednault, M. Thibeault-Martel, I. Grenon, D. Laprise, M. Boulianne and J-G. Girard for their technical support and A. Garside for checking the English text.

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Tables and figures

Table 1.1 : 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 1.2 : F-Value resulting from GLM with repeated measurements using treatment and site as model factors, for cell number, cell area and cell wall thickness; and from GLIMMIX for the proportion of latewood. One and two asterisks indicate differences at $p < 0.05$ and $p < 0.01$, respectively.

	Cell number	Lumen area	Cell wall thickness	Latewood proportion
Year	3.35	6.13*	0.71	0.14
Treatment	0.61	1.51	8.90**	1.29
Site	7.43**	1.74	0.38	1.15
YearxTreatment	0.17	4.27*	1.42	0.08
YearxSite	10.01**	0.83	0.63	0.11
YearxTreatmentxSite	0.52	0.28	2.64	1.06

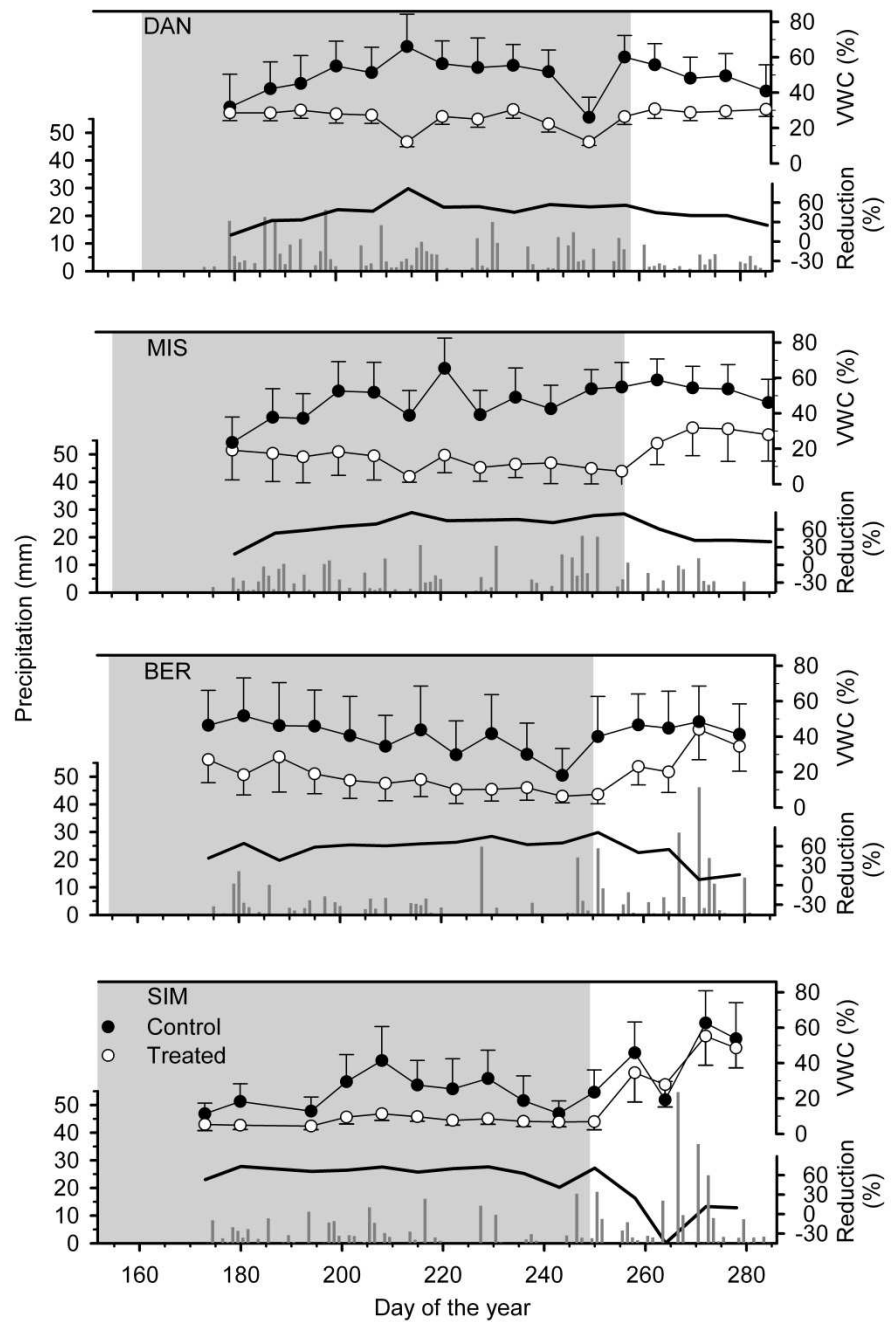


Figure 1.1 : Volumetric water content of the soil (VWC) in the treated (white dots) and control (black dots) plots during (grey background) and after the rain exclusion treatment. Vertical bars represent the standard deviation. Grey bars represent precipitation (mm).

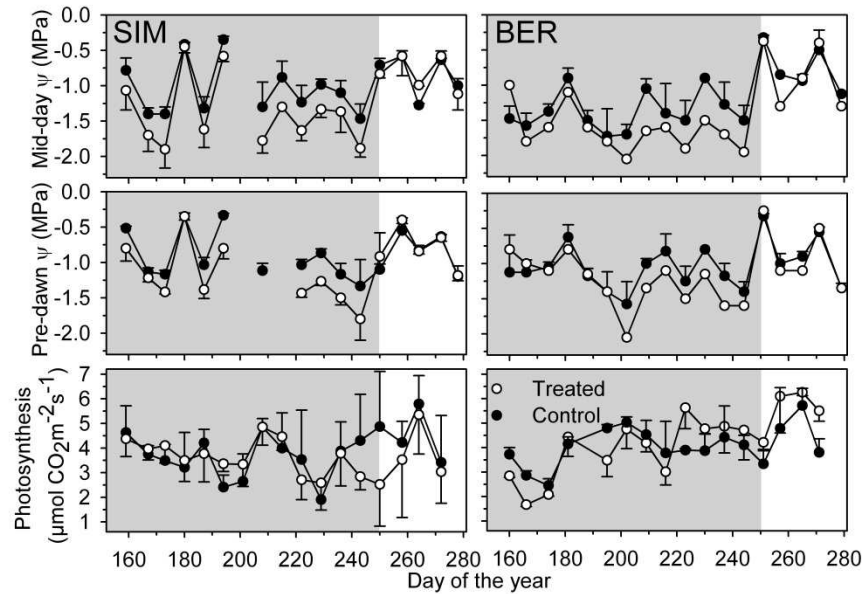


Figure 1.2 : Ψ_{md} , Ψ_{pd} and photosynthetic rate of treated (white dots) and control (black dots) trees during (grey background) and after the rain exclusion treatment. Vertical bars represent the standard deviation.

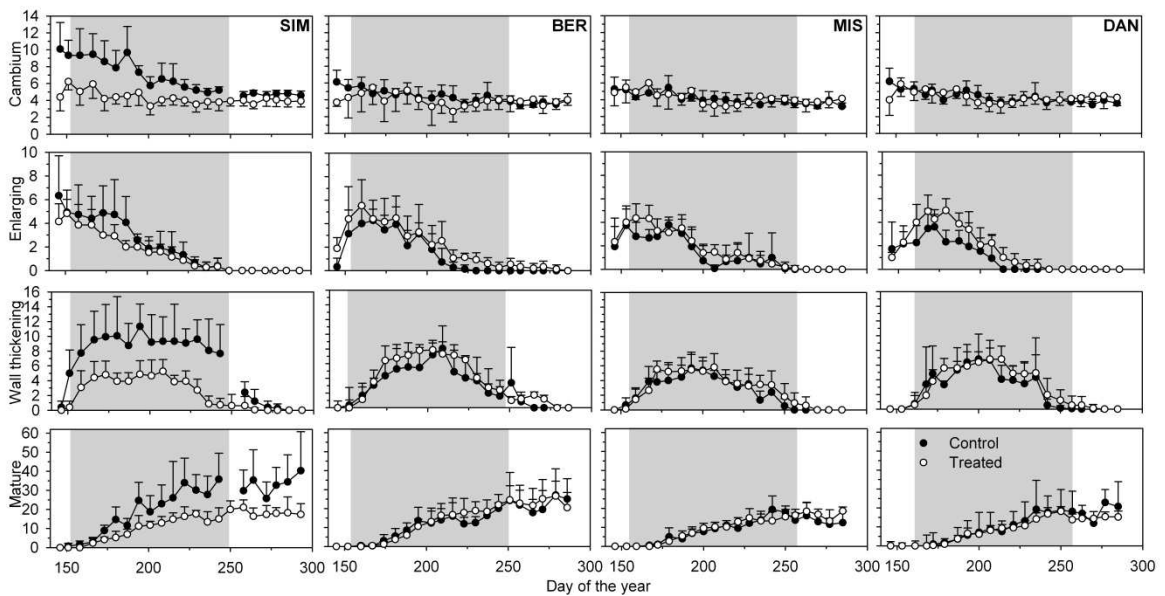


Figure 1.3 Radial number of cells, enlarging, wall thickening and mature cells in developing xylem of treated (white dots) and control trees (black dots) before, during (grey background) and after the rain exclusion treatment. Vertical bars represent the standard deviation.

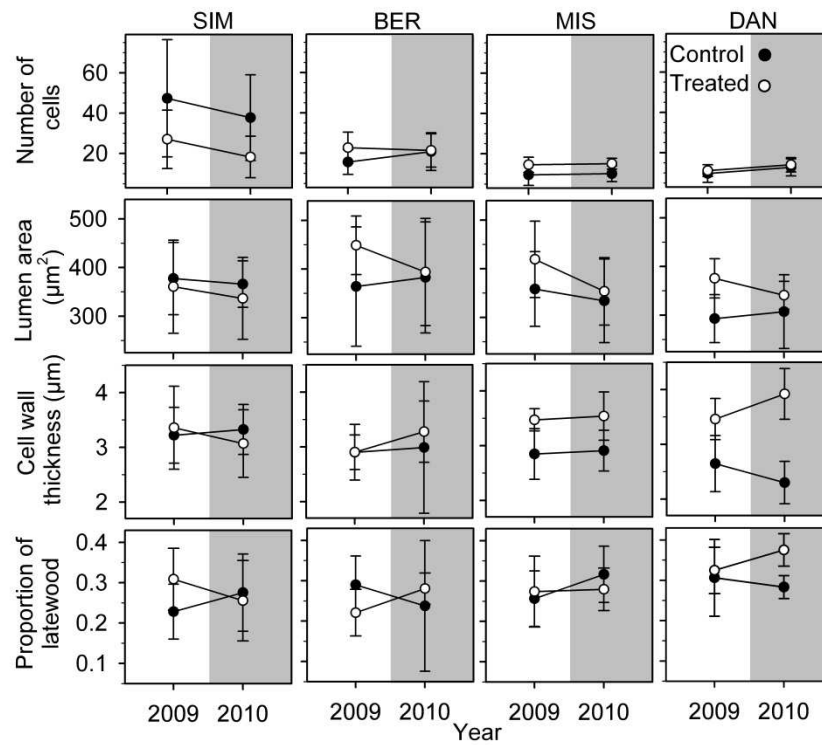


Figure 1.4 : Number of cells, cell area, cell wall thickness and proportion of latewood in the treated (white dots) and control (black dots) trees in 2009 (the year before treatment) and 2010 (the year of treatment, grey background). Vertical bars represent the standard deviation.

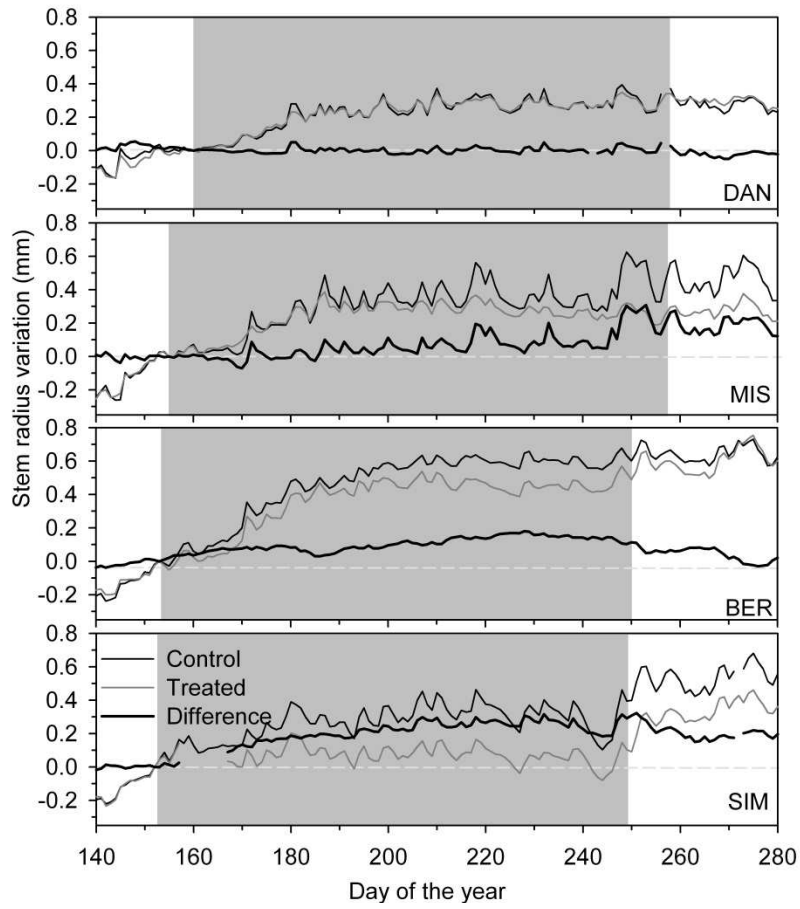


Figure 1.5 : Mean daily stem radius variations (mm) in control (black line) and treated trees (grey line) before, during and after the rain exclusion treatment (grey window). The thicker black line shows the difference between treated and control trees

CHAPTER 2. : HIGH RESOLUTION
ANALYSIS OF STEM RADIUS
VARIATIONS IN BLACK SPRUCE (*PICEA
MARIANA* (MILL.) BSP) SUBJECTED TO
RAIN EXCLUSION FOR THREE
SUMMERS

Published in : *Trees*, 1-9. doi: 245 10.1007/s00468-014-1011-4 (2014)

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High resolution analysis of stem radius variations in black spruce (*Picea mariana* (Mill.) BSP) subjected to rain exclusion for three summers

Abstract

Future climate warming is expected to produce more severe and frequent periods of drought with consequent water stresses for boreal species. In this paper, we present a high resolution analysis of stem radius variations in black spruce under rain exclusion. All summer long rain exclusions were applied for three consecutive summers to mature trees on four sites along a latitudinal gradient. The stem radius variations of control and treated trees were monitored year-round at an hourly resolution with automatic point dendrometers. The seasonal patterns of shrinking and swelling were analyzed using a sequential analysis technique and the daily patterns of contraction and expansion were extracted. Overall, the treated trees followed their diurnal cycles of contraction and expansion during the rain exclusions and no significant cumulative difference in stem expansion between control and treated trees was observed over the three years. In the third year trees subjected to rain exclusion showed larger stem contractions in summer on three out of four sites and larger winter contractions were observed on the northern sites. This study shows that repeated summer rain exclusion does not necessarily lead to a direct evident stress reaction, showing the resilience of the boreal forest.

Keywords: *Picea mariana*, Point dendrometers, Drought, Boreal forest, Climate change, Diurnal cycles

Key message: A rain exclusion repeated for three years resulted in larger summer stem contractions in three of the sites in the third year of the experiment and in larger winter contractions in the northern sites. However, there was no pronounced stress reaction in the stem radius variations of mature black spruce since total stem expansion was not reduced.

Introduction

The eastern boreal forest is generally characterized by cold temperatures and low evaporation with soils that often remain wet throughout the growing season. Growth of boreal species is strongly related to abundant water supply and the trees are adjusted to living in high moisture conditions (Hofgaard et al., 1999, Huang et al., 2010). These conditions could partially change in the future as climatic models for eastern Canada predict increases in temperature and precipitation in the next 50 years (Plummer et al., 2006, IPCC, 2007). The increase in precipitation should mainly occur in winter in the form of snowfall, while drought events should be experienced in spring and summer, when the main plant growth processes take place (Zhang et al., 2000, Burke et al., 2006). It is likely that an increase in winter precipitation may not be sufficient to balance out the increasing summer temperatures and evaporation, resulting in soil moisture content decline and more frequent and longer droughts during the growing season (Easterling et al., 2000, Motha and Baier, 2005). The predicted droughts may co-occur with other forest disturbances such as insect outbreaks or fires and may dramatically contribute to an increase in tree mortality in the Canadian forest (Peng et al., 2011).

Numerous studies were done on the drought effects on tree growth in natural and controlled environments throughout the world (Eilmann et al., 2009, Rossi et al., 2009, Swidrak et al., 2011, D'Orangeville et al., 2013). Despite this improving knowledge about the global reaction of forests to drought and changing precipitation (Beier et al., 2012, Choat et al., 2012) the impacts of repeated summer droughts on the diurnal cycles of mature black spruce (*Picea mariana* (Mill.) BSP) still remain largely unknown. Black spruce is the conifer characterizing the transcontinental boreal forest of Canada. It has the ability to grow on a wide range of soil conditions and is well adapted to conditions of low nutrient availability and waterlogged soils (Lamhamedi and Bernier, 1994).

Mature black spruce trees seem resistant to one summer of rain exclusion and cell production was not affected (Belien et al., 2012); moreover they are able to adapt to water deficit by increasing its fine root production (Major et al., 2012). It is not known how they will react when water availability is reduced several years in a row. Because of its high latitude, the response of the boreal forest may be different from that of other biomes in the world (Soja et al., 2007).

Automatic dendrometers can provide useful information on the seasonal and diurnal patterns of stem contraction and expansion and can be used to study water relations and drought responses of trees (Améglio et al., 2001, Giovannelli et al., 2007, Drew and Downes, 2009). Seasonal changes in stem radius variations have been classified according to rehydration patterns, showing a period of stem shrinking in winter, followed by rehydration and growth in spring and summer (Tardif et al., 2001, Turcotte et al., 2009). In summer, diurnal stem radius variations are mainly driven by transpiration and soil water content; shrinking takes place during the day, followed by expansion due to rehydration at night. Daily variations in winter are driven by temperature changes, when shrinking takes place during cooling and expansion during temperature increases (Sevanto et al., 2006). It was hypothesized by Giovannelli et al. (2007) that, at the beginning of drought periods, there is a decline in tree water status as shown by larger stem contractions. Daily stem expansion is positively related to the amount of precipitation (Deslauriers et al., 2003, Deslauriers et al., 2007, Turcotte et al., 2011) while a decrease in soil water content resulted in larger daily amplitudes and more stem shrinking (Sevanto et al., 2005, Intrigliolo and Castel, 2006). Stem shrinkage can be a signal of an internal water deficit, which may later alter different physiological processes. For example, stomatal conductance may be inhibited and photosynthesis and cell division decline after a continued hydrological stress (Abe et al., 2003, Chaves et al., 2003). In previous work, it was shown that an exclusion of precipitation affected daily

stem radius variations of black spruce (Belien et al., 2012), but the origin of the differences between control and treated trees was not yet explored and only one year of treatment was regarded.

To our knowledge, an exclusion experiment over three consecutive years with all year round monitoring was never performed before. In this paper, we present an analysis of stem radius variation chronologies collected over three years at four sites located along a latitudinal gradient on a sample of mature trees living in their natural environment. A rain exclusion was applied each summer on four study sites, to mimic a climate change scenario with reduced summer rainfall. This allowed us to study the effects of rain exclusion both during its occurrence and in the following winter and spring. We analysed the seasonal patterns of shrinking and rehydration throughout the years using a sequential analysis technique (Page, 1961). At a higher resolution, the daily patterns of contraction and expansion were extracted (Deslauriers et al., 2011) and compared between the trees subjected to rain exclusions and a control group. It was expected that a repeated summer drought applied to mature black spruce trees would affect (I) the seasonal stem radius variations by decreasing the total stem expansion and (II) the characteristics of the daily cycles by increasing the amplitude of contraction.

Methodology

Study sites

The study was conducted on black spruce (*Picea mariana* (Mill.) BSP) in the boreal forest of the Saguenay-Lac-Saint-Jean region, Quebec, Canada (see Belien et al. 2012 for more details on the sites). Four permanent plots [Simoncouche (abbreviated as SIM), Bernatchez (BER), Mistassibi (MIS) and Camp Daniel (DAN)] were installed in mature stands of 80-100 years old located along a latitudinal and altitudinal gradient to cover a wide range of tree growth dynamics (Table I). A weather station was present in a canopy gap within 100m of the study plots on all sites. Rainfall and temperature data were recorded every 15 min and stored as hourly sums or means respectively using CR10X dataloggers (Campbell Scientific Corporation). The volumetric water content (VWC) of the soil was measured weekly with a portable device (Fieldscout TDR 100) in four points at a distance of 1 m from the collar of each tree and continuously with a fixed sensor at the weather station at a depth between 12-20 cm using a time domain reflectometry soil moisture meter (TDR). All VWC measurements were divided by the maximum observed value of the site to standardize results.

At all study sites, soils were 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. At the other sites, the organic layer was deeper and attained 20-40 cm. Throughout the study period the climate conditions were typical for the boreal zone with cold winters and cool summers with abundant precipitation (Table I). The mean annual temperature ranged from 0.90 °C in the northern site (DAN) to 4.16 °C in the southern site (SIM). Rain occurred regularly throughout the summer (DOY 150-250) on all sites, with SIM having the highest and BER the lowest total amount (Fig. I). However, soil moisture content in BER was higher than at the other sites due to the

higher water holding capacity of the soil (Fig. 1e).. The lowest values of soil moisture content were observed in SIM during the summer (DOY 150-250) of 2010; overall this site had the highest seasonal variations in soil water content. MIS and DAN, the two northern sites, had intermediate soil moisture contents with fewer fluctuations, indicating a more constant water supply in the soil. A clear increase in soil moisture content can be seen during snowmelt from the end of March, followed by a decrease with fluctuations during summer (DOY 150-250). In winter there was a gradual reduction of the soil water content, with MIS attaining the lowest values. (Fig. 1e).

Experimental design

In each site, ten dominant or co-dominant trees with upright stems and similar diameters were chosen, five control and five treated trees (details of tree selection are reported in Belien et al. 2012). The selection was based on proximity among the treated trees to allow the installation of the equipment for rain exclusion on the same group of individuals. Trees with polycormic stems, partially dead crowns, reaction wood or evident damage due to parasites were avoided. Plastic transparent under-canopy roofs were installed during late May-early June to exclude the treated trees from precipitation in 2010, 2011 and 2012. The majority of the root system of black spruce is localized within a distance of 90-200 cm from the stem collar. Accordingly, the plastic roofs extended for at least three meters from the stem of each tree and drained the rain into sinking points in the soil to avoid water flowing back towards the stem. The plastic roofs were removed in September to avoid freezing of the roots during winter due to lack of insulation by the snow. The control trees were left untreated.

Data collection

Stem radius variations were measured with automatic point dendrometers (Agricultural Electronics Corp., Tucson, Arizona) from May 2010 to October 2012. The dendrometers were

placed at about 1.3m height, to avoid interference with micro-coring. Dendrometers were based on a precision linear variable differential transducer enclosed in an aluminum housing and fixed on the tree with stainless steel rods having a thermal linear expansion coefficient of $17 \mu\text{m}\cdot\text{m}^{-1}\cdot^{\circ}\text{C}^{-1}$. 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 where the sensing rod touched the tree before their installation to minimize error due to hygroscopic thickness variations. As the stem changed in size, the core of the transducer moved and translated the displacement in an electrical signal. Measurements were taken every 15 min and stored in CR-10X dataloggers (Campbell Scientific Corporation) providing precise and high-resolution data of radius variation over time.

Data analysis

Hourly means of the stem radius variations were taken and irregularities, like abnormal increases or decreases, were removed from the raw data with a 4-degree smoothing using the EXPAND procedure in SAS (Deslauriers et al., 2011). The difference between the total stem expansion of treated and control trees was tested using a simple t-test for the dendrometer value at the last day of the measurements, at the end of the third summer.

Diurnal means were taken from the chronology of daily stem radius variations and the growth trend was removed by subtracting the previous value from each value (Anderson, 1977). Positive and negative shifts in the daily mean stem radius variations over the three years were studied using cumulative sum (CUSUM) charts, which represents a running total of deviations from

a reference value, set at zero in this case (Page, 1961). The positive and negative cumulative sum of the deviations of the sample values greater than k standard errors from the target mean are calculated and plotted. An upward or downward out-of-control state is detected when the CUSUM chart exceeds the decision interval of six and represents a moment when the values are either below or above the overall average. CUSUM chart chronologies were drawn up for the three consecutive growing seasons.

The stem-cycle approach was used to determine and characterize the phases of contraction and expansion, where contraction was defined as the period from the first maximum of the cycle to the minimum and expansion was the total period from the minimum to the next maximum. The amplitude of contraction and expansion was extracted for each cycle and weekly means were used to study the cycle characteristics. Differences between the control and treated plots were tested with a sliced ANOVA, which tests for the simple main effects of the treatment for each week on the time series of amplitudes, for each site.

Results

Weather and site characteristics

During the three periods of rain exclusion the soil moisture content around the treated trees decreased quickly after the installation of the roofs and then stayed at continuously low values until the removal of the roofs (Fig. I). The two southern sites became the driest during the periods of exclusion. The soils had recuperated up to the same values as the control plots at the start of the second and third season of exclusion. Snow usually melted first in SIM at the end of April and then in May on the other sites.

Stem radius variations

Over the three years, all sites showed characteristic seasonal patterns in treated and control trees. The stem radius gradually increased as from the end of April until mid-July, when stem increase reduced and a plateau was reached. In winter stem shrinking occurred roughly from November until mid-March, this period of winter shrinking started earlier in the northern sites, MIS and DAN (Fig. II). The cumulative stem expansion after the three years of the experiment ranged from 2.07mm in SIM to 0.89mm in MIS and was lower in the rain excluded trees as compared with the control trees (Table I). No clear latitudinal or altitudinal trend was observed. The percentage difference between treated and control trees was larger in SIM (38%) and smaller in MIS (8%). This difference was not significant on any of the sites (t-test). In MIS and DAN the divergence between the control and treated trees appeared when the winter shrinking started, whereas in BER and SIM both curves already separated during the summer drought (Fig II). On all sites and treatments, amplitudes of expansion and contraction were largest in summer; they became smaller during winter and started increasing again in early spring. During the summers of 2011 and 2012 the control trees in DAN showed larger upward shifts compared with the treated trees (Fig. II).

During the last summer of rain exclusion, the contractions in MIS and BER were significantly larger in the treated trees, but expansion was the same as in the control trees (Fig. III). Also in SIM, there were significantly larger summer contractions in the treated trees, but towards the end of the exclusion the amplitude of the contraction decreased (Fig. III). More upward shifts were seen in the control trees in SIM during summer, but in winter both treatments largely followed the same pattern (Fig. II).

The drought treatment did not only affect the stem radius variations during the growing season but also had an impact in the following winter. In the northern sites, DAN and MIS, winter expansion and contraction were larger in the treated trees than in the control trees (Fig. III). This also happened sporadically during the second winter in SIM. The higher winter fluctuations in the CUSUM charts show that in DAN and MIS the winter shrinkage was stronger in the treated trees (Fig. II). This stronger shrinkage was followed by a larger rehydration in spring meaning that winter stem dehydration was greater in the north, for the excluded trees.

Discussion

The rain exclusion did not cause a significant difference in the cumulative stem expansion of black spruce over the three years. Larger stem contractions were observed in summer during the third year in three out of the four sites, in both winters in MIS and DAN and a few times in the second winter in SIM.

An increase in daily stem contraction in summer may be a reliable indicator of early water stress (Intrigliolo and Castel, 2006, Giovannelli et al., 2007). In the first and second year of the rain exclusion there was no significant difference in the amplitude of contraction and expansion between the control and treated trees along the latitudinal gradient. The increase in stem contraction during the third year indicates that there could be a cumulative effect of rain exclusion on the water status of the plant. When soil water is unlimited stem contractions can be explained by changes in vapour pressure deficit (Devine and Harrington, 2011). However, stem shrinking becomes a function of water availability when the soil water content is inadequate to recharge the stem overnight. Stem contraction occurs when water is lost from the bark tissues due to transpiration, but not immediately replaced via soil water uptake. The water loss takes place mainly in the living, physiologically active parts of the stem (Zweifel et al., 2000). In summer this can have a potential impact on cell division and development and other metabolic processes.

No increase in summer shrinking was observed in DAN, the northern site. This site had the lowest temperatures, a shorter growing season than the other three sites and high soil water table was observed throughout the summer (Boulouf Lugo et al., 2012). These conditions may result in a lower transpirational demand and slower tree growth. It was shown by McLaughlin et al. (2003) that slower growing trees may be less sensitive to drought than fast growing trees.

In SIM, the stem contractions, but also the expansions, of the treated trees decreased towards the end of the third exclusion period, resulting in a lower daily fluctuation. This may imply that the trees could rehydrate less during the night, meaning that there was less water available to lose during transpiration.

We showed that winter contraction was larger in the treated trees than in the control trees of the northern sites (MIS and DAN). As in summer, winter shrinking indicates water loss and is thus an indicator of the tree water status (Loris et al., 1999, Zweifel and Häsler, 2000). Winter stem shrinking is driven by temperature (Sevanto et al., 2006) and occurs during the night, when water leaves the elastic bark tissues to prevent cell damage due to freezing (Zweifel and Häsler, 2000). The drier soils in the treated sites are expected to be colder because of their lower heat conductance (Yun and Santamarina, 2008), this may reduce water uptake and the trees may have a lower internally stored water reserve due to the rain exclusion treatment, this may explain the larger stem shrinkage during winter. Water losses that occurred in winter are usually compensated during early spring rehydration, when cycles contain a freeze-induced contraction and a thaw induced expansion (Turcotte et al., 2009). Consequently the treated trees in DAN and MIS, which had larger winter losses, had a larger spring rehydration.

It is not expected that differences in root architecture may play a role in the different reactions among sites, because black spruce generally has a superficial root system (Strong and La Roi, 1983). It is however possible that after one year of drought there was an allocation of biomass investment towards the roots at the expense of aboveground biomass (Major et al., 2012). It was found earlier that the amount of cells produced and the cell size were not affected by the drought treatment in MIS, BER and DAN (Belien et al., 2012). The cumulative difference in stem expansion between the treatments is therefore mainly water related. For SIM it should be kept in mind that the

growth of the treated trees was already lower before the start of the experiment (Belien et al., 2012). This was accounted for in the analysis of shrinking and expansion patterns by removing the growth trend.

Possible reductions in photosynthesis and growth due to reduced water availability may be mitigated by rising temperatures that may stimulate photosynthesis and induce an earlier start of the growing season (Sevanto et al., 2006). On the other hand, higher temperatures may lead to higher evaporation in winter, resulting in even more water loss and stem desiccation.

The northern sites have thicker organic soil layers, giving them generally wetter conditions but making them more susceptible to climatic events such as droughts (Drobyshev et al., 2010). The soil of the southern site, SIM has a lower water holding capacity due to a thinner organic soil layer. The results of our investigation demonstrated how important it is to include different sites when studying water relations, since environmental factors may play a role in the effect of the rain exclusion treatment on the stem radius variations. Stem radius variations are not only influenced by the amount of soil water available, but also by vapour pressure deficit, solar radiation and maximum temperature (Deslauriers et al., 2003, Deslauriers et al., 2007, Devine and Harrington, 2011). These environmental variables were not altered in this experiment, even though they are also expected to differ under future climate change.

Conclusion

Larger stem contractions were observed in summer during the third year in three out of four sites, in both winters in MIS and DAN and a few times in the second winter in SIM. There was no significant difference in total stem expansion. In spite of the repeated summer rain exclusion treatments, there was surprisingly no clear overall reaction of the trees to the treatment. Moreover, they kept following diurnal and seasonal cycles of hydration and dehydration. We showed that trees

may not only react during periods of rain exclusion, but that there may also be a response in the following winter, depending on the site conditions. When studying mature trees' reaction to a modification of the environment such as rain exclusion, it is important to repeat the experiment for several years on the same trees to monitor the long-term effects on stem radius variations and water status.

Acknowledgments

This work was funded by the Natural Sciences and Engineering Research Council of Canada and the Consortium Ouranos. The authors thank F. Gionest and J-G. Girard for their technical support and A. Garside for checking the English text. The authors declare that they have no conflict of interest.

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Table and figures

Table 2.1 : Location and altitude of the four study sites and cumulative stem expansion (mm) with t-test results after the three years of rain exclusion.

Site	Latitude	Longitude	Altitude (m above sea level)	Cumulative expansion(mm)		F-Value	p
				Control	Treated		
DAN	50°41'N	72°11'W	487	1.96±0.44	1.47±0.97	1.65	0.6246
MIS	49°43'N	71°56'W	342	1.10±0.24	0.89±0.31	1.28	0.8167
BER	48°51'N	70°20'W	611	1.31±0.33	1.21±0.37	4.87	0.1541
SIM	48°12'N	71°14'W	338	2.07±0.36	1.28±0.88	5.81	0.1168

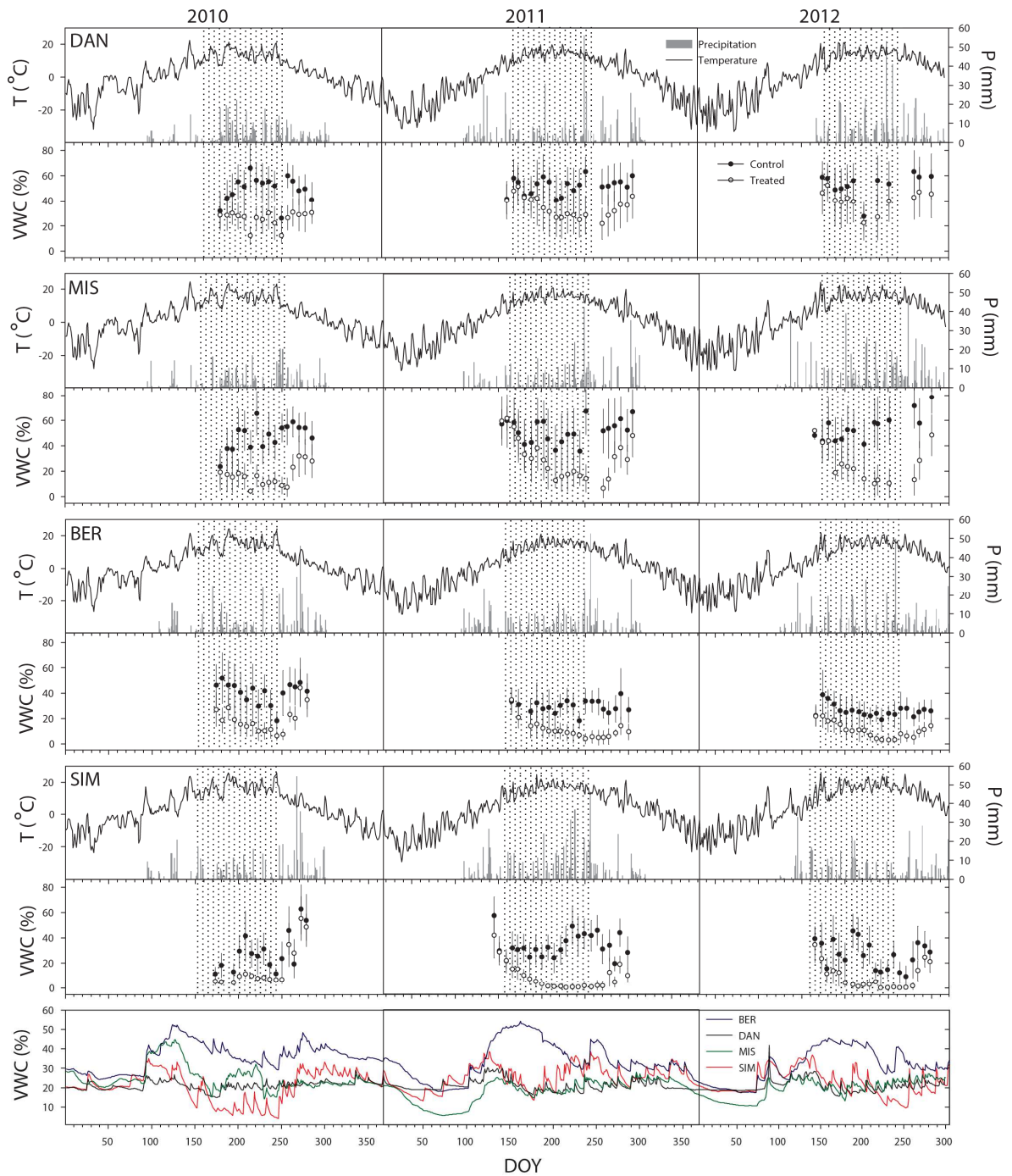


Figure 2.1 a-d : Daily air temperature (T, black curve) and precipitation (P, grey bars) for each site. Volumetric water content of the soil (VWC) in the treated (open circles) and control (solid circles) plots with the standard deviation. The shaded background represents the periods of the rain exclusion. 1e: Volumetric water content of the control sites over the entire study period.

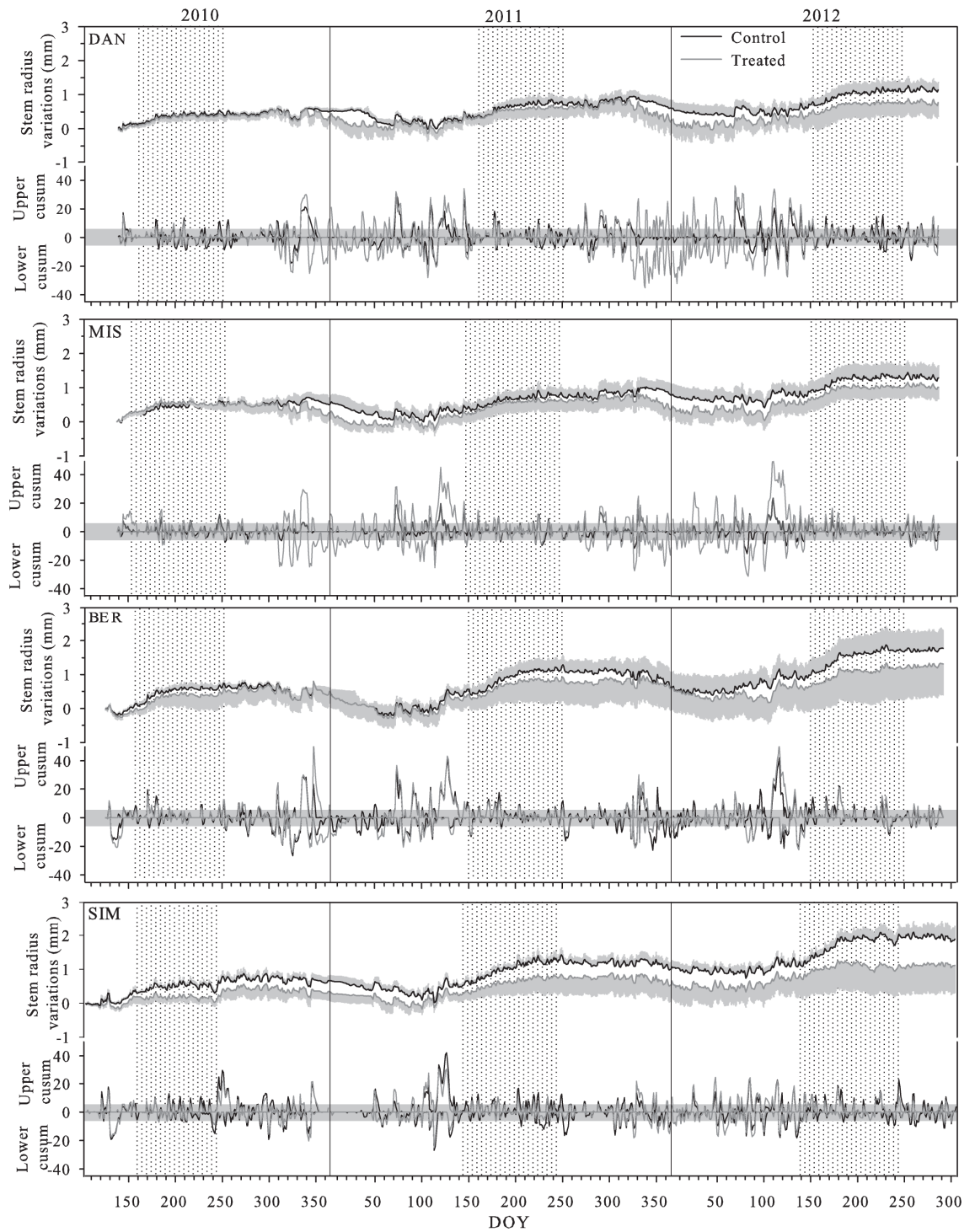


Figure 2.2 : Daily means and standard deviations (grey bars) of the stem radius variations of the treated (grey lines) and control trees (black lines). High and low-sided CUSUM charts for the daily stem radius variations, where the high sided only uses the positive values and the low-sided only the negative values, for control (black lines) and treated (grey lines) trees. The grey

zone indicates the decision interval h (6), the shaded background represents the periods of rain exclusion.

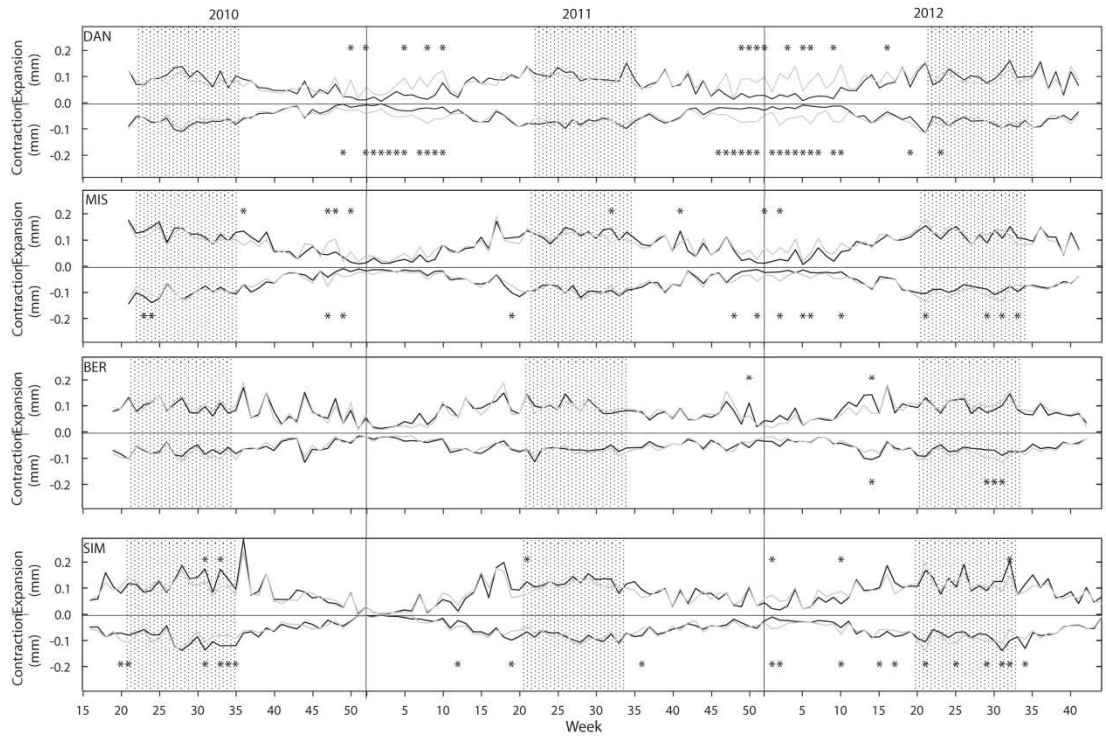


Figure 2.3 : Weekly means of the daily contraction and expansion for the treated (grey lines) and control (black lines) trees. Significant differences ($p < 0.05$) are indicated with an asterisk. The shaded background indicates the periods of rain exclusion

CHAPTER 3. FOLIAR ABSORPTION IN
BLACK SPRUCE [*PICEA MARIANA*
(MILL.) BSP] SAPLINGS: DOES IT EXIST?

In press at American Journal of Experimental Biology (2014)

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Foliar absorption in black spruce [*Picea mariana* (mill.) bsp] saplings:
does it exist?

Abstract

Foliar absorption is a known water acquisition mechanism in many species and ecosystems. Experiments in the field showed that mature black spruce [*Picea mariana* (Mill.) BSP] can surprisingly sustain periods of summer drought. A possible explanation for this phenomenon is that this species is able to rehydrate via its needles. In this study, we explored if black spruce saplings are able to absorb water via the needles or to increase their water potential and photosynthesis after needle wetting. Forty saplings were used, of which half were excluded from irrigation until water potential of -2.70 MPa. For the first part of the experiment, the saplings were sprayed at night with a colorant solution and water potential was measured the following day. No colorant was absorbed by the saplings and no difference in water potential was found between irrigated and non-irrigated individuals. The experiment was then repeated, spraying saplings with normal water and measuring water potential and photosynthesis. Once again there was no increase in water potential or photosynthesis following the canopy spraying. The results of this study show no evidence of foliar absorption in black spruce saplings. However this does not exclude the occurrence of foliar absorption via passive or active mechanisms in mature trees, which grow under different circumstances.

Keywords: Foliar absorption; *Picea mariana*; Boreal forest; Drought

Introduction

Throughfalling rainwater absorbed by the root system is usually considered to be the primary water supply in forest ecosystems. However, part of this water is intercepted by the canopy where it may be absorbed by the leaves (Barbier et al., 2009). The phenomenon of foliar absorption is being increasingly studied because the contribution of foliar absorption to the water status of trees may be particularly important during periods of water deficit (Breshears et al., 2008, Eller et al., 2013). Moreover, trees may be able to relocate the absorbed water to belowground organs and tissues when the soil is dry (Eller et al., 2013). Conifers are known to intercept a higher quantity of rainwater than broadleaves and deciduous trees, possibly due to differences in leaf area index and needle arrangement (Keim et al., 2006, Barbier et al., 2009), and this can occur in many species and ecosystems. In the Mediterranean region, dew absorption by the evergreen shrubs *Lavandula stoechas* and *Rosmarinus officinalis* was studied by Munné-Bosch et al. (1999), in redwood forests 80% of the dominant species benefit from foliar absorption (Limm et al., 2009). Other examples include species such as *Picea abies* that can absorb water via its twigs (Katz et al., 1989), *Juniperus* spp. (Breshears et al., 2008) and *Pinus strobus* (Boucher et al., 1995) are also known to absorb water via their needles.

Rain, dew or fog may reduce plant water loss via transpiration (Reinhardt and Smith, 2008) and may also directly improve the water content of the foliage (Carter Berry and Smith, 2014). Water can diffuse directly into the leaves via the cuticle, twigs and branches (Katz et al., 1989, Boucher et al., 1995, Limm et al., 2009). Foliar absorption can quickly improve a plant's water status by increasing its water potential. Other benefits are improved stomatal conductance, plant growth and survival (Boucher et al., 1995, Breshears et al., 2008, Limm et al., 2009). Although canopy wetting may negatively affect photosynthesis and growth due to the presence of a water film

on the leaf surface that reduces CO₂ transport (Brewer and Smith, 1997), other studies point out ecophysiological improvements. Transpiration may decrease due to a lower vapour pressure deficit. In *Abies fraseri*, photosynthetic activity and water relations were strongly related with cloud conditions (Reinhardt and Smith, 2008) and fog interception resulted in better leaf water potential and photosynthesis for *Sequoia sempervirens* (Simonin et al., 2009).

In a previous study, it was shown that black spruce [*Picea mariana* (Mill.) BSP] withstood repeated periods without direct water supply to the root system (Belien et al., 2012; 2014). In that experiment plastic under-canopy roofs were installed on mature black spruce trees for three consecutive summers. The canopy thus remained exposed to frequent rainfall, which may have allowed the trees to cope with the soil drought. Black spruce is one of the most common tree species in the boreal forest of Eastern North America and it is more sensitive to water stress than other boreal conifers (Grossnickle and Blake, 1986). In spite of its importance, the water relations of black spruce are not yet well-understood. If root water is not directly available, foliar absorption is one alternative way to obtain water. The objective of this study was to explore if foliar absorption is a possible water absorption mechanism in black spruce saplings. We tested the hypothesis that black spruce is capable of absorbing rainwater and using it to improve its water relations and vital physiological processes. As it was expected that the foliar absorption would be more evident in saplings that were water-stressed, we measured water potential and photosynthesis in five-year old black spruce seedlings submitted to a drought treatment and nocturnal canopy wetting.

Methodology

Experimental setup

The experiment was conducted on 40 black spruce saplings selected at the end of summer 2012. The five-year old saplings then passed the winter outside and were put into a heated greenhouse in January 2013. It was ensured that the saplings were in maximum growth during the experiment, which was conducted in March-April 2013. Day/night temperatures in the greenhouse were kept at 25/13 °C and relative humidity was 5-25% during the day and 30-40% at night. For the first part of the experiment, 20 experimental saplings were excluded from irrigation on DOY 73-95, while 20 control saplings were irrigated weekly with drip irrigation. On the evenings of DOY 92 and 94, half of the irrigated and non-irrigated saplings were sprayed with normal water and the other half with a solution of water and a non-toxic water-soluble food colorant using a manual spray bottle. Fifty-six ml of food colorant composed of Brilliant blue (Blue 1) and Allura red (Red 40) (McCormick, London, Canada) was dissolved per liter of water. For the second part, the experiment was repeated on the same saplings that were deprived of irrigation on DOY 122-149 and sprayed with normal water on the evenings of DOY 146-148. This time no colorant was used.

Each sapling was sprayed with approximately 0.5 l or until saturation of its needles (about five minutes per sapling), whichever was reached first. To avoid water simply being taken up by the roots, the stem base and root system of each sapling was covered with a plastic sheet sealed to the pot and stem.

Before the experiment, the roots of young black spruce saplings were plunged in the colorant overnight to test if the colorant could be absorbed by and observed in the plant tissues. These saplings were cut open lengthwise after 24 hours in the colorant solution and checked

visually for colorant. The inside of branches and needles showed blue staining of the tissues, indicating that the colorant could be absorbed and transported within the plant.

Measurements

The volumetric water content (VWC) of the soil was measured with a time domain reflectometry soil moisture meter, shoot water potential was measured with a pressure chamber (model 610 Pressure Chamber, PMS instruments), and photosynthesis was measured using the LI-6400XT Portable Photosynthesis System (LICOR Biosciences, Lincoln, Nebraska) with the 6400-22L Lighted Conifer Chamber. Measurements were taken bi-weekly at mid-day as from one week before the irrigation exclusion until the end of the experiment, and every day after canopy wetting. Measurements were done on 5 plants per group (irrigated/non-irrigated) per sampling in the first part of the experiment and on 3 plants in the second part. The needles were completely dry during measurements to ensure that there was no indirect effect of the canopy wetting, such as a reduction in transpiration. At the end of the experiment, all saplings were abundantly irrigated and water potential was measured once again to verify that they had survived the treatment.

Statistics

The effect of the drought treatment and canopy wetting on water potential and photosynthesis before (DOY 92 and DOY 145) and after the canopy wetting (DOY 93-95 and DOY 147-149) was tested with a general linear model (GLM) followed by pairwise post-hoc t-tests in SAS (SAS institute Inc., Cary, North Carolina).

Results

In both parts of the experiment, the VWC of non-irrigated saplings decreased below 10% about 4 days after interruption of the irrigation (Figure 1). The VWC in irrigated plants fluctuated according to the times of irrigation. No increase in VWC was observed after the canopy wetting. When irrigation resumed at the end of the experiment, the soil rehydrated attaining the pre-treatment values.

The saplings that were sprayed with the colorant did not show any signs of staining of the tissues. After 19 and 11 days of irrigation exclusion in the respective experiments, the water potential of the non-irrigated saplings dropped below -2.70 MPa. During the first part of the experiment, water potential never exceeded -2.29 Mpa and went as low as -3.45 Mpa after the spraying. The spraying in the second part of the experiment resulted in an increase in water potential up to -1.89 Mpa, but during the following two days, after multiple wetting events, the water potential dropped again to lower values. The results of the GLM demonstrated significant differences ($F=48.88$, $p<0.001$ and $F=13.05$, $p<0.0001$ for the first and second experiment respectively, Figure 2). Post-hoc tests showed that there was a significant difference in water potential between the irrigated and non-irrigated saplings. However, there was no difference between the pre- and post-canopy wetting water potential within each treatment (Figure 2).

Photosynthesis decreased in the non-irrigated saplings to $0.12 \mu\text{mol CO}_2\text{m}^{-2}\text{s}^{-1}$ after 17 days of treatment, whereas the irrigated saplings had a photosynthetic rate of $11.33 \mu\text{mol CO}_2\text{m}^{-2}\text{s}^{-1}$ on the same day. The GLM showed significant effects of the irrigation treatments and canopy wetting ($F=15.84$, $p<0.001$), the post-hoc tests showed that there was no significant effect of the canopy wetting. There was a significant difference between irrigated and non-irrigated saplings after the canopy-wetting (Figure 2c). In both experiments, the saplings recovered water potentials

and photosynthetic activity similar to the irrigated saplings within one week after irrigation was resumed.

Discussion

The results of this experiment showed no evidence of foliar absorption by black spruce saplings subjected to water deficit. The first experiment showed that there was no significant change in water potential, and no absorption of the blue colorant via the needles. It was then considered that even though there was no direct water absorption, and thus no improvement in water potential, the saplings might have been able to recuperate enough water to maintain basic physiological processes such as photosynthesis. The second experiment however confirmed canopy wetting did not affect water potential and photosynthesis. Even though the colorant could be absorbed and transported by the plant tissues, no staining was found in the xylem. The hypothesis that black spruce would be able to absorb rainwater through needles and use it to improve its water relations was definitely rejected by this experiment.

Absorption

The blue food colorant could be absorbed by the plant tissues of young black spruce as demonstrated by the preliminary test. Katz et al. (1989) observed that dye could be absorbed via the twig xylem, but not via the needles after canopy wetting. In our experiment however, the colorant was not found in any plant tissues after canopy wetting.

The absorption of water and solutes into the leaves depends on several factors, like the wettability of the cuticle. The systematic build-up of aerosol depositions on the leaf surfaces decreases their hydrophobicity, and thus increases their wettability (Grantz et al., 2003). Certain

species have specialized morphological structures to facilitate water uptake like hydathodes or trichomes. In species that lack these structures, such as black spruce, water can diffuse into the leaf via the cuticle (Gouvra and Grammatikopoulos, 2003) or via the stomata. Hydraulic activation of stomata can then take place when a continuous thin water film forms on the stomatal walls, enabling the bidirectional transport of water and solutes between the leaf interior and its surface (Burkhardt, 2010). On the other hand, the presence of a water film on the leaf may reduce its photosynthetic carbon uptake (Brewer and Smith, 1997).

Duration of canopy wetting event and timing of measurements

The plants were sprayed until water started dripping down from the needles, and it was ensured that all leaves and branches were wetted. It is considered that this is sufficient to have an effect on the plant water status, as previous experiments showed the occurrence of foliar absorption in *Pinus strobus* and *Juniperus spp.* after a short-time canopy wetting (Boucher et al., 1995, Breshears et al., 2008). The concentration of suspended materials on leaves is highest at the beginning of rain events, which may quickly facilitate uptake by the plant (Grantz et al., 2003). Black spruce exhibits nighttime stomatal conductance that is strongly dependent on decreasing temperature. It is therefore possible that there was a short-term improvement in the plant water potential, but no effect during the following days. Breshears et al. (2008) took measurements immediately after canopy wetting but hypothesized that the change in water potential could persist over more than a day. Plants were allowed to dry, as in Boucher et al. 1995, to avoid an indirect effect of the canopy wetting such as reduced transpiration. An increase in gas exchange was also to be expected after leaf drying (Gouvra and Grammatikopoulos, 2003, Simonin et al., 2009).

Drought treatment

No different reaction was observed between irrigated and non-irrigated saplings, however it was expected that there would be a larger foliar water uptake in plants with lower water potential (Katz et al., 1989, Breshears et al., 2008). The water potential of the saplings dropped below -2.70 MPa, which is defined as severe water deficit for black spruce (Balducci et al., 2013). When soil water potential drops below plant water potential, water can theoretically move down the soil-plant-atmosphere continuum through the passive conduits of roots and stems, which may facilitate the foliar absorption (Nadezhdina et al., 2010). Black spruce often keeps its stomata open, even during drought stress (Stewart et al., 1995). However, extreme water stresses may limit foliar uptake when the epidermis and cuticle contract due to dehydration (Burgess and Dawson, 2004, Limm et al., 2009).

Age of saplings

In earlier experiments, it was shown that young black spruce saplings had a reaction to drought, whereas mature trees surprisingly seemed more resistant to its effects (Belien et al., 2012, Balducci et al., 2013). Young needles have higher physiological activity, like a higher stomatal conductance because there is less wax accumulated in stomata (Ludlow and Jarvis, 1971, Rayment and Jarvis, 1999); this may facilitate foliar absorption in younger plant parts. However, the young needles are more hydrophobic than older ones because less particulate matter has been built up on the surfaces (Grantz et al., 2003). In adult plants in a natural environment however, there may be other factors promoting the occurrence of foliar absorption. The presence of mosses and lichens, in particular on twigs and branches, increases the water holding capacity in the forest. Low vapour pressure deficit during rainfall may contribute to an improved plant water status irrespective of stomatal effects (Berry and Smith, 2013) and cloud formation may reduce transpiration. In

particular for late successional species, like black spruce, the amount of throughfalling rainwater is lower, whereas the amount of water intercepted by the canopy is higher than in earlier successional species (Barbier et al., 2009).

Although foliar absorption occurs in different species and ecosystems, black spruce may not have developed this mechanism because root water is always available and is a much more efficient water acquisition strategy. Other reasons for not exploiting foliar absorption are that accumulation of leaf water may favour pathogen attacks and inhibit photosynthesis. It is possible that, despite the lack of active absorption of water via the needles, a passive absorption mechanism may be present in mature trees.

This study consists of a greenhouse experiment in which black spruce saplings were excluded from irrigation and then sprayed at night to test if foliar absorption occurred. The results showed no evidence of foliar absorption or improvement of water potential and photosynthesis in black spruce saplings. There was no difference in the reaction to foliar wetting between saplings that were water-stressed or well-irrigated. Although the saplings did not show foliar absorption, there is the possibility that mature trees in natural conditions may benefit from foliar wetting because of specific environmental circumstances.

Acknowledgements

This work was funded by the Natural Sciences and Engineering Research Council of Canada and the Consortium Ouranos. The authors thank D. Gagnon and B. Allaire for their technical support and A. Garside for checking the English text.

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Tables and Figures

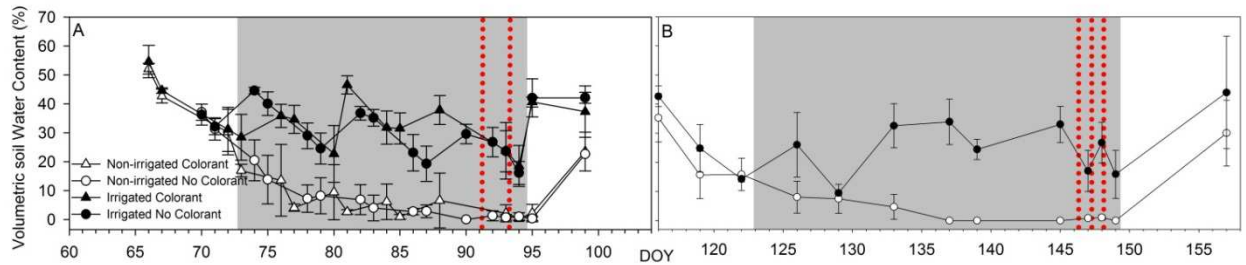


Figure 3.1 : Volumetric soil water content during the first (A) and second (B) part of the experiment in the non-irrigated (open) and irrigated (solid) plants with (circles) and without (triangles) colorant reported as means with the standard deviations. The shaded background represents the periods with no irrigation. The dotted vertical lines show the times of canopy wetting.

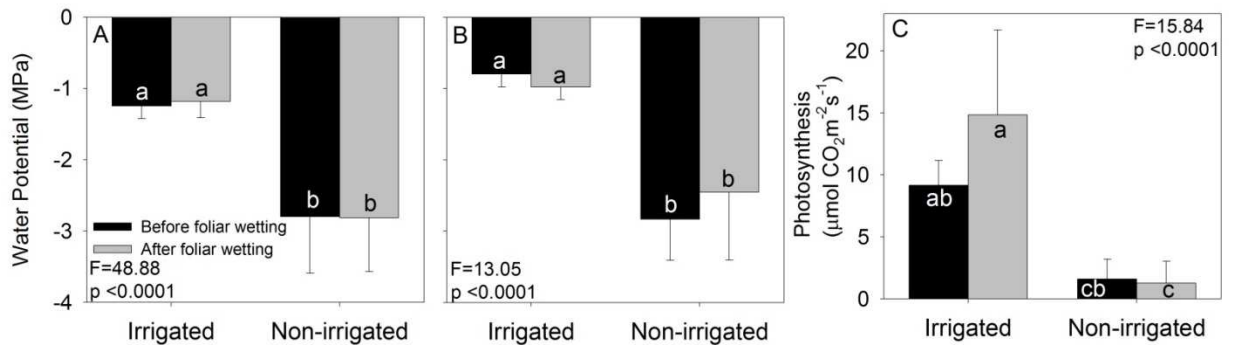


Figure 3.2: The stem water potential before (black bars) and after (grey bars) the canopy wetting in irrigated and non-irrigated plants during the first (A) and second (B) part of the experiment. Plus photosynthetic rate (C) before (black bars) and after (grey bars) the canopy wetting in irrigated and non-irrigated plants during the second part of the experiment. Vertical lines show the standard deviation, letters indicate significant differences according to the post-hoc test ($p < 0.05$), F and p-values of the GLM are given in each graph.

CHAPTER 4. WOOD ANATOMY OF
BLACK SPRUCE SUBJECTED TO
REPEATED RAIN EXCLUSION

Belien Evelyn, Rossi Sergio, Morin Hubert, Deslauriers Annie

WOOD ANATOMY OF BLACK SPRUCE SUBJECTED TO REPEATED RAIN EXCLUSION

Abstract

The climate models for Eastern Canada predict increases in temperature and precipitation in the next 50 years; the higher latitudes are 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. The experiment was repeated for three years. The objective was to evaluate the impact of an artificial drought period on cell characteristics and to explain black spruces hydraulic strategies by constructing a xylem vulnerability curve. Micro-cores were extracted from the trees at the end of each growing season and cell characteristics measured for the three experimental and two preceding years. Ring width and the number of cells followed the same pattern as the control trees over the five measured years. There was an overall effect of the treatment and the year on cell size and cell wall thickness. During the first year of the experiment the lumen area of the treated trees decreased, whereas the cell wall thickness increased. In the second and third year of the exclusion the cell size increased again and cell wall thickness reduced. The effect on cell wall thickness is less pronounced in the most southern site. The results demonstrate that a reduction in water availability altered the cell characteristics, but that repeated summer droughts do not enhance the effect on wood anatomy of mature black spruce. Mature black spruce trees have a rather conservative hydraulic strategy and do not seem to be susceptible to the short-term negative consequences of reduced direct water availability to the roots.

Key words: Black spruce, drought, cell production, cell wall thickness, cell size

Introduction

The climate models for Eastern Canada predict increases in temperature and precipitation in the next 50 years, with the precipitation occurring mainly in winter as snow (Zhang et al., 2000, Burke et al., 2006). Extreme drought conditions are expected in summer and a reduction of 20-40% of soil moisture is projected during the growing season (Houle et al., 2012). In combination with the higher temperatures this will increase the frequency and intensity of droughts in the boreal forest of Quebec. This ecosystem usually has low evaporation, low temperatures and generally humid conditions during the summer. Boreal plants are adapted to live with abundant water. Changes in these conditions may lead to potentially severe water stresses in the high-latitude forests (Easterling et al., 2000). Black spruce is a common tree species in the boreal forest of Québec, it has a high economic value due to its good fiber quality. The wood is highly appreciated for paper production and construction. Black spruce is, like many boreal species, adapted to living in an environment with abundant precipitation. Its root system is normally superficial, with maximum depths up to 30cm and can be found within 3m from the tree base (Strong and La Roi, 1983).

Water deficits may affect several physiological processes in plants, starting with a reduction in turgor pressure and in photosynthesis and transpiration (Hsiao, 1973). A reduced water potential will then affect cell expansion, cell wall synthesis and eventually cell division (Abe and Nakai, 1999, Abe et al., 2003, Woodruff et al., 2004). Trees suffering from drought are known to produce xylem cells with an altered anatomy, like cells with smaller diameters and thicker cell walls (Abe et al., 2003, Rossi et al., 2009, Jyske et al., 2010, D'Orangeville et al., 2013) in order to decrease hydraulic conductivity under drought and reduce desiccation. This is a normal defence mechanism of the trees to reduce the risk of cavitation (Sperry et al., 2006). For mature black spruce, it was found that during one single summer of imposed drought, the trees produced smaller tracheids with thicker cell walls (Belien et al., 2012).

Drought responses on trees are often delayed, showing reductions of growth and modifications of wood anatomy in the years following the drought event (Granier et al., 2007). Repeated droughts may affect growth, weaken the trees, make them more sensitive to biotic and abiotic stress and eventually increase the risk of tree mortality (Pedersen, 1998, Bigler et al., 2006). Most of the preceding drought experiments were done in rather short periods of time, but long term drought experiments are recommended to better understand the consequences of precipitation changes (Beier et al., 2012). Moreover it is important to include drought experiments

on mature trees because they do not necessarily react in the same way as seedlings (McDowell et al., 2008).

A reduced water potential may trigger cavitation of the stem xylem and thus lead to loss of hydraulic conductivity. To better understand a species' protection to drought it is useful to produce xylem vulnerability curves. A vulnerability curve describes the relationship between xylem pressure and percentage loss of conductivity, which indicates the plants resistance to embolism. When the water potential would drop below the point where 50% of the conductivity is lost (P50), the water transport function of the xylem is strongly reduced, increasing the risk of embolism which can result in tissue damage and plant death (Brodribb and Cochard, 2009). The difference between the lowest measured water potential and P50 represents the safety margin. Plants with low or negative safety margins experience a lot of embolism and run a higher risk of hydraulic failure. Conifers are known to have greater safety margins than angiosperms (Choat et al., 2012, Johnson et al., 2012), but the percentage loss of conductivity of black spruce trees has never been measured before.

In this study we present a three-year rain exclusion experiment on mature black spruce trees in their natural environment. The objective was to evaluate the impact of an artificial drought period on the cell characteristics and to better understand the cavitation resistance of black spruce by constructing a xylem vulnerability curve. It was hypothesized that the reduction of soil moisture would lead to a decrease in cell production and cell size, and an increase in cell wall thickness. It was expected that this effect would increase with repeated episode of drought throughout the three years of the experiment.

Materials and methods

Study sites and experimental design

Four permanent research plots in the boreal forest of the Saguenay-Lac-Saint-Jean region, Quebec, Canada were used for this study [Simoncouche (abbreviated as SIM), Bernatchez (BER), Mistassibi (MIS) and Camp Daniel (DAN)] (see Belien et al., 2012 for more details on the sites). The climate on these sites is typical for the boreal forest, with cold winters and cool summers with abundant precipitation. During the study period the mean annual temperature ranged from 0.90 °C in the northern site (DAN) to 4.16 °C in the southern site (SIM). There was regular rainfall on the

sites during summer (June-September), with SIM having the highest and BER the lowest total amount (Belien et al., 2014).

On each site five control trees and five experimental trees were selected based on their proximity to facilitate installation of the experiment. Trees on slopes and trees with polycormic stems, partially dead crowns, reaction wood or evident damage due to parasites were avoided. Plastic under-canopy roofs were installed on the five experimental trees in late May-early June in 2010, 2011 and 2012 and they were removed each September to ensure winter survival of the trees. The roofs extended for at least three meters from the stem of each tree to cover the entire root system (Strong and La Roi, 1983), and drained into sinking points to avoid water flowing back to the stem. The control trees were left untreated.

Sampling

Micro-cores were collected in October, at the end of the growing season between 90 and 170 cm above ground using a Trephor (Rossi et al., 2006). The samples were embedded in paraffin and cut into sections of 7 μm with a rotary microtome and fixed on slides. After staining with safranin numerical images were recorded with an optical microscope at a magnification of 20x. Cell area and cell wall thickness were measured and the number of cells was counted in the tree rings of 2008-2012 along three radial files per section using Wincell (Regent Instruments) and averaged for each tree and year.

Data treatment

A repeated-measures analysis of variance was used because the measurements were repeated in time on the same experimental units. A general linear model (GLM) was used with the year in the repeated statement and with treatment as a model factor for each site to evaluate if there was a significant interaction between year and treatment.

Vulnerability curve

To construct a xylem vulnerability curve eight straight branches of 35-40cm long and 1cm in diameter were cut from the trees. Leaves and side branches were removed to avoid transpiration and cavitation and the branches were wrapped in wet papers for transport. The percentage loss of conductivity was then determined during centrifugation using the 'Cavitron' method (Cochard, 2002). A nonlinear sigmoidal regression was applied to the data to construct the vulnerability curve

and to characterize P50, which is the tension in MPa causing 50% loss of hydraulic conductivity. The cavitation resistance can be estimated for mature black spruce by the calculation of the safety margin ($\Psi_{\min} - \Psi_{50}$).

Results

Cell production was highest in the control trees of SIM, with 47 ± 29 cells formed in 2009; the least productive site was the control plot of DAN in 2009 with only 10 ± 4 cells formed. The control trees in SIM always had a larger cell production than the treated trees and this difference was already present in the years before the treatment. In none of the sites, the treatment resulted in a significant difference between the number of cells produced by control and treated trees.

Cell area was largest in treated trees of MIS in 2008 ($478 \pm 80 \mu\text{m}$) and smallest in control trees of DAN 2011 ($283 \pm 103 \mu\text{m}$). There was a significant effect of treatment on cell area in BER ($p=0.0225$). As can be seen in fig 1 there was a reduction of cell area of the treated trees during the first year of the treatment, whereas the control trees underwent a slight increase of cell area. In the second and third year cell size recuperated to pre-treatment values. In MIS and DAN, and to a lesser extent in SIM, the same effect could be observed, however it was not significant.

The largest cell walls were formed in SIM in 2011 ($4.32 \pm 0.57 \mu\text{m}$) and thinnest cell walls were formed in BER in 2011 ($2.34 \pm 0.62 \mu\text{m}$). In DAN, MIS and BER there was a significant effect of the treatment on cell wall thickness, cells had larger cell walls in 2010, the first year of the treatment. Before the treatment the curves of treated and control trees had the same tendencies and in the second and third year of the treatment cell walls reduced to pre-treatment values. In SIM no effect of the treatment was found.

A vulnerability curve was made for black spruce to estimate cavitation resistance of mature black spruce samples (fig 2). Hydraulic conductivity strongly starts to decrease as from tensions higher than -3MPa ; this is the point where the curve becomes steep and a small increase in pressure quickly causes a large loss in hydraulic conductivity. The point at which 50% loss of conductivity occurs (P50) for black spruce was found at -3.9MPa . The lowest measured water potential in our study trees was -2.2MPa (unpublished results) which means that the safety margin is of 1.6MPa .

Discussion

We presented the effects of a manipulative rain exclusion experiment on mature black spruce trees in their natural environment. In contrast with the hypothesis, cell production was not affected by the rain exclusion, and rather stayed constant throughout the years. Cell area was significantly decreased during the first year in BER; in the other sites the effect was not significant. The hypothesis of increased cell wall thickness was confirmed during the first year of the experiment for three out of four sites, however in contrast to the expectation this effect was not maintained in the following years.

Cell production was not affected by the treatment on any of the sites. It could have been expected that cell production would have decreased in the second and third year, as was the case for balsam fir (D'Orangeville et al., 2013). Cambium cell division is normally water-dependent and a reduction in water availability should lead to a reduction in radial growth. Since photosynthesis, another water-dependent process, was not affected either by the rain exclusion (Belien et al. 2012), the trees probably did not suffer an internal water deficit or stress.

The change in tracheid characteristics, smaller cells with thicker cell walls, in the first year are an expected adaptive response to dry conditions in order to improve the mechanical strength of the conduits and reduce the risk of xylem cavitation (Hacke et al., 2001). Previous studies showed that repeated droughts may have significantly larger effects on tree growth and render them more susceptible to eventual mortality (Jenkins and Pallardy, 1995, Bigler et al., 2006). Surprisingly, in the present study the effect of the rain exclusion on wood characteristics was not maintained during the second and third year of the experiment, showing the resistance of mature black spruce. It is not clear why the trees did not show the same reaction during the second and third year of the rain exclusion. It is possible that, for a short period after the installation of the experiment, the trees had a strong root growth to access more or different water sources (Krause and Morin, 2005). However, black spruce is known to have a rather superficial root system (Strong and La Roi, 1983).

Effects of drought may occur immediately during the period of drought, or with a few years to decades of lag. Mortality or growth reductions occurring later than a few years after a drought event are more usually difficult to clarify. In this study no data was available to estimate the long term effect on these trees.

The vulnerability curve helps to explain these results. Safety margins and vulnerability curves are being studied for many species and ecosystems (Willson and Jackson, 2006, Cai and Tyree, 2010, Choat et al., 2012) however, in none of these studies boreal species were included. We demonstrate that, like many conifers species, black spruce has a rather large safety margin. The positive safety margin, with water potentials never below P50, implies that large amounts of embolism are unlikely to occur (Johnson et al., 2012), black spruce is thus employing a rather conservative hydraulic strategy. The tracheid measurements also confirm that black spruce is not particularly susceptible to cavitation or risk of hydraulic failure.

It is generally known that species from dry climates are more resistant to cavitation than species from mesic regions (Choat et al., 2012, Cochard et al., 2013). Since the boreal forest of Quebec is a generally humid ecosystem, with enough rain water available, it was not expected that black spruce would exhibit a good cavitation resistance. Xylem embolisms can however not only be caused by water deficit, but the same mechanism may occur during freeze-thaw events (Mayr et al., 2007). Black spruce is a slow growing species, adapted to short growing seasons and long cold winters. Pittermann and Sperry (2003) determined a cavitation threshold for tracheid diameter, above which embolisms increase, in any of the years in this experiment, tracheids were never larger than this threshold. Due to the specific boreal climate, the trees seem to be already equipped with a large safety margin. Resistance to freezing induced cavitation does however not guarantee a resistance to drought induced cavitation. When water potential is already low, a freezing event can still induce cavitation, even in narrow tracheids, and thus jeopardize water transport (Sparks et al., 2001, Willson and Jackson, 2006).

The first-year effect on tracheid characteristics was not found on SIM, the most southern site which is known to have faster tree growth (Boulouf Lugo et al., 2012). In spite of contradictory results (Ouedraogo et al., 2013), it is known that faster growing black spruce better maintains its physiological processes and recovers more rapidly after drought, compared with slow growing stands (Tan and Blake, 1997). For the three northern sites, the effect of the rain exclusion treatment may have been enhanced in 2010, because on these sites this was the year with the lowest rainfall (Table 1), thus resulting in a generally dryer environment for the trees.

Conclusion

It was shown with a three years repeated rain exclusion experiment that black spruce did not reduced cell production, nor a persisting reduction of cell area or an increase in cell wall thickness. In three out of four sites a decrease in cell area was found in the first year. For the first time a xylem vulnerability curve, describing the relationship between water status and the percentage loss of hydraulic conductivity, was constructed for mature black spruce trees. Like many other conifers the trees seemed to have a strong trade-off between tracheid size and protection against cavitation. This is unusual for a species growing in a non-water-limited environment, but its adaptations to survive the long, cold winters and to prevent embolism due to freezing may have led to a lower susceptibility to hydraulic failure. Mature black spruce, growing in its' natural environment, seems to be not prone to the short-term negative consequences of reduced water availability.

Acknowledgments

This work was funded by the Natural Sciences and Engineering Research Council of Canada and the Consortium Ouranos. The authors thank F. Gionest, C. Soucy, S. Pednault, M. Thibeault-Martel, I. Grenon, D. Laprise, M. Boulianne and J-G. Girard for their technical support

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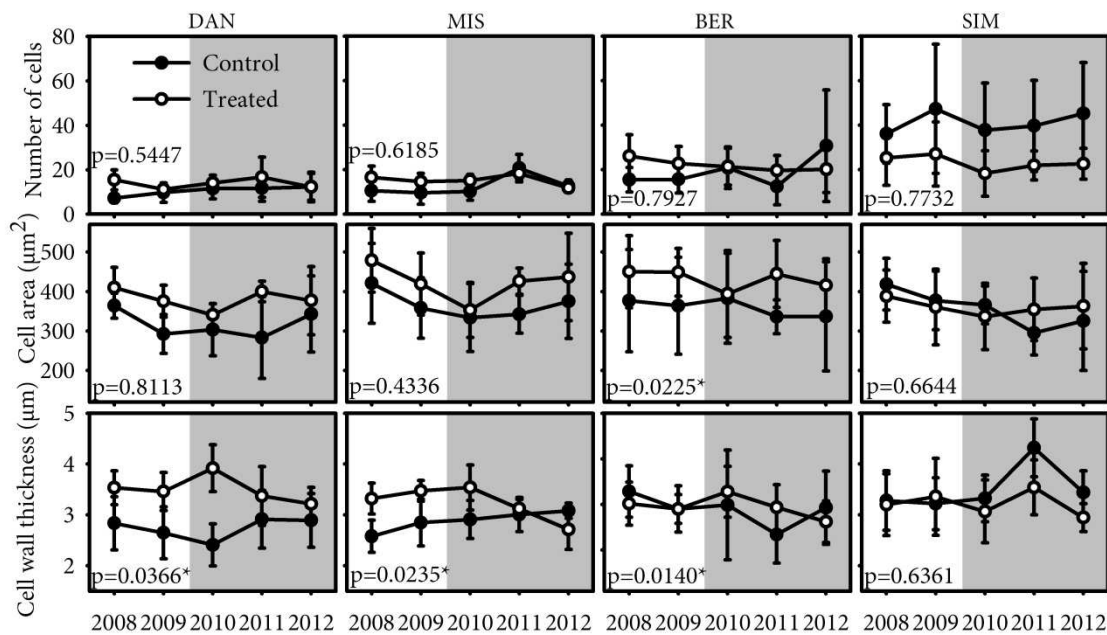
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Tables and figures

Table 4.1 : Location and climatic characteristics of the four study sites in each experimental year in order of decreasing latitude.

Site	Latitude	Longitude	Altitude (m above sea level)	Summer Temperature			Summer Precipitation		
				2010	2011	2012	2010	2011	2012
DAN	50°41'N	72°11'W	487	11.54	11.43	12.2	448	496.7	567.6
MIS	49°43'N	71°56'W	342	13.12	13.05	13.65	413.7	438.1	675.9
BER	48°51'N	70°20'W	611	12.48	11.95	12.72	455.8	501.7	474.2
SIM	48°12'N	71°14'W	338	14.21	14.08	14.95	497.3	656.8	418.8



2008 2009 2010 2011 2012 2008 2009 2010 2011 2012 2008 2009 2010 2011 2012 2008 2009 2010 2011 2012

Figure 4.1 : Number of cells, cell area and cell wall thickness in the treated (open circles) and control trees (solid circles) from 2008 to 2012. The shaded background indicates the years of the treatment. Vertical bars represent the standard deviation.

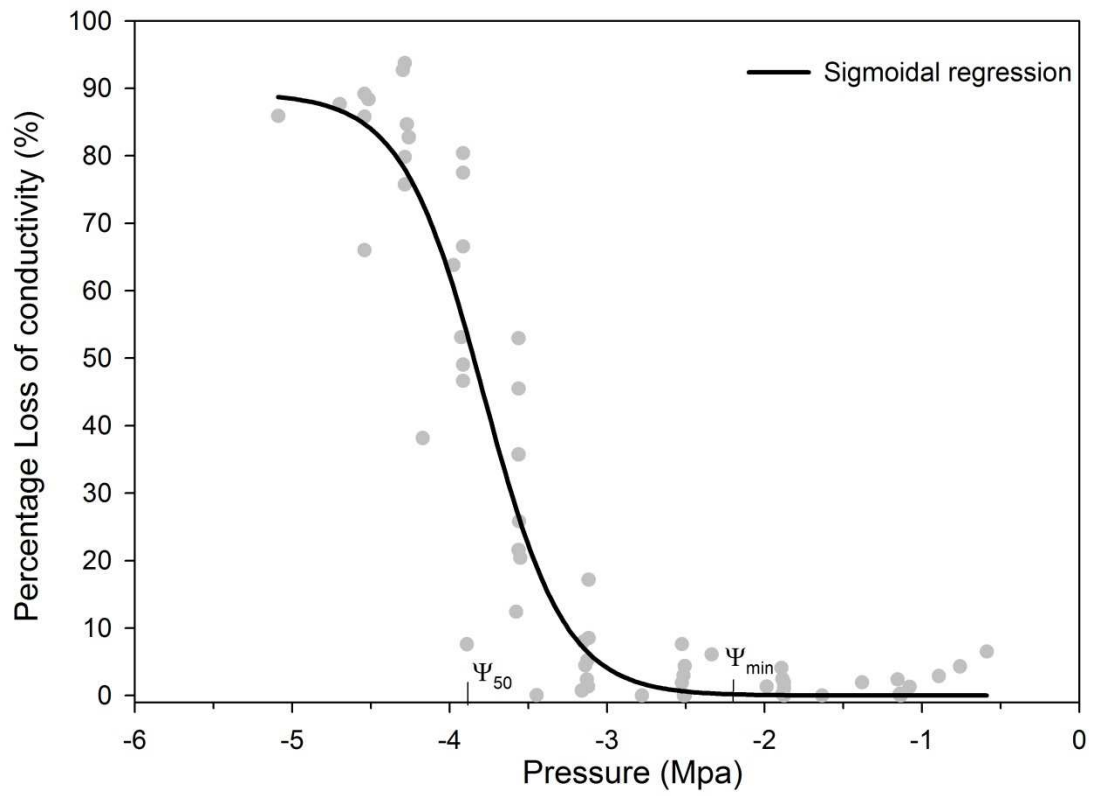


Figure 4.2 : Vulnerability curve, grey dots represent the percentage loss of conductivity of eight black spruce samples. The black line represents a nonlinear sigmoidal regression. Ψ_{50} and Ψ_{min} are indicated.

GENERAL CONCLUSION

The objective of this doctorate was to study the effects of an artificially induced rain exclusion on radial increment and radial variation, cambium phenology, physiology and tracheid properties of mature black spruce trees. The objective was attained through a multidisciplinary experimental approach. This work is innovative in that it shows the results of a three year rain exclusion experiment on mature black spruce trees, in their natural environment.

Earlier studies found that drought generally results in lower leaf water potential, reduced photosynthesis and growth (McDowell et al., 2008) leading to hydraulic failure and tree mortality, even in regions that are normally not at drought risk (Choat et al., 2012). Black spruce on the other hand seems to be dealing rather well with rain exclusion. Our trees did not experience a reduction of photosynthesis or water potential, meaning that there is no direct physiological effect of the rain exclusion. Consequently, wood anatomy did not show the expected reduction on cell size and increase in cell wall thickness, except in the first year, when a small effect was found in some of the sites. In addition to these results on physiology and anatomy, the analysis of stem radius variations confirmed that the trees were perfectly capable of following their diurnal cycles of contraction and expansion. However, in three out of four sites the trees subjected to rain exclusion showed larger stem contractions in the summer and larger winter contractions were observed on the northern sites. An alternative water absorption strategy was explored during this project, but no evidence was found for foliar absorption in black spruce.

Limitations of the study

Even though this study gives a good idea of the reaction of black spruce to rain exclusion events, the study trees were mature. Since young forests are less resistant to climate change, these

results may underestimate the effects of climate change on black spruce in general (Luo and Chen, 2013).

The rain exclusion was applied for three consecutive summers, and for about 100 days per summer. This implies an extreme limitation of precipitation on the research sites who normally receive between 401 and 532mm of summer precipitation. Moreover the study was based on individual trees, and not on stand level. Both facts implement that the applied experiments is not necessary a good representation of realistic climate change.

The only environmental factor that was altered in this study is the amount of rainfall on the root system; however in the light of future climate change also temperature may rise, resulting in higher evaporation and thus increasing the effect of drought. On the other hand, CO₂ levels may rise leading to an increased water use efficiency (Keenan et al., 2013), thus mitigating the effect of reduced carbon uptake due to drought. As is clear from the results, the trees did not suffer intense drought stress, and no signs of hydraulic failure were found. However, repeated and long duration droughts may result in carbon starvation, when stomatal closure decreases carbon uptake and the trees starve because of a continuous demand for carbohydrates (McDowell et al., 2008). Since our study was done on a relatively short term, the evaluation of long term effects like carbon starvation was not possible.

For rain exclusion experiments in natural environment it is generally recommended to apply trenching around the trees to avoid lateral water supply from outside the study plots. In our study this was not done due to the specific field conditions with rocky soils and to avoid root damage (Beier et al., 2012). The rain exclusion was installed after snowmelt, meltwater was thus available and the trees may have had a good chance to rehydrate in spring before the start of the rain exclusion. The plastic sheets for rain exclusion were installed at a height of about 2m, allowing the

entire canopy to be exposed to rainfall and dew. The potential effects of this exposure were studied in the greenhouse experiment on foliar absorption (chapter 3). The greenhouse experiment on foliar absorption was done on younger saplings. It should be noted that this does not necessarily reflect the actual field conditions. Notably the presence of mosses and lichens on mature trees in their natural environment may increase the proneness of the tree to foliar absorption.

Opportunities for further research and alternative explanations

As was clear from the results of this study, black spruce was very well able to survive and maintain growth during three consecutive summers of rain exclusion. Since only the root system was excluded from precipitation, it is possible that there are other sources or mechanisms of water absorption. The hypothesis of foliar absorption was tested and rejected as described in chapter 3, but there may be other possibilities. During this project a try-out was done using stable isotopes of oxygen to determine possible water sources of the trees (unpublished results, see appendix 1). No clear difference in water sources was found between treated and control trees, but further experiments could be done using isotopic tracers. Isotopic tracers could also be used to verify if functional root grafts exist between neighboring trees.

Root grafts between trees are known to have the capacity to transfer water and nutrients (Pregent and Y., 2001). As was found by Fraser et al. (2010) root grafts are used by *Pinus contorta* to cope with stresses like, in that case, shading. Tarroux and Desrochers (2006) showed that root grafting occurs and is functional for transporting water and nutrients in *Pinus banksiana*. In black spruce, however, root grafting takes place, but the functionality of the grafts has never been demonstrated.

Implications of the results

An increase in the number of extreme and severe drought events is a very realistic threat to ecosystems all over the world (Burke et al., 2006). Therefore there is a great need for experimental studies on the effects of drought in different environmental conditions (Hartmann, 2011).

The stem radius variations analysis showed that trees do not only react during the periods of rain exclusion, but that there may be a delayed reaction in the following winter. It was shown that wood anatomy was influenced the first year, but not in the following years of the experiment. This implies that when studying mature trees' reactions to an environmental modification, it is important to replicate the experiment over several years and to do year-round measurements.

This project contributes to the general knowledge about tree-drought relations by showing the effects of drought on black spruce at different sites throughout the boreal forest and through different methods. Even though questions remain about black spruce's water relations, this study shows that black spruce does not necessarily show an evident stress reaction and is resistant when excluded from summer precipitation.

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Appendix 1: Isotopes

Isotopes

Isotopic compositions (D and ^{18}O) of different water pools in an ecosystem (soil, plant and atmospheric) can describe interactions between these water pools and biological and hydrological processes (Ehleringer and Dawson, 1992, Dawson et al., 2002). No hydrogen or oxygen isotope fractionation, changes in the isotope abundance, takes place during water uptake through roots (Wershaw et al., 1966). This means that water taken up and transpired by plant roots has the same isotopic composition as the water source. The soil water is taken up by plants and the isotopes can thus be used as a natural tracer of plant water sources. The study of oxygen isotopes of rain water and parts of the trees may improve the understanding of the trees' water relations.

Hypothesis

Earlier results (Belien et al., 2012; 2014) showed that the black spruce trees, excluded from summer precipitation were able to cope very well with the occurring soil desiccation. It is possible that during the first year of drought, root development took place, resulting in access to alternative water sources, like deeper soil layers. Roots in deeper soil layers may have higher water uptake efficiency in desiccated soils (Plamboeck et al., 1999), and fine root development may be modified during periods of drought (Feil et al., 1988, Konôpka and Lukac, 2013). Also site characteristics, like the soils' water holding capacity may have influenced the trees' reaction to the drought treatment.

Methodology

Rain water was sampled 5 (SIM) or 6 (BER) times between DOY 180 and 260 using pluviometers with a small funnel opening to prevent evaporation, the pluviometers were emptied weekly. Branch and stem xylem were sampled between DOY 200 and 260 with a telescopic lopper and an increment corer. The bark and phloem of these samples were removed, of the stem samples only the youngest part of the xylem was kept for analysis. On both sites three lysimeters were installed at 60 cm in the control and treated plots, and three 30 cm lysimeters were additionally installed in the control plots. Note that in SIM there was no water available at 60 cm in the treated plots, in the control plots at 30 cm at only two of the sampling days. The water was extracted from the wood samples at PSI institute in Switzerland, and the oxygen isotope ratio of all water samples was determined at the WSL in Switzerland.

Results

Precipitation values correspond to normal values for this region. The lower values at the beginning of measurements may suggest some left over storage of snow melt water, which is usually more depleted of heavy isotopes. It was clear from the water samples that the samples at 30cm depth were more influenced by the rain water than the samples at 60cm depth, which are more depleted in heavy isotopes. No clear difference in isotope composition could be seen between the xylem of control and treated trees, suggesting that they use the same water sources (Fig 1 a-b).

Figure

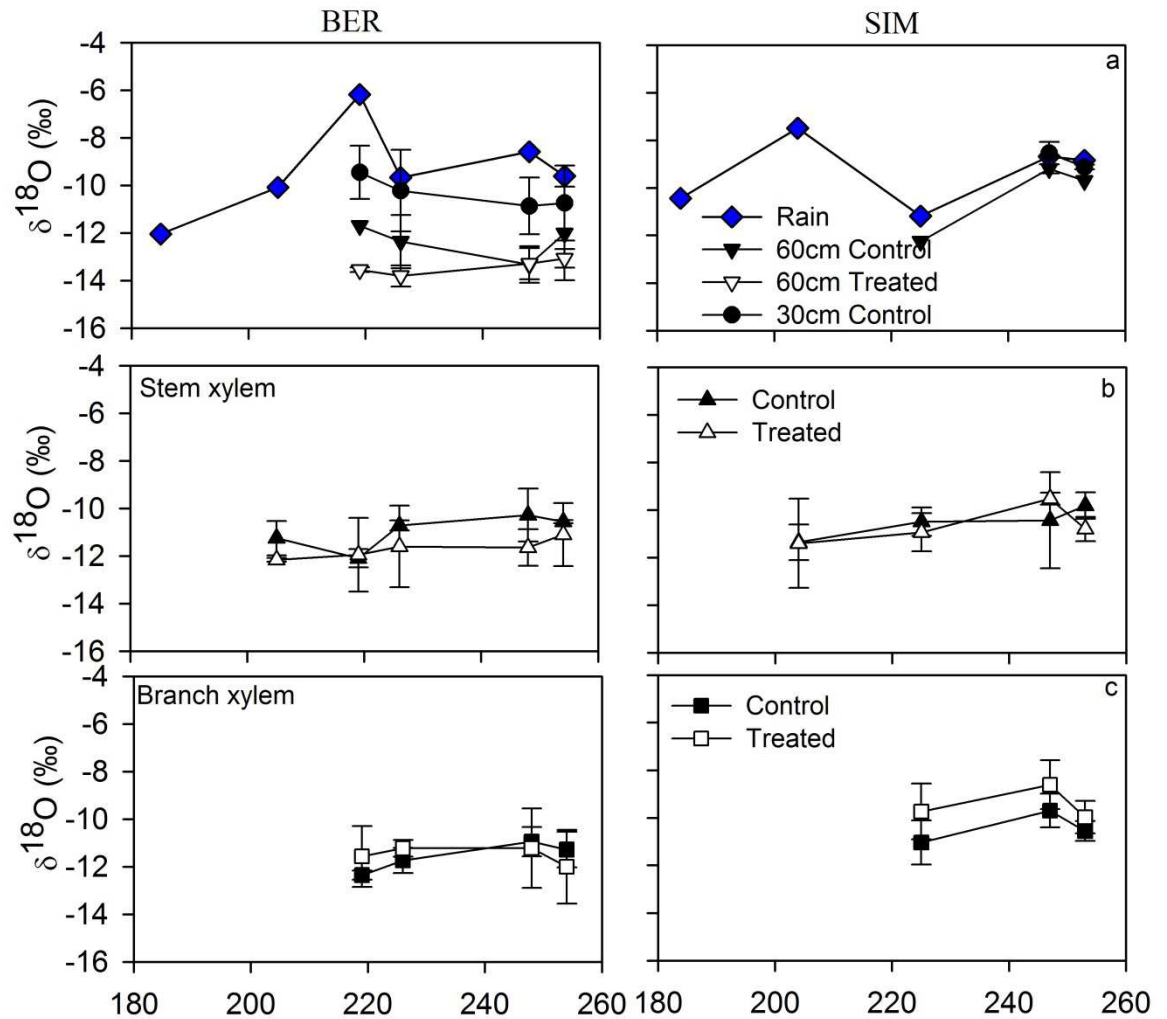


Figure 0.1 : Signals of $\delta^{18}\text{O}$ (‰ versus VSMOV) on BER and SIM for The different water sources (a), the stem xylem (b) and the branch xylem (c). Bars represent the standard deviation.

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