UNIVERSITÉ DU QUÉBEC À CHICOUTIMI

EFFETS DU DÉFICIT HYDRIQUE SUR LA PHÉNOLOGIE DU CAMBIUM, LA XYLOGÉNÈSE ET LA DENSITÉ DU BOIS DES SEMIS D'ÉPINETTES NOIRES SOUMIS À DIFFÉRENTS RÉGIMES THERMIQUES

THÈSE
PRESENTÉE
COMME EXIGENCE PARTIELLE
DU DOCTORAT EN SCIENCES DE L'ENVIRONNEMENT

PAR

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2015

Résumé

En forêt boréale, la température est considérée comme un important facteur abiotique qui règle l'activité cambiale et la production des cellules, mais la sécheresse est aussi connue pour avoir un effet direct sur la largeur des cernes de croissance. Selon les dernières prévisions climatiques, une augmentation de la température de l'ordre de 2 à 6°C est attendue avec un effet plus prononcé aux latitudes élevées. Cela favorisera l'évaporation de l'eau et la récurrence des phénomènes de sécheresse. Ces changements climatiques pourraient avoir une incidence sur la croissance des jeunes plants ainsi que sur les mécanismes physiologiques qui y sont associés. Au Canada, une augmentation de la mortalité des arbres suite à la sécheresse a déjà été enregistrée dans les dernières décennies. Dans un tel contexte, il est donc primordial de comprendre comment et dans quelle mesure les jeunes plants réagiront aux stress environnementaux, comme les vagues de chaleur et la sécheresse, suite à leur mise en terre. Le but de cette thèse est d'étudier les effets de différents régimes thermiques et hydriques sur la croissance radiale et la formation du bois de l'épinette noire [*Picea mariana* (Mill.) BSP] en milieu contrôlé, où des conditions environnementales déterminées par des modèles de prévision climatique ont été appliquées. Des expériences ont eu lieu durant les saisons de croissance 2010 à 2012 dans les serres expérimentales de l'Université du Québec à Chicoutimi (Québec, Canada). Les plants ont été cultivés à des températures plus élevées pendant le jour et pendant la nuit par rapport au contrôle. Les épinettes ont également subi deux différents régimes hydriques, soit une irrigation en continu et une période ou des périodes de déficit hydrique durant le maximum de croissance. L'activité cambiale, la xylogénèse, les relations hydriques, les échanges gazeux, l'assimilation de CO_2 et les réserves en amidon ont été déterminés chez les jeunes plants d'épinettes noires afin de pouvoir définir les limites physiologiques associées à la croissance sous les scénarios climatiques futurs. Les plants ont atteint un déficit hydrique sévère lorsqu'ils rejoignaient des valeurs proches de -3 MPa, correspondant au point d'entrée de l'air suite à une augmentation des embolies dans le xylème. Pendant le déficit hydrique, l'activité cambiale et la formation du bois ont été ralenties ou stoppées par le manque d'eau. Après la relance de l'irrigation, la reprise de l'activité cambiale s'étendait sur 2 à 4 semaines avec des reprises plus longues à des températures plus chaudes. Lors d'une augmentation de la température entre 2 et 6 °C, les plants non-irrigués présentaient un taux de mortalité de 5 à 20% respectivement alors que la mortalité était près de 0% à température ambiante. De plus, ce pourcentage de mortalité était beaucoup plus important lors d'une augmentation de la température de 6°C pendant le jour seulement. Les résultats ont permis d'élucider les mécanismes physiologiques qui sont impliqués dans la mortalité des jeunes plants comme l'inaccessibilité de l'eau et le manque de carbone.

Chez les plants non-irrigués, une diminution de la densité du bois a été observée. Les sous-processus associés à la xylogénèse, soit la durée et les taux d'élargissement et de déposition de la paroi cellulaire, ont permis de comprendre la mécanique de formation du cerne et de la densité sous les différents scénarios climatiques testés. Durant le déficit hydrique, le taux d'expansion des cellules a diminué de 28% mais a été compensé par une augmentation de la durée de cette phase qui était 21% plus longue. Similairement, le taux de formation de la paroi a été de 37% inférieur chez les plants non-irrigués. Pendant le déficit hydrique, la durée de la formation de la paroi a été en moyenne plus courte de 22% et 24% à températures plus élevées. La modification des sous-processus durant la formation du bois a grandement contribué aux variations de la densité du bois observées sous les différentes conditions de stress hydrique et de température. Ces résultats ont permis de développer de façon plus approfondie la dynamique de la xylogénèse et de mettre en évidence un mécanisme de compensation minimisant les impacts des stress sur l’anatomie du bois. Chez les plants non-irrigués, l'effet du stress récurrent a conduit à une durée de contraction et d’expansion radiale plus longue dans les cycles diurnes de la tige, mesurés à l’aide de dendromètres automatiques. L'intensité et la durée du déficit hydrique, combinée à des températures nocturnes plus chaudes, ont une influence importante sur les variations radiales de la tige. Cette thèse a permis de mettre en évidence la sensibilité de l'épinette noire à la disponibilité hydrique ainsi que la grande plasticité dans les mécanismes de formation du cerne. Les processus de formation du bois sont déterminants pour la largeur du cerne (xylogénèse) et de sa structure (anatomie du bois), la première étant très plastique et la seconde très conservatrice.

Mots-clés : activité cambiale, dynamique de la xylogénèse, anatomie du bois, densité du bois, relations hydriques, réchauffement, déficit hydrique, *Picea mariana*, jeunes plants.

Remerciements

En premier lieu, je tiens à remercier ma directrice, Annie Deslauriers, et mon codirecteur, Alessio Giovannelli, de m'avoir accordé leur confiance dans la réalisation de ce projet et pour leur encadrement dans ce cheminement. Merci pour votre encouragement, votre soutien et votre grande disponibilité au-delà du décalage horaire, tout cela m'a permis de me surpasser.

Je remercie le Conseil de recherches en sciences naturelles et en génie du Canada (CRSNG), Hubert Morin, le Consortium Ouranos et la Fondation de l'Université du Québec à Chicoutimi pour leur support financier tout au long de ce projet.

Depuis le début, cette thèse a impliqué plusieurs personnes pour le travail acharné en serre, lorsque je n'étais pas encore arrivée au Québec. Merci aux personnes qui ont effectué ce travail : Jacques Allaire, Daniel Gagnon, François Gionest, Benoit Allaire, Vanessa Tremblay, Pierre-Yves Plourde, Laurie Caron, Pascal Lapointe et Maria Laura Traversi.

Je remercie particulièrement Sergio Rossi, qui a été déterminant à mon arrivée à Chicoutimi. J'ai grandement apprécié son aide généreuse pour la logistique, les leçons de statistiques et de rédaction de manuscrits.

C'est dans un cadre méditerranéen et napolitain plutôt que boréal et québécois que plusieurs collaborations ont fleuri au cours de ce grand projet. Je remercie Henri Cuny pour ses explications sur le logiciel R (...faites sur un escalier en marbre !), une personne d'une patience et d'une disponibilité sans égard. Je remercie également Cyrille Rathgeber pour son éthique et sa rigueur, qui s'amalgament avec un grand humour et de bonnes idées. Je remercie Sylvain Delzon pour son savoir-faire, sa contribution et sa générosité pour ses conseils. Ces personnes ont donné un nouvel élan à cette thèse, je les remercie infiniment.

J'exprime ma gratitude aux membres du comité pour avoir bien voulu évaluer ma thèse, dont les critiques constructives m'ont permis de regarder ma thèse avec un autre œil.

Je remercie deux grands, Germain Savard et Daniel Gagnon, pour leur grande disponibilité et bonne humeur contagieuse. J'en ai appris avec eux !!!

Pour toutes les analyses de laboratoire, je remercie mes collègues pour leur attention et leur enseignement sur les analyses cellulaires comme sur le Québec : Sonia Pednault et Caroline Soucy, les souriantes spécialistes du microtome et des cellules ! Merci aussi à Maxime Thibault-Martel pour ses conseils précieux dans l'apprentissage de la xylogénèse. Pour la vie entre la serre et le laboratoire, je tiens à remercier Maria Laura Traversi pour toutes les suggestions et l'aide au niveau des mesures de physiologie végétale. Merci également à Pierre Gelhaye, qui au laboratoire s'est occupé de mesures de densité et qui m'a fait découvrir les facettes secrètes des radios et ses programmes. Je remercie aussi pour l'aide aux expériences en serre Marilène Beaulieu la sucrée sarcastique, Maryse Labrecque pour la rapidité d'esprit, ainsi que Camélia Dubois-Bouchard, Catherine Boivin, Philippe Cournoyer-Farley et Winna H. Landry

Je remercie Jacqueline Boulouf-Lugo, Carlo Lupi, Ernesto Cairo, Francisco Espin, et Jérôme Lévesque pour les découvertes et les discussions presque philosophiques sur l'épinette noire et la forêt boréale. Je remercie mes amis Carlo Lupi, Daniele De Barba et Angelo Fierravanti pour les belles discussions sur divers sujets et surtout les amours de jeunesse, c'est-à-dire la foresterie ! Je souhaite aussi remercier Hibat Allah Bouzidi, la gentillesse et la grâce en personne, et Isabelle Allie pour son efficacité constante et son humour.

À mes collègues de bureau, merci d'avoir partagé ces années doctorales et cette ambiance multiculturelle. Merci à Émilie Pamerleau-Couture, Maxime Tremblay, Audrey Lemay, Carlo Lupi, Boris Dufour, Joana Vieira, Evelyn Belien, Fabio Bosio, Daniele De Barba, Rocio Urrutia-Jalabert, Emanuele Ziaco, Madjelia Dao Some, Serena Antonucci, Lionel Navarro, Maxence Martin, Magali Perrin et Miguel Montoro.

Dans le cadre administratif, je tiens à remercier la merveilleuse Dominique Simard, qui a su me guider avec humour dans l'apprentissage de la machine de l'UQAC et de l'UQAM. Je remercie aussi la Vie Étudiante de l'UQAC et son équipe géniale, Patrice Seguin, Myriam Tremblay et Sandra Côté.

L'UQAC m'a aussi permis de croiser les routes d'autres personnes teintées d'une grande générosité, humanité, ouverture et sagesse. Je les remercie pour leur amitié et présence : Lili, Marga, Lyne, Maud et Audrey. Merci aussi à toute ma bande de filles chanteuses, mes amis(es) judokas et mes étudiants des cours d'italien. Un merci tout spécial et chaleureux à mes amis(es) et ma parenté éparpillés dans le *Bel Paese*... l'Italie !

Je remercie ma famille, mes parents et mon frère qui ont toujours été présents. Merci pour leur grand amour et belle patience, pour leur éducation nourrie d'intelligence et de respect envers le prochain et la nature. Enfin, merci à mon copain, Patrick pour tout ce qu'il apporte à chaque moment, pour l'amour et le soutien dans ces années d'étude, il a aussi contribué à ce succès. Merci, O Soli miei!

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INTRODUCTION GÉNÉRALE

1.1 MISE EN CONTEXTE

Les plus longues séries d'observations mesurées de l'Est du Canada indiquent un réchauffement moyen de la température de surface de 2 °C depuis 1876 au Québec (Bonsal *et al.* 2001; Vincent et Mekis 2006; Houle, Moore et Provencher 2007). D'ici 2100, les scénarios actuels projettent que les changements climatiques seront responsables d'une augmentation globale des températures de 2-4 °C, d'importantes sécheresses dans certaines régions (Seager *et al.* 2007; Christensen *et al.* 2013; Park *et al.* 2013), ainsi que de l'augmentation de la fréquence et la gravité des sécheresses extrêmes, des extrêmes de température et des vagues de chaleur (Sterl *et al.* 2008; IPCC 2013). Il est connu que ces changements climatiques affecteront inégalement les régions des hautes latitudes. En effet, au Québec les changements attendu seront plus importants en hiver et au nord du 48^{e} parallèle (IPCC, 2013; Ouranos, 2014) avec des conséquences sur la composition et la croissance des forêts. Dans les dernières décennies, il a été observé que l'augmentation de la croissance des forêts dans l'hémisphère nord est en partie attribuable au réchauffement du climat (Boisvenue et Running 2006; Bonan 2008). Une étude sur la forêt québécoise décrit qu'il y a eu une augmentation du volume de bois marchand de 25 m^{3}/ha en raison du réchauffement engendré par les changements climatiques (Rainville *et al.* 2014). Toutefois, les changements dans les températures et régimes de précipitations sont considérés à la base de l'augmentation de la vulnérabilité des arbres dans plusieurs écosystèmes (Allen *et al.* 2010a). D'autres études rapportent que la hausse des températures pourrait amplifier les effets de la sécheresse sur la mortalité des arbres (van Mantgem *et al.* 2009; Peng *et al.* 2011).

Au Québec, le reboisement représente une des stratégies adoptées dans un contexte de développement durable, afin de diminuer la pression sur les forêts naturelles et satisfaire les besoins économiques (Lamhamedi *et al.* 2003). En 2007, plus de 34 millions de plants produits dans les pépinières gouvernementales ont été mis en terre (MRNF, 2009), mais le gouvernement du Québec vise à déterminer la vulnérabilité des jeunes plants destinés au reboisement dans le contexte des changements climatiques [PACC 2013-2020, Gouvernement du Québec (2012)]. Comme l'épinette noire [*Picea mariana* (Mill.) B.S.P.] est l'une des espèces les plus communes de la forêt boréale du Canada et du Québec, des recherches sont souhaitées afin d'intégrer les effets anticipés des changements climatiques sur la gestion sylvicole de cette espèce. Il est donc important de déterminer et d'évaluer la survie des jeunes plants exposés à certains stress environnementaux, comme la sécheresse et les vagues de chaleur, suite à la mise en terre. Ce projet de recherche a pour objectif d'étudier les effets de différents régimes thermiques et hydriques sur la croissance radiale et la formation du bois de l'épinette noire [*Picea mariana* (Mill.) B.S.P.] en milieu contrôlé, c'est-à-dire où sont appliquées des conditions environnementales déterminées par les modèles de prévision climatique.

1.2 PROBLÉMATIQUE

La voie pour la circulation de l'eau sol-plantes-atmosphère peut être représentée par une série de résistance en phase liquide et vapeur, dans laquelle l'approvisionnement en eau est d'une importance centrale pour la croissance et la survie des plantes (Zimmermann *et al.* 1994; Tyree 1997). Les modèles climatiques prédisent une plus grande évaporation résultant en une diminution de l'humidité du sol et une augmentation de l'intensité des sécheresses (IPCC 2013; Villarini, Smith et Vecchi 2013), se traduisant en une modification de la consommation d'eau des plantes. Également les plantes contribuent à la réduction de l'eau dans le sol, en absorbant l'eau nécessaire pour la croissance, le métabolisme et en faisant du stockage d'eau dans différents compartiments (aubier, parois cellulaires, vaisseaux inactifs, cellules vivantes de feuilles, phloème avec ces types de cellules et rayons de bois). La tolérance au déficit hydrique dépend de la durée et de l'intensité même du déficit (McDowell *et al.* 2008). La diminution de la pression de turgescence hydrostatique dans la cellule induit des réponses de la perception du stress des plantes (Savidge 2001). La structure du xylème est donc impliquée dans l'équilibre hydrique des plantes et peut être d'une grande importance dans le mécanisme de tolérance aux déficits hydriques (Brodribb et Cochard 2009). Cette tolérance des plants dépend aussi de la capacité d'établir rapidement un lien fort entre les racines et le sol environnant (Bigras et Dumais, 2005).

Le réchauffement devrait avoir un effet au niveau physiologique sur la photosynthèse et à la fois sur la respiration. Les températures élevées pourraient avoir un impact sur la photosynthèse à la fois positive et à la fois négative (Way et al., 2008). L'acclimatation de la photosynthèse à une augmentation de la température représente un des mécanismes positives impliqués dans l'adaptation pour réduire les effets négatifs de cette hausse de température (Chaves *et al.* 2002; Flexas *et al.* 2004; Chaves, Flexas et Pinheiro 2009). Il est connu que chez les semis d'épinettes noires l'acclimatation thermique est causée par une réduction de la respiration et des investissements d'azote dans les aiguilles (Way et Sage

2008). L'eau constitue également une exigence principale pour la photosynthèse et est la principale composante de la majorité des cellules végétales. Les contraintes, imposées par le déficit hydrique sur la photosynthèse, ont été traditionnellement considérées en termes de limites « stomatique » et « non stomatique » (Gunderson, J. et Wullschleger 2000); de sorte que les effets de la sécheresse peuvent être directs (comme la disponibilité réduite de CO_2 causée par la limitation de la diffusion à travers les stomates et le mésophile) (Flexas *et al.* 2004), ou indirects (sous forme de stress oxydatif) (Chaves *et al.* 2002; Chaves et Oliveira 2004; Chaves, Flexas et Pinheiro 2009). McDowell (2011) suggère que pendant un déficit hydrique, la croissance des plantes peut être réduite avant la diminution de l’assimilation de CO_2. Lorsqu’un déficit hydrique se prolonge, plusieurs interactions complexes surviennent pouvant compromettre l’utilisation des réserves par le métabolisme et la défense ainsi que pour le maintien de la turgescence des cellules et l’assimilation de CO_2 (McDowell 2011). Dans des conditions environnementales plus chaudes, l'incapacité de balancer le gain de carbone photosynthétique et la perte de carbone par la respiration pourrait limiter la croissance des arbres, jusqu'à en compromettre la survie (McDowell 2011). Toutefois, ces mécanismes de réaction sont encore en discussion et appellent à un besoin de recherche.

Les conditions hydriques ont une grande influence sur les phases de la croissance radiale (division et élargissement des cellules) (Abe et Nakai 1999; Abe *et al.* 2003) et la température détermine le début de la croissance (Rossi *et al.* 2006). Ceci est essentiel à considérer afin de séparer les effets interactifs de la hausse des températures et du déficit de vapeur de pression d’eau, en parallèle avec la sévérité de la sécheresse dans le sol

(Allen *et al.* 2010b), sur les échanges gazeux du jeune plant (processus stomatique, flux d'eau jour-nuit, assimilation du CO_2) et sur la croissance radiale. La consommation d'eau n'est pas liée seulement à l'anatomie de la feuille, mais aussi à l'anatomie du xylème (Sperry *et al.* 2002; Fichot *et al.* 2009). L'acclimatation des propriétés structurelles du xylème aux sécheresses peut être un mécanisme important permettant aux plants de coordonner la quantité d'eau fournie à la canopée avec les conditions environnementales (Mencuccini 2003). Toutefois, il manque encore des connaissances sur les effets des facteurs de stress environnementaux sur la croissance radiale et la formation du bois chez l'épinette noire.

1.2.1 Facteurs de stress environnementaux sur la croissance et la formation du bois

Dans les environnements froids, la formation du cerne de croissance est limitée à une certaine période de l'année et est sous le contrôle de plusieurs facteurs endogènes, comme les glucides (Kozlowski et Pallardy 1997), les hormones, dont l'auxine (Uggla *et al.* 2001) et l'éthylène (Love *et al.* 2009), l'âge cambial (Rossi *et al.* 2008a) et les facteurs exogènes, comme la température et les précipitations (Deslauriers et Morin 2005; Giovannelli *et al.* 2007; Thibeault-Martel *et al.* 2008). Des études sur le développement intra-annuel du cerne de croissance de l'épinette noire en forêt boréale montrent que la période disponible pour la formation des cellules du bois est très courte (Deslauriers et Morin 2005; Rossi, Deslauriers et Anfodillo 2006; Deslauriers *et al.* 2008; 2008b; 2009). Chez les arbres matures, la majorité du cerne annuel est formé en moins d'un mois et demi, soit entre le début juin et la mi-juillet. Le maximum de production cellulaire se produit aux environs du solstice d’été (Rossi *et al.* 2006). La division cellulaire et l’élargissement des trachéides sont des processus influencés par les conditions hydriques (Abe et Nakai 1999; Savidge 2001). La pression hydrostatique agit sur le cambium, qui règle l’activité mitotique des cellules, et sur les cellules en différenciation (Abe et Nakai 1999; Giovannelli *et al.* 2011; Rathgeber *et al.* 2011). Lors de la première phase de différenciation, avec l’augmentation de la turgescence due à l’absorption d’eau dans le cytoplasme, les parois primaires des cellules sont étirées et augmentent le diamètre radial et la surface du lumen (Kramer 1964). Après la phase d’élargissement, la différenciation cellulaire se poursuit avec la formation des parois secondaires. La dernière phase se traduit par la mort cellulaire, c'est-à-dire l’autolyse du cytoplasme (Plomion, Leprovost et Stokes 2001; Rathgeber *et al.* 2011). Le processus de différenciation d’une dérivée cambiale nécessite une période allant de quelques jours à plusieurs semaines et varie aussi selon les espèces, le type de cellules (par exemple, vaisseaux versus trachéides) et le moment du développement au cours de la saison végétative (Kutscha, Hyland et Schwarzmann 1975; Rossi et al., 2008b; 2011).

Des recherches ont démontré qu’un déficit hydrique peut contribuer à ralentir la croissance chez les jeunes plantations de conifères (Burdett, Herring et Thompson 1984; Bernier 1993). Dans les premiers stades d’un déficit hydrique, l’élargissement cellulaire est d’abord inhibé et, quand le déficit commence à être plus sévère, la division cellulaire est elle aussi inhibée (Abe et Nakai 1999; Abe *et al.* 2003; Jyske *et al.* 2010). Cette prolongation du déficit hydrique produit une cascade d'interactions complexes pouvant compromettre l'utilisation des réserves pour le métabolisme, la défense, le maintien de la turgescence (McDowell, 2011). Toutefois, ces mécanismes de réaction sont encore en discussion.

Une diminution de la disponibilité hydrique conjuguée à des températures plus élevées pourrait donc augmenter l'évapotranspiration des plantes et causer un assèchement des sols. Ces changements pourraient avoir des conséquences plus marquées sur la croissance des forêts. La température est un autre facteur influençant la formation du cerne de croissance. La majorité des études traitent de l'importance de la température printanière sur la réactivation cambiale après la phase de dormance hivernale (Seo *et al.* 2008; Thibeault-Martel *et al.* 2008; Gruber *et al.* 2010). La relation entre la croissance et la température de l'air n'est pas nécessairement linéaire et elle pourrait dépendre d'un seuil de température printanier en dessous duquel les divisions cellulaires ne débutent pas. En effet, selon Rossi *et al.* (2006), un seuil de température relativement constant de 8 °C est nécessaire pour la réactivation de la productivité cambiale au printemps, et ce seuil serait commun chez plusieurs espèces de conifères des milieux tempérés et boréaux (Deslauriers *et al.* 2008; Rossi *et al.* 2008b; Swidrak *et al.* 2011). Chez l'épinette de Norvège (*Picea abies*), Gričar *et al.* (2007) ont constaté que des températures plus élevées que 8 °C (23-25 °C) ont été favorables aux divisions du cambium au tout début de la saison de croissance et les cellules en lignification et en maturation ont été observées plus tôt comparativement à des arbres soumis à des températures plus basses (9-11°C). Cela nous porte à croire qu'une hausse des températures de croissance, prévue par les scenarios des changements climatiques, peut engendrer des conséquences significatives sur l'épinette noire, encore plus que ces augmentations seront plus rapides pendant la nuit que le jour. À ce sujet, certaines études mentionnent que la formation de la paroi cellulaire est l’un des processus de la croissance radiale qui se produit surtout pendant la nuit (Saveyn et al., 2007; Turcotte et al., 2011). Dans le même sens, des études plus récents confirment l'hypothèse que les processus de croissance se produisent majoritairement pendant la nuit de concert avec l'état hydrique et la pression de turgescence (Pantin, Simonneau et Muller 2012; Steppe, Sterck et Deslauriers 2015). L'accroissement des températures nocturnes pourrait donc affecter les mécanismes physiologiques et affecter le stockage de l'eau pendant la journée. La réponse de la dynamique de la région cambiale par rapport aux températures pendant les différentes phases de croissance, en combinaison avec un déficit hydrique, reste donc à déterminer.

1.2.2 La dynamique dans les flux et le stockage d'eau

Les cycles circadiens de déshydratation et de réhydratation contrôlent le rétrécissement et le gonflement des cellules vivantes du phloème, du cambium et du xylème en formation (Turcotte *et al.* 2011). En raison des propriétés élastiques des tissus et selon le continuum sol – plante – atmosphère du bilan hydrique des arbres, les cycles circadiens causent des variations quantifiables dans la taille de la tige pouvant être mesurées avec des dendromètres automatiques (Deslauriers *et al.* 2003; Giovannelli *et al.* 2007). Ces derniers sont aussi utilisés pour mesurer la variation intra-annuelle de croissance radiale du tronc en continu (Deslauriers *et al.* 2003; Deslauriers, Rossi et Anfodillo 2007; Giovannelli *et al.* 2007; Turcotte *et al.* 2011). Par contre, les mesures fournies par les dendromètres sont fortement influencées par la variation de l’état hydrique des tissus, en particulier ceux du phloème et du liber (Zweifel et Hasler 2001). En effet, le début de la croissance radiale du tronc au printemps est souvent masqué par la réhydratation de la tige (Downes, Beadle et Worledge 1999; Zweifel et Hasler 2001; Mäkinen, Nöjd et Saranpää 2003). Les variations radiales de l'épaisseur des tissus du tronc sont à la fois irréversibles (division et élargissement cellulaire) et réversibles (cycle journalier d'hydratation et de déshydratation). Les semis et les jeunes arbres peuvent être atteint plus rapidement aux conditions de stress hydrique, car ils ont une capacité moindre de stocker l'eau dans la tige, les feuilles et les racines que les arbres matures. La quantité d'eau stockée que les arbres peuvent transpirer dépendra de l'équilibre entre la perte de l'eau de la surface foliaire et l'écoulement de l'eau à partir du système conducteur et de la superficie de bois de l'aubier (Domec et al., 2012). Les mécanismes d'adaptation aux sécheresses mis en place dans un court, moyen et long terme pourrait être à la fois physiologiques (i.e. contrôle stomatique, ajustement osmotique) et à la fois morphologiques (i.e. changements dans le taux de croissance, surface foliaire) (Domec et al., 2012). Toutefois, ces aspects ne sont pas toujours faciles à identifier en milieu naturel. Cependant, il est connu que lors des sècheresses, la coordination entre l'approvisionnement en eau, la surface foliaire et la surface de l'aubier et la transpiration foliaire est l'élément nécessaire pour maintenir l'efficacité du système hydraulique (Mencuccini, 2001). Dans les environnements méditerranéens, il est connu que des variations dans les cycles circadiens d'hydratation et déshydratation sont observables pendant une sécheresse estivale (Vieira *et al.* 2013). Il y a présentement un nombre limité d'études concernant la réponse des jeunes arbres en milieu contrôlé, ainsi que la dynamique de la croissance intra-annuelle des jeunes arbres suivis avec les dendromètres automatiques.

1.2.3 Effets de la température et du déficit hydrique sur la structure du xylème : implications sur la fonctionnalité du système

La sécheresse estivale a un effet sur la largeur des cernes de croissance (Eilmann *et al.* 2006; 2011) causant une diminution des diamètres des cellules (Abe et al., 2003) et ce, expliqué par le contrôle direct de la disponibilité en eau requise par l'activité cambiale (Steppe *et al.* 2006; Zweifel *et al.* 2006). Ces réductions de la croissance radiale dépendent d'une diminution du nombre de cellules (Rossi et al. 2009) ou de la production de trachéides plus petites lors de périodes estivales sèches (Corcuera, Camarero et Gil-Pelegrín 2004). De plus, l'effet d'un déficit hydrique peut aussi affecter les propriétés des trachéides et par le fait même, du bois. Le diamètre cellulaire et le diamètre et l'épaisseur de la paroi sont d'importantes propriétés des trachéides qui influencent la densité du bois (Rathgeber, Decoux et Leban, 2006). En effet, cette dernière est un trait fonctionnel important et dépend de la proportion du bois final et du diamètre des trachéides du bois initial. Il est connu qu'une augmentation de la densité du bois est provoquée par l'épaississement des parois cellulaires et la diminution du diamètre radial des cellules (Nicholls 1984; Rathgeber, Decoux et Leban, 2006). De plus, les propriétés hydrauliques du xylème sont liées à certaines caractéristiques anatomiques, par conséquent à la densité du bois (Rathgeber, Decoux et Leban, 2006). Rossi *et al.* (2009) ont observé que la réponse des semis d'*Abies balsamea* à une période de 20 jours de déficit hydrique consistait en une réduction de 50% de la surface du lumen et du diamètre des trachéides et aussi une augmentation de l'épaisseur des parois cellulaires du bois final. Parallèlement, une autre étude sur *Picea abies* a montré que l'exclusion d'eau de pluie, durant 60-75 jours pendant cinq ans, a provoqué une augmentation de la densité du bois (Jyske *et al.* 2010). Suite à ces changements au niveau de l’anatomie et de la densité du bois, il faut souligner qu’il peut y avoir des variations dans les propriétés du xylème et l’efficacité d'utilisation de l’eau. Les ajustements physiologiques et fonctionnels déterminent le caractère adaptatif d'une espèce, par exemple, une réduction de la surface foliaire, une réduction de la conductivité stomatique ou, encore, une perte de conductivité (Corcuera, Camarero et Gil-Pelegrín 2004). Celle-ci est considérée comme une propriété hydraulique, liée à la vulnérabilité à la cavitation et par conséquent à la sécheresse (Brodribb et Cochard, 2009). Des études sur des espèces ligneuses des régions arides de l'Ouest américain et de l'Asie centrale ont démontré que la réduction de la dimension des cellules et l'augmentation de la densité du bois correspondent à une plus grande efficacité d'utilisation de l'eau (Kocacinar et Sage 2004). Toutefois, l'effet est différent pour les feuillus de la Méditerranée où les sécheresses extrêmes causant une réduction des diamètres des vaisseaux sont responsables d'une diminution de la conductance hydraulique, de la surface foliaire et du rapport surface foliaire : aubier (Corcuera, Camarero et Gil-Pelegrín 2004). Toutefois, il reste à déterminer les effets conjoints d’un déficit hydrique et d’une augmentation de la température sur l’anatomie, la formation du bois et la densité du bois à des latitudes élevées.

En résumé, les effets de la sècheresse et de l’augmentation de la température sur la formation et la densité du bois sont peu connus sur les espèces boréales. L’épinette noire est l'une des espèces commerciales les plus communes et importantes dans l’est du Canada (Gagnon et Morin 2001). En raison des implications écologiques et socio-économiques considérables, une évaluation plus détaillée du rôle de ces facteurs environnementaux est nécessaire afin de comprendre de quelle façon la phénologie du cambium, la xylogénèse et la densité du bois de l’épinette noire pourraient se modifier dans les années à venir en lien avec les changements climatiques.

1.3 APPROCHE MÉTHODOLOGIQUE

Cette thèse aborde pour la première fois les effets combinés des stress abiotiques du déficit hydrique et de l'augmentation de la température en utilisant des expériences effectuées en milieu contrôlé. Ce projet a été réalisé sur des jeunes arbres d'épinettes noires (*Picea mariana*) de 4 ans et s'est déroulé dans un complexe de serres de recherche comprenant trois zones complètement indépendantes et gérées individuellement par un système de contrôle (Priva Maximizer) (Tableau 1.1).

Au printemps de chaque année et pendant 3 ans, 1104 jeunes arbres d'épinettes noires en racines nues ont été transplantés dans des pots en plastique (volume 4.5 l) remplis de mousse de tourbe de sphaigne avec perlite et vermiculite. Les jeunes arbres ont été cultivés à l'extérieur pendant chaque été précédant l'année d'expérience (Tableau 1.1). Le printemps suivant, les pots ont été irrigués par un système d'irrigation à gouttes et répartis dans les trois différentes zones du complexe de serres. Les plants ont été maintenus à la capacité au champ, afin de prévenir l'état des sols anaérobies en fournissant une quantité d'eau égale au poids des pertes quotidiennes, soit de 500 ml d'eau par plant par jour. Chaque semaine durant environ 6 mois, 36 semis ont été récoltés (3 traitements de température × 2 traitements hydriques × 6 semis pris au hasard). Ceci représente au total 900 semis (25 semaines × 36 semis) (Tableau 1.1, Figure 1.1).

La zone contrôle de la serre a été maintenue à une température similaire à la température externe, alors que les deux zones traitées (Figure 1.1) ont subi une augmentation de la température par rapport à la zone contrôle (Tableau 1.1, Figure 1.1). Les modifications de la température ont été réalisées d’avril à novembre en suivant les températures normales historique pour la région du Saguenay (Figure 1.2), pendant une période de croissance plus longue de trois mois que celle observée pour l’épinette noire à la même latitude (Dufour et Morin 2006; Thibeault-Martel *et al.* 2008). Durant la première année, la modification de la température dans les zones réchauffées (Figure1.1), par rapport à la zone contrôle, a été appliquée durant toute la journée. La deuxième et la dernière année, la modification de la température a été produite seulement la nuit, du coucher du soleil à l’aube. Pour l’application de l'irrigation, les plants de chacune des zones ont été divisés en deux régimes différents. La moitié des plants, les témoins, ont été maintenus à la capacité du champ pendant toute la période de croissance. Le déficit hydrique a été réalisé sur la deuxième moitié des plants en cessant l’irrigation pendant une période variant entre deux semaines et un mois durant les deux premières années. La troisième année, trois cycles de déficit hydrique ont été effectués en correspondance avec trois phases de différenciation des trachéides (division cellulaire, élargissement et lignification).

Tableau 1.1 Brève description des expériences réalisées durant cette thèse avec leurs chapitres respectifs dans lesquels elles sont présentées en détail. Toutes les expériences ont été réalisées en milieu contrôlé aux serres de l'Université du Québec à Chicoutimi (48°25′N, 71°04′W, 150 m au niveau de la mer) sur l'épinette noire [*Picea mariana* (Mill.) B.S.P.].

Expérience	**Traitement**	**Durée de l'expérience**	**Chapitre**
Effets du réchauffement et du déficit hydrique sur l'activité cambiale et la formation du bois	Contrôle Réchauffement de +2 et +5 °C 1 déficit hydrique de 32 jours	6 mois en 2010	II
Effets du déficit hydrique et du réchauffement sur la survie des plants et les caractéristiques du bois	1 déficit hydrique de 25 jours Contrôle Réchauffement de +6 °C le jour Réchauffement de +6 °C la nuit	6 mois en 2011	III
L'atténuation des effets du déficit hydrique et du réchauffement par des mécanismes compensatoires	1 déficit hydrique de 25 jours Contrôle Réchauffement de +6 °C le jour Réchauffement de +6 °C la nuit	6 mois en 2011	IV
Effets combinés de la sécheresse et du réchauffement nocturne et diurne sur les variations radiales des tiges	Contrôle Réchauffement de +6 °C le jour Réchauffement de +6 °C la nuit 1 déficit hydrique de 25 jours 3 cycles de déficit hydrique	6 mois en 2011 6 mois en 2012	V

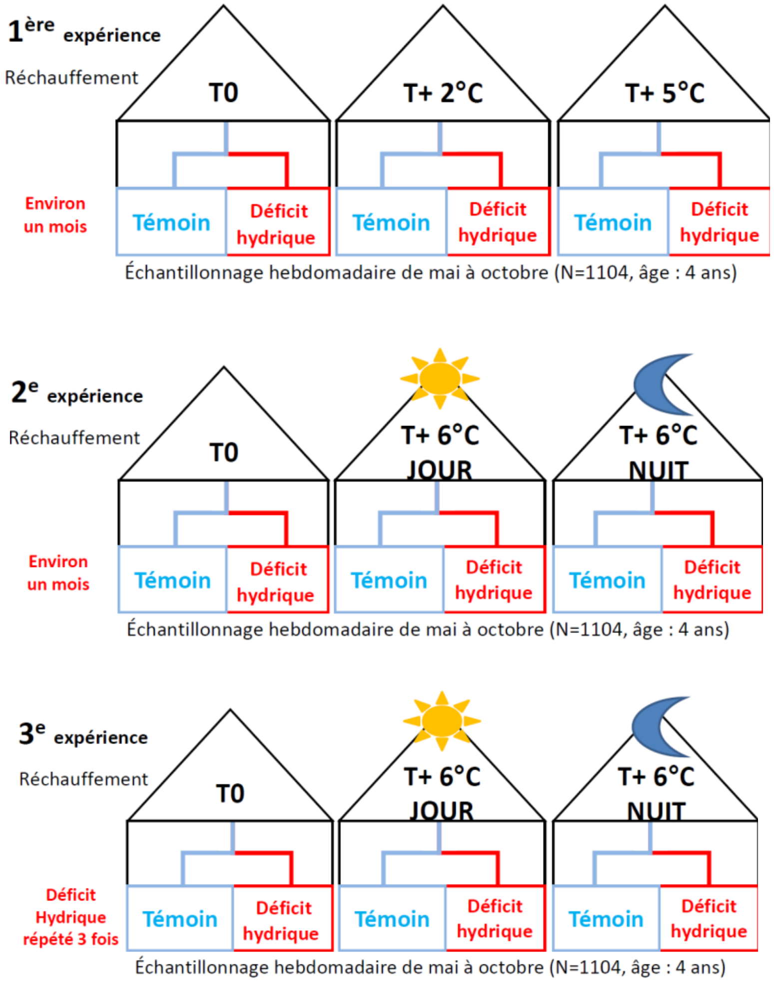

Figure 1.1 Design expérimental des expériences réalisées durant cette thèse. Toutes les expériences ont été réalisées en milieu contrôlé aux serres de l'Université du Québec à Chicoutimi (48°25′N, 71°04′W, 150 m au niveau de la mer) sur l'épinette noire de 4 ans [*Picea mariana* (Mill.) B.S.P.]. Ces expériences correspondent à 3 traitements de température × 2 traitements hydriques sur 6 semis/chapelle pris au hasard à chaque semaine parmi 1104 épinette. T0 correspond à la température contrôle, les autres deux traitements thermiques correspondent au réchauffement appliqué.

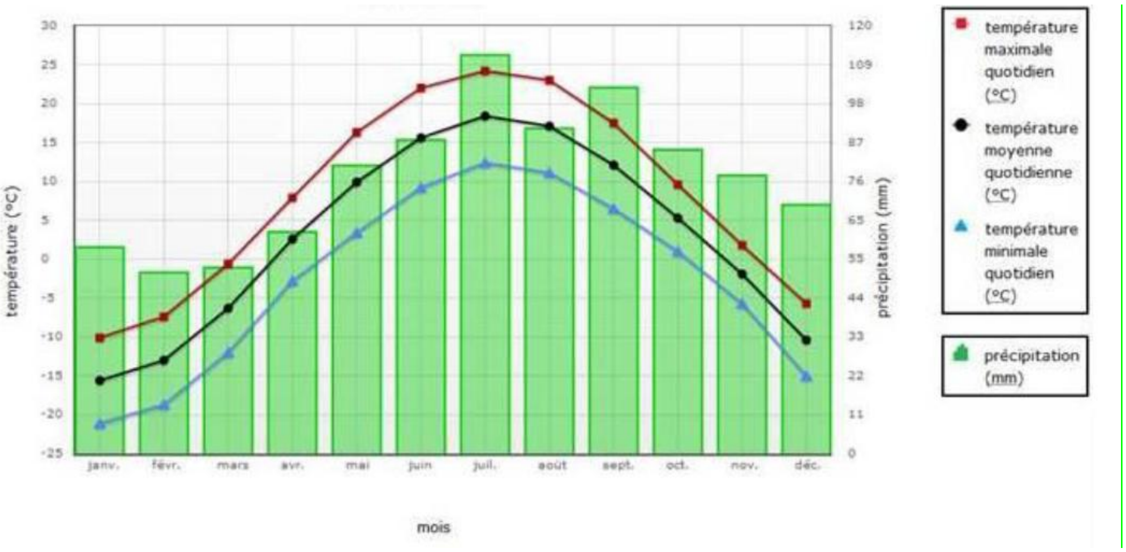

Figure 1.2 Données climatiques historiques des températures et des précipitations pour les normales climatiques du Canada de 1981 à 2010 de la station météorologique de Bagotville (source Environnement Canada).

1.4 OBJECTIFS ET HYPOTHÈSES

Le projet a pour but d'étudier les effets d’une combinaison des régimes thermiques et hydriques sur la croissance radiale et la formation du bois de jeunes arbres d’épinettes noires en milieu contrôlé. La formation du cerne de croissance a été suivie à l'aide des analyses histologiques pendant la saison de croissance (phénologie) et après la fin de la différenciation des cellules du bois (anatomie). La formation du bois a été étudiée en relation avec (1) le statu hydrique des plants, (2) les échanges gazeux et l'assimilation de CO_2, (3) la croissance des méristèmes primaires, (4) l’état hydrique de la tige, par des analyses des variations radiales circadiennes de la tige, et (5) la structure du xylème. Les jeunes arbres utilisés dans les expériences ont été soumis à deux traitements simultanés en milieu contrôlé afin de simuler les scénarios climatiques futurs : (1) un traitement de hausse de la température pendant le jour et/ou la nuit et (2) un traitement de déficit hydrique appliqué sur des périodes de durées variables selon les expériences (cycles courts de sécheresse, 25 et 32 jours).

Plusieurs études se sont penchées sur la question de l’activité cambiale et la xylogénèse dans les environnements froids montrant que la température représente un élément important pour la croissance (Deslauriers *et al.* 2008; Rossi *et al.* 2013). Toutefois, peu d’études ont analysé l’influence du climat sur l’activité du cambium chez les jeunes arbres de la forêt boréale. Or, pour comprendre l’impact des changements climatiques (réchauffement climatique et augmentation de la fréquence et de la sévérité des sècheresses), il est fondamental d'interpréter les mécanismes physiologiques qui affectent la xylogénèse et les liens avec la production du bois. Par exemple, la sécheresse peut affecter plusieurs caractéristiques de croissance telles que l'anatomie du xylème et l'accroissement radial (Abe *et al.* 2003; Corcuera, Camarero et Gil-Pelegrín 2004; Arend et Fromm 2007). Il est connu que les semis d'épinettes noires démontrent une résistance à la sécheresse grâce à une acclimatation de la conductance stomatique et du taux de photosynthèse (Zine El Abidine *et al.* 1994), cependant ces derniers paramètres sont strictement liés à une augmentation de la température (Sage, Way et Kubien 2008). La densité du bois, un paramètre clé pour les estimations de stockage de carbone (Chaves et Oliveira 2004; Chaves, Flexas et Pinheiro 2009), dépend des caractéristiques des trachéides (Rathgeber, Decoux et Leban 2006). Dans le contexte des changements climatiques, différents régimes thermiques et hydriques pourraient modifier la croissance ainsi que les propriétés cellulaires. Les hypothèses suivantes ont été posées (*voir Chapitre II*) :

1. Le déficit hydrique sévère pourrait induire une réduction de la croissance du cerne annuel en réponse à la diminution des réserves en eau, des échanges gazeux et de l'assimilation de CO_2 ;
2. L'activité du cambium pourrait être modifiée par le ralentissement de la division cellulaire et de la durée de l'expansion cellulaire en réponse à la sévérité progressive du déficit hydrique qui est fonction de la température ;
3. Le déficit hydrique combiné à l'augmentation des températures de croissance pourrait induire la formation de parois cellulaires plus minces ou des cellules plus petites.

Dans les environnements froids, la température joue un rôle fondamental au début de la saison de croissance (Rossi *et al.* 2011; Boulouf Lugo, Deslauriers et Rossi 2012). Il faut d'abord rappeler que les températures ne devraient pas changer également pendant la nuit et le jour avec les changements climatiques (IPCC 2013). Il est connu que le réchauffement nocturne provoque des impacts sur les mécanismes physiologiques liés au stockage de carbone et aux relations hydriques (Sage 2002; Turnbull, Murthy et Griffin 2002; Turnbull *et al.* 2004; Sage, Way et Kubien 2008). L'hypothèse posée est que le déficit hydrique accrue et l'augmentation des températures pendant la nuit et le jour peuvent modifier l'équilibre de l'eau et du carbone dans la plante, ce qui conduit à (*voir Chapitre III*) :

1. Une réduction de stockage de carbone dans la tige pendant la nuit et une augmentation au cours de la journée ;
2. Une altération de l'anatomie et la densité du bois (comme une augmentation structurelle de l'investissement de carbone) ;
3. Une augmentation de la mortalité des jeunes arbres.

Après avoir décrit la xylogénèse et mis en évidence les relations entre les variables environnementales étudiées, il sera possible d'analyser l'impact de la modification artificielle des facteurs environnementaux d'intérêt pour cette étude sur la dynamique propre de la xylogénèse et les mécanismes de réponse des jeunes arbres. De nombreuses études fournissent une indication sur la formation du bois et l'influence des conditions environnementales, telles que la sécheresse (Gričar et Čufar 2006; Gruber *et al.* 2010), le réchauffement du sol (Lupi *et al.* 2011) et l'effet combiné du réchauffement et de la sécheresse chez les feuillues (Fonti *et al.* 2013). Ces études sont limitées à des variables statiques qui sont insuffisantes pour décrire les processus dynamiques qui se produisent pendant la croissance secondaire, incluant le cambium. Les hypothèses posées sont que le déficit hydrique et le réchauffement modifieront la vitesse et la durée de la xylogénèse, induisant des changements dans les caractéristiques anatomiques des trachéides. Ainsi, les réchauffements pendant le jour et la nuit combinés à une absence de l'irrigation peuvent induire un changement dans la cinétique de la xylogénèse (*voir Chapitre IV*).

Pour terminer, on intégrera dans notre étude les variations radiales de la tige mesurées par les dendromètres en suivant le cycle diurne et nocturne du tronc. Les processus d'élargissement et de rétrécissement sont principalement influencés par les conditions nocturnes de l'état hydrique et de la température. Des études ont rapporté qu'il existe une relation positive entre le taux d'expansion de la tige et la température au cours de la phase d'expansion (Downes, Beadle et Worledge 1999; Deslauriers *et al.* 2003). L'état hydrique de la plante, la disponibilité en eau dans le sol et l'évapotranspiration peuvent influencer les variations de la tige (Deslauriers *et al.* 2007; Giovannelli *et al.* 2007). Les dendromètres fournissent aussi un signal quotidien de variation de la tige à évaluer en conditions de sécheresse (Vieira *et al.* 2013). Les hypothèses posées sont que (*voir Chapitre V*) :

1. La perte de l'eau, par l'augmentation de la transpiration, augmentera pendant la journée (augmentation de la contraction) et la réhydratation diminuera au cours de la nuit (diminution de l'expansion de la tige) en fonction de l'augmentation de la température diurne et nocturne ;

2. La durée et l'amplitude des processus (contraction et extension) changeront en fonction de l'intensité du déficit hydrique et de l’augmentation de la température. Le régime thermique provoquera un changement de la dynamique d’épuisement et de reconstitution des réserves en eau dans le tronc.

1.5 STRUCTURE DE LA THÈSE

Ce mémoire de doctorat présente le travail réalisé pendant 4 années, au Laboratoire d’écologie végétale et animale de l’Université du Québec à Chicoutimi. À travers une revue bibliographique, l’introduction générale (Chapitre I) pose le contexte général et les objectifs de cette thèse et propose ensuite une description exhaustive du dispositif expérimental mis en place. Les quatre chapitres de résultats suivants (Chapitre II à V) présentent les découvertes majeures réalisées. Enfin, la conclusion générale (Chapitre VI) présente une synthèse du travail.

La thèse se présente sous le format d’une thèse par publications. L’introduction générale, la revue des connaissances sur le contexte québécois des changements climatiques et la formation du bois, la description du dispositif expérimental et la conclusion sont rédigées en français. En revanche, les quatre parties de résultats sont rédigées en anglais, car chacune constitue un article scientifique publié ou en préparation pour publication dans une revue scientifique internationale avec comité de révision par des pairs. Les références bibliographiques sont listées à la fin de chaque partie. Cette thèse a été financée d’octobre 2010 à janvier 2015 par deux subventions du

Conseil de recherches en sciences naturelles et en génie du Canada (CRSNG), le Consortium Ouranos et par une subvention de la Fondation de l’Université du Québec à Chicoutimi.

Le chapitre II porte sur les effets du déficit hydrique et de l'augmentation de la température sur l’activité du cambium, les traits anatomiques du xylème et les relations hydriques. La partie méthodologique de ce chapitre constitue une description de l’état physiologique, des analyses cellulaires et anatomiques des jeunes arbres soumis à des températures de +2 et +5 °C supérieurs à la température du contrôle (Tableau 1.1). Les jeunes arbres sont soumis à deux régimes d'irrigation pour étudier les effets d'un déficit hydrique de 32 jours (mai - juin 2010). Les moments importants (avant, pendant et après le déficit hydrique) pour la physiologie, le développement cellulaire et l’anatomie du cerne de croissance de l’année ont été mis en évidence. *Ce chapitre est le fruit du travail de la candidate. Annie Deslauriers, Alessio Giovannelli, Sergio Rossi et Cyrille B.K. Rathgeber ont participé à l'élaboration et l’écriture des idées de base de ce chapitre.*

Le chapitre III aborde la physiologie, l’anatomie et la densité du bois, mais évalue comment les ressources de carbone et la conductivité hydraulique de la tige déterminent la capacité des plants à survivre à un réchauffement durant le jour ou la nuit en combinaison avec une période de sécheresse. Les relations hydriques des plants, la dynamique des glucides non structuraux et de l'amidon, le taux de mortalité des plants, l'anatomie et la densité du bois des plants ont été comparés entre les arbres soumis aux traitements de réchauffement jour/nuit et traitements hydriques en 2011 (Tableau 1.1). Ceci a permis de mettre en évidence les effets des régimes hydriques et de la température du jour par rapport à la température de nuit. *Ce chapitre est le fruit du travail de la candidate. Annie Deslauriers, Alessio Giovannelli, Marilène Beaulieu, Sylvain Delzon, Cyrille B.K. Rathgeber et Sergio Rossi ont participé à l'élaboration et l'écriture des idées de base de ce chapitre.*

Le chapitre IV aborde la formation du cerne de croissance, en utilisant les processus dynamiques impliqués dans la formation de bois sous les effets du réchauffement et de la sécheresse. La xylogénèse, les échanges gazeux, les relations hydriques et l'anatomie du bois résultant des jeunes arbres ont été suivis pendant l'expérience de 2011 où la température est augmentée pendant le jour ou la nuit (6 °C), conjointement à une période de sécheresse (Tableau 1.1). Un mécanisme de compensation a été montré entre le taux et la durée du processus de différenciation cellulaire qui atténue les effets de la sécheresse et du réchauffement sur la structure des cernes. *Ce chapitre est le fruit du travail de la candidate. Henri E. Cuny a participé à la réalisation d'une partie des analyses statistiques. Henri E. Cuny, Cyrille B.K. Rathgeber, Annie Deslauriers, Alessio Giovannelli et Sergio Rossi ont participé à l'élaboration et l'écriture des idées de base de ces chapitres.*

Le chapitre V aborde aussi la formation du cerne de croissance selon des mesures enregistrées à partir de dendromètres électroniques (Tableau 1.1). Les données horaires journalières ont été extraites et comparées à l'aide de régressions logistiques. Les conditions environnementales influençant les processus d'hydratation et déshydratation ainsi qu'un effet de la température ont été identifiées. *Ce chapitre est le fruit du travail de la candidate. Annie Deslauriers, Alessio Giovannelli et Sergio Rossi ont participé à l'élaboration et l'écriture des idées de base de ces chapitres.*

Les articles de cette thèse sont présentés différemment du format de publication adopté par les différents journaux. Le lecteur peut obtenir les manuscrits dans leur forme publiée en se reportant aux références indiquées dans les chapitres suivants.

1.6 RÉFÉRENCES

Abe H et Nakai T. 1999. Effect of the water status within a tree on tracheid morphogenesis in *Cryptomeria japonica* D. Don. Trees, 14 : 124–129.

Abe H, Nakai T, Utsumi Y et Kagawa A. 2003. Temporal water deficit and wood formation in *Cryptomeria japonica*. Tree Physiology, 23 : 859–863.

Allen CD, Macalady AK, Chenchouni H, Bachelet D, McDowell N, Vennetier M, Kitzberger T, Rigling A, Breshears DD, Hogg EHT, Gonzalez P, Fensham R, Zhang Z, Castro J, Demidova N, Lim J-H, Allard G, Running SW, Semerci A et Cobb N. 2010a. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. Forest Ecology and Management, 259 : 660-684.

Allen CD, Macalady AK, Chenchouni H, Bachelet D, McDowell N, Vennetier M, Kitzberger T, Rigling A, Breshears DD, Hogg EHT, Gonzalez P, Fensham R, Zhang Z, Castro J, Demidova N, Lim J-H, Allard G, Running SW, Semerci A et Cobb N. 2010b. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. Forest Ecology and Management, 259 : 660-684.

Arend M et Fromm J. 2007. Seasonal change in the drought response of wood cell development in poplar. Tree Physiology, 27 : 985–992.

Bernier PY. 1993. Comparing natural and planted black spruce seedlings. I. Water relations and growth. Canadian Journal of Forest Research, 23 : 2427-2434.

Bigras F.J. & Dumais D. (2005) Root-freezing damage in the containerized nursery: impact on plantation sites – A review. *New Forests*, **30**, 167–184.

Boisvenue C et Running SW. 2006. Impacts of climate change on natural forest productivity - Evidence since the middle of the 20th century. Global Change Biology, 12 : 862-882.

Bonan GB. 2008. Forests and climate change : Forcings, feedbacks, and the climate benefits of forests. Science, 320 : 1444-1449.

Bonsal BR, Zhang X, Vincent LA et Hogg WD. 2001. Characteristics of daily and extreme temperatures over Canada. Journal of Climate, 14 : 1959-1976.

Boulouf Lugo J, Deslauriers A et Rossi S. 2012. Duration of xylogenesis in black spruce lengthened between 1950 and 2010. Annals of Botany, 110 : 1099-1108.

Brodribb TJ et Cochard H. 2009. Hydraulic failure defines the recovery and point of death in water-stressed conifers. Plant Physiology, 149 : 575.

Burdett AN, Herring LJ et Thompson CF. 1984. Early growth of planted spruce. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere, 14 : 644-651.

Chaves MM et Oliveira MM. 2004. Mechanisms underlying plant resilience to water deficits: prospects for water-saving agriculture. Journal of Experimental Botany, 55: 2365-2384.

Chaves MM, Flexas J et Pinheiro C. 2009. Photosynthesis under drought and salt stress: Regulation mechanisms from whole plant to cell. Annals of Botany, 103: 551-560.

Chaves MM, Pereira JS, Rodrigues J, Ricardo ML, Osório CPP, Carvalho ML, Faria I et Pinheiro C. 2002. How plants cope with water stress in the field. Photosynthesis and growth. Annals of Botany, 89 : 907-916.

Christensen JH, Krishna Kumar K, Aldrian E, An S-I, Cavalcanti IFA, de Castro M, Dong W, Goswami P, Hall A, Kanyanga JK, Kitoh A, Kossin J, Lau N-C, Renwick J, Stephenson DB, Xie S-P et Zhou T. 2013. Climate Phenomena and their Relevance for Future Regional Climate Change. Dans : Stocker TF, *et al.* éds. Climate Change 2013: The Physical Science Basis Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, p. 1217–1308.

Corcuera L, Camarero JJ et Gil-Pelegrín E. 2004. Effects of a severe drought on Quercus ilex radial growth and xylem anatomy. Trees - Structure and Function, 18 : 83-92.

Deslauriers A et Morin H. 2005. Intra-annual tracheid production in balsam fir stems and the effect of meteorological variables. Trees - Structure and Function, 19 : 402-408.

Deslauriers A, Rossi S et Anfodillo T. 2007. Dendrometer and intra-annual tree growth: What kind of information can be inferred? Dendrochronologia, 25 : 113-124.

Deslauriers A, Morin H, Urbinati C et Carrer M. 2003. Daily weather response of balsam fir (*Abies balsamea* (L.) Mill.) stem radius increment from dendrometer analysis in the boreal forests of Quebec (Canada). Trees - Structure and Function, 17 : 477–484.

Deslauriers A, Anfodillo T, Rossi S et Carraro V. 2007. Using simple causal modeling to understand how water and temperature affect daily stem radial variation in trees. Tree Physiology, 27 : 1125-1136.

Deslauriers A, Rossi S, Anfodillo T et Saracino A. 2008. Cambial phenology, wood formation and temperature thresholds in two contrasting years at high altitude in southern Italy. Tree Physiology, 28 : 863-871.

Domec J.-C. & Johnson D.M. (2012) Does homeostasis or disturbance of homeostasis in minimum leaf water potential explain the isohydric versus anisohydric behavior of Vitis vinifera L. cultivars? *Tree Physiology*, **32**, 1-4.

Downes G, Beadle C et Worledge D. 1999. Daily stem growth patterns in irrigated *Eucalyptus globulus* and *E. nitens* in relation to climate. Trees - Structure and Function, 14 : 102-111.

Dufour B et Morin H. 2006. Focusing modelling on the tracheid development period – An alternative method for treatment of xylogenesis intra-annual data. Dendrochronologia, 25 : 125-133.

Eilmann B, Weber P, Rigling A et Eckstein D. 2006. Growth reactions of *Pinus sylvestris* L. and *Quercus pubescens* Willd. to drought years at a xeric site in Valais, Switzerland. Dendrochronologia, 23 : 121-132.

Eilmann B, Zweifel R, Buchmann N, Graf Pannatier E et Rigling A. 2011. Drought alters timing, quantity, and quality of wood formation in Scots pine. Journal of Experimental Botany, 62 : 2763-2771.

Fichot R, Laurans F, Monclus R, Moreau A, Pilate G et Brignolas F. 2009. Xylem anatomy correlates with gas exchange, water-use efficiency and growth performance under contrasting water regimes: evidence from *Populus deltoides* × *Populus nigra* hybrids. Tree Physiology, 29 : 1537-1549.

Flexas J, Bota J, Loreto F, Cornic G et Sharkey TD. 2004. Diffusive and metabolic limtations to photosynthesis under drought and salinity in C3 plants. Plant Biology, 6 : 269-279.

Fonti P, Heller O, Cherubini P, Rigling A et Arend M. 2013. Wood anatomical responses of oak saplings exposed to air warming and soil drought. Plant Biology (Stuttg), 15 Suppl 1 : 210-219.

Gagnon R et Morin H. 2001. Les forêts d'épinettes noires du Québec: dynamique, perturbations et biodiversité. Le Naturaliste Canadien, 125 : 26-35.

Giovannelli A, Emiliani G, Traversi ML, Deslauriers A et Rossi S. 2011. Sampling cambial region and mature xylem for non structural carbohydrates and starch analyses. Dendrochronologia, 29 : 177-182.

Giovannelli A, Deslauriers A, Fragnelli G, Scaletti L, Castro G, Rossi S et Crivellaro A. 2007. Evaluation of drought response of two poplar clones (*Populus*×*canadensis* Mönch 'I-214' and *P. deltoides* Marsh. 'Dvina') through high resolution analysis of stem growth. Journal of Experimental Botany, 58 : 2673-2683.

Gouvernement du Québec. 2012. Plan d'action sur les changements climatiques 2013-2020, "le Québec en action Vert 2020". Gouvernement du Québec, MDDEP, Bibliothèque et Archives nationales du Québec.

Gričar J et Čufar K. 2006. Seasonal dynamics of phloem and xylem formation in silver fir and Norway spruce as affected by drought. Russian Journal of Plant Physiology, 55 : 538-543.

Gričar J, Zupančič M, Čufar K et Oven P. 2007. Regular cambial activity and xylem and phloem formation in locally heated and cooled stem portions of Norway spruce. Wood Science and Technology, 41 : 463-475.

Gruber A, Strobl S, Veit B et Oberhuber W. 2010. Impact of drought on the temporal dynamics of wood formation in *Pinus sylvestris*. Tree Physiology, 30 : 490-501.

Gunderson CA, J. NR et Wullschleger SD. 2000. Acclimation of photosynthesis and respiration to simulated climatic warming in northern and southern populations of *Acer saccharum*: laboratory and field evidence. Tree Physiology, 20 : 87-96.

Houle D, Moore J-D et Provencher J. 2007. Ice bridges on the St. Lawrence River as an index of winter severity from 1620 to 1910. Journal of Climate, 20 : 757-764.

IPCC. 2013. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Dans: Cambridge University Press, Cambridge, UK and New York, NY, USA, p. 1535.

Jyske T, Kaakinen S, Nilsson U, Saranpaa P et Vapaavuori E. 2010. Effects of timing and intensity of thinning on wood structure and chemistry in Norway spruce. Holzforschung, 64 : 81-91.

Kocacinar F et Sage RF. 2004. Photosynthetic Pathway Alters Hydraulic Structure and Function in Woody Plants. Oecologia, 139: 214-223.

Kozlowski TT et Pallardy SG. 1997. Growth control in woody plants. Academic Press, San Diego, Calif./Toronto, xiii, 641 pp.

Kramer PJ. 1964. The role of water in wood formation. Dans :, In: The formation of wood in forest trees (Zimmermann MH ed) Academic Press, New York, pp. 519-532.

Kutscha NP, Hyland F et Schwarzmann JM. 1975. Certain seasonal changes in balsam fir cambium and its derivatives. Wood Science and Technology, 9: 175-188.

Lamhamedi MS, Margolis H, Renaud M, Veilleux L et Auger I. 2003. Effets de differentes regies d'irrigation sur la croissance, la nutrition minerale et le lessivage des elements nutritifs des semis d'epinette noire (1+0) produits en recipients à parois ajourees en pepiniere forestiere. Canadian Journal of Forest Research, 33: 279-291.

Love J, Bjorklund S, Vahala J, Hertzberg M, Kangasjarvi J et Sundberg B. 2009. Ethylene is an endogenous stimulator of cell division in the cambial meristem of *Populus*. *Proceedings of the National Academy of Sciences of the United States of America*, 106: 5984–5989.

Lupi C, Morin H, Deslauriers A et Rossi S. 2011. Xylogenesis in black spruce : does soil temperature matter? Tree Physiology, 32 : 74–82.

Mäkinen H, Nöjd P et Saranpää P. 2003. Seasonal changes in stem radius and production of new tracheids in Norway spruce. Tree Physiology, 23 : 959-968.

McDowell N, Pockman WT, Allen CD, Breshears DD, Cobb N, Kolb T, Plaut J, Sperry J, West A, Williams DG et Yepez EA. 2008. Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? New Phytologist, 178 : 719–739.

McDowell NG. 2011. Mechanisms linking drought, hydraulics, carbon metabolism, and vegetation mortality. Plant Physiology, 155 : 1051.

Mencuccini M. 2003. The ecological significance of long-distance water transport: short-term regulation, long-term acclimation and the hydraulic costs of stature across plant life forms. Plant, Cell and Environment, 26 : 163–182.

Mencuccini M. & Bonosi L. (2001) Leaf/sapwood area ratios in Scots pine show acclimation across Europe. *Canadian Journal of Forest Research*, **31**, 442-456.

Ministère des Ressources naturelles et de la Faune. 2009. Ressources et industries forestières portrait statistique. http://www.mrn.gouv.qc.ca/publications/forets/connaissances/stat_edition_complete/complete.pdf [consulté le 6 juin 2009].

Nicholls, J.W.P., 1984. Tracheid cell dimensions and density relationships for normal and abnormal wood of *Pinus radiata* (Don) D. Australian Forest Research 14, 85-98.

Ouranos (2014) Vers l’adaptation. Synthèse des connaissances sur les changements climatiques au Québec. Partie 1 : Évolution climatique au Québec. Édition 2014. Montréal, Québec : Ouranos, 79 p.

Pantin F, Simonneau T et Muller B. 2012. Coming of leaf age: Control of growth by hydraulics and metabolics during leaf ontogeny. New Phytologist, 196 : 349-366.

Park WA, Allen CD, Macalady AK, Griffin D, Woodhouse CA, Meko DM, Swetnam T, Rauscher SA, Seager R, Grissino-Mayer HD, Dean JS, Cook ER, Gangodagamage C, Cai M et McDowell N. 2013. Temperature as a potent driver of regional forest drought stress and tree mortality. Nature Climate Change, 3 : 292-297.

Peng C, Ma Z, Lei X, Zhu Q, Chen H, Wang W, Liu S, Li W, Fang X et Zhou X. 2011. A drought-induced pervasive increase in tree mortality across Canada’s boreal forests. Nature, 1 : 467–471.

Plomion C, Leprovost G et Stokes A. 2001. Wood Formation in Trees. Plant Physiology, 127 : 1513–1523.

Rainville A, Beaulieu J, Langevin L, Logan T et Lambert M-C. 2014. Prédire l'effet des changements climatiques sur le volume marchand des principales espèces résineuses plantées au Québec, gràce à la génétique forestière. In: *Mémoire de recherche forestière n 174* (ed. M.d.F. Gouvernement du Québec, de la Faune et des Parcs, Direction de la recherche forestière), pp. 58.

Rathgeber CBK, Decoux V et Leban J-M. 2006. Linking intra-tree-ring wood density variations and tracheid anatomical characteristics in Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco). Annals of Forest Science, 63 : 699–706.

Rathgeber CBK, Longuetaud F, Mothe F, Cuny H et Le Moguédec G. 2011. Phenology of wood formation: Data processing, analysis and visualisation using R (package CAVIAR). Dendrochronologia, 29 : 139–149.

Rossi S, Deslauriers A et Anfodillo T. 2006. Assessment of cambial activity and xylogenesis by microsamplings tree species: an example at the alpine timberline. IAWA Journal, 27 : 383–394.

Rossi S, Deslauriers A, Anfodillo T et Carraro V. 2006. Evidence of threshold temperatures for xylogenesis in conifers at high altitudes. Oecologia, 152 : 1-12.

Rossi S, Deslauriers A, Anfodillo T et Carrer M. 2008a. Age-dependent xylogenesis in timberline conifers. New Phytologist, 177 : 199–208.

Rossi S, Deslauriers A, Gričar J, Seo Jw, Rathgeber CB, Anfodillo T, Morin H, Levanic T, Oven P et Jalkanen R. 2008b. Critical temperatures for xylogenesis in conifers of cold climates. Global Ecology and Biogeography, 17 : 696-707.

Rossi S, Hubert M, Deslauriers A et Plourde P-Y. 2011. Predicting xylem phenology in black spruce under climate warming. Global Change Biology, 17 : 614–625.

Rossi S, Simard S, Rathgeber CBK, Deslauriers A et De Zan C. 2009. Effects of a 20-day-long dry period on cambial and apical meristem growth in *Abies balsamea* seedlings. Trees - Structure and Function, 23 : 85-93.

Rossi S, Deslauriers A, Gričar J, Seo Jw, Rathgeber CB, Anfodillo T, Morin H, Levanic T, Oven P et Jalkanen R. 2008b. Critical temperatures for xylogenesis in conifers of cold climates. Global Ecology and Biogeography, 17 : 696-707.

Rossi S, Anfodillo T, Čufar K, Cuny HE, Deslauriers A, Fonti P, Frank D, Gričar J, Gruber A, King GM, Krause C, Morin H, Oberhuber W, Prislan P et Rathgeber CBK. 2013. A meta-analysis of cambium phenology and growth: linear and non-linear patterns in conifers of the northern hemisphere. Annals of Botany, 112 : 1911-1920.

Sage RF. 2002. How terrestrial organisms sense, signal, and respond to carbon dioxide. Integrative and Comparative Biology, 42 : 469–480.

Sage RF, Way DA et Kubien DS. 2008. Rubisco, Rubisco activase, and global climate change. Journal of Experimental Botany, 59 : 1581–1595.

Seager R, Ting M, Held I, Kushnir Y, Lu J, Vecchi G, Huang HP, Harnik N, Leetmaa A, Lau NC, Li C, Velez J et Naik N. 2007. Model projections of an imminent transition to a more arid climate in southwestern North America. Science, 316: 1181-1184.

Seo J-W, Eckstein D, Jalkanen R, Rickebusch S et Schmitt U. 2008. Estimating the onset of cambial activity in Scots pine in northern Finland by means of the heat-sum approach. Tree Physiology, 28: 105-112.

Sperry JS, Hacke UG, Oren R et Comstock JP. 2002. Water deficits and hydraulic limits to leaf water supply. Plant, Cell and Environment, 25 : 251–263.

Steppe K, Sterck F et Deslauriers A. 2015. Diel growth dynamics in tree stems: linking anatomy and ecophysiology. Trends Plant Sci.

Steppe K, De Pauw DJW, Lemeur R et Vanrolleghem PA. 2006. A mathematical model linking tree sap flow dynamics to daily stem diameter fluctuations and radial stem growth. Tree Physiology, 26 : 257–273.

Sterl A, Severijns C, Dijkstra H, Hazeleger W, van Oldenborgh GJ, van den Broeke M, Burgers G, van den Hurk B, van Leeuwen PJ et van Velthoven P. 2008. When can we expect extremely high surface temperatures? Geophysical Research Letters, 35.

Swidrak I, Gruber A, Kofler W et Oberhuber W. 2011. Effects of environmental conditions on onset of xylem growth in *Pinus sylvestris* under drought. Tree Physiology, 31 : 483-493.

Thibeault-Martel M, Krause C, Morin H et Rossi S. 2008. Cambial activity and intra-annual xylem formation in roots and stems of *Abies balsamea* and *Picea mariana*. Annals of Botany, 102 : 667–674.

Turcotte A, Rossi S, Deslauriers A, Krause C et Morin H. 2011. Dynamics of depletion and replenishment of water storage in stem and roots of black spruce measured by dendrometers. Frontiers in Functional Plant Ecology, 2 : 21.

Turnbull MH, Murthy R et Griffin KL. 2002. The relative impacts of daytime and night-time warming on photosynthetic capacity in *Populus deltoides*. Plant, Cell and Environment, 25 : 1729-1737.

Turnbull MH, Tissue DT, Murthy R, Wang X, Sparrow AD et Griffin KL. 2004. Nocturnal warming increases photosynthesis at elevated CO_2 partial pressure in *Populus deltoides*. New Phytologist, 161 : 819-826.

Tyree MT. 1997. The Cohesion–Tension theory of sap ascent: current controversies. Journal of Experimental Botany, 48 : 1753–1765.

Uggla C, Magel E, Moritz T et Sundberg B. 2001. Function and Dynamics of Auxin and Carbohydrates during Earlywood/Latewood Transition in Scots Pine. Plant Physiology, 125 : 2029–2039.

van Mantgem PJ, Stephenson NL, Byrne JC, Daniels LD, Franklin JF, Fulé PZ, Harmon ME, Larson AJ, Smith JM, Taylor AH et Veblen TT. 2009. Widespread Increase of Tree Mortality Rates in the Western United States. Science, 323 : 521-524.

Vieira J, Rossi S, Campelo F, Freitas H et Nabais C. 2013. Seasonal and daily cycles of stem radial variation of *Pinus pinaster* in a drought-prone environment. Agricultural and Forest Meteorology, 180 : 173– 181.

Villarini G, Smith JA et Vecchi GA. 2013. Changing frequency of heavy rainfall over the central United States. Journal of Climate, 26 : 351-357.

Vincent LA et Mekis É. 2006. Changes in daily and extreme temperature and precipitation indices for Canada over the twentieth century. Atmosphere - Ocean, 44 : 177-193.

Way DA et Sage RF. 2008. Thermal acclimation of photosynthesis in black spruce [*Picea mariana* (Mill.) B.S.P.]. Plant, Cell and Environment, 31 : 1250-1262.

Zimmermann U, Meinzer FC, Benkert R, Zhu JJ, Schneider H, G. G, Kuchenbrod E et Haase A. 1994. Xylem water transport: is the available evidence consistent with the cohesion theory? Plant, Cell and Environment, 17 : 1169-1118.

Zine El Abidine A, Bernier PY, Stewart JD et Plamondon AP. 1994. Water stress preconditioning of black spruce seedlings from lowland and upland sites. Canadian Journal of Botany, 72 : 1511-1518.

Zweifel R et Hasler R. 2001. Dynamics of water storage in mature subalpine Picea abies: temporal and spatial patterns of change in stem radius. Tree Physiology, 21 : 561-569.

Zweifel R, Zimmermann L, Zeugin F et Newbery DM. 2006. Intra-annual radial growth and water relations of trees: implications towards a growth mechanism. Journal of Experimental Botany, 57 : 1445–1459.

CHAPITRE II

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

RESEARCH PAPER

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

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Balducci L., Deslauriers A., Giovannelli A., Rossi S. & Rathgeber C.B.K. (2013) Effects of temperature and water deficit on cambial activity and woody ring features in *Picea mariana* saplings. *Tree Physiology,* **33**, 1006–1017.

2.1 ABSTRACT

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

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

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

2.2 INTRODUCTION

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

In boreal ecosystems, temperature is the most important factor for tree growth (Körner 2003*a*, 2003*b*). Cambial activity and cell differentiation are determined by temperature (Oribe et al. 2001, Begum et al. 2007, Rossi et al. 2007, 2008*b*). Recent studies have estimated temperature thresholds regulating different phases of xylem phenology in mature black spruce [*Picea mariana* (Mill.) B.S.P.], linking the passage between thermally favorable and unfavorable periods (Rossi et al. 2011). Other research confirmed the influence of cambial age or tree size on radial growth (Rossi et al. 2008*a*, Rathgeber et al. 2011). It is also documented in different species that the climatic sensitivity of radial growth changes with tree age (Rozas et al. 2009, Vieira et al. 2009). However, information is lacking about climatic influence on cambial activity in young trees of the boreal forest. Water availability is another important influencing factor linked to cambial activity and wood formation (Giovannelli et al.2007, Camarero et al. 2010). In the stem, cambium cell division and expansion of newly formed tracheids are processes highly sensitive to the plant’s water status (Abe and Nakai 1999, Savidge 2000, Rossi et al. 2009). Past research in 1-year-old seedlings of black spruce evidenced that drought tolerance was mostly through an acclimation of the stomatal conductance and photosynthetic rate (Zine el Abidine et al. 1994), which are strictly linked to an increase in temperature (Sage et al. 2008). Several studies exist on the relation between water conditions and xylem growth (Larson 1963, Shepherd 1964). Saplings can be vulnerable to drought due to the decrease in their ability to uptake soil resources, as observed in past research that evidenced the water-deficit effect on root growth in young plantations of black spruce (Burdett et al. 1984, Bernier 1993). Nevertheless, an evaluation of the combined effects of warming and water deficit on cambial activity in conifer saplings has recently received great interest (de Luis et al. 2011), even if a clear picture is far from being reached, especially in the boreal environment.

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

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

The aim of this paper was to evaluate the effects of three thermal conditions and irrigation regimes on (i) plant water status, gas exchange and CO_2 assimilation, (ii) cambial activity and (iii) wood anatomy in black spruce saplings growing in greenhouses. Three thermal conditions were chosen according to the possible future scenarios drawn by recent climate models (Zhang et al. 2000, Rossi et al. 2011). We tested the following hypotheses: (i) the imposition of severe water deficit could induce a reduction in xylem growth as a response to change in leaf water potential, gas exchange and CO_2 assimilation; (ii) the cambium could display different sensitivity in terms of decrease in cell division and differentiation rate in response to gradual water-deficit severity according to temperature; and (iii) water deficit under warm conditions could induce the formation of thinner cell walls or smaller cells.

2.3 MATERIAL AND METHODS

2.3.1 Experimental design

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

2.3.2 Sapling mortality

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

2.3.3 Water relations, gas exchange and CO_2 assimilation

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

2.3.4 Xylem development

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

2.3.5 Wood anatomy and density

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

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

2.3.6 Statistical analysis

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

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

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

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

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

2.4 RESULTS

2.4.1 Growth conditions and saplings mortality

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

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

2.4.2 Water relations, gas exchange and CO_2 assimilation

After the withholding of irrigation on DOY 142, it was possible to define two stages of water-deficit intensity on the bases of water potential, gas exchange and CO_2 assimilation. From DOY 142 to DOY 158 (mild water deficit), all saplings showed optimal water conditions. Both irrigated and non-irrigated saplings exhibited Ψ_{pd} ranging between −0.4 and −0.7 MPa and Ψ_{md} ranging from −0.5 to −1.4 MPa (Figure 2.2). No marked difference in leaf water potential was observed among the thermal regimes. In these conditions, Amax ranged between 2 and 7 μmol CO_2 m^{-2} s^{-1}, although higher average values of 9 and 14 μmol CO_2 m^{-2} s^{-1} were measured in T+5 and T+2 on DOY 158, respectively (Figure 2.2). From DOY 159 to 173 (severe water deficit), the Ψ_{pd} and Ψ_{md} values dropped dramatically in non-irrigated saplings, reaching higher values on DOY 173 (−2.7 MPa Ψ_{pd} and −2.9 MPa Ψ_{md}) without evident differences between thermal regimes. Accordingly, in non-irrigated saplings, A_{max} ranged between 0.1 and 0.4 μmol CO_2 m^{-2} s^{-1} and g_s was <0.1 mol m^{-2} s^{-1} in T0, T+2 and T+5 (DOY 161) (Figure 2.2). On DOY 179, 1 week after re-watering, all values of non-irrigated saplings were similar to those observed in irrigated ones, showing that the saplings were able to recover an optimal water status. These conditions persisted for the rest of the summer (Figure 2.2).

2.4.3 Xylem development

Cell division was active from the first sampling date, as shown by the six to nine cells observed in the cambial zone on DOY 124 (Figure 2.3). The number of cells in the cambium decreased until the beginning of June, and then increased with a peak at the beginning of July, on DOY 187. This peak was observed only in irrigated saplings (Figure 2.3). A progressive reduction of cambial activity was observed in the irrigated and non- irrigated saplings at the end of August (DOY 237). The number of cells in the cambial zone decreased synchronously in irrigated and non-irrigated saplings and attained the corresponding number of three to four cells at the beginning of September. During water deficit, the patterns of the number of cambial cells in division were similar among the three thermal conditions, while differences were observed between irrigation regimes ($P < 0.05$), especially after the rehydration (DOY 174) for 2 weeks in T0, and for ~4 weeks in T+2 and +5. At the end of August, the number of cells in the cambial zone decreased to four, which indicated the end of cell production (Figure 2.3).

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

The Gompertz function adequately fitted the data through- out the growing period, and was always able to reach an asymptote by the end. Statistical tests detected significant differences in the total number of cells among groups of treatments (*F*=7.30, *P*<0.0001) (Table 2.2). The highest total numbers of cells were observed in irrigated saplings, with values of 123.4, 109.9 and 109.7 tracheids in T0, T+2 and T+5, respectively (Table 2.2, Figure 2.3). Fewer were produced in non-irrigated saplings of T+2 and T+5, showing an average of 90.7 and 87.7 cells along the tree rings, respectively. Another comparison of the total number of cells between the irrigation regimes revealed a significant difference in the saplings (*F* = 32.73, *P* < 0.0001). However, no significant difference was detected in the comparison among total cells between the thermal conditions (*F* = 0.82, *P* = 0.59) (Table 2.2).

2.4.4 Wood anatomy and density

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

From the initial tracheids in earlywood to the last ones in latewood, there was a decrease in the cell length between irrigation regimes in all thermal conditions. However, no significant difference was found (Figure 2.4). Only in the 40–60% portions of the tree ring, the cell length of earlywood was lower on average in non-irrigated saplings

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

2.5 DISCUSSION

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

2.5.1 Saplings mortality

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

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

2.5.2 Needle water relations under water deficit and warming

In our experiment, water deficit greatly influenced the water relations of saplings. During the first phase of the water deficit (from DOY 142 to 158, mild water stress), the Ψ_{pd} value did not drop below −0.7 MPa, and the non-irrigated saplings were able to maintain gas exchange and the photosynthetic rate similar to those of the irrigated ones under all thermal conditions. Stewart et al. (1994) showed that photosynthetic capacity of black spruce seedlings under drought conditions responded positively at Ψ_{pd}= −1.5 MPa. Tan et al. (1992) and Tan and Blake (1997) showed that in drought-stressed saplings (Ψ_{pd} ranging from −1.0 to −1.4 MPa) faster-growing black spruce progeny tolerated, and instead postponed, momentary dehydration. From DOY 159 to 173, when severe water deficit was reached, the Ψ_{pd} value of non-irrigated saplings reached −2.2 MPa. At maximum water deficit (DOY 173), g_s and A_{max} were strongly reduced (<80% with respect to the non-irrigated saplings). These results suggested that the efficiency of water translocation was greatly compromised, probably as a cumulative effect of hydraulic failure through cavitations. Under severe water deficit, the non-irrigated saplings were unable to maintain CO_2 assimilation and stomatal conductance. Our results confirmed that stomatal conductance was strongly reduced at leaf Ψ_{pd} of −1.0 MPa, as observed by Bernier (1993) and Stewart et al. (1994). Also, Stewart et al. (1994) showed that stomatal limitation increased to ~40% during a period of intensive water stress (Ψ_{pd} −1.5 MPa), although photosynthesis capacity remained unaffected by drought treatment. In our case, photosynthesis followed the decrease of stomatal conductance, coupling at Ψ_{pd} −2.2 MPa.

A week after the resumption of irrigation, non-irrigated saplings had completely recovered their plant water balance. As observed by Stewart et al. (1994), after re-watering, stomatal conductance and photosynthesis rate recovered rapidly even after three cycles of drought. After severe water deficit, the recovery follows two processes: a first stage of leaf re-watering and stomata re-opening (Kirschbaum 1987, 1988), and a second stage, after 10 days of re-watering with the partial recovery (40–60%) of maximum photosynthesis (Bogeat-Triboulot et al. 2007). The different thermal conditions did not influence the recovery of the water status of the needle, while the reduction in root allocation induced by the high temperature could expose black spruce in extreme soil drying events (Way and Sage 2008).

2.5.3 Rate of xylem growth: a matter of water?

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

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

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

Our results showed that xylem anatomy was not affected by water deficit at all thermal conditions, while wood density presented only sporadic changes. The third hypothesis was therefore rejected. The lumen area of earlywood tracheids of non-irrigated saplings was not affected by water deficit at all thermal conditions, even if the withholding of irrigation sporadically affected intra-annual variation in density, as a resilience effect of cells to water deficit. In contrast, the cell size of balsam fir saplings was shown to be quickly affected by a dry period (Rossi et al. 2009), so this could demonstrate the resistance of black spruce saplings. The absence of any effect of irrigation regime on cell length at all thermal conditions could be due to the recovery from water deficit of cell expansion. Cell-wall thickening was also not influenced by the irrigation regime at all thermal conditions; the presence of trends only in the 40–65% portion of the tree ring at the higher temperature may indicate a possible influence on the apposition of secondary wall and lignification in response to environmental conditions (De Micco et al. 2007). This could reflect an indirect adjustment of wood anatomy (Fonti et al. 2010) and would confirm the high plasticity of black spruce saplings.

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

2.6 CONCLUSION

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

2.7 ACKNOWLEDGMENTS

This study was funded by the Natural Sciences and Engineering Research Council of Canada and the Consortium Ouranos (Consortium on Regional Climatology and Adaptation to Climate Change). We thank H. Morin, J. Allaire, D. Gagnon, M. Thibeault-Martel, S. Pennault, G. Savard, F. Gionest, C. Soucy, P. Lapointe, V. Tremblay, L. Caron, L. St-Gelais, C. Lupi for their practical help and laboratory analyses. Additional thanks for Maria Laura Traversi (IVALSA-CNR) for the water relations, gas exchange and CO_2 assimilation, P. Gelhaye (INRA-Nancy Champenoux) for wood density analyses and A. Garside for checking the English text. The authors are grateful to the anonymous reviewers for their constructive comments.

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

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

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

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

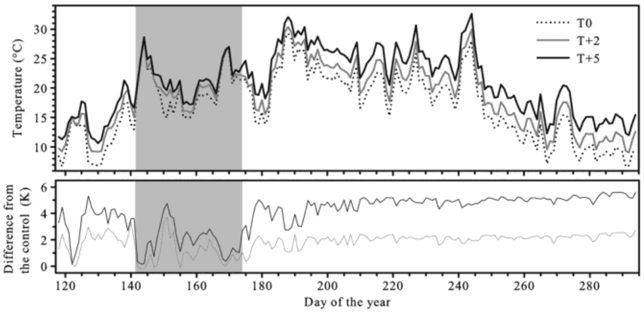

Figure 2.1 Daily temperatures experienced by black spruce saplings of the three thermal conditions before, during and after the water-deficit period (grey background) during the experiment in the greenhouse.

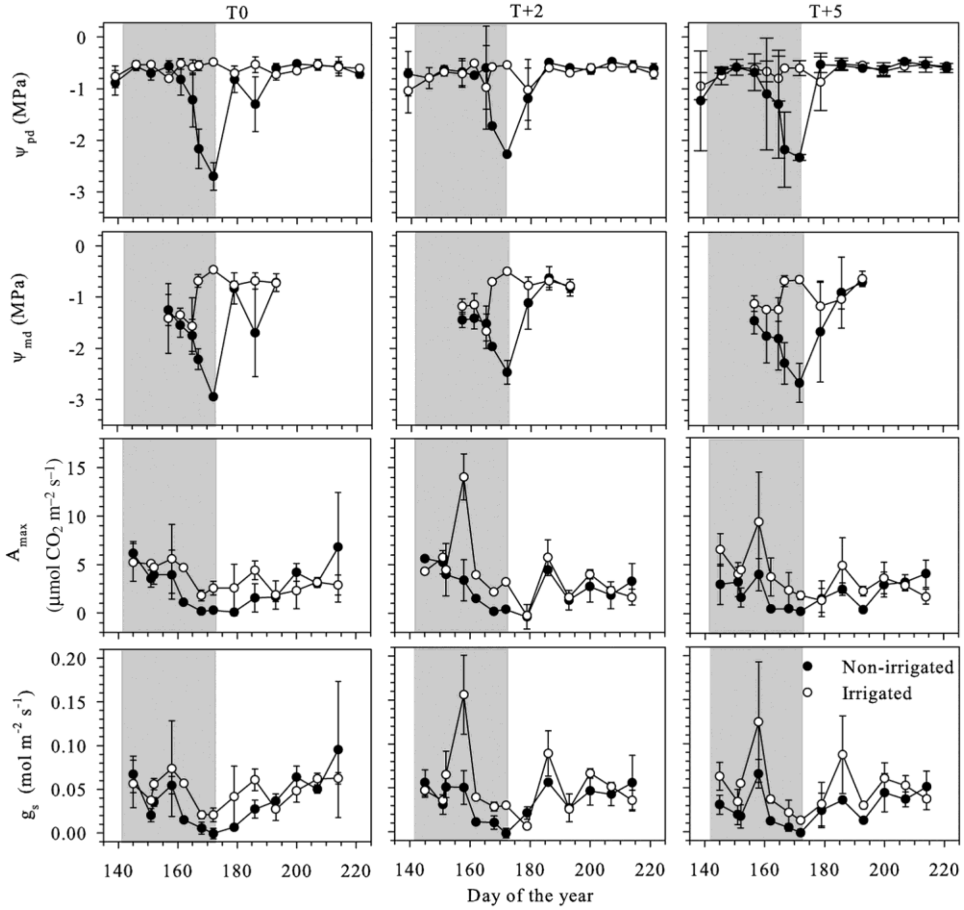

Figure 2.2 Water relations, gas exchange and CO_2 assimilation of black spruce saplings before, during and after the water-deficit period (grey background) at three thermal conditions.

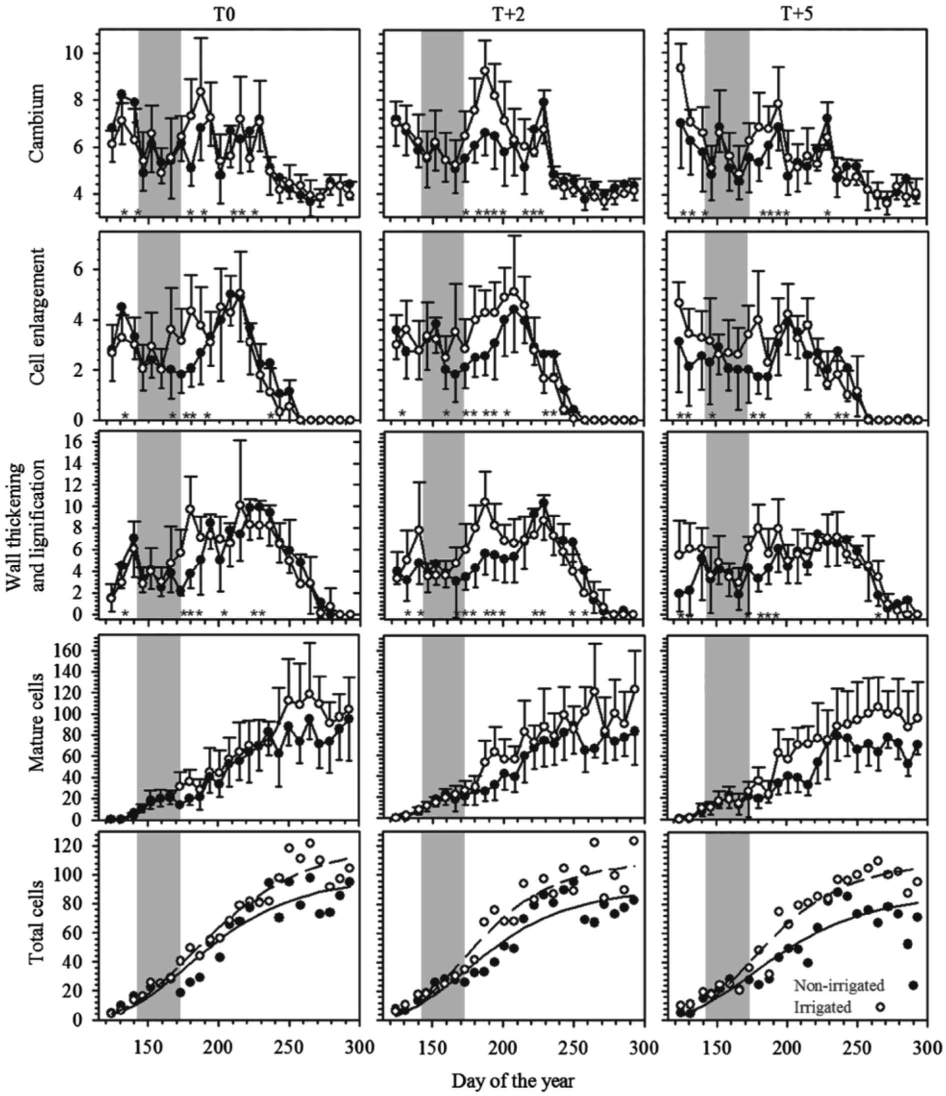

Figure 2.3 Radial number of cambial, enlarging, wall thickening and lignification, and mature cells counted in black spruce saplings before, during and after the water-deficit period (grey background) at three thermal conditions. Vertical bars correspond to the standard deviation. Black and grey curves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

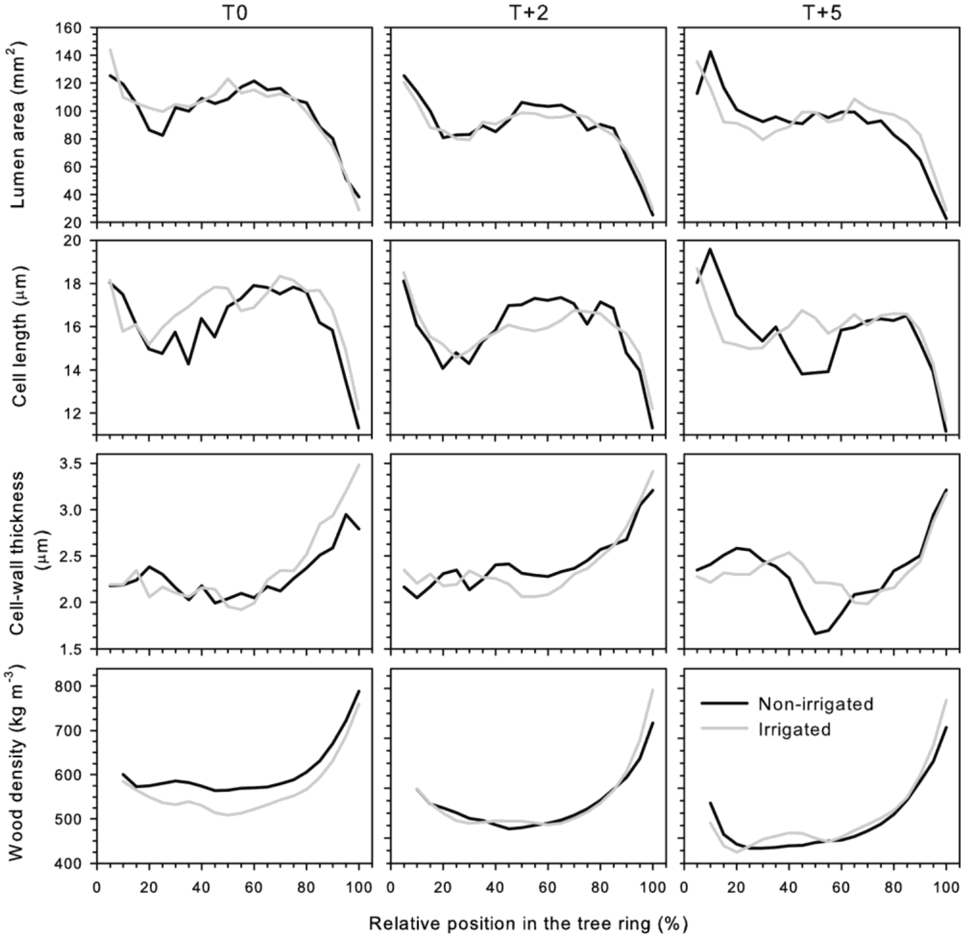

Figure 2.4 Cell features and wood density of the tracheids produced by irrigated and non-irrigated black spruce saplings at three thermal conditions (T0, T+2, T+5) along portions of an annual tree ring. Black and grey curves indicate two irrigation regimes.

2.8 REFERENCES

Abe H, Nakai T (1999) Effect of the water status within a tree on tracheids morphogenesis in *Cryptomeria japonica* D. Don. Trees 14:124–129.

Abe H, Nakai T, Utsumi Y, Kagawa A (2003) Temporal water deficit and wood formation in *Cryptomeria japonica*. Tree Physiology 23:859–863.

Anderegg WRL, Berry Joseph A, Field CB (2012) Linking definitions, mechanisms, and modeling of drought-induced tree death. Trends Plant Science 17:693–700.

Antonova GF, Shebeko VV (1981) Applying cresyl violet in studying wood formation. Khim Drev 4:102–105.

Arend M, Fromm J (2007) Seasonal change in the drought response of wood cell development in poplar. Tree Physiology 27:985–992.

Begum S, Nakaba S, Oribe Y, Kubo T, Funada R (2007) Induction of cambial reactivation by localized heating in a deciduous hardwood hybrid poplar (*Populus sieboldii* × *P. grandidentata*). Annals of Botany 100:439–447.

Belien E, Rossi S, Morin H, Deslauriers A (2012) Xylogenesis in black spruce subjected to rain exclusion in the field. Canadian Journal Forest Research 42:1306–1315.

Bergeron O, Lamhamedi MS (2004) Irrigation control and physiological responses of nursery-grown black spruce seedlings (1 + 0) cultivated in air-slit containers. HortScience, 39:599–605.

Bernier PY (1993) Comparing natural and planted black spruce seedlings. I. Water relations and growth. Canadian Journal Forest Research 23:2427–2434.

Bernier PY, Raulier F, Stenberg P, Ung C (2001) Importance of needle age and shoot structure on canopy net photosynthesis of balsam fir (*Abies balsamea*): a spatially inexplicit modeling analysis. Tree Physiology 81:815–830.

Bogeat-Triboulot MB, Brosche M, Renaut et al. (2007) Gradual soil water depletion results in reversible changes of gene expression, protein profiles, ecophysiology, and growth performance in *Populus euphratica*, a poplar growing in arid regions. Plant Physiology 143:876–892.

Bouriaud O, Leban J-M, Bert D, Deleuze C (2005) Intra-annual variations in climate influence growth and wood density of Norway spruce. Tree Physiology 25:651–660.

Brodribb TJ, Cochard H (2009) Hydraulic failure defines the recovery and point of death in water-stressed conifer. Plant Physiology 149:575–584.

Burdett AN, Herring L J, Thompson CF (1984) Early growth of planted spruce. Canadian Journal Forest Research 14:644–651.

Camarero JJ, Olano JM, Parras A (2010) Plastic bimodal xylogenesis in conifers from continental Mediterranean climates. New Phytologist, 1 85:471–480.

Chave J, Muller-Landau HC, Baker TR, Easdale TA, Steege H, Campbell OW (2006) Regional phylogenetic variation of wood density across 2456 neotropical tree species. Ecological Application 16:2356–2367.

Chave J, Lewis SL, Swenson NG, Zanne AE (2009) Towards a world-wide wood economics spectrum. Ecology Letter 12:351–366.

Chaves MM, Flexas J, Pinheiro C (2009) Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Annals of Botany 103:551–560.

Corcuera L, Camarero JJ, Gil-Pelegrín E (2004) Effects of a severe drought on *Quercus ilex* radial growth and xylem anatomy. Trees 18:83–89.

de Luis M, Novak K, Raventós J, Gričar J, Prislan P, Čufar K (2011) Cambial activity, wood formation and sapling survival of *Pinus halepensis* exposed to different irrigation regimes. Forest Ecology Management 262:1630–1638.

De Micco V, Saurer M, Aronne G, Tognetti R, Cherubini P (2007) Variations of wood anatomy and δ13C within tree rings of coastal *Pinus pinaster* showing intra annual density fluctuations. IAWA J 28:61–74.

Denne MP (1988) Definition of latewood according to Mork (1928). IAWA Bull 10:59–62.

Domec JC, Gartiner BL (2002) Age- and position-related changes in hydraulic versus mechanical dysfunction of xylem: inferring the design criteria for Douglas-fir wood structure. Tree Physiology 22:91–104.

Fonti P, von Arx G, García-Gonzáles EB, Sass-Klaassen U, Gärtner H, Eckstein D (2010) Studying global change through investigation of the plastic responses of xylem anatomy in tree rings. New Phytologist 185:42–53.

Galmés J, Ribas-Carbó M, Medrano H, Flexas J (2011) Rubisco activity in Mediterranean species is regulated by the chloroplastic CO_2 concentration under water stress. Journal of Experimental Botany 62:653–665.

Gindl W, Grabner M (2000) Characteristics of spruce (*Picea abies* L. Karst) latewood formed under abnormal low temperature. Holzforschung 54:9–11.

Gindl W, Grabner M, Wimmer R (2000) The influence of temperature on latewood lignin content in treeline Norway spruce compared with maximum density and ring width. Trees 14:409–414.

Giovannelli A, Deslauriers A, Fragnelli G, Scaletti L, Castro G, Rossi S, Crivellaro A (2007) Evaluation of drought response of two poplar clones (*Populus* × *canadensis* Mönch ‘I-214’ and *P. deltoides* Marsch.‘Dvina’) through high resolution analysis of stem growth. Journal of Experimental Botany 58:2673–2683.

Giovannelli A, Emiliani G, Traversi ML, Deslauriers A, Rossi S (2011) Sampling cambial region and mature xylem for non-structural carbohydrates and starch analyses. Dendrochronologia 29:177–182.

Gričar J, Čufar K, Oven P, Schmitt U (2005) Differentiation of terminal latewood tracheids in silver fir trees during autumn. Annals of Botany 95:959–965.

Hoch G, Richter A, Körner C (2003) Non-structural carbon compounds in temperate forest trees. Plant Cell and Environment 26:1067–1081.

IPCC (2007) Climate change 2007 – impacts, adaptation and vulnerability: contribution of working group II to the fourth assessment report of the IPCC. Cambridge University Press, London, UK.

Johnson DM, McCulloh KA, Meinzer FC, Woodruff DR, Eissenstat DM, Philips N (2011) Hydraulic patterns and safety margins, from stem to stomata, in three eastern US tree species. Tree Physiology 6:659–668.

Jyske T, Hölttä T, Mäkinen H, Nöjd P, Lumme I, Spiecker H (2010) The effect of artificially induced drought on radial increment and wood properties of Norway spruce. Tree Physiology 30:103–115.

Kirschbaum MUF (1987) Water-stress in *Eucalyptus pauciflora* – comparison of effects on stomatal conductance with effects on the mesophyll capacity for photosynthesis, and investigation of a possible involvement of photoinhibition. Planta 171:466–473.

Kirschbaum MUF (1988) Recovery of photosynthesis from water stress in *Eucalyptus pauciflora* – a process in two stages. Plant Cell and Environment 11:685–694.

Körner C (2003*a*) Carbon limitation in trees. Journal of Ecology 91:4–17.

Körner C (2003*b*) Alpine plant life: functional plant ecology of high mountain ecosystems. 2nd edn. Springer, Berlin.

Kutscha NP, Hyland F, Schwarzmann JM (1975) Certain seasonal changes in Balsam fir cambium and its derivatives. Wood Science Technology l9:175–188.

Lamhamedi M, Gagnon J (2003) Nouvelle technologies de production de plants forestiers au Québec et leur intégration dans les programmes de reboisement des pays en voie de développement. Note de recherche n°120 Bibliothèque National du Québec. Gouvernement du Québec, Ministère des Ressources naturelles, de la Faune et des Parcs. 2004-3032.

Lamhamedi M, Margolis H, Renaud M, Veilleux L, Auger I (2003) Effets de différentes régies d'irrigation sur la croissance, la nutrition minérale et le lessivage des éléments nutritifs des semis d'épinette noire (1 + 0) produits en récipients à parois ajourées en pépinière forestière. Canadian Journal Forest Research 33:279–291.

Larson PR (1963) The indirect effect of drought on tracheid diameter in red pine. Forest Science 9:52–62.

Logan T, Charron I, Chaumont D, Houle D (2011) Atlas de scénarios climatiques pour la forêt québécoise. Mars 2011 Ouranos. http://www.ouranos.ca/media/publication/162_AtlasForet2011-Sans-nnexes.pdf.

Lupi C, Morin H, Deslauriers A, Rossi S (2011) Xylogenesis in black spruce: does soil temperature matter? Tree Physiology 32:74–82.

Lupi C, Morin H, Deslauriers A, Rossi S, Houle D (2012) Increasing nitrogen availability and soil temperature: effects on xylem phenology and anatomy of mature black spruce. Canadian Journal Forest Research 42:1277–1288.

Lutz JA, Halpern CB (2006) Tree mortality during early forest development: a long term study of rates, causes and consequences. Ecology Monography 76:257–275.

Mayor JE, Johnsen KH (1999) Shoot water relations of mature black spruce families displaying a genotype × environment interaction in growth rate. II. Temporal trends and response to varying soil water conditions. Tree Physiology 19:375–382.

McDowell NG (2011) Mechanisms linking drought, hydraulics, carbon metabolism and vegetation, mortality. Plant Physiology 155:1051–1059.

Ministère des Ressources naturelles et de la Faune (2009) Ressources et industrie forestière Portrait Statistique. Edition 2009. ISBN 978-2-550-65558-9, Gouvernement du Québec, Ministère des Ressources naturelles et de la Faune.

Mothe F, Duchanois G, Zannier B, Leban J-M (1998) Analyse micro densitométrique appliquée au bois: méthode de traitement des don- nées utilisée à l'Inra-ERQB. Annals Forest Science 55:301–313.

Oribe Y, Funada R, Shibagaki M, Kubo T (2001) Cambial reactivation in locally heated stems of the evergreen conifer *Abies sachalinensis* (Schmith) Masters. Planta 212:684–691.

Peng C, Ma Z, Lei X et al. (2011) A drought-induced pervasive increase in tree mortality across Canada's boreal forests. Nature Climate Change 1:467–471.

Phillips NG, Ryan G, Bond BJ, McDowell NG, Hinckley. TM, Cèmák J (2003) Reliance on stored water increases with tree size in three species in the Pacific Northwest. Tree Physiology 23:237–245.

Plummer DA, Caya D, Frigon A, Côté H, Giguère M, Paquin D, Biner S, Harvey R, De Elia R (2006) Climate and climate change over North America as simulated by the Canadian RCM. Journal of Climate 19:3112–3132.

Polge H (1978) Fifteen years of wood radiation densitometry. Wood Science Technology 12:187–196.

Potvin C, Lechowicz MJ, Tardif S (1990) The statistical analysis of eco-physiological response curves obtained from experiments involving repeated measures. Ecology 71:1389–1400.

Proseus TE, Boyer JS (2005) Turgor pressure moves polysaccharides into growing cell walls of *Chara corallina*. Ann Bot 95:967–979.

Quinn GP, Keough MJ (2002) Experimental design and data analysis for biologists. Cambridge University Press, Cambridge, UK, 527 pp.

Rathgeber CBK, Decoux V, Leban J-M (2006) Linking intra-tree-ring wood density variations and tracheid anatomical characteristics in Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco). Annals of Forest Science 63:699–706.

Rathgeber CBK, Longuetaud FL, Mothe F, Cuny H, Le Mongédec G (2011) Phenology of wood formation: data processing, analysis and visualisation using R (package CAVIAR). Dendrochronologia 29:139–149.

Rossi S, Morin H (2011) Demography and spatial dynamics in balsam fir stands after a spruce budworm outbreak. Canadian Journal Forest Research 41:1112–1120.

Rossi S, Deslauriers A, Morin H (2003) Application of the Gompertz equation for the study of xylem cell development. Dendrochronologia 21:33–39.

Rossi S, Deslauriers A, Anfodillo T (2006*a*) Assessment of cambial activity and xylogenesis by microsampling tree species: an example at the Alpine timberline. IAWA J 27:383–394.

Rossi S, Deslauriers A, Anfodillo T, Morin H, Saracino A, Motta R, Borghetti M (2006*b*) Conifers in cold environments synchronize maximum growth rate of tree-ring formation with day length. New Phytologist 170:301–310.

Rossi S, Deslauriers A, Anfodillo T, Carraro V (2007) Evidence of threshold temperatures for xylogenesis in conifers at high altitudes. Oecologia 152:1–12.

Rossi S, Deslauriers A, Anfodillo T, Carrer M (2008*a*) Age-dependent xylogenesis in timberline conifers. New Phytologist 177:199–208.

Rossi S, Deslauriers A, Gričar J et al. (2008*b*) Critical temperatures for xylogenesis in conifers of cold climate. Global Ecology and Biogeography 17: 696–707.

Rossi S, Simard S, Rathgeber CBK, Deslauriers A, De Zan C (2009) Effects of a 20-day-long dry period on cambial and apical meristem growth in *Abies balsamea* seedlings. Trees 23:85–93.

Rossi S, Morin H, Deslauriers A, Plourde P-Y (2011) Predicting xylem phenology in black spruce under climate warming. Global Change Biology17:614–625.

Rozas V, DeSoto L, Olano JM (2009) Sex-specific, age-dependent sensitivity of tree-ring growth to climate in the dioecious tree *Juniperus thurifera.* New Phytologist 182:687–697.

Ruel J-C, Doucet R, Boily J (1995) Mortality of balsam fir and black spruce advance growth 3 years after clear-cutting. Canadian Journal Forest Research 25:1528–1537.

Sage RF, Way DA, Kubien DS (2008) Rubisco, Rubisco activase, and global climate change. Journal of Experimental Botany 59:1581–1595.

Sala A, Piper F, Hoch G (2010) Physiological mechanisms of drought–induced tree mortality are far from being resolved. New Physiologist 186:274–281.

Sala A, Woodruff DR, Meinzer F (2012) Carbon dynamics in trees: feast or famine? Tree Physiology 32:764–775

Savidge RA (2000) Intrinsic regulation of cambial growth. Journal of Plant Growth Regulation 20:52–77.

Seager R, Ting M, Held et al. (2007) Model projections on an imminent transition to a more arid climate in south-western North America. Science 316:1181–1184.

Shepherd KR (1964) Some observations on the effect of drought on the growth of *Pinus radiata* D. Don. Aust For 28:7–22.

Sterl A, Severijns C, Dijkstra et al. (2008) When can we expect extremely high surface temperatures? Geophysical Research Letters 35:L14703.

Stewart JD, Zine el Abidine A, Bernier PY (1994) Stomatal and mesophyll limitations of photosynthesis in black spruce seedlings during multiple cycles of drought. Tree Physiology 15:57–64.

Tan W, Blake TJ (1997) Gas exchange and water relations responses to drought of fast- and slow-growing black spruce. Canadian Journal of Botany 75: 1700–1706.

Tan W, Blake TJ, Boyle TJB (1992) Drought tolerance in faster- and slower-growing black spruce (*Picea mariana*) progenies: II. Osmotic adjustment and changes of soluble carbohydrates and amino acids under osmotic stress. Physiology Plant 85:645–651.

van Mantgem PJ, Stephenson NL, Byrne JC et al. (2009) Widespread increase of tree mortality rates in the western United States. Science, 323:521–524.

Vieira J, Campelo F, Nabais C (2009) Age-dependent responses of tree-ring growth and intra-annual density fluctuations of *Pinus pinaster* to Mediterranean climate. Trees 23:257–265.

Way DA, Sage RF (2008) Elevated growth temperature reduce the carbon gain of black spruce [*Picea mariana* (Mill.) B.S.P.]. Global Change Biology 14:624–636.

Zhang X, Vincent LA, Hogg WD, Niitsoo A (2000) Temperature and precipitation trends in Canada during the 20th century. Atmosphere - Ocean 38:395–429.

Zine el Abidine A, Bernier PY, Stewart JD, Plamondon AP (1994) Water stress preconditioning of black spruce seedlings from lowland and upland sites. Canadian Journal of Botany 72:1511–1518.

CHAPITRE III

How do drought and warming influence survival and wood traits of *Picea mariana* saplings?

RESEARCH PAPER

Title: How do drought and warming influence survival and wood traits of *Picea mariana* saplings?

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Balducci L., Deslauriers A., Giovannelli A., Beaulieu M., Delzon S., Rossi S. & Rathgeber C.B.K. (2014) How do drought and warming influence survival and wood traits of *Picea mariana* saplings? *Journal of Experimental Botany*, **66**, 377-389.

3.1 ABSTRACT

Warming and drought occur with increased frequency and intensity at high-latitudes in the future. How heat and water stress can influence tree mortality remains partially unknown. The aim of this study was to evaluate how C-resources, stem hydraulics, wood anatomy and density determine the ability of black spruce saplings to survive daytime or nighttime warming (+ 6 °C in comparison with control) in combination with a drought period. We monitored plant water relations, the dynamics of non-structural carbohydrates and starch, mortality rate, wood anatomy and density of saplings. Warming, in conjunction with 25 days of water deficit, increased sapling mortality (10 and 20% in nighttime and daytime warming, respectively) compared with the control conditions (0.8%). Drought substantially decreased gas exchange, and also predawn and midday leaf water potential to values close to -3 MPa which likely induced xylem embolism (xylem air entry pressure, P_{12}, being on average around -3 MPa for this species). In addition, the recovery of gas exchange never reached the initial pre-stress levels, suggesting a possible loss of xylem hydraulic conductivity associated with cavitation. Consequently, the mortality may be due to xylem hydraulic failure. Warmer temperatures limited the replenishment of starch reserves after their seasonal minimum. Lighter wood was formed during the drought period, reflecting a lower carbon allocation to cell wall formation, preventing the adaptation of the hydraulic system to drought. Saplings of black spruce experienced difficulty in adapting under climate change conditions, which might compromise their survival in the future.

Key words: Climate change, drought, warming, survival, carbon balance, wood anatomy, wood density

Symbols/abbreviations and units:

T0, greenhouse with a temperature equal to the external air temperature; T+Day, greenhouse with temperature of 6 °C higher than T0 during the day; T+Night, greenhouse with temperature of 6 °C higher than T0 during the night; pre-dawn leaf water potential $\Psi_{pd,}$ MPa; midday leaf water potential $\Psi_{md,}$ MPa; stomatal conductance, g_s, mol m^{-2} s^{-1} ; maximum photosynthesis rate, A_{max}, µmol m^{-2} s^{-1}; xylem air entry point, P_{12}, MPa; pressure inducing 50% loss of hydraulic conductance, P_{50}, MPa; slope of the vulnerability curve of the stem, S, % MPa^{-1}; minimum leaf water potential, Ψ_{min}, MPa; PLC_p, predicted native embolism; vulnerability curve, VC; day of the year, DOY; volumetric water content of soil, VWC, %; water deficit period, WDp.

3.2 INTRODUCTION

In boreal ecosystems, an increase in temperature of about 2-4 °C is expected by 2060 due to global warming (Perkins *et al.*, 2012). The projected change in climate extremes could have important consequences on tree growth and survival. Recent increases in tree mortality were assumed to be caused by drought and heat stress associated with global change (Allen *et al.*, 2010, Breshears *et al.*, 2005), with repercussions at higher latitudes (Hogg & Bernier, 2005). In the last decades, Peng *et al.* (2011) found that regional drought increased tree mortality in mature stands of the Canadian boreal forest. Drought conditions particularly affect growth and survival of stand regeneration (Hogg & Schwarz, 1997, Payette & Filion, 1985), because young trees are more vulnerable to root embolism and stomatal closure (Domec *et al.*, 2004, Mueller *et al.*, 2005). The increases in temperature occurring during drought also lead to a quicker dehydration of young trees because of a higher evapotranspiration demand (Angert *et al.*, 2005).

Recent research has emphasized the importance of carbon storage in the physiological response of trees under global environmental changes, such as the increase of CO_2 (Körner, 2003), temperature (Adams *et al.*, 2009), or drought (McDowell *et al.*, 2008). Tree mortality due to drought is a critical component in North-American boreal forests (Peng *et al.*, 2011). Three main hypotheses have been proposed concerning the physiological mechanisms leading to tree mortality during drought: (1) the *carbon starvation hypothesis*, suggesting that a prolonged stomatal closure leads to a depletion of carbohydrate reserves (McDowell *et al.*, 2008), (2) the *hydraulic failure hypothesis*, reflecting a strong alteration of water transport (Brodribb *et al.*, 2010, Urli *et al.*, 2013) and (3) *biotic disturbance*, indicating that pathogens and insects amplify the effects of the two previous mechanisms (McDowell *et al.*, 2008). Recurrent droughts could affect plant carbon balance, while severe droughts result in xylem embolism, both leading to increased mortality in forest stands (McDowell *et al.*, 2008). However, an intriguing debate on the occurrence of carbon starvation (Leuzinger *et al.*, 2009, Sala, 2009, Sala *et al.*, 2010) or a possible coupling of the first two hypotheses (McDowell, 2011) suggests that more studies are needed to elucidate the complex mechanisms involved in tree survival under environmental constraints.

Temperatures are not expected to change equally during the night and day: between 1950 and 1993, the nighttime air temperature increased at about twice the rate of maximum air temperature (IPCC, 2001). This tendency was confirmed for the boreal forest in Canada (Bonsal *et al.*, 2011, Bonsal *et al.*, 2001). Nocturnal warming has several impacts on physiological mechanisms, such as carbon storage and water relations (Sage, 2002, Turnbull *et al.*, 2002). Nighttime water uptake and stem sap flow depend on the availability of water in the soil and on the previous day’s plant transpiration (Fuentes *et al.*, 2013). High nighttime temperature limits the plant water recovery, impairing water balance of the next day, leading to higher stem water shortage; especially during summer drought, when the nights are shorter (Fuentes *et al.*, 2013, Zeppel *et al.*, 2012). During fatal water status, when the plant does not recover from water stress, water transport is reduced by low stem hydraulic conductivity due to the presence of embolisms (Brodribb *et al.*, 2010). High nighttime temperature also alters the carbon balance: nocturnal warming increases maintenance respiration

(Turnbull et al., 2004, Turnbull et al., 2002) leading to a faster degradation of the transitory starch in the chloroplast, thus decreasing the carbon intended to support respiration and growth at night and during the following day.

Wood density is highly sensitive to environmental conditions (Bouriaud *et al.*, 2005, Rozenberg *et al.*, 2002), especially to low or high temperatures. During the growing season, an early temperature decline can induce a reduction in lignin content within terminal tracheids of the latewood (Gindl *et al.*, 2000), showing a marked effect of temperature on the lignification processes. In black spruce, the inhibition of latewood formation can lead to the development of a light ring, due to shortened growing season and low temperature caused by volcanic eruptions (Filion *et al.*, 1986). In a recent study, we showed that wood density was also susceptible to warmer conditions (Balducci *et al.*, 2013). Because wood density mainly depends on cell dimensions and the quantity of structural carbon (cellulose, hemicelluloses and lignin) forming the secondary cell wall (Emiliani *et al.*, 2011, Gindl *et al.*, 2000) the variations in wood density are reflected in the hydraulic architecture of plants. According to the Hagen-Poiseulle law, cells with higher lumen area are more efficient in water transport than smaller tracheid elements with thicker cell walls, but less resistant to embolism at high (less negative) leaf water potential (Domec & Gartiner, 2002). Wood density is the final balance of carbon investment during wood formation (soluble carbon converted in structural carbon) and is a key factor to defining the final proportion between cell-wall and lumen area (Chave *et al.*, 2006, Dalla-Salda *et al.*, 2011, Rathgeber *et al.*, 2006). Consequently, wood density could help to explain the efficiency *versus* safety of the xylem. A new challenge could therefore be to understand how sugars and wood density may influence cavitation and survival in response to temperature and drought.

The aim of this study was to evaluate how combined water deficit and temperature increase affected the dynamics of water, sugar and starch in the stem, the resulting wood anatomy and density, and saplings survival in black spruce. We tested the hypothesis that water deficit coupled with increased night and day temperatures can alter the water and carbon balance of the plant, which results in (i) a reduction in carbon storage in the stem during the night and an increase during the day, (ii) an altered wood anatomy and density (i.e. an increased structural carbon investment), and (iii) exacerbated sapling mortality.

3.3 MATERIALS AND METHODS

3.3.1 Experimental design

The experiment was conducted during the 2011 growing season in Chicoutimi, Canada (48° 25’ N, 71° 04’ W, 150 m above sea level) on 4-year-old black spruce saplings [*Picea mariana* (Mill.) B.S.P.]. In summer 2010, before the beginning of the experiment, the saplings were transplanted into plastic reversed-conic pots (4.5 l in volume) and grown in an open field until the next spring. In April 2011, 1104 saplings of homogeneous size (53.01 ± 8.8 cm in height and 10.43± 1.79 mm in diameter at the collar) were randomly selected and fertilized with 1 g l^{-1} of NPK (20-20-20) dissolved in 500 ml of water to avoid nutrient deficiency. The saplings were arranged in three adjacent greenhouses, where they were grown until October 2011. During the experiment, sapling growth (368 saplings per treatment) was investigated under three different thermal conditions: control (named T0), corresponding to external air temperature; and two warming conditions (T+Day and T+Night), which were 6 °C warmer than T0 during the day (from 7.00 am to 19.00 pm) and during the night (from 19.00 pm to 7.00 am), respectively. In addition, during the maximum xylem growth, when saplings are more susceptible to dry conditions (Rossi *et al.*, 2006), two irrigation regimes were applied: (1) control (named, irrigated saplings), consisting of maintaining the soil water content at approximately 80% of field capacity; (2) water deficit (named, non-irrigated saplings), in which irrigation was withheld for 25 days in June (from day of the year 158 to 182) in 184 saplings per thermal condition.

3.3.2 Water relations, gas exchange and CO_2 assimilation

Water relations, gas exchange and CO_2 assimilation were measured from May to August on branches of the first whorl of 18 saplings per week (3 saplings × 3 thermal conditions × 2 irrigation regimes) (Supplementary Fig. S3.1). In each sapling, pre-dawn [Ψ_{pd}] and midday [Ψ_{md}] leaf water potential were measured using a pressure chamber (PMS Instruments, Corvalis, OR). The minimum leaf water potential [Ψ_{min}] was considered as the daily minimum predawn and midday water potential, [$\Psi_{min\ pd}$] and [$\Psi_{min\ md}$], respectively. They were recorded for each irrigation regime under different thermal conditions (Meinzer *et al.*, 2009). In each plant, gas exchange (stomatal conductance g_s, mol m^{-2} s^{-1}) and CO_2 assimilation (maximum photosynthesis rate, A_{max}, µmol m^{-2} s^{-1}) were measured from 10:00 to 13:00 under saturating irradiance conditions (1000 µmol m^{-2} s^{-1}) using a portable photosynthesis system (Li-6400, LI-COR Inc., Lincoln, NB). Air temperature, vapor pressure deficit, CO_2 concentration and irradiance inside the chamber were maintained at 25 °C, 2.2 ± 0.7 kPa, 400 µmol mol^{-1} and 1000 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD), respectively. Measurements were expressed according to the specific needle surface area computed as a ratio of needle dry mass per unit of needle surface area and using a regression according to Bernier *et al.* (2001). For the same plants, the volumetric water content (VWC) of the soil was measured weekly by time domain reflectometry (TDR Fieldscout 300). The measurements were taken at 7 cm depth in each pot and replicated twice (Topp *et al.*, 1984).

Sapling stems of homogeneous size (49.5 ± 8.3 cm in height and 6.7 ± 0.8 mm in diameter at the collar), straight and without needles, were selected. They were collected in early morning to minimize xylem tension. We used the centrifuge method to measure the vulnerability of branch xylem to water stress induced cavitation caused by air seeding (Delzon *et al.*, 2010). The xylem hydraulic conductivity (k_s, m² MPa^{-1} s^{-1}), embolism vulnerability (P_{12}, xylem air entry point, MPa; P_{50}, pressure inducing 50% loss of hydraulic conductance, MPa) and slope of the vulnerability curve (S, % MPa^{-1}) of the stem were measured using the CAVITRON technique on 5 control saplings (Cochard *et al.*, 2005, Delzon *et al.*, 2010). The centrifugation-based technique was used to establish negative pressure in the xylem and to provoke water-stress-induced cavitation, using a custom-built honeycomb rotor (Precis 2000, Bordeaux, France) mounted on a high-speed centrifuge (Sorvall RC5, Asheville, NC, USA) (Delzon *et al.*, 2010). We calculated the difference between [Ψ_{min}] and the xylem pressures at which PLC=50% (Ψ50), this corresponds to a safety margin for the saplings (Choat *et al.*, 2012, Meinzer *et al.*, 2009) For irrigated saplings the predicted native embolism (PLC$_p$) was estimated from minimum midday water potential [Ψ_{min}] and the vulnerability curve (VC) (Delzon *et al.*, 2010, Urli *et al.*, 2013).

3.3.3 Mortality

Sapling mortality was monitored weekly from May to October and assessed according to the total number of experimental plants (1104) (Supplementary Fig. S3.1). Every week, the mortality percentage was calculated on the total number of saplings that had died per irrigation regime and thermal condition, excluding saplings randomly selected every week from each treatment for the analysis of total non-structural carbohydrates and starch. Sapling mortality was determined by complete needle wilting and stem necrosis.

3.3.4 Wood anatomy and density

Stem disks of 36 saplings (6 saplings × 3 thermal conditions × 2 irrigation regimes), were randomly collected during the last three weeks of October (Supplementary Fig. S3.1). Wood sections were stained with safranine (1% in water) and fixed on slides with histological mounting medium. Digital images were recorded using a camera mounted on a microscope to measure xylem features along three paths using WinCell^{TM} (Regent Instruments Inc., Canada). For each cell along the paths, lumen area, radial diameter and wall thickness were measured. For each anatomical section, earlywood and latewood were identified according to Mork's formula, which classifies all cells with lumen smaller than twice a double cell wall as latewood (Denne, 1988). The stem disks were air-dried until 12% moisture content and X-rayed together with a calibration wedge following standard techniques (Polge & Nicholls, 1972). Radiographs were digitalized using a scanner, and the acquired digital images were treated using semiautomatic procedures in order to produce tree-ring microdensity profiles (Mothe *et al*., 1998). Each tree ring was divided into ten equal parts size considering the relative percentage distance from the beginning of the ring (Mothe *et al*., 1998).

3.3.5 Analysis of non-structural carbohydrates (NSC) and starch

The cambium and xylem tissues of 18 saplings (3 saplings × 3 thermal conditions × 2 irrigation regimes) were collected every two weeks (Supplementary Fig. S3.1) and non-structural carbohydrates (NSC) were extracted following the procedure described in Giovannelli et al. (2011). An Agilent 1200 series HPLC with a RID and a Shodex SC 1011 column and guard column, equipped with an Agilent Chemstation for LC systems program, was used for soluble carbohydrates assessment. A calibration curve was created for each carbohydrate using standard sucrose, raffinose, glucose, fructose (Canadian Life Science) and D-pinitol (Sigma-Aldrich). Total non-structural carbohydrates (total NSC) were calculated as the sum of soluble carbohydrates concentrations (raffinose, sucrose, glucose, pinitol and fructose). Xylem powder was also used for starch extraction, performed according to Chow and Landhäusser (2004). The starch was solubilized with NaOH 0.1M and acetic acid 0.1M and was hydrolyzed enzymatically with an alpha-amylase solution at 2000U/mL and amyloglucosidase 10U/mL. PGO-color reagent and H_2SO_4 75% were added to the solution 24 hours later. Starch was assessed using a spectrophotometer at 533 nm (Chow & Landhäusser, 2004).

3.3.6 Statistical analyses

The means of xylem anatomy and density were compared using two-way analysis of variance (ANOVA) with Tukey's test ($P<0.05$). The comparisons among thermal conditions were performed using slice option procedure in SAS (SAS Institute, Cary, NC). For each sample, a sigmoid function (Pammenter & Willigen, 1998, Urli *et al.*, 2013) was fitted to the vulnerability curve using proc NLIN in SAS (SAS Institute, Cary, NC) according to the equation (1):

$$PLC = \frac{100}{1 + exp^{\frac{s}{25(Pi - P_{50})}}} \qquad (1)$$

Where *P50* is the pressure inducing 50% loss of hydraulic conductance, MPa, slope of the vulnerability curve (S, % MPa^{-1}) of the stem at the inflection point (Urli *et al.*, 2013). For each thermal condition, the total soluble sugars in cambium and xylem were compared, for each day, between irrigation regimes by using Wilcoxon non-parametric analysis (P=0.05). Wilcoxon non-parametric starch comparisons were conducted using NPAR1WAY procedure in SAS. However, due to a very low number of samples on some days of the year (DOY) (N<3), some tests could not be performed.

3.4 RESULTS

3.4.1 Growth conditions

During the experiment, mean temperatures T+Day and T+Night were, on average, 4.5 and 5.2 °C warmer than T0, as heating was applied from 7.00 am to 19.00 pm in T+Day and from 19.00 pm to 7.00 am in T+Night (Fig. 3.1). During the water deficit period, temperature in T0 varied between 14 °C and 22 °C. Maximum temperatures of ~24°C were reached in July for T0. A gradual decrease in temperature was then observed from the end of August, with a minimum of 3.8 °C in October (Fig. 3.1).

During the period of water deficit, the volumetric water content (VWC) of non-irrigated saplings decreased in all thermal conditions. After the drought period, VWC increased quickly and field capacity was reached on DOY 200, 20 days after the resumption of irrigation (Fig. 3.2). After DOY 183, the VWC was maintained at field capacity until the end of experiment.

3.4.2 Sapling mortality

The treatments generated a high mortality rate of saplings, especially after the drought period (during June). In the irrigated treatments, all trees survived at the three thermal conditions (Table 3.1). In the non-irrigated saplings, the rate of mortality proportionally increased with higher nighttime and daytime temperatures. One week after the end of water deficit, the mortality was 0.8% in T0, 10.48% in T+Night and 19.55% in T+Day. At the end of July (DOY 202, 3 weeks after re-watering), mortality persisted in all thermal conditions but was much lower in T+Day and T0, with values of 0.44% and 0.8%, respectively. Higher sapling mortality was still observed in T+Night with the value reaching 1.78% (Table 3.1).

3.4.3 Saplings water relations, gas exchange and CO_2 assimilation

Similar patterns of gas exchange were observed in the irrigated regimes, with a small increase of A_{max} at higher daytime temperature: the value of A_{max} was 7 μmol CO_2 m^{-2} s^{-1} for T0 compared with 8 and 9 μmol CO_2 m^{-2} s^{-1} in T+Day (Fig. 3.3). During the drought period, A_{max} ranged between 4 and -0.04 μmol CO_2 m^{-2} s^{-1} in non-irrigated saplings. A faster decrease was observed in T+Day and T+Night, starting on DOY 166 until the end of the drought period. Similar patterns were observed for g_s, which was lower than 0.02 (values) in T0 under high temperature (DOY 166) (Fig. 3.3).

Overall, the recovery of gas exchange after rewatering was much slower at higher daytime and nighttime temperatures. Indeed, the gas exchange values of non-irrigated saplings were similar to those of irrigated saplings after one week in T0, two weeks in T+Day and four weeks in T+Night. In addition, gas exchange never recovered to pre-stress levels in both temperature treatments, likely due to the relatively slow recovery of xylem hydraulic conductivity following rewatering.

In April and May, predawn and midday leaf water potential showed optimal water status for all treatments (Fig. 3.3). During the period when irrigation was withheld, predawn leaf water potentials (Ψ_{pd}) gradually dropped, with a more pronounced decrease at higher nighttime temperature. The Ψ_{pd} of irrigated saplings were close to zero (ranging between −0.3 to −0.4±0.1 MPa) demonstrating an optimal plant water status. In non-irrigated saplings, Ψ_{pd} gradually dropped with increasing daytime and nighttime temperature, with values of − 0.7±0.4 MPa for T0, −1.63±1.1 MPa for T+Day and −2.8±0.2 MPa for T+Night. From DOY 171 to 181, lower values of Ψ_{md} were observed in non-irrigated saplings, with values reaching -2.1±0.5 MPa in T0, -1.95±0.5 MPa and -2.38±0.1 MPa in T+Day and T+Night, respectively. The minimum leaf water potential values ($\Psi_{min\ md}$) were also low with values of −2.1 MPa and −1.97 MPa in T0 and T+Day, respectively, and the lowest values observed in T+Night ($\Psi_{min\ pd}$, −2.8 MPa). After resumption of irrigation, the recovery of plant water status differed between the thermal conditions. The leaf water potential did not differ between the irrigated and non-irrigated saplings at T0. However, saplings growing at T+Day and T+Night showed a slower plant water status recovery with a delay of 2 and 4 weeks, respectively. The non-irrigated saplings needed one week for the recovery of midday leaf water potential at all thermal conditions.

The xylem pressure inducing 50% loss of conductance (*P50*) reached average values of −4.26 MPa and air point entry (*P12*) reached average values of −2.95 MPa in irrigated saplings (Fig. 3.4, Table 3.2). The slope of the vulnerability curve (S) was 41.71% MPa^{-1} (Table 3.2). The difference between Ψ_{min} and $\Psi 50$ was 2.59 MPa for irrigated saplings. The values of PLC_p ranged from 4.8% to 8.0%.

3.4.4 Wood anatomy and density

Both treatments affected cell features and wood density. Along the tree rings, cell lumen area of T0 progressively decreased from about 300 μm^2 to 20 μm^2 (Fig. 3.5). At warmer conditions, statistical differences between treatments were observed, cell lumen remained stable in the central portion of the tree ring with values ranging from 150 to

100 μm^2, and then it decreased to minimal values of 20-29 μm^2 at high temperature conditions at the end of the annual ring.

Cell-wall thickness was affected by water deficit at all thermal conditions. In the first 20% of the ring, cell-wall thicknesses were quite similar between all treatments (with values ranging between 1.5 and 1.7 µm, Fig. 3.5). However, statistical differences were observed between irrigation regimes in all thermal treatments (Table 3.4). In non-irrigated saplings, cell-wall thickness of the cells located in 50-85% of the tree ring remained at about 2 µm (Fig. 3.5). In comparison, cell-wall thickness of irrigated saplings kept increasing and reached values of about 2.5 to 3 µm in T+Day and T+Night. The maximum cell-wall thickness was reached in latewood with values of 3.6 µm at 90% of the tree ring for non-irrigated saplings and 4 µm for irrigated ones at 85-95% of the tree ring.

Wood density was affected by water deficit and nighttime temperature. Wood density profiles increased along the annual tree ring, with higher values found in latewood (Fig. 3.5). The average values of wood density in the different thermal conditions were 643, 630 and 648.70 kg m^{-3} at T0, T+Day and T+Night, respectively (Table 3.3). At 50-85% portions of the annual ring, a significant difference was observed between the irrigation regimes, with irrigated saplings showing higher density values than non-irrigated ones ($P<0.05$) (Table 3.4). In the warming conditions, significant differences were observed at T+Night ($P<0.0001$). In irrigated saplings, the values of maximum wood density were similar at all thermal conditions, ranging from 982 to 991 kg m^{-3}. A progressive decrease of maximum wood density at high thermal conditions was observed in the non-irrigated saplings (Table 3.3). In irrigated saplings, the minimum wood density increased with warming, while in non-irrigated ones the density decreased with warming (Table 3.3). Fluctuations in wood density were recorded in the 50-85% portions of the annual ring especially at increased nighttime temperatures, with irrigated saplings showing higher values than non-irrigated ones (Fig. 3.5, Table 3.4).

In irrigated saplings, earlywood (EW) represented about 60% of the tree ring at T0 while the proportion increased to about 74% and 68% at T+Day and T+Night, respectively. In non-irrigated saplings the EW values ranged between 70-73%. Consequently latewood (LW) proportion was greater in irrigated saplings at T0. On average, the EW width of the irrigated saplings increased at T+Day and, in non-irrigated ones, increased at T+Night (Table 3.3). Statistical differences were found in EW width at T+Day and in LW width at T+Night (Table 3.3, 3.4).

3.4.5 Dynamics of total NSC and starch

During the growing season, similar concentrations of total NSC were observed in the cambium at all thermal conditions (Fig. 3.6). At the beginning of the experiment (DOY 125), average values of total NSC ranged from 40 to 50 mg/g in irrigated saplings. In non-irrigated saplings the value was 34 mg/g at T0 while it was 49 and 97 mg/g at T+Day and T+Night, respectively. From DOY 139 to DOY 181, an increase was observed in the total NSC, with values ranging from 100 to 200 mg/g in all treatments. Two weeks after re-watering (DOY 196), a synchronous and drastic drop of NSC was found in all treatments. On DOY 209, total NSC in cambium was again high, with mean values ranging from 150 to 200 mg/g and it then decreased gradually at the end of September (Fig. 3.6). In the xylem, total NSC changed in a similar manner during the growing season in all treatments (Fig. 3.6). Similar concentrations were observed at the beginning and end of the experiment, with higher amounts observed on DOY 195 for all thermal conditions, except in non-irrigated saplings for T0.

At the beginning of the experiment, similar amounts of starch were observed between the irrigation regimes (Fig. 3.6). Starch reached its maximum values on DOY 118 and 139 (ranging from 4 to 6 mg/g) and dropped to zero at the end of water deficit (DOY 181) at all thermal conditions (Fig. 3.6). After re-watering, starch showed a similar pattern among the irrigation regimes, ranging from 0.24 to 0.23 mg/g. However, a slower increase was observed after the summer minimum at warmer conditions with lower concentration in T+Day (0.47 mg/g) and T+Night (0.48 mg/g) compared with T0 (0.62 mg/g). A significant difference was found in the starch concentration between temperature treatments, while no difference was found between irrigation regimes and their interaction (Supplementary Table S3.1).

3.5 DISCUSSION

3.5.1 Mechanisms of sapling survival

An increase in air temperature in conjunction with 25 days of water deficit induced a significant increase in sapling mortality (about 10% in T+Night and 20% T+Day). Mortality persisted as long as three weeks after the resumption of irrigation, especially at higher nighttime temperature. Our observations were in agreement with the mortality observed in 3 year-old black spruce seedlings on regenerated cutover (Ruel *et al.*, 1995). Previous research showed that juvenile mortality in black spruce ranged from 10% to 21% according to stem height and the presence of stem wounds, as the root system cannot withstand drought (Ruel *et al.*, 1995). Another study observed that warmer temperatures during growth triggered a higher percentage of mortality in black spruce seedlings with consequent growth compensation (Way & Sage, 2008b). During the water deficit, stomatal conductance and CO_2 assimilation in non-irrigated saplings were lower compared with irrigated saplings at high temperature, as found by Way and Sage (2008a).

During water stress, stomatal conductance was strongly reduced when leaf Ψ_{pd} ranged from -1.0 to -1.5 MPa (Bernier, 1993, Stewart *et al.*, 1994) and damage to the root system of black spruce was observed when Ψ_{pd} reached -2.5 MPa (Johnsen & Major, 1999). Our results showed that a higher percentage mortality occurred in T+Day when Ψ_{pd} reached a level of -1.6 MPa and $\Psi_{min\ md}$ was -1.97 MPa. The mortality was lower in

T+Night, even when $\Psi_{min\ pd}$ reached -2.8 MPa and Ψ_{md} -2.38 MPa. However, it was more persistent in T+Night, with a percentage of 1.78% observed three weeks after re-watering. The physiological mechanisms involved in tree mortality occur at different timescales (Anderegg *et al.*, 2012) and are linked to species-specific vulnerability to cavitation (Delzon *et al.*, 2010). In trees, Ψ_{min} is a relevant parameter to understand stem xylem cavitation and to define the thresholds of hydraulic failure (Brodribb *et al.*, 2010, Urli *et al.*, 2013). In our findings, the minimum leaf water potential reached values close to -3 MPa that likely induced xylem embolism (xylem air entry pressure, P_{12}, being on average around -3 MPa for this species). Moreover, in view of the standard deviation of both P_{12} and P_{50} it is possible that some individuals even reached their lethal cavitation threshold, explaining the mortality rate observed in our experiment. In sapling stems, the xylem tension inducing 50% loss of conductivity (*P50*) was -4.26 MPa on average. All together, these results suggest that our saplings had a narrow safety margin under drought conditions and thus a high risk of hydraulic failure (Choat *et al.*, 2012). This result is in line with recent studies reporting that daily cycles of cavitation and successive repair are not habitual events for trees (Cochard & Delzon, 2013, Sperry, 2013, Wheeler *et al.*, 2013), as cavitation might only occur under severe drought (Delzon & Cochard, 2014). On the basis of our results, we considered that the hydraulic functionality of xylem would not be completely or irreversibly compromised for control saplings. However, our results were based on the response of a limited number of saplings and dead individuals were not considered. In addition, the increase of mortality in non-irrigated saplings could be explained by a reduction in leaf hydraulic conductivity at warmer temperature. Indeed, during the post-drought period, saplings had dramatically lower rates of photosynthesis and stomatal conductance than those of pre-stress and controls. This slow recovery phase might be due to a loss in leaf hydraulic conductivity associated with xylem cavitation. This could explain and significantly to the death of conifer saplings, as reported in Brodribb and Cochard (2009).

The higher mortality rate under warmer conditions could be due to temperature-sensitivity when incomplete restoration of carbon reserves was reached, as observed in recent studies on conifers (Adams *et al.*, 2013, Hartmann *et al.*, 2013, Sala *et al.*, 2012). During water deficit, leaf parameters g_s and A_{max} declined to zero at all thermal conditions. A decrease in the maximum photosynthetic rate could normally be associated with a negative carbon gain, meaning that less sucrose would be translocated in the phloem and unloaded in cambium. However, NSC concentrations in both cambium and xylem were similar between the irrigated and non-irrigated saplings. The intra-annual pattern of NSC showed an inverse trend in the soluble sugar content between cambium (decline) and xylem (increase) around DOY 160 when starch in the xylem was near zero suggesting the presence of strong seasonal dynamics, as observed in other conifers (Gruber *et al.*, 2012, Schaberg *et al.*, 2000). This seasonal scenario was often reported in spring and winter when starch to sugars conversion occurs (Bucci *et al.*, 2003, Schaberg *et al.*, 2000). The slower replenishment in starch reserves observed under warming, for both irrigated and non-irrigated saplings, could suggest an active role of starch, not only for the allocation of carbon resources for growth and metabolic demands, but also for the recovery of plants after drought.

We hypothesize that the higher percentage of mortality at higher daytime and nighttime temperatures, as well as the prolonged mortality at T+Night, could be related to the lower starch reserves after their seasonal minimum. The lower starch amount could be caused by a lower accumulation in the xylem due to a decrease in photosynthesis induced by water deficit. We suppose that the day and night daily fluctuation of starch in the stem could be analogous to that in the leaves. Thus, the lower recovery of starch in the xylem could reflect a change in the partitioning during the day and night. Reduction of C-storage in the stem could be caused by (1) diminution of the fraction of carbon stored for later use or (2) immediate use required to meet the higher metabolic demand at higher temperature. The co-occurrence of abiotic stresses thus limits the pools of stored carbon, possibly from lower sugars translocation by the phloem (Galiano *et al.*, 2011, Sala *et al.*, 2012, Woodruff & Meinzer, 2011). Recent researches showed that under moderate drought, plant water conditions required for C-remobilization sustained the survival of saplings, while severe drought strongly reduced the ability of saplings to utilize starch reserves, which did not assure sapling survival (Hartmann *et al.*, 2013). Even if our study is limited and precludes information on NSC and starch pattern in other sink tissues (roots and leaves), we cannot invoke the carbon starvation hypothesis.

3.5.2 Does the modification in wood anatomy make plants more resistant?

Under warmer conditions, xylem anatomy was modified by water deficit. Drought can induce the development of latewood cells in earlywood, which is a typical reaction in species growing in the Mediterranean area (Cherubini *et al.*, 2003, de Luis *et al.*, 2011). In this study however, at warmer temperatures, the observed plateau of cell-wall thickness could represent the incapacity of black spruce to allocate sufficient C-resource to build thicker cell walls. Moreover, a higher decrease (or lower plateau) was observed in non-irrigated saplings, clearly indicating a lower C allocation to cell-wall development. The effect was also amplified as the water deficit occurred during the period of maximum cell production and differentiation. The co-occurrence of drought and warming that limited photosynthetic acclimation, with a consequent reduction in carbon (Way & Sage, 2008b), could influence the synthesis of cell-wall components and produce thin cell walls (Luomala *et al.*, 2005). The most important consequence of such combined stress effects was the formation of wood with a lower density, which generally reflects a high hydraulic conductivity (Bucci *et al.*, 2004). This strategy does not allow the adaptation of black spruce toward a more efficient hydraulic system but probably decreases plant survival under warming and drought stress. Wood density is strongly correlated to drought-induced embolism (Hoffmann *et al.*, 2011, Pittermann *et al.*, 2006), because a low hydraulic conductivity may be an element of great drought resistance (Hacke *et al.*, 2001), but the relationship between wood density and resistance to cavitation is not direct. A lower wood density was recently proposed as a strategy to avoid catastrophic embolism after severe water deficit (Rosner *et al*., 2013; Hoffmann *et al*., 2011). The lower wood density could be caused by a change in the C-allocation as (1) more C is required to meet the higher respiration demand at higher temperature, especially during the night (Amthor, 2000); (2) the C-resources are mobilized for osmoregulation and are not available for cell wall building (Muller *et al.*, 2011, Pantin *et al.*, 2012).

3.6 CONCLUSION

This experiment emphasizes the importance of investigating sapling responses to multifactor stress in order to reveal the effects on individual survival and xylem performance. Our findings showed that the recovery of gas exchange never reached the initial pre-stress levels, indicating a loss in xylem hydraulic conductivity compared to pre-stress levels that could explain the hydraulic failure and death of individuals at warmer conditions. The consequences of drought under warming can improve our understanding of the role of wood density and carbon storage for sapling survival. This study underlined the importance of considering the active role of C-storage and its utilization during tree growth under harsh environmental conditions. Although depletion carbon reserves did not take place during prolonged water deficit, the carbon-water relations changed and were important for the survival process in saplings.

3.7 ACKNOWLEDGEMENTS

This study was funded by the Natural Sciences and Engineering Research Council of Canada and the Consortium Ouranos. We thank H. Morin, M.J. Gagnon, D. Gagnon, G. Savard, C. Soucy and all personal of LEVA and LASEVE laboratories for their support and laboratory analyses. Additional thanks for M.L. Traversi, P. Gelhaye and P. Rozenberg for support in wood density analyses, P. Bouche for cavitating measures and A. Garside for checking the English text. The authors are grateful to the anonymous reviewers for their constructive comments.

Table 3.1 Percentage of mortality one and three weeks after the water deficit period (WDp).

	% Sapling mortality		
Irrigated	**T0**	**T+Day**	**T+Night**
1 week after WDp	0	0	0
3 weeks after WDp	0	0	0
Non-irrigated			
1 week after WDp	0.81	19.76	10.48
3 weeks after WDp	0.89	0.45	1.79

Table 3.2 Mean values and SD of xylem pressure inducing 50% loss in conductance (P_{50}, MPa), P_{12}, xylem air entry point, (MPa) and vulnerability curve slope of stem (S, % MPa^{-1}) measured on black spruce saplings.

	***Picea mariana* saplings**
Parameters	Irrigated
P_{50} (MPa)	-4.27±0.1
P_{12} (MPa)	-2.95±0.05
Slope (% MPa-1)	41.72±16.70

Table 3.3 Wood properties (mean and SD standard deviation) of black spruce saplings at three thermal conditions during the greenhouse experiment in 2011). Significant effects between irrigation regimes (*P≤ 0.05)* are in bold.

Wood density (Kg/m^3)	**Irrigation regimes**	**T0**	**T+Day**	**T+Night**
Mean	Non-irrigated	638 ± 86	598 ± 79	621 ± 99
	Irrigated	650 ± 79	657 ± 79	676 ± 95
Minimum	Non-irrigated	481 ± 90	435 ± 61	470 ± 73
	Irrigated	438 ± 59	478 ± 86	478 ± 96
Maximum	Non-irrigated	954 ± 153	917 ± 141	936 ± 145
	Irrigated	991 ± 120	985 ± 109	982 ± 100
Earlywood	Non-irrigated	566 ± 87	541 ± 60	577 ± 88
	Irrigated	540 ± 52	598 ± 80	590 ± 99
Latewood	Non-irrigated	832 ± 125	785 ± 146	811 ± 121
	Irrigated	850 ± 108	844 ± 104	825 ± 99
Ring Width (mm)				
Earlywood	Non-irrigated	0.56 ± 0.2	0.59 ± 0.2	0.71 ± 0.3
	Irrigated	0.64 ± 0.2	0.81 ± 0.2	0.59 ± 0.2
Latewood	Non-irrigated	0.22 ± 0.2	0.23 ± 0.2	0.15 ± 0.1
	Irrigated	0.33 ± 0.1	0.28 ± 0.2	0.33 ± 0.2
Proportion (%)				
%Earlywood	Non-irrigated	71.8	73.6	70.8
	Irrigated	60.5	74.0	68.8
%Latewood	Non-irrigated	28.2	26.4	29.2
	Irrigated	39.5	26.0	31.2

Table 3.4 *P* values for wood density, cell-wall thickness and lumen area along relative portion of tree ring (%) in black spruce saplings calculated between irrigation regimes (I), among thermal conditions (T) and interaction between irrigation regimes and thermal conditions (I × T) and DOY (Day of the year). Significant effects (*P≤ 0.05)* are in bold.

Relative portion of tree ring (%)	**Lumen area**			**Cell-wall thickness**			**Wood density**		
	I	**T**	**T × I**	**I**	**T**	**T × I**	**I**	**T**	**T × I**
5%	0.0734	0.0125	0.0086	0.018	0.6222	0.9311	0.863	0.2488	0.9572
10%	0.1833	<.0001	0.0368	0.0201	0.0227	0.9393	0.6981	0.569	0.5622
15%	0.0374	<.0001	0.0005	0.0041	0.0012	0.2776	0.7275	0.6738	0.3188
20%	<.0001	<.0001	<.0001	0.0003	0.0005	0.2431	0.7871	0.5327	0.2653
25%	0.037	<.0001	<.0001	<.0001	0.0044	0.1173	0.8825	0.3263	0.224
30%	0.0162	<.0001	<.0001	<.0001	<.0001	0.013	0.8295	0.1777	0.1457
35%	0.6011	<.0001	0.0103	<.0001	<.0001	0.0152	0.5039	0.1032	0.1128
40%	0.1043	0.0002	<.0001	<.0001	0.0065	0.0155	0.2266	0.0798	0.1274
45%	0.8612	0.0193	<.0001	<.0001	0.0022	0.1574	0.0641	0.131	0.1314
50%	0.0132	0.5606	<.0001	<.0001	<.0001	0.5906	0.0148	0.3645	0.1224
55%	<.0001	0.0007	0.0021	<.0001	<.0001	0.0151	0.0048	0.666	0.1765
60%	<.0001	0.0008	<.0001	<.0001	<.0001	0.0005	0.0035	0.5406	0.3595
65%	0.0076	<.0001	0.0119	<.0001	<.0001	<.0001	0.0038	0.3249	0.6323
70%	0.0761	<.0001	<.0001	<.0001	<.0001	<.0001	0.0053	0.2262	0.7925
75%	0.118	<.0001	<.0001	0.0202	<.0001	<.0001	0.0076	0.1696	0.7991
80%	0.1289	<.0001	<.0001	0.0247	<.0001	<.0001	0.0134	0.1391	0.8223
85%	0.9564	<.0001	<.0001	0.0157	<.0001	<.0001	0.0281	0.1453	0.924
90%	0.0207	<.0001	<.0001	0.0643	<.0001	0.0018	0.0569	0.2142	0.9931
95%	0.6998	<.0001	<.0001	0.0453	0.0033	0.7037	0.0987	0.4413	0.9516
100%	0.0268	<.0001	<.0001	0.3345	0.0076	0.0023	0.0987	0.8289	0.8944

Supplementary Table S3.1

Means and P values for total non-structural carbohydrates (NSCs) in cambium and in xylem (mg/g_{dw}) and starch in xylem (mg/g_{dw}) in black spruce saplings calculated between irrigation regimes (I), among thermal conditions (T) and interaction between irrigation regimes and thermal conditions (I × T). Significant effects ($P \leq 0.05$) are in bold.

	Irrigation regimes						**Prob**			
	Irrigated			**Non-irrigated**			**I**	**T**	**I x T**	**DOY**
	T0	**T+Day**	**T+Night**	**T0**	**T+Day**	**T+Night**				
Total NSC in cambium	144.43	115.79	138.73	135.63	137.75	139.18	0.44	0.05	0.05	**<0.001**
Total NSC in xylem	9.21	8.67	8.90	8.79	9.27	8.87	0.90	0.96	0.54	**<0.001**
Starch in xylem	4.17	3.09	3.09	4.46	2.71	3.42	0.14	**<0.001**	0.67	**<0.001**

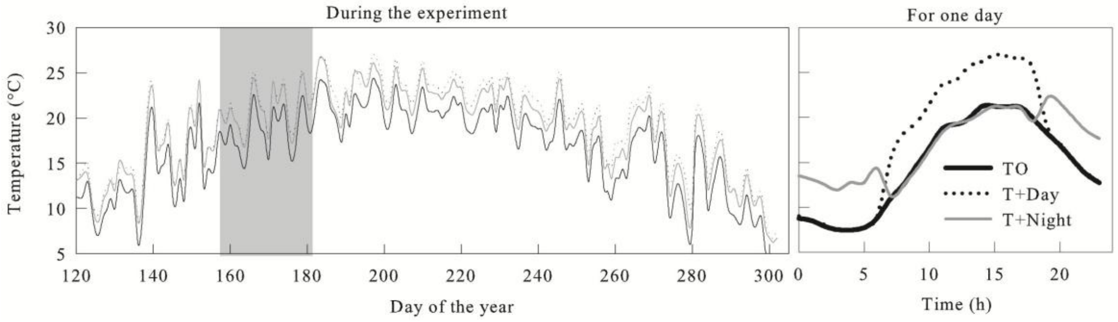


Figure 3.1 Daily temperatures experienced by black spruce saplings in the three thermal conditions (T0, control temperature; T+Day, temperature increase during the day; T+Night, temperature increase during the night) during the greenhouse experiment from April to October. Grey background corresponds to water deficit period during June.

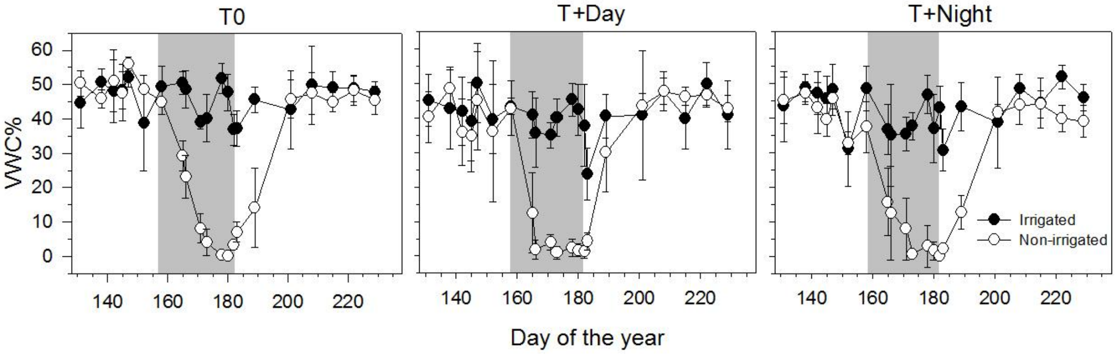


Figure 3.2 Volumetric water content (VWC) of soil in irrigated saplings (black circles) and non-irrigated saplings (white circles) before, during and after the water deficit period (grey background) at three thermal conditions (T0, control temperature; T+Day, temperature increase during the day; T+Night, temperature increase during the night) during the greenhouse experiment in 2011. Vertical bars represent standard deviation.

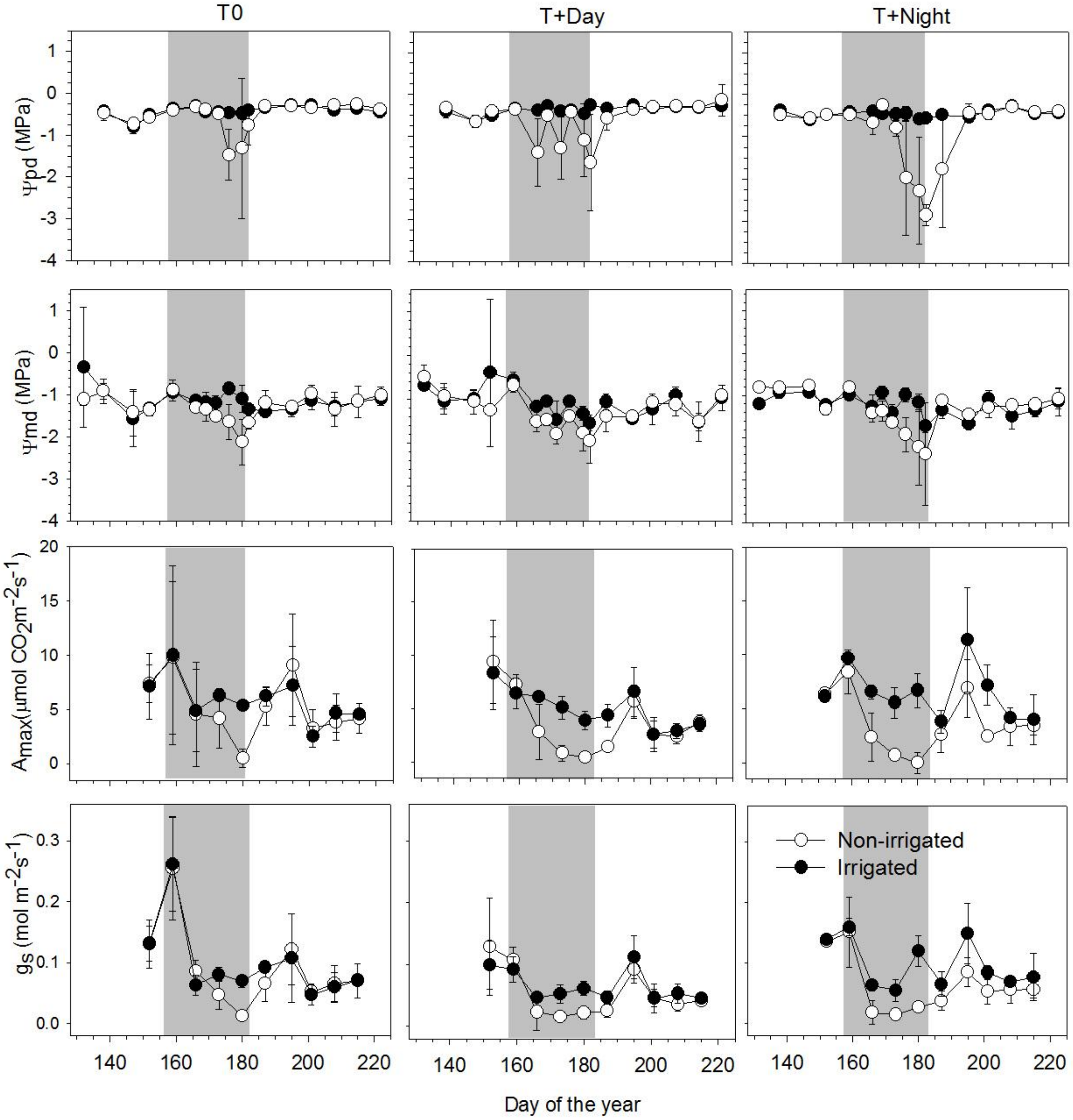


Figure 3.3 Pre-dawn leaf water potential (Ψ_{pd}, MPa), midday leaf water potential (Ψ_{md}, MPa), CO_2 assimilation (maximum photosynthesis rate, A_{max}, μmol m^{-2} s^{-1}) and gas exchange (stomatal conductance g_s, mol m^{-2} s^{-1}) of black spruce saplings before, during and after the water deficit period (WDp) at three thermal conditions during the greenhouse experiment in 2011.

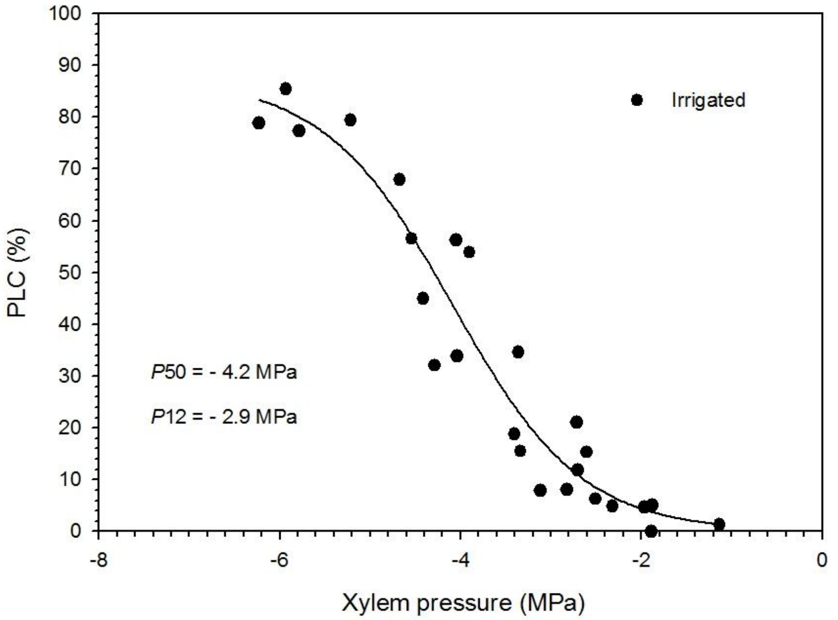


Figure 3.4 Mean percentage loss of hydraulic conductance (PLC%) versus xylem pressure (MPa) for black spruce saplings. Vulnerability curve was obtained with CAVITRON technique.

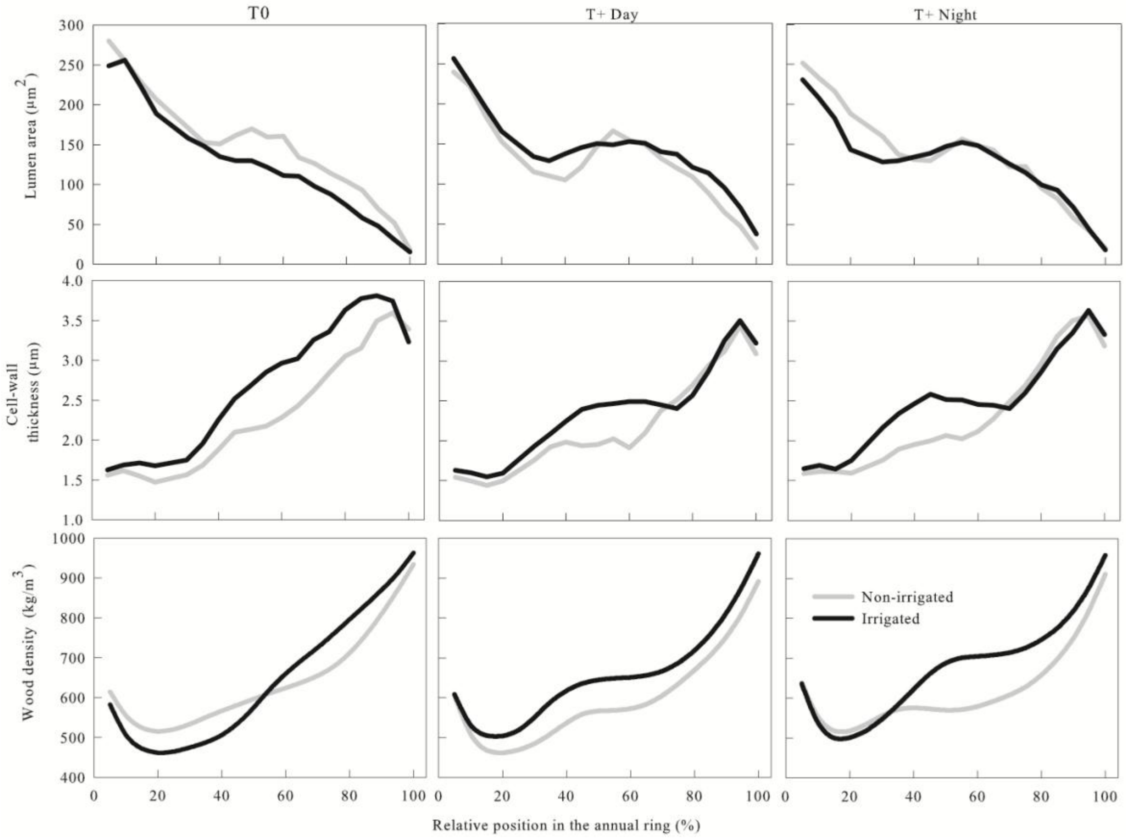


Figure 3.5 Cell features and wood density of the tracheids produced by irrigated (black curves) and non-irrigated (grey curves) black spruce saplings at three thermal conditions (T0, control temperature; T+Day, temperature increase during the day; T+Night, temperature increase during the night) along portions of an annual tree ring.

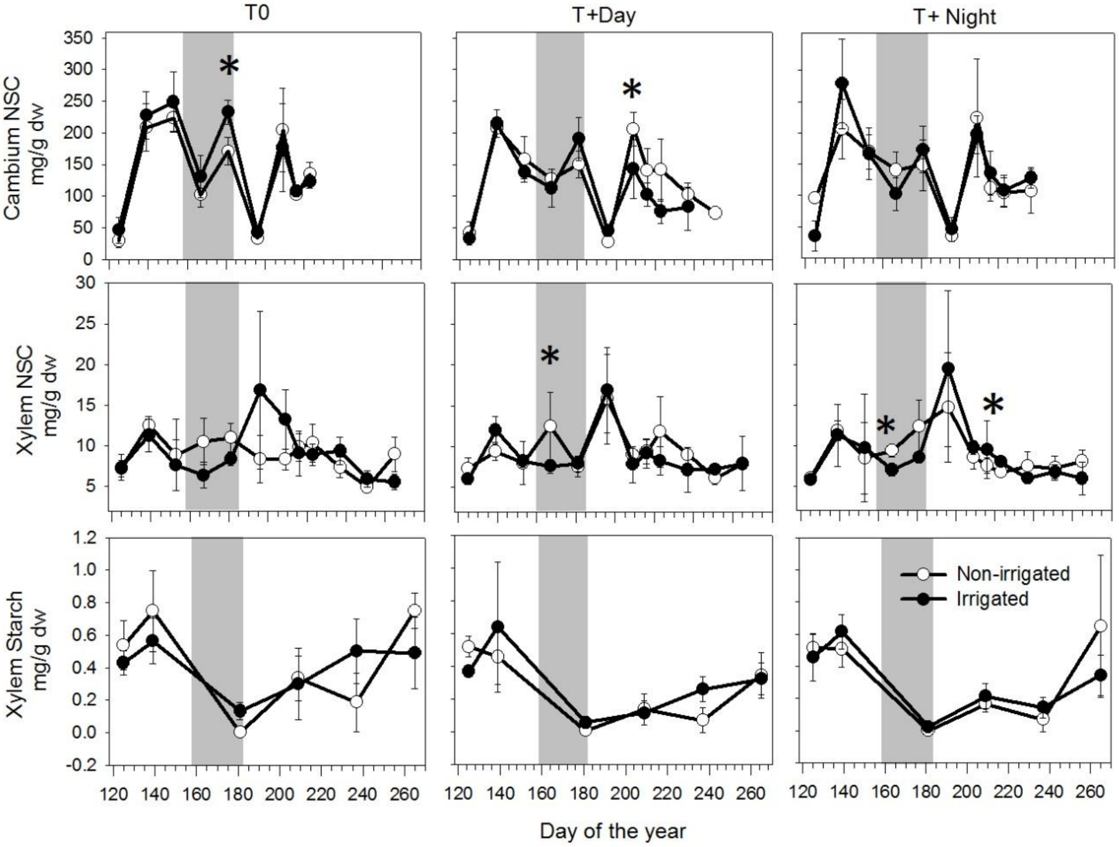
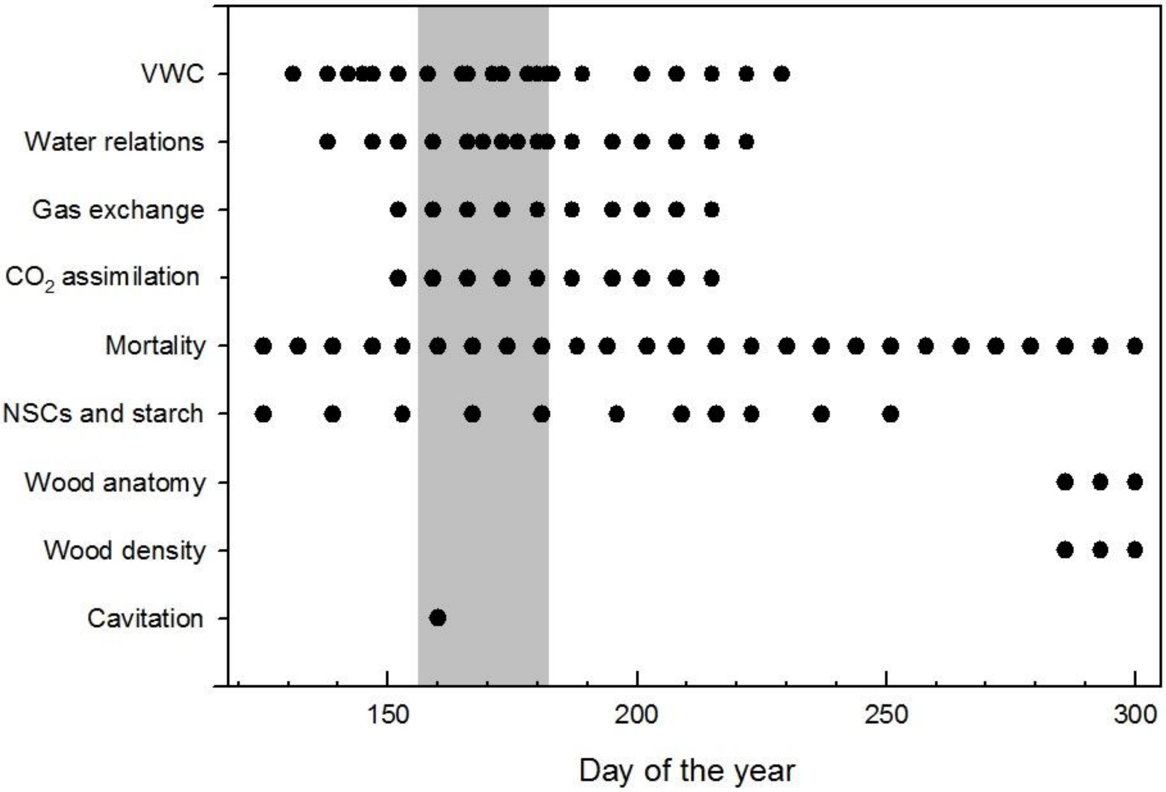


Figure 3.6 Non-structural carbohydrates (NSC) in mg/g$_{dw}$ in cambium and in xylem and starch concentration in xylem (mg/g$_{dw}$) in black spruce saplings before, during and after the water deficit period (grey background) at three thermal conditions (T0, control temperature; T+Day, 6 °C higher daytimg temperature; T+Night, 6 °C higher nighttime temperature). Black and white circles indicate the two irrigation regimes. *Asterisks* indicate statistically significant differences between the two irrigation regimes (Wilcoxon test, ($P \leq 0.05$).



Supplementary Figure S3.1 Sampling timetable of black spruce saplings. Black circles correspond to sampling days before, during and after the water deficit period (grey background). VWC correspond to volumetric water content of soil and NSCs correspond to non-structural carbohydrates.

3.8 REFERENCES

Adams HD, Germino MJ, Breshears DD, Barron-Gafford GA, Guardiola-Claramonte M, Zou CB, Huxman TE. 2013. Nonstructural leaf carbohydrate dynamics of *Pinus edulis* during drought-induced tree mortality reveal role for carbon metabolism in mortality mechanism. *New Phytologist* **197**, 1142–1151.

Adams HD, Guardiola-Claramonte M, Barron-Gafford GA, Villegas JC, Breshears DD, Zoug CB, Troch PA, Huxman TE. 2009. Temperature sensitivity of drought-induced tree mortality portends increased regional die-off under global-change-type drought. *Proceedings of the National Academy of Sciences of the United States* **106**, 7063-7066.

Allen CD, Macalady AK, Chenchouni H, Bachelet D, McDowell N, Vennetier M, Kitzberger T, Rigling A, Breshears DD, Hogg EHT, Gonzalez P, Fensham R, Zhang Z, Castro J, Demidova N, Lim J-H, Allard G, Running SW, Semerci A, Cobb N. 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management* **259**, 660-684.

Amthor JS. 2000. Direct effect of elevated CO_2 on nocturnal in situ leaf respiration in nine temperate deciduous tree species is small. *Tree Physiology* **20**, 139-144.

Anderegg WRL, Berry JA, Smith DD, Sperry JS, Anderegg LDL, Field CB. 2012. The roles of hydraulic and carbon stress in a widespread climate-induced forest die-off. *Proceedings of the National Academy of Sciences of the United States* **109**, 233-237.

Angert A, Biraud S, Bonfils C, Henning CC, Buermann W, Pinzon J, Tucker CJ, Fung I. 2005. Drier summers cancel out the CO_2 uptake enhancement induced by warmer springs. *Proceedings of the National Academy of Sciences of the United States* **102**, 10823-10827.

Balducci L, Deslauriers A, Giovannelli A, Rossi S, Rathgeber CBK. 2013. Effects of temperature and water deficit on cambial activity and woody ring features in *Picea mariana* saplings. *Tree Physiology* **33**, 1006–1017.

Bernier PY. 1993. Comparing natural and planted black spruce seedlings. I. Water relations and growth. *Canadian Journal of Forest Research* **23**, 2427-2434.

Bernier PY, Raulier F, Stenberg P, Ung C-H. 2001. Importance of needle age and shoot structure on canopy net photosynthesis of balsam fir (*Abies balsamea*): a spatially inexplicit modeling analysis. *Tree Physiology* **21**, 815–830.

Bonsal BR, Wheaton EE, Meinert A, Siemens E. 2011. Characterizing the Surface Features of the 1999–2005 Canadian Prairie Drought in Relation to Previous Severe Twentieth Century Events. *Atmosphere-Ocean* **49**, 320-338.

Bonsal BR, Zhang X, Vincent LA, Hogg WD. 2001. Characteristics of daily and extreme temperatures over Canada. *Journal of Climate* **14**, 1959-1976.

Bouriaud O, Leban JM, Bert D, Deleuze C. 2005. Intra-annual variations in climate influence growth and wood density of Norway spruce. *Tree Physiology* **25**, 651-660.

Breshears DD, Cobb NS, Rich PM, Price KP, Allen CD, Balice RG, Romme WH, Kastens JH, Floyd ML, Belnap J, Anderson JJ, Myers OB, Meyer CW. 2005. Regional vegetation die-off in response to global-change-type drought. *Proceedings of the National Academy of Sciences of the United States* **102**, 15144-15148.

Brodribb TJ, Bowman D, Nichols S, Delzon S, Burlett R. 2010. Xylem function and growth rate interact to determine recovery rates after exposure to extreme water deficit. *New Phytologist* **188**, 533-542.

Brodribb TJ, Cochard H. 2009. Hydraulic failure defines the recovery and point of death in water-stressed conifers. *Plant Physiology* **149**, 575.

Bucci SJ, Scholz FG, Goldstein G, Meinzer FC, Hinojosa JA, Hoffmann WA, Franco AC. 2004. Processes preventing nocturnal equilibration between leaf and soil water potential in tropical savanna woody species. *Tree Physiology* **24**, 1119–1127.

Bucci SJ, Scholz FG, Goldstein G, Meinzer FC, Stenberg LDSL. 2003. Dynamic changes in hydraulic conductivity in petioles of two savanna tree species: factors and mechanisms contributing to the refilling of embolized vessels. *Plant, Cell & Environment* **26**, 1633–1645.

Chave J, Muller-Landau HC, Baker TR, Easdale TA, Steege H, Campbell OW. 2006. Regional and phylogenetic variation of wood density across 2456 neotropical tree species. *Ecological Applications* **16**, 2356-2367.

Cherubini P, Gartner BL, Tognetti R, Brker OU, Schoch W, Innes JL. 2003. Identification, measurement and interpretation of tree rings in woody species from mediterranean climates. *Biological Reviews of the Cambridge Philosophical Society* **78**, 119-148.

Choat B, Jansen S, Brodribb TJ, Cochard H, Delzon S, Bhaskar R, Bucci SJ, Feild TS, Gleason SM, Hacke UG, Jacobsen AL, Lens F, Maherali H, Martinez-Vilalta J, Mayr S, Mencuccini M, Mitchell PJ, Nardini A, Pittermann J, Pratt RB, Sperry JS, Westoby M, Wright IJ, Zanne AE. 2012. Global convergence in the vulnerability of forests to drought. *Nature* **491**, 752-755.

Chow PS, Landhäusser SM. 2004. A method for routine measurements of total sugar and starch content in woody plant tissues. *Tree Physiology* **24**, 1129 –1136.

Cochard H, Damour G, Bodet C, Tharwat I, Poirier M, Améglio T. 2005. Evaluation of a new centrifuge technique for rapid generation of xylem vulnerability curves. *Physiologia plantarum* **124**, 410–418.

Cochard H, Delzon S. 2013. Hydraulic failure and repair are not routine in trees. *Annals of Forest Science* **70**, 659-661.

Dalla-Salda G, Martinez-Meier A, Cochard H, Rozenberg P. 2011. Genetic variation of xylem hydraulic properties shows that wood density is involved in adaptation to drought in Douglas-fir (*Pseudotsuga menziesii* (Mirb.)). *Annals of Forest Science* **68**, 747–757.

de Luis M, Novak K, Raventós J, Gričar J, Prislan P, Čufar K. 2011. Climate factors promoting intra-annual density fluctuations in Aleppo pine (*Pinus halepensis*) from semiarid sites. *Dendrochronologia* **29**, 163-169.

Delzon S, Cochard H. 2014. Recent advances in tree hydraulics highlight the ecological significance of the hydraulic safety margin. *New Phytologist*.

Delzon S, Douthe C, Sala A, Cochard H. 2010. Mechanism of water-stress induced cavitation in conifers: bordered pit structure and function support the hypothesis of seal capillary-seeding. *Plant, Cell & Environment* **33**, 2101-2111.

Denne MP. 1988. Definition of latewood according to Mork (1928). *IAWA Bullettin* **10**, 59-62.

Domec JC, Gartiner BL. 2002. Age- and position-related changes in hydraulic versus mechanical dysfunction of xylem: inferring the design criteria for Douglas-fir wood structure. *Tree Physiology* **22**, 91–104.

Domec JC, Warren J, Meinzer M, Brooks F, Coulombe C, Coulombe J, Coulombe R. 2004. Native root xylem embolism and stomatal closure in stands of Douglas-fir and ponderosa pine: mitigation by hydraulic redistribution. *Oecologia* **141**, 7-16.

Emiliani G, Traversi ML, Anichini M, Giachi G, Giovannelli A. 2011. Transcript accumulation dynamics of phenylpropanoid pathway genes in the maturing xylem and phloem of picea abies during latewood formation. *Journal of Integrative Plant Biology* **53**, 783-799.

Filion L, Payette S, Gauthier L, Boutin Y. 1986. Light rings in Subartic conifers as a dendrochronological tool. *Quaternary Research* **26**, 272-279.

Fuentes S, Mahadevan M, Bonada M, Skewes MA, Cox JW. 2013. Night-time sap flow is parabolically linked to midday water potential for field-grown almond trees. *Irrigation Science* **31**, 1-12.

Galiano L, Martinez-Vilalta J, Lloret F. 2011. Carbon reserves and canopy defoliation determine the recovery of Scots pine 4 yr after a drought episode. *New Phytologist* **190**, 750-759.

Gindl W, Grabner M, Wimmer R. 2000. The influence of temperature on latewood lignin content in treeline Norway spruce compared with maximum density and ring width. *Trees* **14**, 409-414.

Giovannelli A, Emiliani G, Traversi ML, Deslauriers A, Rossi S. 2011. Sampling cambial region and mature xylem for non structural carbohydrates and starch analyses. *Dendrochronologia* **29**, 177-182.

Gruber A, Pirkebner D, Florian C, Oberhuber W. 2012. No evidence for depletion of carbohydrate pools in Scots pine (*Pinus sylvestris* L.) under drought stress. *Plant Biology (Stuttg)* **14**, 142–148.

Hacke UG, Sperry JS, Pockman WT, Davis SD, McCulloh KA. 2001. Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. *Oecologia* **126**, 457–461.

Hartmann H, Ziegler W, Kolle O, Trumbore S. 2013. Thirst beats hunger – declining hydration during drought prevents carbon starvation in Norway spruce saplings. *New Phytologist* **200**, 340–349.

Hoffmann WA, Marchin RM, Abit P, Lau OL. 2011. Hydraulic failure and tree dieback are associated with high wood density in a temperate forest under extreme drought. *Global Change Biology* **17**, 2731-2742.

Hogg EH, Schwarz AG. 1997. Regeneration of planted conifers across climatic moisture gradients on the Canadian prairies: implications for distribution and climate change. *Journal of Biogeography* **24**, 527-534.

Hogg EHT, Bernier PY. 2005. Climate change impacts on drought-prone forests in western Canada. *Forestry Chronicle* **81**, 675-682.

IPCC. 2001. Climate Change 2001: scientific basis. In: Change IPoC, ed. Cambridge University Press.

IPCC. 2013. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge, UK and New York, NY, USA: Cambridge University Press, 1535.

Johnsen KH, Major JE. 1999. Shoot water relations of mature black spruce families displaying a genotype ×environment interaction in growth rate. I. Family and site effects over three growing seasons. *Tree Physiology* **19**, 367-374.

Körner C. 2003. Carbon limitation in trees. *Journal of Ecology* **91**, 4-17.

Leuzinger S, Bigler C, Wolf A, Korner C. 2009. Poor methodology for predicting large-scale tree die-off. *Proceedings of the National Academy of Sciences of the United States* **106**.

Luomala EM, Laitinen K, Sutinen S, Kellomaki S, Vapaavuori E. 2005. Stomatal density, anatomy and nutrient concentrations of Scots pine needles are affected by elevated CO_2 and temperature. *Plant Cell and Environment* **28**, 733-749.

McDowell N, Pockman WT, Allen CD, Breshears DD, Cobb N, Kolb T, Plaut J, Sperry J, West A, Williams DG, Yepez EA. 2008. Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytologist* **178**, 719–739.

McDowell NG. 2011. Mechanisms linking drought, hydraulics, carbon metabolism, and vegetation mortality. *Plant Physiology* **155**, 1051.

Meinzer FC, Johnson DM, Lachenbruch B, McCulloh KA, Woodruff DR. 2009. Xylem hydraulic safety margins in woody plants: coordination of stomatal control of xylem tension with hydraulic capacitance. *Functional Ecology* **23**, 922-930.

Mothe F, Duchanois G, Zaannier B, Laeban J-M. 1998. Analyse microdensitométrique appliquée au bois : méthode de traitement des données utilisée à l'Inra-ERQB (programme Cerd). *Annals of Forest Science* **55**, 301-313.

Mueller RC, Scudder CM, Porter ME, Trotter RT, Gehring CA, Whitham TG. 2005. Differential tree mortality in response to severe drought: evidence for long-term vegetation shifts. *Journal of Ecology* **93**, 1085-1093.

Muller B, Pantin F, Génard M, Turc O, Freixes S, Piques M, Gibon Y. 2011. Water deficits uncouple growth from photosynthesis, increase C content, and modify the relationships between C and growth in sink organs. *Journal of Experimental Botany* **62**, 1715-1729.

Pammenter NW, Willigen CV. 1998. A mathematical and statistical analysis of the curves illustrating vulnerability of xylem to cavitation. *Tree Physiology* **18**, 589-593.

Pantin F, Simonneau T, Muller B. 2012. Coming of leaf age: Control of growth by hydraulics and metabolics during leaf ontogeny. *New Phytologist* **196**, 349-366.

Payette S, Filion L. 1985. White spruce expansion at the tree line and recent climatic change. *Canadian Journal of Forest Research* **15**, 241-251.

Peng C, Ma Z, Lei X, Zhu Q, Chen H, Wang W, Liu S, Li W, Fang X, Zhou X. 2011. A drought-induced pervasive increase in tree mortality across Canada’s boreal forests. *Nature* **1**, 467–471.

Pittermann J, Sperry JS, Wheeler JK, Hacke UG, Sikkema EH. 2006. Mechanical reinforcement of tracheids compromises the hydraulic efficiency of conifer xylem. *Plant, Cell & Environment* **29**, 1618–1628.

Plummer DA, Caya D, Frigon A, Côté H, Giguère M, Paquin D, Biner S, Harvey R, De Elia R. 2006. Climate and Climate Change over North America as Simulated by the Canadian RCM. *Journal of Climate* **19**, 3112-3132.

Polge H, Nicholls J. 1972. Quantitative radiography and the densitometric analysis of wood. *Wood Science* **5**, 51-59.

Rathgeber CBK, Decoux V, Leban J-M. 2006. Linking intra-tree-ring wood density variations and tracheid anatomical characteristics in Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco). *Annals of Forest Science* **63**, 699–706.

Rossi S, Deslauriers A, Anfodillo T. 2006. Assessment of cambial activity and xylogenesis by microsamplings tree species: an example at the alpine timberline. *IAWA Journal* **27**, 383–394.

Rozenberg P, Van Loo J, Hannrup B, Grabner M. 2002. Clonal variation of wood density record of cambium reaction to water deficit in Picea abies (L.) Karst. *Annals of Forest Science* **59**, 533-540.

Ruel J-C, Doucet R, Boily J. 1995. Mortality of balsam fir and black spruce advance growth 3 years after clear-cutting. *Canadian Journal of Forest Research* **25**, 1528-1537.

Sage RF. 2002. How terrestrial organisms sense, signal, and respond to carbon dioxide. *Integrative and Comparative Biology* **42**, 469–480.

Sala A. 2009. Lack of direct evidence for the carbon-starvation hypothesis to explain drought-induced mortality in trees. *Proceedings of the National Academy of Sciences of the United States* **106**, E68.

Sala A, Piper F, Hoch G. 2010. Physiological mechanisms of drought-induced tree mortality are far from being resolved. *New Phytologist* **186**, 274-281.

Sala A, Woodruff DR, Meinzer FC. 2012. Carbon dynamics in trees: feast or famine? *Tree Physiology* **32**, 764-775.

Schaberg PG, Snyder MC, Shane JB, Donnelly JR. 2000. Seasonal patterns of carbohydrate reserves in red spruce seedlings. *Tree Physiology* **20**, 549-555.

Sperry JS. 2013. Cutting-edge research or cutting-edge artifact? An overdue control experiment complicates the xylem refilling story. *Plant, Cell & Environment* **36**, 1916–1918.

Stewart JD, Zine El Abidine A, Bernier PY. 1994. Stomatal and mesophyll limitations of photosynthesis in black spruce seedlings during multiple cycles of drought. *Tree Physiology* **15**, 57-64.

Topp GC, Zebchuk WD, Davis JL, Bailey WG. 1984. The measurement of soil water content using a portable TDR hand probe. *Canadian Journal of Soil Science* **64**, 313-321.

Turnbull MH, Murthy R, Griffin KL. 2002. The relative impacts of daytime and night-time warming on photosynthetic capacity in *Populus deltoides*. *Plant, Cell & Environment* **25**, 1729-1737.

Urli M, Porté AJ, Cochard H, Guengant Y, Burlett R, Delzon S. 2013. Xylem embolism threshold for catastrophic hydraulic failure in angiosperm trees. *Tree Physiolgy* **33**, 672–683.

Way DA, Sage RF. 2008a. Elevated growth temperatures reduce the carbon gain of black spruce [*Picea mariana* (Mill.) B.S.P.]. *Global Change Biology* **14**, 624–636.

Way DA, Sage RF. 2008b. Thermal acclimation of photosynthesis in black spruce [*Picea mariana* (Mill.) B.S.P.]. *Plant, Cell & Environment* **31**, 1250-1262.

Wheeler JK, Huggett BA, Tofte AN, Rockwell FE, Holbrook MN. 2013. Cutting xylem under tension or supersaturated with gas can generate PLC and the appearance of rapid recovery from embolism. *Plant and Cell Environment* **36**, 1938–1949.

Woodruff DR, Meinzer FC. 2011. Water stress, shoot growth and storage of non-structural carbohydrates along a tree height gradient in a tall conifer. *Plant, Cell & Environment* **34**, 1920-1930.

Zeppel MJB, Lewis JD, Chaszar B, Smith RA, Medlyn BE, Huxman TE, Tissue DT. 2012. Nocturnal stomatal conductance responses to rising [CO_2], temperature and drought. *New Phytologist* **193**, 929-938.

CHAPITRE IV

Compensatory mechanisms mitigate the effect of warming and drought on wood formation

RESEARCH PAPER

Title: Compensatory mechanisms mitigate the effect of warming and drought on wood formation

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Accepted in Plant Cell and Environment (DOI: 10.1111/pce.12689)

4.1 ABSTRACT

Due to global warming, high-latitude ecosystems are expected to experience increases in temperature and drought events. Wood formation will have to adjust to these new climatic constraints to maintain tree mechanical stability and long-distance water transport. The aim of this study is to understand the dynamic processes involved in wood formation under warming and drought. Xylogenesis, gas exchange, water relations and wood anatomy of black spruce [*Picea mariana* (Mill.) B.S.P.] saplings were monitored during a greenhouse experiment where temperature was increased during daytime or night-time (+6 °C) combined with a drought period. The kinetics of tracheid development expressed as rate and duration of the xylogenesis sub-processes were quantified using generalized additive models. Drought and warming had a strong influence on cell production, but little effect on wood anatomy. The increase in cell production rate under warmer temperatures, and especially during the night-time warming at the end of the growing season, resulted in wider tree-rings. However, the strong compensation between rates and durations of cell differentiation processes mitigates warming and drought effects on tree-ring structure. Our results allowed quantification of how wood formation kinetics is regulated when water and heat stress increase, allowing trees to adapt to future environmental conditions.

Key words: global warming, water deficit, xylogenesis, tree-ring structure, wood anatomy.

Symbols/abbreviations and units:

T0, greenhouse with a similar temperature to that of external air temperature; T+day, greenhouse with temperature 6 °C higher than T0 during the day; T+night, greenhouse with temperature 6 °C higher than T0 during the night; $\Psi_{pd,}$ pre-dawn leaf water potential; $\Psi_{md,}$ midday leaf water potential; g_s, stomatal conductance; A_{max}, maximum photosynthesis rate; DOY, day of the year; VWC, volumetric water content of soil; radial number of cambial (n_C), enlargement (n_E), lignification and wall thickening (n_W), mature (n_M), and total cells (n_{Total}); mean durations of cell enlargement (d_E, days) and wall thickening (d_W, days); cell production rate (r_C, cells day^{-1}); mean cellular rates of radial diameter enlargement (r_E, µm day^{-1}) and wall deposition (r_W , µm^2 day^{-1}); mean absolute error (MAE, cell), mean absolute percentage error (MAPE, %), model efficiency (EF, %).

4.2 INTRODUCTION

Since the mid-20th century, frequency, duration and magnitude of heat waves have increased (Perkins *et al.*, 2012), and recent climate projections predict temperatures of 3 to 6 °C higher by 2100 (IPCC, 2013). Several studies have reported that the recent warming is more pronounced at high latitudes (Vincent *et al.*, 2012, Wang *et al.*, 2013). In these regions, the night-time temperatures are changing faster than daytime temperatures (Donat *et al.*, 2013). In the early century, these trends were recorded for the Canadian boreal forest, with increases in night-time summer temperatures being reported by some authors (Bonsal *et al.*, 2001, Casati & De Elía, 2014). How these changes could influence tree growth and forest stand productivity in these regions remains an open question (Price *et al.*, 2013) in particular for the widely distributed black spruce [*Picea mariana* (Mill.) B.S.P.], the most important tree species of the North American boreal forest. Dendrochronological studies hypothesized that black spruce located at high latitude could benefit from global warming because of a lengthening of the growing season, while radial growth in the southern regions might be negatively affected by warming (Huang *et al.*, 2010).

An increase of regional precipitations has been observed in North America, but higher temperatures can lead to faster and greater evaporation with a consequent change in soil moisture and drought intensity (Donat *et al.*, 2013, Seneviratne *et al.*, 2012, Villarini *et al.*, 2013). In plants, water deficit induces a stress response that is modulated at different levels in tissues and organs depending on intensity and duration of the stress. Water deprivation provokes turgor loss in developing tissues. The decreasing of hydrostatic turgor pressure within the cell induces plant stress perception and responses (i.e. stomatal closure and/or decreasing of photosynthate production), and intermediate physiological changes (i.e. lower plant water potential and decrease in cell volume) (Kranner *et al.*, 2010, Savidge, 2001), finally resulting in a reduction of plant growth. Recent research showed that the response of secondary meristem to soil water depletion is faster and stronger than that of primary meristem (Berta *et al.*, 2009, Bogeat-Triboulot *et al.*, 2007, Giovannelli *et al.*, 2007). During plant development, the negative effect of abiotic stresses (multiple stresses) could be compensated by the recovery of stem growth and cambial activity or other wood traits. In this context, the plant meristem (i.e. cambium) displays different sensitivity according to the duration and intensity of stress, as well as different recovery capacity after a stress. The capacity of the cambium to resume normal activity after stress determines the capacity of the plant to compensate for the depressing effect of the stress on growth. Compensatory changes among architectural and structural traits and properties can result in tissue functions remaining unchanged, with the plant retaining the same level of performance (Lachenbruch & McCulloh, 2014). A comprehensive investigation of the relative importance of cambial activity and xylem traits under climate change thus appears crucial.

Wood formation (xylogenesis) is a complex and highly dynamic process characterized by successive phases of cell production and differentiation: cell division, cell radial enlargement, secondary cell-wall formation and wall lignification (hereafter regrouped under the term “wall thickening”), and programmed cell death (Denne & Dodd, 1981). This mechanism is fundamental for lignophyte functioning, because xylem structure provides mechanical stability and long-distance water transport. Regarding this, the number of studies on wood formation in adult trees has increased considerably in recent years (Gričar *et al.*, 2007). Many studies provide interesting insights into wood formation and the influence of environmental conditions, such as drought (Gričar *et al.*, 2006, Moser *et al.*, 2010, Oberhuber & Gruber, 2010), warming and drought (Balducci *et al.*, 2013, Fonti *et al.*, 2013) and ozone (Richet *et al.*, 2012). However, such intra-annual investigations, although accurate, are often limited to static views of the processes, which are insufficient to describe the dynamic processes occurring during secondary growth and to unravel the finer mechanisms of environmental influences. An accurate quantification of the dynamics of xylogenesis indeed appears to be a necessary endeavor to better understand environmental influences, as it comprises the mechanisms through which climatic factors are recorded in tree-ring structure (Cuny *et al.*, 2014). For example, it is the duration and rate of cell enlargement that determine the final cell radial diameter, while the duration and rate of wall-material deposition determine the amount of secondary cell-wall. In turn, the ratio between the total amount of wall material deposited for one cell and the final size of this cell, determine the changes in lumen dimension (diameter and area), wall thickness, and wood density profile along conifer tree-rings (Cuny *et al.*, 2014). A precise assessment of the kinetics (i.e. timings, durations and rates) of cell development is therefore crucial to obtain a mechanistic understanding of how stresses influence wood anatomy and, as a result, plant functioning and performance. In this sense, recent works have demonstrated that

Generalized Additive Models (GAMs) are particularly suitable to precisely and fully characterize wood formation dynamics (Cuny *et al.*, 2013), opening a door to the mechanistic understanding of how climatic variations, extreme events and, more generally, environmental stresses influence wood formation and the resulting tree-ring structure.

Among environmental factors, temperature plays a crucial role, because it is the main abiotic driver directly influencing cambium resumption in the stem (Begum *et al.*, 2010, Begum *et al.*, 2013, Oribe *et al.*, 2001). However, the well-known temperature effects are limited in time and restricted to cambium reactivation within the stem. Recent researches have described the various effects on whole plants: warming decreased carbon storage in the xylem and the imbalance of plant water status during xylogenesis can influence the sugars available for radial growth (Deslauriers *et al.*, 2014). Photosynthesis (i.e. the main carbon source) can be greatly reduced during drought, and it has been shown that this is more marked under higher temperatures (Balducci *et al.*, 2015). In this context, the need to understand the detailed mechanisms of xylogenesis under controlled drought and warming becomes a priority. The hydromechanical response to water stress implies first a reduction in cell expansion (Proseus *et al.*, 2000), and then, under prolonged water stress, a decline in cambial cell division (Abe *et al.*, 2003, Muller *et al.*, 2011, Pantin *et al.*, 2013). Saplings can be especially vulnerable to drought (de Luis *et al.*, 2011, Rossi *et al.*, 2009) and major susceptibility is observed in boreal species (Balducci *et al.*, 2015, Peng *et al.*, 2011). Past studies considered that the rate of growth is influenced by environment, in particular the total cell production

(Denne & Dodd, 1981, Horáček *et al.*, 2003). A recent study showed that radial growth and cambium activity were highly sensitive to water imbalance, but, after re-watering, surviving plants were able to resume radial growth showing certain resilience to abiotic stresses (Balducci *et al.*, 2013). All these studies highlighted the effect of temperature and water stress on xylogenesis but without clarifying in detail which sub-processes were more altered and which were more involved in the growth recovery.

The aim of this study is to unravel the mechanisms by which water deficit in combination with night and day warming affect the sub-processes of xylogenesis and to understand how these influences are recorded in the tree-ring structure. We monitored the dynamics of wood formation, assessed the kinetics of cell differentiation and measured the resulting tree-ring structure in saplings of black spruce. Preliminary results have shown that in a multi-stress experiment (drought and warming) cambium was more affected by drought during stress imposition whilst warmer temperature determined higher cambium activity in the irrigated condition. We tested the following hypotheses that i) water deficit and warming will modify the rate and duration of xylogenesis (cell division, enlargement and cell wall thickening), inducing lower changes in the tracheid lumen, diameter and cell wall thickness and ii) daytime and night-time warming can limit the kinetics of xylogenesis after the resumption of irrigation in stressed plants, determining a compensatory effect of stem growth and mitigating the effect of water stress on xylem traits.

4.3 MATERIALS AND METHODS

4.3.1 Experimental design

The experiment was conducted during the 2011 growing season in Chicoutimi, QC, Canada (48° 25’ N, 71° 04’ W, 150 m above sea level) on 4-year-old black spruce saplings. Saplings were of local provenance (LU3NEPN07-G41) obtained from « Pépinière de Sainte-Luce », a public forest nursery in Sainte-Luce, QC, Canada (48° 33' N 68° 23' W). In the summer of 2010, the saplings were put into plastic reversed-conic pots (volume 4.5 l) filled with peat moss, perlite and vermiculite and maintained in the open air close to a greenhouse until spring 2011. Saplings were watered regularly to limit the impact of the transplanting. In April 2011, 1104 saplings of uniform size (53 ± 8.8 cm in height and 10.4 ± 1.8 mm in diameter at the collar) were selected, fertilized (with 1 g l^{-1} of NPK 20-20-20 fertilizer dissolved in 500 ml of water), and transferred into three greenhouses. During the experiment, the saplings were grown in the same plastic reversed-conic pots (volume 4.5 l) under natural daylight and local photoperiod. Inside the greenhouses, dew point was maintained with an automatic control system between approximately 11 and 14 °C. Three thermal treatments were applied: the control (named T0 and in which greenhouse temperature was equal to external air temperature), and two warming treatments named T+day and T+night, with temperature 6 °C warmer than T0 during the day (7.00 am - 19.00 pm) and during the night (19.00 pm - 7.00 am), respectively. Two irrigation treatments were applied for each thermal condition: (i) control (irrigated saplings), in which soil water content was maintained above 80% of field capacity; and (ii) water deficit (non-irrigated saplings) in which irrigation was withheld for 25 days in June. So in total there were six combinations of treatments (3 thermal conditions × 2 irrigation regimes).

4.3.2 Water relations, gas exchanges and CO_2 assimilation

Water relations, gas exchange and CO_2 assimilation were measured from May to August on branches of the first whorl of 3 saplings for the 6 treatments. Pre-dawn [Ψ_{pd}] and midday [Ψ_{md}] leaf water potential were measured using a pressure chamber (PMS Instruments, Corvalis, OR). Gas exchange and CO_2 assimilation (stomatal conductance g_s, mol m^{-2} s^{-1} and maximum photosynthesis rate, A_{max}, µmol m^{-2} s^{-1}) were measured from 10.00 am to 13.00 pm under saturating irradiance conditions (1000 µmol m^{-2} s^{-1}) using a portable photosynthesis system (Li-6400, LI-COR Inc., Lincoln, NB). Air temperature, vapor pressure deficit, CO_2 concentration and irradiance inside the chamber were maintained at 25 °C, 2.2 kPa (except at 29 °C when it reached 3.7 kPa), 400 µmol mol^{-1} and 1000 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD), respectively. Measurements were expressed according to the specific needle surface area computed as ratio of needle dry mass per unit of needle surface area and using a regression (Bernier *et al.*, 2001). For the same saplings, the volumetric water content (VWC) of the soil was measured weekly by time domain reflectometry (TDR Fieldscout 300). The measurements were taken twice per pot at 7 cm depth in the same moment (Topp *et al.*, 1984).

4.3.3 Wood formation dynamics

A destructive sampling of 6 saplings, randomly selected each week from every treatment (i.e. 36 saplings per week), was performed from May to October. Stem disks were collected 2 cm above the root collar of each sapling. The wood sections were stained with cresyl violet acetate (0.16% in water) and examined within 10-25 min with visible and polarized light at 400-500× magnification to distinguish the differentiation of xylem according to four distinct phases. For each section, the radial number of cambial (n_C), enlarging (n_E), wall thickening (n_W), and mature cells (n_M) were counted along three radial files. The total number of xylem cells was calculated as the sum of differentiating and mature cells ($n_{Total} = n_E + n_W + n_M$). In the cambial zone, the cells were characterized by thin cell walls and small radial diameters (Rossi *et al.*, 2006). Enlarging cells were bigger than cambial cells but were still surrounded by thin primary walls. Cells in the wall thickening zone undergo secondary wall formation and lignification. Criteria for discriminating wall thickening in cells were the birefringence under polarized light, which indicated the presence of secondary cell wall, and the coloration due to the reaction of cresyl violet acetate with lignin, which produced a color change from violet to blue when lignification was complete (Rossi *et al.*, 2006). Thus, when a homogeneous blue color was present over the whole cell wall, cells were considered mature (Rossi *et al.*, 2006).

4.3.4 Characterization of the dynamics of wood formation

In order to accurately characterize the dynamics of wood formation, a statistical approach was used based on generalized additive models (GAMs) developed by Cuny *et al.* (2013). A GAM is a generalized linear model (GLM) in which the linear predictor partly depends on some unknown smooth functions (Wood, 2006). The strength of GAMs lies in their flexibility, i.e. their ability to deal with non-linear and non-monotonic relationships between the response and the set of explanatory variables (GAMs are referred to as being data-driven). Because of this flexibility, GAMs have proved to be far more appropriate than parametric approaches (e.g. GLMs) to model the complex non-linear and non-monotonous patterns that characterize wood formation dynamics (Cuny *et al.*, 2013).

For each treatment, GAMs were fitted to the number of cells recorded in the cambial, enlargement, wall thickening, and mature phases using the mgcv package (Wood, 2006) of the R statistical software (R Development Core Team, 2011). The average cell numbers predicted by the GAMs were then used to quantify the kinetics (timing, duration and rate) of the developmental phases (cell enlargement and cell-wall thickening) for the different tracheids produced. First, the dates of entry of tracheids into each development phase (cell enlargement, wall thickening, and mature phases) were computed. From these dates, the durations of cell enlargement (d_E, days) and wall thickening (d_W, days) were calculated for the successive tracheids. The mean rates of cell differentiation (radial diameter enlargement rate r_E in µm day^{-1}, and wall deposition rate r_W in $µm^2$ day^{-1}) were then estimated for each tracheid by dividing its final dimensions (cell radial diameter and wall cross-sectional area, respectively) by the time it spent in the corresponding phases (d_E and d_W, respectively). In addition, the daily rate of cell production was calculated as the difference between the total numbers of cells predicted by GAMs for two consecutive days.

4.3.5 Woody cells features

In order to measure cell features, stem disks of 36 saplings (6 saplings × 3 thermal conditions × 2 irrigation regimes), randomly collected during the last three weeks in October, were stained with safranine (1% in water) and fixed on slides with a mounting medium. Digital images were recorded using a camera mounted on a microscope to measure xylem features along three paths using WinCell^{TM} (Regent Instruments Inc., Canada). For each cell along the paths, lumen radial diameter, lumen area, and cell-wall radial thickness were measured. The cell radial diameter was calculated as the sum of the lumen radial diameter and the double cell-wall radial thickness, and the wall cross-sectional area was estimated based on a constant ratio of 1.2 between the tangential and radial wall thickness (Rathgeber *et al.*, 2006, Skene, 1972) and assuming rectangular-shaped tracheids (see Cuny *et al.* (2014), for detailed explanations). To highlight the cell anatomical patterns in tree-ring structure by removing high-frequency variability, cell diameter, cell wall area, lumen area, and wall thickness were smoothed with GAMs using the mgcv package (Wood, 2006) of the R statistical software (R Development Core Team, 2011).

4.3.6 Statistical analysis

Means of predawn leaf water potential, stomatal conductance and maximum photosynthesis rate were compared between irrigation regimes with t-tests before, during and after water deficit. The comparisons among thermal conditions were conducted using GLM procedure in SAS (SAS Institute, Cary, NC, USA). Multiple means comparison was performed using Tukey's test ($P < 0.05$), and PDIFF option of the GLM procedure (Quinn & Keough, 2002).

The goodness of fit of the GAMs was assessed using the mean absolute error (MAE) (Cuny *et al.*, 2013, Willmott & Matsuura, 2005), and the mean absolute percentage error (MAPE, %). In addition to this, the model efficiency (EF, %) was computed (Cuny *et al.*, 2013).

The means of cell number between the different zones (cambial, enlargement, wall thickening and mature zone), the duration and rate of the developmental phases (cell production, enlargement and cell-wall thickening), and also woody cells features were compared using two-way analysis of variance (ANOVA procedure in SAS) with Tukey's test ($P \leq 0.05$).

Spearman’s rank correlations were used to assess the monotonic relationship between the mean rates of cell differentiation (cell production rate by cambial zone, r_C cells day^{-1}; radial diameter enlargement rate, r_E, µm day^{-1}, and wall deposition rate, r_W , µm^2 day^{-1}) and daily mean temperature (°C) (Quinn & Keough, 2002).

4.4 RESULTS

4.4.1 Environmental conditions

At the beginning of the experiment, the average daily air temperature in T0 greenhouse was 7 °C and increased to 12 °C in spring (April-May) (Fig. 1). Daily mean temperatures T+day and T+night were 4.5 and 5.2 K warmer on average than the T0 (external air temperature) treatment, and followed an evolution parallel to the T0 during all the experiment. Maximum temperatures in T0 (around 24 °C) were reached in July. The temperature then gradually decreased to a minimum of 3.8 °C in October (Fig. 1). During the water deficit period, which lasted 25 days (from DOY 158 to 182), temperature in T0 ranged between 14 °C and 22 °C.

Volumetric water content (VWC) in non-irrigated saplings decreased in all thermal conditions during the water deficit period (Fig. 2). From DOY 183, VWC increased quickly after the resumption of irrigation, reaching field capacity on DOY 200 (Fig. 2). After this, the VWC remained at field capacity until the end of October.

4.4.2 Saplings water relations, gas exchanges and CO_2 assimilation

From April to May, all saplings showed optimal water status (predawn leaf water potential at -0.5 MPa (Table 1). A similar pattern was observed in irrigated regimes for gas exchange and CO_2 assimilation: mean average stomatal conductance (g_s) was 0.13 mol m^{-2} s^{-1} and maximum photosynthesis rate (A_{max}) 7.4 µmol m^{-2} s^{-1} (Table 1). About twenty days after withholding irrigation, the predawn leaf water potential of non-irrigated saplings was lower than control saplings (-2.2 MPa versus -0.04 MPa, respectively) (with significant differences between irrigation regimes during the dry period, $P < 0.001$, Table 1). From DOY 171 to 181, lower values of Ψ_{pd} were observed in non-irrigated saplings, with values reaching -2.1 MPa in T0, -1.97 MPa and -2.38 MPa in T+day and T+night, respectively. During the dry period, significant differences were observed between T0 and T+night ($P < 0.001$) (Table 1). On DOY 166 until the end of the dry period (DOY 182), A_{max} of non-irrigated saplings decreased from about 9.42 to -0.03 μmol CO_2 m^{-2} s^{-1} in all thermal conditions. A faster decline of A_{max} was observed at higher day and night temperatures (T+day and T+night) with significant differences between irrigation regimes and among temperature treatments ($P < 0.0001$) (Table 1). During the dry period, g_s values declined, and were 0.02 fold lower under warmer conditions than control ($P < 0.0001$, Table 1). Average values of g_s ranged from 0.13 to 0.03 mol m^{-2} s^{-1}, for irrigated and non-irrigated saplings, respectively. After the resumption of irrigation the timing of the recovery of saplings was significantly affected by thermal regime. The non-irrigated saplings needed one week for the recovery of midday leaf water potential, optimal gas exchange, and CO_2 assimilation in control conditions (T0). The recovery of predawn leaf water potential took two weeks in T+day and four weeks in T+night. In non-irrigated saplings, A_{max} started to increase after one week in T0, two weeks in T+day and after four weeks in T+night. However, in non-irrigated saplings gas exchange never recovered pre-stress levels in either temperature treatment.

4.4.3 Wood formation dynamics

GAMs functions gave a good fit of the change in cell numbers in the different development phases with model efficiency varying between 53 and 78% (EF, Table 2). The MAE increased with the stage of cell differentiation (i.e. from cambium to mature cell) reflecting the variance increase in the number of formed cells (N_m and N_{Total}) among saplings (Table 2 and Fig. 3). In May, when the experiment started, cell division was already active in all treatments as 6-7 cells were observed in the cambial zone (Fig. 3). The GAMs showed a curved shape with a peak at the end of May and a progressive decrease around the end of August and beginning of September at warmer temperatures and T0, respectively. The end of cell production was reached when the number of cells in the cambial zone returned to similar values to that recorded during the dormant state based on anatomical observations. The number of cambial cells did not change significantly between irrigation regimes, but temperature was found to have a significant effect during the water deficit period (Table 3). The number of cells in enlargement increased rapidly from the beginning of the experiment to the end of May in all treatments. The peak of cell enlargement ranged from the end of May to the beginning of June at warmer temperature and T0, respectively, but without significant effect. During water deficit imposition, the number of cells in enlargement decreased significantly faster for non-irrigated seedlings, and then remained stable for 3-4 weeks after water resumption in all thermal conditions, suggesting that fewer tracheids were entering differentiation (Fig. 3). After water resumption, the number of cells in enlargement showed significant differences.

In all thermal conditions, the seasonal dynamics of the number of cells in the wall thickening phase in non-irrigated saplings showed bimodal curves with a first peak at the end of May and a second higher peak after the water deficit period (Fig. 3). In contrast, the number of cells undergoing secondary wall formation and lignification reached a maximum at the beginning of July in irrigated saplings (Fig. 3). Significant differences in the number of cells in wall thickening were detected between irrigation regimes (Table 3). In non-irrigated saplings, the number of mature cells increased in the same manner as irrigated ones until the beginning of June, but the increase slowed down for several weeks during the water deficit. Significant differences were observed in mature and total cells between irrigation regimes and among thermal conditions (Table 3).

4.4.4 Kinetics of tracheid development

In non-irrigated saplings, the rate of cell production by cambium varied in time, showing a bimodal pattern. The rate followed a pronounced and significant decrease throughout the drought period (Table 4) even reaching 0 at the end, but was able to resume as soon as irrigation restarted. The second drop corresponded to the normal end of the growing season (Fig. 4). The significant drop in cell production rate during drought explained the slowdown in the cell increment patterns of non-irrigated plants (Table 4): the total number of cells stopped increasing during drought while it continued to increase for the irrigated saplings (Fig. 3). Irrigated saplings presented three changes in the growth rate (Fig. 4). A first peak was observed at the end of May, in correspondence to the culmination of the number of cells in cambium and in enlargement. A second major peak occurred at the beginning of July when the number of cells in the wall thickening phase increased. The small dip at the end of August (T0) was more pronounced in T+day at a temperature higher than 23 °C, and a third peak occurred at the beginning of September in T+night, when night-time temperature ranged between 19 and 24 °C (Fig. 4).

The warming and drought treatment affected the duration and rates of cell enlargement. The d_E values of irrigated saplings exhibited very few changes during the growing season and reached a maximum of 8-9 days in mid-July, while d_E values of non-irrigated plants increased about 3 fold (from 5 to 18 days) during water deficit, and then returned to the initial value (around 5 days) two weeks after the resumption of irrigation (Fig. 4). During the dry period, the increase of d_E was more pronounced in non-irrigated saplings at T0 than warmer temperatures, although this difference was not statistically significant. The significant decrease in d_E was more pronounced for T+night (19%) than in T0 and T+day (Table 4). During the water deficit, however, r_E of non-irrigated saplings dropped about 3 fold (from 3 to 1 µm day^{-1}) and was significantly lower than in irrigated saplings. Significant increases were observed in r_E of T+night and T+day compared with T0, with 38% and 18% of change, respectively. Significant differences were observed between irrigation regimes for the duration of the enlargement phase: d_E in non-irrigated saplings was on average 21% longer than in irrigated saplings (5.17 days versus 4.29 days, respectively), but the rate of enlargement decreased by 11% (4.23 versus 4.76 µm day^{-1}, Table 4).

During the drought period, the duration of cell-wall thickening (d_W) in non-irrigated saplings more than doubled (from 10 to 20-28 days) with a less pronounced increase at warmer day and night conditions, whereas it stayed constant at the beginning (around 11 days) in irrigated saplings, increasing specially at the end of growing season (Fig. 4). Two weeks after the resumption of irrigation, the d_W in non-irrigated saplings returned to being similar to control ones (around 10 days). However, while d_W increased in non-irrigated saplings during water deficit, the rate of cell wall deposition (r_W) decreased about 2 to 3 fold (from 15 to 5 μm^2 day^{-1}) and was 37% lower than irrigated saplings. During daytime and night-time warming, d_W values showed a significant effect of temperature and was on average 22% and 24% shorter than T0, respectively (Table 4). The cell wall deposition rate was 8% lower in non-irrigated than in irrigated saplings (17.7 vs. 16.2 μm^2 day^{-1}, respectively, $P<0.0001$, Table 4). The irrigated saplings growing in the warmer conditions (T+night and T+day) showed similar patterns of r_W with 2 abrupt peaks in mid-July and mid-September, while temperature increased ranging between 19 and 25 °C. Positive correlation was observed between day warming and cell wall deposition rate (Table 6). During day and night warming significant differences were observed in $r_{W,}$ which was 14% and 28% faster than in T0 on average (Table 2).

4.4.5 Woody ring features

In general, mature xylem cells in irrigated saplings were only 4% larger than in non-irrigated saplings (on average, 16.7 vs. 16.0 µm in radial diameter, respectively). Before water deficit imposition, cell diameter exhibited similar patterns in all treatments (Fig. 5). Twenty days after re-watering, only non-irrigated saplings in warmer conditions presented lower values than irrigated ones. From the end of July until the end of October, saplings growing in warmer conditions showed higher values than T0.

Cells of irrigated saplings exhibited 12% more cell wall area on average than those of non-irrigated saplings (167 vs. 148 µm^2 in wall cross area, respectively). The patterns of variation in cell wall area were similar among thermal conditions until the first week of water deficit imposition. At the end of water deficit (from DOY 170 to 181), the wall cross area of tracheids in non-irrigated saplings was significantly lower than that in irrigated saplings (Table 5). In addition, significant differences were observed in wall cross area according to thermal conditions ($P<0.0001$, Table 5). At T0, irrigated saplings reached 200 µm^2 at the end of July and then decreased to around 50 µm^2. Under warmer temperature, the cell wall area of irrigated saplings presented a bimodal pattern with two peaks in mid-July and the end of August (Fig. 5). In non-irrigated saplings, cell wall area ranged from 130 to 150 µm^2 from the middle of the water deficit period until the end of July, and then showed maximum values shifted to September.

Lumen area was slightly but significantly different between irrigation regimes ($P<0.0001$, Table 5). Because they contained more wall material, cells of irrigated saplings had a lumen area 5% lower on average than cells of non-irrigated saplings (130 vs. 136 µm^2, respectively). The cells lumen area of the irrigated saplings at T0 progressively decreased from 250 µm^2 to 20 µm^2 or less along tree ring. In warmer conditions, cell lumen area of non-irrigated saplings remained stable during water deficit with values ranging around 150 μm^2 until mid-July, and then reduced to 20-30 μm^2 at warmer conditions at the end of autumn (Fig. 5).

Cell wall thickness also differed slightly but significantly according to the irrigation regime ($P < 0.0001$, Table 5). Indeed, because they contained more wall material for almost the same size, cells of irrigated saplings had walls about 11% thicker than those of non-irrigated saplings. From DOY 158 to DOY 166, cell wall thickness was quite similar between all treatments (reaching around 2 µm, Fig. 5). From DOY 170, the values remained at about 2 µm without increasing for non-irrigated saplings. By comparison, cell wall thickness of irrigated saplings kept increasing (T0) or reached values of about 2.5 to 3 µm in T+day and T+night. The maximum cell wall thickness was reached at the end of October, and was 3.6 µm in non-irrigated saplings and 4 µm in irrigated saplings.

4.4.6 Effects of temperature on rates of cell differentiation

The value of cell production rate (r_C) increased with the increasing of temperature during the growing season with correlation ranging between 0.37 and 0.52 depending of the treatments (Table 6). The highest correlations in r_C were found for T+night of irrigated saplings followed by T+day in non-irrigated saplings. The highest correlation in r_E was found for T+day of irrigated saplings. No significant correlation was observed in r_E for T+night in irrigated saplings. In irrigated saplings, wall deposition rate was positively correlated only with T+day, while in non-irrigated saplings r_W was correlated with T+night.

4.5 DISCUSSION

4.5.1 Warming and drought influences on xylem cell production

The imposition of 25 days of water deficit strongly decreased cell production rate in non-irrigated saplings at all thermal conditions. Cell division was affected by water deficit, as observed in previous studies on young trees (de Luis *et al.*, 2011, Rossi *et al.*, 2009). We demonstrated that under reduced water availability, cell production was strongly inhibited. At the end of water deficit, the rate of cell production was reduced by 86% in non-irrigated saplings. In situ observations show that conifers in dry environments produce fewer cells and so thinner tree-rings (Camarero *et al.*, 2010, Vieira *et al.*, 2013). Some of them, like Scots pine, can even stop cambial activity earlier when drought is too severe (Eilmann *et al.*, 2011, Gruber *et al.*, 2010, Ren *et al.*, 2015). However, during our experiment, we observed that cambium is highly plastic and can resume cambial activity as soon as conditions again become favorable for growth. For mature black spruce, Rossi *et al.* (2014) observed a greater variability in rate of cell production due to the site (from 0.2 to 1.1 cells day^{-1}), where a thermal latitudinal gradient differently affected the duration of cell differentiation and number of formed cells. Although those values appear similar to the results obtained in this study, we demonstrate that the application of a combination of abiotic stresses (temperature and water availability) strongly influenced cell production rate in a specific time of the growing season, suggesting a synchronic balance between rate and duration in building the tree-ring.

Given that growth processes, such as cell division, do not respond linearly to temperature (Körner, 2006), we found that the rate of cell production at T+night and under water deficit did not decrease till zero. A very low rate of cell production was maintained, allowing a faster increase in cell division after the water deficit period. Previous studies proved that cell production occurs during the night (Saveyn *et al.*, 2007, Turcotte *et al.*, 2011) and the present study confirmed this trend. In addition, the irrigated saplings grown under warmer conditions maintained a high cell production rate at the end of the growing season, which was even doubled during night-time warming. This could explain the higher correlations observed between radial growth and night temperature.

4.5.2 Xylem cell differentiation compensation under drought and warming

The saplings responded to water deprivation by considerably decreasing the rates of all sub-processes of xylogenesis (cell division, cell enlargement and wall deposition), but these rate reductions were compensated by increases in the durations of the corresponding sub-processes. While the rates of enlargement and wall deposition decreased by about 3 fold during water deficit, the durations of these processes increased by 2-3 fold, thus this mitigated the negative impact of water deficit on tree-ring structure. The occurrence of such a compensatory effect could be caused by the necessity to maintain leaf efficiency and functionality, ensuring that enough water reaches the foliage for maintaining leaf physiological processes. The existence of compensatory mechanisms that limit the impact of biotic stress on plants has been already demonstrated. For example, when defoliation occurs, growth is suppressed and growth compensation has also been proposed to sustain physiological process like photosynthesis, stomatal conductance and senescence (Collin *et al.*, 2000, Iqbal *et al.*, 2012, Striker *et al.*, 2008).

For the first time, our investigations have unraveled how wood formation kinetics respond to abiotic stress. When water stress occurs, cell production rates fall, and newly produced cells no longer push differentiating cells out of their zone. Consequently, differentiating cells get "stuck" in the differentiation stages of expansion and wall thickening (Figure 3-4). Seen this way, the compensatory mechanism could be a consequence of the internal dynamics of the wood forming tissues and would be activated in order to complete, as well as possible, cell development when optimal water conditions resume. Turgor maintenance is required in all growth processes, from cell division to the deposition of cell wall polysaccharide (Pantin *et al.*, 2011, Proseus *et al.*, 2000). In the case of cell enlargement, an adequate turgor pressure for irreversible cell wall expansion was estimated at about 0.9 MPa (Génard *et al.*, 2001), a value representing the wall yielding threshold. In the absence of water deficit, this threshold is normally reached after sunset when the water again flows from the xylem to the living cells near the cambium (Steppe *et al.*, 2015). Although we did not measure turgor pressure, its values follow the same decreasing trend as water potential. As our measurements of both predawn and midday leaf water potential indicate values close to -2.2 MPa (see Table 1), the drop observed in the rate of cell enlargement could thus reflect the direct effect of low turgor pressure. These values were consistent with the physiological reaction observed in black spruce seedlings (Stewart & Bernier, 1995, Walsh *et al.*, 2015). The low water potential values are related with low osmotic potential caused by solute accumulation in the living cells (i.e. sugars, ions and proteins) (Major & Johnsen, 1999, Proseus *et al.*, 2000). In black spruce, the accumulation of raffinose, an osmotically active sugar, started at a water potential lower than -1 Mpa (Deslauriers *et al.*, 2014), possibly indicating the value at which the water started to flow from the living cells to xylem and the reduction of turgor pressure. Therefore, the cells "stuck" in the enlargement phase possibly increase the duration of this phase "awaiting" the adequate turgor pressure for cell growth.

In our study, drought decreased gas exchange during and after re-watering (g_s and A_{max} decline to zero at all thermal conditions, Table 1). The observed reductions in photosynthetic production in non-irrigated saplings thus implied a decrease in carbon availability, as observed in Deslauriers *et al.* (2014). Carbohydrates play a central role in the modulation of xylogenesis (Ainsworth & Bush, 2011); indeed sugars represent the primary resource for cell wall deposition (Deslauriers *et al.*, 2014, Giovannelli *et al.*, 2007, Pantin *et al.*, 2013). Carbon constraints could explain the compensatory effect occurring during cell wall formation. During prolonged water deficit, sugars are more likely to be sequestered for osmoregulation and cannot fully sustain cell wall deposition (Deslauriers *et al.*, 2014, Pantin *et al.*, 2013). Therefore, the non-availability of carbon for maintaining a high cell wall deposition rate is possibly compensated by an increase in process duration, ensuring the building of optimal cell walls.

We showed that the compensatory mechanisms, represented here by an increase in the duration over rate, mitigated the drought impact on xylem anatomy. Cuny *et al.* (2014) highlighted the relative contribution of duration and rate of xylogenesis sub-processes in the resulting tree-ring anatomy: cell enlargement duration contributed to 75% of change in cell diameter, while changes in cell wall thickness were principally attributed to changes in cell size (67%). Thus, cell diameter and lumen area were only slightly modified, while cell wall cross area and thickness partially decreased because the drop in the rate of wall deposition was not fully compensated by an increase in the duration of the process. This impact was evidenced in wood density by Balducci *et al.* (2015). However, the change in rate and duration of wall deposition contributes less (about 33%) to the wall thickness and wood density than cell size (Cuny *et al.*, 2014). This confirmed that the compensation in cell enlargement contributes to largely mitigate the impact of drought on wood anatomy. Under stressed conditions, the adaptation of the hydraulic system to drought involved the tree ring morphology. Indeed, under water deficit and warming, lighter value of wood density can reflect a lower carbon allocation for cell wall formation (Balducci *et al.*, 2015).

4.6 CONCLUSION

Innovative analyses of tree-ring development were performed in this study, providing new insights into the influence of water stress and warming on xylem formation and structure in black spruce. We demonstrate that xylogenesis sub-processes cope with changing water and thermal conditions in order to maintain the tree-ring anatomy. Indeed, we found the existence of compensatory mechanisms occurring in the wood formation process in response to abiotic stress. These compensatory effects limit the negative effects of multistress on xylem structure, a crucial trait of plant functioning providing both mechanical stability and water transport along the tree stem. This study highlights the contrasting sensitivity of tree-ring width and structure; the former being highly plastic and the latter very conservative. This strategy appears to be valuable for recovery after a water deficit. Further researches are needed to confirm these findings and these should focus on the carbon and turgor pressure threshold at which the compensatory mechanism starts to occur. In particular, it needs to be verified if the compensatory mechanism is a feature of adaptation in trees as in other species living in drier areas – such as some temperate and Mediterranean species – where smaller cells with a thick cell wall (De Micco *et al.*, 2007) and even a false ring (Battipaglia *et al.*, 2010) are formed during summer drought.

4.7 ACKNOWLEDGEMENTS

This study was funded by the Natural Sciences and Engineering Research Council of Canada and the Consortium Ouranos. We thank H. Morin, D. Gagnon, G. Savard, B. Allaire, C. Soucy, C. Bouchard, M. Beaulieu, M. Labrecque for their practical help and laboratory analyses. We thank C. Krause, associate editor and the anonymous reviewer for helpful comments. Additional thanks to A. Garside for checking the English text.

Table 4.1 Ecophysiological measurements conducted in black spruce saplings before, during and after the water deficit period at three thermal conditions (T0 control temperature; T+day, 6 °C higher daytime temperature; T+night, 6 °C higher night-time temperature). Mean (±SD) are reported for pre-dawn leaf water potential (Ψpd, MPa), CO_2 assimilation (maximum photosynthesis rate, Amax, µmol m^{-2} s^{-1}) and gas exchange (stomatal conductance gs, mol m^{-2} s^{-1}). *P* values of ecophysiological parameters were calculated between irrigation regimes (Water), among thermal conditions (Temp) and for interaction between irrigation regimes and thermal conditions (Water × Temp) and DOY (Day of the year). Significant effects (*P≤ 0.05)* are highlighted in bold.

		Mean values								
		Irrigated			Non-irrigated			*Effet (P value)*		
	Time (DOY)	T0	T+day	T+night	T0	T+day	T+night	Water	Temp	Water × Temp
Ψpd (MPa)	Before (152)	-0.50±0.10	-0.50±0.10	-0.48±0.02	-0.58±0.04	-0.4±0.10	-0.48±0.02	0.6194	0.1455	0.2633
	During (180)	-0.50±0.10	-0.50±0.06	-0.58±0.06	-1.30±0.70	-1.09±0.80	-2.28±1.20	**<.0001**	0.0905	0.166
	After (208)	-0.40±0.08	-0.30±0.08	-0.28±0.05	-0.29±0.00	-0.28±0.05	-0.28 ±0.15	**0.0001**	**0.0121**	**0.0332**
Amax	Before (152)	7.41±4.20	8.34±4.50	6.13±0.06	7.15 ±2.00	9.42 ±5.40	6.38 ±0.05	0.5078	**0.0231**	0.8371
(µmol m^{-2} s^{-1})	During (180)	5.37±0.30	3.90±1.10	6.70±1.80	0.51 ±0.90	0.41 ±0.50	-0.03 ±1.00	**<.0001**	**<.0001**	**<.0001**
	After (208)	7.20±2.50	6.57±0.90	11.37±0.40	9.08 ±1.90	5.66 ±0.90	6.9 ±1.90	**0.006**	**<.0001**	0.8099
gs	Before (152)	0.13±0.04	0.09±0.05	0.14±0.00	0.13 ±0.00	0.13 ±0.10	0.14 ±0.00	0.5322	0.2607	0.387
(mol m^{-2} s^{-1})	During (180)	0.07±0.01	0.02±0.01	0.12±0.02	0.01 ±0.00	0.06 ±0.01	0.03 ±0.00	**<.0001**	**<.0001**	**<.0001**
	After (208)	0.06±0.03	0.05±0.02	0.07±0.00	0.07 ±0.03	0.04 ±0.01	0.06 ±0.02	**0.0401**	**0.0002**	0.402

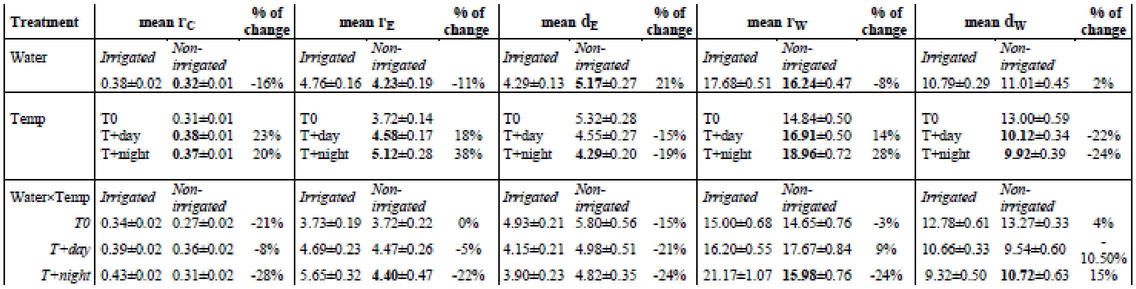
Table 4.2 Mean absolute error (MAE, cell), mean absolute percentage error (MAPE, %) and model efficiency (EF, %) calculated from the fittings of generalized additive model (GAMs) on the number of cells in the cambial, enlargement, wall thickening and mature zones for black spruce saplings before, during and after the water deficit at three thermal conditions (T0 control temperature; T+day, 6 °C higher daytime temperature; T+night, 6 °C higher night-time temperature).

Treatment	Zone	MAE (cell)	MAPE (%)	EF (%)
T0 × Irrigated	**Cambial**	1.00	17	62
	Enlargement	0.86	51	53
	Wall thickening	1.53	36	66
	Mature	8.26	23	78
T+day × Irrigated	**Cambial**	0.98	17	60
	Enlargement	0.81	48	55
	Wall thickening	1.53	36	53
	Mature	10.29	25	77
T+night × Irrigated	**Cambial**	1.06	18	58
	Enlargement	0.78	49	57
	Wall thickening	1.41	36	62
	Mature	11.11	27	74
T0 × Non-irrigated	**Cambial**	1.09	18	56
	Enlargement	0.80	50	58
	Wall thickening	1.41	40	59
	Mature	8.12	26	74
T+day × Non-irrigated	**Cambial**	1.10	18	51
	Enlargement	0.88	46	52
	Wall thickening	1.20	35	60
	Mature	7.46	26	75
T+night × Non-irrigated	**Cambial**	0.99	17	63
	Enlargement	0.84	53	51
	Wall thickening	1.34	39	55
	Mature	8.17	26	73
Overall Means	**Cambial**	1.04	18	58
	Enlargement	0.83	50	54
	Wall thickening	1.40	37	59
	Mature	8.90	26	75

Table 4.3 *P* values calculated between irrigation regimes (Water), among thermal conditions (Temp) and interaction between irrigation regimes and thermal conditions (Water × Temp) for cell number observed in the different zones of differentiation (cambial, enlargement, wall thickening, mature zone and total cells) in black spruce saplings before, during and after water deficit Significant effects (*P≤ 0.05)* are highlighted in bold.

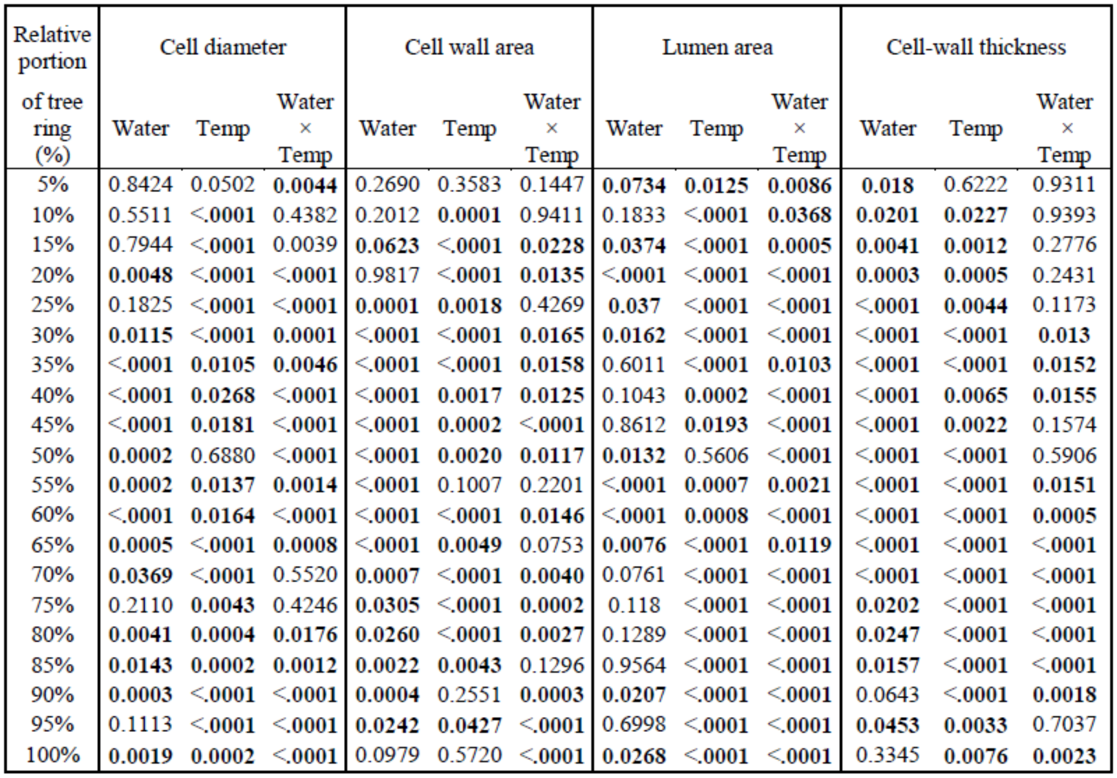
		***P* values**		
n (Cells)	**Time**	**Water**	**Temp**	**Water × Temp**
Cambial cells	Before	0.2936	0.5452	0.6711
	During	0.2514	**0.0100**	0.4005
	After	0.9459	0.394	0.5754
Enlargement cells	Before	0.6773	0.7151	0.186
	During	0.0755	0.4547	0.3951
	After	0.8011	**0.0067**	0.2515
Wall-thickening cells	Before	0.0726	0.0816	0.1279
	During	**0.0397**	0.4321	0.0595
	After	**<.0001**	**0.0017**	0.6267
Mature cells	Before	0.2652	**0.0253**	0.1019
	During	**0.0218**	0.2324	0.2214
	After	**<.0001**	**0.0006**	**0.0375**
Total cells	Before	0.1274	0.0542	0.1517
	During	0.2725	0.3637	0.2162
	After	**<.0001**	**0.0010**	0.0556

Table 4.4 Mean rate (±SE) of cell radial production by cambium (r_C, cell day^{-1}), cell enlargement (r_E, µm day^{-1}) and cell wall deposition (r_W µm^2 day^{-1}) before, during and after the water deficit period (Water, W) in black spruce saplings at three thermal conditions (Temperature, T). The mean duration is also reported for the phases of cell enlargement (d_E, days) and cell wall deposition (d_W, days). The percentages of change (%) were calculated between irrigation regimes (Water), among thermal conditions (Temp) and interaction between irrigation regimes and thermal conditions (Water × Temp). The percentage of change (%) indicates positive or negative changes on the rate and duration of wood formation. Significant effects (*$P \leq 0.05$)* are highlighted in bold.



Treatment	**mean r_C**		**% of change**	**mean r_E**		**% of change**	**mean d_E**		**% of change**	**mean r_W**		**% of change**	**mean d_W**		**% of change**
Water	*Irrigated*	*Non-irrigated*		*Irrigated*	*Non-irrigated*		*Irrigated*	*Non-irrigated*		*Irrigated*	*Non-irrigated*		*Irrigated*	*Non-irrigated*	
	0.38±0.02	**0.32**±0.01	-16%	4.76±0.16	**4.23**±0.19	-11%	4.29±0.13	**5.17**±0.27	21%	17.68±0.51	**16.24**±0.47	-8%	10.79±0.29	11.01±0.45	2%
Temp	T0	0.31±0.01		T0	3.72±0.14		T0	5.32±0.28		T0	14.84±0.50		T0	13.00±0.59	
	T+day	**0.38**±0.01	23%	T+day	**4.58**±0.17	18%	T+day	4.55±0.27	-15%	T+day	**16.91**±0.50	14%	T+day	**10.12**±0.34	-22%
	T+night	**0.37**±0.01	20%	T+night	**5.12**±0.28	38%	T+night	**4.29**±0.20	-19%	T+night	**18.96**±0.72	28%	T+night	**9.92**±0.39	-24%
Water×Temp	*Irrigated*	*Non-irrigated*		*Irrigated*	*Non-irrigated*		*Irrigated*	*Non-irrigated*		*Irrigated*	*Non-irrigated*		*Irrigated*	*Non-irrigated*	
T0	0.34±0.02	0.27±0.02	-21%	3.73±0.19	3.72±0.22	0%	4.93±0.21	5.80±0.56	-15%	15.00±0.68	14.65±0.76	-3%	12.78±0.61	13.27±0.33	4%
T+day	0.39±0.02	0.36±0.02	-8%	4.69±0.23	4.47±0.26	-5%	4.15±0.21	4.98±0.51	-21%	16.20±0.55	17.67±0.84	9%	10.66±0.33	9.54±0.60	-10.50%
T+night	0.43±0.02	0.31±0.02	-28%	5.65±0.32	**4.40**±0.47	-22%	3.90±0.23	4.82±0.35	-24%	21.17±1.07	**15.98**±0.76	-24%	9.32±0.50	**10.72**±0.63	15%

Table 4.5 *P* values for cell diameter, cell-wall area, lumen area and cell-wall thickness along relative portion of tree ring (%) in black spruce saplings during the experiment in 2011calculated between irrigation regimes (Water), among thermal conditions (Temp) and interaction between irrigation regimes and thermal conditions (Water × Temp). Significant effects ($P\leq 0.05$) are highlighted in bold.



Relative portion of tree ring (%)	Cell diameter			Cell wall area			Lumen area			Cell-wall thickness		
	Water	Temp	Water × Temp	Water	Temp	Water × Temp	Water	Temp	Water × Temp	Water	Temp	Water × Temp
5%	0.8424	0.0502	**0.0044**	0.2690	0.3583	0.1447	**0.0734**	**0.0125**	**0.0086**	**0.018**	0.6222	0.9311
10%	0.5511	<**.0001**	0.4382	0.2012	**0.0001**	0.9411	0.1833	<**.0001**	**0.0368**	**0.0201**	**0.0227**	0.9393
15%	0.7944	<**.0001**	0.0039	**0.0623**	<**.0001**	**0.0228**	**0.0374**	<**.0001**	**0.0005**	**0.0041**	**0.0012**	0.2776
20%	**0.0048**	<**.0001**	<**.0001**	0.9817	<**.0001**	**0.0135**	<**.0001**	<**.0001**	<**.0001**	**0.0003**	**0.0005**	0.2431
25%	0.1825	<**.0001**	<**.0001**	**0.0001**	**0.0018**	0.4269	**0.037**	<**.0001**	<**.0001**	<**.0001**	**0.0044**	0.1173
30%	**0.0115**	<**.0001**	**0.0001**	<**.0001**	<**.0001**	**0.0165**	**0.0162**	<**.0001**	<**.0001**	<**.0001**	<**.0001**	**0.013**
35%	<**.0001**	**0.0105**	**0.0046**	<**.0001**	<**.0001**	**0.0158**	0.6011	<**.0001**	**0.0103**	<**.0001**	<**.0001**	**0.0152**
40%	<**.0001**	**0.0268**	<**.0001**	<**.0001**	**0.0017**	**0.0125**	0.1043	**0.0002**	<**.0001**	<**.0001**	**0.0065**	**0.0155**
45%	<**.0001**	**0.0181**	<**.0001**	<**.0001**	**0.0002**	<**.0001**	0.8612	**0.0193**	<**.0001**	<**.0001**	**0.0022**	0.1574
50%	**0.0002**	0.6880	<**.0001**	<**.0001**	**0.0020**	**0.0117**	**0.0132**	0.5606	<**.0001**	<**.0001**	<**.0001**	0.5906
55%	**0.0002**	**0.0137**	**0.0014**	<**.0001**	0.1007	0.2201	<**.0001**	**0.0007**	**0.0021**	<**.0001**	<**.0001**	**0.0151**
60%	<**.0001**	**0.0164**	<**.0001**	<**.0001**	<**.0001**	**0.0146**	<**.0001**	**0.0008**	<**.0001**	<**.0001**	<**.0001**	**0.0005**
65%	**0.0005**	<**.0001**	**0.0008**	<**.0001**	**0.0049**	0.0753	**0.0076**	<**.0001**	**0.0119**	<**.0001**	<**.0001**	<**.0001**
70%	**0.0369**	<**.0001**	0.5520	**0.0007**	<**.0001**	**0.0040**	0.0761	<**.0001**	<**.0001**	<**.0001**	<**.0001**	<**.0001**
75%	0.2110	**0.0043**	0.4246	**0.0305**	<**.0001**	**0.0002**	0.118	<**.0001**	<**.0001**	**0.0202**	<**.0001**	<**.0001**
80%	**0.0041**	**0.0004**	**0.0176**	**0.0260**	<**.0001**	**0.0027**	0.1289	<**.0001**	<**.0001**	**0.0247**	<**.0001**	<**.0001**
85%	**0.0143**	**0.0002**	**0.0012**	**0.0022**	**0.0043**	0.1296	0.9564	<**.0001**	<**.0001**	**0.0157**	<**.0001**	<**.0001**
90%	**0.0003**	<**.0001**	<**.0001**	**0.0004**	0.2551	**0.0003**	**0.0207**	<**.0001**	<**.0001**	0.0643	<**.0001**	**0.0018**
95%	0.1113	<**.0001**	<**.0001**	**0.0242**	**0.0427**	<**.0001**	0.6998	<**.0001**	<**.0001**	**0.0453**	**0.0033**	0.7037
100%	**0.0019**	**0.0002**	<**.0001**	0.0979	0.5720	<**.0001**	**0.0268**	<**.0001**	<**.0001**	0.3345	**0.0076**	**0.0023**

Table 4.6 Spearman correlations coefficients between the mean rates of cell differentiation (cell production rate by cambium, r_C cells day^{-1}; radial diameter enlargement rate, r_E, µm day^{-1}, and wall deposition rate, r_W , µm^2 day^{-1}) and daily mean temperature (°C) during the greenhouse experiment in 2011. Thermal conditions are presented as control (T0), temperature increase during the day (T+day) and temperature increase during the night (T+night). Significant correlations ($P \leq 0.05$) are highlighted in bold.

Rate	Irrigated			Non-irrigated		
	T0	T+day	T+night	T0	T+day	T+night
r_C	0.48	0.34	0.52	0.47	0.49	0.37
r_E	0.33	0.55	0.27	0.39	0.33	0.42
r_W	0.05	0.40	0.18	-0.15	0.22	0.29

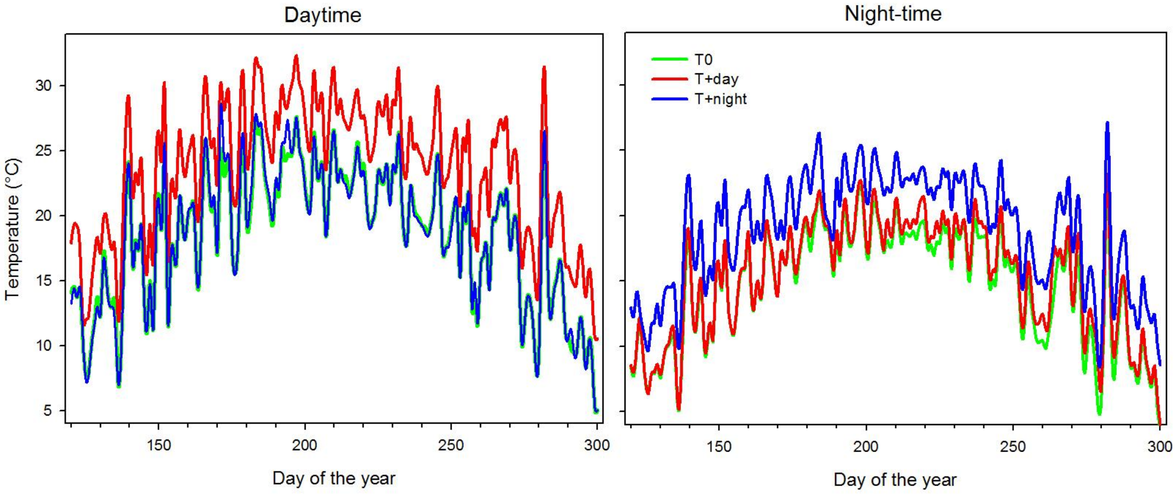


Figure 4.1 Mean of daytime (*left figure*) and night-time (*right figure*) temperatures experienced by black spruce saplings at the three thermal conditions during the greenhouse experiment.

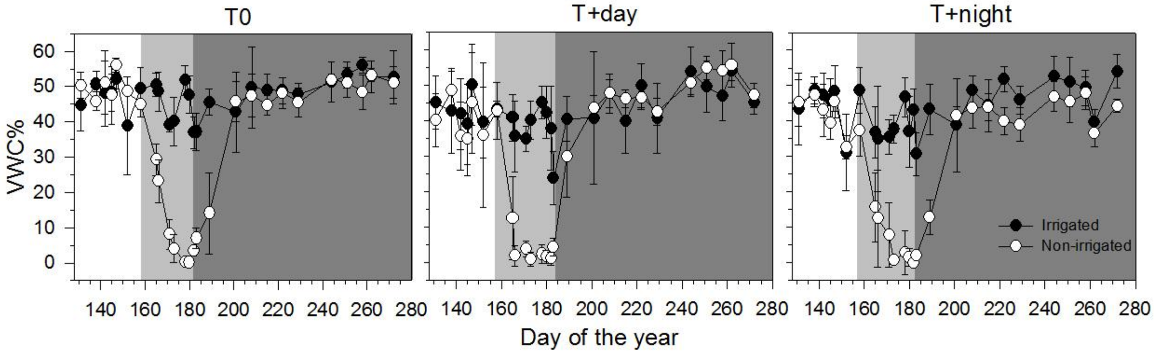


Figure 4.2 Volumetric water content (VCW) of soil in irrigated (I) and non-irrigated saplings (NI) before, during and after the water deficit period (*white, light grey and dark grey background*) at three thermal conditions (T0 control temperature; T+day, 6 °C higher daytime temperature; T+night, 6 °C higher night-time temperature) during the greenhouse experiment in 2011.

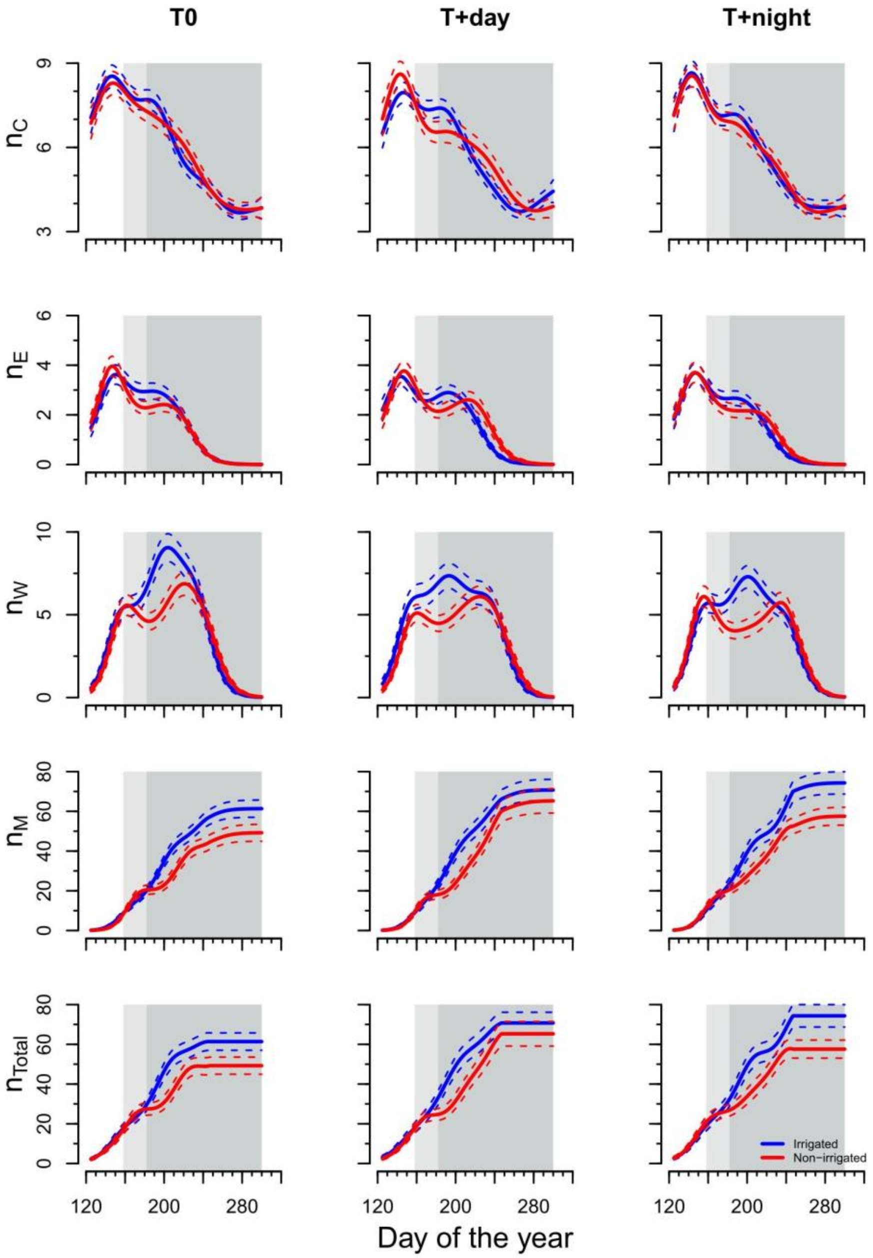


Figure 4.3 Radial number of cambial (n_C), enlargement (n_E), wall thickening (n_W), mature (n_M) and total cells (n_{Total}) in black spruce saplings before, during and after the water deficit period weekly (*white, light grey and dark grey)*. Blue and red curves indicate the mean number of cells in irrigated and non-irrigated saplings, respectively. Dotted blue and red curves delimit the 90% confidence intervals for irrigated and non-irrigated saplings, respectively.

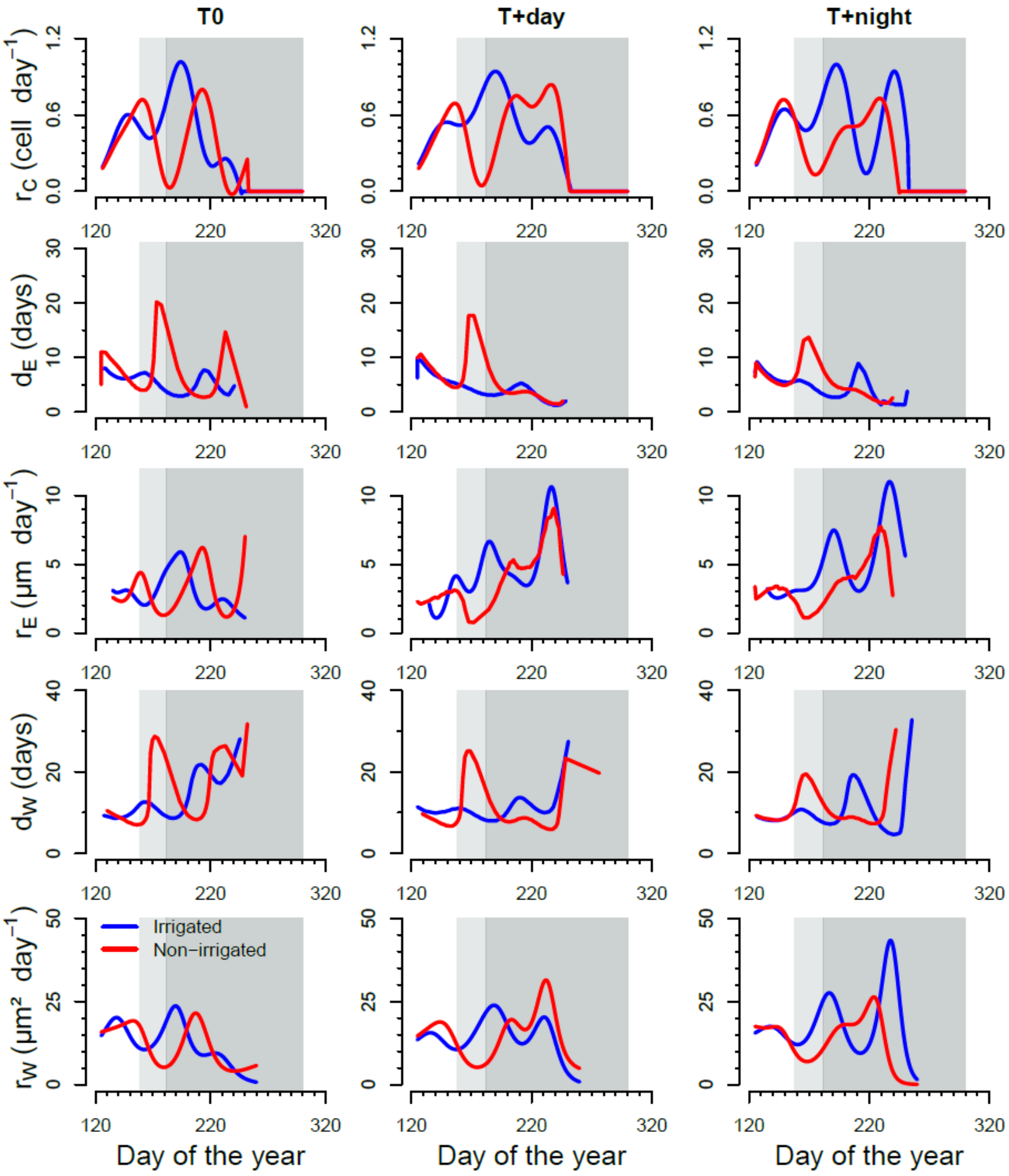


Figure 4.4 Rate of cell radial production (r_C) in the cambial zone, rates of enlargement (r_E) and rate of wall deposition (r_W), duration of enlargement (d_E) and duration of wall deposition (d_W) as computed from generalized additive models (GAMs). Blue and red curves indicate the number of cells in irrigated and non-irrigated saplings, respectively. White, light grey and dark grey background correspond to before, during and after the water deficit period, respectively.

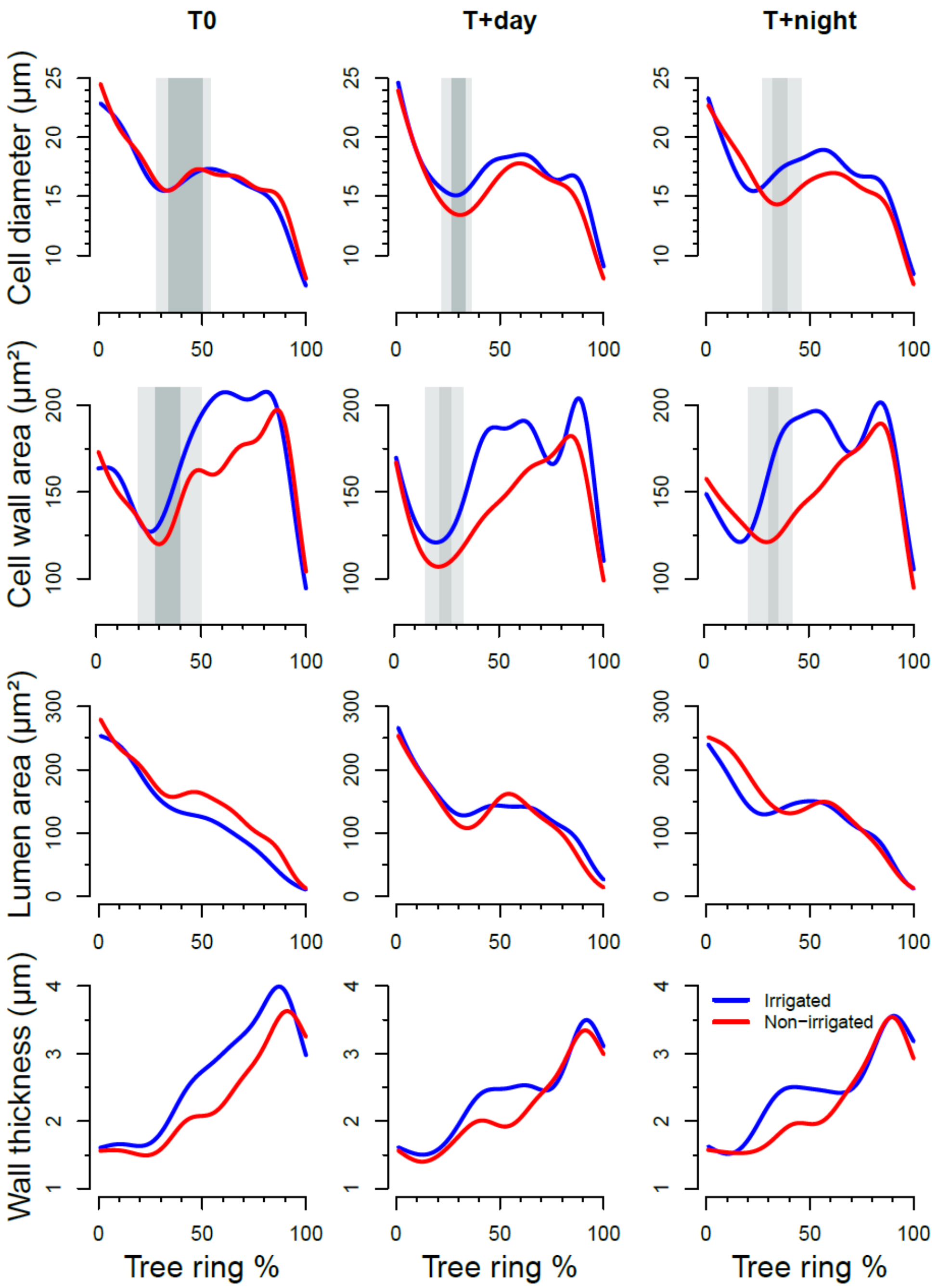


Figure 4.5 Cell features of the tracheids produced by irrigated and non-irrigated black spruce saplings at three thermal conditions (T0 control temperature; T+day, 6 °C higher daytime temperature; T+night, 6 °C higher night-time temperature) before, during and after the water deficit period. Blue and red curves indicate two irrigation regimes. Dark grey corresponds to cells with their entire enlargement (for curve of cell diameter) or their entire wall thickening (for curve of wall cross area) during water deficit.

4.8 REFERENCES

Abe H., Nakai T., Utsumi Y. & Kagawa A. (2003) Temporal water deficit and wood formation in *Cryptomeria japonica*. *Tree Physiology*, **23**, 859–863.

Ainsworth E.A. & Bush D.R. (2011) Carbohydrate export from the leaf: a highly regulated process and target to enhance photosynthesis and productivity. (Update on Carbohydrate Export from the Leaf) (Report). *Plant Physiology*, **155**, 64.

Balducci L., Deslauriers A., Giovannelli A., Beaulieu M., Delzon S., Rossi S. & Rathgeber C.B.K. (2015) How do drought and warming influence survival and wood traits of *Picea mariana* saplings? *Journal of Experimental Botany*, **66**, 377-389.

Balducci L., Deslauriers A., Giovannelli A., Rossi S. & Rathgeber C.B.K. (2013) Effects of temperature and water deficit on cambial activity and woody ring features in *Picea mariana* saplings. *Tree Physiology*, **33**, 1006–1017.

Battipaglia G., De Micco V., Brand W.A., Linke P., Aronne G., Saurer M. & Cherubini P. (2010) Variations of vessel diameter and δ^{13}C in false rings of *Arbutus unedo* L. reflect different environmental conditions. *New Phytologist*, **188**, 1099-1112.

Begum S., Nakaba S., Oribe Y., Kubo T. & Funada R. (2010) Cambial sensitivity to rising temperatures by natural condition and artificial heating from late winter to early spring in the evergreen conifer *Cryptomeria japonica*. *Trees*, **24**, 43–52.

Begum S., Nakaba S., Yamagishi Y., Oribe Y. & Funada R. (2013) Regulation of cambial activity in relation to environmental conditions: understanding the role of temperature in wood formation of trees. *Physiologia Plantarum*, **147**, 46–54.

Bernier P.Y., Raulier F., Stenberg P. & Ung C.-H. (2001) Importance of needle age and shoot structure on canopy net photosynthesis of balsam fir (*Abies balsamea*): a spatially inexplicit modeling analysis. *Tree Physiology*, **21**, 815–830.

Berta M., Giovannelli A., Potenza E., Traversi M.L. & Racchi M.L. (2009) Type 3 metallothioneins respond to water deficit in leaf and in the cambial zone of white poplar (*Populus alba*). *Journal of Plant Physiology*, **166**, 521—530.

Bogeat-Triboulot M.B., Brosche M., Renaut J., Jouve L., Le Thiec D., Fayyaz P., Vinocur B., Witters E., Laukens K., Teichmann T., Altman A., Hausman J.F., Polle A., Kangasjarvi J. & Dreyer E. (2007) Gradual soil water depletion results in reversible changes of gene expression, protein profiles, ecophysiology, and growth performance in *Populus euphratica*, a poplar growing in arid regions. *Plant Physiology*, **143**, 876-892.

Bonsal B.R., Zhang X., Vincent L.A. & Hogg W.D. (2001) Characteristics of daily and extreme temperatures over Canada. *Journal of Climate*, **14**, 1959-1976.

Camarero J.J., Olano J.M. & Parras A. (2010) Plastic bimodal xylogenesis in conifers from continental Mediterranean climates. *New Phytologist*, **185**, 471–480.

Casati B. & De Elía R. (2014) Temperature Extremes from Canadian Regional Climate Model (CRCM) Climate Change Projections. *Atmosphere-Ocean*, **52**, 191-210.

Collin P., Epron D., Alaoui-Sossé B. & Badot P.M. (2000) Growth responses of common ash seedlings (*Fraxinus excelsior* L.) to total and partial defoliation. *Annals of Botany*, **85**, 317-383.

Cuny H.E., Rathgeber C.B.K., Frank D., Fonti P. & Fournier M. (2014) Kinetics of tracheid development explain conifer tree-ring structure. *New Phytologist*, **203**, 1231-1241.

Cuny H.E., Rathgeber C.B.K., Senga Kiessé T., Hartmann F.P., Barbeito I. & Fournier M. (2013) Generalized additive models reveal the intrinsic complexity of wood formation dynamics. *Journal of Experimental Botany*, **64**, 1983-1994.

de Luis M., Novak K., Raventós J., Gričar J., Prislan P. & Čufar K. (2011) Cambial activity, wood formation and sapling survival of *Pinus halepensis* exposed to different irrigation regimes. *Forest Ecology and Management*, **262**, 1630–1638.

De Micco V., Saurer M., Aronne G., Tognetti R. & Cherubini P. (2007) Variations of wood anatomy and δ^{13}C within-tree rings of coastal *Pinus pinaster* showing intra-annual density fluctuations. *IAWA Journal*, **28**, 61–74.

Denne M.P. & Dodd R.S. (1981) The environmental Control of Xylem Differentiation. In: *Xylem Cell Development*, pp. 255.

Deslauriers A., Beaulieu M., Balducci L., Giovannelli A., Gagnon M.-J. & Rossi S. (2014) Impact of warming and drought on carbon balance related to wood formation in black spruce. *Annals of Botany*, **114**, 335-345.

Donat M.G., Alexander L.V., Yang H., Durre I., Vose R., Dunn R.J.H., Willett K.M., Aguilar E., Brunet M., Caesar J., Hewitson B., Jack C., Klein Tank A.M.G., Kruger A.C., Marengo J., Peterson T.C., Renom M., Oria Rojas C., Rusticucci M., Salinger J., Elrayah A.S., Sekele S.S., Srivastava A.K., Trewin B., Villarroel C., Vincent L.A., Zhai P., Zhang X. & Kitching S. (2013) Updated analyses of temperature and precipitation extreme indices since the beginning of the twentieth century: The HadEX2 dataset. *Journal of Geophysical Research: Atmospheres*, **118**, 1-16.

Eilmann B., Zweifel R., Buchmann N., Graf Pannatier E. & Rigling A. (2011) Drought alters timing, quantity, and quality of wood formation in Scots pine. *Journal of Experimental Botany*, **62**, 2763-2771.

Fonti P., Heller O., Cherubini P., Rigling A. & Arend M. (2013) Wood anatomical responses of oak saplings exposed to air warming and soil drought. *Plant Biology (Stuttg)*, **15 Suppl 1**, 210-219.

Génard M., Fishman S., Vercambre G., Huguet J.G., Bussi C., Besset J. & Habib R. (2001) A biophysical analysis of stem and root diameter variations in woody plants. *Plant Physiology*, **126**, 188.

Giovannelli A., Deslauriers A., Fragnelli G., Scaletti L., Castro G., Rossi S. & Crivellaro A. (2007) Evaluation of drought response of two poplar clones (*Populus×canadensis* Mönch ‘I-214’ and *P. deltoides* Marsh. ‘Dvina’) through high resolution analysis of stem growth. *Journal of Experimental Botany*, **58**, 2673-2683.

Gričar J., Zupančič M., Čufar K., Koch G., Schmitt U. & Oven P. (2006) Effect of local heating and cooling on cambial activity and cell differentiation in the stem of Norway spruce (*Picea abies*). *Annals of Botany*, **97**, 943-951.

Gričar J., Zupančič M., Čufar K. & Oven P. (2007) Regular cambial activity and xylem and phloem formation in locally heated and cooled stem portions of Norway spruce. *Wood science and technology*, **41**, 463-475.

Gruber A., Strobl S., Veit B. & Oberhuber W. (2010) Impact of drought on the temporal dynamics of wood formation in *Pinus sylvestris*. *Tree Physiology*, **30**, 490-501.

Horáček P., Šlezingerová J. & Gandelová L. (2003) Analysis of cambial activity and formation of wood in *Quercus robur* under conditions of a floodplain forest. *Journal of Forest Science*, **49**, 412-418.

Huang J., Tardif J.C., Bergeron Y., Denneler B., Berninger F. & Girardin M.P. (2010) Radial growth response of four dominant boreal tree species to climate along a latitudinal gradient in the eastern Canadian boreal forest. *Global Change Biology*, **16**, 711-731.

IPCC (2013) Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. pp. 1535. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Iqbal N., Masood A. & Khan N. (2012) Analyzing the significance of defoliation in growth, photosynthetic compensation and source-sink relations. *Photosynthetica*, **50**, 161-170.

Körner C. (2006) Significance of temperature in plant life. Plant Growth and Climate Change (eds J. I.L. Morison & M.D. Morecroft), pp. 48–69. Blackwell Publishing, Oxford.

Kranner I., Minibayeva F.V., Beckett R.P. & Seal C.E. (2010) What is stress? Concepts, definitions and applications in seed science. *New Phytologist*, **188**, 655-673.

Lachenbruch B. & McCulloh K.A. (2014) Traits, properties, and performance: how woody plants combine hydraulic and mechanical functions in a cell, tissue, or whole plant. *New Phytologist*, **204**, 747-764.

Major J.E. & Johnsen K.H. (1999) Shoot water relations of mature black spruce families displaying a genotype × environment interaction in growth rate. II. Temporal trends and response to varying soil water conditions. *Tree Physiology*, **19**, 375--382.

Moser L., Fonti P., Büntgen U., Esper J., Luterbacher J., Franzen J. & Frank D. (2010) Timing and duration of European larch growing season along altitudinal gradients in the Swiss Alps. *Tree Physiology*, **30**, 225-233.

Muller B., Pantin F., Génard M., Turc O., Freixes S., Piques M. & Gibon Y. (2011) Water deficits uncouple growth from photosynthesis, increase C content, and modify the relationships between C and growth in sink organs. *Journal of Experimental Botany*, **62**, 1715-1729.

Oberhuber W. & Gruber A. (2010) Climatic influences on intra-annual stem radial increment of *Pinus sylvestris* (L.) exposed to drought. *Trees - Structure and Function*, **24**, 887-898.

Oribe Y., Funada R., Shibagaki M. & Kubo T. (2001) Cambial reactivation in locally heated stems of the evergreen conifer *Abies sachalinensis* (Schmidt) masters. *Planta*, **212**, 684-691.

Pantin F., Monnet F., Jannaud D., Costa J.M., Renaud J., Muller B., Simonneau T. & Genty B. (2013) The dual effect of abscisic acid on stomata. *New Phytologist*, **197**, 65-72.

Pantin F., Simonneau T., Rolland G., Dauzat M. & Muller B. (2011) Control of Leaf Expansion: A Developmental Switch from Metabolics to Hydraulics. *Plant Physiology*, **156**, 803-815.

Peng C., Ma Z., Lei X., Zhu Q., Chen H., Wang W., Liu S., Li W., Fang X. & Zhou X. (2011) A drought-induced pervasive increase in tree mortality across Canada's boreal forests. *Nature*, **1**, 467–471.

Perkins S.E., Alexander L.V. & Nairn J.R. (2012) Increasing frequency, intensity and duration of observed global heatwaves and warm spells. *Geophysical Research Letters*, **39**, L20714.

Price D.T., Alfaro R.I., Brown K.J., Flannigan M.D., Fleming R.A., Hogg E.H., Girardin M.P., Lakusta T., Johnston M., McKenney D.W., Pedlar J.H., Stratton T., Sturrock R.N., Thompson I.D., Trofymow J.A. & Venier L.A. (2013) Anticipating the consequences of climate change for Canada's boreal forest ecosystems. *Environmental Reviews*, **21**, 322–365.

Proseus T.E., Zhu G. & Boyer J.S. (2000) Turgor, temperature and the growth of plant cells: using *Chara corallina* as a model system. *Journal of Experimental Botany*, **51**, 1481-1494.

Quinn G. & Keough M. (2002) *Experimental Design And Data Analysis For Biologists*. Cambridge University Press, Cambridge, UK.

Rathgeber C.B.K., Decoux V. & Leban J.-M. (2006) Linking intra-tree-ring wood density variations and tracheid anatomical characteristics in Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco). *Annals of Forest Science*, **63**, 699–706.

Ren P., Rossi S., Gricar J., Liang E. & Cufar K. (2015) Is precipitation a trigger for the onset of xylogenesis in Juniperus przewalskii on the north-eastern Tibetan Plateau? *Annals of botany*, **115**, 629.

Richet N., Afif D., Tozo K., Pollet B., Maillard P., Huber F., Priault P., Banvoy J., Gross P., Dizengremel P., Lapierre C., Perre P. & Cabane M. (2012) Elevated CO_2 and/or ozone modify lignification in the wood of poplars (*Populus tremula* x *alba*). *Journal of Experimental Botany*, **63**, 4291-4301.

Rossi S., Deslauriers A. & Anfodillo T. (2006) Assessment of cambial activity and xylogenesis by microsamplings tree species: an example at the alpine timberline. *IAWA Journal*, **27**, 383–394.

Rossi S., Girard M.J. & Morin H. (2014) Lengthening of the duration of xylogenesis engenders disproportionate increases in xylem production. *Global Change Biology*, **20**, 2261-2271.

Rossi S., Simard S., Rathgeber C.B.K., Deslauriers A. & De Zan C. (2009) Effects of a 20-day-long dry period on cambial and apical meristem growth in *Abies balsamea* seedlings. *Trees - Structure and Function*, **23**, 85-93.

Saveyn A., Steppe K. & Lemeur R. (2007) Daytime depression in tree stem CO_2 efflux rates: Is it caused by low stem turgor pressure? *Annals of Botany*, **99**, 477–485.

Savidge R. (2001) Intrinsic regulation of cambial growth. In: *J. Plant Growth Regul.*, pp. 52-77.

Seneviratne S.I., Nicholls N., Easterling D., Goodess C.M., Kanae S., Kossin J., Luo Y., Marengo J., McInnes K., Rahimi M., Reichstein M., Sorteberg A., Vera C. & Zhang X. (2012) Changes in climate extremes and their impacts on the natural physical environment. In: IPCC Special Report on Extremes, 109-230.

Skene D. (1972) The kinetics of tracheid development in *Tsuga canadensis* carr. And its relation to tree vigour. *Annals of Botany*, **36**, 179-187.

Steppe K., Vandegehuchte M.W., Tognetti R. & Mencuccini M. (2015) Sap flow as a key trait in the understanding of plant hydraulic functioning. *Tree Physiology*, **35**, 341-345.

Stewart J.D. & Bernier P.Y. (1995) Gas exchange and water relations of 3 sizes of containerized Picea mariana seedlings subjected to atmospheric and edaphic water stress under controlled conditions. *Annals of Forest Science*, **52**, 1-9.

Striker G.G., Insausti P. & Grimoldi A.A. (2008) Flooding effects on plants recovering from defoliation in *Paspalum dilatatum* and *Lotus tenuis*. *Annals of botany*, **102**, 247-254.

Topp G.C., Zebchuk W.D., Davis J.L. & Bailey W.G. (1984) The measurement of soil water content using a portable TDR hand probe. *Canadian Journal of Soil Science*, **64**, 313-321.

Turcotte A., Rossi S., Deslauriers A., Krause C. & Morin H. (2011) Dynamics of depletion and replenishment of water storage in stem and roots of black spruce measured by dendrometers. *Frontiers in Plant Science*, **2**, 21.

Vieira J., Rossi S., Campelo F., Freitas H. & Nabais C. (2013) Seasonal and daily cycles of stem radial variation of *Pinus pinaster* in a drought-prone environment. *Agricultural and Forest Meteorology*, **180**, 173– 181.

Villarini G., Smith J.A. & Vecchi G.A. (2013) Changing frequency of heavy rainfall over the central United States. *Journal of Climate*, **26**, 351-357.

Vincent L.A., Wang X.L., Milewska E.J., Wan H., Yang F. & Swail V. (2012) A second generation of homogenized canadian monthly surface air temperature for climate trend analysis. *Journal of Geophysical Research*, **117**.

Walsh D., Rossi S. & Lord D. (2015) Size and age: intrinsic confounding factors affecting the responses to a water deficit in black spruce seedlings. *iForest – Biogeosciences and Forestry*, **8**, 401-409.

Wang X.L., Feng Y. & Vincent L.A. (2013) Observed Changes in One-in-20 Year Extremes of Canadian Surface Air Temperatures. *Atmosphere-Ocean*, **52**, 1-10.

Willmott C. & Matsuura K. (2005) Advantages of the mean absolute error (MAE) over the root mean square error (RMSE) in assessing average model performance. *Climate Research*, **30**, 79-82.

Wood S.N. (2006) *Generalized Additive Models: an introduction with R*, Boca Raton, FL: Chapman and Hall/CRC.

CHAPITRE V

Combined effects of droughts and nocturnal and diurnal warming on stem radius variations in conifer saplings

RESEARCH PAPER

Title: Combined effects of droughts and nocturnal and diurnal warming on stem radius variations in conifer saplings

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In preparation

5.1 ABSTRACT

Recent droughts and warming in boreal regions represent the multiple dimensions of climate events. Continuous monitoring of plant water status has important potential to detect processes related to short- and long-term dynamics of tree stem depletion and replenishment under climate change. This study investigates the stem radial variations of *Picea mariana* saplings growing in a greenhouse. In 2011 and 2012, four-year-old plants were grown in warmer conditions during the day (*Diurnal+T*) or during the night (*Nocturnal+T*) at a temperature 6 °C higher than air temperature (*T0*) with a dry period of about 1 month in June 2011 and three cyclic droughts at the end of May until mid-July in 2012. High-resolution analysis of stem radius variations with automatic point dendrometers and leaf water relations were monitored from May to October of each year. This study evidenced that stem radius variations were not influenced just by water soil availability or water deficit intensity and duration, but that temperature was an important driver of stem size oscillation. The stem water balance demonstrated an important dependence between midday water potential and temperature. A prolonged water deficit caused a greater effect on stem contraction than recurrent droughts. The presence of a carry-over effect at the end of the recurrent drought cycles could influence the climatic signal strength in evergreen species of the boreal forest.

Keywords: Dendrometer, temperature, water deficit, water relations, young trees.

Symbols/abbreviations and units:

T0, greenhouse with a similar temperature to that of external air temperature; *Diurnal+T*, greenhouse with temperature 6 °C higher than T0 during the day; *Nocturnal+T*, greenhouse with temperature 6 °C higher than T0 during the night; $\Psi_{pd,}$ pre-dawn leaf water potential; $\Psi_{md,}$ midday leaf water potential; g_s, stomatal conductance; A_{max}, maximum photosynthesis rate; DOY, day of the year; VWC, volumetric water content of soil; (h) duration in hours of each cycle and phase.

5.2 INTRODUCTION

Favorable water transport conditions in trees mostly depend on hydraulic architecture, which define the functionality and conductivity of the xylem. In trees, the conditions of non-stationary or dynamic water flow are subjected to a time lag between the water loss through canopy transpiration and water absorption by roots (Goldstein et al., 1998, Schulze et al., 1984, Steppe et al., 2002). Transpiration is a passive process controlled by stomata and influenced by microclimatic conditions such as solar radiation, air saturation deficit, and wind speed (Kramer, 1964). In most ecosystems, transpiration commonly follows a diurnal pattern, with water loss from the leaves during the day partly drained from the stem storage compartments [ray parenchyma and phloem (Kavanagh et al., 2007)]. The water storage in the stem compartment can buffer the difference in plant water content that is created as a result of canopy transpiration and root absorption (Goldstein et al., 1984, Phillips et al., 2009). The water loss is correlated with a decreasing of stem water storage, which corresponds to 12-15% of daily transpiration during the summer (Loustau et al., 1996, Phillips et al., 2010). Movement of water into the tree is correlated with the capacity of sapwood cells to store it and release it during transpiration (Meinzer et al., 2003). The hydraulic capacitance of the stem may have significant implications for plant safety in response to a perturbation (Meinzer et al., 2009, Phillips et al., 2009) or in mitigating the effect of environmental stresses on plant water balance.

Several studies have shown that automatic dendrometers provide an important proxy to assess daily signal of stem radius variations linked to the dehydration/rehydration cycle of the stem (Turcotte et al., 2011, Zweifel et al., 2010). In the last decades, the water storage dynamics of trees have received a lot of attention; indeed stem radius variation measured by automatic dendrometer can better document stem water status as well as short-term growth responses (Deslauriers et al., 2003, Downes et al., 1999, Herzog et al., 1995). These measurements reveal that stem water relations are influenced by variations in soil moisture availability and evaporative demand (Cocozza et al., 2012, Giovannelli et al., 2007). Similarly, precipitation regime can influence the dynamics of depletion and replenishment of the stem water storage compartment in a water limited environment (Biondi and Rossi, 2014). It is postulated that recurrence of water deficit caused by the expected future environmental changes could cause a decrease in soil water availability, which might reflect in a reduction in water reserves, thus increasing the vulnerability of the water transport system in plants.

Warming and drought limit tree growth. The recent droughts in boreal regions illustrated the multiple dimensions of climate events, including negative impacts on tree survival and productivity (Peng et al., 2011). A reduction in precipitation combined with warmer temperature may induce negative effects on growth as a result of increased evaporative demand (Lindner et al., 2002, Price et al., 2013). However, recent evidence showed that in mature black spruce growing in boreal ecosystems, artificial drought conditions during three consecutive summers did not affect stem water status and radial growth (Belien et al., 2014). This may be due to compensatory mechanisms used by black spruce to drought and warming (Balducci et al., 2015b), however, in other biomes, such as in tropical and Mediterranean regions, drought was the main limiting factor for growth (Cocozza et al., 2015, Urrutia-Jalabert et al., 2015, Vieira et al., 2013).

Continuous monitoring of plant water status with dendrometers has important potential to detect processes related to short- and long-term dynamics of tree stem water depletion and replenishment under climate change. As the response of trees largely depends on species and local conditions, the assessment of stem water status and tree growth could elucidate trees sensitivities to climate change.

Daily changes in stem radius are a result of periodicity and can be associated to circadian rhythms (Turcotte et al., 2009). In the literature, daily stem radius variations were divided into three distinct phases: contraction, expansion and increment (Turcotte et al., 2009, Downes et al., 1999) and it was postulated that the circadian rhythms were generated in response to a dehydration/rehydration cycle within the stem (Tognetti et al., 2009). Some studies found that the circadian cycles of dehydration and rehydration occur mainly in the living cells (cambium, phloem, bark, rays parenchyma) (Zweifel and Hasler, 2001, Steppe et al., 2006). The stem water reserves decrease during the day and are refilled during the night (Zweifel and Hasler, 2001). In non-limiting water conditions, stem water refilling can take place during the night when transpiration is minimized (Čermák et al., 2007, Goldstein et al., 1998). Night-time stem water replenishment is an important mechanism by which a consistent proportion of the water lost by transpiration during the day is restored within the storage tissues; the daily water storage generally contributes 15–25% of daily transpiration, but it can be up to 50% during drought periods (Goldstein et al., 1998). Therefore, an important factor for the full replenishment of the water storage compartments, and as a result radial growth, is the duration of night-time refilling.

At northern latitudes, the shorter and warmer nights during the summer may be insufficient for complete stem water replenishment even when water is not a limiting factor (Kavanagh et al., 2007). Nocturnal water transport within the stem could differ substantially from daytime water transport under important ecological drivers such as temperature and water. In the climate change scenarios, night-time temperature is predicted to increase more than daytime (Casati and de Elia, 2014, IPCC, 2013), thus it could affect the physiological mechanisms and stem water storage within the plant more than daytime temperature. Because nocturnal water fluxes are considered significant for plant water relations (Dawson et al., 2007, Zeppel et al., 2010) and plant growth (Muller et al., 2011), a crucial question arises about how stem water relations will be affected by future nocturnal and diurnal warming in combination with drought.

This study investigated the effects of warming and drought on stem radius variations (duration and amplitude) of black spruce saplings using dendrometers. We tested the following hypotheses:

(i) Under a diurnal warming, the higher water loss during the day will increase stem contraction. At night, rehydration will increase stem expansion to compensate for the water loss during the day.

(ii) Under a nocturnal warming, the lower water loss during the day will decrease stem contraction. At night, rehydration will decrease during reducing stem expansion.

(iii) Both duration and amplitude of stem contraction and expansion will change according to the intensity of water deficit.

5.3 MATERIALS AND METHODS

5.3.1 Experimental design

Two independent experiments were conducted in 2011 and 2012 on 4-year-old black spruce [*Picea mariana* (Mill.) B.S.P.] saplings growing in a greenhouse at Chicoutimi, Canada (48°25′N, 71°04′W, 150 m above sea level). Saplings were collected from a local the public nursery in Sainte-Luce, QC, Canada. In summer 2010 and 2011, before the beginning of the experiment, the saplings were transplanted into plastic reversed-conic pots (4.5 litres in volume) and grown in an open field until the following spring. In April 2011 and 2012, 18 saplings of homogeneous size (53.01 ± 8.8 cm in height and 10.43 ± 1.79 mm in diameter at the collar) were randomly selected. Different irrigation and temperature regimes were applied in three sections of the greenhouse. In the control thermal regime (named T0) plants were grown under external air temperature, while the other two sections were subjected to specific thermal regimes. In 2011 and 2012, plants were grown in warmer conditions during the day *Diurnal+T* (from 07.00 to 19.00 h) or during the night *Nocturnal+T* (from 19.00 to 07.00 h) at a temperature 6 °C higher than T0. During both experiments, continuous heating was applied in greenhouses with electronic system (computer and electronic thermostat, Harnois's System, QC, Canada) and processed according to Balducci et al., (2013, 2015). During maximum xylem growth, when saplings are more susceptible to dry conditions, two irrigation regimes were applied: (i) control (named, irrigated saplings), consisting of maintaining the soil water content at ~80% of field capacity; and (ii) water deficit (named, non-irrigated saplings), in which irrigation was withheld for 25 days in June 2011 for 3 saplings per thermal condition and processed according to Balducci et al., (2013, 2015). In 2012, from the end of May until mid-July, three cyclic droughts were applied by withholding irrigation. The first and second drought periods were followed by a 6 and 5 days recovery period, respectively, with the resumption of the irrigation, while all saplings were watered until the end of October after the third drought cycle. In each drought cycle irrigation was resumed when the soil water content reached 10% (threshold values).

5.3.2 Data collection

Leaf water potential was measured from May to August on branches of the first whorl of 18 saplings (3 saplings × 3 thermal conditions × 2 irrigation regimes per week). Pre-dawn [Ψ_{pd}] and midday [Ψ_{md}] leaf water potentials were measured using a pressure chamber (PMS Instruments, Corvalis, OR). To exclude vibrations to the dendrometers anchored on the saplings, the volumetric water content (VWC) of the soil was measured weekly by time domain reflectometry for 6 other plants per treatment grown in the same three sections of the greenhouse (TDR Fieldscout 300). The measurements were taken at 7 cm depth in each pot, replicated twice at the same time and processed according to Balducci et al., (2013, 2015) and Topp et al., (1984).

Stem radius variations (µm) were monitored using automatic point dendrometers. Dendrometers were appositely constructed for saplings with a sensing rod held against the outer surface of the bark by a constant force (Fig. 5.1). The rod was made of stainless steel with a thermal linear expansion coefficient of 2.5 mm^{-1} 8 C^{-1}, the macro sensor PR750 (Pennsauken, NJ) ranges from 1.25 mm to 250 mm and offers a core-to-bore radial clearance of 0.25 mm with the standard core supplied. The PR750 sensor output V1/Vx ratio is converted into the value (length of sensor, mm) using a linear calibration regression equation (Loggernet software, Campbell Scientific, Inc., Logan, Utah). The instrument consists of a displacement transducer that is anchored to a plastic holder fixed with four screws into the soil (Fig. 5.1). Dendrometers were installed on 18 saplings at a height of about 5 cm above the collar from spring to autumn (May–October) 2011 and 2012. Stem size variation was recorded every 15 min and averaged over each hour (Deslauriers et al., 2003). The temperature variation does not affect the sensor measurements due to the thermal expansion of the frame.

5.3.3 *Stem variation phases*

Stem cycle extraction was performed using a three-step procedure composed of two SAS routines (SAS Institute, Inc.) specially developed to analyze hourly automatic dendrometer data (Vieira et al., 2013). The procedure divides the series into distinct phases: (1) contraction the period between the first maximum radius and the next minimum; (2) recovery, the period from the minimum until the position of the previous maximum value or when the stem reverts to a contraction phase (Turcotte et al., 2009, Turcotte et al., 2011). The SAS routines calculated the amount of stem radial variation and its relative duration (Deslauriers et al., 2011). The duration in hours (h) of each cycle and phase was also calculated.

5.3.4 Statistical analyses

Across water and thermal treatments, Spearman's rank correlations were used to assess the monotonic relationship between the pre-dawn (Ψ_{pd}) and midday Ψ_{md} leaf water potential and mean daily temperature during the two experiments in 2011 and 2012 (Quinn and Keough, 2002).

Logistic regression analyses for the duration of contraction and expansion in black spruce saplings were calculated between irrigation regimes and among thermal conditions. The proportion between the duration of the contraction and expansion phase was compared using PROC LOGISTIC ($P<0.05$). The comparisons among thermal conditions and irrigation regimes were then performed using slice option procedure in SAS (SAS Institute, Cary, NC) and were executed separately for three periods in 2011 and for seven periods in 2012 to determine the influence of treatments on the amplitude of stem variation and circadian stem cycle duration. A general linear model (GLM) was used to perform the univariate analysis of split plot repeated measured data of amplitude for each phase (contraction and expansion) (Moser and Saxton, 1990). In order to compare these phases between periods and treatments, we grouped separately three periods in 2011 and seven periods in 2012. Contrasts were applied to investigate the difference between irrigation regimes and among thermal conditions within periods (Moser and Saxton, 1990).

5.4 RESULTS

5.4.1 Growth conditions

During 2011, *Diurnal+T* and *Nocturnal+T* were, on average, 4.5 and 5.2 °C warmer than T0, while in 2012 they were 4.6 and 4.3 °C warmer (Fig. 5.2). In both years, during the water deficit, daily temperature in T0 varied between 14 °C and 22 °C. Maximum temperatures of ~24°C for T0 were reached in July. A gradual decrease in temperature was then observed from the end of August, until a minimum of about 4 °C.

During the 25 days of water deficit in 2011, the volumetric water content (VWC) of non-irrigated saplings decreased in all thermal conditions. After the drought period, VWC increased quickly and field capacity was reached on DOY 200, 20 days after the resumption of irrigation (Fig. 5.3). After DOY 183, the VWC was maintained at field capacity until the end of the experiment. During the experiment in 2012, VWC of irrigated saplings ranged from 40 to 50% in all thermal conditions (Fig. 5.3). During three cycles of water deficit, VWC of non-irrigated saplings ranged from 8 to 11%. In all treatments, non-irrigated saplings recovering from the third drought cycle showed a similar trend to irrigated saplings. VWC of non-irrigated saplings was generally lower in warmer conditions than in the control (25% vs. 40%), and rapidly increased in the second and third rehydration periods (Fig. 5.3).

5.4.2 Relationship between temperature and plant water potential

The midday leaf water potential (Ψ_{md}) significantly decreased with increasing temperature (Table 5.1). During water deficit, lower values of Ψ_{md} were observed in non-irrigated saplings, with values reaching –2.1 MPa, –1.95 MPa, and –2.38 MPa in T0, *Diurnal+T* and *Nocturnal+T*, respectively. The value of Ψ_{md} decreased with the increasing of diurnal and nocturnal warming with correlation coefficients ranging from -0.66 to -0.52 depending on the treatments. In both years the highest correlation was found for *Nocturnal+T* of irrigated sampling followed by non-irrigated samplings. No significant correlation was observed for T0 in 2011. The signs of the correlation of Ψ_{md} were negative in 2011 and 2012, whilst no relationships were found with predawn leaf water potential (Ψ_{pd}) in either year (Table 5.1).

5.4.3 Stem radius increase in 2011 and 2012

During both experiments, the stem radius increase in irrigated saplings ranged from 0.47 to 0.65 mm on average (Fig. 5.4). In 2011 and 2012, stem radius of irrigated saplings progressively increased from the end of April to May with a plateau detected in June, and stem growth then expanded continuously until October (Fig. 5.4). However, the thermal treatments led to different results between years. During 2011, small differences in stem radius increase were observed from mid-July among thermal conditions with higher values observed under warming conditions (0.60-0.74 mm) compared with T0 (0.39 mm). During the water deficit, non-irrigated saplings were partially damaged, and one non-irrigated sapling in T0 and one in *Diurnal+T* were seriously damaged with complete needle wilting and stem necrosis on DOY 207 and DOY 234, respectively, and this could explain the erratic trend of stem variation in 2011 for non-irrigated saplings in T0. During 2012, the stem radius increase in irrigated saplings was lower in *Diurnal+T* compared with T0. Similar stem radius increase was measured in *Nocturnal+T* and T0 with values of around 0.9 mm.

In non-irrigated saplings, a pronounced decrease was observed in the long-term drought (2011), from mid-June until the end of the watering suspension, with values of about 0.21 mm (Fig. 5.4). On DOY 190, one week after rehydration, the stem radius increment of non-irrigated saplings was only partially restored and progressively increased until the end of the experiment (on average 0.3 in non-irrigated saplings versus 0.8 mm in irrigated ones) (Fig. 5.4).

After three water deficit cycles, the decrease in stem radius of non-irrigated saplings (2012) was slighter than that recorded in response to prolonged water deficit (2011). During the second and third drought cycle, differences in stem radius increase were observed between irrigation regimes and higher values of stem radius were measured in T0 (about 0.40 mm), while in *Nocturnal+T* and *Diurnal+T*, lower values of stem radius increase were measured with values of 0.32 and 0.28 mm, respectively (Fig. 5.4).

5.4.4 Circadian stem cycle: duration and amplitude

Throughout the 2011 experiment, the warmer conditions induced significant differences in the duration of the phases of contraction and expansion (Fig. 5.5, Table 5.2). The duration of contraction in irrigated saplings displayed similar values during the experiment, while duration of contraction in non-irrigated saplings increased (from 7 to 13 hours) in response to water deficit. Before and after water deficit, the duration of contraction was similar between irrigation regimes (*pre-water deficit,* Fig. 5.5). On the contrary, during the water deficit, an increase in stem cycle contraction was measured with significant differences between irrigation regimes ($P<0.001$) (Table 5.2) with the highest increase in *Diurnal+T* in non-irrigated saplings, but under *Nocturnal+T* changes in irrigated saplings were not significant. The duration of expansion commonly ranged between 6 and 13 hours. Before water deficit, significant differences were observed in duration of expansion between irrigation regimes and both warmer regimes (Table 5.2). Then during water deficit the longer durations of expansion were observed in *Diurnal+T*, ranging from 10 to 13 hours, respectively ($P<0.001$). After rehydration the durations of expansion also showed significant differences between irrigation regimes and between warmer conditions ($P < 0.001$).

In 2012, thermal conditions induced significant differences in duration of contraction at the beginning of the experiment and after the third recovery, while the duration of expansion showed significant differences among thermal conditions in all three drought cycles (Table 5.2). No statistical differences were observed in the first cycle of drought between irrigation regimes at all thermal conditions. During the second drought cycle, the temperature did not influence the duration of contraction, but irrigation regimes had sporadic effects. Duration of expansion in saplings at *Nocturnal+T* increased during the second drought cycle with 11 hours and then decreased to about 8 hours during the third, while in *Diurnal+T* the duration of expansion was reduced to about 6 hours in the third water deficit (Fig. 5.5). Only in the third drought cycle and during the third rehydration, durations of contraction and expansion showed significant differences between irrigation regimes and thermal conditions (Table 5.2). In the third irrigation period, the duration of expansion in irrigated saplings was longer in *Nocturnal+T*, around 9 hours, while the shorter duration of expansion was measured in non-irrigated saplings at T0, with 7 hours (Fig. 5.5). Thus, during the third cycle of drought and its respective rehydration, there was a decrease in duration of the contraction and expansion cycle due to faster contraction and expansion phases at warmer temperatures than T0.

During the water deficit (2011), significant differences were found in the amplitude of contraction and expansion at the different thermal conditions (Fig. 5.6, Table 5.3). Before the water deficit, the amplitude of contraction and expansion was similar between irrigation regimes. The amplitude of contraction of non-irrigated saplings increased during water deficit at T0 and *Nocturnal+T*, and then returned to similar initial values. The highest amplitude of contraction was measured during water deficit at T0 and *Nocturnal+T* (0.06 and 0.04, respectively). During the rehydration period, the values of amplitude of contraction were 0.02 mm in irrigated versus 0.03 mm in non-irrigated and significant differences in the contraction amplitude were observed between irrigation regimes ($P<0.05$) (Fig. 5.6, Table 5.3).

In 2012, no statistical differences in amplitude of contraction and expansion were observed in the first cycle of drought and rehydration between irrigation regimes and thermal conditions. The highest amplitudes of contraction and expansion were observed in *Diurnal+T* during the second drought cycle (0.06 and 0.07 mm, respectively) (Fig. 5.6). There were significant differences in amplitude of contraction and expansion among thermal conditions during the second recovery period ($P<0.001$ and $P<0.05$, respectively) (Table 5.3). During the second irrigation, values of contraction amplitude were 0.02 versus 0.03 mm in warmer conditions, respectively. Lower values of amplitude of expansion were observed in *Nocturnal+T* with about 0.02 mm, compared with 0.03 mm in T0 and 0.04 mm in *Diurnal+T*, during the second recovery period. Significant differences of amplitude of contraction and expansion were observed in the interaction between irrigation regimes and thermal conditions only in the third cycle of water deficit and its recovery, (Fig. 5.6, Table 5.3).

5.5 DISCUSSION

This study evidenced that stem radius variations were not influenced just by soil water availability, but that temperature was an important driver of the stem size oscillation. The concomitant imposition of warming combined with prolonged water deficit caused significant differences in stem radius variation in 2011. In 2012, the circadian stem cycle of saplings subjected to recurrent droughts responded mainly to the warming. Water deficit had significant effect on duration of contraction in the first and second periods because of their shorter duration of their cycles. However no significant effect of water deficit was observed on amplitude of cycles in 2012. During water deficit, an increase in amplitude of stem contraction is known to be an indicator of water stress intensity in the plant (Deslauriers et al., 2007, Giovannelli et al., 2007). This was not completely observed during our experiments and the differences in the effects of water deficit between 2011 and 2012 could be explained by the ability of black spruce to regulate water lost by transpiration and to reduce fluctuations in compartments water potential (Meinzer et al., 2008). Indeed, when leaf water potential of black spruce saplings dropped below -2.70 MPa for 20-30 consecutive dry days in 2011, plants were in severe water deficit, as observed in previous studies (Balducci et al., 2013, Stewart and Bernier, 1995); this threshold was not observed in 2012. During the recurrent cycles of water deficit (2012), the changes in temperature and water soil availability provoked a carry-over effect on the plant water balance that could be detected by evaluation of the duration and amplitude of the stem cycle.

In 2011, during recovery until the autumn, the amplitude of stem contraction showed a marked decrease under *Nocturnal+T*, while in 2012 this decrease was observed in *Diurnal+T,* suggesting that stem shrinkage could be influenced by warmer temperature according the intensity of drought. The first hypothesis was refuted for both 2011 and 2012 experiments, as the influence of diurnal temperature was more important on duration than amplitude. Past studies recorded the influence of temperature on soil water uptake during winter and autumn (Sevanto et al., 2006), which occurred mainly during the night (Zweifel and Hasler, 2001, Zweifel et al., 2000). The second hypothesis was accepted for the 2011experiment, but refuted for the 2012experiment, as the influence of temperature was more important on duration than amplitude. Dendrometers gave extra information on the changes in stem size to different effects of driving factors, mainly air temperature, as separated effect of nocturnal and diurnal warming, according the duration and intensity of drought.

During prolonged water deficit (2011), the stem contraction lasted longer under higher diurnal temperatures compared with the other thermal conditions. An incomplete refilling during the night could explain this result. The stem expansion in *Diurnal+T* was shorter during the night and in the early morning, leading to a longer duration of stem contraction. In 2011, circadian cycles did not have the same duration in response to different thermal regimes, evidencing that the proportion between shrinkage and expansion could represent a compensation to balance incomplete water refilling in warming conditions. During hot and dry days, stem contraction occurred when water lost by transpiration was not fully compensated by root absorption. Thus, the soil water uptake was probably insufficient to recharge stem water storage tissues', indicating that the fraction of stem water storage lost during the day was not completely refilled at night (Devine and Harrington, 2011, Kavanagh et al., 2007). Evaporative demand, transpiration and soil water content has been demonstrated to affect phloem water status and consequently stem radius variations (Cuevas et al., 2010, Ortuño et al., 2010).

During the recurrent water deficits (2012), we observed a carry-over effect (i.e. delayed and amplified effect) of successive water deficits on duration and a sporadic effect on amplitude of the phases of the diurnal stem cycle. The duration of the contraction increased only in the second drought cycle indicating that water content and turgor pressure were maintained over a short water deficit period, as observed in Giovannelli et al. (2007). During water deficit, lower values of Ψ_{md} were observed in non-irrigated saplings, with values reaching –2.1 MPa in T0, –1.95 MPa, and –2.38 MPa in *Diurnal+T* and *Nocturnal+T*, respectively. Black spruce is a typical isohydric species, which under water stress prevents dehydration with early stomatal closure (Domec and Johnson, 2012) and osmotic adjustments (Deslauriers et al., 2014). In this study, midday water potential was negatively correlated with temperature (i.e. Ψ_{md} decreases with increasing temperature) with increasing correlation in warmer conditions, especially those occurring during the night. The midday leaf water potential depends on soil water uptake and is maintained by stomatal control of the transpiration (Domec and Johnson, 2012). Significant relationships existed between night-time water uptake and midday water potential measured the previous day (Zeppel et al., 2010). On the contrary, no significant correlations were found between temperature and pre-dawn water potential meaning that the equilibrium between leaf and soil water potential did not necessarily take place at the end of the night (Bucci et al., 2004, Bucci et al., 2005, Donovan et al., 2001, Donovan et al., 1999), but the contribution of rehydration is at the beginning and during the night. For this reason, black spruce saplings could be able to resist a short period (two weeks) of water stress. However, the durations of stem contraction and expansion were affected at the third drought period, indicating a carry-over effect on the duration of the stem cycle phases. As for the one water deficit in 2011, the longer stem shrinkage observed at the third cycle of dehydration can be explained by incomplete refilling at night.

In 2012, *Diurnal+T* warming had a sporadic and delayed effect of on the amplitude of stem contraction and expansion at the second drought cycle. This contrasts with the prolonged drought in 2011 and another study when the amplitude of contraction was also influenced by higher temperature: in July, a rise of 10 °C in temperature rise increased the contraction amplitude in spruce and larch (King et al., 2013). Warmer temperatures in daytime can deplete stored water in the stem faster and warming could have an indirect effect mediated by pressure turgor on the stem radial variation. Stem diameter shrinkage changed with the internal water status, which was influenced by three different compartments and by other sink organs of the plant (needles and roots). The results obtained in this study help to support that prolonged drought and warming have a marked influence on stem radial variations, whereas stem water loss in concomitance to recurrent and short droughts and diurnal warming leads to a temporary decrease of stored water in the stem. The effect of heat stress interacts with the severity of drought.

5.6 CONCLUSION

Our results confirmed that stem variation reflects the influence of both water availability and temperature. Temperature is an important driver of stem size variation, directly by increasing diurnal water loss and indirectly by decreasing soil water content via evapotranspiration. The stem water balance during the day, represented by the midday water potential, was linked with the daytime air temperature. How the water availability changes seasonally – from short recurrent to one prolonged water deficit – differently influence the patterns of stem radial variations. A prolonged water deficit caused a greater effect on stem contraction than recurrent droughts, confirming the importance of water regulation in the long term in a drought prone environment. The presence of a weak and carry-over effect at the end of the recurrent drought cycles on duration and amplitude of the stem cycle could influence the climatic signal strength in evergreen species of the boreal forest.

5.7 ACKNOWLEDGEMENTS

This study was funded by the Natural Sciences and Engineering Research Council of Canada, Consortium Ouranos and Université du Québec à Chicoutimi. We thank H. Morin, D. Gagnon, G. Savard, F. Gionest, D. Walsh and all personnel of LEVA laboratories for their support. We thank C. Krause, associate editor and anonymous reviewers for helpful comments. Additional thanks to A. Garside for checking the English text.

Table 5.1 Spearman correlation coefficients between leaf water potential (pre-dawn Ψ_{pd} and midday Ψ_{md}, MPa) and temperature (°C) during the greenhouse experiment in 2011 and 2012. Thermal conditions are presented as control T0; temperature increase during the day, *Diurnal+T*; temperature increase during the night *Nocturnal+T*. Significant correlations ($P \leq 0.05$) are in bold.

			Year	**2011**			
		Irrigated				**Non-irrigated**	
	T0	Diurnal+T	Nocturnal+T		T0	Diurnal+T	Nocturnal+T
Ψ_{pd}	0.33	0.19	-0.14		0.24	-0.27	-0.10
Ψ_{md}	-0.32	**-0.51**	**-0.65**		-0.30	**-0.58**	**-0.58**
			Year	**2012**			
		Irrigated				**Non-irrigated**	
	T0	Diurnal+T	Nocturnal+T		T0	Diurnal+T	Nocturnal+T
Ψ_{pd}	0.17	0.22	0.04		0.23	0.06	0.00
Ψ_{md}	**-0.37**	**-0.22**	**-0.59**		**-0.40**	**-0.54**	**-0.55**

Table 5.2 Logistic regression analysis for duration of stem radius contraction and expansion in black spruce saplings calculated between irrigation regimes (Water), among thermal conditions (Temp) and interaction between irrigation regimes and thermal conditions (Water × Temp) during the greenhouse experiments in 2011 and 2012. Significant effects ($P \leq 0.05$) are in bold.

Year 2011	Water	Temp	Water × Temp
DF	1	2	2
Pre water deficit			
contraction	**0.0163**	**<.0001**	**0.0006**
expansion	**<.0001**	**<.0001**	0.0497
Water deficit			
contraction	**<.0001**	**<.0001**	0.6114
expansion	**0.0107**	**0.0002**	**0.0034**
Post water deficit			
contraction	0.2063	**<.0001**	**<.0001**
expansion	**<.0001**	**<.0001**	**<.0001**
Year 2012			
DF	1	2	2
Pre water deficit			
contraction	0.7577	**<.0001**	0.109
expansion	0.0535	**0.0263**	0.2326
1 cycle water deficit			
contraction	0.0775	0.273	0.3698
expansion	0.5475	**0.0123**	0.5561
1 Irrigation period			
contraction	0.3119	0.9146	0.9513
expansion	0.293	0.473	0.0742
2 cycle water deficit			
contraction	**<.0001**	0.0503	0.2177
expansion	0.6619	**0.0005**	0.252
2 Irrigation period			
contraction	0.1094	0.0595	0.7037
expansion	0.069	0.4611	**0.0054**
3 cycle water deficit			
contraction	0.0511	**0.0002**	0.6705
expansion	**0.0086**	**0.0027**	**<.0001**
3 Irrigation period			
contraction	0.273	**<.0001**	**<.0001**
expansion	**<.0001**	**<.0001**	**0.0027**

Table 5.3 Logistic regression analysis for amplitude of contraction and expansion in black spruce saplings calculated between irrigation regimes (Water), among thermal conditions (Temp) and interaction between irrigation regimes and thermal conditions (Water × Temp) during the greenhouse experiments in 2011 and 2012. Significant effects ($P \leq 0.05$) are in bold.

Year 2011	Water	Temp	Water × Temp
DF	1	2	2
Pre water deficit			
contraction	0.1175	0.6079	**0.0403**
expansion	0.1744	0.8612	0.0965
Water deficit			
contraction	0.4302	**0.0261**	**0.0312**
expansion	0.9100	0.1044	**0.0372**
Post water deficit			
contraction	**0.0341**	0.8721	0.1096
expansion	0.3239	0.7185	0.1984
Year 2012			
DF	1	2	2
Pre water deficit			
contraction	0.2188	0.1279	0.1884
expansion	0.3324	0.1888	0.1115
1 cycle water deficit			
contraction	0.9188	0.1237	0.2178
expansion	0.7645	0.0585	0.1795
1 Irrigation period			
contraction	0.7487	0.8363	0.0990
expansion	0.6793	0.8995	0.1833
2 cycle water deficit			
contraction	0.1648	0.4352	0.3658
expansion	0.4721	0.3707	0.2385
2 Irrigation period			
contraction	0.3626	**0.0066**	0.0895
expansion	0.5046	**0.0229**	0.2829
3 cycle water deficit			
contraction	0.1791	0.0529	**0.0317**
expansion	0.6861	0.0765	**0.0212**
3 Irrigation period			
contraction	0.2822	0.0738	**0.0145**
expansion	0.5075	0.1561	**0.0248**

Figure 5.1 Dendrometer (showed from different angles) installed on the surface of the bark of a black spruce sapling during the greenhouse experiments in 2011 and 2012.

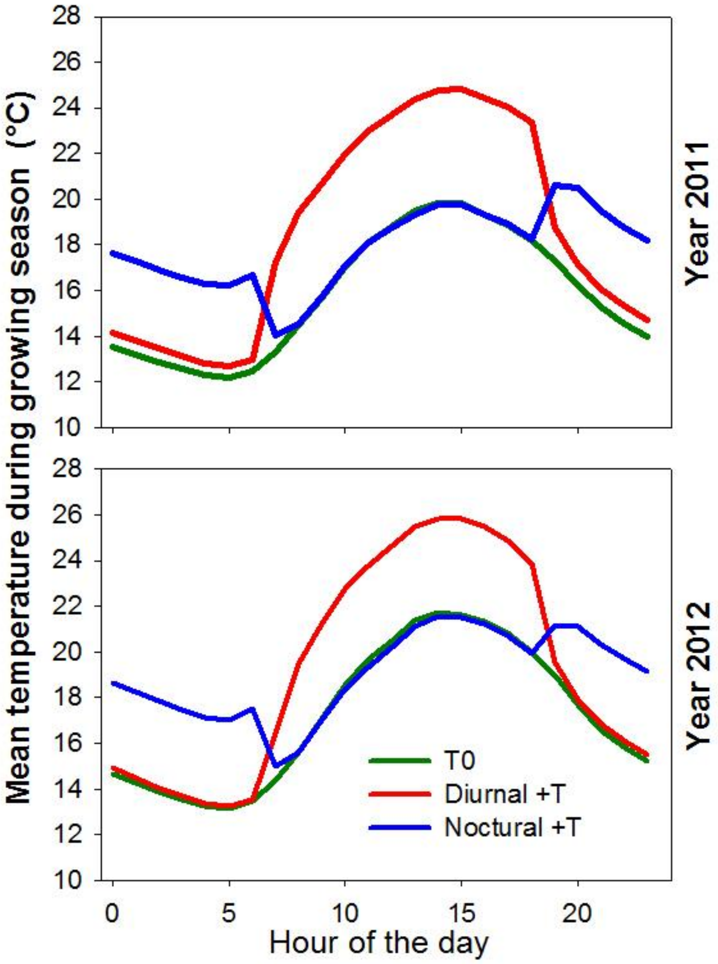

Figure 5.2

Mean temperatures experienced by black spruce saplings in the three thermal conditions (T0, control temperature (green curve); *Diurnal+T*, temperature increase during the day (red curve); *Nocturnal+T*, temperature increase during the night (blue curve) during the greenhouse experiments from April to October 2011 and 2012.

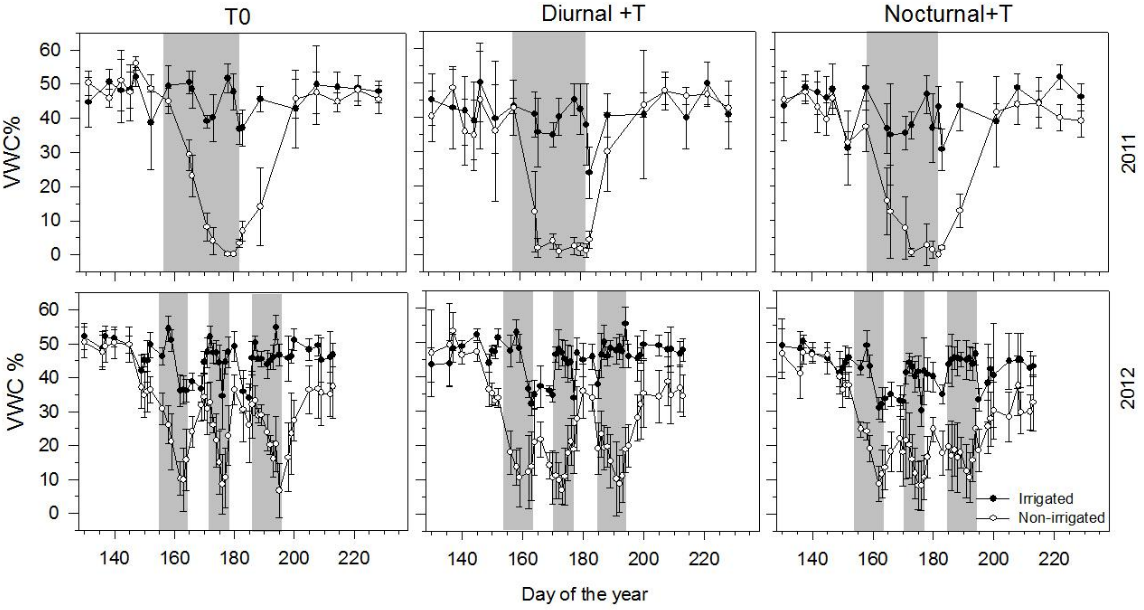

Figure 5.3
Volumetric water content (VWC) of soil in irrigated (black circles) and non-irrigated saplings (white circles) before, during and after the water deficit period (grey background) at three thermal conditions (T0, control temperature; *Diurnal+T*, temperature increase during the day; *Nocturnal+T*, temperature increase during the night) during the greenhouse experiments in 2011 and 2012. Vertical bars represent standard deviation.

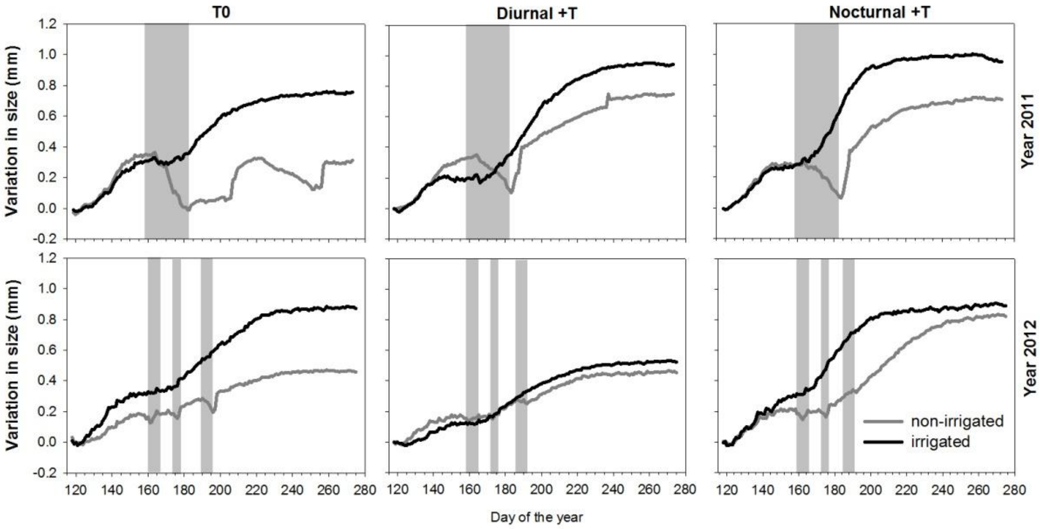

Figure 5.4 Time series of radius variation for black spruce saplings between April and October 2011 and 2012. Black and dark grey curves represent irrigation regimes, before, during and after the water deficit period (grey background) at three thermal conditions (T0, control temperature; *Diurnal+T*, temperature increase during the day; *Nocturnal+T*, temperature increase during the night) during the greenhouse experiments.

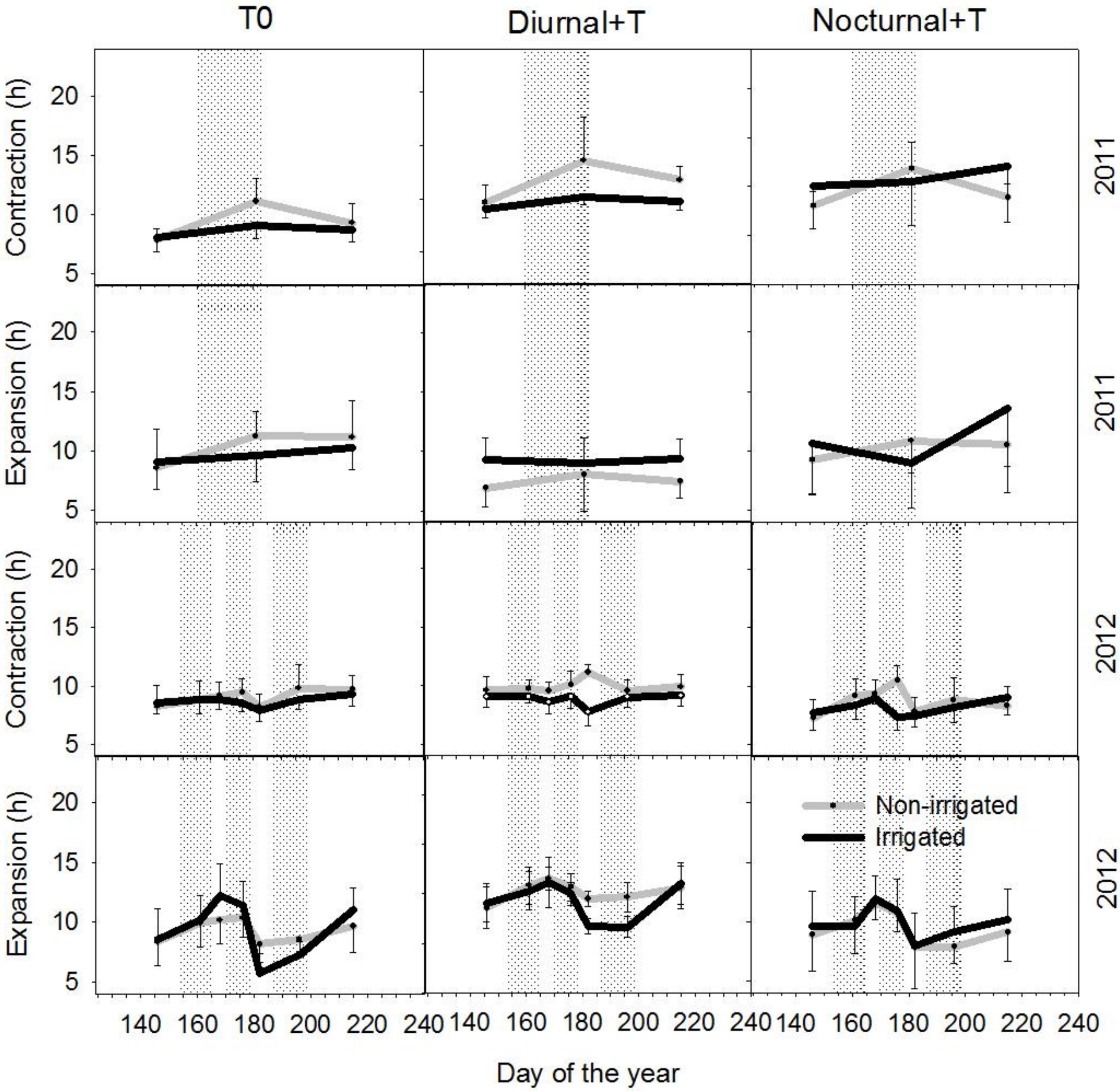

Figure 5.5 Mean duration of contraction (± SD) in stem of black spruce saplings before, during and after the water deficit period (dotted grey background) at three thermal conditions (T0, control temperature; *Diurnal+T* , temperature increase during the day; *Nocturnal+T,* temperature increase during the night) during the greenhouse experiments in 2011 and 2012. Differences between irrigation regimes (black and grey bars) and periods are reported in Table 5.2.

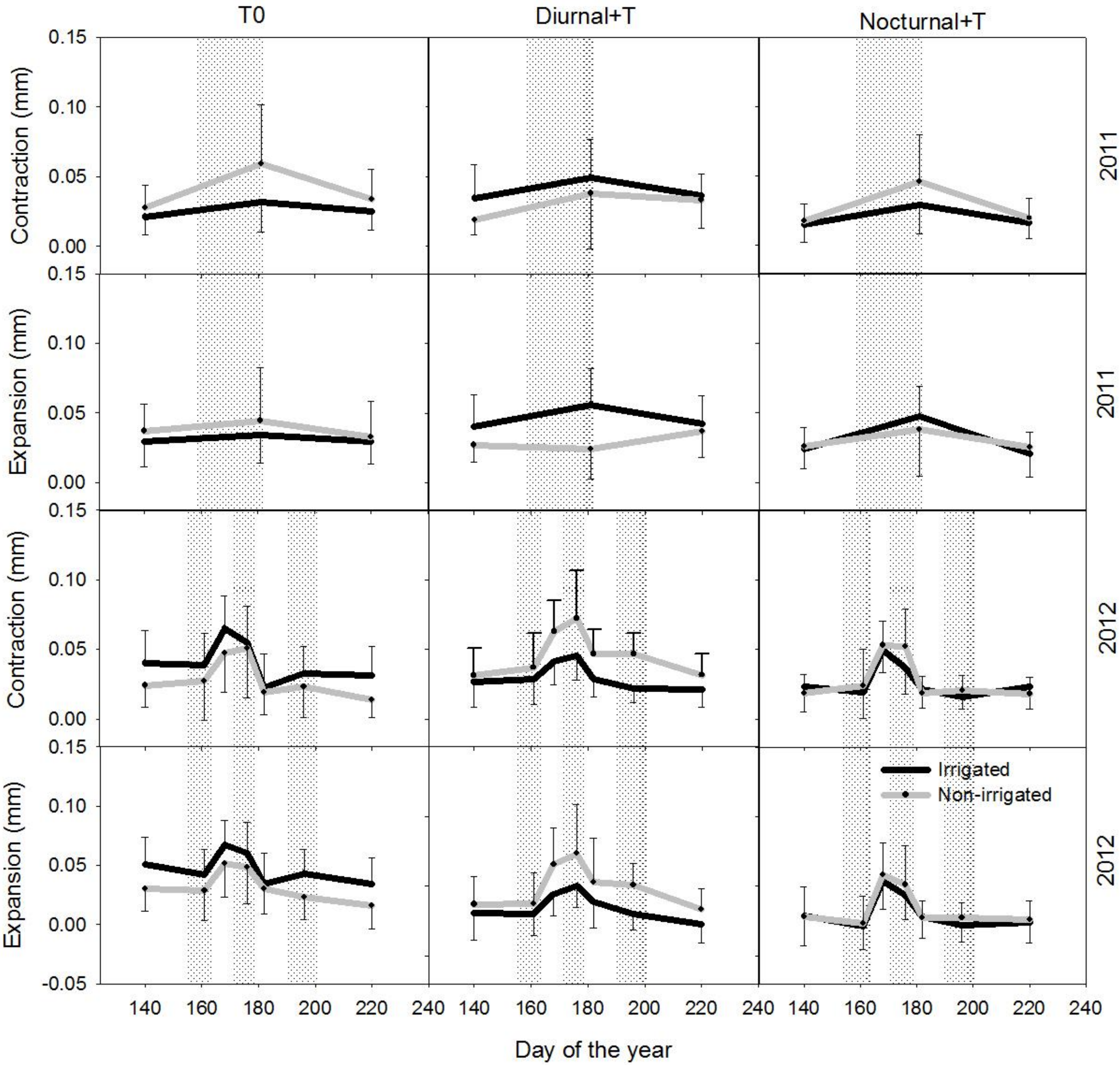

Figure 5.6 Mean amplitude of contraction and expansion (± SD) in stem of black spruce saplings in 3 cycles of water deficit period (dotted grey background) and rehydration at three thermal conditions (T0, control temperature; *Diurnal+T*, temperature increase during the day; *Nocturnal+T*, temperature increase during the night) during the greenhouse experiments in 2011 and 2012. Differences between irrigation regimes (black and grey bars) and periods are reported in Table 5.3.

5.8 REFERENCES

Balducci L, Cuny HE, Rathgeber CBK, Deslauriers A, Giovannelli A, Rossi S. 2015b. How do drought and warming influence survival and wood traits of *Picea mariana* saplings? *Plant Cell and Environment,* DOI: 10.1111/pce.12689

Balducci L, Deslauriers A, Giovannelli A, Beaulieu M, Delzon S, Rossi S, Rathgeber CBK. 2015. How do drought and warming influence survival and wood traits of *Picea mariana* saplings? *Journal of Experimental Botany,* **66**: 377-89.

Balducci L, Deslauriers A, Giovannelli A, Rossi S, Rathgeber CBK. 2013. Effects of temperature and water deficit on cambial activity and woody ring features in *Picea mariana* saplings. *Tree Physiology,* **33**: 1006–1017.

Belien E, Rossi S, Morin H, Deslauriers A. 2014. High-resolution analysis of stem radius variations in black spruce [*Picea mariana* (Mill.) BSP] subjected to rain exclusion for three summers. *Trees-Structure and Function,* **28**: 1257-1265.

Biondi F, Rossi S. 2014. Plant-water relationships in the Great Basin Desert of North America derived from *Pinus monophylla* hourly dendrometer records. *International Journal of Biometeorology*.

Bucci SJ, Goldstein G, Meinzer FC, Franco AC, Campanello P, Scholz FG. 2005. Mechanisms contributing to seasonal homeostasis of minimum leaf water potential and predawn disequilibrium between soil and plant water potential in Neotropical savanna trees. *Trees,* **19**: 296–304.

Bucci SJ, Scholz FG, Goldstein G, Meinzer FC, Hinojosa JA, Hoffmann WA, Franco AC. 2004. Processes preventing nocturnal equilibration between leaf and soil water potential in tropical savanna woody species. *Tree Physiology,* **24**: 1119–1127.

Casati B, de Elia R. 2014. Temperature Extremes from Canadian Regional Climate Model (CRCM) Climate Change Projections. *Atmosphere-Ocean,* **52**: 191-210.

Čermák J, Kučera J, Hinckley TM, Bauerle WL, Phillips N. 2007. Tree water storage and its diurnal dynamics related to sap flow and changes in stem volume in old-growth Douglas-fir trees. *Tree Physiology,* **27**: 181-198.

Cocozza C, Giovannelli A, Lasserre B, Cantini C, Lombardi F, Tognetti R. 2012. A novel mathematical procedure to interpret the stem radius variation in olive trees. *Agricultural and Forest Meteorology,* **161**: 80– 93.

Cocozza C, Marino G, Giovannelli A, Cantini C, Centritto M, Tognetti R. 2015. Simultaneous measurements of stem radius variation and sap flux density reveal synchronisation of water storage and transpiration dynamics in olive trees. *Ecohydrology,* **8**: 33-45.

Cuevas MV, Torres-Ruiz JM, Alvarez R, Jimenez MD, Cuerva J, Fernandez JE. 2010. Assessment of trunk diameter variation derived indices as water stress indicators in mature olive trees. *Agricultural Water Management,* **97**: 1293-1302.

Dawson TE, Burgess SSO, Tu KP, Oliveira RS, Santiago LS, Fisher JB, Simonin KA, Ambrose AR. 2007. Nighttime transpiration in woody plants from contrasting ecosystems. *Tree Physiology,* **27**: 561-575.

Deslauriers A, Anfodillo T, Rossi S, Carraro V. 2007. Using simple causal modeling to understand how water and temperature affect daily stem radial variation in trees. *Tree Physiology,* **27**: 1125-1136.

Deslauriers A, Beaulieu M, Balducci L, Giovannelli A, Gagnon M-J, Rossi S. 2014. Impact of warming and drought on carbon balance related to wood formation in black spruce. *Annals of Botany,* **114**: 335-45.

Deslauriers A, Morin H, Urbinati C, Carrer M. 2003. Daily weather response of balsam fir (*Abies balsamea* (L.) Mill.) stem radius increment from dendrometer analysis in the boreal forests of Quebec (Canada). *Trees-Structure and Function,* **17**: 477–484.

Deslauriers A, Rossi S, Turcotte A, Morin H, Krause C. 2011. A three-step procedure in SAS to analyze the time series from automatic dendrometers. *Dendrochronologia,* **29**: 151-161.

Devine WD, Harrington CA. 2011. Factors affecting diurnal stem contraction in young Douglas-fir. *Agricultural And Forest Meteorology,* **151**: 414-419.

Domec J-C, Johnson DM. 2012. Does homeostasis or disturbance of homeostasis in minimum leaf water potential explain the isohydric versus anisohydric behavior of Vitis vinifera L. cultivars? *Tree Physiology,* **32**: 1-4.

Donovan L, Linton M, Richards J. 2001. Predawn plant water potential does not necessarily equilibrate with soil water potential under well-watered conditions. *Oecologia,* **129**: 328-335.

Donovan LA, West DJ, Pappert JB, Grisé RA, Alder NN, Richards JH. 1999. Predawn disequilibrium between plant and soil water potentials in two cold-desert shrubs. *Oecologia,* **120**: 209-217.

Downes G, Beadle C, Worledge D. 1999. Daily stem growth patterns in irrigated *Eucalyptus globulus* and *E. nitens* in relation to climate. *Trees - Structure and Function,* **14**: 102-111.

Giovannelli A, Deslauriers A, Fragnelli G, Scaletti L, Castro G, Rossi S, Crivellaro A. 2007. Evaluation of drought response of two poplar clones (*Populus×canadensis* Mönch ‘I-214’ and *P. deltoides* Marsh. ‘Dvina’) through high resolution analysis of stem growth. *Journal of Experimental Botany,* **58**: 2673-2683.

Goldstein G, Andrade JL, Meinzer FC, Holbrook NM, Cavelier J, Jackson P, Celis A. 1998. Stem water storage and diurnal patterns of water use in tropical forest canopy trees. *Plant, Cell and Environment,* **21**: 397-406.

Goldstein G, Meinzer F, Monasterio M. 1984. The role of capacitance in the water balance of Andean giant rosette species. *Plant, Cell and Environment,* **7**: 179-186.

Herzog KM, Häsler R, Thum R. 1995. Diurnal changes in the radius of a subalpine Norway spruce stem: Their relation to the sap flow and their use to estimate transpiration. *Trees - Structure and Function,* **10**: 94-101.

IPCC. 2013. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge, United Kingdom and New York, NY, USA: Cambridge University Press; 2013. p. 1535.

Kavanagh KL, Pangle R, Schotzko AD. 2007. Nocturnal transpiration causing disequilibrium between soil and stem predawn water potential in mixed conifer forests of Idaho. *Tree Physiology,* **27**: 621-629.

King G, Fonti P, Nievergelt D, Büntgen U, Frank D, King G, Fonti P, Nievergelt D, Büntgen U, Frank D. 2013. Climatic drivers of hourly to yearly tree radius variations along a 6°C natural warming gradient. *Agricultural and Forest Meteorology,* **168**: 36-46.

Kramer PJ. 1964. The role of water in wood formation. In: The formation of wood in forest trees (Zimmermann MH ed) Academic Press, New York, pp. 519-532.

Lindner M, Sohngen B, Joyce AL, Price DT, Bernier PY, Karjalainen T. 2002. Integrated forestry assessments for climate change impacts. *Forest Ecology and Management,* **162**: 117-136.

Loustau D, Roumagnac P, Berbigier P, Arruda-Pacheco C, David JS, Ferreira MI, Pereira JS, Tavares R. 1996. Transpiration of a 64-year-old maritime pine stand in Portugal. 1. Seasonal course of water flux through maritime pine. *Oecologia,* **107**: 33-42.

Meinzer FC, James SA, Goldstein G, Woodruff D. R. MFC, McCulloh K. A. 2003. Whole-tree water transport scales with sapwood capacitance in tropical forest canopy trees. *Plant, Cell and Environment,* **26**: 1147–1155.

Meinzer FC, Johnson DM, Lachenbruch B, McCulloh KA, Woodruff DR. 2009. Xylem hydraulic safety margins in woody plants: coordination of stomatal control of xylem tension with hydraulic capacitance. *Functional Ecology,* **23**: 922-930.

Meinzer FC, Woodruff DR, Domec J-C, Goldstein G, Campanello PI, Gatti MG, Villalobos-Vega R. 2008. Coordination of leaf and stem water transport properties in tropical forest trees. *Oecologia,* **156**: 31–41.

Moser EB, Saxton AM. 1990. Repeated measures analysis of variance: application on tree research. *Canadian Journal Forest Research,* **20**: 524-535.

Muller B, Pantin F, Génard M, Turc O, Freixes S, Piques M, Gibon Y. 2011. Water deficits uncouple growth from photosynthesis, increase C content, and modify the relationships between C and growth in sink organs. *Journal of Experimental Botany,* **62**: 1715-1729.

Ortuño MF, Conejero W, Moreno F, Moriana A, Intrigliolo DS, Biel C, Mellisho CD, Pérez-Pastor A, Domingo R, Ruiz-Sánchez MC, Casadesus J, Bonany J, Torrecillas A. 2010. Could trunk diameter sensors be used in woody crops for irrigation scheduling? A review of current knowledge and future perspectives. *Agricultural Water Management,* **97**: 1-11.

Peng C, Ma Z, Lei X, Zhu Q, Chen H, Wang W, Liu S, Li W, Fang X, Zhou X. 2011. A drought-induced pervasive increase in tree mortality across Canada’s boreal forests. *Nature,* **1**: 467–471.

Phillips N, Lewis JD, Logan BA, Tissue D. 2010. Inter- and intra-specific variation in nocturnal water transport in Eucalyptus. *Tree Physiology,* **30**: 586-596.

Phillips N, Scholz F, Bucci S, Goldstein G, Meinzer F. 2009. Using branch and basal trunk sap flow measurements to estimate whole-plant water capacitance: comment on Burgess and Dawson (2008). *Plant and Soil,* **315**: 315–324.

Price DT, Alfaro RI, Brown KJ, Flannigan MD, Fleming RA, Hogg EH, Girardin MP, Lakusta T, Johnston M, McKenney DW, Pedlar JH, Stratton T, Sturrock RN, Thompson ID, Trofymow JA, Venier LA. 2013. Anticipating the consequences of climate change for Canada’s boreal forest ecosystems. *Environmental Reviews,* **21**: 322–365.

Quinn G, Keough M. 2002. *Experimental Design And Data Analysis For Biologists.* Cambridge, UK: Cambridge University Press.

Schulze E-D, Turner NC, Glatzel G. 1984. Carbon, water and nutrient relations of two mistletoes and their hosts: A hypothesis. *Plant, Cell and Environment,* **7**: 293-299.

Sevanto S, Suni T, Pumpanen J, Gronholm T, Kolari P, Nikinmaa E, Hari P, Vesala T. 2006. Wintertime photosynthesis and water uptake in a boreal forest. *Tree Physiology,* **26**: 749-757.

Steppe K, De Pauw DJW, Lemeur R, Vanrolleghem PA. 2006. A mathematical model linking tree sap flow dynamics to daily stem diameter fluctuations and radial stem growth. *Tree Physiology,* **26**: 257–273.

Steppe K, Lemeur R, Samson R. 2002. Sap flow dynamics of a beech tree during the solar eclipse of 11 August 1999. *Agricultural and Forest Meteorology,* **112**: 139-149.

Stewart JD, Bernier PY. 1995. Gas exchange and water relations of 3 sizes of containerized Picea mariana seedlings subjected to atmospheric and edaphic water stress under controlled conditions. *Annals of Forest Science,* **52**: 1-9.

Tognetti R, Giovannelli A, Lavini A, Morelli G, Fragnito F, d’Andria R. 2009. Assessing environmental controls over conductances through the soil–plant–atmosphere continuum in an experimental olive tree plantation of southern Italy. *Agricultural and Forest Meteorology,* **149**: 1229-1243.

Topp GC, Zebchuk WD, Davis JL, Bailey WG. 1984. The measurement of soil water content using a portable TDR hand probe. *Canadian Journal of Soil Science,* **64**: 313-321.

Turcotte A, Morin H, Krause C, Deslauriers A, Thibeault-Martel M. 2009. The timing of spring rehydration and its relation with the onset of wood formation in black spruce. *Agricultural and Forest Meteorology,* **149**: 1403-1409.

Turcotte A, Rossi S, Deslauriers A, Krause C, Morin H. 2011. Dynamics of depletion and replenishment of water storage in stem and roots of black spruce measured by dendrometers. *Frontiers in Plant Science,* **2**: 21.

Urrutia-Jalabert R, Rossi S, Deslauriers A, Malhi Y, Lara A. 2015. Environmental correlates of stem radius change in the endangered *Fitzroya cupressoides* forests of southern Chile. *Agricultural and Forest Meteorology*, **200**: 209–221.

Vieira J, Rossi S, Campelo F, Freitas H, Nabais C. 2013. Seasonal and daily cycles of stem radial variation of *Pinus pinaster* in a drought-prone environment. *Agricultural and Forest Meteorology*, **180**: 173– 181.

Zeppel M, Tissue D, Taylor D, Macinnis-Ng C, Eamus D. 2010. Rates of nocturnal transpiration in two evergreen temperate woodland species with differing water-use strategies. *Tree Physiology*, **30**: 988-1000.

Zweifel R, Eugster W, Etzold S, Dobbertin M, Buchmann N, Häsler R. 2010. Link between continuous stem radius changes and net ecosystem productivity of a subalpine Norway spruce forest in the Swiss Alps. *New Phytologist*, **187**: 819-830.

Zweifel R, Hasler R. 2001. Dynamics of water storage in mature subalpine Picea abies: temporal and spatial patterns of change in stem radius. *Tree Physiology*, **21**: 561-569.

Zweifel R, Item H, Häsler R. 2000. Stem radius changes and their relation to stored water in stems of young Norway spruce trees. *Trees-Structure and Function*, **15**: 50–57.

CHAPITRE VI

CONCLUSION GÉNÉRALE

Le but de cette thèse était de comprendre de quelle manière l'activité cambiale et la formation du cerne de croissance des jeunes arbres d'épinettes noires [*Picea mariana* (Mill.) B.S.P.] pourraient varier dans le futur sous l'influence des changements climatiques. L'analyse de la croissance intra-annuelle et les manipulations des conditions environnementales des jeunes plants d'épinettes noires n'avaient jamais été abordées auparavant. Cette thèse présente une étude détaillée sur l'activité cambiale et la xylogénèse à l'aide de mesures directes de la croissance radiale et à l'aide de dendromètres électroniques, ainsi que des mesures de l'état hydrique et des échanges gazeux. Cette thèse aborde aussi les liens existants entre la croissance radiale, les sucres solubles totaux, les réserves d'amidon et la survie des plants. La densité et l'anatomie du bois ont été étudiées. Les techniques utilisées ont permis d'approfondir les connaissances sur la réponse des jeunes arbres pendant la saison de croissance et les relations avec les conditions environnementales.

6.1 ÉVALUATION DE LA DYNAMIQUE INTRA-ANNUELLE DE LA FORMATION DU CERNE DE CROISSANCE

L'activité cambiale et la xylogénèse ont été étudiées aux chapitres II, III et IV. L'activité cambiale et la formation du bois sont des processus biologiques très représentatifs et sensibles au déficit hydrique et à l'augmentation de la température. Dans les chapitres II et III, nous avons associé l'écophysiologie à la phénologie de la xylogénèse. D'un côté, l'écophysiologie a montré que les plants d'épinettes noires réagissent à la sécheresse selon des seuils spécifiques dans le temps. Les résultats obtenus ont permis de définir des seuils de déficit hydrique « modéré » (rejoint à un potentiel hydrique de -1.4 MPa) et « sévère » (rejoint à un potentiel hydrique de -2.9 MPa), ces résultats sont en accord avec plusieurs études sur l'épinette noire (Blake, Bevilacqua et Zwiazek 1991; Zine El Abidine *et al.* 1994; Major et Johnsen 1999). Sous un déficit hydrique sévère, les plants non-irrigués ont été incapables de maintenir l'assimilation du CO_2 et la conductance stomatique, qui ont été fortement réduites (<80% par rapport aux plants irrigués). Ces conditions suggèrent que l'efficacité de la translocation de l'eau a été fortement compromise, probablement par des cavitations irréversibles dans le xylème. De plus, nous avons montré qu'il existe une similitude dans le développement et la récupération du stress foliaire des plants non-irrigués à température plus élevée. Cela indique que le réchauffement seul ne peut pas complètement aggraver l'état hydrique des aiguilles, si les plants n'atteignent pas des seuils critiques de potentiel hydrique. Le potentiel hydrique de pré aube et de mi-journée arrivaient à des valeurs proches de -3 MPa avec l'augmentation des embolies dans le xylème. De plus, que la reprise des échanges gazeux n'a jamais atteint les niveaux de pré-déficit hydrique, suggérant une perte de conductivité hydraulique du xylème associée à la cavitation (Chapitre III). Pour cette espèce, nous avons trouvé que le point d'entrée d'air dans le xylème (P_{12}) était en moyenne -3 MPa, tandis que la pression subie par le xylème induisant le 50% de perte de conductivité hydraulique (P_{50}) était de -4.2 MPa. La conductance stomatique et l'assimilation du CO_2 récupèrent rapidement lorsque le rétablissement des conditions physiologiques optimales est complété (Stewart, Zine El Abidine et Bernier 1994; Bernier *et al.* 2001). La récupération de l'état hydrique et de la conductance surviennent en premier et sont suivies par l’assimilation du CO_2, qui a une reprise partielle de l’ordre de 40-60% (Bogeat-Triboulot *et al.* 2007).

D’un autre côté, nous avons associé la xylogénèse des plants soumis au déficit hydrique à différents seuils de déficit. Dans le stade du déficit hydrique qualifié de « modéré », l’élargissement des cellules est d’abord inhibé, la progression du déficit hydrique affecte la division cellulaire, tel qu’observé par plusieurs études (Abe *et al.* 2003; Jyske *et al.* 2010; de Luis *et al.* 2011). Nos résultats ont montré que l’activité cambiale et la différenciation cellulaire ont subi un ralentissement graduel à la fin du déficit hydrique qualifié de « sévère » et pendant plusieurs semaines après la reprise de l’irrigation. Par contre, l'augmentation de la température influençait grandement la période de récupération des plants soumis au déficit hydrique. Le développement cellulaire a été ralenti dans le temps avec une augmentation des températures, déterminant différentes sensibilités du cambium et des phases du développement cellulaire. Cette réponse concorde avec celle d'autres études dont l'application localisée de la température sur la tige peut provoquer des altérations de l'activité cambiale (Oribe *et al.* 2001; Gričar *et al.* 2007; Begum *et al.* 2013). Cela signifie que l'effet du déficit hydrique sur les plants peut être maintenu plus longtemps sous des températures plus élevées. Ces résultats sont en accord avec ceux de Arend et Fromm (2007) qui ont observé une réduction de la croissance radiale, spécialement de l’élargissement cellulaire, de l’épaississement et la formation de la paroi suite à une sécheresse, ainsi qu’une augmentation de la mortalité.

Les résultats suggèrent que les mesures intra-annuelles du chapitre II, bien que précises, se limitent souvent à des variables statiques, telles que le nombre de cellules. Par contre, cela peut s’avérer insuffisant afin de décrire correctement les processus dynamiques sous-jacents qui se produisent pendant la croissance secondaire. Dans la littérature, la plupart des études se concentrent sur la phénologie de la formation du bois, en caractérisant les différences dans le début, la fin et la durée de la forêt boréale et tempérée (Lupi *et al.* 2010; Moser *et al.* 2010; Rossi *et al.* 2011; Rossi, Girard et Morin 2014). Dans les environnements froids, ces différences se reflètent par une influence de la température sur la phénologie du développement cellulaire (Rossi *et al.* 2011; Lupi 2012). Beaucoup moins d'études se penchent sur la cinétique (Cuny *et al.* 2013; Cuny *et al.* 2014). En effet, les nouvelles cellules du xylème se différencient selon une cinétique particulière – caractérisée par une vitesse et une durée spécifique – entraînant des changements dans l'anatomie du bois le long du cerne de croissance (Cuny *et al.* 2014). En analysant les résultats aux chapitres II et IV, nous avons mis en évidence les liens dynamiques existants entre les taux et la durée de la production du bois, qui atténuent les impacts des stress environnementaux sur la structure du cerne de croissance.

Nos résultats concordent avec les recherches qui ont étudié les effets de la disponibilité en eau sur la croissance intra-annuelle des jeunes arbres dans les environnements méditerranéens et tempérés (Rossi *et al.* 2009; de Luis *et al.* 2011). L'arrêt de l'activité cambiale est plus précoce lorsque la sécheresse est plus importante (Gruber *et al.* 2010; Eilmann *et al.* 2011) et affecte la production du bois menant à la formation de cernes de croissance plus étroits (Giovannelli *et al.* 2007; Čufar *et al.* 2008). Les résultats présentés au chapitre IV représentent un avancement important et unique par rapport au chapitre II et aux dernières recherches, car l’intégration de la cinétique a permis de calculer la vitesse et la durée de la croissance du xylème. Cet avancement a permis de comprendre comment la cinétique de la formation du bois fonctionne sous contraintes hydriques et thermiques.

L'augmentation des températures a accéléré la vitesse de production des cellules (plants irrigués vs. non-irrigués) selon une évolution bimodale suite à la sécheresse où la vitesse était doublée dans les nuits chaudes. Les données de la littérature relatives aux taux de production cellulaire dans les environnements froids suggèrent que pour l'épinette de Norvège (*Picea abies*) le taux de production cellulaire était de 0.5 à 1 cellule jour^{-1} (Mäkinen, Nöjd et Saranpää 2003), ce qui est similaire aux taux trouvés en forêt boréale (Deslauriers et Morin 2005). Pour des arbres matures d'épinettes noires, Rossi, Girard et Morin (2014) ont trouvé une plus grande variabilité des taux dus au site (de 0.2 à 1.1 cellules jour^{-1}). Bien que ces valeurs semblent très similaires à celles que nous avons obtenues, nous avons démontré que l'application d'une combinaison de contraintes abiotiques influençait fortement la vitesse de production cellulaire dans un moment précis de la saison de croissance.

Les trachéides effectuent leur différenciation en débutant avec l'élargissement radial et, ensuite, avec la formation de la paroi secondaire. Nous avons calculé que la phase d'élargissement chez des plants non-irrigués durait en moyenne 21% de plus que chez plants irrigués. Par contre, le taux d'élargissement a diminué de 28%, compensant pour l’augmentation de la durée. Par exemple, sur les arbres matures, Rossi *et al.* (2006) ont montré que la durée de l'élargissement peut varier d'une à quatre semaines chez diverses espèces de conifères dans les Alpes, tandis que Deslauriers, Morin et Bégin (2003) ont calculé une durée d'élargissement d'une semaine chez le sapin. Dans la présente étude, des durées différentes selon le régime d'irrigation ont été calculées : les plants irrigués présentaient très peu de changements au cours de la saison de croissance avec des valeurs variant de 8-9 jours, tandis que la durée d'élargissement des plants non-irrigués augmentait de trois fois (de 5-18 jours de plus). La production chez les plants non-irrigués a chuté de 28% pour balancer cette plus longue durée d'élargissement.

En ce qui concerne la vitesse du dépôt de la paroi cellulaire, elle était inférieure de 8% chez les plants non-irrigués par rapport aux irrigués (16,2 vs 17,7 µm² jour^{-1}, respectivement). À des températures plus élevées, les plants non-irrigués ont montré des vitesses de déposition de la paroi plus rapides de jour et de nuit (de 14% et 28% de plus respectivement par rapport au contrôle). Le même mécanisme de compensation était aussi observable pour la durée de formation de la paroi, de sorte que les plants non-irrigués nécessitaient de 10 à 20-28 jours de plus pour former la paroi secondaire.

Dans cette thèse, nous avons associé les deux composantes de la cinétique (durée et vitesse) à des mécanismes de régulation. D'un côté, la pression de turgescence et la disponibilité en carbone pourraient expliquer la compensation entre la durée et la vitesse des processus de xylogénèse (Proseus, Zhu et Boyer 2000). Le maintien de la turgescence est nécessaire dans tous les processus de croissance, de la division cellulaire au dépôt des polysaccharides formant la paroi cellulaire (Proseus, Zhu et Boyer 2000). Les glucides représentent la ressource principale pour la division cellulaire et le dépôt de la paroi (Ainsworth et Bush 2011). La réduction de la photosynthèse durant un déficit hydrique implique une diminution de l'allocation en carbone pour la croissance provoquant donc des mécanismes de compensation qui se manifestent par une augmentation de la durée, assurant ainsi un taux de déposition approprié pour la construction de parois cellulaires optimales.

6.2 ÉVALUATION DES CARACTÉRISTIQUES DU XYLÈME

Les chapitres II, III et IV, montrent que l'anatomie du bois des plants d'épinettes noires est généralement résistante à la sécheresse et au réchauffement. L'anatomie du bois est formée en réponse aux conditions environnementales de la saison (Vaganov, Hughes et Shashkin 2006) et la contribution des sous-processus associés à la xylogénèse est fondamentale pour la définition de la structure du cerne (Cuny *et al.* 2014). Cette thèse a démontré que par une forte compensation entre les taux et les durées des sous-processus de différenciation cellulaire, les effets de la sécheresse et du réchauffement sont atténués sur la structure des cernes. Dans l'ensemble de ces résultats, les dimensions des cellules n'ont pas été influencées par les différents traitements imposés. Cependant, l'épaisseur de la paroi est l'unique caractéristique anatomique à se modifier partiellement suite aux conditions de stress. Dans ce cas, la variabilité de l'épaisseur de la paroi est en grande partie attribuée à la durée de l'élargissement (67%) et dans une moindre partie, attribuable à la déposition de la paroi (33%) (Cuny *et al.* 2014), cela signifie que le mécanisme de compensation entre la durée et le taux se fait principalement pendant l'élargissement et moins pendant la phase de déposition de la paroi. Les glucides non-structuraux représentent la ressource principale contribuant au dépôt de la paroi (Giovannelli *et al.* 2011; Pantin *et al.* 2013; Deslauriers *et al.* 2014) et lorsqu'ils sont partiellement épuisés ou non disponibles, comme nous l'avons montré dans le chapitre III, cela peut influencer l'épaisseur de la paroi cellulaire. Ce résultat montre l'influence majeure des glucides sur la déposition de la paroi et confirme leur rôle central dans la modulation de la xylogénèse.

Plusieurs considèrent la densité du bois comme le portrait final de l'investissement en carbone au cours de la formation du bois (le carbone soluble est converti en carbone structural) (Rathgeber, Decoux et Leban 2006). En effet, la densité du bois dépend des dimensions des cellules et de la quantité de carbone (la cellulose, les hémicelluloses et la lignine) pour la formation de la paroi cellulaire secondaire (Gindl, Grabner et Wimmer 2000; Emiliani *et al.* 2011). On considère même qu'il y a des relations entre les dimensions des trachéides et la densité du bois. Ces relations sont établies par la proportion finale entre la paroi cellulaire et le lumen cellulaire (Chave *et al.* 2006; Rathgeber, Decoux et Leban 2006). On peut donc affirmer que les variations de la densité du bois sont reflétées dans l'architecture hydraulique des plants. En effet, au niveau anatomique, des auteurs considèrent que la présence du bois final, qui est caractérisé par des petites cellules avec des parois plus épaisses, détermine une densité du bois plus élevée (Larson 1964; Begum *et al.* 2012). Cependant, quelques auteurs avaient déjà trouvé que la proportion du bois final n'est pas complètement responsable de l'augmentation de la densité du bois (Mäkinen, Saranpää et Linder 2002). Des études ont également montré que la densité du bois des conifères peut être strictement corrélée aux conditions environnementales (Chave *et al.* 2006), en particulier, à la température (Gindl, Grabner et Wimmer 2000; Grabner *et al.* 2006). En ce sens, les résultats des chapitres II et III ont montré que la température influence la densité du bois. Même si dans le chapitre II aucune différence significative n’était mise en évidence entre les régimes hydriques et les conditions thermiques, on observait un effet sporadique de la température et une réduction importante de la densité du bois à haute température. Au chapitre III, les résultats ont démontré que la densité du bois a été affectée par le déficit hydrique et par l'augmentation de la température pendant la nuit, influençant les profils de densité du bois des plants irrigués et non-irrigués. L'effet positif de l'augmentation de la température se manifeste par une augmentation de la largeur du bois initial en réponse au réchauffement pendant le jour, tandis qu'une augmentation de la largeur du bois final est observée sous réchauffement pendant la nuit. Cet effet de la température est présent dans la plupart des études en dendrochronologie où on retrouve une forte corrélation de la température estivale avec l'augmentation de la densité du bois maximale (Wang, Payette et Bégin 2002; Esper *et al.* 2008; Esper *et al.* 2012). Toutefois, Cuny *et al.* (2014) affirment que la contribution relative des changements de la superficie de la section de la paroi sur la variation de la densité du bois est de 25%, comparativement à 75% pour la taille des cellules. Ceci dit, les résultats de la présente étude suggèrent que la réduction de la densité du bois chez les plants non-irrigués est causée seulement en partie par un changement dans la répartition du carbone, qui est alloué en priorité à la respiration cellulaire au lieu de la formation des parois (Amthor 2000; Duan *et al.* 2013) lors d’une augmentation de la température. Ces résultats concordent avec ceux de Pamerleau-Couture (2011) chez les arbres matures d'épinettes noires où une diminution de la densité du bois est attribuée à une diminution de l'épaisseur des parois. Si la taille des cellules est majoritairement responsable de la densité du bois (Cuny *et al.* 2014), la récurrence des contraintes hydriques amène une plus faible densité du bois. Une réduction de la densité du bois est donc un bon indicateur d'une stratégie d’adaptation du xylème afin d’éviter une défaillance hydraulique (cavitation) suite à un déficit hydrique sévère (Rosner *et al.* 2014; Ruiz Diaz Britez *et al.* 2014).

6.3 IMPLICATIONS SUR LA SURVIE DE JEUNES ARBRES D'ÉPINETTES NOIRES

Cette étude (Chapitres II et III) a aussi mis en évidence qu’au cours de la formation du bois, l'effet combiné de l'augmentation des températures avec un déficit hydrique ne limitait pas seulement l'activité cambiale, mais aussi la survie des plants. L'augmentation de la température de +2 à +5 °C entraînait un taux de mortalité de 5 à 12%, respectivement. Lors d'une augmentation de la température de +6 °C pendant la nuit et le jour, les taux atteignaient des valeurs de 10 et 20% par rapport au contrôle (mortalité de 0.8%). Toutefois, ces taux de mortalité ne peuvent pas représenter la mortalité réelle observée en raison du prélèvement destructif effectué parmi les jeunes arbres afin de suivre la xylogénèse. Les résultats obtenus peuvent constituer une sous-estimation de la mortalité face aux conditions environnementales modifiées. Dans les écosystèmes méditerranéens, les conditions de sécheresse au printemps et pendant l'été sont fortement liées à la survie (de Luis *et al.* 2011). De plus, des observations sur des jeunes plantations en milieu boréal témoignent de l'effet du déficit hydrique sur la croissance racinaire (Burdett, Herring et Thompson 1984; Bernier 1993), car les plants peuvent être plus vulnérables à la sécheresse en raison de leur capacité réduite d'absorption des ressources minérales et nutritives. Lors de l'enlèvement du couvert végétal après une coupe, la survie de régénération préétablie d'épinettes noires est diminuée chez les petites tiges (hauteur ≤ 30 cm) et les tiges opprimées (hauteur <1 m) (Ruel 1989; Ruel, Doucet et Boily 1991). Dans le cas des petites tiges, la mortalité est autour de 19,7%. Pour celles de mauvaise qualité, suite aux blessures d'exploitation par exemple, la mortalité atteindrait 50% (Ruel, Doucet et Boily 1991). Si l'établissement de la régénération en nombre élevé représente une condition importante pour le renouvellement du peuplement, sa protection après coupe ne les met pas à l'abri de la sécheresse et du stress thermique (Breshears *et al.* 2009; Allen *et al.* 2010).

6.4 ÉVALUATION DE LA CROISSANCE ET DES RÉSERVES DE CARBONE : ENTRE LA DÉFAILLANCE HYDRAULIQUE ET L'ÉPUISEMENT DU CARBONE

Cette thèse a confirmé en partie le rôle actif des réserves de carbone sur l'activité cambiale, les mécanismes métaboliques ainsi que la reprise après la sécheresse. Dans le chapitre III, aucune différence n'a été montrée dans le contenu en glucides totaux non-structuraux entre les plants irrigués et non-irrigués, aux différentes températures. La variation des glucides était similaire à celle observée chez d'autres conifères (Gruber *et al.* 2012). Par contre, les réserves en amidon dans le xylème, représentant le stockage à plus long terme du carbone, étaient significativement plus basses lorsque les plants étaient exposés aux températures élevées. Des recherches ont démontré l’importance du stockage en carbone dans la réponse physiologique des arbres face aux changements climatiques, comme la variation du niveau de CO_2 (Arnone III *et al.* 2000; Hoch et Körner 2008), la température (Adams *et al.* 2009) et la sécheresse (McDowell *et al.* 2008; Adams *et al.* 2013). La gravité de ces divers phénomènes de stress s'exacerbe à l'échelle régionale entraînant une augmentation du taux de mortalité de plants (Peng *et al.* 2011) et affectant probablement la balance en carbone. Cette thèse a montré que l'augmentation des températures a ralenti le processus de reconstitution des réserves en amidon après le minimum saisonnier, ce qui suggèrerait une différente répartition de l'amidon entre le jour et la nuit. Après leur minimum saisonnier, cette baisse des réserves d'amidon pourrait être liée au pourcentage plus élevé de mortalité observé à des températures diurnes et nocturnes plus élevées et pourrait être responsable du taux de mortalité prolongé aux hautes températures nocturnes. Les effets négatifs de la sécheresse et de l'augmentation des températures sur l'approvisionnement et le stockage de l’amidon, ainsi que leur accessibilité (glucose dérivé de l’hydrolyse de l’amidon) lors de la relance de l'irrigation, pourraient ralentir et/ou arrêter la croissance, jusqu'à être déterminants pour la survie (Hartmann *et al.* 2013; Hartmann, McDowell et Trumbore 2015).

6.5 L'EFFET COMBINÉ DES STRESS ABIOTIQUES SUR LE PLANT ENTIER

À travers les chapitres II, III, IV et V, nous avons pu confirmer le rôle fondamental du déficit hydrique et de l'augmentation de la température pour la xylogénèse et la production du bois. Avec le réchauffement climatique, il est possible que la sécheresse s’intensifie en forêt boréale avec l’accélération de l'évapotranspiration. L'effet combiné des conditions environnementales sur les plants est souvent plus important, car la multiplication des conditions de stress peut causer un dysfonctionnement des processus physiologiques. D'abord, aux chapitres II et III, l'impact combiné du déficit hydrique et des températures élevées a été identifié pour les relations hydriques, l'assimilation du

CO_2 et la conductance stomatique des aiguilles. Lors d’une sécheresse, l'activité cambiale et les phases de la xylogénèse peuvent être inhibées avant la photosynthèse (Abe *et al.* 2003; McDowell 2011). L’état de turgescence des plants agit donc comme première contrainte pour certaines phases de développement (Woodruff et Meinzer 2011) comme la division et l’élargissement. Sous l'effet des températures élevées, le réchauffement nocturne augmente la respiration, provoquant un effet important sur le métabolisme de la plante (Turnbull, Murthy et Griffin 2002; 2004; Way 2013). De plus, les résultats des chapitres II et III ont montré que les jeunes arbres ont une mortalité plus élevée, qui est induite par la sécheresse et supérieure lorsque les plants se développent à des températures plus élevées, s'expliquant par une réduction de la conductance stomatique de la feuille. En effet, dans la période post-sécheresse, la reprise très lente des paramètres écophysiologiques (potentiels hydriques et échanges gazeux) peut être due à une perte de conductivité hydraulique du xylème associée à la cavitation, comme l’ont démontré Brodribb et Cochard (2009).

Au chapitre II, il a été démontré que la croissance radiale et l'activité du cambium étaient très sensibles à un déficit hydrique, mais, après la relance de l'irrigation, les plants survivants ont pu reprendre la croissance radiale, montrant une certaine résilience aux multi stress abiotiques, soit le déficit hydrique et l'augmentation de la température. Au chapitre IV, les sous-processus de la xylogénèse les plus altérés par l'effet des stress hydriques et thermiques ont été mis en évidence. De ce fait, cette thèse a clarifié les mécanismes de compensation mis en place lors de la combinaison de deux stress abiotiques (augmentation de la température et déficit hydrique).

Au chapitre V, il a été démontré que les variations radiales de la tige ne sont influencées que par la disponibilité en eau dans le sol, définie par l'intensité et la durée du déficit hydrique, mais que la température était un moteur important des cycles diurnes de la tige. L'imposition concomitante d’un réchauffement et d’une période unique de déficit hydrique (2011) a provoqué une augmentation significative de la durée et l’amplitude de la contraction. Chez les plants non-irrigués en 2011, la durée de la contraction a presque doublé lors du déficit hydrique. Par contre, lorsque les plants étaient soumis à des sécheresses de plus courtes durées, mais répétitives (2012), l’effet du stress hydrique sur les cycles diurnes était significatif seulement lors de la troisième période de stress : la durée de la contraction et de l’expansion étaient plus rapides à des températures plus élevées que pour les plants contrôles. En général, l'amplitude de la contraction était plus grande, tandis que l'amplitude de l’expansion était plus petite dans le traitement de réchauffement nocturne. L'imposition concomitante du réchauffement et d'un déficit hydrique unique en 2011 provoque des variations radiales des tissus du tronc qui sont liées aux variations irréversibles de la division et l'élargissement cellulaire, déjà observées pendant le déficit hydrique dans les chapitres II et IV. En 2012, cependant, les sécheresses récurrentes provoquaient un effet de report sur l'équilibre hydrique des plants non-irrigués, indiquant que la teneur en eau et la turgescence ont été maintenues sur des courtes périodes. Ceci illustre le fait que les jeunes épinettes sont en mesure de résister à une première phase de stress hydrique, c'est-à-dire de « stress modéré », tel qu’observé au chapitre II.

6.6 CONTRIBUTIONS MAJEURES DE LA THÈSE

Les résultats des chapitres II à V ont permis de connaître la phénologie du cambium, la dynamique intra-annuelle du développement du cerne de croissance, les caractéristiques du xylème et la survie de jeunes arbres d'épinettes noires, nécessaires à l'interprétation des réponses aux scénarios climatiques futurs. Cette thèse a donc clarifié les effets de la combinaison de facteur de stress abiotique et leurs contributions individuelles. En analysant l'ensemble des quatre chapitres, les contributions majeures de la thèse sont les suivantes :

- Les sécheresses fréquentes et intenses d’environs un mois, associées au réchauffement, compromettent la survie des plants. L'effet combiné de l'augmentation journalière de la température de l’air avec un déficit hydrique a augmenté la mortalité des plants de 5 à 12%. Dans des conditions de réchauffement appliquées pendant le jour et la nuit (+6°C), la mortalité des jeunes arbres était plus exacerbée pendant le jour (20%), en raison d'une défaillance hydraulique dans le xylème. Par contre, la mortalité était plus prolongée dans le temps sous des conditions de réchauffement nocturne, en raison de la restauration incomplète des réserves de carbone.
- Pour les plants survivants, un ralentissement de la croissance et de l'activité cambiale pendant le déficit hydrique et un maintien de cet effet lors de la reprise de l'état hydrique optimale à température élevée ont été observés. L'impact du déficit hydrique sur la formation du bois a une influence à très court terme (arrêt de la division cellulaire peu de temps après le début du stress hydrique) alors que l’influence de la température semble agir à plus long terme, tout au long de la saison de croissance.

- Les effets combinés de l'augmentation journalière de la température et du déficit hydrique n'ont pas démontré d'impact évident sur les caractéristiques du xylème, étant fortement conservées ou peu modifiées suite à ces changements environnementaux. Toutefois, lors du réchauffement appliqué seulement pendant le jour ou la nuit et combiné à un déficit hydrique, l'effet sur l'anatomie et sur la densité du bois a été plus important à cause d’une insuffisance en carbone.
- La cinétique de la formation de bois est déterminante pour l’anatomie et par conséquent, de la densité du bois. Cette cinétique est grandement modifiée lors d’un réchauffement et d’une sécheresse. La réduction de la vitesse est compensée par une augmentation de la durée des sous-processus de la xylogénèse (élargissement et formation des parois). Ces mécanismes compensatoires atténuent fortement l'impact de la sécheresse et du réchauffement sur l'anatomie du bois.
- L'étude des phases de contraction et d'expansion, des paramètres physiologiques, ainsi que des relations hydriques à différentes températures et échelles temporelles, ont montré que les variations radiales ne sont pas seulement influencées par la disponibilité en eau, mais aussi par la température. Notamment, la température nocturne se comporte comme un facteur déterminant dans l'équilibre hydrique.

6.7 PERSPECTIVES DE RECHERCHE

Dans les dernières années, un nombre croissant d'études soulignent l'importance de considérer l’effet multiplicatif des facteurs de stress abiotiques sur la croissance et leurs implications au niveau écophysiologique sur l'équilibre hydrique et le cycle du carbone.

Dans un contexte d'augmentation des changements climatiques, un changement de la dynamique de la phénologie du cambium pourrait jouer un rôle dans la dynamique des réserves de carbone. En effet, ces réserves sont nécessaires pendant la saison de croissance ainsi que pendant la période de repos végétatif. La question concernant la croissance et la survie de la régénération naturelle et artificielle est très pertinente face aux changements climatiques. Les mesures utilisées dans cette thèse seront importantes pour de futures recherches en serre et en pépinière, car celles-ci nécessitent une bonne compréhension de l'interaction entre les stress abiotiques afin de déterminer la dynamique de l'utilisation de l'eau et le stockage de carbone. Notre design expérimental mis en place pourrait être déterminant pour examiner l'effet à court et à moyen terme des stress abiotiques qui auraient un impact majeur dans les pépinières, mais aussi dans les plantations d'épinettes noires.

L'épinette noire constitue l'essence la plus utilisée dans le reboisement, car chaque année cette espèce compte pour 54% des plants mis en terre au Québec (Gouvernement du Québec 2013). L'épinette noire est aussi parmi les conifères qui enregistrent une augmentation de la mortalité due aux sécheresses régionales (Peng *et al.* 2011). Dans un contexte de changement climatique et suite à nos résultats sur le taux de mortalité (Chapitre II et III), le risque de mortalité lors du reboisement représente un enjeu pour les jeunes plantations. Pour ce faire, des études ultérieures en plantation sont nécessaires : on propose par exemple de développer des expériences qui consistent dans l'application de chambres permanentes en milieu naturel afin d'évaluer les effets d'une augmentation de la température de jour/nuit. De plus, une étude de ce genre en milieu naturel pourrait permettre de définir les implications physiologiques (respiration du sol et de la plante, photosynthèse) et structurales (formation du bois) liées aux stress abiotiques.

Compte tenu des nouveaux résultats au niveau de la dynamique de la xylogénèse et de l'effet des sous-processus dans l'anatomie (Chapitre IV), il serait aussi intéressant d'élargir l'étude à d'autres espèces, en milieu naturel (en plantation). En effet, la cinétique de différenciation cellulaire et ses effets sur l'anatomie et la densité sont inconnue des autres conifères d'intérêt commercial en forêt boréale comme le pin gris, l'épinette blanche, le sapin baumier et le mélèze. Cela pourrait aider à comprendre quand, à quelle vitesse et combien de temps les processus de la xylogénèse sont responsables de l'adaptation des conifères boréaux aux nouvelles conditions environnementales.

L'irrigation précise est essentielle pour le succès de la production et la performance des plants. Cet élément, combiné à la fertilisation, est important pour assurer les teneurs en eau dans le sol nécessaire pour la survie et la croissance racinaire. La combinaison de ces deux facteurs est importante, surtout dans les premières années de vie afin d'éviter le lessivage des engrais dans les cultures extérieures, surtout dans les périodes critiques de la croissance racinaire, radiale et apicale. Les résultats obtenus sur la phénologie et la cinétique du cambium de l'épinette noire (Chapitre IV) pourraient s'intégrer dans une étude sur la phénologie de l'allongement des racines fines et à leur mortalité associée à certains traitements d'irrigation et de fertilisation. Une telle étude pourrait aider à comprendre comment la disponibilité en eau et la fertilité du sol influencent la répartition du carbone entre le flux aérien et souterrain chez l'épinette noire.

6.8 RÉFÉRENCES

Abe H, Nakai T, Utsumi Y et Kagawa A. 2003. Temporal water deficit and wood formation in Cryptomeria japonica. Tree Physiology, 23 : 859–863.

Adams HD, Germino MJ, Breshears DD, Barron-Gafford GA, Guardiola-Claramonte M, Zou CB et Huxman TE. 2013. Nonstructural leaf carbohydrate dynamics of *Pinus edulis* during drought-induced tree mortality reveal role for carbon metabolism in mortality mechanism. New Phytologist, 197 : 1142–1151.

Adams HD, Guardiola-Claramonte M, Barron-Gafford GA, Villegas JC, Breshears DD, Zoug CB, Troch PA et Huxman TE. 2009. Temperature sensitivity of drought-induced tree mortality portends increased regional die-off under global-change-type drought. Proceedings of the National Academy of Sciences of the United States, 106 : 7063-7066.

Ainsworth EA et Bush DR. 2011. Carbohydrate export from the leaf: a highly regulated process and target to enhance photosynthesis and productivity.(Update on Carbohydrate Export from the Leaf)(Report). Plant Physiology, 155 : 64.

Allen CD, Macalady AK, Chenchouni H, Bachelet D, McDowell N, Vennetier M, Kitzberger T, Rigling A, Breshears DD, Hogg EHT, Gonzalez P, Fensham R, Zhang Z, Castro J, Demidova N, Lim J-H, Allard G, Running SW, Semerci A et Cobb N. 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. Forest Ecology and Management, 259 : 660-684.

Amthor JS. 2000. Direct effect of elevated CO_2 on nocturnal in situ leaf respiration in nine temperate deciduous tree species is small. Tree Physiology, 20 : 139-144.

Arend M et Fromm J. 2007. Seasonal change in the drought response of wood cell development in poplar. Tree Physiology, 27 : 985–992.

Arnone III JA, Zaller JG, Spehn EM, Niklaus PA, Wells CE et Körner C. 2000. Dynamics of root systems in native grasslands: effects of elevated atmospheric CO_2. New Phytologist, 147 : 73–85.

Begum S, Nakaba S, Yamagishi Y, Oribe Y et Funada R. 2013. Regulation of cambial activity in relation to environmental conditions: understanding the role of temperature in wood formation of trees. Physiologia plantarum, 147 : 46–54.

Begum S, Nakaba S, Yamagishi Y, Yaman eK, Islam MA, Oribe Y, Ko J-H, Jin H-O et Funada R. 2012. A rapid decrease in temperature induces latewood formation in artificially reactivated cambium of conifer stems. Annals of Botany, 110 : 1-11.

Bernier PY. 1993. Comparing natural and planted black spruce seedlings. II. Nutrient uptake and efficiency of use. Canadian Journal of Forest Research, 23 : 2435-2442.

Bernier PY, Raulier F, Stenberg P et Ung C-H. 2001. Importance of needle age and shoot structure on canopy net photosynthesis of balsam fir (*Abies balsamea*): a spatially inexplicit modeling analysis. Tree Physiology, 21 : 815–830.

Blake TJ, Bevilacqua E et Zwiazek JJ. 1991. Effects of repeated stress on turgor pressure and cell elasticity changes in black spruce seedlings. Canadian Journal of Forest Research, 21 : 1329-1333.

Bogeat-Triboulot MB, Brosche M, Renaut J, Jouve L, Le Thiec D, Fayyaz P, Vinocur B, Witters E, Laukens K, Teichmann T, Altman A, Hausman JF, Polle A, Kangasjarvi J et Dreyer E. 2007. Gradual soil water depletion results in reversible changes of gene expression, protein profiles, ecophysiology, and growth performance in *Populus euphratica*, a poplar growing in arid regions. Plant Physiology, 143 : 876-892.

Breshears DD, Myers OB, Meyer CW, Barnes FJ, Zou CB, Allen CD, McDowell NG et Pockman WT. 2009. Tree Die-Off in Response to Global Change-Type Drought: Mortality Insights from a Decade of Plant Water Potential Measurements. Frontiers in Ecology and the Environment, 7 : 185-189.

Brodribb TJ et Cochard H. 2009. Hydraulic failure defines the recovery and point of death in water-stressed conifers. Plant Physiology, 149 : 575-584.

Burdett AN, Herring LJ et Thompson CF. 1984. Early growth of planted spruce. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere, 14 : 644-651.

Chave J, Muller-Landau HC, Baker TR, Easdale TA, Steege H et Campbell OW. 2006. Regional and phylogenetic variation of wood density across 2456 neotropical tree species. Ecological Applications, 16 : 2356-2367.

Čufar K, Prislan P, Luis M et Gričar J. 2008. Tree-ring variation, wood formation and phenology of beech (*Fagus sylvatica*) from a representative site in Slovenia, SE Central Europe. Trees, 22 : 749-758.

Cuny HE, Rathgeber CBK, Frank D, Fonti P et Fournier M. 2014. Kinetics of tracheid development explain conifer tree-ring structure. New Phytologist, 203 : 1231-1241.

Cuny HE, Rathgeber CBK, Senga Kiessé T, Hartmann FP, Barbeito I et Fournier M. 2013. Generalized additive models reveal the intrinsic complexity of wood formation dynamics. Journal of Experimental Botany, 64 : 1983-1994.

de Luis M, Novak K, Raventós J, Gričar J, Prislan P et Čufar K. 2011. Cambial activity, wood formation and sapling survival of Pinus halepensis exposed to different irrigation regimes. Forest Ecology and Management, 262 : 1630–1638.

Deslauriers A et Morin H. 2005. Intra-annual tracheid production in balsam fir stems and the effect of meteorological variables. Structure and Function, 19 : 402-408.

Deslauriers A, Morin H et Bégin Y. 2003. Cellular phenology of annual ring formation of *Abies balsamea* in the Quebec boreal forest (Canada). Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere, 33 : 190-200.

Deslauriers A, Beaulieu M, Balducci L, Giovannelli A, Gagnon M-J et Rossi S. 2014. Impact of warming and drought on carbon balance related to wood formation in black spruce. Annals of Botany, 114 : 335-345.

Duan H, Amthor JS, Duursma RA, O’Grady AP, Choat B et Tissue DT. 2013. Carbon dynamics of eucalypt seedlings exposed to progressive drought in elevated [CO_2] and elevated temperature. Tree Physiology, 33 : 779–792.

Eilmann B, Zweifel R, Buchmann N, Graf Pannatier E et Rigling A. 2011. Drought alters timing, quantity, and quality of wood formation in Scots pine. Journal of Experimental Botany, 62 : 2763-2771.

Emiliani G, Traversi ML, Anichini M, Giachi G et Giovannelli A. 2011. Transcript accumulation dynamics of phenylpropanoid pathway genes in the maturing xylem and phloem of picea abies during latewood formation. Journal of Integrative Plant Biology, 53 : 783-799.

Esper J, Niederer R, Bebi P et Frank D. 2008. Climate signal age effects—Evidence from young and old trees in the Swiss Engadin. Forest Ecology and Management, 255 : 3783-3789.

Esper J, Büntgen U, Timonen M et Frank DC. 2012. Variability and extremes of northern Scandinavian summer temperatures over the past two millennia. Global and Planetary Change, 88-89 : 1-9.

Gindl W, Grabner M et Wimmer R. 2000. The influence of temperature on latewood lignin content in treeline Norway spruce compared with maximum density and ring width. Trees, 14 : 409-414.

Giovannelli A, Emiliani G, Traversi ML, Deslauriers A et Rossi S. 2011. Sampling cambial region and mature xylem for non structural carbohydrates and starch analyses. Dendrochronologia, 29 : 177-182.

Giovannelli A, Deslauriers A, Fragnelli G, Scaletti L, Castro G, Rossi S et Crivellaro A. 2007. Evaluation of drought response of two poplar clones (*Populus×canadensis* Mönch 'I-214' and *P. deltoides* Marsh. 'Dvina') through high resolution analysis of stem growth. Journal of Experimental Botany, 58 : 2673-2683.

Gouvernement du Québec. 2013. Ressources et industries forestières, portrait statistique 2013. Ministère des Ressources naturelles, 2013.

Grabner M, Cherubini P, Rozenberg P et Hannrup B. 2006. Summer drought and low earlywood density induce intra-annual radial cracks in conifers. Scandinavian Journal of Forest Research, 21 : 151-157.

Gričar J, Zupančič M, Čufar K et Oven P. 2007. Regular cambial activity and xylem and phloem formation in locally heated and cooled stem portions of Norway spruce. Wood Science and Technology, 41 : 463-475.

Gruber A, Strobl S, Veit B et Oberhuber W. 2010. Impact of drought on the temporal dynamics of wood formation in *Pinus sylvestris*. Tree Physiology, 30 : 490-501.

Gruber A, Pirkebner D, Florian C et Oberhuber W. 2012. No evidence for depletion of carbohydrate pools in Scots pine (*Pinus sylvestris* L.) under drought stress. Plant Biology, 14 : 142–148.

Hartmann H, McDowell NG et Trumbore S. 2015. Allocation to carbon storage pools in Norway spruce saplings under drought and low CO_2. Tree Physiology, 35 (3): 243-252.

Hartmann H, Ziegler W, Kolle O et Trumbore S. 2013. Thirst beats hunger – declining hydration during drought prevents carbon starvation in Norway spruce saplings. New Phytologist, 200 : 340–349.

Hoch G et Körner C. 2008. Growth and carbon relations of tree line forming conifers at constant vs. variable low temperatures. Journal of Ecology, 97 : 57-66.

Jyske T, Kaakinen S, Nilsson U, Saranpaa P et Vapaavuori E. 2010. Effects of timing and intensity of thinning on wood structure and chemistry in Norway spruce. Holzforschung, 64 : 81-91.

Larson PR. 1964. Contribution of different-aged needles to growth and wood formation of young red pines. Forest Science, 10 : 224-238.

Lupi C, Morin H, Deslauriers A, Rossi S & Houle D. 2012. Increasing nitrogen availability and soil temperature: effects on xylem phenology and anatomy of mature black spruce1. Canadian Journal Forest Research, 42 : 1277–1288.

Lupi C, Morin H, Deslauriers A et Rossi S. 2010. Xylem phenology and wood production: resolving the chicken-or-egg dilemma. Plant, Cell and Environment, 33 : 1721–1730.

Major JE et Johnsen KH. 1999. Shoot water relations of mature black spruce families displaying a genotype × environment interaction in growth rate. II. Temporal trends and response to varying soil water conditions. Tree Physiology, 19 : 375--382.

Mäkinen H, Saranpää P et Linder S. 2002. Wood-density variation of Norway spruce in relation to nutrient optimization and fibre dimensions. Canadian Journal Forest Research, 32 : 185–194.

Mäkinen H, Nöjd P et Saranpää P. 2003. Seasonal changes in stem radius and production of new tracheids in Norway spruce. Tree Physiology, 23 : 959-968.

McDowell N, Pockman WT, Allen CD, Breshears DD, Cobb N, Kolb T, Plaut J, Sperry J, West A, Williams DG et Yepez EA. 2008. Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? New Phytologist, 178 : 719–739.

McDowell NG. 2011. Mechanisms linking drought, hydraulics, carbon metabolism, and vegetation mortality. Plant Physiology, 155 : 1051.

Moser L, Fonti P, Büntgen U, Esper J, Luterbacher J, Franzen J et Frank D. 2010. Timing and duration of European larch growing season along altitudinal gradients in the Swiss Alps. Tree Physiology, 30 : 225-233.

Oribe Y, Funada R, Shibagaki M et Kubo T. 2001. Cambial reactivation in locally heated stems of the evergreen conifer *Abies sachalinensis* (Schmidt) masters. Planta, 212 : 684-691.

Pamerleau-Couture É. 2011. Impact de trois interventions sylvicoles sur la croissance et la qualité du bois de l'épinette noire en forêt boréale. Mémoire de maîtrise, Université du Québec à Chicoutimi.

Pantin F, Monnet F, Jannaud D, Costa JM, Renaud J, Muller B, Simonneau T et Genty B. 2013. The dual effect of abscisic acid on stomata. New Phytologist, 197 : 65-72.

Peng C, Ma Z, Lei X, Zhu Q, Chen H, Wang W, Liu S, Li W, Fang X et Zhou X. 2011. A drought-induced pervasive increase in tree mortality across Canada’s boreal forests. Nature, 1 : 467–471.

Proseus TE, Zhu G et Boyer JS. 2000. Turgor, temperature and the growth of plant cells: using *Chara corallina* as a model system. Journal of Experimental Botany, 51 : 1481-1494.

Rathgeber CBK, Decoux V et Leban J-M. 2006. Linking intra-tree-ring wood density variations and tracheid anatomical characteristics in Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco). Annals of Forest Science, 63 : 699–706.

Rosner S, Světlík J, Andreassen K, Børja I, Dalsgaard L, Evans R, Karlsson B, Tollefsrud MM et Solberg S. 2014. Wood density as a screening trait for drought sensitivity in Norway spruce. Canadian Journal of Forest Research, 44 : 131105133611006.

Rossi S, Girard MJ et Morin H. 2014. Lengthening of the duration of xylogenesis engenders disproportionate increases in xylem production. Global Change Biology, 20 : 2261-2271.

Rossi S, Deslauriers A, Anfodillo T et Carraro V. 2006. Evidence of threshold temperatures for xylogenesis in conifers at high altitudes. Oecologia, 152 : 1-12.

Rossi S, Hubert M, Deslauriers A et Plourde P-Y. 2011. Predicting xylem phenology in black spruce under climate warming. Global Change Biology, 17 : 614–625.

Rossi S, Simard S, Rathgeber CBK, Deslauriers A et De Zan C. 2009. Effects of a 20-day-long dry period on cambial and apical meristem growth in *Abies balsamea* seedlings. Trees - Structure and Function, 23 : 85-93.

Ruel J-C. 1989. Régénération de peuplements d'épinette noire après coupe à blanc mécanisée résultats préliminaires. Ministère de l'énergie et des ressources Forêts, Direction de la recherche et du développement, Québec, 1 ressource en ligne (18 pp.).

Ruel J-C, Doucet R et Boily J. 1991. Étude de la mortalité initiale de la régénération préétablie de sapin et d'épinette noire après une coupe avec protection de la régénération. Direction de la recherche, Ministère des forêts, Sainte-Foy, 1 ressource en ligne (9 pp).

Ruiz Diaz Britez M, Sergent A-S, Martinez Meier A, Bréda N et Rozenberg P. 2014. Wood density proxies of adaptive traits linked with resistance to drought in Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco). Trees, 28 : 1289-1304.

Stewart JD, Zine El Abidine A et Bernier PY. 1994. Stomatal and mesophyll limitations of photosynthesis in black spruce seedlings during multiple cycles of drought. Tree Physiology, 15 : 57-64.

Turnbull MH, Murthy R et Griffin KL. 2002. The relative impacts of daytime and night-time warming on photosynthetic capacity in *Populus deltoides*. Plant, Cell and Environment, 25 : 1729-1737.

Turnbull MH, Tissue DT, Murthy R, Wang X, Sparrow AD et Griffin KL. 2004. Nocturnal warming increases photosynthesis at elevated CO_2 partial pressure in *Populus deltoides*. New Phytologist, 161 : 819-826.

Vaganov EA, Hughes MK et Shashkin AV. 2006. Growth dynamics of conifer tree rings- images of past and futures environments. Introduction and Factors Influencing the Seasonal Growth of Trees. Dans : Springer, Heidelberg, p. 1-20.

Wang L, Payette S et Bégin Y. 2002. Relationships between anatomical and densitometric characteristics of black spruce and summer temperature at tree line in northern Quebec. Canadian Journal of Forest Research, 32 : 477–486.

Way DA. 2013. Will rising CO_2 and temperatures exacerbate the vulnerability of trees to drought? Tree Physiology, 33 : 775–778.

Woodruff DR et Meinzer FC. 2011. Water stress, shoot growth and storage of non-structural carbohydrates along a tree height gradient in a tall conifer. Plant, Cell and Environment, 34 : 1920-1930.

Zine El Abidine A, Bernier PY, Stewart JD et Plamondon AP. 1994. Water stress preconditioning of black spruce seedlings from lowland and upland sites. Canadian Journal of Botany, 72 : 1511-1518.