UNIVERSITÉ DU QUÉBEC EN ABITIBI-TÉMISCAMINGUE

# CROISSANCE ET NUTRITION DE L'ÉPINETTE NOIRE EN LIEN AVEC LE DÉVELOPPEMENT DE RACINES ADVENTIVES

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

PAR
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MAI 2019

# REMERCIEMENTS

Tout d'abord je voudrais remercier ma directrice, Annie DesRochers, de son accueil lors de mon périple abitibien, de m'avoir initiée au monde fabuleux de l'épinette, et de la pertinence de ses remarques, aussi bien lors de l'élaboration des expériences que lors de la rédaction des manuscrits. Je remercie également mon co-directeur, Nelson Thiffault, pour avoir pris le relai et m'avoir accueillie à son tour à Québec, ainsi que de sa bonne humeur permanente. Merci à tous les deux de m'avoir fait confiance en me laissant une grande autonomie dans la réalisation de ce doctorat et pour m'avoir guidée durant toutes ces années.

Je voudrais également remercier mon coach de fin de doctorat, Mathieu Bouchard, pour m'avoir soutenue durant la périlleuse période de rédaction. J'en profite pour également remercier la Direction de la Recherche Forestière pour m'avoir hébergée durant toutes ces années.

Je remercie tous mes collègues et collaborateurs qui m'ont apporté leur soutien et leur aide à un moment ou un autre de cette thèse : Lyne Blackburn, Sylvie Carles, Thiphaine Després, Sandrine Gautier-Éthier, Vincent Maurin, Pierre Périnet, Linda Perron-Beauchemin, Émilie Tarroux, Sophie Thériault, Véronique Simard, Pauline Suffice et tous ceux que j'ai probablement oubliés.

Merci à tous mes amis de près ou de loin qui m'ont aidée à avancer!

Enfin un merci tout particulier à mes proches : mes parents Solange et Régis, qui, même s'ils ne comprennent pas toujours tout ce que je fais, me soutiennent inconditionnellement; ma sœur Anne-Laure et mon beau-frère Jean-Charles, qui ont tellement 'financé' toutes ces années d'étude qu'ils sont un sponsor officiel de cette thèse 'Sapinette'; et enfin mes BFF les Laur-Lambert qui, contre vents et marées, sont toujours là pour moi!! Enfin merci à Louise, juste d'être mon mini rayon de soleil!

DÉDICACE

À la fondation Pernot-Petit-Laur-Lambert
pour leur soutien indéfectible.

# AVANT-PROPOS

Cette thèse est composée de trois articles scientifiques rédigés en anglais, encadrés par une introduction et une conclusion générales rédigées en français.

Chapitre II : Pernot C., Thiffault N., DesRochers A., 2019a. Contribution of adventitious *vs* initial roots to growth and physiology of black spruce seedlings. *Physiologia Plantarum*, doi:10.1111/ppl.12735.

Chapitre III : Pernot C., Thiffault N., DesRochers A., 2019b. Root system origin and structure influence planting shock of black spruce seedlings in boreal microsites. *Forest Ecology and Management*, doi:10.1016/j.foreco.2018.11.043.

Chapitre IV : Pernot C., Thiffault N., DesRochers A., 2019c. Influence of root system characteristics on black spruce seedling responses to limiting conditions. *Plants*, doi:10.3390/plants8030070.

Je suis l'auteure principale des trois articles, ayant élaboré les protocoles, récolté et analysé les données, et rédigé les premières versions des manuscrits. Ma directrice Annie DesRochers et mon co-directeur Nelson Thiffault m'ont soumis la question de recherche, m'ont conseillée à chaque étape et ont contribué à l'amélioration des articles.

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# LISTE DES ABRÉVIATIONS, DES SIGLES ET DES ACRONYMES

| | |
|---|---|
| AR | Adventitious roots |
| BR | Bareroot seedlings |
| C | Containerized seedlings |
| Ca | Calcium |
| $CO_2$ | Carbon dioxide |
| DP | Deeply planted containerized seedlings |
| FC | Field capacity |
| Fig. | Figure |
| $g_s$ | Stomatal conductance |
| IR | Initial roots |
| K | Potassium |
| Mg | Magnesium |
| N | Nitogen |
| P | Phosphate |
| PBS | Phosphate-buffered saline |
| PFA | Paraformaldehyde |

| | |
|---|---|
| $P_n$ | Net photosynthesis |
| RGR | Relative growth rate |
| SD | Standard deviation |
| SE | Standard error |
| SEM | Standard error of the mean |
| $^{15}N$ | Nitrogen 15 |
| $\delta^{13}C$ | Ratio of stable isotopes $^{13}C : ^{12}C$ |

# LISTE DES SYMBOLES ET DES UNITÉS

| | |
|---|---|
| cm | Centimeter |
| $cm^3$ | Cubic centimeter |
| dm | Decimeter |
| $dm^3$ | Cubic decimeter |
| g | Gram |
| h | Hour |
| km | Kilometer |
| kg | Kilogram |
| l | Liter |
| m | Meter |
| $m^2$ | Square meter |
| mg | Milligram |
| min | Minute |
| ml | Milliliter |
| mm | Millimeter |

| | |
|---|---|
| mmol | Millimole |
| MPa | Megapascal |
| s | Second |
| µm | Micrometer |
| µmol | Micromole |
| °C | Degré Celsius |
| % | Percent |
| ‰ | Permille |

# RÉSUMÉ

L'épinette noire (*Picea mariana* [Mill.] BSP) possède une spécificité racinaire : elle développe majoritairement un système racinaire adventif. Les racines adventives émergent à la base de la tige et remplacent progressivement les racines initiales issues de la germination de la graine, jusqu'à devenir prépondérantes au stade mature de l'arbre. Bien que ce phénomène ait été longtemps observé, il reste encore largement inexpliqué. Cette étude visait ainsi à déterminer le rôle des racines adventives lors de la croissance de l'épinette noire au stade de semis, en se focalisant sur leurs aptitudes à capter l'eau et les nutriments, et sur leur capacité d'adaptation à des conditions limitantes en ressources. L'hypothèse générale était que les adventives parviendraient à mieux capter l'eau et les nutriments que les racines initiales et que leur développement permettrait aux semis d'avoir une plus forte croissance et de meilleures performances physiologiques. Pour essayer d'y répondre, cette étude a été réalisée en trois volets.

Lors du premier volet (Chapitre II), les contributions des racines adventives et initiales à la croissance de semis âgés de 4 ans ont pu être testées grâce à un système de double-pot permettant une irrigation et une fertilisation indépendantes pour chaque type de racines. Après une saison de croissance en environnement contrôlé, la plupart des paramètres mesurés (croissance, physiologie et nutrition) ont été semblables pour un même traitement au niveau des racines adventives ou initiales. La principale différence a été observée au niveau de la biomasse racinaire avec un développement de racines adventives favorisé lorsque les semis ont reçu un apport en eau ou en nutriments important, excepté en cas d'irrigation exclusive aux racines initiales qui a résulté en un développement équivalent de racines initiales et adventives.

Le deuxième volet (Chapitre III) consistait à tester l'impact du système racinaire sur la croissance de semis de 2 ans lors d'une plantation en forêt boréale. La comparaison des semis avec ou sans racines adventives a permis d'observer une croissance semblable mais une plus forte captation de nutriments de la part des semis avec racines adventives, en particulier de l'azote marqué ajouté au début de la deuxième saison post-plantation.

Enfin le troisième volet (Chapitre IV) a permis de tester l'influence d'un système racinaire composé de racines adventives et initiales ou uniquement de racines initiales, sur la croissance de semis de 4 ans en conditions limitantes en eau, nutriments ou oxygène. Les semis ayant développé des racines adventives ont eu une plus forte croissance en hauteur que les semis n'ayant que des racines initiales, associée à de meilleures performances physiologiques, en particulier lorsque la disponibilité en eau a été restreinte. Les racines adventives ont présenté une plus grande plasticité cellulaire en conditions limitantes en eau et en oxygène par raport aux racines initiales.

En conclusion, cette étude a permis de relier la présence de racines adventives à de meilleures performances en termes de croissance, de physiologie et de nutrition, chez l'épinette noire au stade de semis. Ces résultats laissent ainsi entrevoir plusieurs pistes de recherches futures mais également de possibles retombées pour la production de semis. En effet, il pourrait être intéressant d'envisager l'enfouissement de la base de la tige lors de la production de semis d'épinette noire, afin de favoriser le développement de racines adventives, en particulier pour les semis destinés à des sites où la disponibilité en eau et/ou en nutriments peut être limitante.

Mots clés : épinette noire, racines adventives, racines initiales, semis, physiologie, nutrition, enfouissement

# CHAPITRE I

# INTRODUCTION

## 1.1 Épinette noire et racines adventives

L'épinette noire (*Picea mariana* [Mill.] BSP) est une des espèces les plus répandues en forêt boréale Nord-Américaine (Viereck et Johnston 1990). Elle est capable de se développer aussi bien sur sites mésiques bien drainés (DesRochers et Gagnon 1997) qu'en tourbières (Lieffers et Macdonald 1990; Islam et Macdonald 2004). Ce large spectre écologique ainsi que la qualité de sa fibre et sa résistance aux insectes et maladies en font une essence prisée par l'industrie forestière. Avec plus de 75 millions de semis mis en terre en 2017, l'épinette noire est l'essence la plus plantée au Québec, Canada (Salmon 2017). Comme de nombreuses espèces du genre *Picea*, elle développe un système racinaire superficiel, localisé dans les 30 premiers centimètres du sol, permettant un bon ancrage et une localisation stratégique pour capter eau, nutriments et oxygène (Sutton 1980; Strong et Roi 1983; Coutts *et al* 1999). Sa spécificité réside dans la composition de son système racinaire : à maturité, l'épinette noire possède un système racinaire majoritairement adventif (LeBarron 1945; McClain 1981). Les racines adventives sont des racines qui se développent à la base de la tige et à l'aisselle des premières branches dès les premières années de l'arbre (Fig. 1.1), et qui progressivement remplacent les racines initiales issues de la germination de la graine (DesRochers et Gagnon 1997; Krause et Morin 1999).

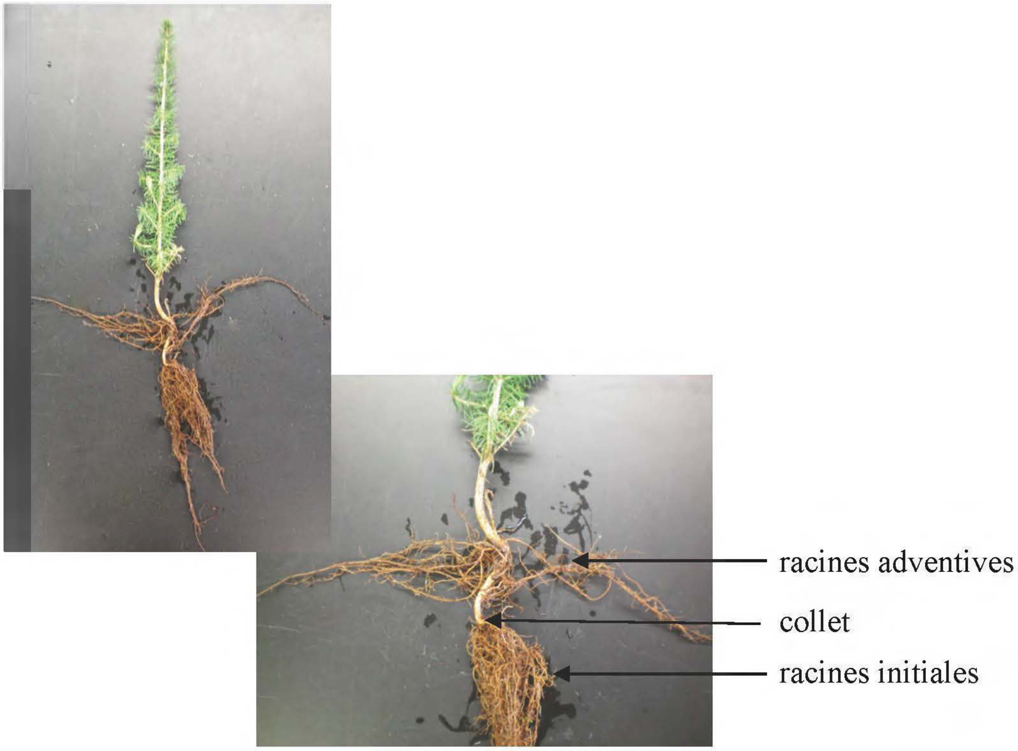

Figure 1.1 Épinette noire de deux ans, avec en-dessous du collet des racines initiales et au-dessus des racines adventives.

Longtemps observé, de nombreuses explications ont été avancées pour ce phénomène : mécanisme d'adaptation à l'enfouissement de la tige par la croissance rapide de sphaignes environnantes (LeBarron 1945), réponse à la plus forte disponibilité en nutriments au niveau de la couche organique que du sol minéral (Sutton 1995) ou développement constitutif dû au contact de la tige avec le sol (Viereck et Johnston 1990). Cependant peu d'études ont été réalisées sur les mécanismes pouvant expliquer la dominance des racines adventives au stade mature de l'arbre. Quelques études préliminaires ont mis en évidence une meilleure croissance des semis ayant des racines adventives par rapport à ceux ne possédant que des racines initiales, sans parvenir toutefois à détecter de différences physiologiques entre les deux types de plants (*e.g.*

concentration foliaire en nutriments, photosynthèse, conductance stomatique, potentiel hydrique) (Yaakoubd 1997; Gagnon 2002).

Chez de nombreuses espèces végétales, les racines adventives sont perçues comme une réponse adaptative de la plante à divers facteurs environnementaux tels que la sècheresse (Liao *et al* 2012), la limitation continue de lumière (Fattorini *et al* 2018), la présence d'ectomycorrhizes (Niemi *et al* 2004) ou encore la faible disponibilité en nutriments (Steffens et Rasmussen 2016). Elles sont également un moyen de propagation, naturel comme lors la formation de marcottes ou de stolons (Nelson et Wilhelm 1957; Lovell et White 1986) mais aussi artificiel, puisqu'il s'agit du principe sur lequel repose le bouturage (Naija *et al* 2008; Da Costa *et al* 2013). Un des facteurs influençant tout particulièrement la formation de racines adventives est le régime hydrique, notamment via l'inondation. En effet, le développement d'un système racinaire adventif fait partie des réponses mises en place par les plantes pour faire face aux conditions anaérobies induites par la submersion, entre autre chez les espèces d'arbres tolérantes à l'inondation (Kozlowski 1997; Steffens et Rasmussen 2016). Lors de la submersion, l'atout des racines adventives provient généralement d'une grande plasticité au niveau de la morphologie cellulaire. Elle peut se présenter sous différentes formes : par la formation d'aérenchyme, un tissu cortical contenant de larges espaces inter-cellulaires afin de faciliter la diffusion d'oxygène comme chez *Oryza sativa* L. ou *Eucalyptus camaldulensis* (McDonald *et al* 2002; Argus *et al* 2015), par un retard de subérisation des cellules par rapport aux racines initiales, permettant un plus grand transport de l'eau par voie apoplastique et ainsi un meilleur équilibre hydrique chez *Larix laricina* [Du Roi] K. Koch (Calvo-Polanco *et al* 2012), ou encore grâce à un plus grand nombre de vaisseaux de xylème (également de plus fort diamètre) que les racines initiales, associé à un retard de maturation engendrant une plus forte conductivité hydraulique des cellules corticales par *Hordeum vulgare* L. (Knipfer et Fricke 2011; Knipfer *et al* 2011). Quelques études portent également sur les capacités des différents types de racines à capter les nutriments, principalement sur des plantes herbacées d'intérêt agricole. Par exemple, en comparaison des racines initiales, les racines adventives ont été capables de capter plus de phosphore en conditions limitantes en phosphore chez le haricot commun (*Phaseolus vulgaris* L.) (Rubio *et al* 2004), et de plus grande quantité de phosphore et potassium en conditions stagnantes de submersion chez le blé (*Triticum aestivum* L.) (Wiengweera et Greenway 2004). Chez *Solanum dulcamara* L., le taux de captation de phosphore est dépendant de la quantité de racines adventives produite en condition d'inondation (Zhang *et al* 2017). Ces observations restent toutefois peu nombreuses et très dépendantes des conditions environnementales; les capacités à capter les nutriments peuvent varier entre les différents types de racines en fonction des concentrations en nutriments dans le sol, comme c'est le cas pour la captation d'azote chez le maïs (*Zea mays* L.) (Yu *et al* 2014; Steffens et Rasmussen 2016).

Les racines adventives semblent ainsi être un avantage chez différentes espèces mais le peu de comparaisons disponibles entre les performances des racines initiales et adventives (en particulier chez les arbres) laisse libre tout un champ d'hypothèses. Quoiqu'il en soit, que ce soit dû à l'augmentation de sa masse durant son développement ou à l'accumulation de matière organique, l'épinette noire développe des racines adventives à la base de sa tige qui se retrouve enfouie, en conditions naturelles comme en plantation (DesRochers 1996; DesRochers et Gagnon 1997; Krause et Morin 2005 Tarroux *et al* 2014). Le système racinaire a un rôle essentiel dans la vie d'un arbre, en particulier en cas de plantation, car de son adaptation au milieu dépend la survie des semis et par conséquent la productivité future du peuplement. Pour une espèce aussi plantée que l'épinette noire, de meilleures connaissances de la performance et de la structure des racines adventives sont importantes car actuellement leur développement n'est pas pris en compte lors de la production et de la plantation de semis.

## 1.2 Plantation et système racinaire

### 1.2.1 Choc de plantation

Le choc de plantation est un phénomène inhérent à toute plantation. Il s'agit de la période nécessaire au semis mis en terre pour s'adapter à son nouvel environnement, *i.e.* entre autres pour développer de nouvelles racines capables de capter les ressources nécessaires à la reprise de croissance (Rietveld 1989; Burdett 1990; Grossnickle 2000). Ainsi, la croissance des semis peut être grandement diminuée durant les premières années post-plantation; chez l'épinette blanche (*Picea glauca* [Moench] Voss), il a été observé que ce ralentissement de croissance pouvait perdurer jusqu'à 10 à 15 ans après plantation (Mullin 1963; South et Zwolinski 1997). Cette stagnation de croissance peut être d'autant plus dommageable que la végétation environnante peut profiter de ce temps pour croitre rapidement et dominer les semis plantés, induisant une forte pression de compétition (Wagner et Robinson 2006; Johansson *et al* 2007; Thiffault *et al* 2012). Cette compétition a lieu couramment pour l'accès à la lumière (Jobidon 2000) et pour la disponibilité en nutriments (Thiffault *et al* 2004; Hébert *et al* 2010).

Le stress hydrique est la première cause du choc de plantation et également de la mortalité des conifères après plantation (Burdett 1990; Margolis et Brand 1990). Si la stagnation de croissance persiste malgré le développement racinaire permettant de puiser l'eau nécessaire à la reprise de croissance, la faible disponibilité en nutriments est évoquée comme un autre facteur possible (Burdett 1990). C'est pourquoi, il est essentiel pour les semis de développer rapidement de nouvelles racines afin d'avoir accès aux ressources du site de plantation (Carlson 1986; Grossnickle 2005). Un fort potentiel de croissance racinaire est un critère important durant la sélection des semis en production, car dans la plupart des cas, il est corrélé avec une meilleure croissance aérienne après plantation (Grossnickle et MacDonald 2018). Plusieurs auteurs suggèrent ainsi de considérer la capacité des semis à produire de nouvelles racines comme une mesure prédictive de leur performance en plantation (Sutton 1980; Burdett *et al* 1983; Feret et Kreh 1985; Johansson *et al* 2007).

### 1.2.2 Stress hydrique et nutrition

L'eau est une composante essentielle à la vie de toute plante. Son transport est fonction de la demande évaporative de l'air, de la transpiration de la plante et la disponibilité en eau du sol. L'équilibre entre biomasse aérienne et souterraine joue alors un rôle important pour la plante : suffisamment de racines absorbantes doivent être mises en place afin de capter l'eau du sol et subvenir aux besoins de transpiration des parties aériennes; s'il y a un débalancement alors la plante tombe en état de stress hydrique, pouvant aller jusqu'à affecter sa survie (Parker 1949; Baldwin et Barney 1976; Haase et Rose 1993; Kozlowski et Pallardy 2002). Cet état de stress hydrique implique toute une cascade de réactions physiologiques, dont une chute du potentiel hydrique qui entraine la fermeture des stomates et donc la réduction des échanges gazeux (Kozlowski et Pallardy 1997). Chez de jeunes *Populus euphratica*, l'installation progressive d'un stress hydrique implique en premier lieu un arrêt de la croissance en diamètre puis en hauteur, avant la fermeture des stomates et une perte de photosynthèse, et en dernier lieu une réduction de la croissance racinaire (Bogeat-Triboulot *et al* 2007). La croissance racinaire a ainsi été maintenue plus longtemps que celle des parties aériennes, entrainant une augmentation du ratio racines/tiges face à un stress hydrique modéré. Ce phénomène est couramment observé chez les arbres et peut être relié à une augmentation de leur potentiel d'extraction en eau du sol pour une même surface foliaire, et donc un meilleur maintien de leur statut hydrique (Sperry *et al* 2002; Bogeat-Triboulot *et al* 2007; Brunner *et al* 2015). Ainsi pour un semis destiné à la plantation, la quantité de racines par rapport à la biomasse aérienne peut être un indicateur du potentiel de résistance à la sècheresse, tout comme son potentiel hydrique dont la mesure avant plantation permet de vérifier l'intégrité du transport de l'eau au sein du plant et donc d'assurer un fonctionnement physiologique optimal dès la mise en terre (Thompson 1985; Grossnickle et Folk 1993; Grossnickle et MacDonald 2018).

Bien que ne représentant que quelques pourcents de la masse totale d'un arbre, les nutriments sont également très importants au niveau physiologique; leur disponibilité est couramment reportée comme étant un élément limitant la croissance des arbres (Kozlowski et Pallardy 1997). Pour la majorité des espèces, l'azote est le nutriment le plus limitant et sa faible disponibilité est généralement reportée comme entrainant une réduction du taux de photosynthèse puisqu'il est le constituant majeur des acides aminés qui forment les protéines végétales, dont certaines enzymes clés de la photosynthèse comme la RuBisCo (Mooney *et al* 1978; Brix 1983; Reich et Schoettle 1988). Toutefois, il peut arriver que d'autres facteurs entrent en jeu et influencent plus fortement le taux de photosynthèse que la disponibilité en azote, comme l'intensité lumineuse, la température, l'âge de l'arbre ou la disponibilité en d'autres nutriments tel le phosphore (Waring *et al* 1985; Teskey *et al* 1994; Warren et Adams 2002; Warren *et al* 2005). L'azote peut provenir directement de la captation par les racines de nitrate, d'ammonium ou d'acides aminés présents dans le sol, mais également des réserves emmagasinées par la plante. Ainsi, les quantités d'azote captées durant une saison de croissance peuvent influencer la croissance en cours mais également celle de l'année suivante. La remobilisation des réserves en azote est entre autres responsable de la croissance des racines absorbantes au printemps, qui peut jouer un rôle clé lors de l'établissement des semis pour faire face au stress hydrique (Villar-Salvador *et al* 2012; Uscola *et al* 2015). Ainsi l'accumulation de réserves en azote, via une importante fertilisation durant la production, peut permettre aux semis nouvellement plantés d'avoir une plus forte croissance racinaire associée à une meilleure absorption des nutriments (Malik et Timmer 1998; Wang *et al* 2016), et parfois également à une augmentation de la photosynthèse (Salifu et Timmer 2003), de la résistance à la sècheresse (Timmer 1997; Andivia *et al* 2014) ou au froid (Islam *et al* 2009).

### 1.2.3 Production de semis

Actuellement, il existe deux types principaux de semis utilisés en plantation qui diffèrent particulièrement par leurs caractéristiques racinaires: les semis en récipients et les semis à racines nues (Fig. 1.2). Les semis en récipients sont des plants ayant grandi en caissettes composées d'alvéoles de faible contenance, engendrant un système racinaire avec une plus forte biomasse que les plants à racines nues mais restreint au volume de la carotte de substrat (Blake et Sutton 1987; Rose et Haase 2005). Les semis à racines nues sont des plants ayant passé au moins leur dernière année de production en pleine terre et dont un maximum de terre est enlevé au niveau des racines pendant l'extraction. Ces plants ont ainsi un système racinaire moins développé que les semis produits en récipients mais non-restreint (Grossnickle et Blake 1986; Grossnickle et El-Kassaby 2016).

Ces deux types de semis sont actuellement produits car ils présentent des performances différentes en fonction du type de site à reboiser. Le fait que les semis en récipients soient mis en terre avec leur carotte de substrat peut être un avantage sur les sites les plus limitants. En effet cette carotte leur sert d'apport initial en eau et en nutriments qui, associé à la grande quantité de racines développées durant la production, permet aux semis en récipients d'avoir un plus fort potentiel de croissance racinaire que les plants à racines nues et donc d'établir plus rapidement un lien avec le site de plantation; l'accès rapide aux ressources du sol leur confère une meilleure résistance au stress hydrique que les plants à racines nues (Nilsson et Örlander 1995; Idris *et al* 2004; Rose et Haase 2005). Ces derniers, du fait de leur production en pleine terre et à une densité plus faible que celle imposée par les caissettes, ont généralement une croissance aérienne supérieure aux semis en récipients qui leur permet d'être plus compétitifs vis-à-vis de la végétation environnante sur les sites où celle-ci peut être un problème (Thiffault 2004; Hytönen et Jylhä 2008).

Semis en récipient

Semis à racines nues

Figure 1.2 Production de semis d'épinettes noires en récipients ou à racines nues et leur influence sur la structure du système racinaire. Les photos du bas illustrent des semis de deux ans.

### 1.2.4 Gauchissement et enfouissement

Actuellement, la production de plants d'épinettes noires au Québec est encadrée par de nombreuses règles visant à obtenir des semis de qualité pour le reboisement. Que ce soit en récipients ou en pleine terre, il est recommandé de planter les semis au niveau du collet c'est-à-dire de la jonction entre les parties aériennes et souterraines. Ce type de plantation favorise la croissance de racines initiales (qui se développent sous le collet) et ne prend pas en compte le développement naturel des racines adventives qui nécessite que la base de la tige soit en contact avec le sol (Aubin 1996; Sutton 1995). Ceci pourrait être une des raisons du ‘gauchissement’ des semis observés durant la production. Le gauchissement est une inclinaison de pousse annuelle par rapport à la verticale, qui apparait durant la phase de croissance exponentielle (Lord *et al* 1991). Une des normes de production indique que les semis présentant un gauchissement de plus de 15° par rapport à la verticale doivent être déclassés (Brouillette *et al* 1987). Il est estimé que jusqu’à 20% des semis sont ainsi rejetés lors de la production en serre ou en tunnel (Lord *et al* 1988; Margolis *et al* 1988). Or en conditions naturelles, la tige des semis présente couramment une période de croissance horizontale durant les premières années (DesRochers et Gagnon 1997). Ce phénomène pourrait ainsi être un positionnement stratégique de la tige au contact du sol, créant des conditions favorables au développement de racines adventives. De plus, à condition que le gauchissement ne soit pas extrême (cime du semis descendante), les semis se redressent en partie naturellement et ne présentent pas de différence de croissance par rapport aux semis au port vertical (Béland et Lapierre 1992; Lamontagne 1993).

L’enfouissement de la tige des semis de quelques centimètres dans le sol durant la production pourrait ainsi facilement induire le développement de racines adventives, une humidité élevée du substrat accélérant d’autant plus leur formation (McClain 1981; Aubin 1996). Bien que signifiant une perte de hauteur initiale au moment de l’enfouissement, plusieurs études ont démontré que les plants enfouis atteignaient une croissance semblable aux semis plantés au niveau du collet (Schwan 1994; Sutton 1995; Paquette *et al* 2011), voire même dans certains cas, avaient une meilleure croissance en hauteur (Sutton 1966; Gagnon 2002; Tarroux *et al* 2014). Bien que la présence de racines adventives ait été observée chez les arbres enfouis, leur rôle dans la croissance des semis n’a pas directement été étudié.

### 1.3 Objectifs : initiales ou adventives?

L'objectif principal de cette thèse était de déterminer le rôle des racines adventives lors de la croissance juvénile de l'épinette noire. Le système racinaire adventif remplaçant progressivement l'initial, nous avons émis l'hypothèse que les racines adventives auraient de meilleures capacités à capter l'eau et les nutriments par rapport aux racines initiales et que leur présence influencerait positivement la croissance et la physiologie des semis.

Le premier volet de cette thèse (Chapitre II) avait pour but de distinguer les contributions des racines initiales et adventives à la croissance de l'épinette noire, via la comparaison de leurs capacités à capter l'eau et les nutriments. Pour se faire, nous avons placé des semis d'épinette noire de 4 ans dans un système de double-pot permettant de séparer les deux systèmes racinaires et ainsi de pouvoir réaliser des apports en eau et en engrais de façon indépendante (Fig. 1.3). Deux régimes hydriques ont été testés, 25% ou 100% de capacité de rétention en eau du sol, déjà connus pour induire des différences significatives de croissance chez des semis d'un an (Aubin 1996); et deux niveaux de fertilisation, avec ou sans apport d'engrais NPK (20-20-20), la quantité de nutriments disponible influençant la croissance des semis (Salifu et Timmer 2001; Idris *et al* 2004). La croissance (accroissement en hauteur et en diamètre, biomasse), la physiologie (taux de photosynthèse, conductance stomatique, potentiel hydrique) et la quantité de nutriments accumulés (contenus foliaires en N, P, K, Ca et Mg) ont été comparés après une saison de croissance en milieu contrôlé. L'hypothèse était que les racines initiales et adventives auraient des capacités à capter l'eau et les nutriments différentes et que cela affecterait différemment le développement des semis.

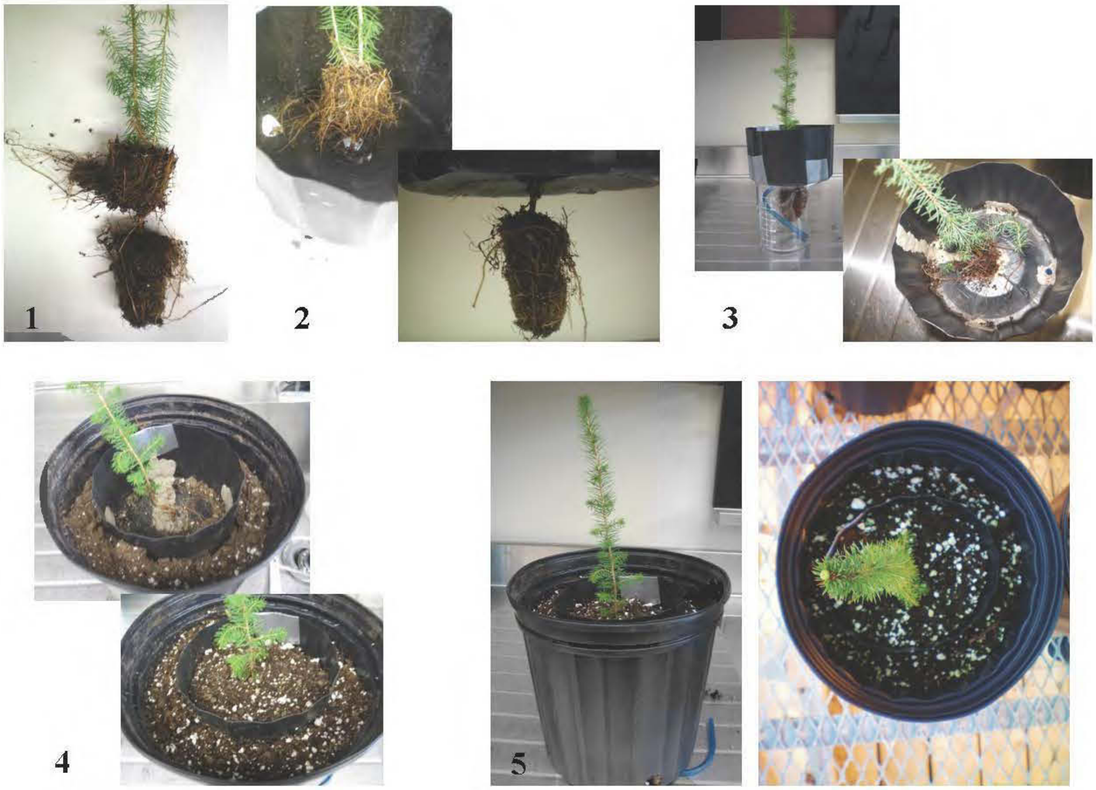

Figure 1.3 Mise en place du système de double-pot. (1) Séparation des racines adventives des initiales; (2) insertion du plant dans le premier pot, avec les racines adventives à l'intérieur et les initiales à l'extérieur; (3) installation d'un tuyau de drainage et calfeutrage du premier pot; (4) mise en place du premier pot à l'intérieur du deuxième et remplissage des deux pots avec du substrat; (5) système de double-pot prêt pour un traitement indépendant en eau et en nutriments des racines adventives et initiales.

Le deuxième volet (Chapitre III) avait pour objectif de tester l'influence de la structure (restreint ou non dans une carotte de substrat, *i.e.* plants en récipients ou racines nues) et l'origine (initial ou adventif, *i.e.* plants mis en terre au niveau du collet ou enfouis) du système racinaire sur la croissance et la physiologie de semis d'épinette noire en plantation en forêt boréale. Nous avons émis l'hypothèse que les caractéristiques racinaires influenceraient l'établissement des semis dès la mise en terre et que la présence de racines adventives serait avantageuse pour une reprise de croissance rapide. Pour tester cette hypothèse, nous avons comparé les performances de trois types de semis âgés de deux ans : (C) des semis en récipients, avec un système racinaire initial très développé, restreint dans la carotte de substrat; (BR) des semis à racines nues, ayant un système racinaire initial moins important mais non restreint; et (DP) des semis en récipient enfouis, possédant un système racinaire initial et adventif restreint dans la carotte de substrat. Ils ont été plantés sur trois types de microsites représentatifs des sites de reboisement en forêt boréale: (i) sur sol minéral, microsite caractéristique de l'environnement de plantation obtenu après scarifiage; (ii) en présence d'éricacées, espèces très présentes dans l'est canadien, qui peuvent rapidement envahir les sites de plantation et qui sont très compétitives au niveau de la captation de nutriments (Mallik 2001; Hébert *et al* 2010); et (iii) en présence de sphaigne et d'éricacées, les sphaignes étant typiques des sites paludifiés et qui par leur accumulation créent une limitation de la disponibilité en nutriments et une activité microbienne réduite (Fenton *et al* 2005; Pacé *et al* 2018). De l'engrais marqué ($^{15}NH_4NO_3$) a été appliqué sur la moitié du dispositif au début de la seconde année post-plantation, afin d'évaluer le potentiel à capter l'azote de chacun des types de semis. À la fin de la deuxième année après plantation, des paramètres de croissance (hauteur, diamètre, biomasse), de nutrition (concentration foliaire en N, P, K, Ca et abondance en $^{15}N$) et de stress hydrique ($\delta^{13}C$) ont été mesurés afin de comparer la réponse au choc de plantation des semis en fonction de leurs caractéristiques racinaires.

Enfin le troisième volet (Chapitre IV) se composait de deux expériences complémentaires : l'une visait à comparer les capacités à absorber l'eau et les nutriments des mêmes trois types de semis utilisés lors du 2$^{ème}$ volet (semis C, BR et DP), cette fois en environnement contrôlé; et la deuxième s'intéressait plus précisément à évaluer la plasticité cellulaire des racines initiales et adventives face à différents régimes hydriques. Ici nous avons supposé que les semis produits en récipients et avec racines adventives seraient capables de mieux s'adapter à des conditions de croissance limitantes en eau, en nutriments et/ou on oxygène que les semis ne possédant que des racines initiales. Pour vérifier cette hypothèse, la première expérience consistait à comparer la croissance (hauteur, diamètre, biomasse), la physiologie (taux de photosynthèse, conductance stomatique, potentiel hydrique) et la concentration foliaire en nutriments (N, P, K, et Ca) des semis C, DP et BR après une saison de croissance en serre durant laquelle ils ont été sujets à la combinaison de deux types d'irrigation (25% ou 100% de capacité de rétention en eau du sol) et de deux niveaux de fertilisation (avec ou sans apport d'engrais NPK 20-20-20). Lors de la seconde expérience, nous avons comparé la morphologie cellulaire des racines initiales et adventives de semis cultivés sous trois régimes hydriques : 25%, 100% de capacités de rétention en eau du sol et en condition d'inondation.

Grâce à cette étude, nous espérions mieux comprendre l'importance du système racinaire adventif chez l'épinette noire lors de différentes conditions environnementales. Naturellement l'épinette noire favorise la croissance de racines adventives par rapport aux initiales, nous avons donc envisagé que ceci puisse être dû à un avantage procuré par celles-ci et qu'il pourrait alors être intéressant de prendre en compte leur développement lors de la production des semis d'épinettes noire, ce qui n'est pas le cas actuellement.

# CHAPITRE II

# CONTRIBUTION OF ADVENTITIOUS VS INTIAL ROOTS TO GROWTH AND PHYSIOLOGY OF BLACK SPRUCE SEEDLINGS

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Publié dans *Physiologia Plantarum*, doi:10.1111/ppl.12735

## 2.1 Résumé

L'épinette noire (*Picea mariana* [Mill.] BSP) est une espèce boréale dont la particularité est le développement d'un système racinaire adventif. Contrairement aux racines initiales issues de la germination de la graine, les racines adventives apparaissent progressivement au-dessus du collet, jusqu'à devenir la majeure partie du système racinaire de l'épinette noire au stade mature. Actuellement, peu de choses sont connues concernant leur rôle physiologique et leur influence sur la croissance de l'arbre, comparé aux racines initiales. Nous avons émis l'hypothèse que les racines adventives présenteraient un avantage pour l'acquisition d'eau et de nutriments par rapport aux racines initiales. Pour tester cette hypothèse, nous avons étudié les capacités d'absorption des deux systèmes racinaires en environnement contrôlé, durant une saison de croissance. Les semis d'épinette noire ont été placés dans un système de double-pots permettant des apports en eau (25% et 100% de capacité de rétention en eau du sol) et en fertilisation (avec ou sans engrais) indépendants aux racines initiales et adventives. Après 14 semaines, des paramètres de croissance (hauteur, diamètre, biomasse), de physiologie (taux de photosynthèse, conductance stomatique, potentiel hydrique) et de nutrition (contenu foliaire en N, P, K, Ca et Mg) ont été comparés. La plupart des paramètres mesurés n'ont pas présenté de différences entre racines adventives ou initiales pour un même traitement, excepté la biomasse racinaire. En effet, les semis d'épinette noire fertilisés ont fortement investi dans la production de racines adventives, deux fois plus qu'au niveau des racines initiales. Cela a également été le cas lorsque l'irrigation était abondante uniquement au niveau des racines adventives, alors que les semis dont les racines adventives ont été soumises à faible régime hydrique ont produit autant de racines initiales que d'adventives. Nous concluons que les semis d'épinette noire performent aussi bien avec des racines adventives qu'avec des initiales, mais que si les ressources sont abondantes, ils favorisent fortement le développement de racines adventives.

## 2.2 Abstract

Black spruce (*Picea mariana* [Mill.] BSP) is a boreal tree species characterized by the formation of an adventitious root system. Unlike initial roots from seed germination, adventitious roots gradually appear above the root collar, until they constitute most of mature black spruce root system. Little is known about the physiological role they play and their influence on tree growth relative to initial roots. We hypothesized that adventitious roots present an advantage over initial roots in acquiring water and nutrients. To test this hypothesis, the absorptive capacities of the two root systems were explored in a controlled environment during one growing season. Black spruce seedlings were placed in a double-pot system allowing irrigation (25% and 100% water container capacity) and fertilization (with or without fertilizer) inputs independent to initial and adventitious roots. After 14 weeks, growth parameters (height, diameter, biomass), physiology (net photosynthetic rate, stomatal conductance, shoot water potential) and nutrient content (N, P, K, Ca and Mg foliar content) were compared. Most measured parameters showed no difference for the same treatment on adventitious or initial roots, except for root biomass. Indeed, fertilized black spruce seedlings invested heavily in adventitious root production, twice as much as initial roots. This was also the case when adventitious roots alone were irrigated, while seedlings with adventitious roots subjected to low irrigation produced initial root biomass equivalent to that of adventitious roots. We conclude that black spruce seedlings perform equally well through adventitious and initial roots, but if resources are abundant they strongly promote development of adventitious roots

## 2.3 Introduction

Black spruce (*Picea mariana* [Mill.] BSP) is one of the most widespread species in North American boreal forests (Viereck and Johnston 1990), where it plays vital economic and environmental roles. With over 70 million seedlings planted annually, it is the most planted tree in Québec, Canada (Salmon 2016). Under natural conditions, mature black spruce trees exhibit exclusively adventitious roots (AR), a shallow root system that develops at the base of the stem and at the axilla of the first branches during seedling growth (McClain 1981, DesRochers and Gagnon 1997, Krause and Morin 2005). These roots gradually replace initial roots (IR) resulting from seed germination. Despite the extent of this phenomenon, it is not considered during nursery production and field plantation of seedlings, which are planted at the root collar level, although the base of the stem has to be in contact with moist soil/humus for AR to be produced (Sutton 1995, Aubin 1996, Gagnon 2002). At the juvenile stage, it is often observed that the base of seedlings' stem grows horizontally (Béland and Lapierre 1992, DesRochers and Gagnon 1997), suggesting that this is a strategy for faster AR development by positioning the base of the stem in more favorable conditions. This phenomenon is also observed in tree nurseries, where about 20% of the seedlings are downgraded due to a basal stem inclination of more than 15% (Lord *et al* 1988, Margolis *et al* 1988).

Adventitious roots may play a significant role for anchoring, water uptake and nutrition, the three functions of the root system (Coutts *et al* 1999). Compared to IR that are located deeper underground, AR develop closer to the soil surface, in the organic layer where conditions are more favorable to growth with greater water and nutrient concentrations (Prescott *et al* 2000). However, although placed in a richer environment than IR, the role of AR and their impact on tree growth remain unclear. Several studies have compared black spruce seedlings planted at the root collar level (IR system only) to deep-planted ones (IR system + AR system), but no clear differences in terms of growth or physiology were observed (Schwan 1994, Sutton 1995, Paquette *et al* 2011). In a recent field experiment, Tarroux *et al* (2014) reported some benefits of deep-planting over planting at the root collar, such as a slightly better height growth and greater root system biomass after 17 years.

Considering these mitigated results, the contribution of AR *vs* IR to tree growth and physiology is not clear. Studies on herbaceous plants of agronomic interest showed some differences between IR and AR in their ability to capture soil nutrients (Steffens and Rasmussen 2016). For example, in common beans (*Phaseolus vulgaris* L.), basal roots (at the base of the hypocotyl) capture more phosphorus than IR, in an P-limiting environment (Rubio *et al* 2004). Under flooding conditions, it was shown that AR in wheat (*Triticum aestivum*) picked up greater amounts of phosphorus and potassium than initial roots (Wiengweera and Greenway 2004). In barley seedlings (*Hordeum vulgare* L.), it was shown that although AR (2 to 3 times fewer than IR) were responsible for only 8% of the water uptake, they had a 3 to 4 times greater cortical cell hydraulic conductivity in the transition zone (*i.e.* not-fully mature zone situated between the root tip and the fully mature zone) and 3 to 4 times greater expression of aquaporins than IR (Knipfer *et al* 2011). However, these studies are few and the findings are highly dependent on environmental conditions and species.

To our knowledge, no study has yet investigated the contribution of AR/IR to tree growth and physiology. We thus hypothesized that both root types have different capacities for water and nutrient uptake, and thus contribute differently to conifer seedling growth, nutrition and physiology. Here, we designed a two-pot system and used it under a semi-controlled environment in order to discriminate between water and nutrient uptake of AR and IR for young black spruces. To test our hypothesis, the two root systems were independently subjected to two water regimes (100 and 25% water container capacity) and NPK fertilization (with, without). After a complete growing season, above- and below-ground growth, physiological and nutrition parameters were recorded and compared among treatment combinations.

## 2.4 Material and Methods

### 2.4.1 Experimental design and treatments

The role of adventitious *vs* initial roots on nutrition and water uptake of black spruce seedlings was investigated using 4-year-old containerized seedlings produced in a provincial nursery from a local seed source (49°49’35’’N, 74°45’32’’W). One year after germination, seedlings were buried down to 5 cm above the root collar using a peat and vermiculite substrate (AGRO MIX® PV20, Fafard, Saint-Bonaventure, Canada) to stimulate the production of adventitious roots over the next 2 years. In February 2013, the 3-year-old dormant seedlings were transferred and cultivated for 1 year into a two-pot system that allowed the addition of water and nutrients independently to IR and AR (Fig. 2.1). The seedling lot used in this study was homogeneous at the onset of the experiment. Mean initial stem diameter was 5.6 ± 0.1 mm (mean ± SD) and mean initial height was 30.3 ± 1.9 cm.

This study was designed as a randomized split plot with six replicate blocks, each containing a replicate of one of the sixteen treatments described in Fig. 2.1A. The two-pot compartmented system allowed us to apply one of sixteen combinations of irrigation × fertilization regime × root origin (initial, adventitious) under semi controlled greenhouse conditions (25/17°C day/night, 16/8 h light/dark photoperiod, natural humidity levels) (Fig. 2.1B). During the 14-week experiment representing a full growing season, irrigation treatments consisted of maintaining either 25 or 100% container capacity of water content. The 100% water container capacity level was determined by saturating the soil substrate with water, then waiting for the excess water to drain out and measuring with a moisture sensor based on time-domain reflectometry (TDR 100; FieldScout, Portland, OR). Soil water content was measured every day in each compartment of every pot with the moisture sensor and tap water was added as needed. The fertilization treatment consisted of either the absence of fertilization or the weekly application of 0.2 g of N-P-K (20-20-20) soluble mineral fertilizer. Fertilizer concentrations of the watering solution were adjusted with respect of the considered compartment and irrigation treatment (*i.e.* plants received either a single dose of 200 ml of 1 g $l^{-1}$ or 100 ml of 2 g $l^{-1}$ in one compartment depending on watering regime, or two half doses in each compartment when both root systems had to be fertilized Fig. 2.1C).

### 2.4.2 Seedling measurements

Seedling height (cm) and ground-level diameter (mm) were measured immediately after seedlings were transferred in the two-pot system and re-measured 14 weeks later. Gas exchange parameters [net photosynthesis ($P_n$) and stomatal conductance ($g_s$, mmol $m^{-2}$ $s^{-1}$)] were also assessed at 14-weeks on 1-year-old twigs located in the upper-half of five to six seedlings per treatment (some died at the beginning of the experiment), using a portable infra-red gas analyzer (LI-6400; LI-COR, Lincoln, NE) equipped with a conifer cuvette in which conditions were controlled at $CO_2$ concentration of 400 µmol $mol^{-1}$; leaf temperature of 24 ± 1°C; 1 000 µmol photons $m^{-2}$ $s^{-1}$ was provided by an halogen lamp placed about the cuvette. Measurements were conducted between 10:00 and 15:00. Needle surface area of the samples used for $P_n$ and $g_s$ was determined using a LI-3100 Leaf Area Meter (LI-COR, Lincoln, NE). At the end of the experiment, shoot water potential was also measured on three similarly exposed shoots collected in the upper-half of the seedling using a Scholander pressure chamber (Model 1000; PMS Instruments, Albany, OR) between 7:00 and 10:00.

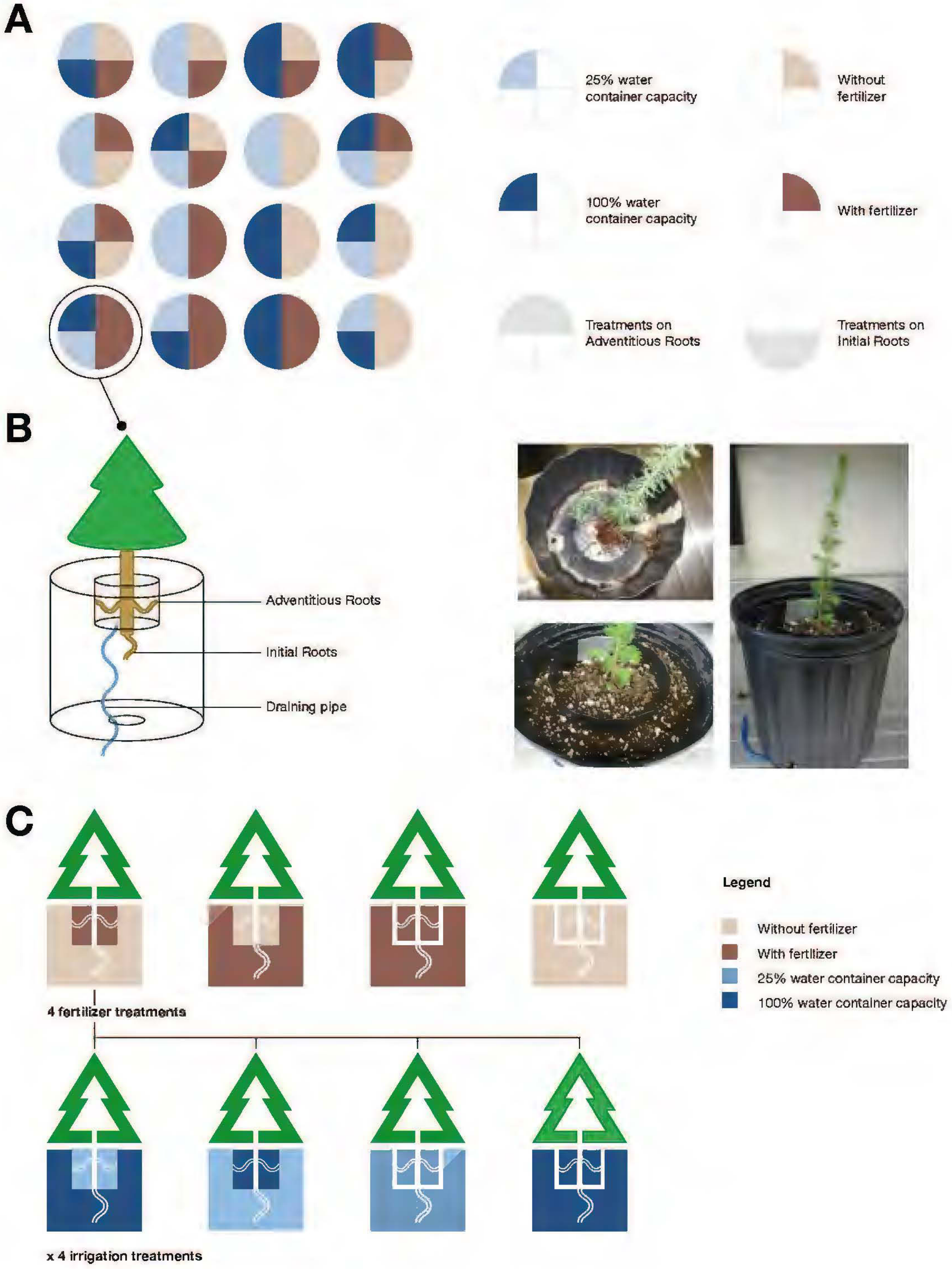

Figure 2.1 Legend on next page.

Figure 2.1 (A) One of the six completely randomized blocks organized in a split-plot design. Each block represented one replicate of all 16 treatments (six blocks for six replicates per treatment). (B) Using a two-pot system, adventitious roots were restrained to the top compartment and initial roots were restrained to the bottom one. The system enabled independent irrigation and fertilization of both compartments. A draining pipe allowed adequate drainage of the top pot while avoiding contamination of the bottom compartment. The pot used for the top compartment was split then sealed to allow insertion of the seedling stem at the root collar level. The top compartment was placed within the bottom one, then both containers were filled with a peat/vermiculite growing substrate. (C) This two-pot system enabled the setup of 16 different treatments based on a combination of two regimes of fertilization (with, without) and/or two regimes of irrigation (maintenance at 100% of container capacity, maintenance at 25% of container capacity) applied to one and/or the other compartmentalized root system.

After 14 weeks, roots, shoots and needles of all seedlings were collected, separated and oven dried for 48 h at 60 ± 5°C to determine dry biomass. Shoots used for gas exchange and water potential measurements were included in biomass determination. For each seedling, a 2 g sub-sample of dried needles was used for nutrient analyses. Tissues were ground using a ball mill (Pulverisette 0, Fritsch, Idar-Oberstein, Germany) and analyzed for macronutrient contents (concentration × total needle mass). Following the approach used by Thiffault and Hébert (2017), samples were submitted to oxidation, combustion at 1350°C and analyzed with a TruMac N for N concentration (LECO Corp., St Joseph, MI). For P, K, Ca and Mg, samples were digested using $H_2SO_4$ + $H_2O_2$ and analyzed by plasma atomic emission spectroscopy (Thermo Jarrel-Ash - ICAP $61^E$, Thermo Fisher Scientific, Waltham, MA).

### 2.4.3 Statistical analyses

Data exploration and statistical analyses were performed using R 3.2.3 programming environment (R Core team 2015). To assess the effect of fertilization and irrigation on black spruce physiology, a linear mixed effects model was carried out using the *lme* function of the package *nlme* (Pinheiro *et al* 2015). Irrigation and/or fertilization of adventitious roots and/or initial roots, as well as their interactions were set as fixed effects, while Blocks and interactions with Blocks were considered as random factors. Dry biomass, height and diameter increments, water potential, net photosynthesis, stomatal conductance and foliar nutrient contents were included as response variables. Prior to analyses, all data were tested for normality and homoscedasticity using standard graphical methods. A significance level of $P \leq 0.05$ was used for all analyses. The *Lsmeans* function of the *lsmeans* package (Lenth 2016) was used to conduct Tukey's HSD post-hoc tests for comparing treatments in the case of a significant F of the ANOVAs.

## 2.5 Results

### 2.5.1 Seedling growth

Height increment was significantly greater for fertilized seedlings (12.5 ± 1.7 cm), regardless of where the fertilizer was applied (IR, AR, or both), compared to unfertilized seedlings (3.8 ± 1.1 cm) (Fig. 2.2A). Irrigation did not affect height increment (Table 2.1). Total seedling and shoot biomass after 14 weeks were also only affected by fertilization (Table 2.1). Total biomass nearly doubled when seedlings were fertilized, compared to unfertilized seedlings (Fig. 2.2B). The interaction between fertilization and irrigation was only significant for diameter increment (Table 2.1), but no specific pattern emerged from this interaction (Annexe A, Fig. S1). Unfertilized seedlings tended to have less diameter growth than fertilized seedlings from either root origin.

AR biomass increased with fertilization and irrigation, while the biomass of IR only responded to the irrigation treatment (Table 2.1). AR biomass increased when fertilizer was applied to IR, AR, or both, whereas IR biomass was similar with or without fertilization (Fig. 2.3A). Two trends emerged for the irrigation treatment: under low irrigation treatment for the two root types or under high irrigation to IR only, seedlings invested as much in IR than in AR. On the other hand, under high irrigation to AR only or to both root systems, AR production was about twice that of the IR (Fig. 2.3B).

### 2.5.2 Physiological parameters

Net photosynthesis significantly increased with irrigation (Table 2.1). The increase in net photosynthesis was greater when the high irrigation treatment was applied to AR; the high irrigation treatment applied only to IR resulted in similar photosynthetic rates to that of seedlings receiving low irrigation treatment on both root systems. (Fig. 2.4A).

Stomatal conductance was affected by irrigation and fertilization treatments (Table 2.1). Seedlings receiving the high irrigation treatment on only one type of root had stomatal conductance rates up to three times higher than that of plants receiving low irrigation on both root types (Fig. 2.4B). Seedlings that received high irrigation on both root types had stomatal conductance levels similar to that of seedlings irrigated at high level on IR only. Stomatal conductance increased from 20 mmol $m^{-2}$ $s^{-1}$ in non-fertilized seedlings to 35 mmol $m^{-2}$ $s^{-1}$ when at least one type of root was fertilized, without distinction between root types (Annexe A, Fig. S2).

Shoot water potential increased (was less negative) with irrigation (Table 2.1). Plants under the low irrigation treatment to AR and IR had shoot water potentials more negative than when seedlings were received high irrigation (on IR and/or AR, Fig. 2.4C).

Table 2.1 ANOVA summary for black spruce seedling growth, physiology and foliar nutrient content, after 14 weeks of fertilization and irrigation treatments in a double-pot system. Values in bold are significant at $\alpha = 0.05$. AR, adventitious roots; IR, initial roots.

| **Variables** | **Fertilization** | | **Irrigation** | | **Fertilization x Irrigation** | |
|---|---|---|---|---|---|---|
| | *F – value* | *P – value* | *F – value* | *P – value* | *F – value* | *P – value* |
| **Growth** | | | | | | |
| Height increment | 12.577 | **<0.001** | 2.621 | 0.057 | 1.402 | 0.204 |
| Diameter increment | 34.637 | **<0.001** | 4.643 | **0.005** | 3.089 | **0.004** |
| Total dry biomass | 11.771 | **<0.001** | 1.656 | 0.185 | 1.134 | 0.352 |
| Shoot dry biomass | 13.237 | **<0.001** | 1.314 | 0.277 | 1.525 | 0.156 |
| AR dry biomass | 5.332 | **0.002** | 3.666 | **0.017** | 0.946 | 0.493 |
| IR dry biomass | 1.344 | 0.267 | 3.229 | **0.028** | 1.719 | 0.102 |
| **Physiology** | | | | | | |
| Net photosynthesis | 1.822 | 0.151 | 3.412 | **0.022** | 0.407 | 0.927 |
| Stomatal conductance | 6.343 | **0.001** | 15.814 | **<0.001** | 1.747 | 0.095 |
| Water potential | 2.075 | 0.111 | 37.684 | **<0.001** | 1.988 | 0.054 |
| **Foliar content** | | | | | | |
| N | 65.019 | **<0.001** | 0.989 | 0.403 | 1.308 | 0.249 |
| P | 44.792 | **<0.001** | 1.569 | 0.205 | 2.417 | **0.019** |
| K | 56.180 | **<0.001** | 17.075 | **<0.001** | 5.585 | **<0.001** |
| Ca | 28.473 | **<0.001** | 0.090 | 0.965 | 1.863 | 0.072 |
| Mg | 17.501 | **<0.001** | 0.446 | 0.721 | 1.348 | 0.229 |

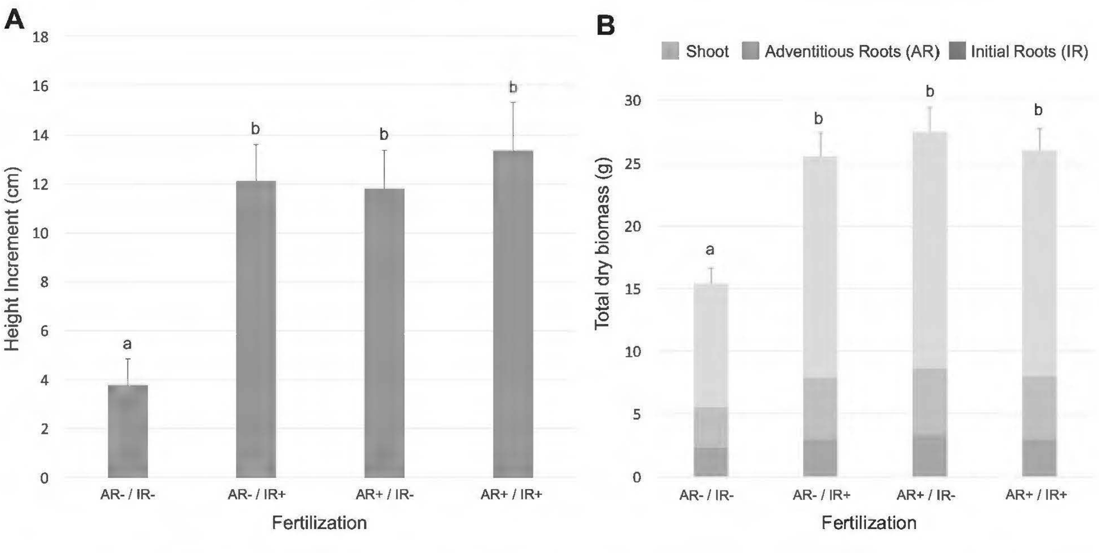

Figure 2.2 Effects of the fertilization treatment on black spruce seedlings (A) mean height increment over a 14 week growing period and (B) total dry biomass for each combination of fertilization (adventitious roots (AR) + (fertilized)/ − (unfertilized) and initial roots (IR) + (fertilized)/ − (unfertilized)). Error bars are standard errors of the mean (SEMs). For a given variable, different letters indicate significant difference at $P \leq 0.05$.

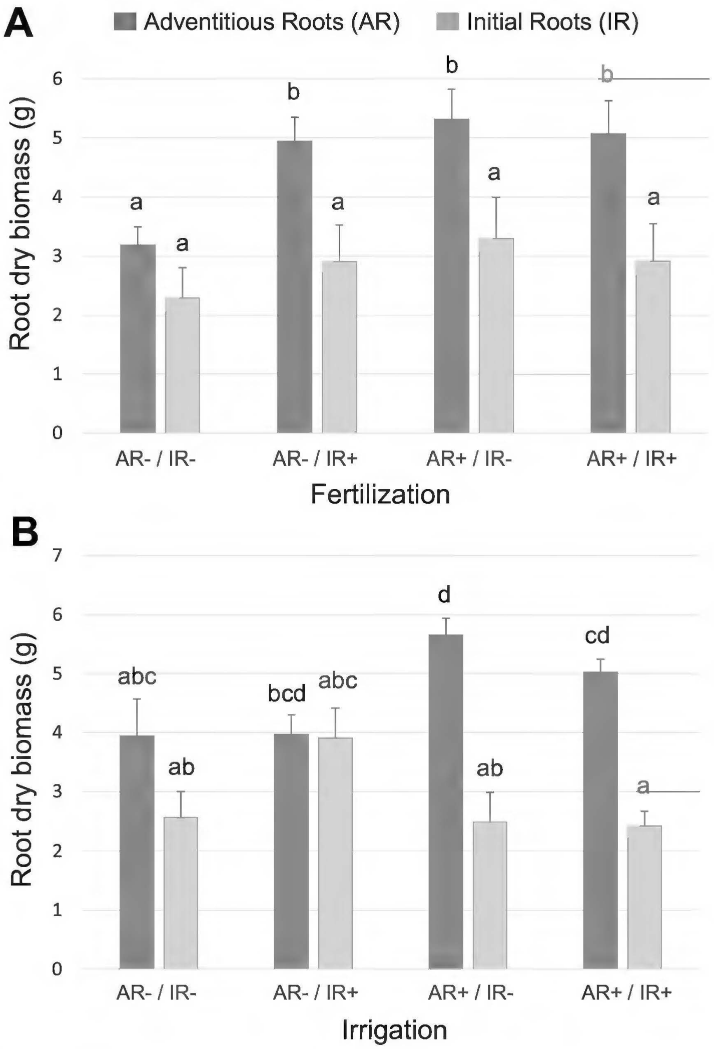

Figure 2.3 Mean dry biomass of black spruce seedlings' adventitious (AR) and initial (IR) roots where AR and/or IR were (A) fertilized (+) or not (−) or (B) irrigated at 100% water container capacity (+) or 25% water container capacity (−). Error bars refer to standard errors of the mean (SEMs). For a given variable, different letters indicate significant difference at $P \leq 0.05$.

### 2.5.3 Foliar nutrient contents

Fertilization had a significant effect on foliar nutrient contents (Table 2.1). Foliar N content increased five times with fertilization, and this effect was greater when both types of roots were fertilized compared to when only initial roots received the fertilizer (Fig. 2.5A). Foliar N content of AR-only fertilized seedlings was similar to that of IR-only and AR+IR-fertilized seedlings.

Foliar Ca content showed the same pattern as foliar N; Fertilization increased Ca content the most when it was applied to both root types compared to non-fertilized and IR-fertilized seedlings. Fertilization increased foliar Mg content similarly when fertilization was applied to either root type or both (Fig. 2.4B). Irrigation had no effect on foliar Ca or Mg contents (Table 2.1).

Fertilization and irrigation interacted to influence foliar P and K contents (Table 2.1). No specific pattern was detected for the interaction of treatments on P foliar contents (Fig. 2.5C). Seedlings that received fertilizer on both IR and AR had significantly higher foliar P contents when high irrigation was restricted to IR, compared to seedlings receiving low irrigation to both root systems. Foliar K contents increased without irrigation for fertilized seedlings, whereas irrigation had no effect on non-fertilized seedlings (Fig. 2.5C). This effect was stronger when adventitious roots received fertilization.

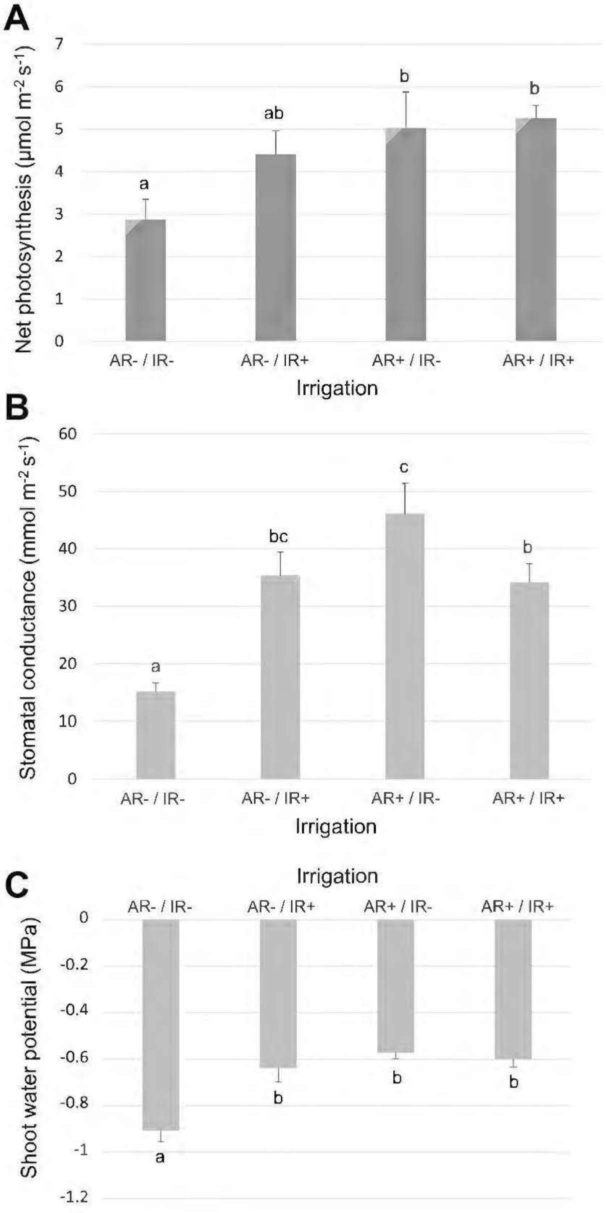

Figure 2.4 Effect of irrigation treatments on black spruce seedlings mean (A) net photosynthesis (μmol $m^{-2}$ $s^{-1}$), (B) stomatal conductance (mmol $m^{-2}$ $s^{-1}$) and (C) shoot water potential (MPa) with adventitious roots (AR) and/or initial roots (IR) irrigated at 100% water container capacity (+) or 25% water container capacity (−). Error bars refer to standard errors of the mean (SEMs). For a given variable, different letters indicate significant difference at $P \leq 0.05$.

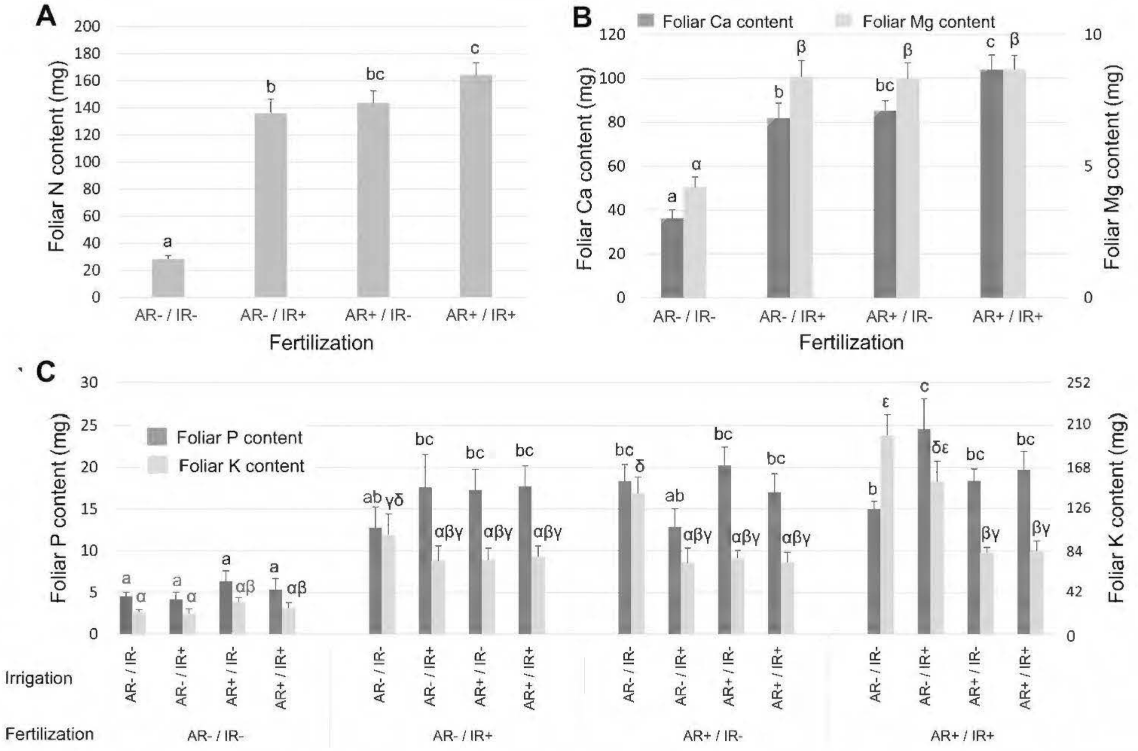

Figure 2.5 Mean foliar (A) nitrogen (N), (B) calcium and magnesium (Ca, Mg) content (mg) of black spruce seedlings with adventitious roots (AR) and/or initial roots (IR) fertilized (+) or not (−). Mean foliar (C) phosphorus (P) and potassium (K) content (mg) of seedlings with AR and/or IR-fertilized (+) or unfertilized (−) and AR and/or IR irrigated at 100% water container capacity (+) or 25% water container capacity (−). Error bars refer to standard errors of the mean (SEMs). For a given variable, different letters indicate significant difference at $P \leq 0.05$.

## 2.6 Discussion

The use of a double-pot system allowed us to test the effect of different regimes of fertilization and irrigation on black spruce seedlings. Adventitious (AR) and initial (IR) roots were treated independently in order to investigate their respective contribution to plant growth, physiology and nutrition status (Fig. 2.1). Adding fertilizer or water almost always enhanced development of AR; only in the case of high irrigation to IR did it lead to seedlings with as many AR than IR (Fig. 2.3). Under natural conditions, black spruce naturally tends to produce many AR, whether seedlings are deep planted (favourable conditions to AR production) or not (planting at the collar level) (Tarroux *et al* 2014). Forest soils in the boreal forest are usually covered by thick humus layers consisting in organic material at different stages of decomposition; hence they are a significant nutrient source for trees. The physical characteristics of the humus also favors high water contents (Prescott *et al* 2000). In that context, our results suggest that the availability of nutrients and water in the organic layer explains the strong development of AR found in boreal conditions (DesRochers and Gagnon 1997, Krause and Morin 2005). In addition, black spruce is known to perform well and to be frequently observed in lowland 'muskegs', *i.e.* very wet and cold environments (Vincent 1965, Elliot-Fisk 1988, Conlin and Lieffers 1993, Patterson *et al* 1997). This could be explained by the development of an AR system, allowing trees to grow well despite difficult conditions. AR are already known to be triggered by flooding conditions and allow better adaptation of tamarack (*Larix laricina* [Du Roi] K. Koch) (Islam and Macdonald 2004, Calvo-Polanco *et al* 2012), a species that is often found growing in boreal peatlands with black spruce (Lieffers and Macdonald 1990).

Among physiological parameters measured, no significant difference was observed between a same irrigation treatment on IR or AR only. However, some measurements such as photosynthesis rate or stomatal conductance, tended to be slightly higher for treatment on AR. These physiological results of AR irrigation could be explained by more efficient water uptake, like barley seedlings whose adventitious roots have cortical cell hydraulic conductivity and some aquaporin expressions 3 to 4 times higher than initial roots (Knipfer *et al* 2011). Although these differences are small and short-term, they suggest better physiological performance of seedlings irrigated on AR only, compared to the other irrigations. This would support the hypothesis that AR present an advantage over IR in acquiring water, being more sensitive to an increase in resource availability, even if the increase is small. Indeed, the 25% water container capacity irrigation level may not have been stressful enough; stomatal closure in black spruce requires conditions that reduce xylem water potential to values below –1.4 MPa (Grossnickle and Blake 1986, Blake and Sutton 1987, Zine El Abidine 1993). Our experimental conditions resulted in a minimum shoot water potential of about –0.9 MPa (Fig. 2.4B), which may explain why the irrigation treatment did not induce significant responses for most variables (Table 2.1).

Black spruce usually responds well to fertilization, especially in terms of nutrient uptake (Malik and Timmer 1995, Malik and Timmer 1998, Houle and Moore 2008). We observed small differences in N and Ca foliar contents in relation to where fertilizer was applied: although fertilization of IR *vs* AR produced similar effects, fertilization of AR tended to increase N and Ca contents to levels similar to those when fertilizing both root systems; it was not the case when only IR were fertilized (Fig. 2.5A,B). Under natural conditions, the shallow development of AR system thus probably plays a significant role in increasing fitness of black spruce under boreal conditions where nitrogen is most limiting to productivity (Tamm 1991, Vitousek *et al* 2002, Maynard *et al* 2014). As a slow growing species that develops primarily in nutrient-poor environments, it was suggested that black spruce may have a strategy of over-consuming and storing available N for future nutrient-poor periods (Patterson *et al* 1997, Lupi *et al* 2013). This could explain why, despite having a higher foliar N content, seedlings with both AR- and IR-fertilized had similar total biomass and height increments as seedlings with only IR-fertilized (Fig. 2.2, Fig. 2.5A).

Overall, little differences were measured in black spruce seedling physiology and growth following irrigation and fertilization of IR *vs* AR. Although our results partly tend to support our hypothesis, we did not identify a clear advantage of adventitious roots over initial roots in acquiring water and nutrients. Both root systems appear to play a similar role in seedling nutrition and water balance, and hence, both are important contributors to growth. However, we observed a major difference in root biomass responses to the treatments at the end of the simulated growing season in a double pot system in a semi-controlled environment: AR production significantly increased under water- and nutrient-rich conditions, whereas IR did not respond to the manipulation of resource availability. This indicates that resource levels are a strong driver of adventitious root production.

## 2.7 Acknowledgements

This research was funded by a doctoral scholarship *Bourse en Milieu Pratique Innovation* provided by the *Fonds de Recherche du Québec – Nature et Technologies* (FRQNT), Natural Sciences and Engineering Research Council of Canada (NSERC) and the NSERC-UQAT-UQAM industrial Chair in Sustainable Forest Management. We thank the University of Quebec in Chicoutimi and the governmental nursery of Trecesson (Quebec, Canada) for providing seedlings for the experiment and their technical assistance. We are also indebted to the staff of the *Laboratoire de chimie organique and inorganique* of the *Ministère des Forêts, de la Faune et des Parcs du Québec* for having performed the chemical analyses, Isabelle Auger for statistical advice, and Lyne Blackburn for technical assistance.

# CHAPITRE III

# ROOT SYSTEM ORIGIN AND STRUCTURE INFLUENCE PLANTING SHOCK OF BLACK SPRUCE SEEDLINGS IN BOREAL MICROSITES

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Publié dans *Forest Ecology and Management*, doi:10.1016/j.foreco.2018.11.043

## 3.1 Résumé

Le choc de plantation peut durer plusieurs années durant lesquelles la croissance du semis peut être significativement réduite. Cette durée dépend du temps nécessaire au système racinaire pour s'acclimater à son nouvel environnement. Nous avons émis l'hypothèse que les caractéristiques du système racinaire seraient des facteurs importants pour l'établissement des semis d'épinette noire (*Picea mariana* [Mill.] BSP). Pour tester cette hypothèse, nous avons comparé la croissance et la physiologie de trois types de plants : des semis en récipients, avec un système racinaire initial dense, restreint au volume de la carotte de tourbe; des semis en récipient enfouis, dont la tige a été enfoui durant la production afin de développer un système racinaire adventif et initial dense; et des semis à racines nues, avec un système racinaire initial moins développé mais non restreint. Les trois types de semis ont été plantés sur trois microsites typiques de sites de reforestation en forêt boréale de l'est canadien : (1) sur sol minéral nu; (2) en présence d'éricacées; ou (3) en présence d'espèces de sphaignes et d'éricacées. De l'engrais composé d'azote marqué ($^{15}N$) a été appliqué à la moitié des semis afin de comparer leur capacité à capter l'azote. Nous avons mesuré la croissance (hauteur, diamètre, biomasse aérienne et souterraine), la nutrition (incluant l'absorption de $^{15}N$) et la discrimination en $^{13}C$ (comme indicateur de stress hydrique) des semis à la fin de la deuxième saison de croissance. Excepté au niveau racinaire, peu d'interactions entre les types de semis et les microsites ont été observées. Pour les microsites fertilisés, nous supposons que les espèces d'éricacées ont capté la plupart des nutriments ajoutés puisque les semis plantés directement sur sol minéral ont eu des accroissements en hauteur et en diamètre plus importants que les semis en présence de végétation concurrente. Sur les microsites non fertilisés, les espèces de sphaigne et d'éricacées ont eu un effet significativement négatif sur la croissance des semis. Au niveau du type de plants, la présence de la carotte de tourbe a permis aux semis en récipients, enfouis ou non, d'avoir un $\delta^{13}C$ plus faible, suggérant un stress hydrique moins important, que les semis à racines nues. L'important développement de racines initiales des semis en récipients leur a permis d'atteindre une plus forte biomasse totale. Nous supposons que c'est la présence de racines adventives qui a permis aux semis en récipients enfouis d'avoir une meilleure absorption des nutriments, en particulier de l'azote. La disponibilité en nutriments étant un des facteurs les plus limitant en forêt boréale, nous suggérons l'utilisation de plants en récipients enfouis afin de réduire le choc de plantation.

Mots clés : épinette noire, choc de plantation, racines adventives, racines initiales, semis en récipient, semis à racines nues, type de plant

## 3.2 Abstract

Planting shock can last for several years during which seedling growth may significantly slow down. This duration depends upon the time that is required for the root system to acclimatize to its new environment. We hypothesized that characteristics of the root system are important factors for black spruce (*Picea mariana* [Mill.] BSP) seedling establishment. To test this hypothesis, we compared growth and physiology of three stock types: containerized seedlings, with a dense initial root system restricted to the volume of a root plug; deeply planted containerized seedlings, with the stem buried during production to develop dense adventitious and initial root systems; and bareroot seedlings, with a less developed but unrestricted initial root system. The three stock types were planted on three microsites that are typical of reforestation sites found in eastern Canadian boreal forest: (1) bare mineral soil; (2) in presence of ericaceous shrubs; or (3) in presence of *Sphagnum* spp. and ericaceous species. Labelled nitrogen fertilizer was added to half of the seedlings to compare nitrogen uptake. We measured seedling growth (height, diameter, above- and belowground biomass), nutrition (including $^{15}N$ uptake) and $^{13}C$ discrimination (as an indicator of water stress) at the end of the second growing season. Except at the root level, there were few interactions between stock types and microsites. For fertilized microsites, we speculate that ericaceous species captured most added nutrients since seedlings that were planted directly on mineral soil had greater height and diameter increments than seedlings with competing vegetation. In unfertilized microsites, *Sphagnum* spp. and ericaceous species had significant negative effects on seedling growth. For stock type, the presence of a root plug allowed containerized and deeply planted seedlings to have a lower $\delta^{13}C$, suggesting less water stress compared to bareroot seedlings. Strong initial root system development of containerized seedlings resulted in greater total biomass. We hypothesized that the presence of adventitious roots in deeply planted seedlings positively influenced nutrient uptake, particularly N. As nutrient availability is one of the most limiting factors in the boreal forest, we suggest using deeply planted containerized seedlings to reduce planting stress.

Keywords: black spruce, planting shock, adventitious root, initial root, containerized seedling, bareroot seedling, stock type

## 3.3 Introduction

Seedling vigour and quality of planting sites partly determine tree productivity. Achieving expected plantation objectives is dependent upon early seedling survival and growth. Growth stagnation of newly planted trees is referred to as "planting shock", *i.e.* the length of time until root systems get established and seedlings resume growth (Burdett, 1990; South and Zwolinski 1997; Grossnickle 2000). In addition to reduced growth, planting shock can threaten plantation success as it allows competing vegetation to overtop planted seedlings (Wagner and Robinson, 2006; Johansson *et al* 2007; Thiffault *et al* 2012). On average, planting shock lasts for 1−3 years before waning when seedlings develop new roots that are adapted to the planting site, which ensures coupling with soil resources (Rietveld, 1989; Carlson and Miller, 1990; Haase and Rose, 1993; Jacobs *et al* 2004). One of the main factors of planting shock and the first cause of mortality of coniferous seedlings after plantation is water stress (Burdett, 1990; Margolis and Brand, 1990). This stressor could be even more damaging given that the effects of climate change on temperature and precipitation regimes in the boreal forest are likely to lead to more frequent and intense drought events (Gauthier *et al* 2015; d'Orangeville *et al* 2018). If growth stagnation persists after roots have become established, it has been suggested that this may be due to poor access to site nutrients (Burdett, 1990), especially on intrinsically poor boreal sites (de Montigny and Weetman 1990). The ability of seedlings to produce an efficient root system rapidly for water and nutrient uptake is thus a critical factor that enables them to survive and resume rapid growth after planting.

Black spruce (*Picea mariana* (Mill.) BSP) is one of the most planted species in Quebec, Canada (Salmon, 2018). Two main stock types are commonly used in plantations, which vary greatly in root system characteristics: bareroot (BR) and containerized seedlings. BR stock consists of seedlings that have spent at least the last year of nursery production in an open field environment, from which soil or growing medium has been removed during harvesting. They are usually grown taller and have greater shoot/root ratios than containerized seedlings (Alm, 1983; Grossnickle and Blake, 1987; Thiffault *et al* 2003). Due to their large size and production under field conditions, BRs have less developed, but more extensive root systems than do containerized seedlings, which are produced in rigid-walled containers with a restricted volume for root development. This containerized production method produces seedlings with a spiralling, but more developed root system than BRs for shoots of similar size (Blake and Sutton, 1987; Barnett and Brissette, 2004; Rose and Haase, 2005). The high root density and protection that is provided by the root plug during handling allow containerized seedlings to maintain a greater root growth potential than BRs and, thus, establish faster root-to-soil contact, giving them greater resistance to water stress (Burdett *et al* 1984; Nilsson and Örlander, 1995; Grossnickle and El-Kassaby, 2016). The performance of each stock type is dependent upon the conditions of the planting sites (Thiffault, 2004). For example, on sites where water is limiting, containerized seedlings, have been shown to have better survival, despite being smaller than BRs due to greater water stress resistance (Burdett *et al* 1984; Nilsson and Örlander, 1995). Yet, BRs are more suited to sites where competing vegetation is important because of their larger size (Hytönen and Jylhä, 2008; Grossnickle and El-Kassaby, 2016).

Black spruce has the ability to develop adventitious roots (LeBarron, 1945; Sutton, 1967). Unlike initial roots originating from seed germination, adventitious roots progressively emerge at the base of the stem and at the axilla of the first branches. Over the years, initial roots gradually disappear and adventitious roots become a dominant feature of mature black spruce trees (McClain, 1981; DesRochers and Gagnon, 1997; Krause and Morin, 2005). This is thought to be an adaptation to humid growing conditions, although exclusively adventitious root systems are also found on trees growing in mesic well-drained sites

(DesRochers and Gagnon, 1997). Under controlled conditions, black spruce seedlings tended to have higher photosynthesis rates and stomatal conductance when adventitious roots were irrigated, compared to seedlings receiving irrigation to initial roots only (Pernot *et al* 2019a). Despite these facts, production techniques in nurseries do not favour adventitious root development since the base of the stem needs to be in contact with moist conditions to stimulate adventitious root production (Sutton, 1995; Aubin, 1996; Gagnon, 2002).

In this context, we hypothesized that the origin (initial *vs* adventitious) and structure of the root system would influence black spruce growth and physiology after planting on boreal sites. To test this hypothesis, we compared three 2-year-old stock types: containerized (C); bareroot (BR); and deeply planted containerized (DP) seedlings, which were also produced in containers, but buried 5 cm after their first growing season to produce adventitious roots prior to outplanting. To test the performance of the different stock types under differing site conditions, seedlings were planted on three microsites that are typical of reforestation sites found in the eastern Canadian boreal forest: (1) bare mineral soil, resulting from mechanical site preparation; (2) in presence of ericaceous shrubs, a family of understory species that generally dominates reforestation sites in eastern Canada (Mallik, 2001; Bloom and Mallik, 2004) on undisturbed organic matter; or (3) in presence of *Sphagnum* spp. and ericaceous shrubs on undisturbed organic matter, *Sphagnum* spp. being characteristics of paludified black spruce stands, which create thick organic matter accumulations (Fenton *et al* 2005) that result in reduced microbial activity and limited soil nutrient availability (Elliott-Fisk, 2000; Pacé *et al* 2018). Since adventitious roots gradually replace initial roots during black spruce development, we considered that this shift could be related to better water or nutrient uptake capacity of these roots, especially in the presence of competitive vegetation. Therefore, DP seedlings should have a post-planting advantage over C or BR stock types.

## 3.4 Material and Methods

### 3.4.1 Study site and experimental design

We established an experimental plantation in the black spruce-feather moss bioclimatic domain of Quebec, Canada (Saucier *et al* 2009), near Lake Aigremont, which is about 80 km south of the city of Chibougamau (49°18'N, 73°49'W). Mean annual temperature is 0.2 °C (± 3.7 °C) and mean annual total precipitation is 995.8 mm (Climate normals 1981-2010, Environment Canada, 2010). The soil is a Humo-Ferric Podzol (Soil Classification Working Group, 1998; USDA, Cryorthod or Haplorthod) that developed from a glacial till with a slope $< 10$ % and is covered by a mor humus that is 10 cm thick. The previous stand was dominated by black spruce before being burned by a wildfire in 2005. Ericaceous shrubs (*Kalmia angustifolia* L., *Rhododendron groenlandicum* (Oeder) Kron & Judd, *Vaccinium angustifolium* Ait.) gradually invaded the site over the following years. The site was scarified in October 2010 using a T26 disk trencher (Bracke Forest AB, Bräcke, Sweden).

Our experiment was established in June 2011, over a 100 m × 50 m area on the scarified site, at least 50 m from the closest forest edge. Within this area, we established two experimental plots, each consisting of 10 "stock type units" for each of the three following planting microsites: (1) Mineral, bare mineral soil within the scarified trenches; (2) Ericaceous, undisturbed humus that was located between scarified trenches and colonized almost exclusively by ericaceous species (30-35 cm tall); and (3) *Sphagnum* spp. + ericaceous, undisturbed humus that was located between scarified trenches and colonized by ericaceous species and a thick layer ($>$ 30-50 cm) of *Sphagnum* spp. (Fig. 3.1). Dry biomass of competing vegetation was estimated over five replicates of 1 $m^2$ each, which were randomly distributed on the site, for the ericaceous shrubs on the ericaceous microsites, and for the ericaceous and *Sphagnum* species for *Sphagnum* spp. + ericaceous microsites. The samples that were weighed following oven-drying (60 °C for 48 h) yielded respective above- and belowground estimates (mean ± SD) of 83.9 g m$^{-2}$ (± 5.6 g m$^{-2}$) and 363.0 g m$^{-2}$ (± 43.2 g m$^{-2}$) in ericaceous microsites, and 71.3 g m$^{-2}$ (± 11.3 g m$^{-2}$) and 191.2 g m$^{-2}$ (± 27 g m$^{-2}$) in *Sphagnum* spp. + ericaceous microsites.

Each stock type unit consisted of three 2-year-old black spruce seedlings spaced that were 2 m apart, randomly distributed among the three stock types: (C) containerized seedlings that were produced in 110 cm$^3$ cavities, and planted at the root collar level (initial root system restricted in a root plug); (DP) deeply planted containerized seedlings, which were produced in 110 cm$^3$ cavities with the root collar buried 5 cm below ground-level at 1-year-old (initial and adventitious root systems restricted in a root plug); and (BR) bareroot seedlings, which were produced in outside growing beds (unrestricted initial root system) (Fig. 2.1). Mean (± SD) initial height was 22.6 ± 2.5 cm, 22.9 ± 3.1 cm and 21.7 ± 3.6 cm and mean (± SD) initial basal diameter was 2.9 ± 0.5 mm, 2.7 ± 0.8 mm and 3.4 ± 0.6 mm for C, DP and BR seedlings, respectively. Due to operational constraints, only six replicates could be planted for the BR stock type. All seedlings were produced from local seed sources (C and DP: 49°49’N, 74°45’W; BR: 48°12’N, 71°29’W).

We selected the first experimental plot (10 stock type units) to receive $^{15}NH_4NO_3$-labelled fertilizer (at 5 % atom%) that was applied over 1 m$^2$ around each seedling at a rate of 50 kg ha$^{-1}$ of N (diluted in 1 L of water). We irrigated all unfertilized seedlings (the second plot) with 1 L of water at the same time as the fertilization treatment. Fertilization was performed at the beginning of the second post-planting growing season (June 2012). To avoid contamination, the 10 fertilized stock type units were located on the same side of the experimental area, thereby precluding formal comparisons between the fertilized/unfertilized seedlings, which was not the objective of the study. Indeed, we either applied or did not apply fertilization to help understand stock type responses in poor natural conditions (unfertilized microsites), while also measuring how they reacted when constraints to growth are lower (fertilized microsites).

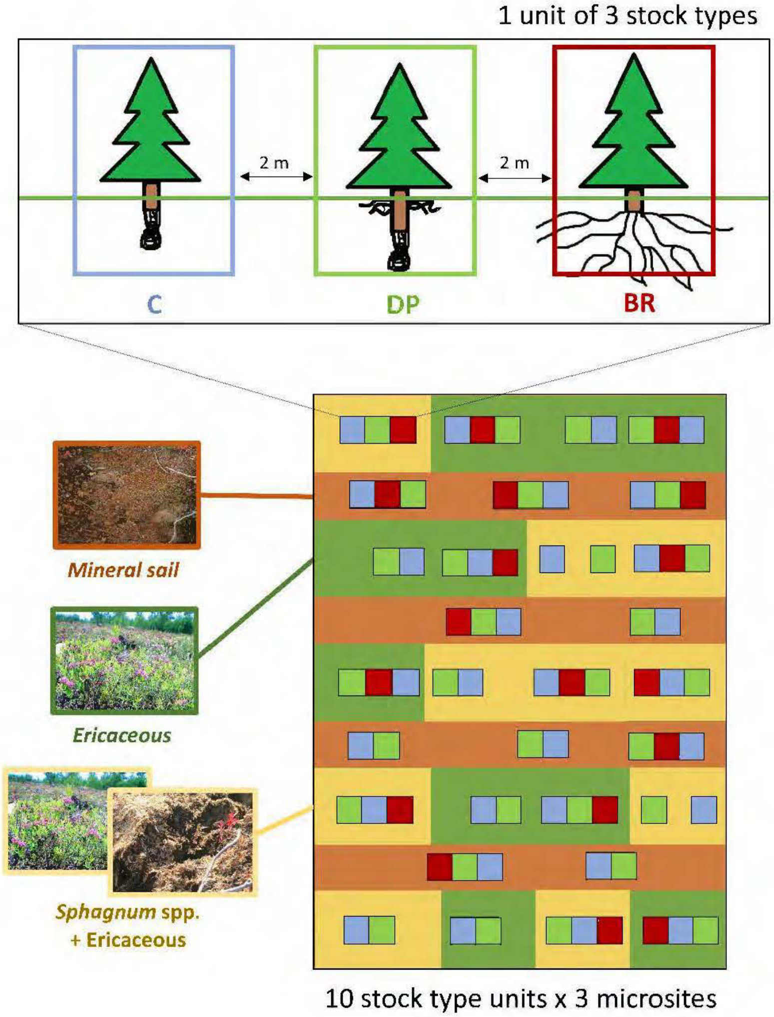


Figure 3.1 Experimental design composed of three 2-year-old black spruce stock types (C) containerized, (DP) deeply planted, and (BR) bareroot seedlings that were planted in three microsites: (1) mineral soil, (2) ericaceous shrubs only, and (3) *Sphagnum* spp. + ericaceous species. One black spruce seedling of each stock type was placed randomly and separated by 2 m from each other into "stock type units". Ten stock type units (replicates) were planted in each microsite (only 6 for BR). This experimental deployment was duplicated, *i.e.*, with or without $^{15}NH_4NO_3$ labelled fertilizer.

### 3.4.2 Seedling measurements

We measured seedling height (cm) and basal diameter (mm) immediately after planting (June 2011) and again at the beginning (June 2012) and at the end (October 2012) of the second growing season. Seedling height was measured from the ground surface using a measuring stick, while basal diameter measurement was taken at ground level with a digital caliper. In October 2012, we harvested all seedlings, separated them into shoots, adventitious roots and initial roots, and oven-dried them (48 h at 60 ± 5 °C) to determine dry biomass. For each microsite type and fertilization treatment (with or without $^{15}N$ fertilization), we randomly selected five seedlings per stock type (C, DP and BR) for nutrient analyses. Dried needles, stems, adventitious and initial roots of all seedlings were ground using a ball mill (Pulverisette 0, Fritsch, Idar-Oberstein, Germany). We analyzed foliar samples for N using a TruMac N (LECO Corporation, St. Joseph, MI, USA) after flash combustion at 1350 °C, and for P, K, Ca and Mg using plasma atomic emission spectroscopy (Thermo Jarrell-Ash - ICAP 61$^{E}$, Thermo Fisher Scientific, Waltham, MA, USA). Needles, stems, adventitious roots and initial roots of seedlings from the $^{15}N$ treatment were analyzed for $^{15}N$ concentration and $\delta^{13}C$ (needles only). Isotope analyses were performed with a PDZ Europa ANCA-GSL elemental analyzer, interfaced with a PDZ Europa 20-20 isotope-ratio mass spectrometer (Sercon Ltd., Crewe, Cheshire, UK) at the Stable Isotope Facility of University of California Davis (Davis, CA, USA). The $\delta^{13}C$ was determined as:

$$\delta^{13}C = ((R_{sample} - R_{standard}) / R_{standard}) \times 1000$$

where $R_{sample}$ and $R_{standard}$ are respectively the $^{13}C/^{12}C$ ratios of the needle sample and the international standard V-PDB (Vienna PeeDee Belemnite). Atom-% $^{15}N$ abundance was determined as:

$$\text{Atom-\%}\ ^{15}\text{N} = [^{15}\text{N} / (\text{Total N})] \times 100 = [^{15}\text{N} / (^{15}\text{N} + ^{14}\text{N})] \times 100$$

Mean total $^{15}N$ abundance was obtained by averaging $^{15}N$ abundances of the different seedling parts, weighted by their biomass.

### 3.4.3 Statistical analyses

We performed all statistical analyses in R v.3.2.3 (R Core Team, 2015), with the threshold for significance set at $P \leq 0.05$. Effects of microsite, stock type and their interaction on response variables (second year height and diameter increments; biomass; concentrations of N, P, K, Ca and Mg; $^{15}N$ abundance and $\delta^{13}C$) were tested according to separate analyses of variance (ANOVAs) for the fertilized and unfertilized seedlings, using the aov function of R. We analyzed 76 fertilized and 72 unfertilized seedlings for growth and biomass parameters and 45 seedlings from each fertilization treatment for nutrient foliar concentrations and isotopic data. No mortality was observed, but several seedlings had broken main stems during the experiment and, therefore, were excluded from analyses. In the case of significant $P$-values, we used post-hoc Tukey's HSD comparisons of treatments. Effect of 'stock type units' was tested for each microsite by fertilization combination to ensure homogeneity within microsites. Assumptions of normality were tested and appropriate transformations were applied when necessary. Height increment, dry biomass (except adventitious roots), and $^{15}N$ abundance (except for needles and total average) were log-transformed. Diameter increment and adventitious root biomass data included many zeros (some seedlings exhibited no diameter increment, and C and BR seedlings did not always have adventitious roots); these data were subsequently transformed to $\log(x + 1)$.

## 3.5 Results

### 3.5.1 Seedling growth

Microsite had a strong effect on the growth of fertilized seedlings ($P < 0.001$; Table 3.1): seedlings planted in mineral soil had height (Fig. 3.2B) and diameter (Fig. 3.2C) increments about twice that of seedlings planted in ericaceous and *Sphagnum* spp. + ericaceous (hereafter, referred to as *Sphagnum*) microsites. All stock types had the same height increment within each microsite (Table 3.1), but C and DP seedlings increased more in girth than BR seedlings (1.29 ± 0.12 mm and 1.25 ± 0.13 mm *vs* 0.69 ± 0.15 mm (mean ± SE) respectively; Fig. 3.2E). Total biomass of fertilized seedlings varied among stock types, depending upon microsite (Table 3.1). Within each microsite, the three stock types had similar biomass; C and DP seedlings growing in mineral soil had greater total biomass than in the presence of competitive vegetation, while for BR seedlings, only *Sphagnum* microsites resulted in a lower biomass gain compared to mineral soil microsites (Fig. 3.2G).

Microsites also affected unfertilized seedlings ($P < 0.05$; Table 3.1), but mineral and ericaceous microsites produced seedlings with similar diameter increments (Fig. 3.2D) and total biomass (Fig. 3.2H), which was greater than in *Sphagnum* microsites. Unfertilized seedlings that were planted in mineral microsites had height increments about 1.5-fold greater than those growing in ericaceous microsites, while seedlings growing in *Sphagnum* had intermediate values (Fig. 3.2B). As was the case for fertilized seedlings, stock type did not affect height growth (Table 3.1), but diameter increments of C and DP seedlings were greater than those of BR (Fig. 3.2F). Unfertilized C seedlings had greater mean biomass than DP seedlings (5.0 ± 0.3 g *vs* 3.8 ± 0.2 g, respectively; Fig. 3.2I).

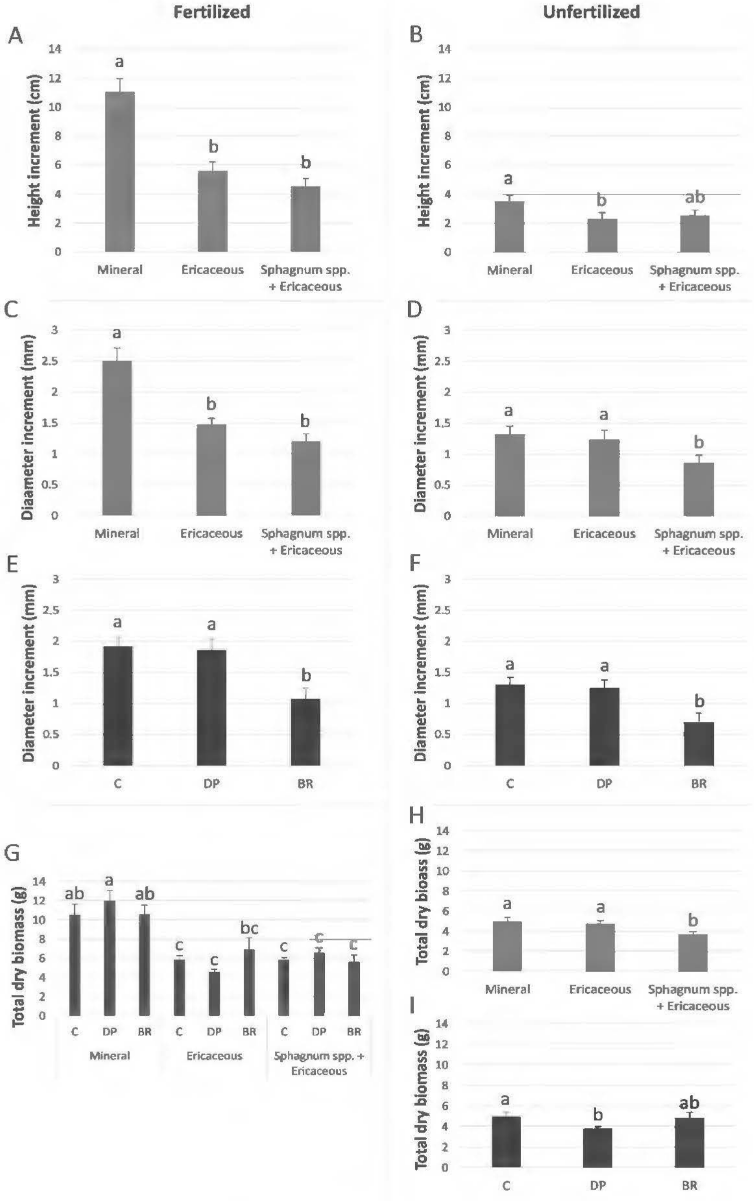


Figure 3.2 Mean height increment of (A) fertilized or (B) unfertilized black spruce seedlings, according to microsite. Mean diameter increment of fertilized (C) and unfertilized (D) seedlings for each microsite, and for stock type of fertilized (E) and unfertilized (F) plots. Mean total dry biomass of fertilized seedlings showing the interaction of microsite × stocktype (G) and of unfertilized seedlings according microsites (H) and stocktypes (I). For each variable, different letters indicate significant differences (Tukey HSD at $P \leq 0.05$). Refer to Fig. 3.1 for stock type descriptions.

Table 3.1. Microsite and stock type effects on growth, foliar nutrient concentrations and isotopic composition of black spruce seedlings. Transformation is indicated in italics. Values in bold are significant at $P \leq 0.05$.

| | Fertilized | | | | | | Unfertilized | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Microsite | | Stock type | | Microsite x Stock type | | Microsite | | Stock type | | Microsite x Stock type | |
| Response Variables | F value | Pr(>F) | F value | Pr(>F) | F value | Pr(>F) | F value | Pr(>F) | F value | Pr(>F) | F value | Pr(>F) |
| **Growth** | | | | | | | | | | | | |
| Height increment *(log)* | 22.54 | **< 0.001** | 1.21 | 0.305 | 0.98 | 0.427 | 3.54 | **0.035** | 0.22 | 0.805 | 0.55 | 0.698 |
| Diameter increment *(log x+1)* | 26.57 | **< 0.001** | 13.21 | **< 0.001** | 1.45 | 0.228 | 4.84 | **0.011** | 8.83 | **< 0.001** | 1.23 | 0.307 |
| Total dry biomass *(log)* | 55.88 | **< 0.001** | 0.03 | 0.967 | 2.83 | **0.032** | 4.76 | **0.012** | 4.42 | **0.016** | 0.15 | 0.962 |
| Shoot dry biomass *(log)* | 48.15 | **< 0.001** | 1.29 | 0.284 | 1.54 | 0.201 | 4.71 | **0.012** | 5.65 | **0.005** | 0.24 | 0.916 |
| Adventitious root dry biomass *(log x+1)* | 28.73 | **< 0.001** | 30.20 | **< 0.001** | 2.24 | **0.048** | 2.59 | 0.090 | 31.13 | **< 0.001** | 2.47 | **0.048** |
| Initial root dry biomass *(log)* | 9.65 | **< 0.001** | 21.33 | **< 0.001** | 4.32 | **0.004** | 2.09 | 0.133 | 20.71 | **< 0.001** | 0.68 | 0.605 |
| Total root dry biomass *(log)* | 41.80 | **< 0.001** | 5.22 | **0.008** | 4.06 | **0.005** | 3.79 | **0.028** | 7.44 | **0.001** | 0.11 | 0.977 |
| **Foliar concentration** | | | | | | | | | | | | |
| N | 13.42 | **< 0.001** | 2.09 | 0.138 | 0.58 | 0.679 | 13.82 | **< 0.001** | 0.80 | 0.457 | 0.52 | 0.721 |
| P | 28.31 | **< 0.001** | 3.50 | **0.041** | 1.53 | 0.214 | 2.99 | **0.046** | 0.87 | 0.427 | 1.11 | 0.369 |
| K | 16.93 | **< 0.001** | 4.12 | **0.025** | 0.74 | 0.572 | 4.73 | **0.016** | 0.86 | 0.434 | 0.17 | 0.952 |
| Ca | 0.20 | 0.818 | 6.67 | **0.003** | 0.07 | 0.990 | 0.00 | 0.998 | 14.61 | **< 0.001** | 0.16 | 0.956 |
| **Isotope** | | | | | | | | | | | | |
| $\delta\,^{13}C$ | 15.16 | **< 0.001** | 3.49 | **0.041** | 0.90 | 0.473 | 4.38 | **0.020** | 7.20 | **0.002** | 1.82 | 0.147 |
| Needle $^{15}N$ at-% | 5.37 | **0.009** | 4.31 | **0.021** | 1.82 | 0.146 | 0.48 | 0.623 | 1.43 | 0.254 | 0.63 | 0.647 |
| Stem $^{15}N$ at-% *(log)* | 5.61 | **0.008** | 3.55 | **0.039** | 1.09 | 0.377 | 0.07 | 0.930 | 0.40 | 0.674 | 1.72 | 0.167 |
| Adventitious root $^{15}N$ at-% *(log)* | 3.00 | **0.049** | 0.12 | 0.888 | 0.29 | 0.882 | 0.07 | 0.929 | 0.39 | 0.683 | 0.23 | 0.916 |
| Initial root $^{15}N$ at-% *(log)* | 2.70 | 0.081 | 0.21 | 0.814 | 1.25 | 0.308 | 0.87 | 0.429 | 0.23 | 0.795 | 1.15 | 0.350 |
| Total $^{15}N$ at-% | 5.60 | **0.008** | 1.66 | 0.204 | 1.29 | 0.292 | 0.39 | 0.682 | 0.72 | 0.496 | 0.19 | 0.940 |

### 3.5.2 Root development

Root biomass of fertilized and unfertilized seedlings was affected by the interaction between stock type and microsite (Table 3.1), such that DP seedlings generally had more adventitious roots than did C and BR on mineral and *Sphagnum* microsites (Fig. 3.3A,B). A similar trend was observed for ericaceous microsites, but only unfertilized DP seedlings produced significantly more adventitious roots than did C seedlings. Fertilized DP seedlings in mineral microsites invested heavily in the development of adventitious roots, with about three to four times more roots being produced than DP planted in the presence of competing vegetation (2.31 ± 0.34 g *vs* 0.59 ± 0.09 g and 0.81 ± 0.07 g for ericaceous and *Sphagnum* microsites, respectively; Fig. 3.3A). All DP seedlings had adventitious roots, while about 65 % of C and BR seedlings had initiated adventitious root production at the end of the experiment, particularly C seedlings on fertilized mineral microsites where resources were likely to be more available and seedlings grew best (Fig. 3.3A). In contrast, fertilized DP seedlings had less initial root biomass than did C or BR seedlings in mineral and ericaceous microsites (Fig. 3.3C). In unfertilized microsites, the biomass of initial roots was only affected by stock type (Table 3.1), with C seedlings having more initial roots than did DP and BR seedlings (Fig. 3.3D).

When biomasses of adventitious and initial roots were combined, fertilized DP seedlings had greater total root biomass than BR in the presence of *Sphagnum*, and tended to have more in mineral microsites as well (Fig. 3.3E). Without fertilization, seedlings that were planted in mineral and ericaceous microsites had greater total root production compared to seedlings in *Sphagnum* microsites (Annexe B, Fig. S3). Unfertilized C seedlings had a greater total root biomass than DP and BR seedlings (1.93 ± 0.09 g *vs* 1.52 ± 0.07 g and 1.47 ± 0.20 g, respectively; Fig. 3.3F).

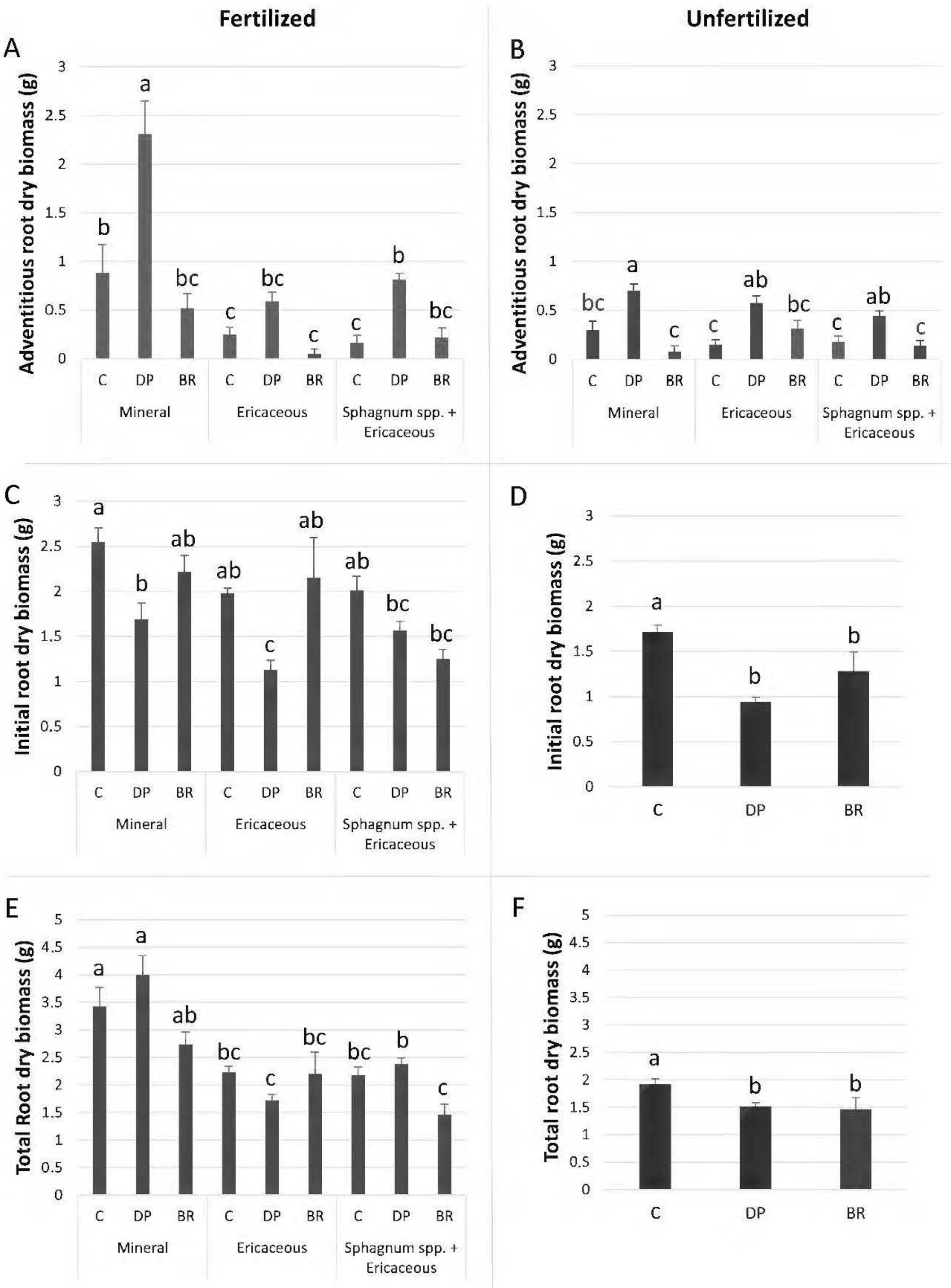


Figure 3.3 Mean dry biomass of (A,B) adventitious, (C,D) initial and (E,F) total roots of fertilized and unfertilized black spruce seedlings, according to microsite and stock type. For a given variable, different letters indicate significant differences at $P \leq 0.05$. Refer to Fig. 3.1 for stock type descriptions.

### 3.5.3 Carbon isotope discrimination

On fertilized microsites, the presence of the humus layer and competing vegetation resulted in seedlings in ericaceous and *Sphagnum* microsites having more negative $\delta^{13}C$ values than those directly planted into the mineral soil, suggesting they were less water stressed (Fig. 3.4A). Trends were similar for unfertilized seedlings, but the difference between mineral and ericaceous microsites was not significant (Fig. 3.4B). Seedlings with root plugs fixed less $^{13}C$ than BR stock type (Fig. 3.4C,D), indicating greater water stress resistance. On fertilized microsites, DP seedlings had intermediate values (Fig. 3.4C).

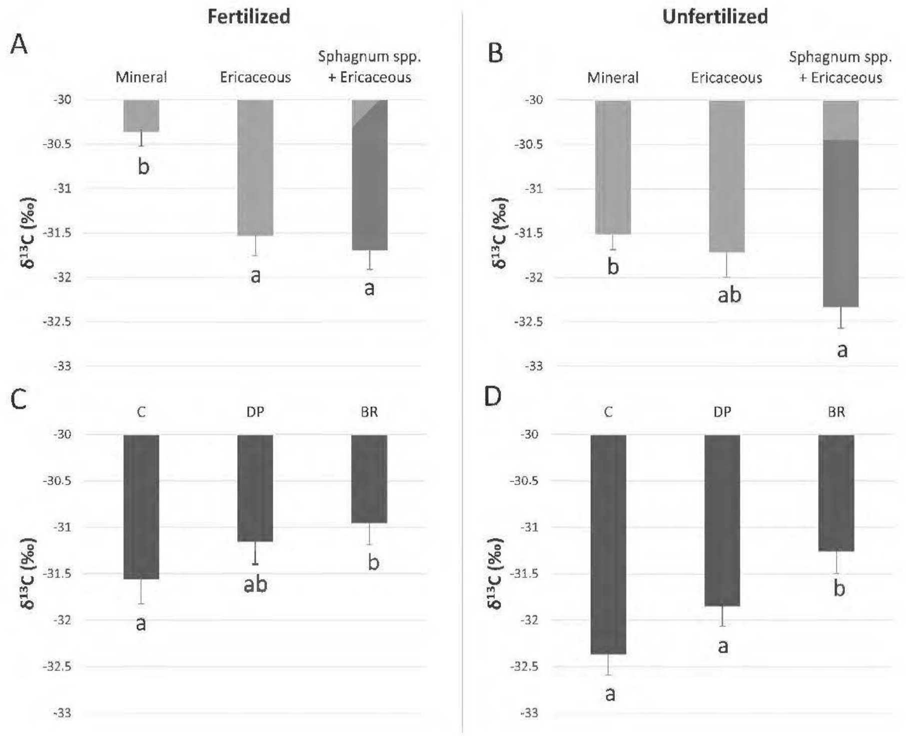


Figure 3.4 Mean $\delta^{13}C$ according to microsites of (A) fertilized and (B) unfertilized seedlings, and according to stock types of (C) fertilized and (D) unfertilized seedlings. For a given variable, different letters indicate significant differences at $P \leq 0.05$. Refer to Fig. 3.1 for stock type descriptions.

### 3.5.4 Nutrient concentrations

Fertilized seedlings that were planted in mineral microsites had higher foliar N, P and K concentrations than seedlings in ericaceous or *Sphagnum* microsites (Fig. 3.5A-C). Trends were similar in unfertilized plots, but differences for foliar P and K were less pronounced (Annexe B, Fig. S4). All stock types had similar N foliar concentrations (Table 3.1). Fertilized DP seedlings had higher foliar P compared to C and BR, and higher foliar K than BR (Fig. 3.5D,E). Foliar Ca was not affected by microsite (Table 3.1). C and DP seedlings had higher foliar Ca than BR seedlings, regardless of fertilization (Fig. 3.5F; Annexe B, Fig. S4).

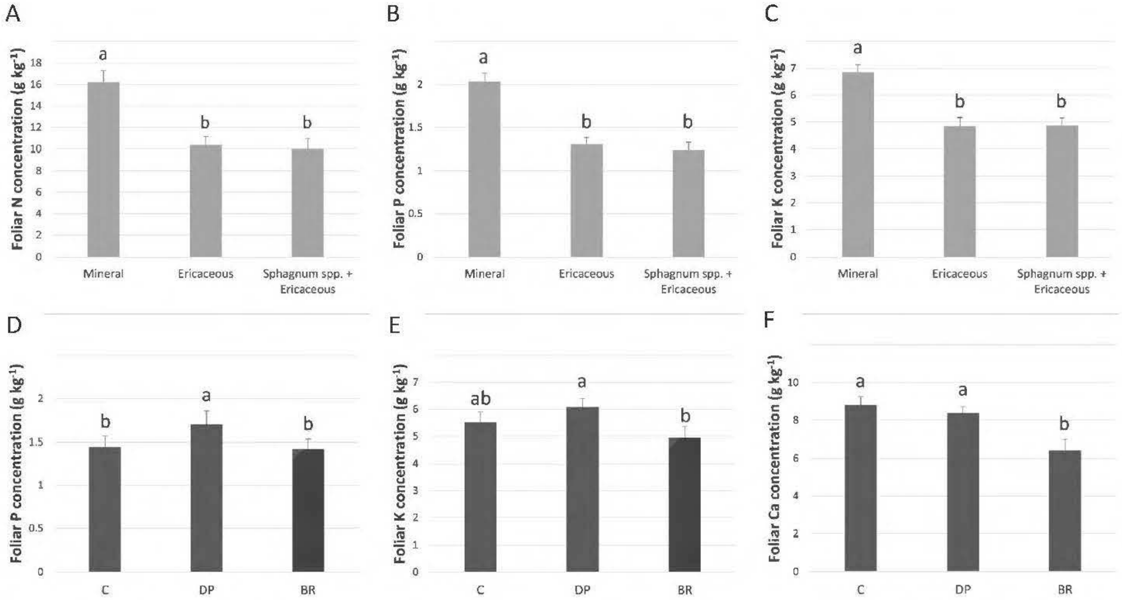


Figure 3.5 Mean foliar concentrations of (A) N, (B) P and (C) K of fertilized seedlings, according to different planting microsites, and foliar (D) P, (E) K and (F) Ca according to different stock types. For a given variable, different letters indicate significant differences at $P \leq 0.05$. Refer to Fig. 3.1 for stock type descriptions.

### 3.5.5 $^{15}N$ abundance

As expected, only fertilized seedlings displayed variation in $^{15}N$ abundances (Table 3.1). In needles, stems, adventitious roots, initial roots, and the average of these components, $^{15}N$ abundance was greater in seedlings that had been planted on mineral microsites compared to those that were planted on ericaceous microsites (Fig. 3.6A; Annexe B, Fig. S5), while seedlings in *Sphagnum* microsites had intermediate values. Abundance of $^{15}N$ was higher in DP needles compared to C and to BR in stems (Table 3.1; Fig. 3.6A,B).

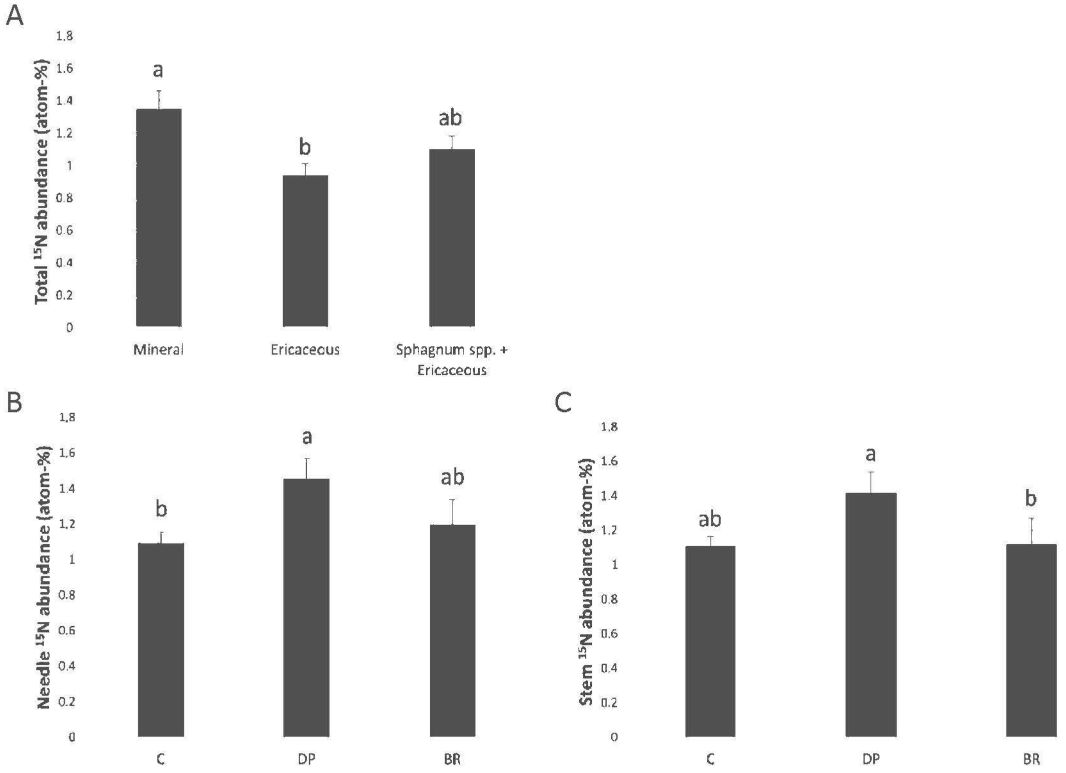


Figure 3.6 Mean total $^{15}N$ abundance of fertilized seedlings, according to microsite (A). Effect of stock type on mean (B) needle and (C) stem $^{15}N$ abundance of fertilized seedlings. For a given variable, different letters indicate significant differences at $P \leq 0.05$. Refer to Fig. 3.1 for stock type descriptions.

## 3.6 Discussion

### 3.6.1 Microsites

After two growing seasons, mineral microsites were the best growth medium for black spruce seedlings. Seedlings in mineral microsites had better growth and higher nutrient concentrations than seedlings growing on microsites with competing vegetation (Fig. 3.2; Fig. 3.5). The effects of microsite appeared to be more strongly related to nutrient limitation than to water availability. Seedlings growing in mineral microsites discriminated less against $^{13}C$, indicating higher water stress during the growing season than seedlings in competition with ericaceous or *Sphagnum* spp. + ericaceous species (Fig. 3.4). This was particularly true for fertilized microsites (Fig. 3.4A), probably because nutrient uptake (especially N) exacerbates water stress (Nilsen, 1995; Patterson *et al* 1997; DesRochers *et al* 2006; Hacke *et al* 2010).

Ericaceous shrubs are known to be strong competitors for nutrients (Thiffault *et al* 2004; Hébert *et al* 2010). Ericaceous species had about 1.5 times more biomass in ericaceous-only than in *Sphagnum* spp. + ericaceous microsites and would have captured a large part of the added fertilizer, as shown by the lower $^{15}N$ abundance that was measured in seedlings there compared to those established in mineral microsites. Seedlings in *Sphagnum* microsites had intermediate abundances (Fig. 3.6). We suggest that it is this greater availability of nutrients that would have allowed seedlings in mineral microsites to attain greater size and biomass than in other microsites. Without fertilization, seedlings in ericaceous microsites had lower height increments than those of seedlings in mineral soil, but their diameter increment and total biomass were similar (Fig. 3.2). It is frequently reported that diameter growth is more strongly affected by competing vegetation than is height growth increment (*e.g.*, Morris *et al* 1990; Jobidon, 2000). However, many of these studies involved competition with trees or fast-growing hardwood shrubs that resulted in reduced light access, particularly during the period of stem thickening, which affects diameter more so than height growth (Shirley, 1945; Lanner, 1985; Brand and Janas, 1988; Groot, 1999). In our study, ericaceous shrubs were only slightly taller than the seedlings at the time of planting (about 30-35 cm *vs* 22 cm) and had reached their maximum height in such ecosystems. This might explain why there was no detectable effect of competition on diameter. Given that our objective was to measure seedling responses to planting shock, we made short-term observations; it is likely that the effect of ericaceous shrubs on seedling diameter increment occurs later following seedling establishment (5 to 8 years after planting; Thiffault *et al* 2012).

*Sphagnum* microsites offered the least favourable conditions for short-term seedling growth, which was partly caused by limited access to nutrients, especially N in unfertilized microsites. Indeed, foliar N concentrations were reduced by half in this environment compared to mineral microsites (Annexe B, Fig. S3). Yet, seedlings that had grown in the presence of ericaceous shrubs, alone or in the presence of *Sphagnum*, showed similar foliar N, P, and K concentrations, suggesting that nutrient availability alone does not explain their lowered growth. It is possible that *Sphagnum* has negative allelopathic effects on tree seedling establishment through chemical interference (Steijlen *et al* 1995; Michel *et al* 2011; Chiapusio *et al* 2013). The negative effect of *Sphagnum* presence could also be attributable to the maintenance of high moisture levels that were induced by the thick layer of *Sphagnum*. Although the ability of the species to retain moisture creates good germination beds, it is not a good substrate for seedling growth, because it can result in sensitivity to frost or needle rust (*Chrysomyxa* spp.) (Ohlson and Zackrisson, 1992; Groot and Adams, 1994; Lavoie *et al* 2006).

### 3.6.2 Stock types – root development and growth response

The three stock types had similar height growth, but containerized seedlings had greater diameter increments than did BRs and appeared less water-stressed, as demonstrate by their lower $\delta^{13}C$ values. This could be due to the presence of a root plug in C and DP seedlings. It has been reported that operationally out-planted containerized seedlings were more protected from pre-planting desiccation and that the plug served as an initial source of moisture and nutrients after planting (Tinus, 1974; Mena-Petite *et al* 2001; Idris *et al* 2004; Jutras *et al* 2007). Therefore, the presence of the root plug can help prevent seedling mortality during 3−4 weeks of drought (Helenius 2005, Helenius *et al* 2002) and allow containerized seedlings to develop new roots more quickly, thereby providing early access to soil resources of the planting sites (Burdett *et al* 1984; Nilsson and Örlander, 1995; Grossnickle and El-Kassaby, 2016). The layout of the experimental design does not allow ruling out, however, the potential confounding effect of seed sources when comparing BR with C and DP seedlings (Pinto *et al* 2011). Although this factor might have played a role in the observed differences, we doubt it was large enough to explain the extent of the effects we measured. Indeed, Benomar *et al* (2016) have shown that seedling height after two years did not vary significantly between spruce planting stock that had been produced from seed sources which were thousands of kilometers apart; planting site was the main factor driving seedling response.

The nature of the root system of each stock type was generally maintained relative to their type of production at the end of the experiment. DP seedlings had more adventitious roots than the other stock types, whereas C and BR seedlings, which were produced and planted at the root collar level, had mostly maintained their initial root system. The presence of pre-established adventitious roots was apparently advantageous for nutrient uptake of seedlings, given that DP seedlings tended to have greater foliar P and K concentrations than C and BR seedlings (Fig. 3.5). DP seedlings also tended to have higher $^{15}N$ abundance in shoots than C and BR seedlings in fertilized microsites (Fig. 3.6). Yet, this did not result in better growth rates, as DP seedlings had similar growth to C seedlings. Black spruce is a species that usually grows in nutrient-poor environments and which favours N luxury consumption and storage (Patterson *et al* 1997; Lupi *et al* 2013). Indeed, the highest $^{15}N$ abundances in DP seedlings were measured in aboveground organs, *i.e.* the shoots and especially the needles, the latter being the primary nitrogen storage tissues for black spruce (Malik and Timmer, 1995, 1998). In addition, as early as 2 years after planting, 65 % of C and BR seedlings had already initiated adventitious root growth (Fig. 3.3). For many species, the development of adventitious roots is an adaptive response (Bellini *et al* 2014; Steffens and Rasmussen, 2016). In black spruce, adventitious root development occurs at the base of the stem and is one of the main mechanisms that allows the species to cope with organic matter accumulation that is typical of boreal forests (LeBarron, 1945; Fenton *et al* 2005). Adventitious root production is one of the main adaptive responses of flood-tolerant species (Kozlowski, 1997), which could explain the strong presence of black spruce in boreal peatlands (Lieffers and Macdonald, 1990); development and rapid cellular adaptation of adventitious roots has already been shown to be a key process for flood adaptation of eastern larch or tamarack (*Larix laricina* [Du Roi] K.Koch), a species also very represented in boreal peatlands (Islam and Macdonald, 2004; Calvo-Polanco *et al* 2012).

Differences in root structure between stock types appears to have influenced total root biomass development. Fertilized containerized stock types, especially DP seedlings, tended to have a total root biomass higher than BRs in mineral and *Sphagnum* microsites. In unfertilized microsites, only C seedlings developed a larger root biomass than BR and DP seedlings. The lower root biomass of DP seedlings compared to C seedlings may explain why there were few differences in nutrient foliar concentrations between stock types without fertilization (Table 3.1). Sufficient numbers of new adventitious roots that are adapted to their environment may be required to affect plant physiology and growth significantly (Zhang *et al* 2017). As good root growth is indicative of the end of planting shock (Rietveld, 1989; Carlson and Miller, 1990;

Grossnickle, 2005), containerized seedlings, *viz.*, C in unfertilized microsites and DP in fertilized microsites, seem to have acclimatized more rapidly to their new environment than did BR seedlings.

### 3.6.3 Management implications

Current management practices in northeastern Canada favour planting of containerized seedlings in bare mineral soil microsites. This combination of treatments resulted in the best growth performance two years after planting in our experiment. The structure of the root system that is restricted in a root plug would be advantageous for limiting planting shock, particularly with respect to water stress. Although DP seedlings grew as well as C seedlings in terms of both height and diameter, they appeared to have been more efficient in terms of nutrient uptake. Developing adventitious roots could benefit long-term growth, especially in boreal forests where access to nutrients is one of the factors most limiting to seedling growth (Tamm, 1991; Lupi *et al* 2013; Maynard *et al* 2014). The better nutrient uptake capacities of DP seedlings apparently did not reduce planting shock compared to C seedlings, as they had similar short-term growth rates. This mirrors the results we have obtained in a controlled environment where black spruce seedlings that were fertilized only through adventitious or initial roots had similar aboveground growth over one growing season (Pernot *et al* 2019a). The strong development of adventitious roots of mature black spruce that has been observed in boreal forests (DesRochers and Gagnon, 1997; Krause and Morin, 2005) could simply be a result of the developing soil organic layer that confers high moisture and nutrient availability (Prescott *et al* 2000), which in turn triggers adventitious root initiation.

Since black spruce production of adventitious roots can easily be initiated by burying the seedling stem base, it would be interesting to produce seedlings with pre-established adventitious roots or by deep-planting the seedlings. Although it represents an initial loss in height, several studies have shown that deep-planting ultimately has no detrimental effect on field performance of common conifer species over the short- or mid-term (Sutton, 1995; Paquette *et al* 2011; Buitrago *et al* 2015; Luoranen and Viiri, 2016). Tarroux *et al* (2014) even observed better height growth of deep-planted black spruce compared to trees that had been originally planted at ground level, a response that was linked with greater production of adventitious roots. Therefore, DP seedlings could be as productive as C seedlings and adventitious roots could confer upon them a long-term advantage in nutrient-poor planting sites.

## 3.7 Conclusion

The origin and structure of the root system influenced growth of black spruce seedlings as early as two years after planting. The root plug of C and DP seedlings likely conferred larger diameter increment and greater water stress resistance on the seedlings, compared to BRs. Given strong initial root system development, C seedlings had greater total biomass than DP seedlings in unfertilized microsites, but this difference disappeared in fertilized ones. Seedlings apparently were more greatly affected by nutrient limitation than by water availability. The presence of humus and competing vegetation, which resulted in lower water stress, negatively affected seedling growth by limiting access to nutrients compared to planting in mineral microsites, which were initially free of competing vegetation. The presence of adventitious roots in DP seedlings apparently improved uptake of nutrients, particularly N. For the purposes of reforestation of boreal sites, burying the stem bases of the seedlings should be considered to initiate the development of adventitious roots and hence, limit nutrient stress.

## 3.8 Acknowledgements

We thank the *Université du Québec à Chicoutimi* and the provincial nursery of Ste. Luce (Quebec, Canada) for providing seedlings and the *Laboratoire de chimie organique et inorganique* of the *Ministère des Forêts, de la Faune et des Parcs du Québec* (MFFPQ) for performing the chemical analyses. We also thank Lyne Blackburn for help and advice in the field and in the laboratory, Dr. Marc Mazerolle for statistical advice, and Evelyne Gaillard (MFFPQ) for technical assistance. We thank Dr. William F.J. Parsons for his help on language revision, Dr. Cosmin Filipescu for his comments on an earlier version of the manuscript, as well as two anonymous reviewers for their constructive advice.

## 3.9 Funding

This research was supported by the *Fonds de Recherche Nature et Technologie du Québec* (FRQNT), Natural Sciences and Engineering Research Council of Canada (NSERC) and NSERC-UQAT-UQAM industrial Chair in Sustainable Forest Management (SFM Chair), in collaboration with the *Direction de la recherche forestière* of MFFPQ (project 142332106).

# CHAPITRE IV

# INFLUENCE OF ROOT SYSTEM CHARACTERISTICS ON BLACK SPRUCE SEEDLING RESPONSES TO LIMITING CONDITIONS

Clémentine Pernot, Nelson Thiffault & Annie DesRochers

Publié dans *Plants*, doi:10.3390/plants8030070

## 4.1 Résumé

Les racines affectent directement l'adaptation des semis plantés aux nouvelles conditions de croissance des sites de reboisement. Pour tester l'influence des caractéristiques racinaires sur la réponse à court terme de semis à la limitation de ressources (eau, nutriments, ou oxygène), nous avons réalisé deux expériences. Nous avons comparé (1) la croissance et la physiologie de trois types de semis d'épinette noire (*Picea mariana* (Mill.) BSP) âgés de 4 ans (en récipient, racines initiales très développées restreintes à la carotte de substrat; à racines nues, racines initiales moins développées mais non restreintes; enfoui en récipient, racines initiales et adventives restreintes) à différentes combinaisons d'irrigation et de fertilisation. Nous avons également examiné (2) la plasticité cellulaire des racines adventives et initiales en réponse à trois régimes hydriques, incluant la submersion. Les semis à racines nues ont eu une meilleure croissance relative en hauteur que les semis en récipients, probablement dû à leur plus grande taille initiale. D'autre part, les semis en récipients ont mieux profité de la fertilisation, en présentant un taux de croissance relative en diamètre supérieur aux semis à racines nues, et ont été moins affectés par la limitation en eau, possiblement dû à la présence de la carotte de substrat offrant une capacité de réserve en eau additionnelle. Pour les semis en récipients, la présence de racines adventives a été bénéfique pour la croissance en hauteur et les performances physiologiques en comparaison des semis avec uniquement des racines initiales. Les racines adventives ont montré une bonne plasticité cellulaire, en particulier en condition de submersion.

Mots clés : racines initiales, racines adventives, type de plant, épinette noire, limitation de ressources, irrigation, fertilisation, submersion

## 4.2 Abstract

Roots directly affect planted seedling adaptation to new growing conditions at reforestation sites. To test the influence of root characteristics on the short-term response of seedlings to limiting resources (water, nutrient, or oxygen), we conducted two experiments. We compared (1) the growth and physiology of three types of four-year-old black spruce (*Picea mariana* (Mill.) BSP) seedlings (Containerized, highly developed initial roots restricted to a plug; bareroot, less developed but unrestricted initial roots; deeply-planted containerized, restricted initial and adventitious roots) to different combinations of irrigation and fertilization. We also investigated (2) the cellular plasticity of adventitious and initial roots to three irrigation regimes including flooding. Bareroot seedlings had better relative growth rates in height than containerized seedlings, probably due to their larger initial size. On the other hand, containerized seedlings took better advantage of fertilization, as shown by a higher relative growth rate in diameter compared to bareroot seedlings and were less affected by water limitation, possibly due to the root plug acting as an additional water reserve capacity. For containerized seedlings, the presence of adventitious roots was beneficial to height growth and physiological performances compared to seedlings with initial roots only. Adventitious roots showed great cell plasticity, particularly under flooding conditions.

Keywords: initial roots, adventitious roots, stock type, black spruce, limiting resources, irrigation, fertilization, flooding

## 4.3 Introduction

Black spruce (*Picea mariana* Mill. BSP) is a dominant species of North American boreal forests. It has a large ecological spectrum (Viereck and Johnston 1990), able to grow in conditions ranging from peatlands (Lieffers and Macdonald 1990; Islam and Macdonald 2004) to mesic well-drained sites (DesRochers and Gagnon 1997). Its plasticity, as well as its fiber quality and resistance to pests and diseases make it the most reforested species in Quebec, Canada (Salmon 2018). With more than 75 million black spruce seedlings planted annually in this province, ensuring the success of plantations is fundamental to support sustainable forest management objectives.

The root system plays a key role in tree establishment (Grossnickle 2005). This is especially important when outplanting seedlings from nursery to field conditions, because the range of changing conditions can be considerable. Seedling ability to develop new roots impacts the time required to establish direct contact with the planting site (Wagner and Robinson 2006; Johansson *et al* 2007; Thiffault *et al* 2012), which is necessary to have direct access to soil resources. Indeed, water stress is the main cause of conifer mortality after planting (Burdett 1990; Margolis and Brand 1990), and limited access to nutrients may cause post-planting growth stagnation (Burdett 1990; de Montigny and Weetman 1990). During nursery production, the ability to produce new roots is thus an important criterion for selecting the most vigorous seedlings (Sutton 1980; Burdett *et al* 1983; Feret and Kreh 1985).

Two stock types are commonly used in plantation: (1) Seedlings produced in rigid-walled containers, resulting in a highly developed root systems restricted in a root plug; and (2) bareroot seedlings grown in open fields, producing unrestricted root systems but of a lower biomass (Grossnickle and Blake 1987; Grossnickle and El-Kassaby 2016). These different root traits can influence seedling aboveground development and their physiology. When reforestation sites present potentially harsh growing conditions, containerized seedlings are often favored since the root plug can act as a source of water and nutrients for some time after planting, allowing them to have a greater root growth potential and to be more resistant to water stress than bareroot seedlings (Idris *et al* 2004; Rose and Haase 2005; Jutras *et al* 2007). On the other hand, bareroot stock is generally preferred for outplanting on sites subjected to high levels of competition as bareroot seedlings are usually larger than container-grown stock (Thiffault 2004).

Containerized and bareroot seedlings however, present root structures that differ from those of naturally regenerated seedlings. Indeed, black spruce planting stock are produced and planted at the root collar level, resulting in root systems exclusively composed of initial roots, *i.e.,* roots formed below the seed germination point. In contrast, the root system of black spruce is predominantly formed of adventitious roots under natural conditions (DesRochers and Gagnon 1997; Krause and Morin 2005; Tarroux *et al* 2014). Adventitious roots develop at the base of the stem from the first years and gradually replace initial roots. In many species, the formation of adventitious roots may allow a better adaptation to harsh environmental conditions, such as flooding (Kozlowski 1997; Steffens and Rasmussen 2016). This is the case of tamarack (*Larix laricina* (Du Roi) K. Koch) which shares the dominance of boreal peatlands with black spruce (Lieffers and Macdonald 1990; Patterson *et al* 1997). The adventitious roots of this species have different cellular morphologies, with less developed endodermis and a delayed suberization compared to initial roots, allowing tamarack seedlings to increase apoplastic water transport and thus maintain water balance despite anaerobic conditions (Calvo-Polanco *et al* 2012). Adventitious roots can be formed with other cellular structures to adapt to flooding, such as aerenchyma – tissues with large intercellular air-filled spaces that facilitate oxygen diffusion (Drew *et al* 1979; Argus *et al* 2015). In some herbaceous species, adventitious roots can also facilitate nutrient uptake. For example, P uptake rate of *Solanum dulcamara* L. was dependent on adventitious root biomass (Zhang *et al* 2017), while *Triticum aestivum* exhibited increased P and K uptake with adventitious nodal roots compared to seminal roots under stagnant flooded conditions (Wiengweera and Greenway 2004). We did not observe significant differences in the ability of black spruce seedlings to uptake nutrients from adventitious or initial roots separated, under greenhouse-controlled conditions (Pernot *et al* 2019a). However, trees with adventitious and initial roots had increased soil nutrients uptake (particularly nitrogen) compared to seedlings with only initial roots under field conditions (Pernot *et al* 2019b).

In this study, we hypothesized that black spruce seedlings with adventitious roots adapt more easily to limiting conditions (water, nutrient, or oxygen) than seedlings with initial roots only, by having (1) better water and nutrient uptake under limited resources availability, and (2) greater cellular plasticity that facilitates adaptation to different irrigation conditions. We tested our hypothesis using two experiments. In Experiment 1, we first tested water and nutrient uptake of three stock types that had previously shown differential responses when outplanted on a boreal site (Pernot *et al* 2019b): (C) Containerized seedlings, the most commonly used in potentially hash planting conditions, with initial roots restricted in a root plug; (DP) deeply-planted containerized seedlings, also produced in container but with the root collar buried 5 cm to develop adventitious roots in addition to the initial roots; and (BR) bareroot seedlings, with a root system without any space restriction, but composed mainly of initial roots. We subjected seedlings to two irrigation treatments (25% or 100% water field capacity) and two levels of fertilization (with or without fertilizer), and compared growth and physiology among stock types (Fig. 4.1). Secondly, knowing that adventitious roots develop both under high water-content (Aubin 1996) and well-drained (DesRochers and Gagnon 1997) conditions, we conducted Experiment 2 by comparing the cellular morphology of adventitious and initial roots under different irrigation regimes: (i) Limited water supply (25% water field capacity); (ii) well-watered condition (100%

water field capacity); and (iii) flooding conditions (Fig. 4.1). Since adventitious roots are often an adaptive response (Geiss *et al* 2009; Bellini *et al* 2014), we anticipated that DP seedlings would be more suitable for planting on sites with harsh growth conditions, such as those submitted to flooding or water and nutrient limitations.

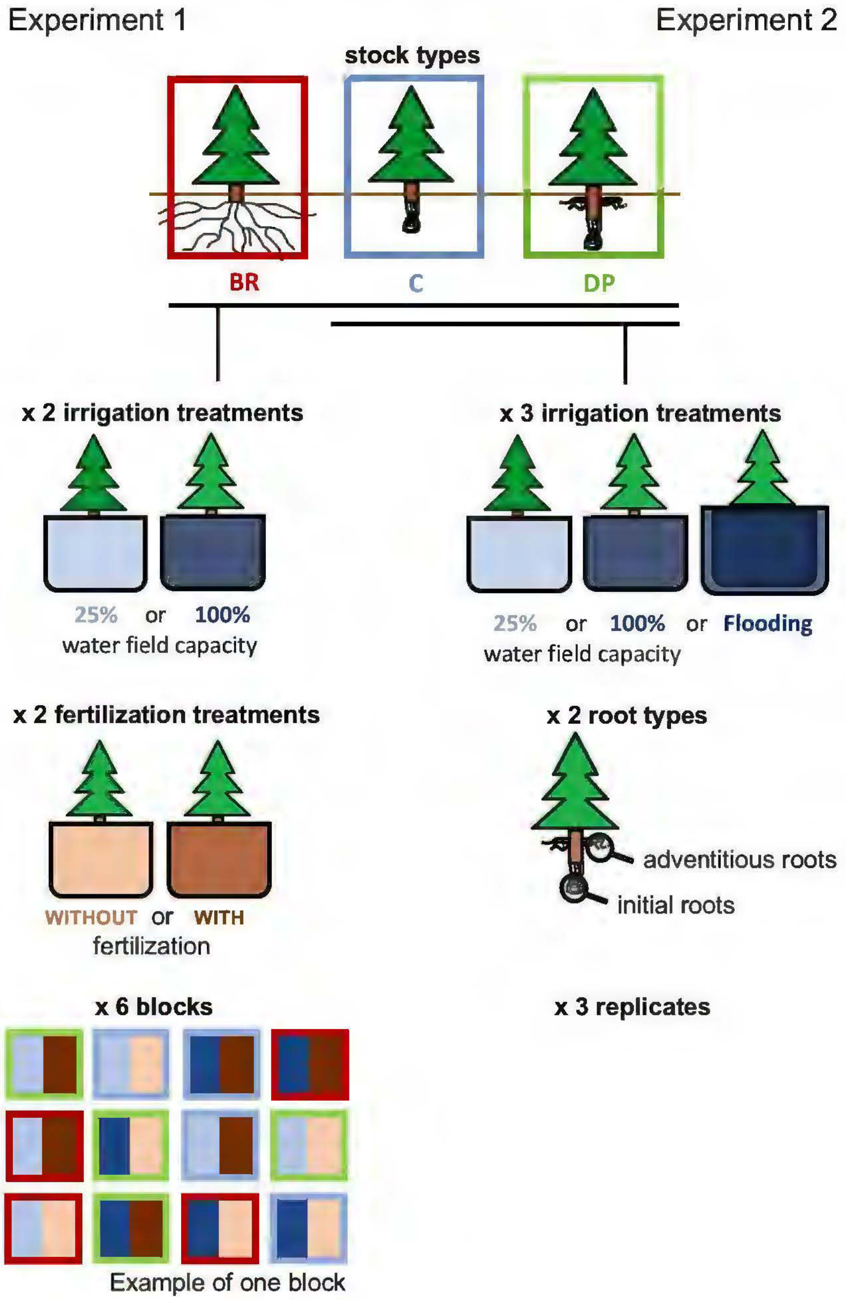


Figure 4.1 Legend on next page.

Figure 4.1 Greenhouse experiments using four-year-old black spruce seedlings. Experiment 1 consisted of three stock types: BR, bareroot seedlings (with an unrestricted root system, mainly composed of initial roots); C, containerized seedlings (with a restricted initial root system); and DP, deeply-planted containerized seedlings (with initial and adventitious roots restricted in a root plug). These were subjected to two irrigation treatments (maintenance of 25% or 100% water field capacity) and two fertilization treatments (with or without fertilization), replicated six times in a factorial design. Experiment 2 consisted of C and DP seedlings subjected to three irrigation treatments (maintenance of 25% or 100% water field capacity, or flooding condition). Cell morphology was observed for initial and adventitious roots (initial roots only for C seedlings) on three replicates per treatment.

## 4.4 Results

### 4.4.1 Experiment 1: Growth and physiological responses to irrigation and fertilization treatments

Bareroot (BR) seedlings had the greatest relative growth rate in height ($RGR_{height}$) after 14 weeks (representing a gain of about 40% of initial height), twice the height growth of containerized (C) seedlings. Deeply-planted (DP) seedlings had intermediate $RGR_{height}$ with an average of 24% increase (Fig. 4.2A). $RGR_{height}$ was also influenced by the interaction between fertilization and irrigation treatments (Table 4.1). Unfertilized seedlings had low and similar $RGR_{height}$ under both irrigation treatments while irrigation increased the height growth of fertilized seedlings (Fig. 4.2B). Fertilization also increased relative growth rate in basal diameter ($RGR_{diameter}$) of C and DP seedlings , while it did not increase diameter growth of BR seedlings, independently of the irrigation treatment (Table 4.1, Fig. 4.2C). The high irrigation level increased $RGR_{diameter}$ of fertilized seedlings only (Fig. 4.2D).

The total biomass of BR seedlings at the end of the experiment was greater than that of C and DP seedlings (Table 4.2). Fertilization increased the total biomass of seedlings while irrigation had no significant effect (Tables 4.1, 4.2). Total root biomass was similar for all treatment combinations (Tables 4.1, 4.2). However, the proportions of initial *vs* adventitious roots differed between the treatments. Root systems of C and BR seedlings were predominantly composed of initial roots, with greater initial root biomass than DP seedlings, except for fertilized seedlings under limited irrigation where differences were not significant (Fig. 4.3A). Conversely, DP seedlings developed more adventitious roots than C and BR seedlings; however, BR seedlings developed a small amount of adventitious roots while C seedlings had exclusively initial roots (Fig. 4.3B). Seedlings had similar final root/shoot ratios among stock types and water regimes (Table 4.1), but unfertilized seedlings invested more in root development than in aerial parts compared to fertilized ones (Table 4.2).

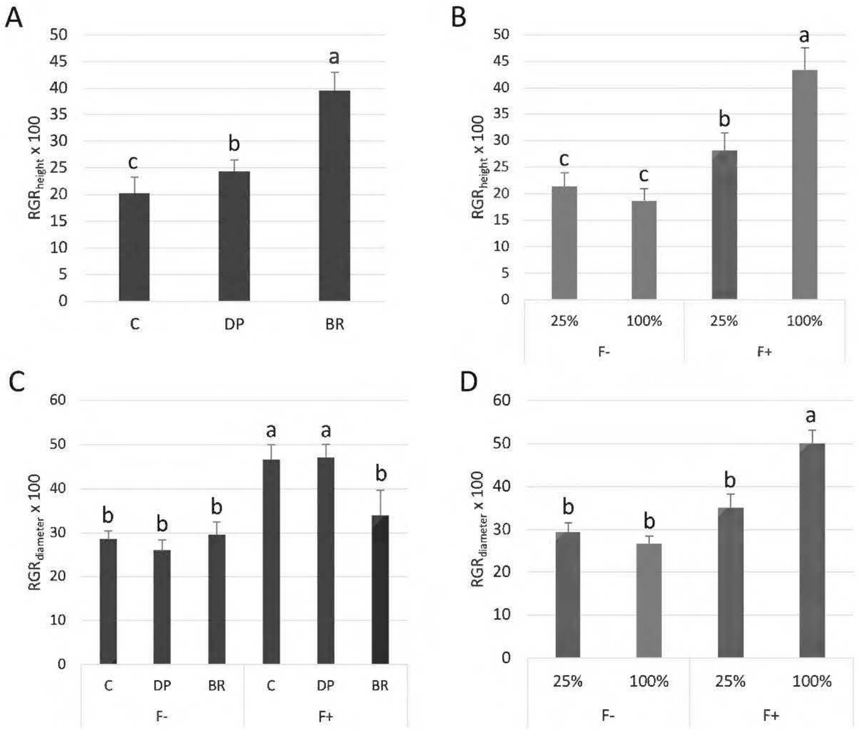


Figure 4.1 Relative growth rate in height ($RGR_{height}$) of black spruce seedlings according to (A) stock types and (B) irrigation (25% or 100% field capacity) × fertilization (F− without and F+ with fertilization) over one growing season. Relative growth rate in diameter ($RGR_{diameter}$) of seedlings according to (C) stock types × fertilization and (D) irrigation × fertilization over one growing season. Bars with the same letter indicate a non-significant difference at $P \leq 0.05$.

Table 4.1 Effects of fertilization, irrigation and stock type on growth, physiology and nutrient concentration of four-year-old black spruce seedlings. When required, analyses were performed on transformed data (indicated in parentheses). Statistically significant values at $P \leq 0.05$ are in bold.

| | Fertilization | | Irrigation | | Stock type | | Fertilization x Irrigation | | Fertilization x Stock type | | Irrigation x Stock type | | Fertilization x Irrigation x Stock type | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *F*-value | *P*-value | *F*-value | *P*-value | *F*-value | *P*-value | *F*-value | *P*-value | *F*-value | *P*-value | *F*-value | *P*-value | *F*-value | *P*-value |
| ***Growth*** | | | | | | | | | | | | | | |
| $\text{RGR}_{\text{height}}$ *(log)* | 43.88 | **<0.001** | 3.68 | **0.060** | 25.89 | **<0.001** | 8.64 | **0.005** | 0.94 | 0.396 | 1.36 | 0.266 | 0.52 | 0.595 |
| $\text{RGR}_{\text{diameter}}$ | 37.52 | **<0.001** | 6.30 | **0.015** | 2.14 | 0.127 | 13.85 | **0.001** | 4.88 | **0.011** | 1.34 | 0.271 | 0.09 | 0.912 |
| Total dry biomass | 21.54 | **<0.001** | 0.49 | 0.486 | 5.21 | **0.009** | 0.79 | 0.379 | 0.59 | 0.560 | 2.07 | 0.137 | 1.67 | 0.198 |
| Shoot dry biomass | 45.52 | **<0.001** | 0.58 | 0.449 | 5.92 | **0.005** | 0.18 | 0.669 | 1.37 | 0.263 | 1.62 | 0.207 | 0.71 | 0.495 |
| Total root dry biomass *(log)* | 0.04 | 0.852 | 0.20 | 0.658 | 2.26 | 0.115 | 1.46 | 0.232 | 0.32 | 0.727 | 2.31 | 0.109 | 2.96 | 0.060 |
| Adventitious root dry biomass *(log x+1)* | 0.01 | 0.993 | 0.80 | 0.376 | 103.28 | **<0.001** | 0.01 | 0.907 | 0.68 | 0.509 | 1.03 | 0.363 | 0.03 | 0.971 |
| Initial root dry biomass *(log)* | 0.31 | 0.581 | 2.39 | 0.128 | 47.84 | **<0.001** | 0.04 | 0.845 | 0.37 | 0.691 | 3.49 | **0.038** | 4.32 | **0.018** |
| Root/Shoot ratio | 35.80 | **<0.001** | 0.01 | 0.937 | 0.57 | 0.567 | 0.82 | 0.369 | 1.24 | 0.298 | 0.20 | 0.815 | 2.33 | 0.107 |
| ***Physiology*** | | | | | | | | | | | | | | |
| Shoot water potential | 22.18 | **<0.001** | 174.92 | **<0.001** | 1.20 | 0.308 | 20.61 | **<0.001** | 1.35 | 0.269 | 0.78 | 0.464 | 3.23 | **0.047** |
| Net photosynthesis | 0.99 | 0.324 | 0.47 | 0.497 | 15.96 | **<0.001** | 1.65 | 0.203 | 4.28 | **0.043** | 3.62 | **0.034** | 2.62 | 0.082 |
| Stomatal conductance *(log)* | 0.05 | 0.833 | 4.75 | **0.034** | 7.25 | **0.002** | 0.01 | 0.944 | 2.96 | **0.060** | 9.87 | **0.000** | 3.89 | **0.027** |
| ***Nutrient concentration*** | | | | | | | | | | | | | | |
| N *(log)* | 1092.67 | **<0.001** | 101.54 | **<0.001** | 0.28 | 0.755 | 0.38 | 0.542 | 2.60 | 0.064 | 1.32 | 0.279 | 0.41 | 0.668 |
| P *(log)* | 313.57 | **<0.001** | 3.40 | 0.071 | 3.15 | **0.050** | 4.02 | **0.048** | 1.73 | 0.187 | 1.29 | 0.285 | 2.82 | 0.069 |
| K *(log)* | 353.91 | **<0.001** | 77.33 | **<0.001** | 2.18 | 0.123 | 36.82 | **<0.001** | 2.37 | 0.104 | 3.91 | **0.044** | 1.29 | 0.285 |
| Ca *(log)* | 85.11 | **<0.001** | 29.90 | **<0.001** | 9.80 | **0.000** | 11.40 | **0.001** | 3.69 | **0.032** | 1.19 | 0.312 | 1.70 | 0.194 |

$\text{RGR}_{\text{height}}$ = Relative growth rate in height; $\text{RGR}_{\text{diameter}}$ = Relative growth rate in basal diameter

Table 4.2 Mean (± SE) of shoot, root and total dry biomass and root/shoot ratios according to fertilization (F− without and F+ with fertilization) and stock type (C, containerized seedlings; DP, deeply-planted containerized seedlings; and BR, bareroot seedlings (refer to Figure 4.1 for description)). Different letters for the same fixed effect (fertilization or stock type) indicate significant differences at $P \leq 0.05$.

| | Fertilization | | Stock type | | |
|---|---|---|---|---|---|
| | F− | F+ | C | DP | BR |
| Shoot dry biomass (g) | 18.4$^{b}$ ± 0.8 | 27.1$^{a}$ ± 1.1 | 20.6$^{b}$ ± 1.1 | 21.9$^{b}$ ± 1.4 | 25.6$^{a}$ ± 1.8 |
| Root dry biomass (g) | 10.6$^{a}$ ± 0.6 | 10.7$^{a}$ ± 0.6 | 10.3$^{a}$ ± 0.8 | 9.8$^{a}$ ± 0.5 | 11.9$^{a}$ ± 0.8 |
| Total dry biomass (g) | 29.0$^{b}$ ± 1.3 | 37.8$^{a}$ ± 1.5 | 30.8$^{b}$ ± 1.7 | 31.7$^{b}$ ± 1.7 | 37.5$^{a}$ ± 2.3 |
| Root/Shoot ratio (g g$^{-1}$) | 0.58$^{a}$ ± 0.03 | 0.40$^{b}$ ± 0.02 | 0.51$^{a}$ ± 0.03 | 0.47$^{a}$ ± 0.02 | 0.50$^{a}$ ± 0.04 |

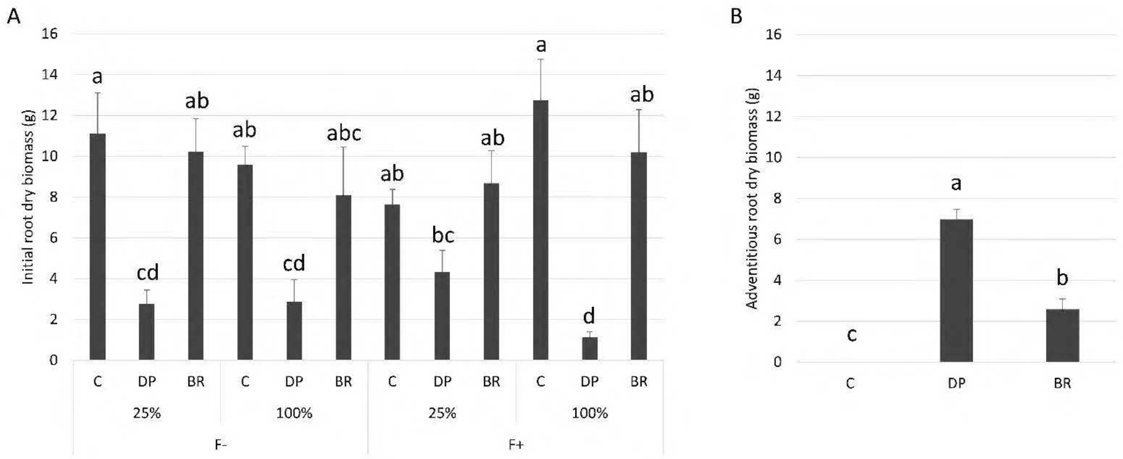


Figure 4.2 Mean dry biomass of black spruce (A) initial roots for each stock type × irrigation (25% or 100% field capacity) × fertilization (F− without and F+ with fertilization) treatments and (B) adventitious roots for each stock type. Bars with the same letter indicate a non-significant difference at $P \leq 0.05$.

The net photosynthesis response of seedlings to fertilization and irrigation differed among stock types (Table 4.1). Unfertilized C and DP seedlings had a higher net photosynthesis than BRs, whereas fertilized DP seedlings had higher net photosynthesis than the other two stock types (Fig. 4.4A). Well irrigated seedlings of all stock types had similar mean net photosynthesis, while DP seedlings had greater net photosynthesis than C and BR seedlings under water-limited conditions (Fig. 4.4B). Similarly, stomatal conductance of BR seedlings was also more reduced by water limitation compared to C and DP seedlings, especially in the presence of fertilization, since this combination of treatments was the only one to have resulted in a significantly greater stomatal conductance in DP seedlings compared to BRs (Fig. 4.4C). As expected, shoot water potential was lowest under limited irrigation conditions, especially for fertilized seedlings (Fig. 4.4D). Stock type or fertilization had no effect on shoot water potential under well watered conditions. Under water limited conditions, fertilization significantly reduced the shoot water potential of all stock types compared to the well-watered condition, while without fertilisation only C and BR seedlings had low shoot water potential, DP seedling shoot water potential being similar to that of well watered seedlings. (Fig. 4.4D).

Needle N concentrations were similar for the three stock types (Table 4.1). Fertilization increased N concentrations in needles (Fig. 4.5A), while irrigation decreased it (Fig. 4.5B). C seedlings had greater P concentrations in needles than DP and BR seedlings (Fig. 4.5C). There was an interaction between irrigation and fertilization treatments such that irrigation had no effect on needle P concentrations of fertilized seedlings while unfertilized seedlings had greater P concentrations under the low irrigation treatment (Annexe C, Fig. S6). Droughted DP seedlings had greater K concentrations than BR seedlings (16.1 ± 2.5 g kg−1 vs. 12.4 ± 2.6 g kg−1; mean ± SE), while C seedlings, having a larger response variability, had an intermediate status (16.7 ± 3.6 g kg−1) (Fig. 4.5D). Fertilized C and DP seedlings had higher Ca concentrations compared to BR seedlings, while this trend was not significant for unfertilized seedlings (Fig. 4.5E). Finally, fertilized seedlings had greater K and Ca concentrations under low irrigation while unfertilized seedlings had low and similar concentrations (Annexe C, Fig. S6).

### 4.4.2 Experiment 2: Root cell morphology

At 25% water field capacity (FC), seedlings produced initial and adventitious roots with a smaller cortex than seedlings at 100% FC or under flooding conditions (Fig. 4.6). Seedlings irrigated at 25% FC exhibited adventitious roots composed of cortical cells with suberized cell walls but that were completely collapsed, forming a thick suberized layer on the periphery of the endodermis. Collapsed suberized cortical cells were also observed in initial roots under limited water supply, but in a less systematic way (Annexe C, Fig. S7). In the case where cortical cells were not collapsed, they still showed suberized cell walls (Fig. 4.6A). For both initial and adventitious roots of seedlings under limited water regime, endodermis appeared more suberized than in roots of seedlings under 100% FC or flooded. At 100% FC, cell morphology of initial and adventitious roots was very similar (Fig. 4.6A). Flooding induced different responses in initial and adventitious roots, adventitious roots had larger cortical cells forming a bigger cortex area than those of seedlings under the other water regimes, whereas the initial roots of flooded seedlings had similar cortex areas to seedlings under 100% FC (Fig. 4.6). The appearance and size of the root cortex were similar in initial roots developed by C or DP seedlings under all irrigation treatments (Fig. 4.6B).

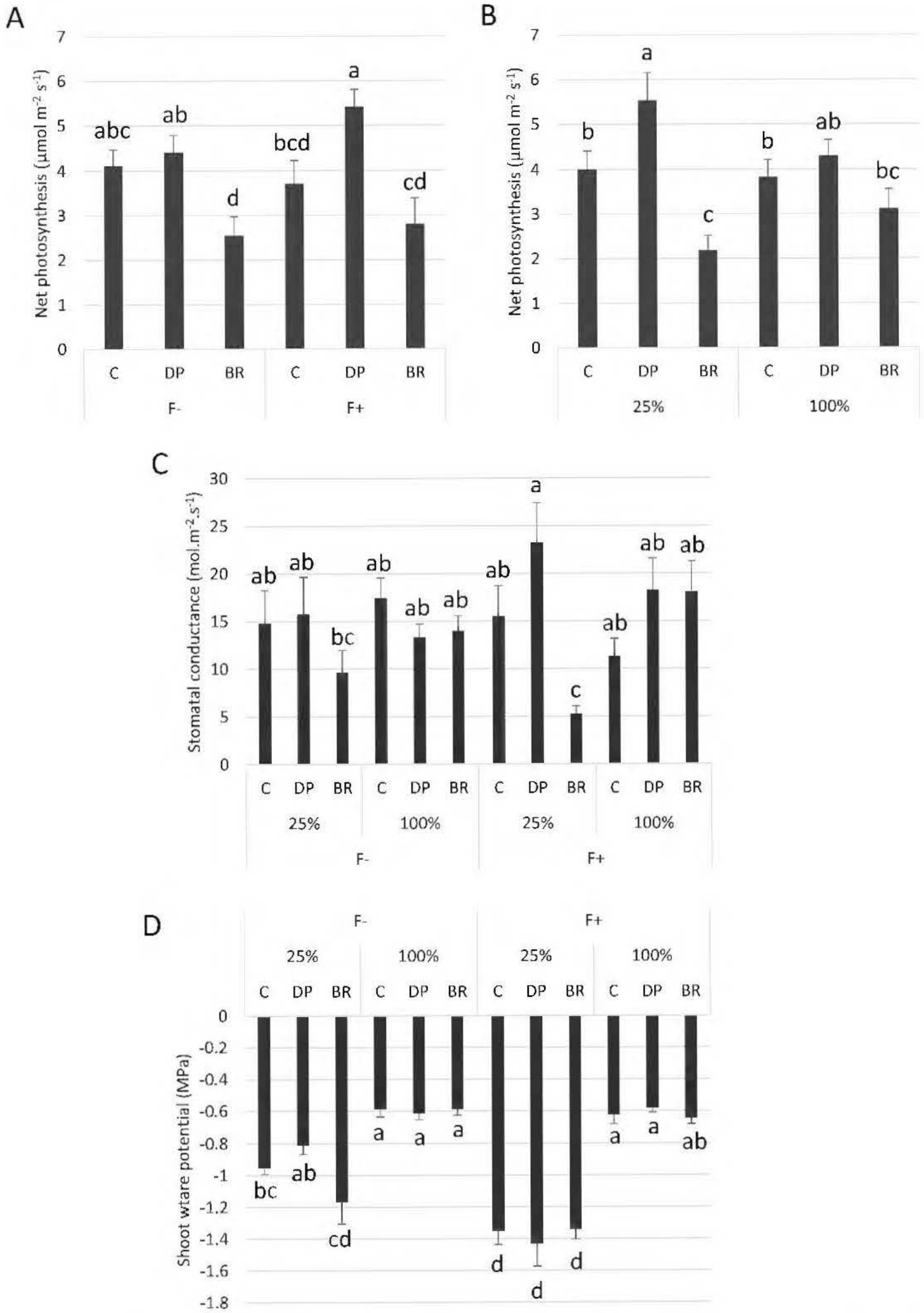


Figure 4.3 Mean net photosynthesis according to (A) stock type × fertilization (F− without and F+ with fertilization) and (B) stock type × irrigation (25% or 100% field capacity). Effects of stock type × irrigation × fertilization on (C) stomatal conductance and (D) shoot water potential. Bars with the same letter indicate a non-significant difference at $P \leq 0.05$.

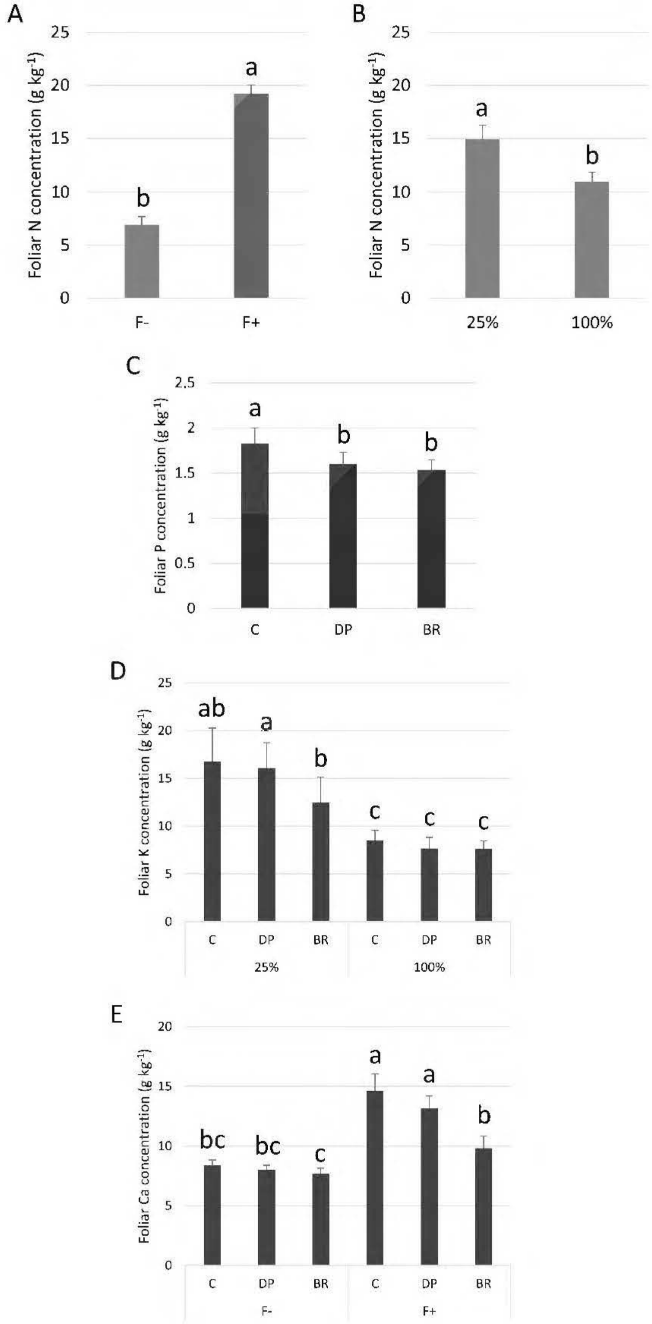
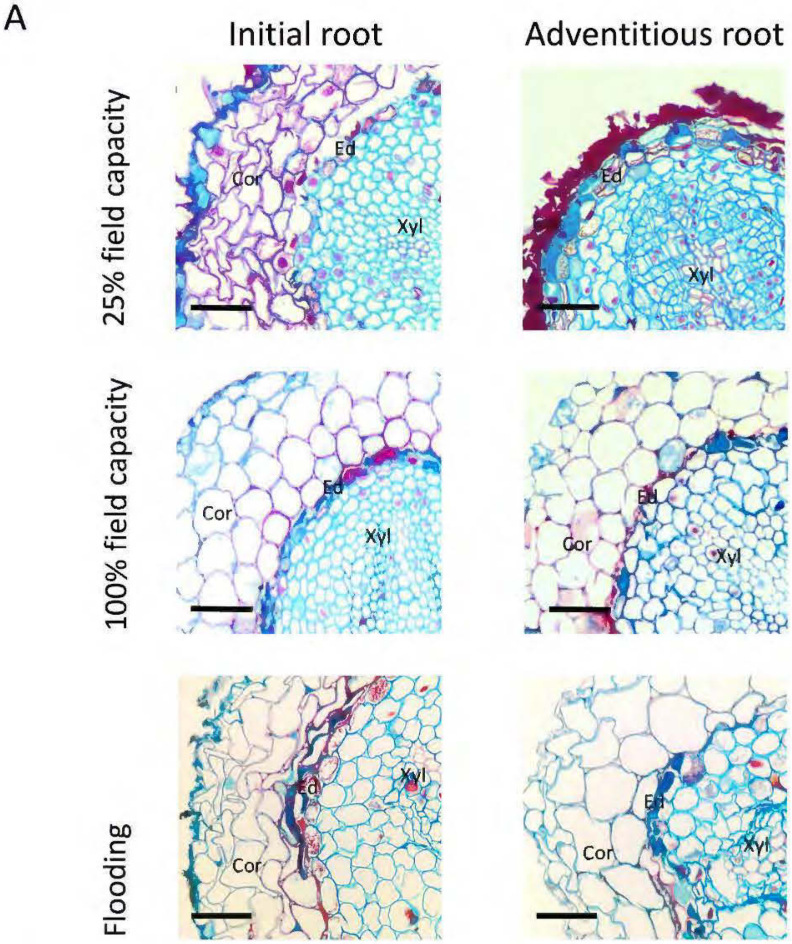


Figure 4.4 Mean foliar nutrient concentrations of black spruce seedlings: N, nitrogen according to (A) fertilization (F− without and F+ with fertilization); (B) irrigation (25% or 100% field capacity); (C) P, phosphorus according stock type; (D) K, potassium according stock type × irrigation; and (E) Ca, calcium according to stock type × fertilization. Bars with the same letter indicate a non-significant difference at $P \leq 0.05$.



B

| | | 25% field capacity | 100% field capacity | Flooding |
|---|---|---|---|---|
| | | **Cortex area (% of the total root surface)** | | |
| C seedling | Initial roots | 35.18$^{a}$ ± 3.92 | 55.61$^{b}$ ± 2.84 | 58.25$^{b}$ ± 2.37 |
| DP seedling | Initial roots | 32.68$^{a}$ ± 3.56 | 50.71$^{b}$ ± 1.99 | 52.48$^{b}$ ± 3.30 |
| | Adventitious roots | 31.64$^{a}$ ± 2.01 | 58.99$^{b}$ ± 2.10 | 65.58$^{c}$ ± 2.20 |
| | | **Average cortical cell size (µm)** | | |
| DP seedling | Adventitious roots | | 34.9$^{a}$ ± 3.3 | 48.5$^{b}$ ±1.5 |

Figure 4.5 Initial and adventitious root morphology of black spruce seedlings under different irrigation regimes. (A) Cross-section of initial and adventitious roots of seedlings irrigated at 25% field capacity, 100% field capacity or flooded. Sections were taken at 20-40 mm from the root tip (Bars = 50 µm; Ed, Endodermis; Cor, Cortex; Xyl, Xylem). (B) Significant measurements of cortex area and average cortical cell size of initial and adventitious roots of seedlings irrigated at 25% field capacity, 100% field capacity or flooded, according their stock types. Values are grand median ± SE. Different letters in the same row indicate significant differences at $P \leq 0.05$.

## 4.5 Discussion

After one growing season, nutrient limitation had more impact on seedling growth than water restriction (Table 4.1, Fig. 4.2B), and nutrient uptake exacerbated water stress (Fig. 4.2B,D; 4.4D) as was found elsewhere (DesRochers *et al* 2006; Hacke *et al* 2010). Black spruce is known to respond well to fertilization in terms of growth and nutrient uptake (Salifu and Timmer 2001; Idris *et al* 2004; Houle and Moore 2008), confirmed here by positive responses in $RGR_{height}$, biomass and nutrient foliar concentrations in fertilized seedlings (Fig. 4.2, 4.5, Annexe C, Fig. S6). Unlike what was observed elsewhere (Mooney *et al* 1978; Brix 1983), fertilization did not increase the net photosynthesis of the different seedling stock types. Some *Pinus* species as well as *Pseudotsuga menziesii* have also been shown to maintain stable photosynthesis rates despite nutrient additions (Brix and Ebell 1969; Teskey *et al* 1994; Warren and Adams 2002; Warren *et al* 2005). Warren et al. (2005) proposed that in these cases, the increase in leaf area and biomass allocation changes are the main responses to fertilization. In our study, fertilized seedlings indeed invested more in aboveground biomass production than those without fertilization (Table 4.2), as reported elsewhere (Cannell 1985; Patterson *et al* 1997). The relatively low impact of irrigation may have been due to the level of water limitation that did not create sufficiently stressful conditions to reduce growth or biomass, especially without fertilization. Indeed, although 25% FC was enough to decrease growth and survival of one-year-old black spruce seedlings (Aubin 1996), here it resulted in average shoot water potentials of -1.43 and -1.17 MPa at the lowest – with or without fertilization, respectively (Fig. 4.4D) – whereas the osmotic potential at turgor-loss point of black spruce is between -1.44 and -2.36 MPa (Fan *et al* 1994; Zine El Abidine *et al* 1995). Nevertheless, based on a water stress criterion defined by a water potential about -1.10 MPa, Patterson et al. (1997) found that one-year-old seedling growth was more affected by irrigation than by fertilization. This may indicate that after a period during which black spruce seedling growth would be highly dependent on water uptake immediately after germination (Patterson *et al* 1997), the trend will be reversed in subsequent years, with a greater need for nutrients. This seems to be in line with observations we have made in the field, in which four-year-old black spruce seedlings had better growth on the richest but driest microsites two years after planting (Pernot *et al* 2019b).

Bareroot (BR) seedlings had better aboveground growth (Fig. 4.2A, Table 4.2) but were more physiologically affected by water restriction compared to containerized (C) and deeply-planted containerized (DP) seedlings (Fig. 4.4B-D). At the beginning of the experiment, BR seedlings were slightly larger in diameter than C and DP seedlings (significantly different at $P \leq 0.06$). Although no biomass measurements were made at this time, we can assume that BR seedlings had a greater biomass (at least aboveground) than the other stock types (Mexal and South 1991; Jinks and Mason 1998), which could have influenced final biomass values. Larger initial diameters are associated with greater height growth, even when assessed over more than one growing season (South *et al* 2005). Indeed, bigger seedlings form buds containing a larger number of needle primordia, which are driving predetermined shoot growth (unaffected by the environmental conditions) during the next growing season (Young and Hanover 1977; Grossnickle 2000). Differences in initial seedling size might thus explain the overall better height growth of BRs relative to other stock types (Grossnickle and El-Kassaby 2016), regardless of resource availability (Fig. 4.2A).

In addition to a generally more developed root system than BRs at the time of planting (Blake and Sutton 1987; Barnett and Brissette 2004; Rose and Haase 2005), containerized seedlings have the advantage of having a root plug, which acts as a source of moisture and nutrients during and just after planting (Mena-Petite *et al* 2001; Idris *et al* 2004; Jutras *et al* 2007). For example, Norway spruce (*Picea abies* L. Karst.) seedlings, with root plugs wet to saturation before planting, had much reduced mortality rates until three to four weeks of drought, compared to dry root plug seedlings (Helenius *et al* 2002; Helenius 2005). In our study, even if submitted to a moderate water stress, BR seedlings were more rapidly impacted by limited irrigation compared to containerized seedlings. We observed a similar trend in response to increased nutrient availability, with containerized seedlings exhibiting higher foliar nutrient concentrations than BRs (although not for N, the most limiting nutrient for the development of black spruce under natural conditions (Fig. 4.5) (Van Cleve *et al* 1990; Lamhamedi and Bernier 1994)). Since the root system of BR seedlings is trimmed when trees are lifted at the nursery before outplanting in the field, initial root/shoot ratios are most likely unbalanced at planting compared to the other stock types (Barnett and Brissette 2004; Renou-Wilson *et al* 2008), which could explain their greater sensitivity to water limitations (DesRochers and Tremblay 2009).

Containerized seedlings had a greater $RGR_{diameter}$ than BR seedlings in the presence of fertilization, independently of the water regime (Fig. 4.2C). High density imposed by containerized seedling production can induced slimmer seedlings due to restricted light assess (Timmis and Tanaka 1976; Simpson 1991), as diameter growth is sensitive to light levels (Brand and Janas 1988; Groot 1999). Once transferred from containers to large pots, C and DP seedlings probably had better light access and invested more in diameter growth, just as trees reaching the canopy submitted to lower light competition are able to invest more in diameter growth than in height under field conditions (Lanner 1985; Wonn and O'Hara 2001). For BRs that are produced in open fields at a generally lower density than containerized seedlings, change of light intensity may have been less critical. However, under limiting nutrient and water conditions, all stock types expressed the same $RGR_{diameter}$ (Fig. 4.2). Diameter growth is more affected by environmental conditions of the current growing season than height growth (Zedaker *et al* 1987; Grossnickle 2000). So, as containerized seedlings are known to perform better than BR seedlings in harsh environments (Idris *et al* 2004; Grossnickle and El-

Kassaby 2016), and in view of the results we obtained using the same stock types planted in boreal microsites (Pernot *et al* 2019b), it appears that our semi-controlled growth conditions were probably only moderately stressful. Limiting irrigation treatment resulted in moderate water stress and, although the nutrient limitation was restrictive, black spruce is a species usually growing in nutrient limited environments (Lamhamedi and Bernier 1994; Lupi *et al* 2013).

The presence of adventitious roots in containerized seedlings has been beneficial to growth, with higher $RGR_{height}$ of DP compared to C seedlings (Fig. 4.2A). This could be explained by slightly improved physiological performances; seedlings with adventitious roots had better net photosynthesis and shoot water potential than the other seedling types under water-limited conditions (Figure 4.4A,B,D). However, this did not translate into greater $RGR_{diameter}$ within the short time frame of our experiment (Figure 4.2C). The higher $RGR_{height}$ of DP seedlings could also be explained by a better morphological adaptation of adventitious roots to their environment compared to the initial roots. First, when submitted to a moderate water stress, both initial and adventitious roots underwent cellular morphological changes (Fig. 4.6). Root cortex death may be one of the root responses to water stress (Jupp and Newman 1987; Spaeth and Cortes 1995; Cuneo *et al* 2016). Although strongly reducing hydraulic root conductance and ion uptake, cortex death associated with suberized endodermis protects the stele from drought and therefore the vascular tissues, thus allowing plants to maintain water transport within the root system (Clarkson *et al* 1968; Jupp and Newman 1987; Enstone *et al* 2002; Cuneo *et al* 2016). Once more favorable water conditions have been restored, bare stele roots are able to resume their elongation and regenerate new lateral roots (Jupp and Newman 1987; Stasovski and Peterson 1991; Spaeth and Cortes 1995). For black spruce seedlings, protection of the stele by suberized endodermis appears to have been enhanced by the maintenance of collapsed cortical cells forming a thick suberin barrier on the endodermis periphery (Fig. 4.6).

This phenomenon has been observed in all adventitious roots and in about two-thirds of initial roots (Annexe C, Fig. S7). This may explain why unfertilized DP seedlings under the low irrigation treatment could maintain similar water potentials than well-irrigated seedlings (Fig. 4.4D). The ability of adventitious roots to change their cell morphology was particularly visible under flooding conditions (Fig. 4.6), with adventitious root formation being a response of flood-tolerant species (Kozlowski 1997; Steffens and Rasmussen 2016). Root morphological adaptations to flooding occur mainly in the cortex area, usually as aerenchyma (McDonald *et al* 2002; Takahashi *et al* 2014), but also in the form of larger cortical cells generally associated with larger intercellular spaces, allowing roots to have better porosity (Angeles *et al* 1986; Pezeshki 1991). These adaptations usually occur within a few days or weeks but may require long-term stressful conditions to affect physiology and seedling growth (Zhang *et al* 2017). Flooding conditions create an oxygen gradient in the soil that promotes root growth close to the soil surface where oxygen availability is highest (Boggie 1977; Kozlowski 1997). In our study, only adventitious roots were able to adapt quickly to flooding, and this would not only be conferred by an advantageous location close to the soil surface, since initial roots of C seedlings also located near soil surface were not able to adapt in the short term.

In conclusion, the presence of adventitious roots was beneficial for containerized black spruce seedlings, resulting in improved physiological performances and greater height growth in DP seedlings relative to C seedlings. Our results show that the physiological advantage provided to seedlings by adventitious roots occurred mainly under limited water conditions and could be related to the greater cellular plasticity of adventitious *vs* initial roots. This better cell plasticity was particularly observed in flooding condition. Thus, seedlings with adventitious roots could perform better in planting sites subjected to drought or flooding events, compared to seedlings with initial roots only. On the other hand, BR seedlings had better relative growth rate in height than containerized seedlings, probably due to bigger initial size, giving them a short-term advantage in sites with moderately stressful conditions.

## 4.6 Material and Methods

Three stock types of 4-year-old black spruce seedlings were used for the experiments: (C) Containerized seedlings produced in 110 cm$^3$ cavities, planted at the root collar level (initial root system restricted in a root plug); (DP) deeply-planted containerized seedlings, produced in 110 cm$^3$ cavities with the root collar buried 5 cm below-ground at 1-year-old (initial and adventitious root systems restricted in a root plug); and (BR) bareroot seedlings, produced in outdoor growing beds (unrestricted initial root system) (Fig. 4.1). C and DP seedlings originated from local seed sources 49°49'N, 74°45'W and BRs from 48°12'N, 71°29'W. One year before the experiments started, seedlings of each stock type were transferred in 10 dm$^3$ containers for Experiment 1 and 4 dm$^3$ containers for Experiment 2. Planting cavities were filled with a peat, perlite, coconut husk fibre substrate (Fafard, AGRO MIX® PV20). For both experiments, seedlings were grown under semi controlled greenhouse environment. Natural light was supplemented with 400 W high pressure sodium S51 lamps (Kavita Canada Inc., Niagara-on-the-Lake, ON, Canada) that provided a photosynthetic flux density of about 400 μmol photon m$^{-2}$ s$^{-1}$ at leaf level; light/dark photoperiod was set to 16 h/8 h, associated with temperatures of 25°C/17 °C, respectively; natural humidity levels were maintained.

### 4.6.1 Experiment 1

In February 2013 and for 14 weeks, seedlings were subjected to a factorial irrigation × fertilization combination of treatments – 100% or 25% FC and with (a weekly addition

0.2 g of NPK soluble mineral fertilizer [20-20-20]) or without fertilization. The experiment was realized as a randomized factorial design with six replicate blocks, each block being composed of the twelve combinations of stock type (BR, C, or DP) × irrigation (100% or 25% FC) × fertilization (with or without fertilizer) (Fig. 4.1).

To avoid confounding the fertilizer treatment with the irrigation treatment, volumes and concentrations of fertilizer were adjusted to 200 ml of 1 g $l^{-1}$ of NPK fertilizer for 100% FC treatment or 100 ml of 2 g $l^{-1}$ of NPK fertilizer for 25% FC treatment. Soil water content was monitored daily with a moisture probe (TDR 100; FieldScout, Portland, OR, USA) and tap water was added as needed.

Physiological measurements were taken at the end of the 14-weeks experiment. Net photosynthesis and stomatal conductance were measured between 10:00 and 15:00 using a LI-6400 portable infra-red gas analyzer (LI-COR, Lincoln, NE, USA) equipped with a conifer cuvette ($CO_2$ concentration of 400 μmol $mol^{-1}$; leaf temperature of 24 ± 1 °C; 1 000 μmol photons $m^{-2}$ $s^{-1}$ with an halogen lamp placed above the cuvette) using 1-year-old twigs from the seedlings' upper-half. Leaf surface area of the measured twigs was determined using a LI-3100 Leaf Area Meter (LI-COR, Lincoln, NE, USA) and gas exchange values were calculated accordingly. Shoot water potential was measured at the end of the experiment between 7:00 and 10:00 using a Scholander pressure chamber (Model 1000; PMS Instruments, Albany, OR, USA) on 3 shoots per seedling, located in the upper-half section of the stem.

Seedling height (cm) and basal diameter (mm) were measured at the beginning and end of the experiment. Initial height and diameter were respectively (mean ± SD): 33.5 ± 4.5 cm and 7.1 ± 0.8 mm for C seedlings; 34.1 ± 5.1 cm and 6.9 ± 1 mm for DP seedlings; and 31.4 ± 4 cm and 7.8 ± 1.2 mm for BR seedlings. Relative growth rates (RGR) in height and basal diameter were estimated using the difference of the natural logarithmic of final and initial measurement of the growing season. After the 14 weeks, seedlings were divided into subparts to determine dry biomass–stems, needles, adventitious roots, and initial roots were oven dried for 48 h at 60 ± 5 °C. Dried needles were ground using a ball mill (Pulverisette 0, Fritsch, Idar-Oberstein, Germany) in order to perform nutrient analyses. Samples were analyzed with a TruMac N (LECO Corp., St Joseph, MI, USA) for N concentrations or with a plasma atomic emission spectrometer (Thermo Jarrel-Ash - ICAP $61^E$, Thermo Fisher Scientific, Waltham, MA, USA) for P, K, Ca concentrations.

### 4.6.2 Experiment 2

In March 2013, C and DP seedlings were subjected to three types of irrigation: (1) Maintenance of 25% water field capacity, (2) 100% water field capacity, or (3) flooding–where containers were placed in bigger waterproof containers and covered with water. Three replicates of irrigation × stock type (C or DP) treatments were done (Fig. 4.1).

Roots were examined after 14 weeks of growth under these conditions. Sampling was realised on C seedlings that only had initial roots and on DP seedlings that had initial and adventitious roots. Roots (5 cm long from the tip, outside the root plug) of each seedling were cut with a razor blade and rapidly placed on ice in PFA (paraformaldehyde 4% (*w*/*v*) in phosphate buffered saline (PBS, pH 7)). To ensure good fixation, several cycles of vacuum infiltration were achieved in a vacuum chamber before being incubated overnight at 4°C. For tissue fixation and embedding, the protocol was adapted from Brewer *et al* (2006) with progressive gradients 20-40-60-80-95-100% of ethanol in PBS (each step during 1 h at room temperature, the 95% step during overnight), 25-50-75-100% of xylene in ethanol at room temperature (each step during 1 to 2 h) then 50-100% of xylene in paraplast at 56 °C (50% for 4 h, 100% overnight).

Serial 7 µm sections were done with a Leica SM2400 sliding microtome (Leica Instruments GmbH, Hubloch, Germany). Sections were dewaxed using two 15-min baths of xylene and rehydrated by gradient 5-min baths of ethanol to water (100-100-95-70-50-0-0%). Double staining was done to increase cell contrast–10 min of 1% aqueous safranin (red staining of lignified tissues) followed by 20 min of 1% aqueous alcian blue (bleu staining of cellulosic tissues). Sections were observed with a ZEISS Axioscope microscope (Carl Zeiss, Don Mills, Ontario, Canada). Measurements and analyses were performed with Fiji software (Schindelin *et al* 2012). All sections were measured to define root surface distribution (cortex area, $n \geq 18$) and cortical cell sizes on a minimum of 6 sections of different roots per treatment.

### 4.6.3 Statistical analyses

Analyses were performed in R v.3.2.3 environment (R Core team 2015). The influence of stock type, irrigation and fertilization on seedling growth and physiology was analyzed through a linear mixed effects model, using the function *lme* of the *nlme* library (Pinheiro *et al* 2015). Response variables were RGR in height and diameter, dry biomass, net photosynsthesis, stomatal conductance, shoot water potential, and foliar nutrient contents. The tested fixed effects were stock types, irrigation and fertilisation treatments and tehir interactions. Blocks were added to the model as a random effect. Data transformations were used when necessary to respect normality and homoscedasticity assumptions. Relative growth rate in height, initial and total root biomass, stomatal conductance and foliar nutrient concentrations were *log*-transformed, while adventitious root biomass was $log(x+1)$-transformed due to the occurrence of many zeros. When fixed effects or their interactions were significant, Tukey's HSD post-hoc tests were performed using *lsmeans* function of the *lsmeans* package (Lenth 2016). When we detected a significant interaction between treatments for a given variable, we focused result presentation and our interpretations on the combination of treatments rather than on the individual effects of the treatments involved in the interaction. Influence of irrigation treatment was tested on cortex area and average cortical cell size of each stock and root type seperatly, using the *aov* function followed by a Tukey's HSD post-hoc tests if treatment was significant. $P \leq 0.05$ was used as significance level for all response variables.

## 4.7 Funding

This research was funded by a doctoral scholarship, '*Bourse en Milieu Pratique Innovation*', provided by the *Fonds de Recherche Nature et Technologie du Québec* (FRQNT), Natural Sciences and Engineering Research Council of Canada (NSERC), and NSERC-UQAT-UQAM industrial Chair in Sustainable Forest Management, in collaboration with Scierie Landrienne.

## 4.8 Acknowledgements

We thank the *University du Québec à Chicoutimi* and the provincial nursery of Sainte Luce (Quebec, Canada) for providing seedlings and their technical assistance; the *Laboratoire de chimie organique and inorganique* of the *Ministère des Forêts, de la Faune et des Parcs du Québec* for nutrient analyses; the Microscopy-Histology Service of Université Laval for their help and advice in histology manipulations; Dr. Alexis Achim for access to equipment; and Sophie Thériault and Lyne Blackburn for technical assistance.

# CHAPITRE V

# CONCLUSION

La formation de racines adventives chez l'épinette noire a depuis longtemps été observée (Bannan 1942; LeBarron 1945). On sait depuis plusieurs années que les racines adventives se développent à tel point qu'elles deviennent la majeure partie du système racinaire de l'épinette noire mature (DesRochers et Gagnon 1997; Krause et Morin 2005). Toutefois, très peu d'études ont été menées concernant les mécanismes sous-jacents pouvant expliquer la prépondérance des racines adventives au détriment des racines initiales. Ainsi, cette thèse avait pour objectif de déterminer le rôle des racines adventives lors de la croissance d'épinettes noires au stade de semis, en particulier en évaluant leur capacité à capter eau et nutriments par rapport aux racines initiales, et en testant leur capacité d'adaptation en conditions limitantes (en eau ou en oxygène).

## 5.1 Contributions des racines adventives

Lors de chacun des trois volets de cette thèse, les différentes conditions testées (*i.e.* irrigation, fertilisation, compétition) ont influencé la croissance et la physiologie des semis d'épinette noire, comme reporté par d'autres études (Aubin 1996; Patterson *et al* 1997; Idris *et al* 2004; Thiffault *et al* 2012), avec entre autres un impact significatif de la disponibilité en nutriments. Ces différents environnements, plus ou moins limitants, ont donc permis de mettre en lumière l'impact à court terme de la présence de racines adventives sur des semis âgés de 4 ans (Fig. 5.1):

- La séparation des racines adventives et initiales grâce au système de double-pot a permis de mieux comprendre l'allocation en biomasse souterraine en fonction des ressources disponibles (Chapitre II). Peu de différences ont été observées entre les semis ayant reçu un même traitement uniquement au niveau des racines adventives ou uniquement au niveau des racines initiales; sans être significatif, quelques paramètres tendaient à être légèrement supérieurs chez les semis traités au niveau des racines adventives, comme le taux de photosynthèse, la conductance stomatique ou le contenu foliaire en azote et calcium. Finalement, la différence majeure a donc été observée au niveau de la biomasse racinaire : lorsqu'il a y eu apport en ressources, les semis ont fortement favorisé le développement des racines adventives par rapport aux initiales; seul le cas d'une irrigation exclusive aux racines initiales a résulté en une production équivalente de racines initiales et adventives, comme en conditions limitantes pour les deux systèmes racinaires.

- En plantation en forêt boréale, la présence de racines adventives a eu un impact positif sur la captation de nutriments en période d'établissement des semis (Chapitre III). En effet, aucune différence de croissance n'a été observée entre les semis en récipients avec ou sans racines adventives deux ans après plantation, mais les semis ayant des racines adventives ont capté davantage de phosphore et de potassium que les semis n'ayant que des racines initiales, et également une plus grande quantité de l'azote marqué ajouté au début de la deuxième saison sur sites.

- Enfin en environnement contrôlé, les semis avec racines initiales et adventives ont eu une croissance plus importante, associée à un avantage physiologique et une plus grande plasticité en conditions limitantes, que les semis n'ayant que des racines initiales (Chapitre IV). En effet, indépendamment des apports en eau ou en nutriments, les jeunes épinettes noires de 4 ans ayant des racines adventives ont eu une croissance en hauteur supérieure, corrélée à un taux de photosynthèse supérieur en présence de nutriments et en condition limitante en eau, ainsi qu'au maintien d'un potentiel hydrique équivalent à celui des semis bien irrigués en conditions limitantes en eau et en nutriments, contrairement aux semis en récipient n'ayant que des racines initiales. Ce dernier volet a également permis de détecter une plus grande plasticité des racines adventives au niveau cellulaire, capables développer de plus grandes cellules corticales en condition anaérobie, contrairement aux racines initiales.

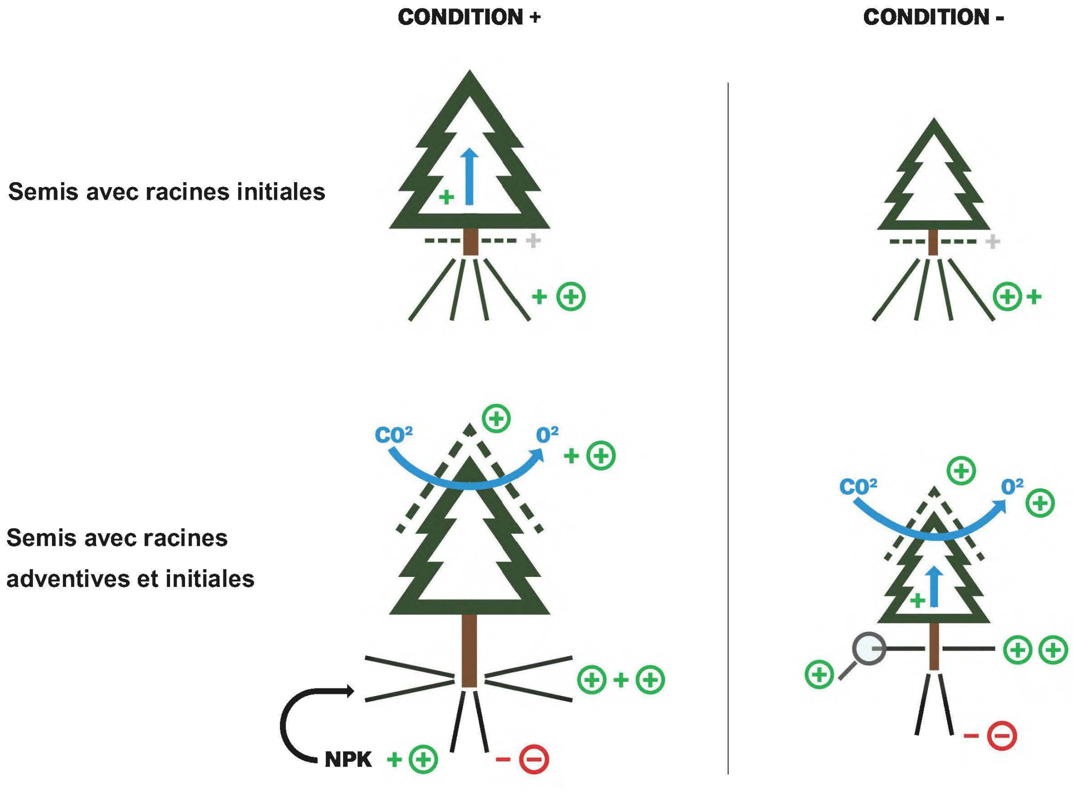


Figure 5.1 Influence du système racinaire sur la croissance et la physiologie des semis d'épinettes noires. Sont représentés les comparatifs des principaux paramètres mesurés lors de cette étude (un signe par volet effectué) entre les semis avec ou sans racines adventives en condition riche (condition +) ou limitante (condition –) en eau ou en nutriments.

\+ , tend à être supérieur; ⊕, significativement supérieur
\- , tend à être inférieur; ⊖ , significativement inférieur

Cette étude permet ainsi un début de réponse aux grandes questions soulevées par cette recherche qui sont : comment et pourquoi les épinettes noires développent des racines adventives?

Le « comment » trouve des réponses dans le Chapitre II. Tout d'abord, toutes les épinettes dont la tige a été enfouie de quelques centimètres dans le sol durant ces expériences ont développé systématiquement des racines adventives, appuyant le fait qu'il s'agit en partie d'une réponse constitutive (Viereck et Johnston 1990; Aubin 1996). Toutefois, ici nous avons pu observer que si les semis avaient un accès important en ressources ils favorisaient largement la production de racines adventives. Le fort développement de racines adventives de l'épinette noire en conditions naturelles (DesRochers et Gagnon 1997; Krause et Morin 2005) pourrait ainsi être expliqué par la couche d'humus présente en surface du sol en forêt boréale, qui par son apport en eau et nutriments (Prescott *et al* 2000) et sa localisation créeraient des conditions favorables à la croissance du système racinaire adventif.

Le « pourquoi » se détecte dans les bénéfices en termes de physiologie et de nutrition. En conditions modérément stressantes (25% de capacité de rétention en eau du sol), les racines adventives semblent avoir une meilleure capacité à capter l'eau permettant aux semis d'avoir une conductance stomatique et un taux de photosynthèse ayant tendance à être plus importants par rapport aux semis irrigués uniquement au niveau des racines initiales (Chapitre II), en particulier en cas de réduction de la disponibilité de l'eau (Chapitre IV). En cas de stress hydrique modéré, cette meilleure capacité à capter l'eau pourrait être dû à la mort systématique des cellules corticales de l'extrémité des racines adventives observée au Chapitre IV. Cueno *et al* (2016) suggèrent que les cellules corticales joueraient un rôle de fusibles hydrauliques, permettant lors des premiers stades d'une sècheresse de préserver l'intégrité du système vasculaire de la plante. Lorsque la disponibilité en eau n'est pas limitante, cela pourrait également être relié à une meilleure captation et un meilleur transport de l'eau par les racines adventives, grâce à certaines aquaporines dont la plus forte expression au sein des racines adventives par rapport aux racines initiales coïncident avec une plus forte conductivité hydraulique des cellules corticales chez l'orge (Knipfer *et al* 2011). Cet avantage serait toutefois limité, puisque lorsque les conditions ont été plus difficiles, comme en plantation (Chapitre III), et qu'il y a eu ajout de fertilisation (qui peut exacerber le stress hydrique (DesRochers *et al* 2006; Hacke *et al* 2010)), les semis en récipients n'ayant que des racines initiales ont eu tendance à être légèrement moins stressés au niveau hydrique que les semis avec racines adventives.

Aperçu au Chapitre II et appuyé au Chapitre III, les semis avec racines adventives ont également été capables de capter davantage de nutriments (azote, phosphore et potassium) que les semis n'ayant que des racines initiales. Dans les deux cas, cette augmentation du contenu en nutriments n'a pas impacté la croissance des semis. Cela pourrait être dû à une stratégie d'adaptation des espèces en milieux pauvres qui consisterait, en cas d'apport important en nutriments, à garder une faible croissance afin de maintenir une concentration interne en azote importante, ce qui se traduit par une croissance stable et une propension à stocker le surplus de nutriments (Clarkson et Hanson 1980; Gezelius et Näsholm 1993; Patterson *et al* 1997; Lupi *et al* 2013). Ceci semble être renforcé lors du Chapitre III par de plus fortes concentrations en azote marqué mesurées au niveau des parties aériennes des semis, qui correspondent aux organes consacrés au stockage chez l'épinette noire (Malik et Timmer 1995; Malik et Timmer 1998). L'accumulation de réserves d'azote peut avoir un impact important sur la future croissance des semis, en permettant par exemple l'année suivante un gain en hauteur et en biomasse supérieur aux semis ayant de plus faibles réserves, ainsi qu'une meilleure captation de nutriments (Malik et Timmer 1998; Wang *et al* 2016). Cette étude se focalisant sur l'impact des caractéristiques racinaires à court terme, il se peut donc que le temps d'observation n'ait pas été suffisamment long pour pouvoir remarquer des bénéfices au niveau de la croissance. Inversement, lors du Chapitre IV, les semis avec racines adventives n'ont pas présenté de concentrations en nutriments plus fortes mais c'est également le seul cas où ces semis ont eu une croissance en hauteur significativement plus importante que les semis n'ayant que des racines initiales. Il se peut donc que les conditions de cette expérience étant moins stressantes que pour les deux premiers chapitres (Chapitre II, lors des comparaisons entre un même traitement sur racines adventives ou initiales, l'autre système racinaire est en conditions limitantes, et Chapitre III, expérience sur le terrain *vs* Chapitre IV en serres et traitement homogène sur l'ensemble des racines), les semis aient investi le surplus de nutriments en croissance plutôt qu'en stockage. Ces résultats vont dans le sens de l'étude préliminaire de Gagnon (2002) qui avait également observé des concentrations en nutriments équivalentes entre semis enfouis ou non, mais avec une plus forte croissance en hauteur des premiers, trois ans après plantation. Quelle que soit la stratégie, l'azote est l'élément le plus limitant la croissance de l'épinette noire en forêt boréale (Lamhamedi et Bernier 1994), ainsi cette plus forte acquisition d'azote par les racines adventives par rapport aux racines initiales seules pourrait être un avantage à long terme pour les semis en plantation. Ceci pourrait expliquer la meilleure croissance en hauteur des épinettes noires enfouies, corrélée à une plus forte production de racines adventives, par rapport à celle des semis mis en terre au niveau du collet, observé 15 ans après plantation par Tarroux *et al* (2014).

## 5.2 Implications pour la production de semis et perspectives

Actuellement la production de semis résineux est encadrée par de nombreuses normes afin d'assurer la meilleure qualité possible des plants mis en terre. Un tiers des critères de triage des semis concerne les racines (avec un tiers pour les parties aériennes et un tiers pour les maladies, sécheresse *etc.*) (Veilleux *et al* 2014). L'insuffisance racinaire est en particulier un problème qui revient dans plusieurs critères. Il pourrait ainsi être envisagé d'enfouir de quelques centimètres la tige des semis durant la production, ce qui aurait pour effet de créer rapidement de nouvelles racines adventives à la base de la tige et permettrait de coloniser uniformément la carotte de substrat. Bien que reporté comme néfaste au moment de la plantation s'il est fait de façon excessive, pour les petits semis en cas de forte pluviométrie ou encore sur sites mal drainés (Mullin 1964; Stroempl 1990; Sutton 1995), l'enfouissement d'une partie de la tige durant la production permettrait de contrôler plus facilement la portion de tige enfouie qu'au moment de la plantation, et le contrôle des apports en eau et nutriments permettrait de favoriser la production de racines adventives tout en conservant une bonne croissance aérienne. De plus, l'enfouissement de la tige pourrait permettre d'enfouir les possibles sinuosités présentes à la base de la tige, ou même de les limiter si celles-ci proviennent d'une stratégie visant à mettre la base de la tige en contact avec le sol pour promouvoir le développement de racines adventives. Plusieurs auteurs ont déjà recommandé l'enfouissement d'une partie de la tige car cela permettrait entre autres une meilleure stabilité des semis et une protection des racines et de la jonction tige/racine contenant des bourgeons dormants capables de produire de nouvelles pousses ou des racines adventives (Stroempl 1990; Sutton 1995). En associant ces critères avec les bénéfices liés aux capacités des racines adventives citées plus haut, la production de semis d'épinettes noires enfouies devrait être envisagée, en particulier pour les sites plus difficiles, par exemple où la disponibilité en eau et/ou en nutriments est faible.

Au cours de cette étude, nous avons également pu observer des différences entre les deux principaux types de productions de semis, en récipients ou à racines nues (Chapitres III et IV). Bien que majoritairement constitué de racines initiales, le système racinaire des épinettes à racines nues comprenait aléatoirement la présence de racines adventives dès la réception des plants, avant le début des expériences. En effet, les semis à racines nues se développent pendant au moins la dernière année de production en pleine terre; bien qu'il soit recommandé de les mettre en terre au niveau du collet, quelques centimètres de tige enfouis au moment de la mise en terre ou l'enfoncement naturel des semis après plantation peuvent suffire à produire des racines adventives. Il serait intéressant pour la suite de comparer les semis à racines nues avec un système strictement composé de racines initiales et d'autres dont la tige serait enfouie de 5 centimètres, pour initier la croissance de racines adventives, comme pour les semis en récipients. De plus, comme reporté par d'autres études (Grossnickle et El-Kassaby 2016), nous avons observé un avantage des plants à racines nues au niveau de la croissance en hauteur en milieu peu à modérément stressant dû à leur plus forte taille (Chapitre IV) mais une plus grande sensibilité au stress hydrique, en particulier en conditions plus difficiles, en forêt boréale (Chapitre III). Les racines adventives étant avantageuses pour la physiologie des semis en cas de stress hydrique modéré pour les semis en récipients, il pourrait être intéressant de tester l'influence des racines adventives, qui pourrait être facilement initiées au moment de transfert en pleine terre, chez les plants à racines nues.

Une autre perspective intéressante serait d'investiguer le rôle des racines adventives dans la résistance des semis à la submersion. Lors du Chapitre IV, nous avons évalué la plasticité des racines adventives et initiales lors de l'inondation des semis alors que celles-ci étaient déjà formées et sans s'attacher à leur impact sur la physiologie et la croissance des semis. Islam et Macdonald (2004) ont comparé les réponses de semis d'épinettes noires et de mélèze laricin à l'inondation, et observé une meilleure adaptation du mélèze via la formation de racines adventives, ce dont n'ont pas été capable les épinettes. Toutefois lors de cette expérience, les semis avaient été mis en terre au niveau du collet, tout comme le niveau d'eau lors du traitement d'inondation. L'épinette noire et le mélèze laricin étant fréquemment observés en tourbière (Lieffers et Macdonald 1990), il se peut que la mise en terre au niveau du collet ait été plus restrictive pour les semis d'épinette que de mélèze et que naturellement les semis s'enfoncent légèrement dans le sol détrempé. Les racines adventives étant reliées à une meilleure absorption de l'eau et de nutriments lors de l'inondation que les racines initiales (Kozlowski et Pallardy 1997), leur présence pourrait être bénéfiques aux semis d'épinettes noires destinés à la plantation sur sites mal drainés, sujets à la paludification.

Cette thèse a ainsi permis de poser les premières bases vers la compréhension du rôle des racines adventives chez l'épinette noire. Jusqu'à présent seules quelques études préliminaires avaient été effectuées sur le sujet, et plus globalement les études comparant les capacités de racines adventives et initiales sont peu nombreuses. Ici, nous avons pu établir que la présence de racines adventives a influencé positivement la physiologie et la croissance de l'épinette noire au stade juvénile, en ayant entre autres une tendance à mieux capter l'eau et les nutriments par rapport aux racines initiales. Cela a permis d'éclaircir de futures pistes de recherche, en particulier en mettant en avant le besoin de suivi à plus long terme, pouvant par exemple expliquer les variations de réponses à l'enfouissement en fonction du temps et du site de plantation (Gagnon 2002; Paquette *et al* 2011; Tarroux *et al* 2014). Avec plusieurs dizaines de millions de semis plantés chaque année, l'épinette noire a une place de choix dans l'industrie forestière au Québec et la qualité de semis est essentielle, en particulier au moment de la mise en terre. L'initiation de racines adventives pourrait facilement être réalisée durant la production en enfouissant la base de la tige des semis lors d'un transfert de récipients (ou lors de la plantation en pleine terre pour les semis à racines nues), et leur présence pourrait être avantageuse pour les semis destinés à une plantation sur sites limitants en eau ou en nutriments.

# ANNEXE A

## CHAPITRE II SUPPLEMENTARY DATA

Figure S1: Mean diameter increment of black spruce seedlings over 14 weeks during which adventitious roots (AR) and/or initial roots (IR) were fertilized (Fertilization +) or not (Fertilization -) and irrigated (Irrigation +, 100% water container capacity) or not (Irrigation -, 25% water container capacity). Error bars are standard errors of the mean (SEMs). Unique letters indicate significant difference at $p \leq 0.05$.

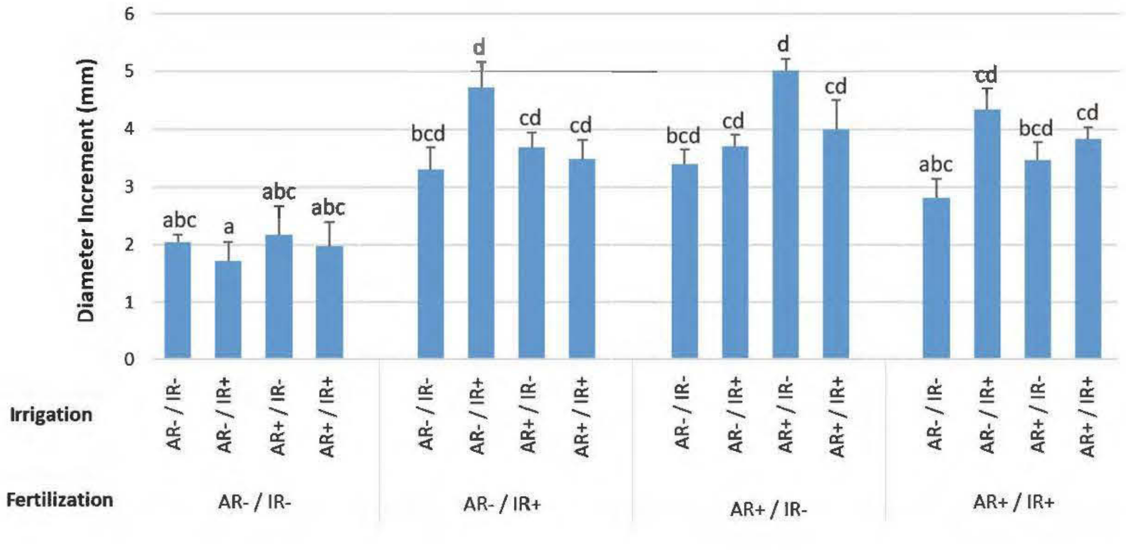
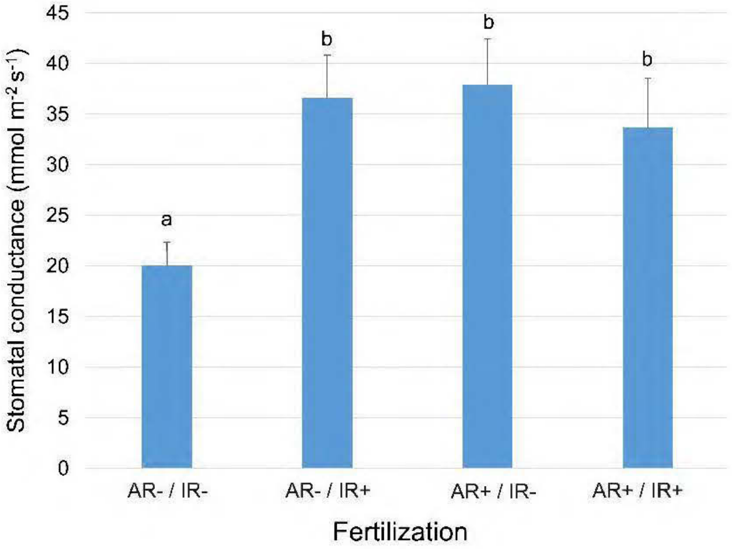


Figure S2: Black spruce seedling stomatal conductance (mmol $m^{-2}$ $s^{-1}$) with adventitious roots (AR) and/or initial roots (IR) being fertilized (+) or not (-). Error bars refer to standard errors of the mean (SEMs). Unique letters indicate significant difference at $p \leq 0.05$.



# ANNEXE B

## CHAPITRE III SUPPLEMENTARY DATA

Figure S3: Mean total root dry biomass of unfertilized seedlings for the three microsites. For a given variable, different letters indicate significant difference at $P \leq 0.05$.

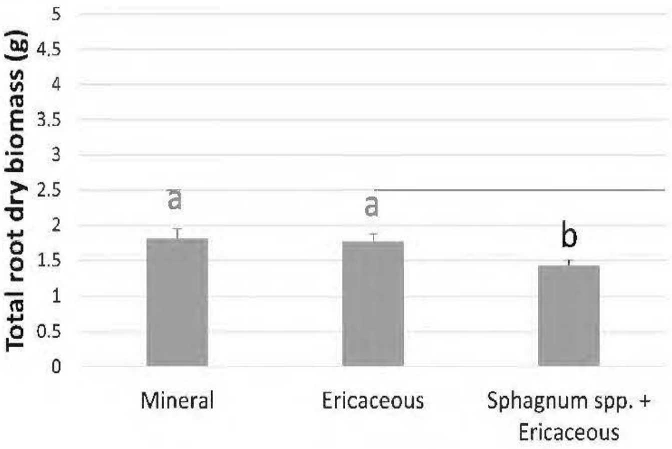


Figure S4: Unfertilized seedlings mean foliar concentration of (a) N, (b) P and (c) K by microsite and of (d) Ca by stock type. For a given variable, different letters indicate significant difference at $P \leq 0.05$.

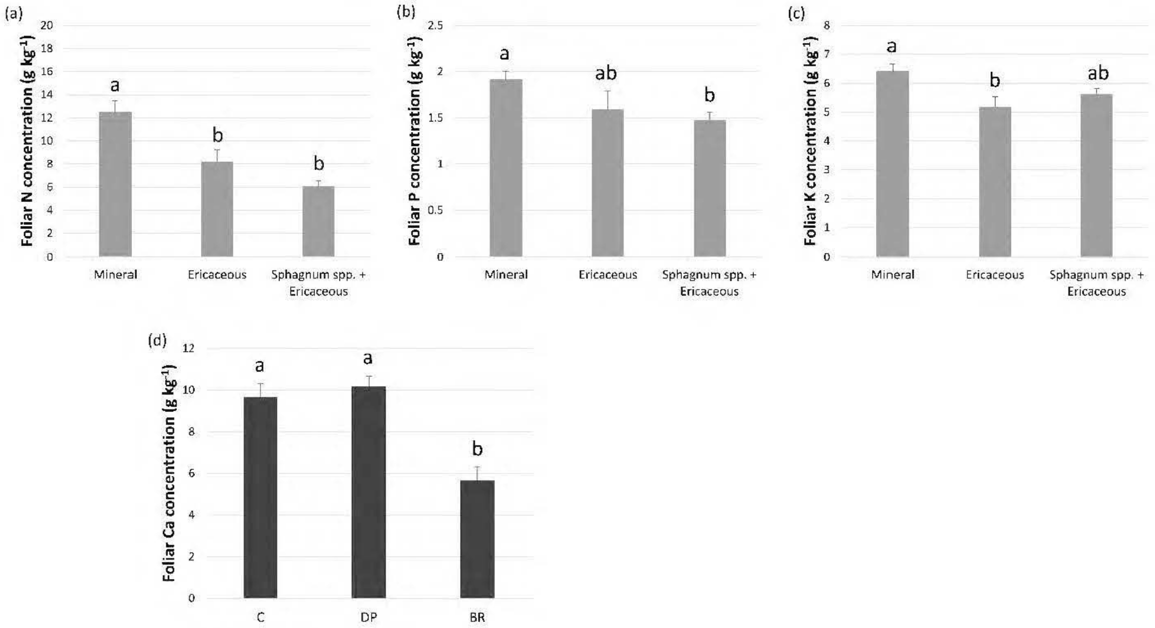
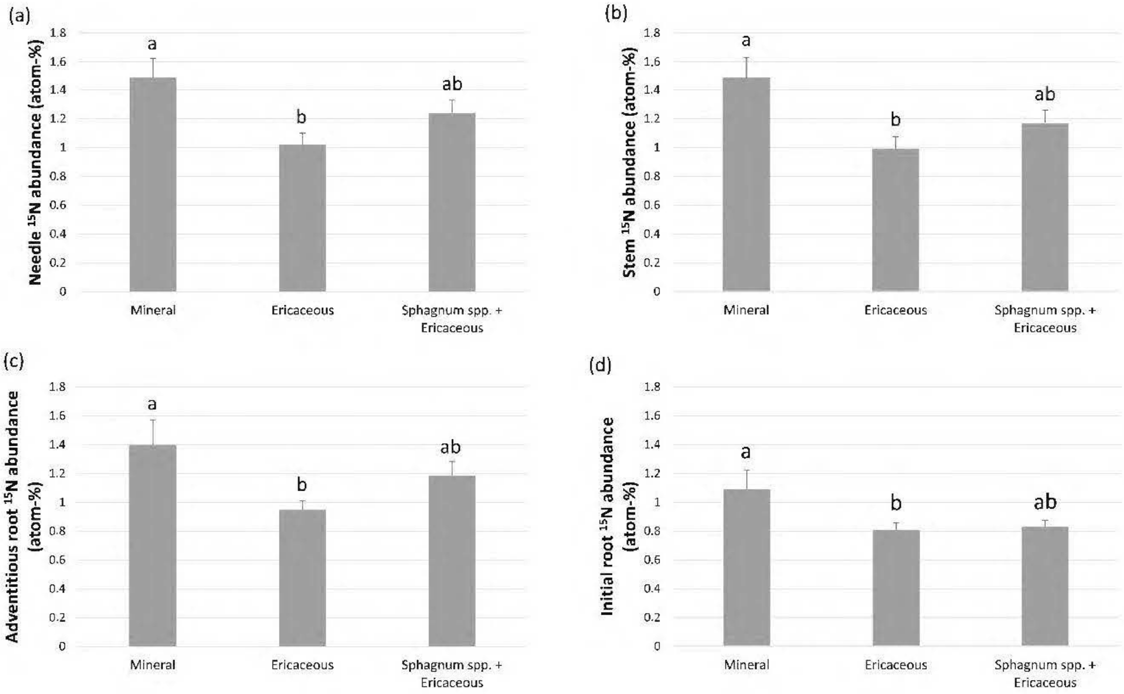


Figure S5: Effect of microsite on atom-% $^{15}$N abundance in (a) needles, (b) stems, (c) adventitious and (d) initial roots of black spruce fertilized seedlings. For a given variable, different letters indicate significant difference at $P \leq 0.05$, except for initial root $^{15}$N abundance where $P \leq 0.1$.



# ANNEXE C

## CHAPITRE IV SUPPLEMENTARY DATA

Figure S6: Mean foliar nutrient concentration of black spruce seedlings: (A) P, phosphorus, (B) K, potassium, (C) Ca, calcium according irrigation (25% or 100% field capacity) x fertilization (F- without, F+ with fertilization). Bars with the same letter indicate a non-significant difference at $P \leq 0.05$.

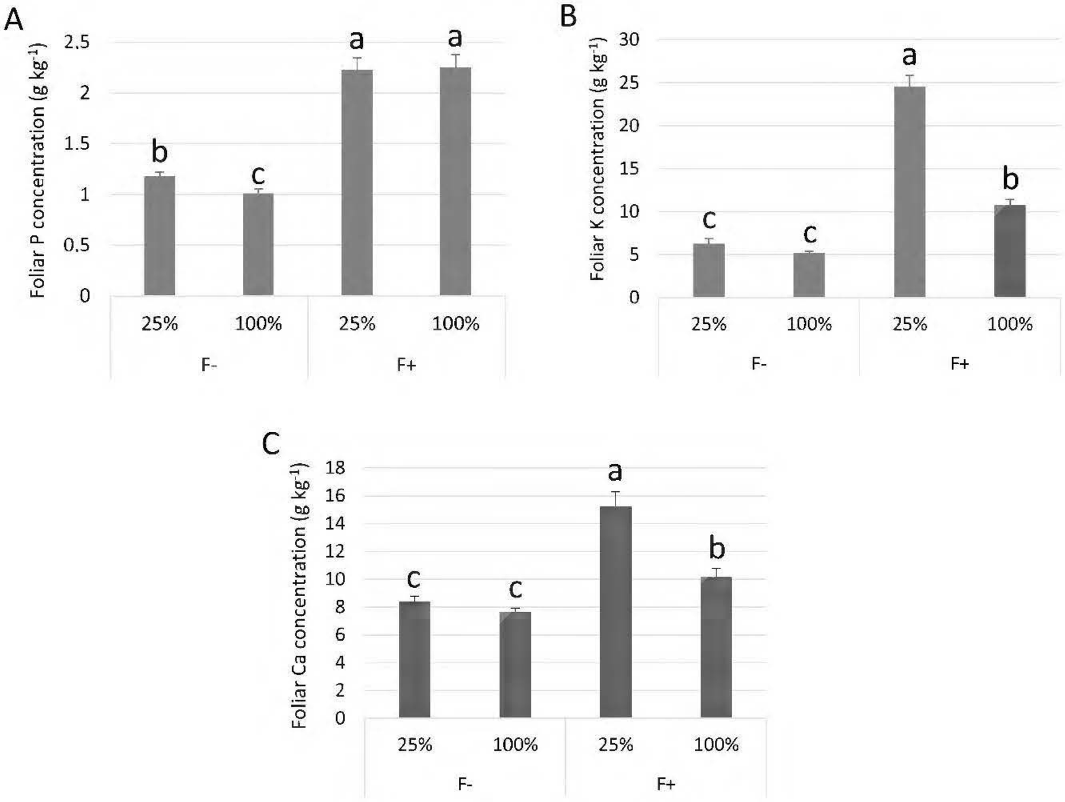


Figure S7: Number of initial and adventitious roots with cortical cells collapsed or not for seedlings irrigated at 25% field capacity. C: containerized seedlings (with a restricted initial root system) and DP: deeply-planted containerized seedlings (with initial and adventitious roots restricted in a root plug).

| | | **Number of roots with collapsed cortical cells** | **Number of roots with non-collapsed cortical cells** |
|---|---|---|---|
| C seedling | Initial roots | 12 | 6 |
| DP seedling | Initial roots | 13 | 5 |
| | Adventitious roots | 18 | 0 |

# BIBLIOGRAPHIE

Alm, A. A. (1983). Black and white spruce plantings in Minnesota: container *vs* bareroot stock and fall *vs* spring planting. *The Forestry Chronicle* 59: 189-191.

Andivia, E., Fernández, M. et Vázquez-Piqué, J. (2014). Assessing the effect of late-season fertilization on Holm oak plant quality: insights from morpho–nutritional characterizations and water relations parameters. *New forests* 45(2): 149-163.

Angeles, G., Evert, R. et Kozlowski, T. (1986). Development of lenticels and adventitious roots in flooded *Ulmus americana* seedlings. *Canadian Journal of Forest Research* 16(3): 585-590.

Argus, R., Colmer, T. et Grierson, P. (2015). Early physiological flood tolerance is followed by slow post-flooding root recovery in the dryland riparian tree *Eucalyptus camaldulensis subsp. refulgens*. *Plant, Cell and Environnement* 38(6): 1189-1199.

Aubin, N. K. (1996). Influence du contenu en eau du substrat et de la profondeur de plantation sur la formation de racines adventives caulinaires, la croissance et l'allocation glucidique de semis d'épinette noire (*Picea mariana* (Mill.) BSP). Mémoire de Maîtrise. Université du Québec à Chicoutimi, Chicoutimi, QC, Canada, ISBN 141230668X.

Baldwin, V. et Barney, C. (1976). Leaf water potential in planted ponderosa and lodgepole pines. *Forest Science* 22(3): 344-350.

Bannan, M. (1942). Notes on the origin of adventitious roots in the native Ontario conifers. *American Journal of Botany* 29(8): 593-598.

Barnett, J. P. et Brissette, J. C. (2004). Stock type affects performance of shortleaf pine planted in the Ouachita mountains through 10 years. Dans *Proceedings of the 12th biennial southern silvicultural research conference.* USDA Forest service, southern research station, Asheville, NC. General technical report SRS-71. p. 420-422.

Béland, M. et Lapierre, J. (1992). Influence du gauchissement de semis d'épinette noire (*Picea mariana* (Mill.) BSP) produits en serre sur leur performance en plantation. *The Forestry Chronicle* 68: 365-370.

Bellini, C., Pacurar, D. I. et Perrone, I. (2014). Adventitious roots and lateral roots: similarities and differences. *Annual Review of Plant Biology* 65: 639-666.

Benomar, L., Lamhamedi, M. S., Rainville, A., Beaulieu, J., Bousquet, J. et Margolis, H. A. (2016). Genetic adaptation *vs* ecophysiological plasticity of photosynthetic-related traits in young *Picea glauca* trees along a regional climatic gradient. *Frontiers in Plant Science* 7: 48.

Blake, T. J. et Sutton, R. F. (1987). Variation in water relations of black spruce stock types planted in Ontario. *Tree Physiology* 3: 331-344.

Bloom, R. G. et Mallik, A. U. (2004). Indirect effects of black spruce (*Picea mariana*) cover on community structure and function in sheep laurel (*Kalmia angustifolia*) dominated heath of eastern Canada. *Plant and Soil* 265: 279-293.

Bogeat-Triboulot, M.-B., Brosché, M., Renaut, J., Jouve, L., Le Thiec, D., Fayyaz, P., ... et Teichmann, T. (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(2): 876-892.

Boggie, R. (1977). Water-table depth and oxygen content of deep peat in relation to root growth of *Pinus contorta*. *Plant and Soil* 48(2): 447-454.

Brand, D. G. et Janas, P. S. (1988). Growth and acclimation of planted white pine and white spruce seedlings in response to environmental conditions. *Canadian Journal of Forest Research* 18: 320-329.

Brewer, P. B., Heisler, M. G., Hejátko, J., Friml et J., Benková, E. (2006). *In situ* hybridization for mRNA detection in *Arabidopsis* tissue sections. *Nature Protocols* 1(3): 1462.

Brix, H. (1983). Effects of thinning and nitrogen fertilization on growth of Douglas-fir: relative contribution of foliage quantity and efficiency. *Canadian Journal of Forest Research* 13(1): 167-175.

Brix, H., Ebell, L.F. (1969). Effects of nitrogen fertilization on growth, leaf area and photosynthesis rate in Douglas-fir. *Forest Science* 15: 189-196.

Brouillette, J., Tétreault, J. et Lortie, J. (1987) Guide d'évaluation de la qualité des plants de reboisement produits en récipients. Gouvernement du Québec Ministère de l'Énergie et des Ressources Service de la régénération forestière 43p.

Brunner, I., Herzog, C., Dawes, M. A., Arend, M. et Sperisen, C. (2015). How tree roots respond to drought. *Frontiers in plant science* 6: 547.

Buitrago, M., Paquette, A., Thiffault, N., Bélanger, N. et Messier, C. (2015). Early performance of planted hybrid larch: effects of mechanical site preparation and planting depth. *New Forests* 46: 319-337.

Burdett, A. N. (1990). Physiological processes in plantation establishment and the development of specifications for forest planting stock. *Canadian Journal of Forest Research* 20: 415-427.

Burdett, A. N., Herring, L. J. et Thompson, C. F. (1984). Early growth of planted spruce. *Canadian Journal of Forest Research* 14: 644-651.

Calvo-Polanco, M., Señorans, J. et Zwiazek, J. J. (2012). Role of adventitious roots in water relations of tamarack (*Larix laricina*) seedlings exposed to flooding. *BMC Plant Biology* 12: 99.

Cannell, M.G.R. (1985) Dry matter partitioning in tree crops. Dans *Attributes ofTrees as Crop Plants* Cannell, M.G.R. (Édition Jackson, J.E.) Institute of Terrestrial Ecology, Abbotts Ripton, UK. p. 160-193.

Carlson, W. C. et Miller, D. E. (1990). Target seedling root system size, hydraulic conductivity, and water use during seedling establishment. Dans *Proceedings, Combined Meeting of the Western Forest Nursery Association Target Seedling Symposium* (Éditions Rose, R., Campbell, S. J. et Landis, T. D.) USDA Forest service, rocky mountain and range experiment station, Ft. Collins, CO. General technical report RM-200 p. 53-65.

Chiapusio, G., Jassey, V. E. J., Hussain, M. I. et Binet, P. (2013). Evidences of bryophyte allelochemical interactions: the case of sphagnum. Dans *Allelopathy* (Édition Cheema, Z., Farooq, M., Wahid, A.) Springer, Berlin, Heidelberg. p. 39-54.

Clarkson, D. T. et Hanson, J. B. (1980) The mineral nutrition of higher plants. *Annual review of plant physiology* 31(1): 239-298.

Clarkson, D. T., Sanderson, J. et Russell, R. S. (1968). Ion uptake and root age. *Nature* 220(5169): 805-806.

Conlin, T. S. S. et Lieffers, V. J. (1993). Anaerobic and aerobic $CO_2$ efflux rates from boreal forest conifer roots at low temperatures. *Canadian Journal of Forest Research* 23: 767-771.

Coutts, M., Nielsen, C. et Nicoll, B. (1999). The development of symmetry, rigidity and anchorage in the structural root system of conifers. *Plant and Soil* 217(1): 1-15.

Cuneo, I., Knipfer, T., Brodersen, C. et McElrone, A. J. (2016). Mechanical failure of fine root cortical cells initiates plant hydraulic decline during drought. *Plant Physiology* 172: 1669-1678.

Da Costa, C. T., De Almeida, M. R., Ruedell, C. M., Schwambach, J., Maraschin, F. D. S. et Fett-Neto, A. G. (2013). When stress and development go hand in hand: main hormonal controls of adventitious rooting in cuttings. *Frontiers in plant science* 4:133.

de Montigny, L. M. et Weetman, G. F. (1990). The effects of ericaceous plants on forest productivity. Dans *The Silvics and Ecology of Boreal Spruce* (Édition Titus, B. D., Lavigne, M. B., Newton, P. F. et Meades, W. J.), Canadian forest service, Forestry Canada, St. John's, Nfld. report N-X-271. p. 83–90.

DesRochers, A. (1996). Détermination de l'âge de l'épinette noire (*Picea mariana*) en sous-étage de peupliers faux-trembles (*Populus tremuloides*) dans la forêt boréale, Québec. Mémoire de Maîtrise. Université du Québec à Chicoutimi, Chicoutimi, QC, Canada, ISBN 1412306930.

DesRochers, A. et Gagnon, R. (1997). Is ring count at ground level a good estimation of black spruce age? *Canadian Journal of Forest Research* 27: 1263-1267.

DesRochers, A., Van den Driessche, R. et Thomas, B. R. (2006). NPK fertilization at planting of three hybrid poplar clones in the boreal region of Alberta. *Forest Ecology and Management* 232: 216-225.

d'Orangeville, L., Houle, D., Duchesne, L., Phillips, R. P., Bergeron, Y. et Kneeshaw, D. (2018). Beneficial effects of climate warming on boreal tree growth may be transitory. *Nature Communications* 9: 3213.

Drew, M., Jackson, M. et Giffard, S. (1979). Ethylene-promoted adventitious rooting and development of cortical air spaces (aerenchyma) in roots may be adaptive responses to flooding in *Zea mays* L. *Planta* 147(1): 83-88.

Elliot-Fisk, D. (1988). The boreal forest. Dans *North American terrestrial vegetation* (Édition Barbour, M. G. et Billings, W. D.) Cambridge University Press, Cambridge, UK. p. 33-62.

Elliott-Fisk, D. L. (2000). The taiga and boreal forest. Dans *North American terrestrial vegetation* (Édition Barbour, M. G. et Billings, W. D.) Cambridge University Press, Cambridge, UK. p. 41-74.

Enstone, D. E., Peterson, C. A. et Ma, F. (2002). Root endodermis and exodermis: structure, function, and responses to the environment. *Journal of Plant Growth Regulation* 21(4): 335-351.

Environment Canada (2010). Normales climatiques canadiennes 1981-2010. Ressource disponible en ligne sur http://climate.weather.gc.ca/climate_normals/index_f.html [dernier accès le 24 novembre 2018].

Fan, S., Blake, T. J. et Blumwald, E. (1994). The relative contribution of elastic and osmotic adjustments to turgor maintenance of woody species. *Physiologia Plantarum* 90(2): 408-413.

Fattorini, L., Hause, B., Gutierrez, L., Veloccia, A., Della Rovere, F., Piacentini, D., Falasca, G. et Altamura, M. M. (2018). Jasmonate promotes auxin-induced adventitious rooting in dark-grown *Arabidopsis thaliana* seedlings and stem thin cell layers by a cross-talk with ethylene signalling and a modulation of xylogenesis. *BMC Plant Biology* 18(1): 182.

Fenton, N., Lecomte, N., Légaré, S. et Bergeron, Y. (2005). Paludification in black spruce (*Picea mariana*) forests of eastern Canada: potential factors and management implications. *Forest Ecology and Management* 213: 151-159.

Feret, P. P. et Kreh, R. E. (1985). Notes: seedling root growth potential as an indicator of Loblolly pine field performance. *Forest Science* 31(4): 1005-1011.

Gagnon, É. (2002). Développement du système racinaire adventif chez des semis d'épinette noire (*Picea mariana* (mill.) BSP) en plantation. Mémoire de Maîtrise. Université du Québec à Chicoutimi, Chicoutimi, QC, Canada, ISBN 1412309891.

Gauthier, S., Bernier, P., Kuuluvainen, T., Shvidenko, A. Z. et Schepaschenko, D. G. (2015). Boreal forest health and global change. *Science* 349, 819-822.

Geiss, G., Gutierrez, L. et Bellini, C. (2009). Adventitious root formation: new insights and perspectives. *Annual plant reviews* 37: 127-156.

Gezelius, K. et Näsholm, T. (1993) Free amino acids and protein in Scots pine seedlings cultivated at different nutrient availabilities. *Tree Physiology* 13(1):71-86.

Groot, A. (1999). Effects of shelter and competition on the early growth of planted white spruce (*Picea glauca*). *Canadian Journal of Forest Research* 29: 1002-1014.

Groot, A. et Adams, M. J. (1994). Direct seeding black spruce on peatlands: fifth-year results. *The Forestry Chronicle* 70: 585-592.

Grossnickle, S. C. (2000). Ecophysiology of northern spruce species: the performance of planted seedlings. NRC Research Press, Ottawa, ON, Canada.

Grossnickle, S. C. (2005). Importance of root growth in overcoming planting stress. *New Forests* 30: 273-294.

Grossnickle, S. C. et Blake, T. J. (1986). Environmental and physiological control of needle conductance for bare-root black spruce, white spruce, and jack pine seedlings on boreal cutover sites. *Canadian Journal of Botany* 64: 943-949.

Grossnickle, S. C. et Blake, T. J. (1987). Water relation patterns of bare-root and container jack pine and black spruce seedlings planted on boreal cut-over sites. *New Forests* 1: 101-116.

Grossnickle, S. C. et El-Kassaby, Y. A. (2016). Bareroot versus container stocktypes: a performance comparison. *New Forests* 47: 1-51.

Grossnickle, S. C. et Folk, R. S. (1993). Stock quality assessment: Forecasting survival or performance on a reforestation site. *Tree Planters' Notes* 44(3): 113-121.

Grossnickle, S. C. et MacDonald, J. E. (2018). Why seedlings grow: influence of plant attributes. *New Forests*: 1-34.

Haase, D. L. et Rose, R. (1993). Soil moisture stress induces transplant shock in stored and unstored 2+0 douglas-fir seedlings of varying root volumes. *Forest Science* 39: 275-294.

Hacke, U. G., Plavcová, L., Almeida-Rodriguez, A., King-Jones, S., Zhou, W. et Cooke, J. E. (2010). Influence of nitrogen fertilization on xylem traits and aquaporin expression in stems of hybrid poplar. *Tree Physiology* 30: 1016-1025.

Hébert, F., Thiffault, N., Ruel, J.-C. et Munson, A. D. (2010). Ericaceous shrubs affect black spruce physiology independently from inherent site fertility. *Forest Ecology and Management* 260: 219-228.

Helenius, P. (2005). Extension of the planting period of norway spruce container seedlings: risks related to the drought-growth stage dynamics and handling practices. Thèse de Doctorat. Université d'Elsinki, Suonenjoki, Finlande.

Helenius, P., Luoranen, J., Rikala, R. et Leinonen, K. (2002). Effect of drought on growth and mortality of actively growing norway spruce container seedlings planted in summer. *Scandinavian Journal of Forest Research* 17: 218-224.

Houle, D. et Moore, J.-D. (2008). Soil solution, foliar concentrations and tree growth response to 3-year of ammonium-nitrate addition in two boreal forests of Québec, Canada. *Forest Ecology and Management* 255: 2049-2060.

Hytönen, J. et Jylhä, P. (2008). Fifteen-year response of weed control intensity and seedling type on norway spruce survival and growth on arable land. *Silva Fennica* 42: 355-368.

Idris, M., Salifu, K. F. et Timmer, V. R. (2004). Root plug effects on early growth and nutrition of container black spruce seedlings. *Forest Ecology and Management* 195: 399-408.

Islam, M. A., Apostol, K. G., Jacobs, D. F. et Dumroese, R. K. (2009). Fall fertilization of *Pinus resinosa* seedlings: nutrient uptake, cold hardiness, and morphological development. *Annals of Forest Science* 66(7): 1-9.

Islam, M. A. et Macdonald, S. E. (2004). Ecophysiological adaptations of black spruce (*Picea mariana*) and tamarack (*Larix laricina*) seedlings to flooding. *Trees* 18: 35-42.

Jacobs, D. F., Rose, R., Haase, D. L. et Alzugaray, P. O. (2004). Fertilization at planting impairs root system development and drought avoidance of douglas-fir (*Pseudotsuga menziesii*) seedlings. *Annals of Forest Science* 61: 643-651.

Jinks, R. et Mason, B. (1998). Effects of seedling density on the growth of corsican pine (*Pinus nigra* var. *maritima Melv.*), scots pine (*Pinus sylvestris* L.) and douglas-fir (*Pseudotsuga menziesii Franco*) in containers. Dans *Annales des sciences forestières*, vol 4. EDP Sciences, p. 407-423.

Jobidon, R. (2000). Density-dependent effects of northern hardwood competition on selected environmental resources and young white spruce (*Picea glauca*) plantation growth, mineral nutrition, and stand structural development – a 5-year study. *Forest Ecology and Management* 130: 77-97.

Johansson, K., Nilsson, U. et Allen, H. L. (2007). Interactions between soil scarification and norway spruce seedling types. *New Forests* 33: 13-27.

Jupp, A. et Newman, E. (1987). Morphological and anatomical effects of severe drought on the roots of *Lolium perenne* L. *New Phytologist* 105(3): 393-402.

Jutras, S., Thiffault, N. et Munson, A. (2007). Comparing large bareroot and container stock: water stress as influenced by peat and soil water availability. *Tree Planters' Notes* 52: 15-18.

Knipfer, T., Besse, M., Verdeil, J.-L. et Fricke, W. (2011). Aquaporin-facilitated water uptake in barley (*Hordeum vulgare* L.) roots. *Journal of Experimental Botany* 62: 4115-4126.

Knipfer, T. et Fricke, W. (2011). Water uptake by seminal and adventitious roots in relation to whole-plant water flow in barley (*Hordeum vulgare* L.). *Journal of Experimental Botany* 62(2): 717.

Kozlowski, T. T. (1997). Responses of woody plants to flooding and salinity. *Tree Physiology* [Monographie] 1: 1-29.

Kozlowski, T. T. et Pallardy, S. G. (1997). Physiology of woody plants. vol Ed. 2. Academic Press.

Kozlowski, T. T. et Pallardy, S. (2002). Acclimation and adaptive responses of woody plants to environmental stresses. *The botanical review* 68(2): 270-334.

Krause, C. et Morin, H. (1999). Root growth and absent rings in mature black spruce and balsam fir, Quebec, Canada. *Dendrochronologia* 16(17): 21–35.

Krause, C. et Morin, H. (2005). Adventive-root development in mature black spruce and balsam fir in the boreal forests of Quebec, Canada. *Canadian Journal of Forest Research* 35: 2642-2654.

Lamhamedi, M. et Bernier, P. (1994). Ecophysiology and field performance of black spruce (*Picea mariana*): a review. *Annales des sciences forestières* 51: 529-551.

Lamontagne, M. (1993). Influence du gauchissement de la tige de l'épinette noire (*Picea mariana* (mill.) BSP) sur le redressement de la tige et la croissance des plants de plus d'un an. Mémoire de Maîtrise. Université du Québec à Chicoutimi, Chicoutimi, QC, Canada, ISBN 1412304741.

Lanner, R. M. (1985). On the insensitivity of height growth to spacing. *Forest Ecology and Management* 13: 143-148.

Lavoie, M., Paré, D. et Bergeron, Y. (2006). Relationships between microsite type and the growth and nutrition of young black spruce on post-disturbed lowland black spruce sites in eastern Canada. *Canadian Journal of Forest Research* 37: 62-73.

LeBarron, R. K. (1945). Adjustment of black spruce root systems to increasing depth of peat. *Ecology* 26: 309-311.

Lenth, R. V. (2016). Least-squares means: the R package lsmeans. *Journal of Statistical Software* 69: 1-33.

Liao, W.-B., Huang, G.-B., Yu, J.-H. et Zhang, M.-L. (2012). Nitric oxide and hydrogen peroxide alleviate drought stress in marigold explants and promote its adventitious root development. *Plant Physiology and Biochemistry* 58: 6-15.

Lieffers, V. J. et Macdonald, S. E. (1990). Growth and foliar nutrient status of black spruce and tamarack in relation to depth of water table in some Alberta peatlands. *Canadian Journal of Forest Research* 20: 805-809.

Lord, D., Allaire, J., Brousseau, H., Gagnon, D., Morissette, S. et Walsh, D. (1988). Le point sur le gauchissement. Dans *Troisième atelier québécois de travail sur la culture des plants forestiers en récipients*, Chicoutimi, QC, Canada, Ministère de l'Énergie et des Ressources du Québec, p. 93-113.

Lord, D., Allaire, J., Walsh, D., Lamontagne, M., Morissette, S., Bouchard, L., … et Walsh, D. (1991) Compréhension et recherche de solutions au problème du gauchissement de la tige de l'épinette noire: rapport final. Projet RDC-661-067/86. Programme de recherche et développement coopératif (RDC). Conseil de recherches en sciences naturelles et en génie. 92 p.

Lovell, P. H. et White, J. (1986). Anatomical changes during adventitious root formation. Dans *New root formation in plants and cuttings*. Springer, p. 111-140.

Luoranen, J. et Viiri, H. (2016). Deep planting decreases risk of drought damage and increases growth of norway spruce container seedlings. *New Forests* 47: 1-14.

Lupi, C., Morin, H., Deslauriers, A., Rossi, S. et Houle, D. (2013). Role of soil nitrogen for the conifers of the boreal forest: a critical review. *International Journal of Plant and Soil Science* 2: 155-189.

Malik, V. et Timmer, V. R. (1995). Interaction of nutrient-loaded black spruce seedlings with neighbouring vegetation in greenhouse environments. *Canadian Journal of Forest Research* 25: 1017-1023.

Malik, V. et Timmer, V. R. (1998). Biomass partitioning and nitrogen retranslocation in black spruce seedlings on competitive mixedwood sites: a bioassay study. *Canadian Journal of Forest Research* 28: 206-215.

Mallik, A. U. (2001). Black spruce growth and understory species diversity with and without sheep laurel. *Agronomy Journal* 93: 92-98.

Margolis, H. A., Bégin, J., Beeson, R. et Bellefleur, P. (1988). The influence of metal halide and high-pressure sodium lamps during photoperiod extension on the allocation of carbon between lignin and cellulose in black spruce seedlings. *Canadian Journal of Forest Research* 18: 962-964.

Margolis, H. A. et Brand, D. G. (1990). An ecophysiological basis for understanding plantation establishment. *Canadian Journal of Forest Research* 20: 375-390.

Maynard, D., Paré, D., Thiffault, E., Lafleur, B., Hogg, K. et Kishchuk, B. (2014). How do natural disturbances and human activities affect soils and tree nutrition and growth in the Canadian boreal forest? *Environmental Reviews* 22: 161-178.

McClain, K. M. (1981). Growth, nutrition and root development of Ontario tubelings, plugs and 3+ 0 bare-root black spruce. Dans *Proceedings of the Canadian Containerized Tree Seedling Symposium*, Department of the Environment Canadian Forestry Service Great lakes forest research centre, Sault Ste. Marie, Ontario, p. 331-341.

McDonald, M., Galwey, N. et Colmer, T. (2002). Similarity and diversity in adventitious root anatomy as related to root aeration among a range of wetland and dryland grass species. *Plant, Cell and Environnement* 25(3): 441-451.

Mena-Petite, A., Ortega-Lasuen, U., González-Moro, M., Lacuesta, M. et Muñoz-Rueda, A. (2001). Storage duration and temperature effect on the functional integrity of container and bare-root *Pinus radiata* D. Don stock-types. *Trees* 15: 289-296.

Mexal, J. G. et South, D. B. (1991). Bareroot seedling culture. Dans *Forest regeneration manual.* Springer, p. 89-115.

Michel, P., Burritt, D. J. et Lee, W. G. (2011). Bryophytes display allelopathic interactions with tree species in native forest ecosystems. *Oikos* 120: 1272-1280.

Mooney, H., Ferrar, P. J. et Slatyer, R. (1978). Photosynthetic capacity and carbon allocation patterns in diverse growth forms of Eucalyptus. *Oecologia* 36(1): 103-111.

Morris, D. M., MacDonald, G. et McClain, K. (1990). Evaluation of morphological attributes as response variables to perennial competition for 4-year-old black spruce and jack pine seedlings. *Canadian Journal of Forest Research* 20: 1696-1703.

Mullin, R. (1963). Planting check in spruce. *The Forestry Chronicle* 39(3): 252-259.

Mullin, R. (1964) Influence of planting depth on survival and growth of red pine. *The Forestry Chronicle* 40(3): 384-391.

Naija, S., Elloumi, N., Jbir, N., Ammar, S. et Kevers, C. (2008) Anatomical and biochemical changes during adventitious rooting of apple rootstocks MM 106 cultured *in vitro*. *Comptes Rendus Biologiques* 331(7): 518-525.

Nelson, P et Wilhelm, S. (1957) Some anatomic aspects of the strawberry root. *Hilgardia* 26(15): 631-642.

Niemi, K., Scagel, C. et Häggman, H. (2004). Application of ectomycorrhizal fungi in vegetative propagation of conifers. *Plant Cell, Tissue and Organ Culture* 78(1): 83-91.

Nilsen, P. (1995). Effect of nitrogen on drought strain and nutrient uptake in norway spruce (*Picea abies* (L.) Karst.) trees. *Plant and Soil* 172: 73-85.

Nilsson, U. et Örlander, G. (1995). Effects of regeneration methods on drought damage to newly planted norway spruce seedlings. *Canadian Journal of Forest Research* 25: 790-802.

Ohlson, M. et Zackrisson, O. (1992). Tree establishment and microhabitat relationships in north swedish peatlands. *Canadian Journal of Forest Research* 22: 1869-1877.

Pacé, M., Fenton, N. J., Paré, D. et Bergeron, Y. (2018). Differential effects of feather and *Sphagnum* spp. mosses on black spruce germination and growth. *Forest Ecology and Management* 415: 10-18.

Paquette, A., Girard, J. P. et Walsh, D. (2011). Deep planting has no short-or long-term effect on the survival and growth of white spruce, black spruce, and jack pine. *Northern Journal of Applied Forestry* 28: 146-151.

Parker, J. (1949). Effects of variations in the root-leaf ratio on transpiration rate. *Plant Physiology* 24(4): 739.

Patterson, T. B., Guy, R. D. et Dang, Q. L. (1997). Whole-plant nitrogen-and water-relations traits, and their associated trade-offs, in adjacent muskeg and upland boreal spruce species. *Oecologia* 110: 160-168.

Pernot, C., Thiffault, N. et DesRochers, A. (2019a). Contribution of adventitious *vs* initial roots to growth and physiology of black spruce seedlings. *Physiologia Plantarum* doi:10.1111/ppl.12735.

Pernot, C., Thiffault, N. et DesRochers, A. (2019b). Root system origin and structure influence planting shock of black spruce seedlings in boreal microsites. *Forest Ecology and Management* 433: 594-605.

Pezeshki, S. (1991). Root responses of flood-tolerant and flood-sensitive tree species to soil redox conditions. *Trees* 5(3): 180-186.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. et Team, R. C. (2015). nlme: Linear and nonlinear mixed effects models. R package version 3.1–120. Ressource disponible en ligne sur https://CRAN.R-project.org/package=nlme [dernier accès le 24 novembre 2018].

Pinto, J. R., Dumroese, R. K., Davis, A. S. et Landis, T. D. (2011). Conducting seedling stocktype trials: a new approach to an old question. *Journal of Forestry* 109: 293-299.

Prescott, C. E., Maynard, D. G. et Laiho, R. (2000). Humus in northern forests: friend or foe? *Forest Ecology and Management* 133: 23-36.

Reich, P. et Schoettle, A. (1988). Role of phosphorus and nitrogen in photosynthetic and whole plant carbon gain and nutrient use efficiency in eastern white pine. *Oecologia* 77(1): 25-33.

Renou-Wilson, F., Keane, M. et Farrell, E. (2008). Effect of planting stocktype and cultivation treatment on the establishment of norway spruce on cutaway peatlands. *New Forests* 36(3): 307-330.

Rietveld, W. (1989). Transplanting stress in bareroot conifer seedlings: its development and progression to establishment. *Northern Journal of Applied Forestry* 6: 99-107.

Rose, R. et Haase, D. L. (2005). Root and shoot allometry of bareroot and container Douglas-fir seedlings. *New Forests* 30: 215-233.

Rubio, G., Sorgonà, A. et Lynch, J. P. (2004). Spatial mapping of phosphorus influx in bean root systems using digital autoradiography. *Journal of Experimental Botany* 55: 2269-2280.

Salifu, K. et Timmer, V. (2001). Nutrient retranslocation response of *Picea mariana* seedlings to nitrogen supply. *Soil Science Society of America Journal* 65(3): 905-913.

Salifu, K. et Timmer, V. (2003). Nitrogen retranslocation response of young *Picea mariana* to nitrogen-15 supply. *Soil Science Society of America Journal* 67(1): 309-317.

Salmon, D. (2016). Ressources et industries forestières du Québec: Portrait statistique, Édition 2016. vol ISBN: 978-2-550-78018-2. Direction du développement de l'industrie des produits du bois, Ministère des Forêts, de la Faune et des Parcs, Québec, QC, Canada.

Salmon, D. (2018). Ressources et industries forestières du Québec: Portrait statistique, Édition 2017. Direction du développement de l'industrie des produits du bois, Ministère des Forêts, de la Faune et des Parcs, Québec, QC, Canada.

Saucier, J., Robitaille, A. et Grondin, P. (2009). Cadre bioclimatique du Québec. Dans *Manuel de foresterie. 2nde edition.* (Édition Doucet, R, et Côté, M.) Ordre des ingénieurs forestiers du Québec, Éditions Multimondes, Québec, QC, Canada. p. 186-205.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., … et Schmid, B. (2012). Fiji: an open-source platform for biological-image analysis. *Nature Methods* 9(7): 676.

Schwan, T. (1994). Planting depth and its influence on survival and growth, a literature review with emphasis on jack pine, black spruce and white spruce. *TR-017* Ontario Ministry of Natural Resources, Northeast Science & Technology.

Shirley, H. L. (1945). Reproduction of upland conifers in the Lake States as affected by root competition and light. *The American Midland Naturalist Journal*: 537-612.

Simpson, D. G. (1991). Growing density and container volume affect nursery and field growth of interior spruce seedlings. *Northern Journal of Applied Forestry* 8(4): 160-165.

Soil Classification Working Group (1998). The canadian system of soil classification. 3ème ed. *Publication 1646.* Agriculture and Agri-Food Canada.

South, D. B., Harris, S. W., Barnett, J. P., Hainds, M. J. et Gjerstad, D. H. (2005). Effect of container type and seedling size on survival and early height growth of *Pinus palustris* seedlings in Alabama, USA. *Forest Ecology and Management* 204(2-3): 385-398.

South, D. B. et Zwolinski, J. B. (1997). Transplant stress index: a proposed method of quantifying planting check. *New Forests* 13: 315-328.

Spaeth, S. C. et Cortes, P. M. (1995). Root cortex death and subsequent initiation and growth of lateral roots from bare steles of chickpeas. *Canadian Journal of Botany* 73(2): 253-261.

Sperry, J., Hacke, U., Oren, R. et Comstock, J. (2002). Water deficits and hydraulic limits to leaf water supply. *Plant, Cell and Environment* 25(2): 251-263.

Stasovski, E. et Peterson, C. A. (1991). The effects of drought and subsequent rehydration on the structure and vitality of *Zea mays* seedling roots. *Canadian Journal of Botany* 69(6): 1170-1178.

Steijlen, I., Nilsson, M.-C. et Zackrisson, O. (1995). Seed regeneration of scots pine in boreal forest stands dominated by lichen and feather moss. *Canadian Journal of Forest Research* 25: 713-723.

Steffens, B. et Rasmussen, A. (2016). The physiology of adventitious roots. *Plant Physiology* 170: 603-617.

Stroempl, G. (1990) Deeper planting of seedlings and transplants increases plantation survival. *Tree Planters' Notes* 41(4): 17-21.

Strong, W. et Roi, G. H. L. (1983). Root-system morphology of common boreal forest trees in Alberta, Canada. *Canadian Journal of Forest Research* 13(6): 1164-1173.

Sutton, R. F. (1967). Influence of planting depth on early growth of conifers. *Commonwealth Forestry Review* 46: 282-295.

Sutton, R. F. (1980). Planting stock quality, root growth capacity, and field performance of three boreal conifers. *New Zealand Journal of Forestry Science* 10(1): 54-71.

Sutton, R. F. (1995). Advantages of deep planting black spruce. *Technical Note 50* Canadian Forest Service, ON, Canada.

Takahashi, H., Yamauchi, T., Colmer, T. D. et Nakazono, M. (2014). Aerenchyma formation in plants. Dans *Low-Oxygen Stress in Plants*. Springer, p. 247-265.

Tamm, C. O. (1991). Nitrogen in terrestrial ecosystems: Questions of productivity, vegetation changes, and ecosystem stability. *Ecological studies* Berlin, Germany, Springer-Verlag.

Tarroux, E., DesRochers, A. et Girard, J.-P. (2014) Growth and root development of black and white spruce planted after deep planting. *Forest Ecology and Management* 318: 294-303.

Teskey, R., Gholz, H. et Cropper, Jr. W. (1994). Influence of climate and fertilization on net photosynthesis of mature slash pine. *Tree Physiology* 14(11): 1215-1227.

Thiffault, N. (2004). Stock type in intensive silviculture: a (short) discussion about roots and size. *The Forestry Chronicle* 80: 463-468.

Thiffault, N. et Hébert, F. (2017). Mechanical site preparation and nurse plant facilitation for the restoration of subarctic forest ecosystems. *Canadian Journal of Forest Research* 47: 926-934.

Thiffault, N., Hébert, F. et Jobidon, R. (2012). Planted *Picea mariana* growth and nutrition as influenced by silviculture × nursery interactions on an ericaceous-dominated site. *Silva Fennica* 46: 667-682.

Thiffault, N., Jobidon, R. et Munson, A. D. (2003). Performance and physiology of large containerized and bare-root spruce seedlings in relation to scarification and competition in Québec (Canada). *Annals of Forest Science* 60: 645-655.

Thiffault, N., Titus, B. D. et Munson, A. D. (2004). Black spruce seedlings in a *Kalmia-Vaccinium* association: microsite manipulation to explore interactions in the field. *Canadian Journal of Forest Research* 34: 1657-1668.

Thompson, B. E. (1985). Seedling morphological evaluation: what you can tell by looking. Dans *Evaluating seedling quality: principles, procedures, and predictive ability of major tests*. Oregon State University, Forest Research Laboratory, Corvallis, p. 59-72.

Timmer, V. (1997). Exponential nutrient loading: a new fertilization technique to improve seedling performance on competitive sites. *New Forests* 13(1-3): 279-299.

Timmis, R. et Tanaka, Y. (1976). Effects of container density and plant water stress on growth and cold hardiness of douglas-fir seedlings. *Forest Science* 22(2): 167-172.

Tinus, R. W. (1974). Characteristics of seedlings with high survival potential. Dans *North American Containerized Forest Tree Seedling Symposium* Denver, CO (Édition Tinus, R. W., Stein, W. I., et Balmer, W. E.) Great plains agricultural council, Lincoln, NE. Publication 68. p. 276-282.

Uscola, M., Villar-Salvador, P., Gross, P. et Maillard, P. (2015). Fast growth involves high dependence on stored resources in seedlings of Mediterranean evergreen trees. *Annals of Botany* 115(6): 1001-1013.

Van Cleve, K., Oechel, W. C. et Hom, J. L. (1990). Response of black spruce (*Picea mariana*) ecosystems to soil temperature modification in interior Alaska. *Canadian Journal of Forest Research* 20: 1530-1535.

Veilleux, P., Allard, J.-Y., Bart, F., Boyer Groulx, D., Gingras, B.-M., Labrecque, D., Marchand, P., Murray, A. et Jean, S. (2014) Guide Terrain, Inventaire de qualification des plants résineux cultivés en récipients. Ministère des Ressources naturelles, Direction générale de la production de semences et de plants forestiers 141p.

Viereck, L. A. et Johnston, W. F. (1990). *Picea mariana* (Mill.) BSP black spruce. Dans *Silvics of North America, vol 1: Conifers*. U.S. Department of Agriculture, Forest Service, Agriculture Handbook 654, Washington, D.C., p. 227-237.

Villar-Salvador, P., Puértolas, J., Cuesta, B., Penuelas, J. L., Uscola, M., Heredia-Guerrero, N. et Benayas, J. M. R. (2012). Increase in size and nitrogen concentration enhances seedling survival in Mediterranean plantations. Insights from an ecophysiological conceptual model of plant survival. *New Forests* 43(5-6): 755-770.

Vincent, A. (1965). Black spruce: a review of its silvics, ecology and silviculture. *Department of Forests*, Canada (no 1100).

Vitousek, P. M., Cassman, K., Cleveland, C., Crews, T., Field, C. B., Grimm, N. B., … et Rastetter, E. B. (2002). Towards an ecological understanding of biological nitrogen fixation. *Biogeochemistry* 57: 1-45.

Wagner, R. G. et Robinson, A. P. (2006). Critical period of interspecific competition for four northern conifers: 10-year growth response and associated vegetation dynamics. *Canadian Journal of Forest Research* 36: 2474-2485.

Wang, J., Yu, H., Li, G. et Zhang, F. (2016). Growth and nutrient dynamics of transplanted *Quercus variabilis* seedlings as influenced by pre-hardening and fall fertilization. *Silva Fennica* 50(2): 1475.

Waring, R., McDonald, A., Larsson, S., Ericsson, T., Wiren, A., Arwidsson, E., … et Lohammar, T. (1985). Differences in chemical composition of plants grown at constant relative growth rates with stable mineral nutrition. *Oecologia* 66(2): 157-160.

Warren, C. R. et Adams, M. A. (2002). Phosphorus affects growth and partitioning of nitrogen to Rubisco in *Pinus pinaster*. *Tree Physiology* 22(1): 11-19.

Warren, C. R., McGrath, J. F. et Adams, M. A. (2005). Differential effects of N, P and K on photosynthesis and partitioning of N in *Pinus pinaster* needles. *Annals of Forest Science* 62(1): 1-8.

Wiengweera, A. et Greenway, H. (2004). Performance of seminal and nodal roots of wheat in stagnant solution: $K^+$ and P uptake and effects of increasing $O_2$ partial pressures around the shoot on nodal root elongation. *Journal of Experimental Botany* 55: 2121-2129.

Wonn, H. T. et O'Hara, K. L. (2001). Height:diameter ratios and stability relationships for four northern rocky mountain tree species. *Western Journal of Applied Forestry* 16(2): 87-94.

Yaakoubd, B. (1997). Relations hydriques et formation des racines adventives caulinaires chez des semis d'épinette noire (*Picea mariana* (Mill.) BSP). Mémoire de Maîtrise. Université du Québec à Chicoutimi, Chicoutimi, QC, Canada, ISBN 141230735X.

Young, E. et Hanover, J. W. (1977). Development of the shoot apex of blue spruce (*Picea pungens*). Canadian Journal of Forest Research 7(4): 614-620.

Yu, P, White, P. J., Hochholdinger, F. et Li, C. (2014). Phenotypic plasticity of the maize root system in response to heterogeneous nitrogen availability. *Planta* 240(4):667-678.

Zedaker, S., Burkhart, H. et Stage, A. (1987). General principles and patterns of conifer growth and yield. Dans *Forest vegetation management for conifer production* (Édition Walstad, J. D. et Kuch, P. J.) John Wiley & Sons, Inc. New York., p. 203-241.

Zhang, Q., Huber, H., Beljaars, S. J. M., Birnbaum, D., de Best, S., de Kroon, H. et Visser, E. J. W. (2017). Benefits of flooding-induced aquatic adventitious roots depend on the duration of submergence: linking plant performance to root functioning. *Annals of Botany* 120: 171-180.

Zine El Abidine, A. (1993). Les relations hydriques et les échanges gazeux de quatre populations d'épinette noire (*Picea Mariana* (Mill.) BSP) en relation avec leur tolérance au stress hydrique. Thèse de Doctorat. Université Laval, Québec, QC, Canada.

Zine El Abidine, A., Stewart, J. D., Plamondon, A. P. et Bernier, P. Y. (1995). Diurnal and seasonal variations in gas exchange and water relations of lowland and upland black spruce ecotypes. *Canadian Journal of Botany* 73(5): 716-722.