UNIVERSITÉ DU QUÉBEC EN ABITIBI-TÉMISCAMINGUE

AOÛTEMENT ET TOLÉRANCE AU GEL DE DIFFÉRENTES SOURCES GÉNÉTIQUES DE L'ÉPINETTE BLANCHE LE LONG D'UN GRADIENT CLIMATIQUE

MÉMOIRE
PRÉSENTÉ
COMME EXIGENCE PARTIELLE
DE LA MAÎTRISE EN ÉCOLOGIE

PAR

CHAFIK ANALY

JUIN 2023

AVANT-PROPOS

Le présent mémoire est divisé en trois chapitres. Le premier chapitre, introduction générale, est consacré à une revue bibliographique en plus du contexte et des objectifs de l'étude. Le deuxième chapitre est présenté sous forme d'un article scientifique qui sera soumis à la revue *Forests* avec comme auteur, « Chafik Analy, Lahcen Benomar, Martin Perron, Julie Godbout, Jean Beaulieu, Yves Bergeron, Jean Bousquet, Mebarek Lamara ». Je suis le principal responsable de la collecte des données, de leur analyse et de la rédaction de l'article. Mes directeurs et membres du comité d'encadrement ont contribué à la conception de l'étude et m'ont assisté dans l'interprétation des résultats. Ils ont aussi révisé de manière critique et constructive le contenu de l'article. Le troisième chapitre est une conclusion générale.

REMERCIEMENTS

Et voilà arrivé à la fin de ma maitrise, une aventure passionnante et enrichissante du point de vue scientifique, mais aussi personnel et professionnel. C'est avec un grand plaisir que j'exprime ma profonde gratitude envers toute personne qui a contribué à l'aboutissement de ce travail.

Je voudrais exprimer tout d'abord ma reconnaissance à mes encadrants, les professeurs MEBAREK Lamara, JEAN Bousquet et YVES Bergeron, pour leur ultime confiance, leur encadrement, leur disponibilité et leur conseils précieux. Qu'ils trouvent ici mes sincères remerciements et respects.

Mes vifs remerciements vont également au Dr. LAHCEN Benomar pour ses orientations et son aide continue qu'il m'a prodigué pendant la collecte des données. Je dois aussi une partie de ce travail à Pr. FABIO Gennaretti et Dr. BENJAMIN Marquis. Je vous remercie pour l'honneur que vous me faites en acceptant de prendre part à mon jury de maitrise, et d'avoir eu l'amabilité d'évaluer ce document. Un grand merci va également au staff du MFFP pour leur soutien technique et logistique consacré à la réalisation de ce travail.

TABLE DES MATIERES

LISTE DES FIGURES

LISTE DES TABLEAUX

RÉSUMÉ

Les changements climatiques affectent tous les écosystèmes de la planète. Au Québec, on s'attend à ce que la température moyenne augmente de 2 °C au cours des prochaines décennies le long du gradient latitudinal. Par conséquent, l'occurrence des extrêmes climatiques sera plus fréquente et causera des dommages importants aux espèces forestières. Étant donné l'importance économique et écosystémique de l'épinette blanche, la migration assistée représente une solution prometteuse pour son adaptation rapide. Dans ce but, six sources de semences provenant de différentes zones climatiques et une source de locale ont été considérées dans cette étude après 7 ans de plantation. La phénologie automnale a été suivie de la mi-juillet à la mi-août 2021. Des échantillons représentatifs ont été prélevés sur chaque source de semences/bloc/site au début du mois de septembre pour évaluer la tolérance au gel. Deux dates d'échantillonnage ont été considérées vers la fin de la saison de croissance pour évaluer la dynamique des hydrates de carbone non-structurel (NSC). Nos résultats ont montré des effets importants du site de plantation, de la source de semences et de leurs interactions sur la formation du bourgeon et la croissance. La probabilité d'occurrence des stades 2, 3 et 4 été significative. La source de semences du sud-est a montré la meilleure croissance dans les conditions chaudes. La tolérance au gel était significativement plus faible au Sud à partir de -12 °C pour toutes les sources de semence. Celles les plus vulnérables partagent des conditions microclimatiques similaires. Quatre NSC ont été détectés : Fructose, glucose, saccharose et pinitol. Ils ont été principalement affectés par le site de plantation. Les trois premiers sucres ont montré des patrons dynamiques significatifs : La teneur en fructose et en glucose a diminué dans le Sud et augmenté dans le Nord entre septembre et octobre, tandis que la teneur en sucrose a enregistré un patron inverse. La source de semence la plus au sud-ouest a enregistré la teneur en sucrose la plus faible. Le climat d'origine et le climat actuel ont montré des associations significatives avec la hauteur, la tolérance au gel et certains composés de NSC. Les sources de semence et le site de plantation de la zone Sud étaient, principalement, caractérisés par le nombre de jours d'été, l'indice d'intensité des précipitations et la durée de la saison de croissance, tandis que les sources de semence et le site de plantation de la zone Nord étaient caractérisés par le nombre de jours de gel et de jours de froid. Les sources de semence des latitudes moyennes étaient principalement décrites par les valeurs quotidiennes minimales et maximales des températures minimales et maximales. Notre étude a permis d'établir une cartographie détaillée de certains traits physiologiques importants nécessaires à une adaptation réussie de l'épinette blanche. Elle peut être utilisée pour identifier les meilleurs sites pour la migration assistée, améliorer la précision des modèles de transfert de semences à travers le Québec, réduire les risques de dommages causés par le gel et maintenir des taux de productivité élevés dans le futur.

Mots clés : Épinette blanche, phénologie automnale, tolérance au gel, croissance, migration assistée, sucres

CHAPITRE I : INTRODUCTION GÉNÉRALE

1.1. Dynamique des populations forestières

Au cours du temps, il a été attesté que les espèces ont réagi individuellement au cours des périodes historiques des changements climatiques par des migrations géographiques vers et depuis des refuges glaciaires uniques (Petit et al., 2008; Gonzales et al., 2009). Des recherches récentes ont démontré que de nombreuses espèces d'arbres subissent déjà des changements de distribution en réponse aux changements climatiques. En effet, différentes études ont mis en évidence le déplacement de certaines espèces vers le pôle et en altitude (Chen et al., 2011), et d'autres se déplacent de l'Est vers l'Ouest pour suivre les changements dans la disponibilité de l'humidité et de température (Feiet et al., 2017). Ceci change en fonction de la population, sa dynamique, et sa position géographique. Ceux dont les besoins en matière d'habitat sont très spécifiques ou dont les aires de répartition sont limitées par des barrières physiques sont entièrement menacées d'extinction ou de disparition (Erickson et al., 2012). Alors que les espèces largement répandues, leur disparition complète ne constitue pas nécessairement un risque, toutefois, les changements climatiques peuvent entraîner une mortalité à grande échelle et la disparition de la population en raison de sa mauvaise adaptation. Ceci peut être causé en grande partie par les extrêmes climatiques, qui ont considérablement augmenté comparé à la période de base 1951-1980 (Hansen et al., 2012). Durant cette période, environ 1 % de la surface terrestre était touchée par des évènements extrêmes de chaleur alors qu'en 2010 a atteint 10 % (Hansen et al., 2012).

1.2. Prévisions des effets de changement climatique

Au Canada, la forêt boréale subira des variations environnementales plus importantes que la moyenne mondiale en ce qui concerne la température et les précipitations (GIEC, 2014). On prévoit des augmentations de température atteignant jusqu'à 6 °C à l'horizon 2100, selon le scénario RCP8.5 (GIEC, 2014), accompagnées d'une augmentation générale des précipitations. Au Québec, l'organisme Ouranos prévoit des augmentations similaires pour les années 2071-2100, alors que les températures annuelles moyennes pourraient augmenter entre 4 °C et 7 °C, étant de plus en plus importantes à mesure que l'on monte vers le Nord québécois. Le régime des précipitations annuelles devrait également augmenter de façon générale pour la province, mais des périodes de sécheresse en été pourraient être observées au Sud du Québec (Collins et al., 2013) (Figure 1.1).
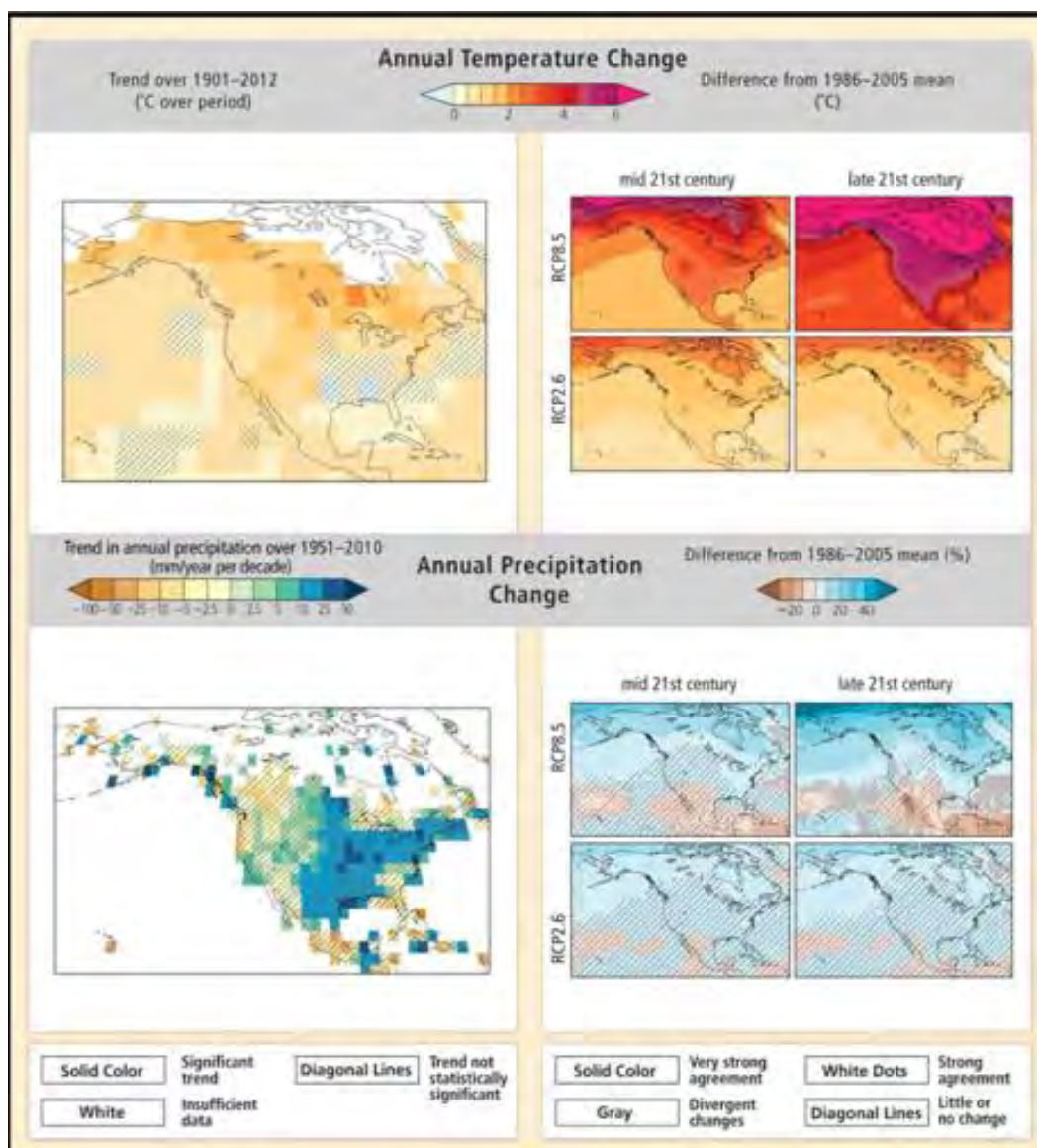
Figure 1.1 Augmentations de température (en haut à droite) et des précipitations (en bas à droite) pour l'Amérique du Nord selon les scénarios RCP2,6 et RCP8,5 et selon l'horizon 2046-2065 et 2081-2100 par rapport aux moyennes de 1986-2005 (Collins et al., 2013).

En tenant compte de l'importance économique de l'industrie forestière au Canada, qui génère 1,25% du produit intérieur brut (PIB) réel (Gouvernement du Canada, 2016a) et le maintien qu'offre à la balance commerciale (exportations des produits forestiers), qui a éteint en 2013, 19,3 milliards de dollars, les changements

climatiques prévus dans l'hémisphère Nord représentent une menace majeure. En effet, il a été souligné qu'il y aura des augmentations de température au futur, accentueront la sécheresse et la disponibilité de l'eau de l'écosystème boréal. Par conséquent, les arbres deviendront plus vulnérables et moins productifs (D'Orangeville et al., 2018).

1.3. Écologie de l'épinette blanche

L'épinette blanche (*Picea glauca* [Moench] Voss) est une espèce largement distribuée dans la forêt boréale nord-américaine. Elle s'étend de la côte Atlantique vers l'océan Pacifique. En Amérique du Nord, cette espèce est répartie au Minnesota, Wisconsin et Maine, et étendu jusqu'à la limite forestière en Alaska (Nienstaedt et Zasada, 1990). Elle se trouve généralement au sein des peuplements mixtes sur des sites bien irrigués et souvent en tant que peuplement pure à l'ouest du Canada (Bergeron et al., 2014). Cette espèce s'établit directement après une perturbation telle qu'un feu de forêt (Greene et al., 1999; Purdy et al., 2002) ou plusieurs décennies après une perturbation (Lieffers et al., 2011) comme les attaques de la tordeuse des bourgeons, qui causent la mortalité d'une grande partie du couvert forestier formée de sapin baumier (*Abies balsamea* (L.) Mill.) (Belle-Isle et Kneeshaw, 2007). L'épinette blanche ne semble pas particulièrement adaptée à la régénération naturelle immédiatement après une perturbation et ne possédant pas des cônes (semi) sérotineux comme l'épinette noire (*Picea mariana* (Mill.) BSP) (Greene et al., 1999). Bien qu'il y ait différentes strates (issue de marcottage des branches) dans certains sites de haute altitude (Nienstaedt et Zasada, 1990), la quasi-totalité de sa régénération provient des graines. La longue espérance de vie, les faibles taux de mortalité, la dispersion périodique des semences, et la tolérance à l'ombre peuvent probablement aider à expliquer la persistance de cette espèce dans les peuplements forestiers et son importance dans la forêt boréale.

1.4. Physiologie et métabolisme des sucres chez les arbres

Les processus physiologiques (croissance, secrétions hormonales, métabolisme des sucres et régulations des phases cycliques) des arbres sont influencés principalement par les facteurs environnementaux (Benomar et al., 2015). Les arbres réagissent différemment aux variations climatiques le long de leurs cycles de développement (Figure 1.2). Par conséquent, des changements rapides et réversibles du métabolisme ou de la morphologie ont lieu; il s'agit de l'acclimatation (El Kayal et al., 2011). Grâce aux capteurs des signaux de température, l'arbre détermine les phases de son cycle de développement. En effet, après la phase de la croissance active, la première phase d'acclimatation automnale, commence l'écodormance. Sachant que cette phase est réversible, l'augmentation de la température vers la fin de l'été peut entrainer une reprise de la croissance active. Ainsi, on parle du débourrement tardif. Ensuite, l'endodormance (approfondissement de la dormance) commence, généralement, entre septembre-novembre et permet d'acquérir la résistance au gel afin de survivre à l'hiver (Prunier et al., 2015). Ce processus est accompagné avec l'allocation des réserves de sucres dans la partie aérienne de l'arbre (Jiang et al., 1994).
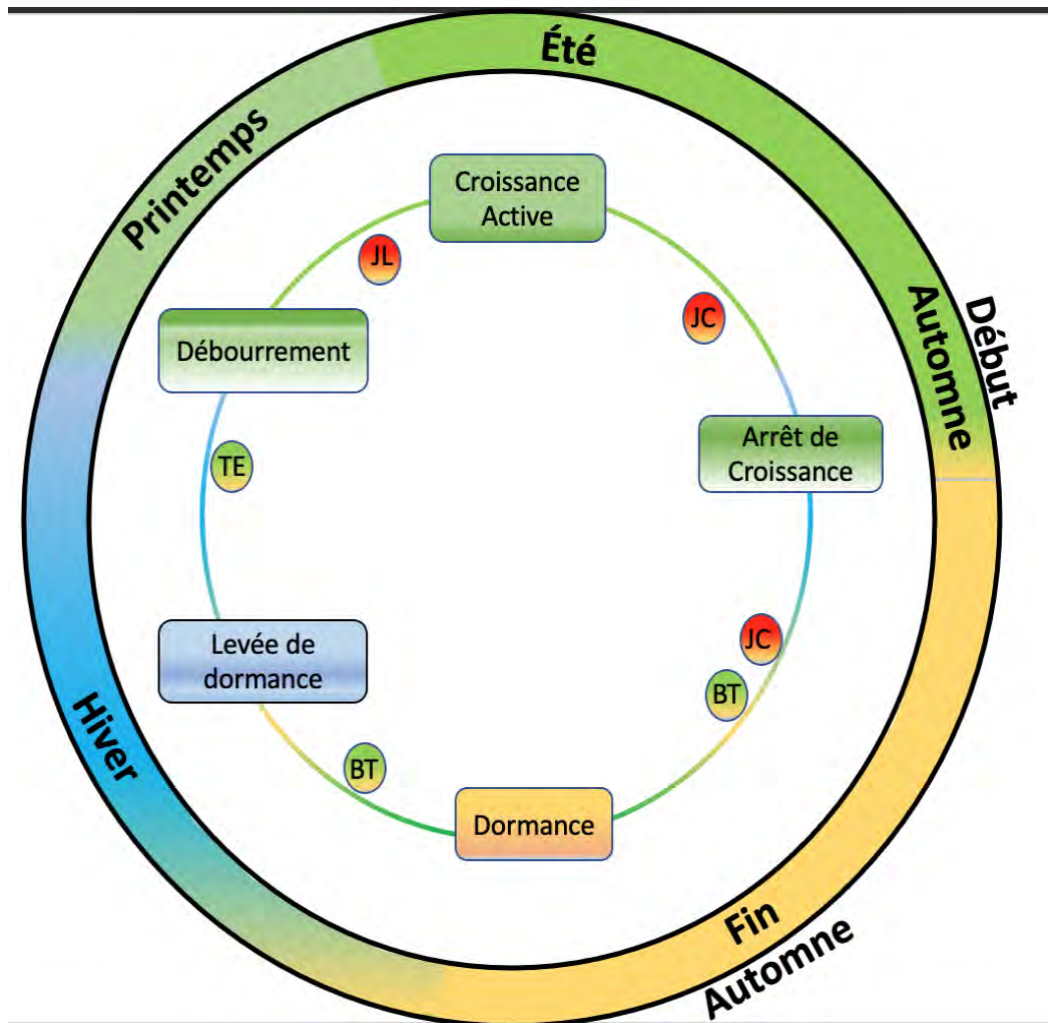
Figure 1.2 Cycle annuel de croissance chez les arbres (Adapté de Singh et al., 2017). JC = jour court; BT=basse température; TE= température élevée; JL=jour long.

La synchronisation de l'entrée en dormance avec la fin de la période propice à la croissance est reliée à la date d'apparition du bourgeon terminal lors de l'aoûtement (Bigras & Colombo, 2001). Cette partie de l'arbre contient une des structures les plus vulnérables au froid qui est le méristème apical. Son développement passe par différents mécanismes physiologiques, citant entre autres, la formation du bourgeon, l'acquisition de la tolérance au gel et la dormance (Rohde & Bhalerao, 2007; Ruttink et al., 2007). Comme l'aoûtement, le niveau de tolérance au gel des arbres n'est pas le même tout au long de l'année. Suivant l'aoûtement, il augmente en effet, progressivement en automne, via un processus actif (Weiser, 1970) appelé

l'endurcissement (Levitt, 1980; Sakai et Sakai, 1987). L'aoûtement est donc une caractéristique phénologique importante de l'acclimatation au froid. Plus tôt le bourgeon terminal apparait, plus tôt l'arbre acquiert la résistance au froid nécessaire pour hiverner sans dommages et plus tôt la croissance s'arrête (Bigras & Colombo, 2001; Howe et al., 2003). L'occurrence des extrêmes climatiques, notamment le gel, entraine des perturbations du cycle de croissance annuel et affecte le développement normal de la croissance des arbres.

1.5. Variabilité génétique et migration assistée de l'épinette blanche

Étant donnée le large domaine écologique de l'épinette blanche au Canada, plusieurs études ont été réalisée sur sa diversité génétique et son adaptation aux changement climatiques. En effet, l'étude d'Andalo et al. (2005) a démontré que l'adaptation locale de l'épinette blanche est un processus complexe sur le plan génétique, impliquant plusieurs composantes indépendantes et leurs interactions. Dans un autre contexte, le développement d'un modèle de transfert de semences d'épinette blanche a témoigné la mésadaptation des sources de semences locales en considérant leurs conditions climatiques d'origines et ceux du site de plantation (Andalo et al., 2005; Hornoy et al., 2015). Cette mésadaptation a été constatée aussi suivant le gradient latitudinal. Les sources génétiques optimales proviennent d'un climat plus méridional alors que les sources locales sont adaptées à un climat moyennement froid que le climat actuel (communication personnelle de Jean Bousquet, 2021). En termes de phénologie, Li et al. (1993) ont souligné la synchronicité du débourrement et la grande variabilité de l'aoûtement de différentes provenances génétiques de l'épinette blanche. En outre, Lu et Man (2011) ont montré que la période de débourrement varie considérablement entre les provenances. Par ailleurs, Beaulieu et Rainville (2005), attestent que les sources de

semences d'épinette blanches sont mieux adaptées aux conditions climatiques là où elles croissent actuellement et atteignent un rendement maximum sous ces conditions.

L'épinette blanche porte un intérêt particulier chez la communauté scientifique en matière d'amélioration génétique et d'aménagement forestier. Ainsi, la migration assistée, qui consiste à déplacer une population par l'humain, de plantes ou d'animaux vers les habitats climatiques auxquels ils sont adaptés (Ste-Marie et al., 2011, Benomar et al., 2022), représente une approche prometteuse d'aide à l'adaptation des espèces forestières. En effet, plusieurs modèles de transfert de semences ont été développés pour différentes espèces, en combinant les données climatiques et les données de croissance des arbres (Rainville et al., 2014). Ces modèles permettent d'estimer la productivité future en volume de bois marchand et déterminer les provenances performantes et mieux adaptées aux conditions futures. Au Canada, certaines provinces ont déjà commencé à adopter la migration assistée comme mesure proactive sur leurs territoires, citant entre autres, le Québec qui utilise les modèles de transfert de Beaulieu et al. (2004) et de Rainville & Beaulieu, (2005). En outre, plusieurs chercheurs (sont impliqués dans le développement des modèles de transfert de semences des principales essences commerciales notamment, le pin gris et l'épinette blanche (Benomar et al., 2015, 2016, 2017; Villeneuve et al., 2016; Lamhamedi et al., 2017) ainsi que l'épinette noire en Ontario (Thomson et al., 2009) et au Québec (Rainville et al., 2014) afin de choisir les sources de semences les mieux adaptées au climat futur à l'échelle provinciale. Ces modèles s'articulent majoritairement sur l'évaluation des contributions génétiques et la plasticité phénotypique des différentes sources de semences. En effet, Marquis et al. (2020) ont souligné l'importance de développer des modèles de transfert de semences qui tiennent en compte les processus physiologiques et les extrêmes climatiques (surtout le gel printanier). En fait, les modèles de transfert ont pour but de prédire la performance des plants selon le climat projeté ou encore selon la différence de climat entre plusieurs sites. Malgré leur importante contribution

dans l'élaboration d'un plan de migration assistée pour le Québec, ces modèles ne prennent toutefois pas en considération certains éléments importants susceptibles d'affecter la survie et la performance des plants (Isaac-Renton et al., 2014), par exemple les traits physiologiques régissant la tolérance des différentes sources génétiques aux sécheresses et au gel. Il a été souligné que les variables de croissance sont fortement associées à la génétique des plants (degré d'adaptation et de plasticité) et à l'environnement (fertilité, sol, eau, température, précipitations, etc.) (Li et al., 2017). Certes, pour réduire les risques des extrêmes climatiques, il est important d'inclure la réponse de certains processus physiologiques aux modèles de transfert (e.g. phénologie et tolérance au gel, acclimatation thermique, nutrition minérale, dynamique des métabolites, etc.) afin d'optimiser la performance des essences forestières en fonction des changements climatiques anticipés (Lamhamedi et al., 2017) et face aux perturbations naturelles (e.g. Feu, tordeuse du bourgeon). Comme il a été mentionné par Kim et al. (2017), le renforcement des anomalies de réchauffement de l'Arctique au cours des dernières décennies a réduit la productivité en Amérique du Nord. De plus, l'occurrence du froid hâtif en 2018 et 2019, avec des températures chutant en dessous de -15 °C à -20 °C et des tempêtes de neige (blizzards) dès le début de novembre (https://www.meteomedia.com/ca, consulté en 2020). Ce phénomène dû aux oscillations vertigineuses du vortex polaire descendant sous les latitudes du Québec étalé sur une grande période en raison de l'affaiblissement du courant jet où le réchauffement accentué du climat au pôle Nord amène le vortex polaire à descendre beaucoup plus vers le sud fréquemment. Ce même phénomène amène aussi des blocages climatiques l'été avec des sécheresses accentuées et plus fréquentes accompagnées d'une remontée plus importante des masses d'air chaudes (Kug et al., 2015; Overland et al., 2016). Ainsi, l'évaluation des traits physiologiques durant la phase juvénile des plants forestiers, après leur mise en terre, est nécessaire puisque la phase d'établissement constitue la période la plus critique en raison de la vulnérabilité des plants aux stress environnementaux et à leurs interactions multiples (Lamhamedi & Bernier, 1994).

C'est dans cette optique où le présent projet a eu lieu en vue de comprendre la relation entre la phénologie automnale de l'épinette blanche et sa tolérance au gel suivant un gradient climatique afin d'évaluer les interactions génotype-environnement et de développer un nouveau modèle de transfert de semences d'épinette blanche. Pour ce faire, les objectifs spécifiques ont été structurés comme suit :

1.6.   Objectifs et hypothèse

1.6.1.   Hypothèse de recherche

La phénologie du bourgeon de l'épinette blanche, sa croissance, sa tolérance au gel et la teneur en sucres solubles varient significativement suivant la source génétique, le gradient climatique et le climat du site de plantation.

1.6.2.   Objectifs spécifiques

➢ Examiner les variations génétiques et la plasticité phénotypique des différentes phases phénologiques d'aoûtement et de croissance.

➢ Déterminer la relation entre la tolérance au gel, le climat du site de plantation et le climat d'origine des sources génétiques.

➢ Comparer la dynamique des sucres solubles à la fin de la saison de croissance des différentes sources génétiques.

CHAPTER II : MANUSCRIPT

BUD SET AND FROST TOLERANCE OF DIFFERENT WHITE SPRUCE SEED SOURCES ALONG A CLIMATIC GRADIENT

Authors:

Chafik Analy[1], Lahcen Benomar[1], Martin Perron[2], Julie Godbout[2], Jean Beaulieu[3], Yves Bergeron[1,4], Jean Bousquet[3], Mebarek Lamara[1*]

[1] Institut de Recherche sur les Forêts, Université du Québec en Abitibi-Témiscamingue, Rouyn-Noranda, QC, Canada

[2] Direction de la Recherche Forestière, Ministère des Ressources naturelles et des Forêts, Québec, QC, Canada

[3] Centre d'Étude de la Forêt et Institut de Biologie Intégrative et des Systèmes, Faculté de Foresterie, de Géographie et de Géomatique, Université Laval, Québec, QC, Canada

[4] Département des sciences biologique, Université du Québec à Montréal, Montréal, Québec, Canada

* Corresponding author

2.1. Abstract

To assess potential impact of climate change on white spruce, six seed sources from different climatic zones and one local seed source were evaluated after 7 years of planting in two sites. Bud set, frost tolerance and non-structural carbohydrates (NSC) content analysis showed important effects of plantation test site, seed source, and their interactions on bud set phenology and growth. The first phase of bud set occurred one week later in the southern site compared to the northern site, whereas the late phase of bud set was similar in both sites. Significant differences were recorded between seed sources for some phenological stages. The frost tolerance was significantly lower in the southern plantation test site below -12 °C for all seed sources. NSC, fructose and glucose content variation were opposite in September between sites. It decreased from September to October in the southern site and increased in the northern site, while Sucrose content recorded the opposite pattern in October. The southwestern seed source had the lowest sucrose content. The NSC content was correlated to frost tolerance. Our study highlighted the important role of NSC in cold hardiness and growth response to early frost and origins climate implication to white spruce adaptation.

Keywords: bud set phenology, *Picea glauca,* assisted migration, cold hardiness, functional traits, growth, non-structural carbohydrates.

2.2. Introduction

During past periods of climate change, natural forest species responded individually with extensive geographic migrations from glacial refugia (Petit et al., 2008; Gonzales et al., 2009; Jaramillo-Correa et al., 2009). Recent research showed that many tree species are already undergoing distributional changes in response to climate change, highlighting the poleward and upper elevation shifts of some species (Chen et al., 2011; Aidenapol et al., 2015; Wu et al., 2015), and others moving in longitude following moisture availability and temperature changes (Fei et al., 2017).

In Canada, it is predicted that the boreal forest will experience greater environmental variation in temperature and precipitation than the global averages (Adam-Poupart et al., 2014). In the eastern part of this ecozone, the temperature will increase by up to 6 °C in the horizon of 2100 under the RCP8.5, the worst-case scenario, coupled with an overall increase in precipitation (IPCC, 2021). For the boreal forest of Québec, the Ouranos climate change monitoring organization has predicted similar increases for the years 2071-2100, where average annual temperatures could rise between 4 °C and 7 °C as one moves northward in Québec. However, the climate change that we are experiencing is shifting the environmental conditions too fast for trees to follow their optimal growing conditions and climate by migration alone (Aitken et al., 2008). Already, signatures of local genetic maladaptation have been recorded with regards to warming temperatures (Andalo et al., 2005; Benomar et al., 2022). Despite the obvious warming trend in average temperature, climate change is also expected to increase the frequency and severity of extreme events, which have a high potential to impact vegetation communities (Lloret et al., 2012; Marquis et al., 2022).

Assisted population migration, also called assisted gene flow, was proposed as a proactive approach to help forest species adaptation to global change. It has also been suggested as a strategy to mitigate the effect of climate change on forests (Pedlar et al., 2012; Aitken & Bemmels, 2016). This approach relies on the calculation of the maximal seed source's transfer distance which is generally within the natural range of the species distribution using growth response and bioclimatic predictors (Beaulieu & Rainville, 2005). However, when moving local seed sources to colder environments, assisted migration could carry the risk of frost damage if seed sources are moved too far, or if expected warmer climate conditions have not yet materialized (Benomar et al., 2022). In spite of the warming climate of the last few decades under northern mid-latitudes, severe frost damage still occurred in natural populations, particularly during the growing season, for instance, after a frost spell in spring 2007 in Eastern North America (Gu et al., 2008; Man et al., 2009), and after another late spring cold spell in 2021 following an early warm spring, where local populations and migrated seed sources suffered severe damages (Benomar et al., 2022). Thus, frost damage and growth limitations become a serious risk for the newly introduced populations.

Moreover, differences between seed sources in bud set and the onset of cold hardiness during late summer and in the fall have been reported to be greater than that for bud flush and the release of cold hardiness in spring (e.g., Li et al., 1993). Thus, latitudinal transfers might have a greater impact on susceptibility to early fall frost occurrences (Aitken & Hannerz, 2001). Many studies have highlighted the necessity of including eco-physiological responses and non-structural carbohydrates metabolism in seed transfer models to reduce frost damage risks (Villeneuve et al., 2016; Marquis et al., 2020; Benomar et al., 2020, 2022). Such models should include extreme weather events, cold hardiness chronology, non-structural carbohydrates dynamics of seed sources, and ecological conditions of plantation test sites at the end of the growing season. Knowing that cold hardiness heavily relies on bud set phenology and the release of cold hardiness, and that

variation for this trait is usually the main factor affecting the length of the growing season (Li et al., 1993), the trade-off between growth and cold hardiness is largely driven by how long trees extend their growing season during the fall (Howe et al., 2003).

In this study, we used six white spruce (*Picea glauca* [Moench] Voss) seed sources originating from different bioclimatic domains in the province of Québec (Canada) and local population controls to assess the importance of adaptive traits that could impact tree productivity in two sites presenting contrasted environmental conditions (warm and cold). Our goal was to disentangle the genetic from the environmental effects and understand their interaction for bud set, frost tolerance and non-structural carbohydrate content, and detect potential clinal variation in those traits.

This study should help minimizing the risks of frost damage related to seed source transfer under assisted migration programs and support the development of new seed transfer models better adapted to predicted climatic conditions, thus maintaining optimal productivity in future reforestation efforts in the context of climate change.

2.3.   Material and methods

2.3.1. Study area: Seed sources and experimental site conditions

Between 2014 and 2015, six seed sources (from orchards) and local seed sources (control) were planted at nine test sites by the MRNF (Ministère des Ressources Naturelles et des Forêts du Québec) distributed between latitudes 45°N and 49°N and longitudes 65°W and 79°W in the province of Québec (Otis-Prud'homme et

al., 2018). These plantations were arranged along an average annual temperature gradient of 5.8 °C. Two of these plantation test sites with most contrasting average annual temperatures were considered in this study, the southern Wendover site (planted in 2014) and the northern Rousseau site (planted in 2015) (Figure 2.1). Each plantation test site thus contained six transferred seed sources and the local seed source (Table 2.1). The photoperiod was estimated for each site based on sites' latitude and longitude. The maximum difference in the daylight period between the northern and southern sites observed during the summer solstice was around half an hour (Annexe A).
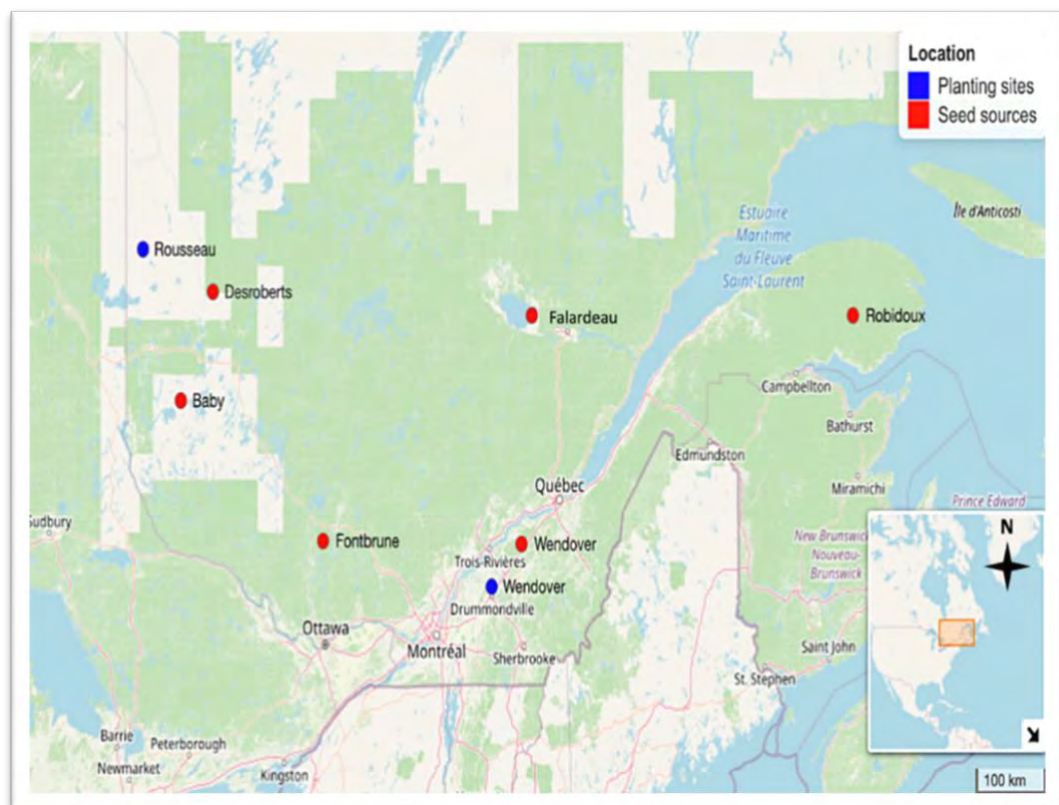
Figure 2.1 Geographical locations of seed sources and plantation test sites.

Table 2.1 Geographical coordinates and bioclimate normals of the six migrated white spruce seed sources origin (timeframe: 1940-1970) and the actual plantation test sites (timeframe: 2010-2020). Climate data was extracted using BIOSIM (Régnière et al., 2014). The table was adapted from (Benomar et al., 2016).

	Latitude (°N)	Longitude (°W)	Altitude (m)	GDD5 (°C)	MAT (°C)	TAP (mm)	FD (days)	SD (days)
Seed sources								
Wendover	46.39	71.94	116	1708	4.16	1093	157	62
Fontbrune	46.43	75.74	354	1588	3.42	989	183	43
Baby	47.75	78.47	321	1480	2.03	920	197	31
Robidoux	48.76	77.86	314	1332	0.84	889	204	14
Desroberts	48.55	65.59	270	1298	2.60	1117	212	32
Falardeau	48.54	71.73	351	1347	1.37	1039	184	35
Plantation test sites								
Wendover	45.98	72.52	71	2141	6.2	1080	156	51
Rousseau	49.15	79.20	292	1430	1.1	925	205	33

GDD5: Number of growing degree-days $>= 5°C$; **MAT:** Mean annual temperature; **TAP:** Total annual precipitation; **FD:** Number of frost days (Days with Tmin < 0°C); **SD:** Number of summer days (days with Tmax > 25°C).

2.3.2. Experimental design

Each site contains 4032 white spruce trees organized in 4 randomized complete blocks (density of 2000 trees/ha; spacing of 2.25 m x 2.25 m). Each block has 7 plots (each being occupied by one of the 7 seed sources), and each plot contains 144 trees (12 × 12). The two outer rows were excluded from the measurements to

avoid the border effect. Thus, only the central 64 trees per plot were considered for sampling and analysis.

2.3.3. Climatic data

Seed source climatic data were obtained using the BIOSIM10 software (Régnière et al., 2014), which allows the extrapolation of temperature and precipitation from data collected in the most adjacent meteorological stations. The climate conditions of seed source origins were assessed using the local climatic normals experienced by the parental generation from 1940 to 1970, while the current plantation test sites' climate was assessed using the climatic normals of plantation test sites from 2010 to 2020. Multiple bioclimatic indices (https://www.climdex.org/learn/indices/) were derived from temperature and precipitation using the climdex R package (Bronaugh, 2010) to assess the underlying mechanisms of adaptation and the interaction effects between seed sources and plantation test sites.

- Odd ratio of frost probability

The odd ratio of annual frost probability during the growing season between plantation test sites (Annexe B) showed an extreme inter-annual variation of frost probability during the after-planting years in the northern plantation test site. For estimation, the growing season was defined as between June 1st and October 31st of each year, and the number of frost days for each year was estimated as the number of days with daily minimal temperature < 0 °C during this period (Annexe B).

2.3.4. Bud set monitoring

A follow-up of the different phenological stages of bud set was carried out on 20 trees/plot/block/site during the growing season of 2021, once a week with a one-week delay for the southern site, from the end of June to early August. For each tree, the chronology of the average bud set stage, on several lateral shoots ranging from 7 to 10 shoots per tree, was recorded considering different geographical exposures and heights. The phenological stages were recorded using the white spruce phenology field guide (Annexe C). The studied bud set stages are described as follows:

Stage 0: no buds. Elongation of the main stem may already be complete. Stage 1: apical bud initiation. Presence of a white bud at the end of the main stem hidden under the needles. Stage 2: beige bud. The scales that begin to cover the bud become beige. The size of the bud has increased. Stage 3: browning bud. The apical bud is completely covered with browning scales. The volume of the apical bud has increased significantly. Stage 4: brown bud. The apical bud is completely brown and clearly visible. The needles of the whorl are beginning to open outwards. Stage 5: open needles. The apical bud is clearly visible, and its brown, opaque and concave scales are well-formed (Dhont et al., 2010). To evaluate the late-frost damage that occurred during the year 2021, the health state of terminal shoots was also recorded considering using a crown damage index, which was defined as the proportion of the crown of the trees affected by the late cold spell. Trees with more than 70% crown damage index were simply avoided.

2.3.5. Evaluation of frost tolerance

A frequency analysis of the first fall frost was carried out over the last 30 years for each plantation test site using the daily minimal temperature. It has allowed us to identify the maximal date before which the first frost fall occurs between the end of the bud setting and the first fall frost. The October 3rd, 2021 was identified as the best late sampling date to avoid fall frost damage. For each seed source/block/site, composite samples of 24 shoots were taken randomly from a few undamaged trees. Shoots were stored in plastic bags previously labelled. Each bag contained a wet absorbent paper to keep a fresh environment for the samples and avoid their deterioration through direct contact with ice when placed in a cooler and sent to the MRNF laboratory in Quebec City for analysis the following day.

The frost tolerance test consisted of applying 6 temperature levels ranging from T_0 = 4 °C (control) to -16 °C with a step of 4 °C using a programmable freezer (Model T20RS Tenney environmental *inc.*, Williamsport, PA, USA). Each temperature level lasts 4 hours, and then the temperature was lowered at a rate of 1 °C·h^{-1}; once the target temperature was attained, it was held constant for 4 hours, and the electrolytic conductivity (EC) was estimated according to the protocol proposed by Lamhamedi et al. (2005, 2022). Then, the temperature was lowered at a rate of 1 °C·h^{-1}, and the EC measurements were repeated after 4 hours with a constant temperature. Briefly, for each site and each freezing temperature, four shoots were used for each seed source in each block. After having undergone the 5 levels of freezing temperature (T_1 = 0 °C, T_2 = -4 °C, T_3 = -8 °C, T_4 = -12 °C, T_5 = -16 °C) as samples of T_0 were not subjected to freezing treatment, the samples were then immersed in 100 ml of demineralized water overnight at a temperature of 4 °C. After saturation, the release of electrolytes (EC measured in µSiemens/cm) in the solution of each sample was measured using a conductivity meter (model 160,

Orion Research Inc., Boston, MA, USA). Two frost tolerance indices were calculated as follow:

REC (%) = EC1/EC2   (Eq. 1)

RECsample = REC/RECo   (Eq. 2)

LDI = (RECsample − RECo)/(1 − RECo))   (Eq. 3)

Where: REC sample: Relative conductivity at a given freezing temperature (Eq. 1), EC1: Measured electrolytic conductivity at a given freezing temperature at the beginning of the test,

EC2: Measured electrolytic conductivity at the end of the test for the same freezing temperature,

RECo: Relative conductivity at reference temperature 4 °C, LDI: Leaf damage index (Eq. 3).

2.3.6. Tree height measurement

To assess the growth performance of seed sources and detect any possible frost effects on their growth, total tree height at 7 years after the plantation was measured in both sites, and mortality was recorded for all seed sources and within each plantation test site. Due to the 1-year difference of trees age, the height was adjusted for 6 years old trees by adding shoot's length of 2021 to the total height of the same year to allow a comparison of height between plantation test sites as a proxy for the same age.

2.3.7. Non-structural carbohydrates (NSC) analyses

To examine variations in non-structural carbohydrate (NSC) content towards the end of the growing season in relation to hardiness and frost tolerance, the sampling of shoots was carried out on two dates in fall of 2021. The first sampling day in early September, and the second in early October 2021. For each seed source, three trees of different height classes (small, medium, and high) were sampled. Synchronizing NSC and sampling for frost tolerance assessment was designed to determine the relationship between frost tolerance and sugar metabolism. After collection, the samples were quickly stored in a cooler containing dry ice. Then they were freeze-dried to preserve the integrity and quality of the sugars. Finally, they were sent to the TransBIOTech laboratory (Levis, QC, Canada) to determine NSC content using an ultra-performance liquid chromatography-evaporative light scattering detector (UPLC-ELSD). These analyses focused primarily on the most important sugars for cold acclimation, that is, raffinose, glucose, sucrose, fructose, and pinitol.

2.3.8. Statistical analyses

To characterize the climate conditions of seed sources origins and actual plantation test sites, the annual odd ratio of frost probability during the growing season and the length of the growing season were calculated using the *climdex.gsl* function from the climdex R package (Bronaugh, 2010). These two variables were fitted separately to simple linear models to evaluate the differences between seed sources and plantation test sites. The significantly different groups were mapped for both variables along the geographic gradient (north south).

Normality and homogeneity of variance assumptions were assessed and validated prior to the analysis of variance (ANOVA) for all fitted linear models in this study. These models were fitted using the *lm and aov* functions from the stats R package (R Core Team 2022). The presence of significant differences was followed by a post hoc analysis using Tukey's test method from stats R package (R Core Team 2022) to identify the best seed sources in terms of growth and frost tolerance. The R-square method was used to estimate the explained percentage of the total variance using the Performance R package (Lüdecke et al., 2021).

The likelihood probability of bud set stage was modeled as a function of day of the year, plantation test site and seed source (Eq. 4) by an ordinal logistic regression using *polr* function from MASS R package (Venables & Ripley, 2002). The results were visualized using the ggeffects R package (Lüdecke, 2018) to highlight the variation of bud set probability over time between seed sources and plantation test sites. The model formula is written as follows:

Bud set stage ~ Site + seed source + site x seed source + day of the year (Eq. 4)
Successive stages correlations were examined as well (Annexe J).

The percentage of damaged terminal shoots and survival rate were fitted to a linear mixed model with fixed effect of seed sources, plantation test site and their interatction and the random effect of the bloc nested within site as follows:

Damaged terminal shoot (%) ~ plantation site + seed source + seed source x plantation site + block (site) (Eq. 6)
Survival rate (%) ~ plantation site + seed source + seed source x plantation site + block (site). (Eq. 7)

To evaluate seed source's frost tolerance, two linear models were fitted to REC and LDI vs. plantation test site, seed source, freezing temperature, and all their interactions. The model formula is written as follow for both frost tolerance indices:

Frost tolerance indice ~ plantation site + seed source + freezing temperature + seed source x plantation site + freezing temperature x plantation site + freezing temperature x seed source + plantation site x seed source x freezing temperature (Eq. 8)

Total height was analyzed using the model in Eq. 6, while NSC content was analyzed using the model in Eq 8 after replacing the freezing temperature by sampling date.

To determine the relationship between the climate of seed sources' origins, the climate of plantation test sites, frost tolerance indices, NSC content, and tree height and bioclimatic variables, correlation analysis was conducted on the average values of seed sources alone, then added the average values of plantation test sites for all 24 variables. Similar correlations were grouped together using the Ward complete hierarchical clustering method (Ward, 1963). The resulting clusters were visualized in two heatmaps (with and without plantation test sites average values) with significance levels using corrplot R package (Taiyun & Viliam, 2021).

All significant correlations of frost tolerance indices were scatter plotted to highlight linear relationships of seed source's origin and the actual plantation test site in terms of bioclimate, frost tolerance, height and NSC content. To determine the discriminating factors of the studied seed sources and plantation test sites, bioclimatic variables were scaled and centered, then, a principal component analysis (PCA) was fitted using the NIPALS (Nonlinear Iterative Partial Least Squares) approach from the pcamethods R package (Stacklies et al., 2007). Factoextra R package (Alboukadel & Fabian, 2020) was used for visualization.

2.4.   Results

2.4.1.   Climate characterization of plantation test sites and seed sources origins

The average length of the growing season, calculated over the reference period, showed that the Wendover seed source had the highest length, around 150 days (Figure 2.2A). However, the northern Baby and Desroberts seed sources exhibited smaller lengths of growing season of around 100 days. Wendover seed source showed significant differences compared to these two north-western seed sources. However, the mid-latitude Robidoux and Falardeau seed sources exhibited a moderate average length of the growing season ranging from 115 to 130 days. These seed sources were not significantly different. On the other hand, plantation test sites showed significant differences in the average length of growing season, with 165 days at the northern site Rousseau, and 200 days at the southern site Wendover (Figure 2.2A).

Regarding the average frost odd ratio probability during the growing season, Wendover, Fontbrune, and Falardeau seed sources showed the lowest frost probability, around 0.1 (Figure 2.2A). It was significantly higher for the Baby seed source (mid-latitude), with an average probability of 0.13. The highest frost probability was exhibited by Robidoux (mid-latitude east) and Desroberts (northwest) seed sources ranging from 0.18 to 0.21. Regarding the plantation test site, significant differences were recorded, with 0.05 average frost probability in the south and 0.16 in the north.

The Fontbrune southern seed source belonged to an intermediate group that combines the c and b Tukey groups for the frost odd ratio probability (Figure 2.2B), which are represented, respectively, by the southern Wendover seed source and the northern Baby seed source.

On the other hand, the Fontbrune, Robidoux and Falardeau seed sources were similar and were clustered into an intermediate group between southern and northern locations for the length of the growing season (Figure 2.2A). The frost odd ratio probability increases from south to north (Figure 2.2B), while the opposite occurs for the length of the growing season (Figure 2.2A).
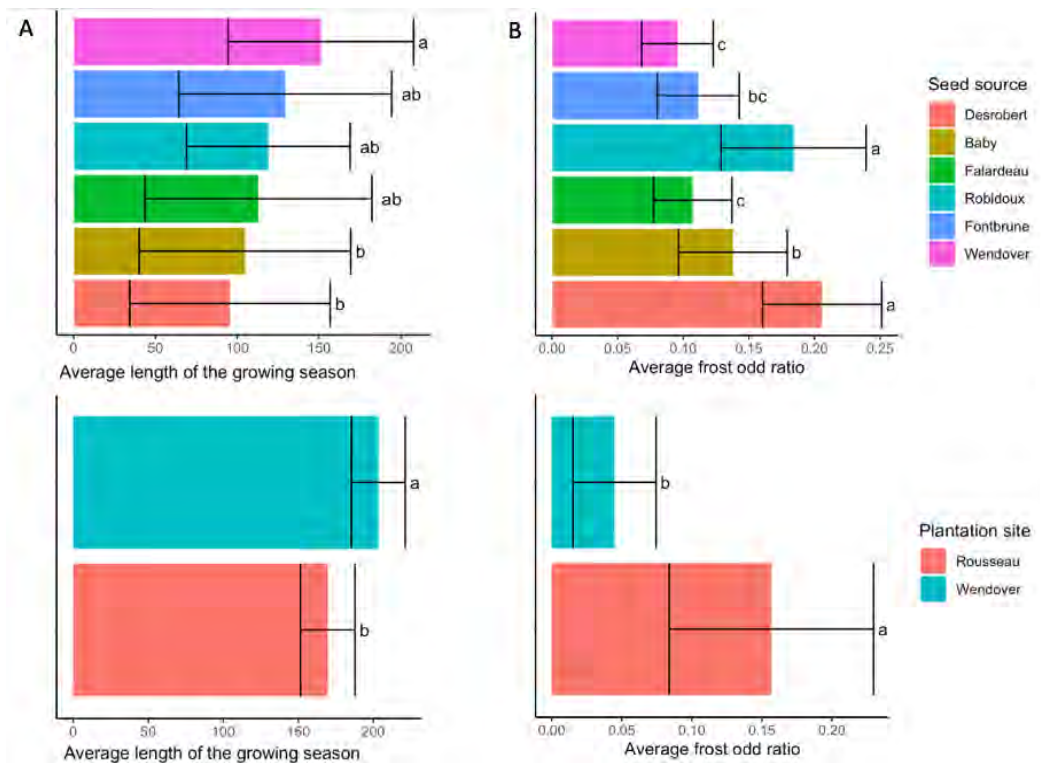
Figure 2.2 Means and standard deviations of the length of growing season (A) and frost odd ratio probability (B) for seed source origins and plantation test sites, with actual climate data (2010-2020) for plantation test sites and data of climates of origins (1940-1970) for seed sources. The beginning of the length of the growing season was determined as 6 consecutive days with temperature $\geq$ 5 °C, from June 1st to October 31st. Seed sources and plantation test sites are listed from north to south.

2.4.2. Chronology of bud set phenology.

The chronology of bud set of the studied seed sources followed a sigmoidal curve starting from 0 (last week of June) to 5 (final bud set stage: 1st week of August). The Wendover plantation test site showed an earlier bud set for all stages and all seed sources compared to those planted in the north, and all seed sources reached the final bud set stage at the same time. However, the last stage of bud set was similar among tested sites. Thus, the bud set period of the studied seed sources was longer at the southern plantation test site. On the other hand, the average normal GDD5 to initiate bud set was higher for the Wendover southern plantation test site, around 700 growing degree-days, versus 490 growing degree-days for the Rousseau northern test site (Figure 2.3).
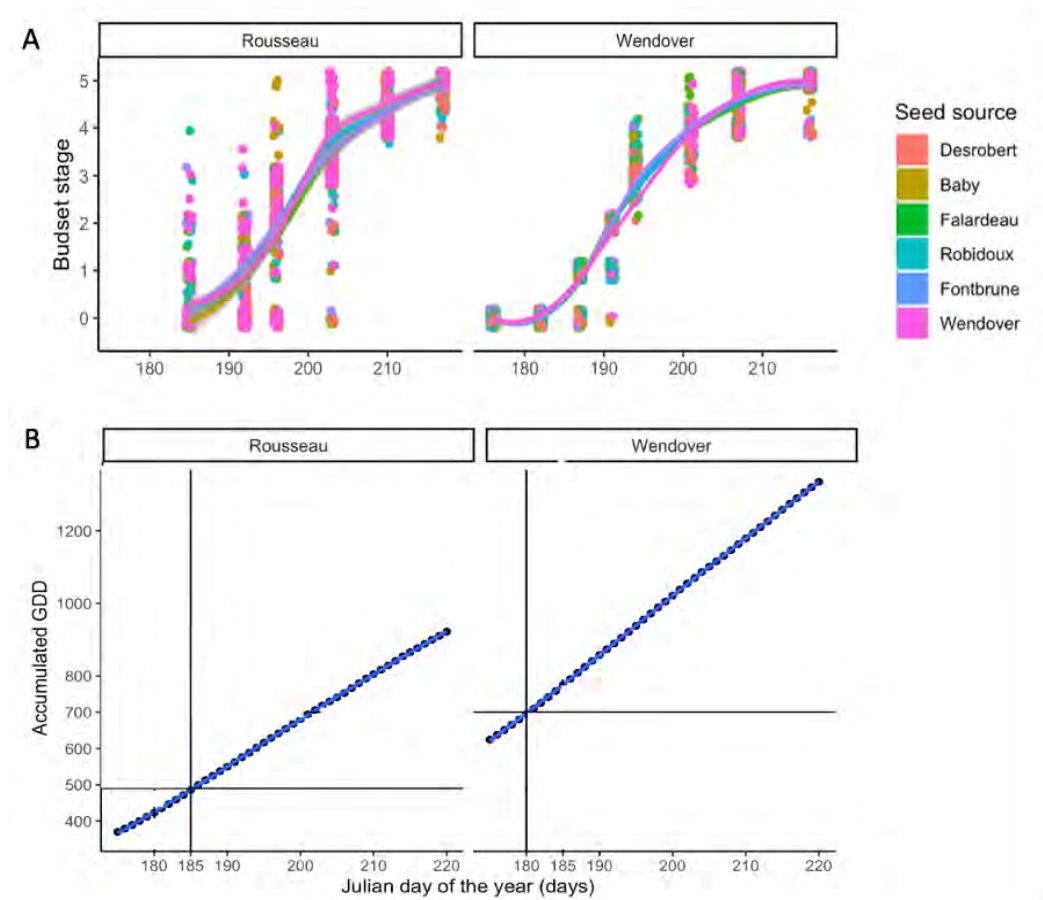
Figure 2.3 The chronology of bud set of the studied seed sources for each of the northern Rousseau and southern Wendover plantation test sites (A) presented with the corresponding average normal GDD (cumulative growing degree-days $\geq$ 5 °C calculated over a 10-year period (2010-2020)) (B). Seed sources are listed from south to north. Note: GDD5=0, if $(T_{min} + T_{max}) < 5°C$; and GDD5 = $(T_{min}+T_{max})/2 - 5$, if $T_{min} + T_{max} \geq 5°C$.

- Modeling bud set phenology of seed source at plantation test sites.

The ordinal logistic regression model of bud set explained 63% of the total variation according to McFadden R2 coefficient (Annex J). Bud set initiation was earlier in the southern plantation test site and for all bud set stages. It highlights a highly significant effect of the day of the year on the bud set transition (Figure 2.4A) and

less interference were recorded for stages 0, 4, and 5. However, stages 1, 2, and 3 showed moderate occurrence probability (with a maximum of 0.5) and higher interference between stages in the time frame between 185 and 205 Julian days of the year (Figure 2.4A). Regarding variation among seed sources, the same pattern was recorded for stages 0, 4, and 5, with higher occurrence probability at the beginning and the end of the bud set cycle. Higher variation was recorded for stages 1, 2, 3, and 4 during the time frame between 190 and 205 Julian days of the year, with moderate probabilities and higher interference between bud set stages (Figure 2.4B). All seed sources coefficients were significant compared to the Wendover seed source except Fontbrune, another southern seed source (Annexe J). There was a highly significant GxE interaction implicating the southern Wendover plantation test site and the seed sources Desroberts, Baby, and the local seed source of that plantation test site (Annexe J).
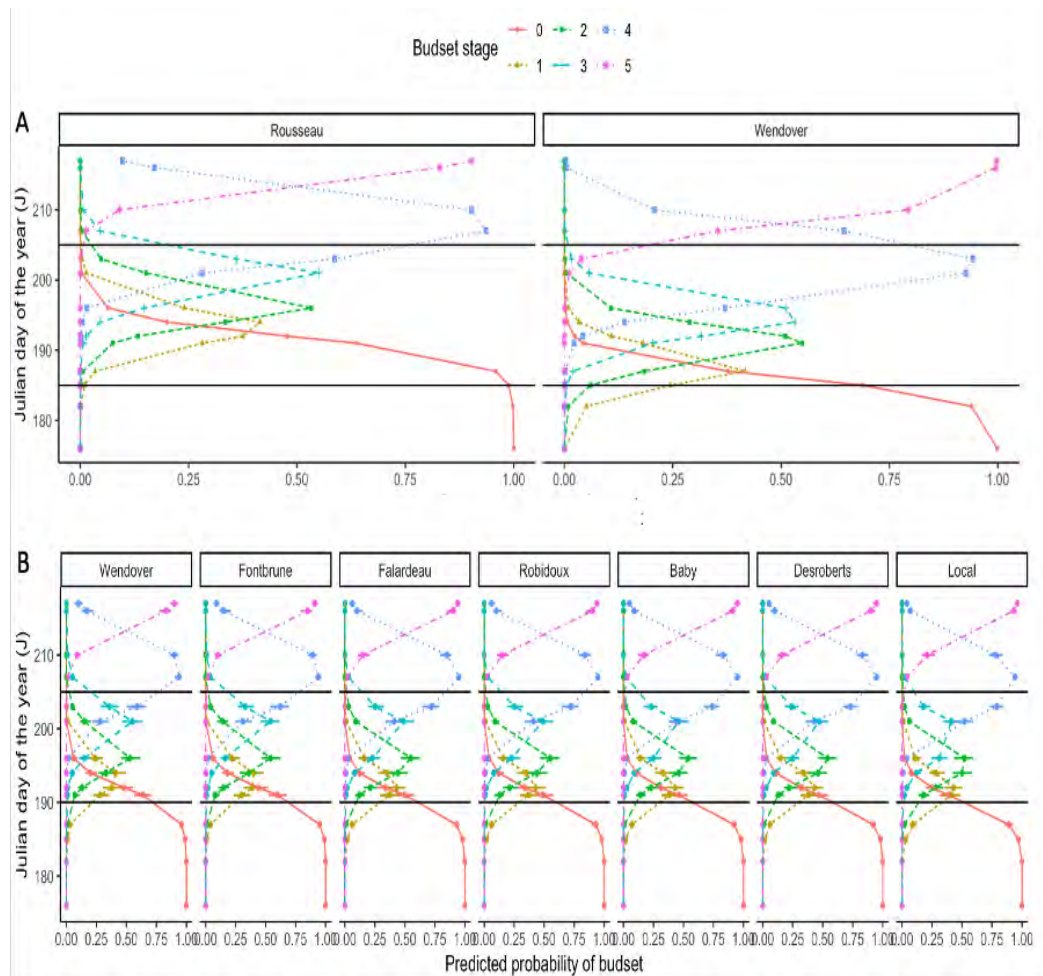
Figure 2.4 Predicted probability distribution and confidence intervals of bud set stages of white spruce by plantation test site and seed source through time: A) northern Rousseau and southern Wendover plantation test sites, and B) the seven seed source including those local to plantation test sites.

2.4.3. Levels of frost tolerance damage of terminal shoots and survival rate of seed sources at plantation test sites

The percentage of trees of each seed source showing shoot frost damage in both plantation test sites following the late cold spell of 2021 (Benomar et al., 2022) is

presented in Figure 2.5. Most seed sources planted in the southern plantation test site showed moderate to high damages to terminal shoots, with values ranging from 50% to 80% (particularly for local seed sources). This site also showed high variability compared to the northern plantation test site where most seed sources had a percentage of damaged terminal shoots around 50%. The lowest level of shoot damage was recorded for the Baby seed source (35%) in the northern plantation test site. Little significant differences were recorded among seed sources and between plantation test sites for shoot frost damage and survival rate (Figure 2.5).
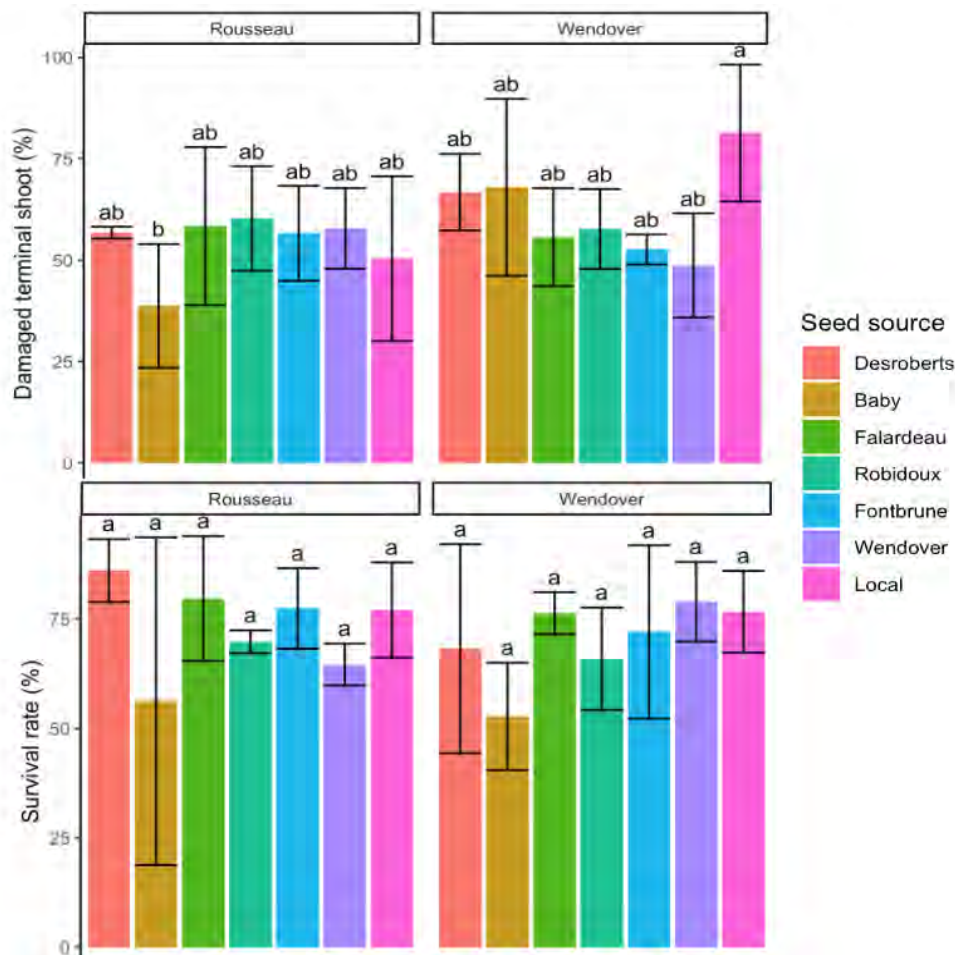
Figure 2.5 Distribution of the average damaged terminal shoots and live trees percentages for all seed sources within each plantation test site. Seed sources are listed from north to south.

2.4.4. Analysis of variance of frost tolerance, height, and detected NSC compounds

Relative electrolytic conductivity (REC) and leaf damage index (LDI) were significantly influenced by the plantation test site, the freezing temperature, and their interaction between both factors. However, only REC showed an additional significant effect of the triple interaction between plantation test site, seed source, and freezing temperature. For height, the seed source, plantation test site, and their interaction effects were highly significant. For NSC compounds, the plantation test site effect was significant for all detected sugars. Seed source and sampling date effects were only significant for sucrose content. However, the interaction between plantation test site and sampling date was significant for fructose, sucrose, and glucose compounds content (Table 2.2).

Table 2.2: Summary statistics of the analysis of variance (ANOVA) of frost tolerance indices, tree's height, and NSC content considering the effects of plantation test site, seed source, freezing temperature, and their possible interactions for frost tolerance. Plantation test site, seed source, freezing temperature, and their possible interactions for frost tolerance. Plantation test site, seed source, and their interaction for height. Plantation test site, seed source, sampling date, and their interaction for NSC content sugars. The mentioned terms are described as follows. DF: degree of freedom, SSQ: Sum of squares errors, MSE: Mean square errors. F-value: Fisher's test value, and p-value: level of significance.

Source of variation	Df	SSQ	MSE	F-val	P-val
A- Relative electrolyte conductivity (REC)					
Plantation test site	1	97.29	97.29	172.65	0.00
Seed source	6	2.07	0.34	0.61	0.72
Temperature	4	176.51	44.13	78.30	0.00
Site x Seed source	6	1.12	0.19	0.33	0.92
Site x temperature	4	122.09	30.52	54.16	0.00
Seed source x temperature	24	21.08	0.88	1.56	0.06
Site x Seed source x temperature	24	42.34	1.76	3.13	0.00
Residuals	135	76.08	0.56		
B- Leaf damage index (LDI)					
Plantation test site	1	445.13	445.13	109.3	0.00
Seed source	6	11.71	1.95	0.48	0.82
Temperature	4	920.59	230.15	56.53	0.00
Site x seed source	6	4.52	0.75	0.19	0.98
Site x temperature	4	550.74	137.69	33.82	0.00
Seed source x temperature	24	45.51	1.90	0.47	0.98
Site x seed source x temperature	24	48.31	2.01	0.49	0.98
Residuals	140	569.98	4.07		

Table 2.2: Continued.

C- 7 years height (cm)

Plantation test site	1	302814.68	302814.6	134.2	0.00
Seed source	6	90743.70	15123.95	6.90	0.00
Block	3	161498.98	53832.99	24.57	0.00
Site x block	3	39565.96	13188.65	6.02	0.00
Site x seed source	6	102863.52	17143.92	7.57	0.00
Residuals	2606	5709230.6	2190.80		

D- Fructose content (%)

Plantation test site	1	15.84	15.84	97.44	0.00
Seed source	6	0.98	0.16	1.01	0.42
Date	1	0.00	0.00	0.00	0.94
Site x seed source	6	0.13	0.02	0.13	0.99
Site x date	1	6.24	6.24	38.40	0.00
Seed source x date	6	0.82	0.14	0.84	0.54
Site x seed source x date	6	0.94	0.16	0.96	0.45
Residuals	220	35.77	0.16		

E- Glucose content (%)

Site	1	11.06	11.06	9.95	0.00
Seed source	6	13.47	2.25	2.02	0.06
Date	1	0.88	0.88	0.79	0.38
Site x seed source	6	5.42	0.90	0.81	0.56
Site x date	1	42.89	42.89	38.57	0.00
Seed source x date	6	9.59	1.60	1.44	0.20
Site x seed source x date	6	2.61	0.44	0.39	0.88
Residuals	220	244.69	1.11		

Table 2.2: Continued.

F- Sucrose content (%)

Site	1	29.61	29.61	27.52	0.00
Seed source	6	23.58	3.93	3.65	0.00
Date	1	16.27	16.27	15.12	0.00
Site x seed source	6	3.68	0.61	0.57	0.75
Site x date	1	128.33	128.33	119.2	0.00
Seed source x date	6	9.14	1.52	1.42	0.21
Site x seed source x date	6	2.26	0.38	0.35	0.91
Residuals	192	206.57	1.08		
G- Pinitol content (%)					
Site	1	6.30	6.30	63.88	0.00
Seed source	6	0.48	0.08	0.81	0.56
Date	1	0.02	0.02	0.18	0.67
Site x seed source	6	0.20	0.03	0.33	0.92
Site x date	1	0.04	0.04	0.37	0.54
Seed source x date	6	0.17	0.03	0.29	0.94
Site x seed source x date	6	0.31	0.05	0.52	0.79
Residuals	220	21.70	0.10		

2.4.5. Frost tolerance indices: Relative electrolytic conductivity (REC) and leaf damage index (LDI) of seed sources at plantation test sites

Significant differences in REC and LDI were detected by ANOVA and more details on the trends observed are presented here. The relative electrolytic conductivity (REC) boxplots of the studied seed sources showed an increasing pattern with high values (low frost tolerance) under -12 °C and -16 °C in the southern Wendover plantation test site (Figure 2.6A). In contrast, most of the seed sources in the northern Rousseau plantation test site showed a more uniform pattern following the decrease of freezing temperature. Thus, all seed sources planted in the north showed low electrolytic conductivity except Falardeau, which reflects their capacity to tolerate higher freezing temperatures. It is well known that low REC values reflect high frost tolerance (Lamhamedi et al., 2005, 2022) and, ultimately, that seed sources are more likely to adapt and grow under these freezing temperatures. Similar patterns were recorded for the leaf damage index (LDI), highlighting its strong correlation with (REC). Seed sources showing values around 2 (black horizontal line, Figure 2.6C) had no apparent frost damage, which was particularly the case for the Desroberts northern seed source and the Robidoux eastern seed source (Figure 2.6B, 2.6C). The identified patterns for REC and LDI were statistically significant (Table 2.2A, 2.2B) and confirmed by Tukey test (Figure 2.6), highlighting three different groups, mainly for the southern plantation test site (Figure 2.6A), with regards to freezing temperature: -16 °C, -12 °C and [-8 °C, 0 °C]. These groups also allowed to identify the most vulnerable seed sources (Falardeau and Wendover) under low-freezing temperatures (Figure 2.6B, 2.6C).
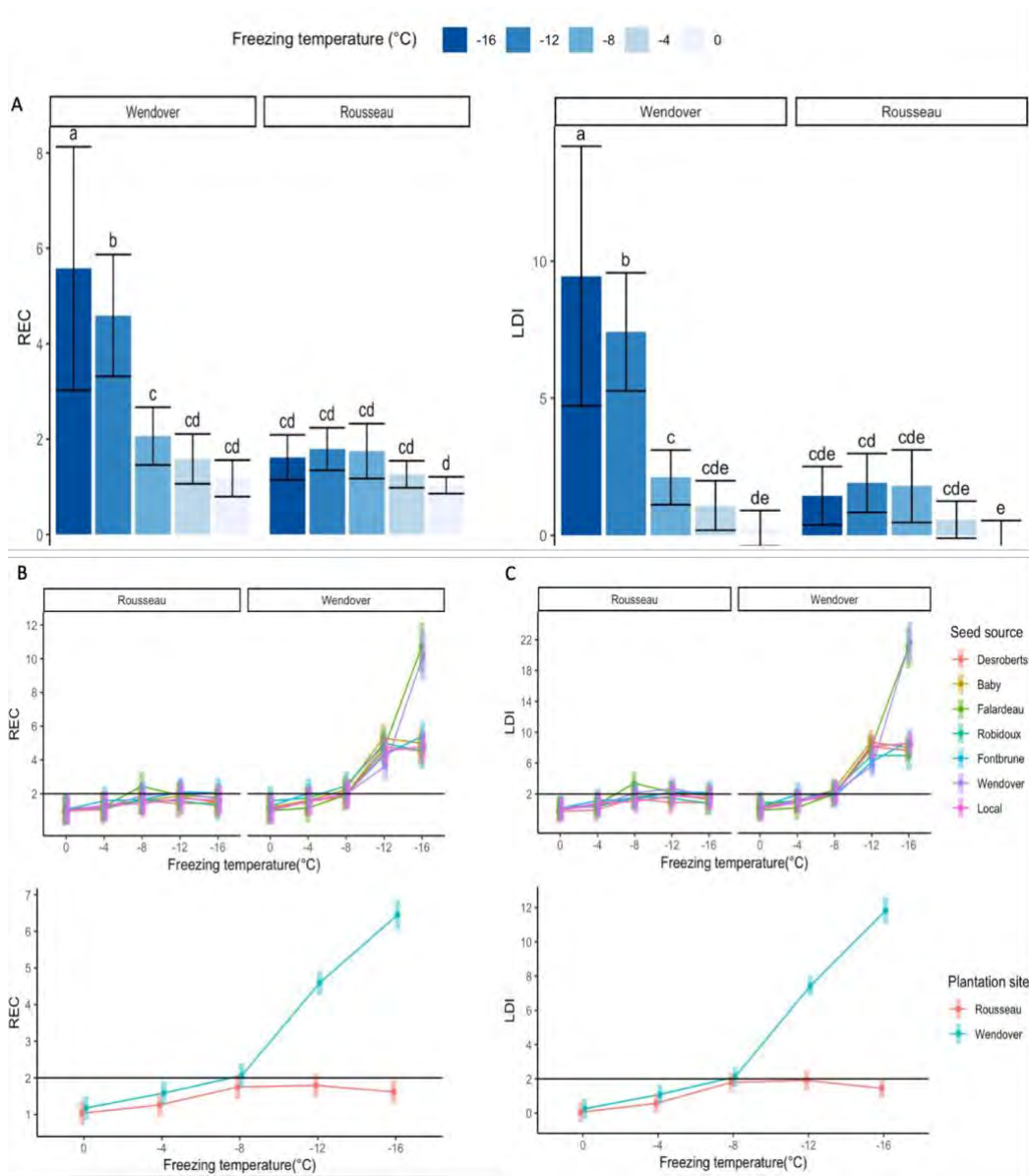
Figure 2.6 Estimated means and standard deviations with confidence intervals of relative electrolytic conductivity (REC) and leaf damage index (LDI) of the studied seed sources within each plantation test site (Rousseau and Wendover) and for the five tested freezing temperatures (A), (B) and (C) represent predicted REC and LDI under the effects of seed source, plantation test site, freezing temperature, and their interactions. Seed sources are listed from north to south.

2.4.6. Height variation within and among seed sources at plantation test sites.

Given that significant effects were detected by ANOVA for 7-year height, we looked at the variation in more details. The distribution of 7-year tree height showed higher variation among seed sources at the Wendover southern plantation test site compared to the Rousseau northern plantation site (Figure 2.7A). The average height in the northern plantation site ranged from 120 cm to 145 cm while it ranged from 130 cm to 185 cm in the southern plantation site. Then mid-latitude east Robidoux seed source recorded the lowest height in the southern plantation test site, while the Fontbrune southern seed source has recorded the maximal height (Figure 2.7B). The latter was significantly higher than the other studied seed sources planted in the south. However, the Desroberts northern and Wendover southern seed sources were approximately similar in terms of height. At the northern plantation test site, no significant differences were recorded between seed sources 7-years height (Figure 2.7A, 2.7B).

Little interaction with sites was observed for the seed sources Wendover, Falardeau, and Baby. The southern Fontbrune seed source showed an important increase in tree height, from 130 cm at the northern Rousseau site to 180 cm at the southern Wendover site, highlighting its positive response to warmer conditions in the south.
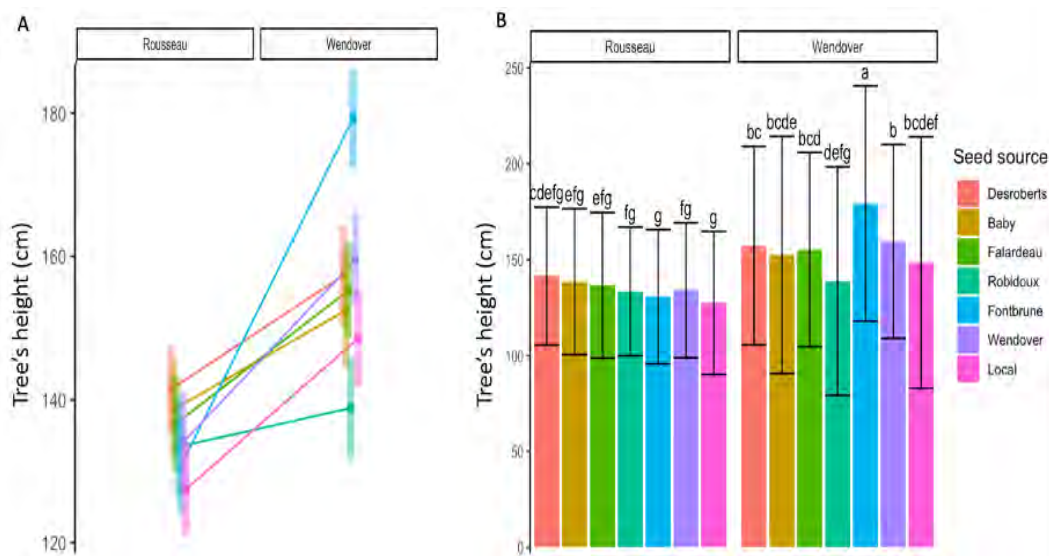
Figure 2.7 Distribution of means, standard deviations, and confidence intervals of 7-years tree height (cm) for all studied seed sources in the northern Rousseau and southern Wendover plantation test sites. A) Represents the confidence intervals of predicted 7-years height from (Table 2) and B) represents the Tukey test results with statistical differences and grouping letters; Seed sources are listed from north to south.

2.4.7. Dynamics of non-structural carbohydrates (NSC)

Given that significant effects were detected by ANOVA for non-structural carbohydrates, we were interested in looking at the variation in more details. The chemical analysis of NSC compounds detected four significant types of sugar: fructose, glucose, sucrose, and pinitol. Following the variation of NSC content, many spatio-temporal patterns were recorded for fructose, glucose, and sucrose content (Figure 2.8A, 2.8B, 2.8C, 2.8E) at both plantation test sites and for both sampling dates. From September to October, fructose and glucose contents increased in the south and decreased in the northern plantation test site (Figure 2.8A, 2.8B, 2.8E), while the opposite of this pattern was observed for sucrose

content (Figure 2.8C and 2.8E). However, pinitol content showed significant differences only between plantation test sites with higher values in the southern plantation test site. Significant seed sources effect was only recorded for sucrose content. The Robidoux seed source had the lowest sucrose content, while Desroberts, Baby, and Wendover seed sources had the highest values. No significant differences were observed between southern and northern seed sources. No significant correlations were recorded between southern and northern seed sources.
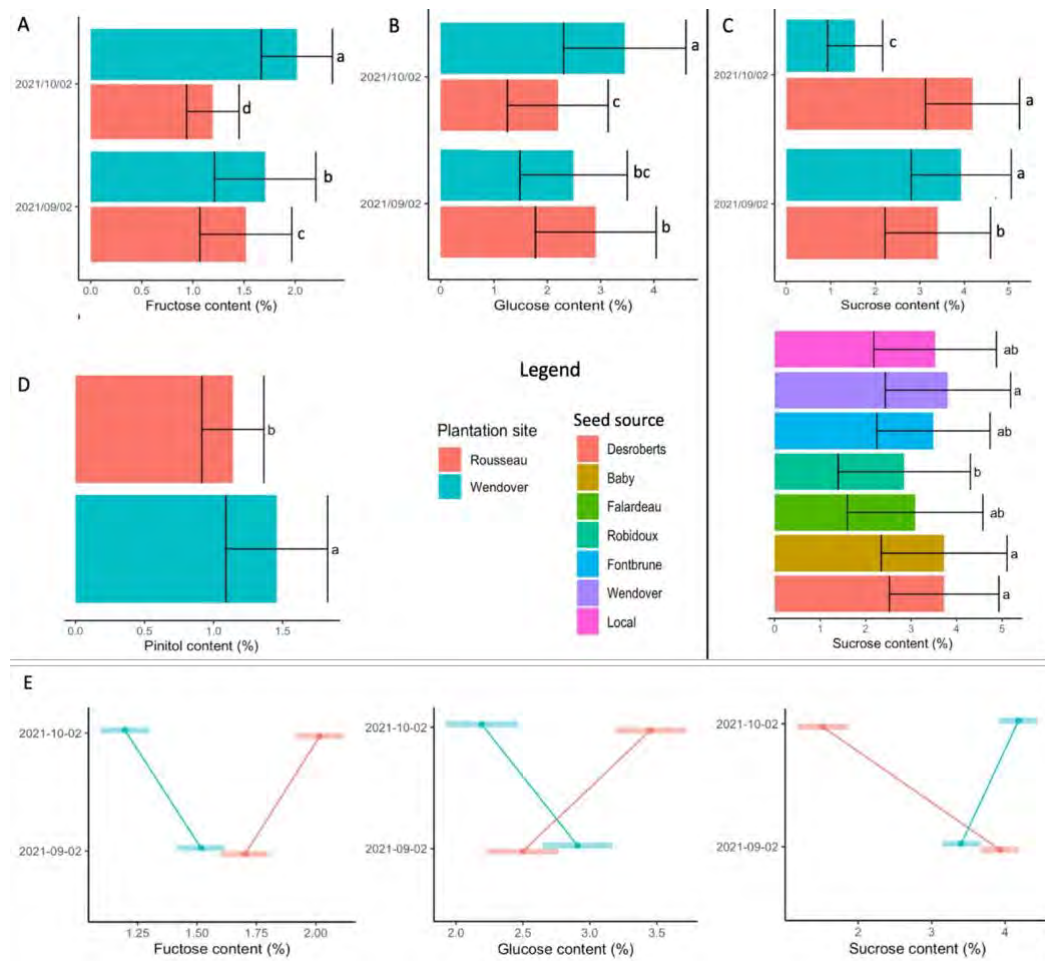
Figure 2.8 Means, standard deviations, and confidence intervals of interactions for all significant differences of non-structural carbohydrates (NSC) content detected between plantation test site, seed source, sampling date, and their interactions. Seed sources and plantation test sites are listed from north to south. The y axis of subplots colored by plantation site represent the sampling date.

2.4.8. Correlation heatmap of non-structural carbohydrates (NSC), height, frost tolerance indices, and bioclimate indices at plantation test site and seed source origin

The correlation analysis of the mean values of all variables taken pairwise showed multiple associations between functional traits, growth parameters, climates of seed source origins, and actual climates at plantation test sites (Figure 2.9A). The hierarchical clustering of similar correlations resulted in six distinct groups, among which four were highly significant clusters (Pearson test with a significance test of 0.05 risk level). The first group was characterized by the number of frost and icing days, the maximum length of the wet spell, and the daily temperature range describing thermal variation and humidity conditions association. The second significant group gathered pinitol and fructose contents, frost tolerance indices, and tree height. It described associations between ecophysiological traits and growth. The third group was more about the climatic conditions during the growing season, described mainly by maximal extremes of daily temperature and the number of summer days, which differentiate well the northern from the southern plantation test sites and seed sources origin climate. The fourth group was characterized mainly by precipitation variables and minimum values of daily maximum and minimum temperature highlighting the thermal amplitude and precipitation association. When excluding the averages of the plantation test sites, many biologically significant correlations were detected, highlighting the important role of ecophysiological plasticity in clustering patterns of associations between variables (Figure 9B).

Figure 2.9 Pearson correlation heatmap of the averages of frost tolerance indices, non-structural carbohydrates content, tree height, bioclimatic variables of seed source origins and plantation test sites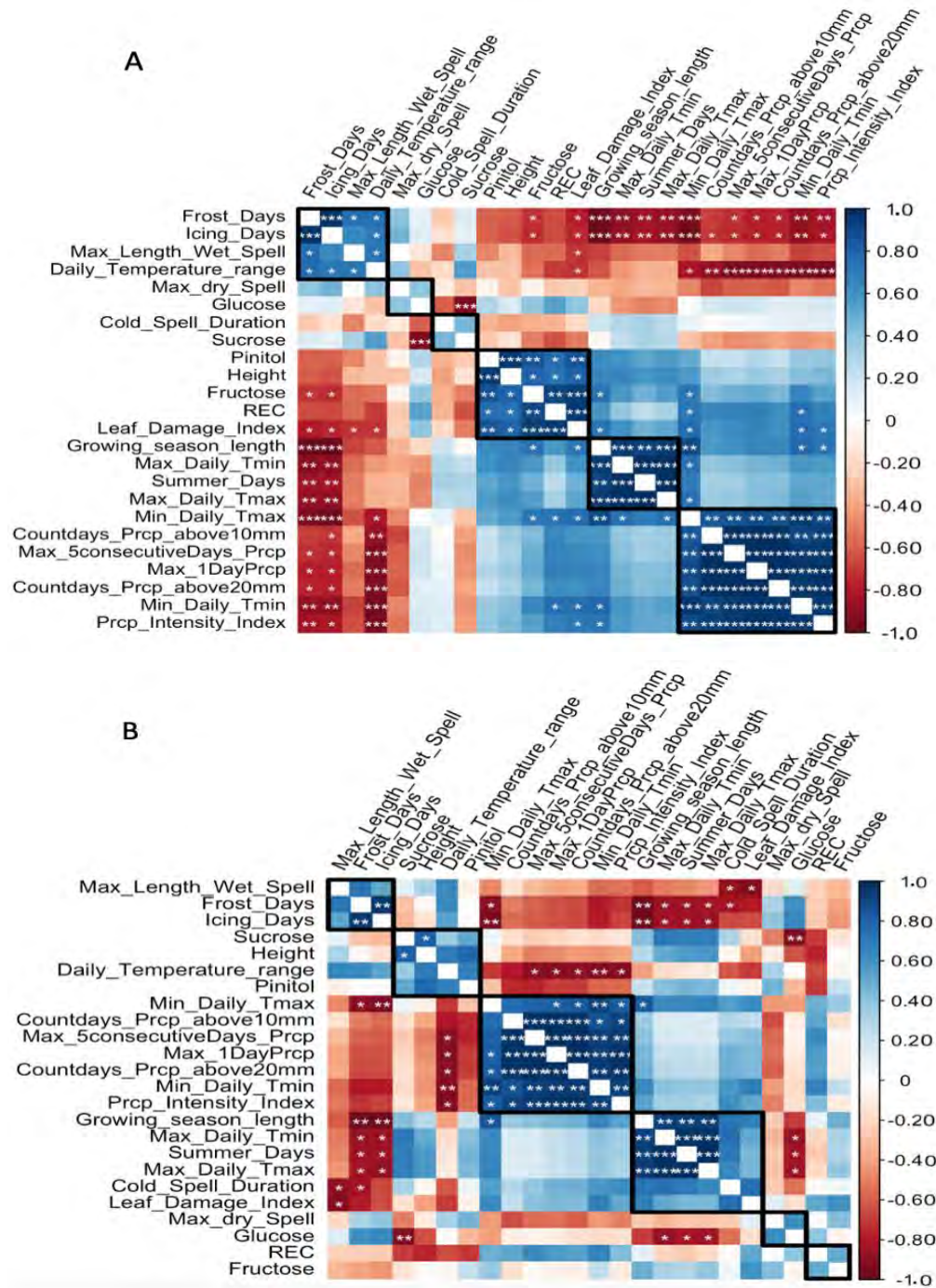 (A) and the same correlation heatmap without the plantation test site averages (B). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

This section reports on all significant pairwise correlations detected between frost tolerance indices, NSC content, tree height, and bioclimatic variables of seed source origins (Figure 2.10). Following the climatic gradient of seed source origins from north to south and from west to east, the average leaf damage index was negatively correlated with tree height. Shorter trees appeared to be more prone to frost damage than taller ones, which is likely due to cold air masses being physically closer to the ground during late cold spells (Benomar et al., 2022).

Fructose content was positively correlated with leaf damage index, with the lowest value being recorded for the northern seed source. The Falardeau from mid-latitude and Wendover southern seed sources had a similar leaf damage index for slightly different fructose content. The average fructose content explained 35% (R^2) of leaf damage index total variance (Figure 2.10).

Regarding the bioclimate variables, the average length of growing season, the minimum values of daily minimal and maximal temperature, and the precipitation intensity index showed positive relationships with the average leaf damage index. They explained, respectively, 46%, 39%, 48%, and 27% of the total variance of leaf damage index. Longer length of growing season was associated with higher leaf damage index (Figure 10), likely indicating a propensy for more southern seed sources to have suffered more damage for the 2021 late cold spell. The average leaf damage index was also positively correlated to the number of frost days and icing days, the maximum length of wet spell, and the daily temperature range value. They explained, respectively, 61%, 41%, 83%, and 48% of leaf damage index total variance. All observed relationships of leaf damage index to seed source origins climate generally followed the same north-south geographic gradient (Figure 2.10). An exception was the Falardeau seed source, which seems to be the most vulnerable and being out of the confidence interval of most of the fitted models (Figure 2.10). The high explained total variance of our models indicates the differential local adaptation of the studied seed sources. The rest of the unexplained variance was attributed mainly to plantation test sites and the plantation test site x freezing

temperature interaction, as identified previously for the leaf damage index (Figure 2.10).
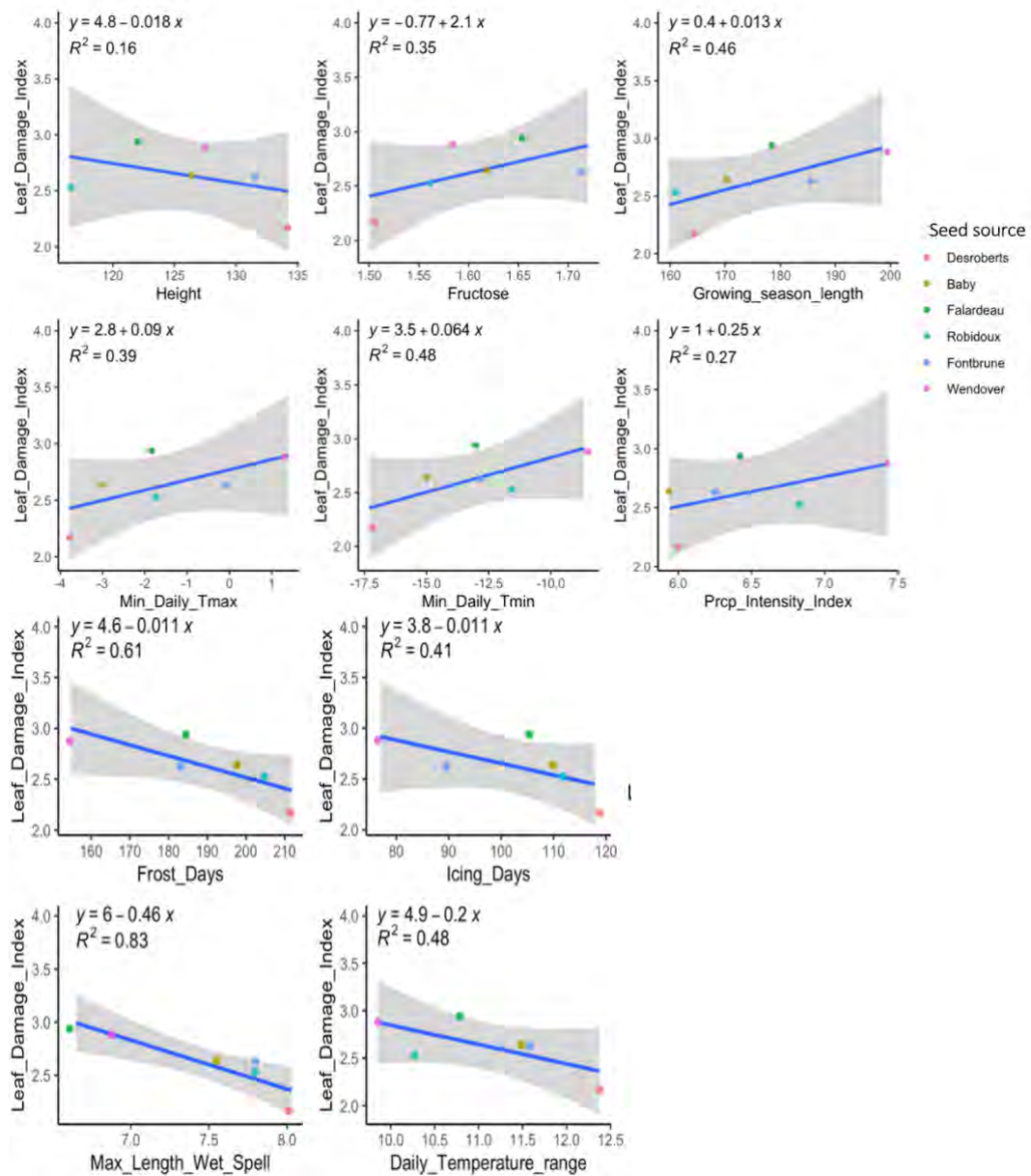
Figure 2.10 Simple linear regressions of average leaf damage index and confidence intervals versus all detected significant correlations with non-structural carbohydrates content, tree height, and bioclimatic variables of seed source origins.

Four significant correlations were recorded for the average REC in relation to NSC content (fructose and pinitol), height, and leaf damage index (Annexe E). An increasing pattern was recorded for fructose content and leaf damage index, explaining, respectively, 25% and 27% of REC's total variance. Knowing that high values of REC reflect low frost tolerance, we can clearly see that Desroberts seed source has exhibited the lowest REC value (highest frost tolerance) among the seed sources. However, Robidoux seed source recorded the highest REC value (lowest frost tolerance) at low to medium values of fructose. Regarding the leaf damage index, Desroberts seed source has recorded the highest frost tolerance and lowest frost damage, while Robidoux showed particularly the lowest frost tolerance with medium frost damage. Wendover and Falardeau have recorded the same leaf damage with medium frost tolerance among seed sources. On the other hand, height and pinitol content have exhibited a decreasing pattern in relation to REC and explaining, respectively, 59% and 49% of REC's total variance. The lowest REC (highest frost tolerance) value was recorded for the most northern Desroberts seed source having the maximum average height, while the most mid-latitude eastern Robidoux seed source has recorded the highest REC value (lowest frost tolerance) with the minimal height among seed sources (Annexe E).

2.5. Discussion

Given the predicted climate change in boreal forest, some species will be exposed to environmental stresses that are likely to cause important damage to trees with low dispersion ability and low plasticity. Thus, becoming maladapted to new conditions and vulnerable to competition against invasive species and climate extremes. Assisted population migration was suggested as a proactive approach to climate change adaptation. It consists of relocating species within their actual

natural range to environments experiencing climatic conditions like those to which they were adapted. In this study, the evaluation of six seed sources on two plantation test sites located along a climatic gradient from southern Wendover to northern Rousseau plantation test sites made it possible to simulate the effect of climatic transfers. Through the study of past and actual climate conditions, bud set phenology, growth, frost tolerance, and non-structural carbohydrates metabolism towards the end of the growing season, it was possible to gain knowledge on the genetic adaptation and plasticity of the studied seed sources in terms of growth and physiological responses to environmental changes.

2.5.1. Length of the growing season and frost probability of seed sources origins and plantation test sites

Going from the southern to northern locations for plantation test sites and seed source origins, the analysis of variance showed a decreasing pattern of the growing season length while the frost probability recorded a significant increase. The mid-latitude most eastern Robidoux seed source has unexpectedly recorded high frost probability (same level of the most northern Desroberts seed source) (Figure 2.4). This association was also confirmed by our principal component analysis of the climate of seed source origins and the actual climate of the plantation test site (Annexe D). Following the geographic gradient from south to north, northern locations were characterized by the number of frost and icing days, while the southern locations were characterized by the growing season length, summer days, and precipitation indices. Our findings about the growing season length and frost occurrence events association are aligned with the findings of Marquis et al. (2022). Besides, Ouranos confirmed these findings by mapping many frosts and growing

season indices under different climate scenarios in Québec on their climate platform (https://www.ouranos.ca/fr/portraits-climatiques, visited in 2022).

2.5.2.   Bud set phenology of seed sources in contrasted environments

Modeling the probability of bud set phenology through time has allowed us to identify the significant effects of the plantation test site, seed sources, and their interactions, in addition to the day of the year effect (Annexe J). The highly significant plantation test site effect highlights the phenotypic plasticity of bud set expression. These results are aligned with Jill et al. (2016), highlighting the earlier initiation of bud set in southern locations. However, other studies attested the early start of bud set in the more northern locations (Beaulieu et al., 2004; Lesser et al., 2004; Li et al., 1993, 1997). These differences might be related to large seed source variation, as well as local photoperiod and temperature, which represent the essential factors to initiate bud formation during the winter and the subsequent dormancy, as highlighted by Delpierre et al. (2015).

Most seed sources recorded highly significant effects compared to Wendover seed source. The exception goes to Fontbrune, reflecting the adaptive capacity of the studied seed sources. Our findings are aligned with previous studies suggesting high genetic control over the marginal effects of environment for the final bud set stages of white spruce (Perrin et al., 2017).

The highly significant effect of the day of the year reflects the temporal aspect of phenology, which is mainly related to temperature and photoperiod. Moreover, large genetic variation in the timing of bud set has been found among natural populations of white spruce in the northeastern part of its natural range, with southern populations showing later bud set and, accordingly, longer growing

season, resulting in larger growth (Li et al., 1993; Jaramillo-Correa et al., 2001). The same adaptive patterns were observed for black spruce in eastern Canada (Beaulieu et al., 2004, Perrin et al., 2017) and interior spruce in western Canada (Liepe et al., 2016).

Significant GxE interactions were recorded for Desroberts, Baby, and local seed sources. These interactions of northwestern and local seed sources suggest that bud set expression changes under warm conditions with long growing seasons. This was observed in the increase of likelihood probability of bud set stages between the 2nd and 4th bud set stages compared to more southern seed sources. Thus, northern seed sources would benefit from longer growing seasons under climate change. This will allow them to increase their growth through the extension of growing season, while maintaining a high level of frost tolerance.

2.5.3. Survival rate of seed sources at plantation test sites

The survival rate of seed sources in the northern Rousseau plantation test site was above 75% for three seed sources: Desroberts, Falardeau (from the northwest and mid-latitude), and Fontbrune (from the south), while the three other seed sources (Baby from the western mid-latitude, Robidoux from the northeast, and Wendover from the south) showed a lower survival rate around 60%. In the southern Wendover plantation test site, most seed sources exhibited a high survival rate above 70%, except for Baby, with a 50% survival rate. Local seed sources showed approximately the same survival rate above 75% in both plantation test sites. These results may underly adaptive genetic variation associated with local climates of seed source origins, given that many significant relationships have been noted between provenance variation and allelic variation at adaptive genes and diverse factors

related to climate in white spruce and other conifers in the region, with climatic variation following mainly a north-south latitudinal trend in temperature and east-west longitudinal trend in precipitation (Li et al., 1997; Beaulieu et al., 2004; Namroud et al., 2008; Prunier et al., 2011, 2013; Hornoy et al., 2015; Villeneuve et al., 2016; Depardieu et al., 2020, 2021). The survival rate was quite lower compared to previous studies of the same seed sources and study area, which reported a 98% of survival rate at the early stage after plantation (Villeneuve et al., 2016; Otis Prud'homme et al., 2018). Besides the increasing occurrence of drought episodes during the growing season in temperate/boreal ecozones at mid-latitudes (Depardieu et al., 2020), which could affect severely the ability of young white spruces to survive and grow (Soro et al., 2022), this decreased survival rate could also be explained, in part, by the increasing occurrence of late spring frosts preceded by warmer springs promoting an early start of the growing season in the south, such as that observed during spring 2021 (Benomar et al., 2022). The proportion of damaged terminal shoots in this study confirmed this hypothesis by recording high damage rates, more than 80%, in the south for all seed sources compared to more than 60% in the north.

2.5.4. Frost damage and tolerance of seed sources in contrasted environments

The relative electrolytic conductivity (REC) and leaf damage index (LDI) of seed sources in both plantation test sites and under different freezing temperatures showed significant effects of site, freezing temperature, their interaction, and the triple interaction (site x seed source x freezing temperature), accounting for more than 85% of the total variance for each index. These two parameters were used as indirect methods in many studies to test for the frost tolerance of many coniferous trees, such as jack pine, black spruce, lodgepole pine, and white spruce (Lamhamedi

et al., 2005, 2022; Man et al., 2021). Our results showed that most seed sources become vulnerable to frost damage under -12 °C when planted in the south. This level of vulnerability remains the same under -16 °C except for the mid-latitude Falardeau seed source and the southern Wendover seed source. These two seed sources have exhibited the highest levels of vulnerability to frost damage (Figure 2.8). However, all seed sources maintained high levels of frost tolerance when planted in the north, even under -12 °C and -16 °C.

These results underline the phenotypic plasticity of frost tolerance following the climatic gradient. Previous studies of the eco-physiological traits of white spruce confirmed that the values of functional traits are significantly different from site to site (Benomar et al., 2016). For example, Schuch et al. (1989a, b) found that the cold hardiness of Douglas fir seed sources was mainly affected by plantation locations in Oregon. Those planted in the north showed the highest frost tolerance and the earliest build-up of cold hardiness. Repo et al. (2000) also reported that the onset of hardening and the increased build-up of frost tolerance was earlier for northern seed sources of Scots pine. For white spruce, Lamhamedi et al. (2005) found that the build-up of frost tolerance is a progressive process varying significantly over time (end of August to mid-October). The general pattern is thus one where during the end of summer and fall seasons, bud formation and the build-up of frost tolerance are intimately related to the decrease of photoperiod accompanied by temperature decrease (Bigras & D'aoust, 1992; Lamhamedi & Bernier, 1994; Johnsen & Skrøppa, 2000; Howe et al., 2003). In the light of our frost tolerance analysis, most seed sources planted in the north acquired frost tolerance under -16 °C earlier and showed no damage to tested samples. Among more southern seed sources, the southwestern Fontbrune seed source showed moderate frost tolerance when planted in the south compared to the Wendover seed source or the mid-latitude Falardeau seed source (same longitude as Wendover seed source). Thus, Fontbrune is likely to be a good candidate for white spruce transfer northward under future climate conditions where it will benefit from warm conditions for growth and moderate frost tolerance. This can be confirmed through

the sampling over many time points, particularly after the end of cold hardiness phenology, to better understand the timing of cold resistance acquisition at the end of the growing season.

2.5.5. Growth performance of different seed sources contrasted environments.

The 7-year height showed highly significant effects of seed sources, plantation test sites, and their interaction. The average height of trees of all seed sources combined was higher in the southern plantation test site, highlighting the effect of the warmer climate conditions on growth productivity. The Wendover southern seed source and Desroberts northern seed source have recorded similar performance in the southern plantation test site. Our results are partially aligned with the findings of Benomar et al. (2016) and Otis Prud'homme et al. (2018) regarding the growth performance of the same white spruce seed sources in contrasting environments. They have reported significant effects of the plantation test site and the studied seed sources at their juvenile stage. However, no significant genotype x environment (GxE) interactions were reported, presumably because the trees were assessed at a very young age (3 to 4 years). However, Lu et al. (2014) reported a significant GxE interactions for different white spruce seed sources' growth performance in contrasted environments. Xie, (2003) has also reported a highly significant GxE interaction effect on 10-year height among 232 open-pollinated interior spruce families in north central interior British Columbia, where altitudinal variation is much more pronounced than in eastern Canada. In our study, the GxE interaction implicating mostly the Fontbrune southern seed source could be explained by genetic adaptation to the local climate of origin, which is characterized by minimal and maximal daily extreme temperatures (T_{min} and T_{max}) together with high values of precipitation variables (maximum 1-day precipitation and max 5 consecutive

days precipitation) (Annexe D). In fact, the climate profile of the Fontbrune seed source origin had similarities with the climate envelopes of both plantation test sites. Combined with the moderate vulnerability of this seed source to frost damage (Figure 2.6), these adaptations could explain the important shift of the Fontbrune tree height when planted in the south. Thus, the Fontbrune seed source would benefit more than others from longer growing seasons under future climate conditions.

2.5.6.   Dynamics of non-structural carbohydrates (NSC)

The highly significant effects of plantation test site were recorded for fructose, glucose, sucrose, and pinitol, suggesting strong environmental effects on non-structural carbohydrate content, which contributed to cold hardiness and thus to the build-up of frost tolerance. Fructose, glucose, and pinitol content decreased northwardly, reflecting their use during the growing season under warm conditions, while sucrose increased northwardly, reflecting their use for the initiation of dormancy and cold resistance acquisition under cold conditions (Figure 2.8). The recorded correlations between the NSC contents reflected their interrelated metabolism. In fact, the ability of plants to synthesize and accumulate sucrose in leaves is mainly determined by the concerted action of three enzymes: sucrose degrading sucrose synthase (SS), acid invertase (AI), and sucrose accumulating sucrose phosphate synthase (SPS) (Rinne et al., 2015). The SS reactions are reversible, while AI reactions, which produce glucose and fructose by hydrolyzing sucrose, are irreversible (Li et al., 2006). Simard et al. (2013) reported sucrose to be the least abundant sugar in conditions of high demand for growth, explaining its increase northwardly in our study. On the other hand, high concentrations of sugars, which have a role in cryoprotection and osmotic adjustments (Hoch & Korner,

2003), were described as a strategy to survive frequent biomass losses caused by extreme climate, mainly during the growing season (Sveinbjörnsson, 2000). Other studies reported high levels of sugars during the growing season in trees growing under cold conditions (Kagawa et al., 2006; Simard et al., 2013), which could explain the observed northward increase of sucrose content used in cold resistance acquisition.

Significant effects of the sampling date were detected for fructose, glucose, and sucrose content which indicate the presence of dynamic metabolism in response to environmental changes. The interaction between plantation test site and sampling date was significant for fructose and glucose content, with an increasing pattern in the south and a decreasing pattern in the north over time. The opposite of these two patterns was recorded for sucrose content. These spatio-temporal interactions suggest the presence of dynamic plasticity of NSC compounds expression. The negative associations of fructose to the numbers of icing days and frost days and its positive association with the growing season length confirmed that fructose is more related to growth and warm conditions (Figure 2.9, A).

Regarding seed sources effects, only sucrose was significantly low for Robidoux most eastern mid-latitude seed source compared to other seed sources reflecting its climate conditions, especially for being exposed to high humidity waves coming from the ocean.

2.5.7. Frost tolerance association with the averages of growth, the NSC compounds content, and the climate of seed sources origins

The leaf damage index (LDI) showed a negative correlation with height and a positive correlation with fructose content. The taller the tree, the less it is exposed to frost damage, and the higher fructose content, the higher frost damage. Indeed, Marquis et al. (2020) have found that taller white spruce trees are less exposed to frost damage. For fructose, Simard et al. (2013) have found that it is related to high growth conditions and tends to be low in colder regions, as identified for the northern Desroberts seed source (Figure 2.10). Besides, previous studies showed that soluble sugars, including sucrose and pinitol, accumulate in leaf tissues to enhance freezing tolerance in pine trees (Strimbeck et al., 2008; Angelcheva et al., 2014).

Regarding the climate of seed source origins, the growing season length, the minimum daily value of maximal temperature, the minimum daily value of minimal temperature, and the precipitation intensity index were positively correlated with the leaf damage index. This pattern was particularly the same going from northern to southern seed source locations. Knowing that the longer the growing season of southern seed sources expose them more to frost damage. The minimum values of daily minimal and maximal temperature characterized mainly the intersection of both climatic southern and northern envelopes as described in the Annexe D. The precipitation intensity index, which is an important growth parameter, was also positively correlated with leaf damage index. Besides, the higher precipitation intensity was also correlated with the growing season length, which explains the higher frost damage for southern seed sources.

On the other hand, negative associations of leaf damage index were recorded with the number of frost days, icing days, the maximum length of the wet spell, and the temperature range. Indeed, our principal component analysis of bioclimate

parameters at plantation test site and seed source origins showed that frost days and icing days characterized northern locations and were negatively correlated to summer days, precipitation intensity and growing season length (Annexe D), which explains the high leaf damage values to the most southern seed source (Wendover). These negatively correlated bioclimatic indices at plantation test site are more likely to induce cold hardiness and dormancy for trees, thus decreasing the propensity for leaf damage. These associations are mainly due to plasticity as found in the correlation heatmap with and without plantation test site averages (Figure 2.9, A & B).

Our findings are aligned with the results of Sebastian-Azcona et al. (2018), indicating a significant relationship between cold hardiness (at -30 °C) and the length of the growing season, coldest monthly temperature, winter temperatures, mean annual temperature, and first frost date.

The REC of seed sources was positively correlated to leaf damage index and fructose content. Knowing that low REC values reflect high frost tolerance levels, white spruce trees tend to have low frost damage when planted in the north. This response is partially due to the adaptive capacity of seed sources to local conditions. On the other hand, negative correlations were recorded for height and pinitol content.

Tall trees recorded low REC values (Annexe E), which could be partly explained by the reduced exposure to cold temperature coming from the soil surface, but also due to the plasticity of frost tolerance as highlighted in our freezing test (Figure 2.6). The pinitol content's negative association with REC (Annexe E) highlights the important role of pinitol in cold resistance acquisition as cited by (Strimbeck et al., 2008; Angelcheva et al., 2014).

2.5.8. Implications for assisted migration

Our findings highlight the local adaptation, environment, and GxE effects on growth and bud set phenology of the studied seed sources in contrasted environments. The temporal factor effect was mainly significant for Non-structural carbohydrates (NSC) and bud set, reflecting the relationship of these two components to white spruce growth cycle. In addition, frost tolerance and NSC content plasticity was highly significant. Our results also showed significant associations between frost tolerance, NSC content, growth, and climate of seed source origins and plantation test sites. These key patterns will help improve seed transfer modeling, investigate potential hotspots for assisted migration at a large scale, and reduce the assisted migration frost risks of white spruce.

2.6. Conclusion

The assessment of bud set phenology, frost tolerance, 7-years tree height, and the non-structural carbohydrates for various seed sources grown in two contrasting environments allowed us to identify the effects of plantation test sites, seed sources, and their interactions, in addition to the timing of sampling dates for non-structural carbohydrate compounds. The following observations should be highlighted.

The onset bud set phenology was earlier in the southern Wendover plantation test site for all seed sources, and most seed sources effects were significant for each stage of bud set transition. The Baby and Desroberts northeastern seed sources and

the two local seed sources at plantation test sites showed significant interactions compared to Wendover seed source.

The frost tolerance showed significant effects of seed sources and plantation test sites, together in interaction with freezing temperature. The central mid-latitude Falardeau and southern seed sources showed the highest vulnerability to frost under -16°C freezing temperature. Thus, they are more likely to suffer from frost damage and have lower growth under future climate conditions if planted in the north. However, the other seed sources showed moderate frost vulnerability while maintaining the same level of frost tolerance under -12 °C and -16 °C, which makes them more likely to grow well under future climate conditions when transferred towards the north.

The significant effects of plantation test site, seed source, and their interactions recorded for 7-year height highlighted the adaptive capacity and plasticity of white spruce seed sources. The Fontbrune seed source outperformed all other seed sources under warmer conditions. This seed source appears to be a good candidate for northwardly transfer, considering its moderate frost tolerance under -16°C and its climate of origin, which overlapped the climatic envelopes of both the Wendover and Rousseau plantation test sites.

The non-structural carbohydrates analysis showed significant plasticity and dynamics. The identified spatio-temporal patterns have suggested that dynamic changes in the carbon pool are taking place at the end of the growing season and play a crucial role in growth, cold hardiness, and frost tolerance.

The climate of seed source origins and the actual climate means at plantation test sites showed significant correlations with the two frost tolerance indices, height, and NSC compounds. These associations have allowed us to map the complex relationships between growth, frost tolerance, and non-structural carbohydrates compounds roles, in growth and cold resistance, in relation to climate conditions.

2.7. Data Availability Statement

The raw data & script supporting the conclusions of this article will be made available by the authors upon request.

2.8. Author Contributions

CA collected data, conducted statistical analyses, and drafted the manuscript. LB collected data, reviewed, and improved the manuscript. MP and JG participated in obtaining funds, providing important support and access to the MFFP laboratory and plantation tests, and reviewed and improved the manuscript. YB and JBe participated in obtaining funds and helped drafting the manuscript. JBo planned and designed the study, drafting the manuscript and funding of this project. ML planned and designed the study, data collection, and drafting the manuscript and funding of this project. All authors reviewed the manuscript and approved the final draft.

2.9. Funding

2.10. Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

2.11. Acknowledgments

CHAPITRE III : CONCLUSION GÉNÉRALE

3.1. Objectifs et conclusions principales

La présente étude, axée sur l'épinette blanche, fournit une cartographie détaillée des effets génétique, environnemental et leur interaction sur la croissance et la tolérance au gel. Il s'en ajoute l'effet temporel sur la phénologie d'aoûtement et la dynamique des sucres non structuraux. Par ailleurs, l'effet du climat d'origine des sources de semence et le climat actuel des sites de plantation ont été explorés également par rapport aux paramètres de croissance et de tolérance au gel. Ainsi, les patrons généraux ont été identifiés et discutés en relation avec la migration assistée comme étant une solution proactive d'adaptation au changement au climatique.

En effet, la croissance des sources de semence testées était supérieure sous des conditions chaudes avec une meilleure performance de Fontbrune. C'est une source de semence provenant du sud-ouest du Québec. Fontbrune a montré une tolérance au gel modérée au Sud entre -12 °C et -16 °C de température de congélation. De 0 °C à -8 °C, aucun dégât de gel n'a été observé pour toutes les sources de semences et dans les deux sites de plantation suggérant la présence d'un seuil minimal commun de résistance au gel (-8 °C). Allant jusqu'à -16 °C de température de congélation, les sources de semences : Wendover (provenant du sud-est) et Falardeau (provenant des latitudes moyennes et la même longitude que Wendover) étaient les plus vulnérables. C'est deux sources de semence sont susceptibles d'enregistrer de plus faibles performances sous les conditions climatiques futures. En effet, l'occurrence des extrêmes climatiques pendant la saison de croissance (notamment le gel automnal et printanier) réduiront significativement leur croissance annuelle (Benomar et al., 2022). Cette réduction pourrait être aggravée

par l'occurrence des épisodes de sécheresse sous un climat future réchauffé (Depardieu et al., 2020). La tolérance au gel élevée aux sites de plantation nordique a été aussi souligné par Repo et al. (2000) pour le pin sylvestre.

La probabilité d'occurrence des stades de formation du bourgeon des sources de semences testées était significativement différente entre elle et entre les sites de plantation, avec un effet significatif du jours de l'année. L'effet du site était trois fois plus important que l'effet génétique. La meilleure source de semence en termes de croissance (Fontbrune) n'a pas montré de différences significatives en termes de formation du bourgeon suggérant sa grande plasticité d'expression, d'où sa meilleure performance. Li et al. (1993) et Jaramillo-Correa et al. (2001) ont souligné l'effet génétique et l'effet du site de plantation sur la formation du bourgeon. De plus, Delpierre et al. (2016) ont trouvé que la formation du bourgeon commence plutôt au Sud qu'au Nord.

Le glucose, le fructose, le sucrose et le pinitol sont les quatre sucres non structuraux détectés. Leurs teneurs étaient significativement différentes entre les sites de plantation confirmant le rôle de l'environnement dans la régulation du métabolisme des sucres de croissance et de résistance au froid. En effet, la teneur des trois premiers sucres était aussi significative entre septembre et octobre. Le fructose et le glucose ont diminué au Sud et ont augmenté au Nord vers la fin de la saison de croissance alors que le sucrose a enregistré un patron opposé avec une diminution importante au Nord et une teneur moyenne supérieur au Sud. La teneur en pinitol était aussi significativement supérieure au sud.

En combinant ces patrons généraux avec les variables climatiques, les paramètres de croissance et de tolérance au gel, on a pu déterminer les sucres associés à la croissance et ceux associés à la résistance au froid. On a également identifié la nature du lien entre la tolérance au gel, le climat d'origine et le climat actuel.

En effet, la teneur en fructose était négativement corrélée au nombre de jours de froid et de gel et positivement corrélé à la longueur de la saison de croissance, la hauteur et l'indice de dommage foliaire sous l'effet principal du climat actuel du site de plantation. Simard et al. (2013) ont aussi trouvé que les teneurs élevées en fructose sont associées à des conditions favorables de croissance alors qu'il est présent à très faibles teneur sous des conditions froides. Ainsi, les teneurs élevées en fructose peuvent être détectées pendant la phase active de croissance où les arbres d'épinette blanche sont plus vulnérables à l'occurrence du gel. Ceci est partiellement vrai pour le pinitol vu ses corrélations positives avec les indices de tolérance au gel et la hauteur. Alors que la teneur en sucrose était négativement corrélée à l'indice de dommage foliaire et la conductivité électrolytique relative (positivement corrélé à la tolérance au gel). D'où le rôle du sucrose en acquisition de la résistance au froid (Kagawa et al., 2006 ; Simard et al., 2013). Concernant la tolérance au gel et sa relation avec le climat, le nombre de jours de froid et de gel, l'amplitude de la température journalière, étaient négativement corrélés à l'indice de dommage foliaire sous l'effet principal du climat actuel du site de plantation.

3.2. Implications et recommandations

L'ensemble de ces résultats permettra d'améliorer la précision des modèles de transfert des semences à l'échelle du Québec et d'orienter les efforts d'amélioration génétique de l'épinette blanche afin de maintenir une meilleure productivité et une bonne adaptabilité aux conditions climatiques futures. En plus, les principaux résultats peuvent servir à approfondir la compréhension des mécanismes d'adaptation de l'épinette blanche à la suite du changement de son environnement en explorant d'avantage les liens entre son écologie et sa physiologie. Par ailleurs,

les liens climatiques identifiés peuvent servir à identifier les meilleurs sites de migration assistée favorisant une productivité élevée et une bonne tolérance au gel.

En guise de conclusion, on recommande d'investiguer d'avantage la tolérance au gel et la dynamique des sucres non-structuraux en relation avec la phénologie d'aoûtement via l'augmentation des dates d'échantillonnage afin de pouvoir superposer les trois composantes sur l'axe du temps et d'espace tout en considérant l'aspect climatique actuel et futur. Ceci permettrait de juger la stabilité des patrons détectés et ajuster la stratégie d'adaptation aux changement climatiques.
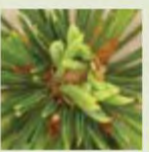
ANNEXE A: PHOTOPERIOD
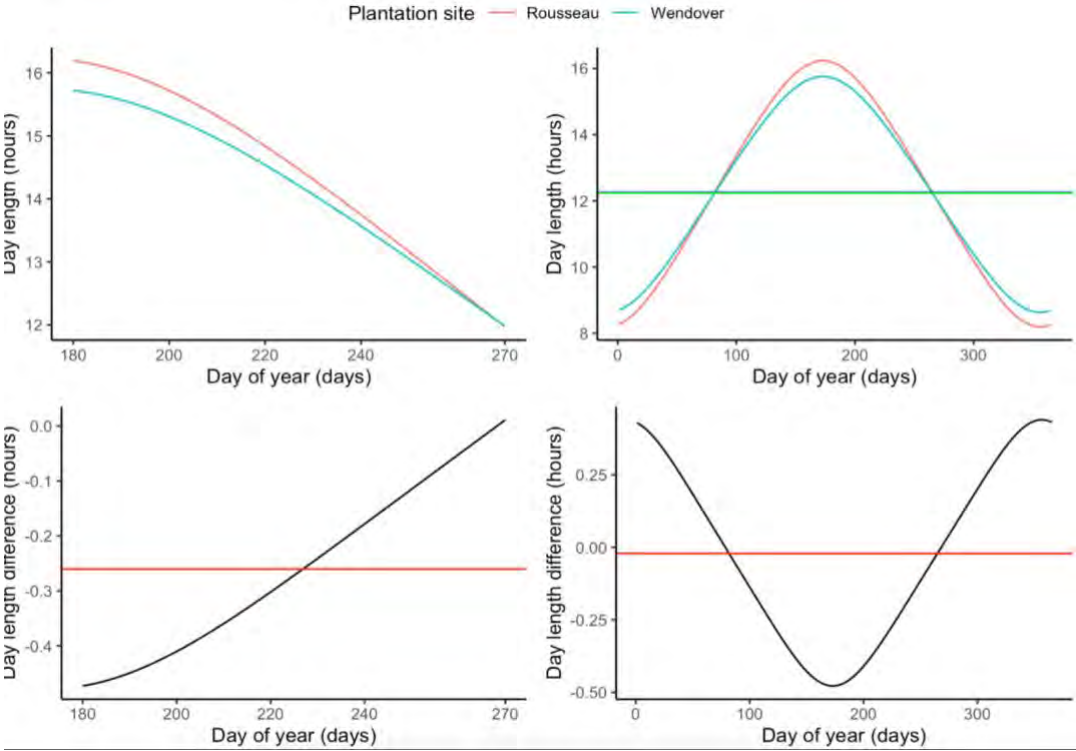
Annexe A Day length distribution over a year for both plantation test sites and the observed differences between them. The distribution was also recorded from the first day of budset to the last day of the year. The red and green horizontal lines represent the average day length and average day length difference between site over the corresponding period.

ANNEXE B: ANNUAL FROST PROBABILITY
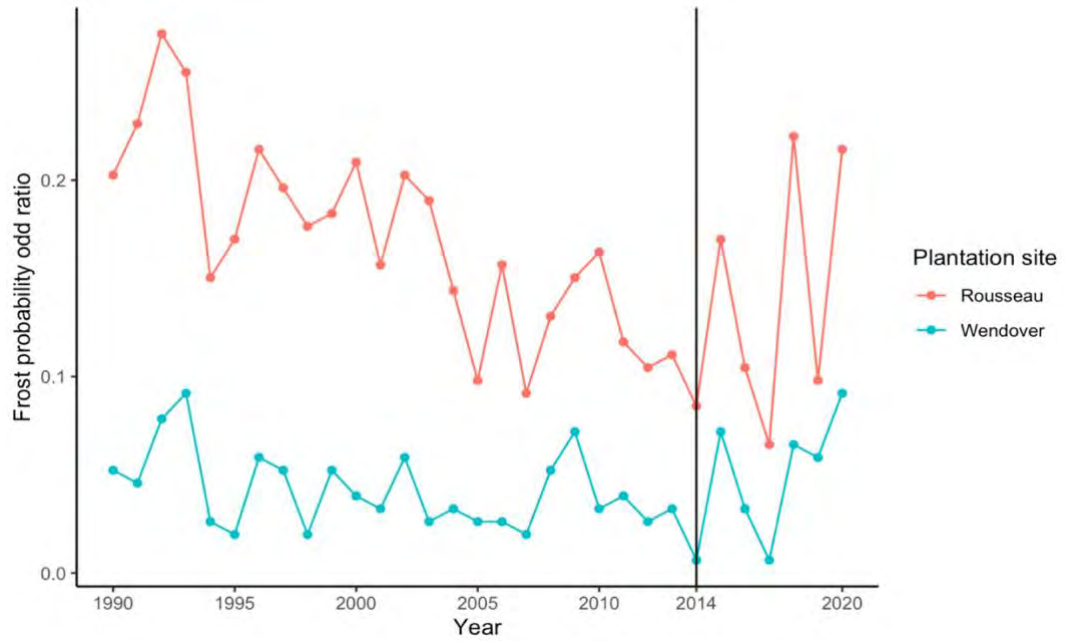
Annexe B Annual odd ratio of frost probability variation recorded during the growing season over a 30-year period for the northern Rousseau and the southern Wendover plantation test sites. The vertical line represents the planting year (2014), where the climate heterogeneity starts.

ANNEXE C: PHENOLOGY FIELD GUIDE

STAGE 0	STAGE 1	STAGE 2	STAGE 3	STAGE 4	STAGE 5
ABSENCE OF BUD	WHITE BUD	BEIGE BUD	BROWNISH BUD	BROWN BUD	SPREAD NEEDLES

Annexe C Illustration of the different phenological stages of bud formation in white spruce (Dhont et al., 2010).
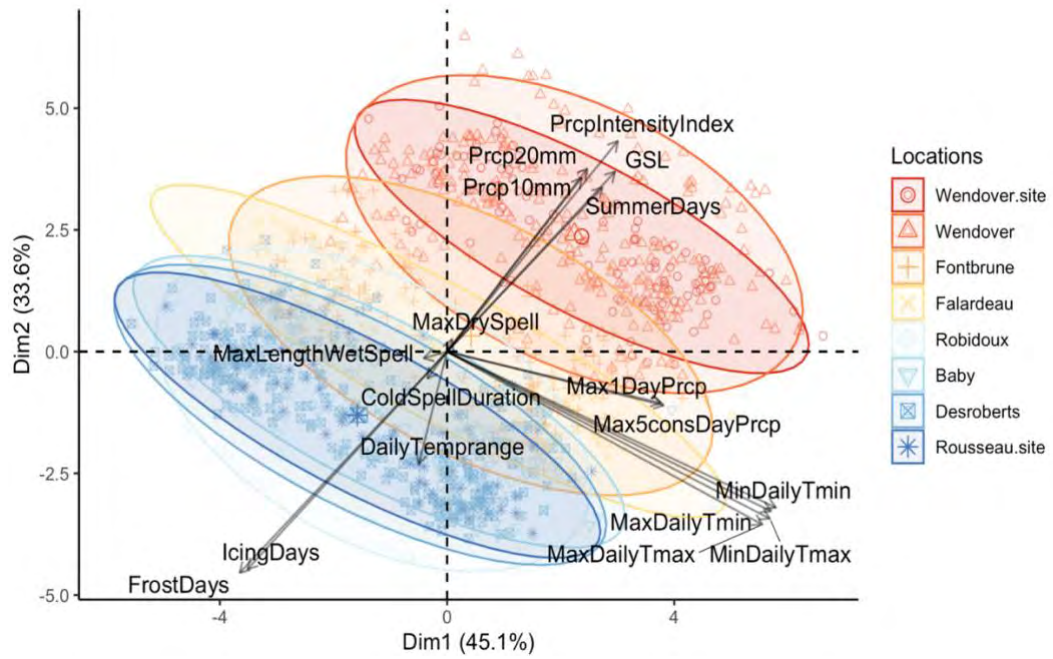
Annexe D Biplot of principal component analysis of bioclimatic variables of seed sources origins and plantation test sites. The locations in the legend are ordered from south (Wendover) to north (Desroberts seed source and Rousseau plan test site.

ANNEXE E: LINEAR MODELS OF AVERAGE FROST TOLERANCE
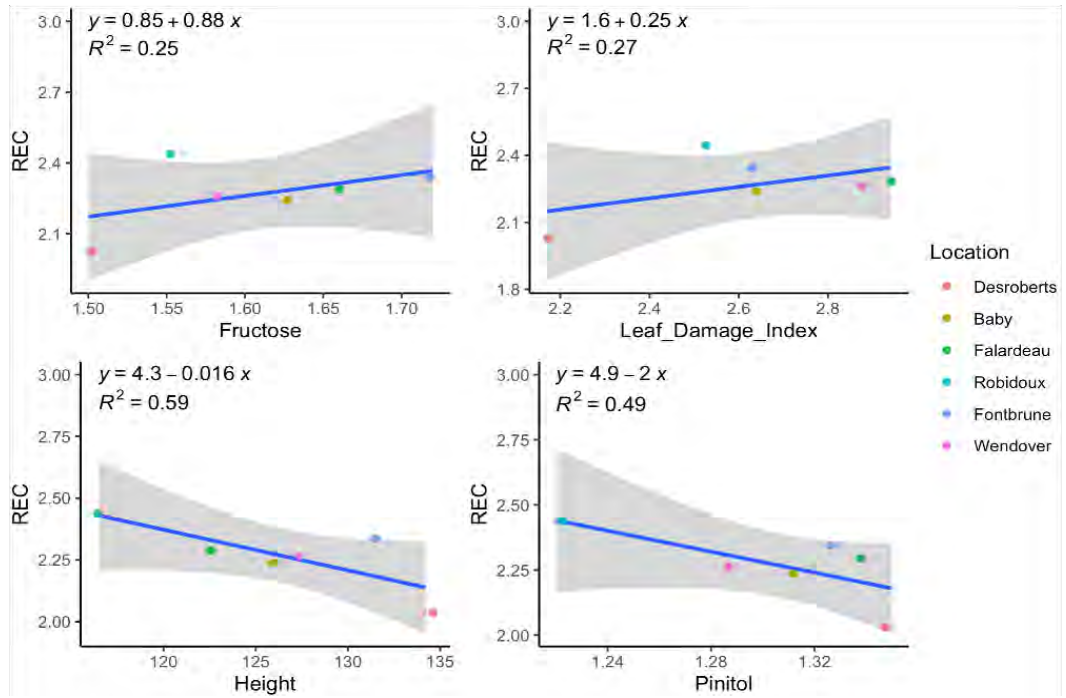
Annexe E Simple linear regressions of mean relative electrolytic conductivity (REC) and confidence intervals versus non-structural carbohydrates content, height, and leaf damage index of seed sources.

ANNEXE F: PRECIPITATION PARAMETERS
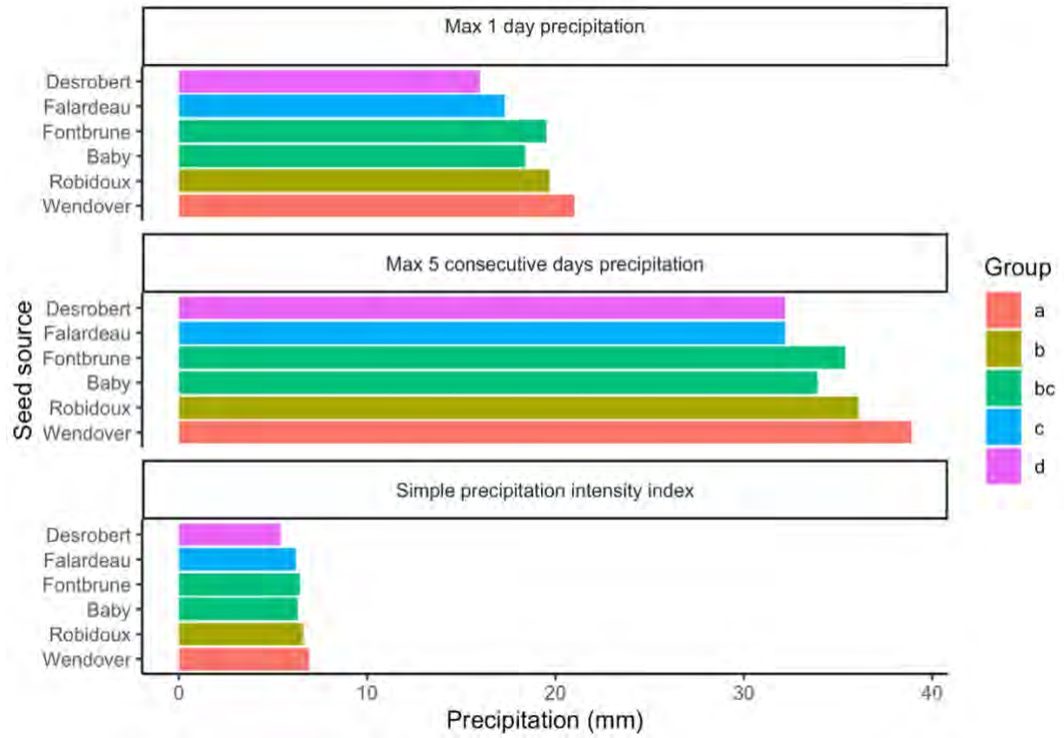
Annexe F Distribution of the annual means of precipitation indices of seed sources calculated for their climate of origin (1940-1970). The groups represent the results of Tukey's honest significant tests. https://www.climdex.org/learn/indices/.
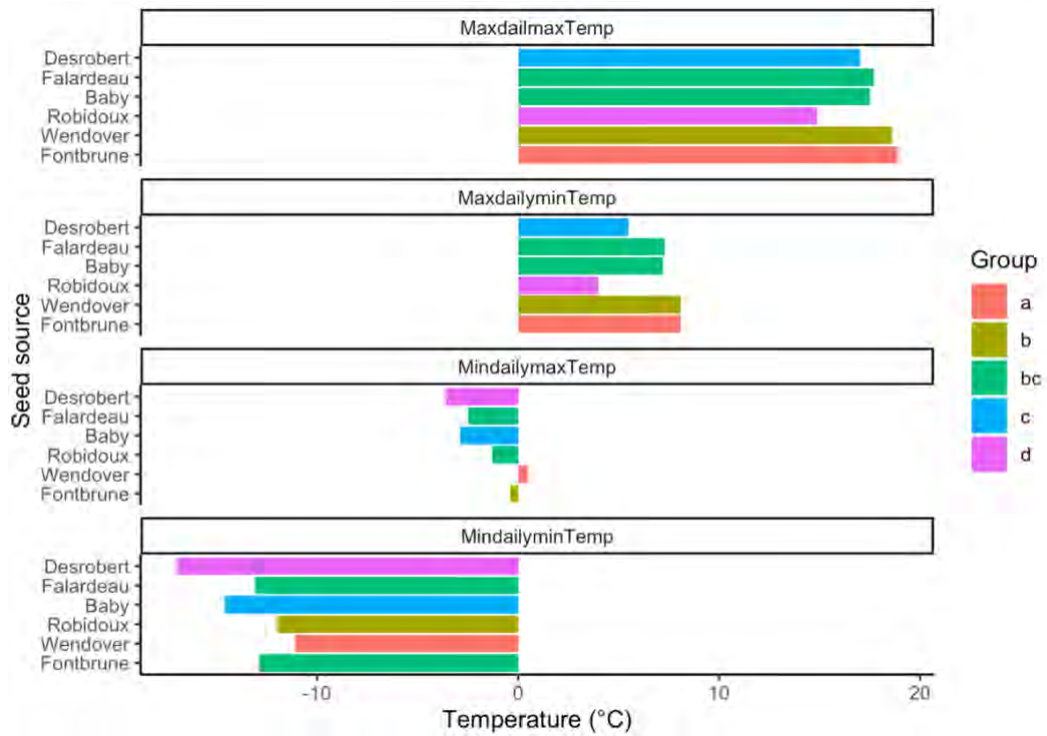
Annexe G Distribution of the indices of annual means of temperature extremes of seed sources calculated for their climate of origin (1940-1970). The groups represent the results of Tukey's honest significant tests. https://www.climdex.org/learn/indices/.
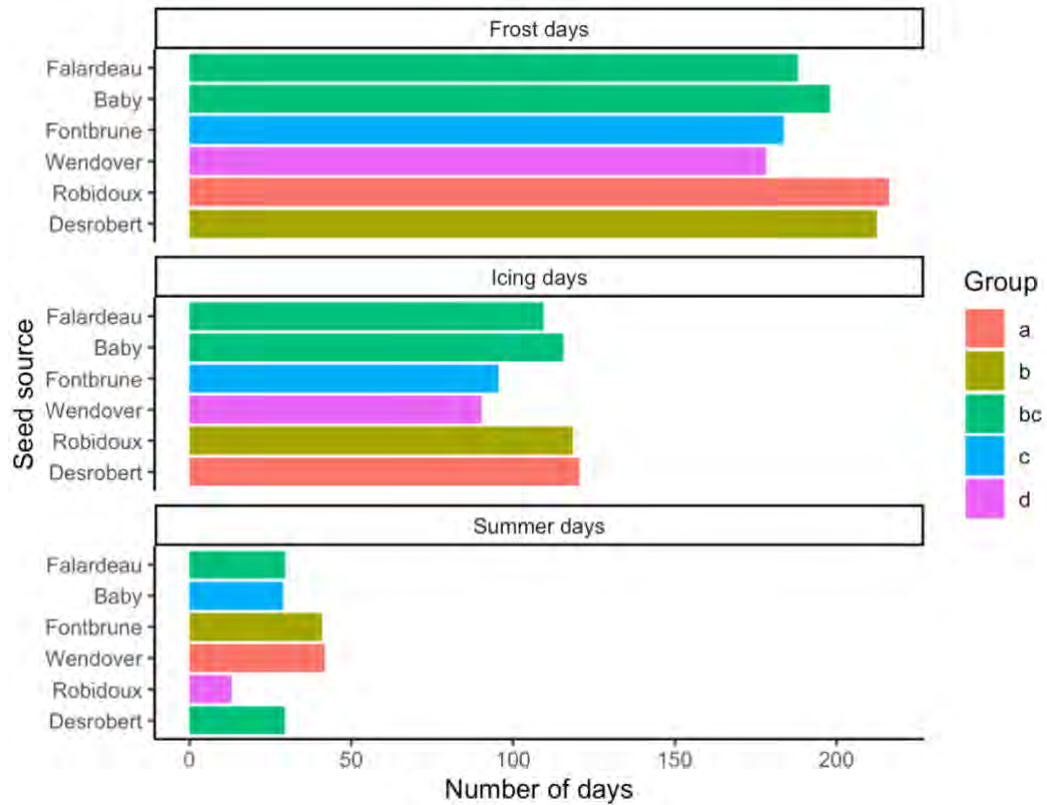
Annexe H Distribution of the annual means of days of cold and thermal indices of seed sources calculated for their climate of origin (1940-1970). The groups represent the results of Tukey's honest significant tests. https://www.climdex.org/learn/indices/.

ANNEXE I: HUMIDITY PARAMETERS
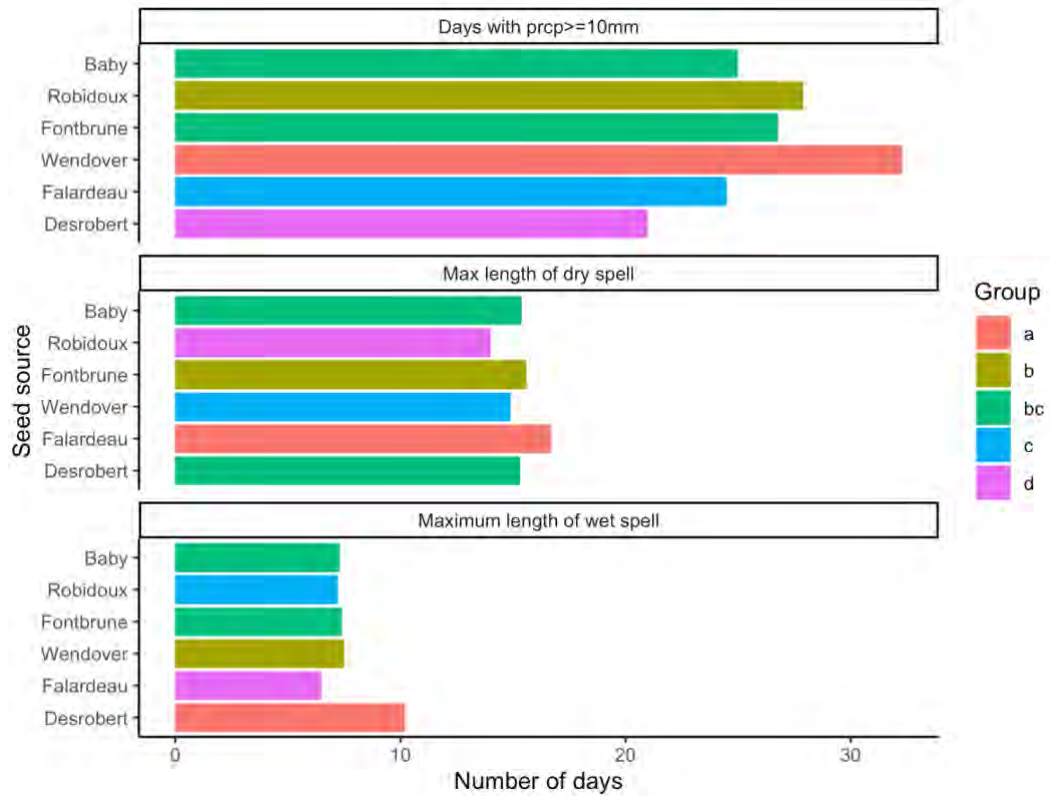
Annexe I: Distribution of the annual average number of days of humidity indices of seed sources calculated for their climate of origin (1940-1970). The groups represent the results of Tukey's honest significant tests. https://www.climdex.org/learn/indices/.

ANNEXE J: PHENOLOGY MODEL SUMMARY

Annexe J The Wendover plantation test site estimates are calculated with Rousseau plantation test site being the reference. For seed sources estimates, the Wendover seed source is the reference. The bud set stages transitions are expressed as follow: Stage n | Stage n+1 (n starting from 0). The R^2 McFadden reflects the total variance explained by the model. It is equivalent to R^2 coefficient for linear models.

Terms	Estimate	Standard error	t-value	p-value
Site Wendover	3.647	0.168	21.760	0.000 ***
Seed source Falardeau	0.576	0.198	2.910	0.004**
Seed source Robidoux	0.582	0.199	2.918	0.004**
Seed source Baby	0.719	0.186	3.872	0.000
Seed source Desroberts	0.672	0.185	3.628	0.000
Seed source Local	1.006	0.188	5.335	0.000
Day of year	0.647	0.001	820.140	0.000
Site Wendover: Seed source Baby	-0.628	0.248	-2.533	0.011
Site Wendover: Seed source Desroberts	-0.496	0.245	-2.025	0.043
Site Wendover: Seed source Local	-0.880	0.255	-3.457	0.001
0|1	124.207	0.019	6668.078	0.000
1|2	126.059	0.069	1824.939	0.000
2|3	128.527	0.085	1515.071	0.000
3|4	131.062	0.102	1281.642	0.000
4|5	138.259	0.107	1293.522	0.000
R^2 McFadden.	0.63			

RÉFÉRENCES

Adam-Poupart, A., Smargiassi, A., Busque, M.A., Duguay, P., Fournier, M., Zayed, J., & Labrèche, F. (2014). Summer outdoor temperature and occupational heat-related illnesses in Quebec (Canada). *Environmental Research*, 134, 339-344.

Aidenapol, A., Denk, T., & Grimm, G. W. (2015). Floristic and physiognomic change in response to climate change: A 23,000-year record from the Iberian Peninsula. *Journal of Biogeography*, 42(9), 1587-1601.

Aitken, S.N. & Hannerz, M. (2001). Genecology and gene resource management strategies for conifer cold hardiness. *In* Conifer Cold Hardiness (Bigras, F.J. & Colombo, S.J., Eds.), Kluwer Academic Publisher, pp. 23-53.

Aitken, S.N., & Bemmels, J.B. (2016). Time to get moving assisted gene flow of forest trees. *Evolutionary Applications*, 9(1), 271-290.

Alboukadel, K., & Fabian, M. (2020). Factoextra: extract and visualize the results. of multivariate data analyses. R package version 1.0. 7.

Andalo, C., Beaulieu, J., & Bousquet, J. (2005). The impact of climate change on growth of local white spruce populations in Quebec, Canada. *Forest Ecology & Management*, 205(1-3), 169-182.

Angelcheva, L., Mishra, Y., Antti, H., Kjellsen, T.D., Funk, C., Strimbeck, R.G., & Schröder, W.P. (2014). Metabolomic analysis of extreme freezing tolerance in Siberian spruce (*P icea obovata*). *New Phytologist*, 204(3), 545-555.

Beaulieu, J., & Rainville, A. (2005). Adaptation to climate change: genetic variation is both a short- and a long-term solution. *Forestry Chronicle*, 81(5), 704-709.

Beaulieu, J., Perron, M., & Bousquet, J. (2004). Multivariate patterns of adaptive genetic variation and seed source transfer in *Picea mariana*. *Canadian Journal of Forest Research*, 34(3), 531-545.

Belle-Isle, J., & Kneeshaw, D. (2007). A stand and landscape comparison of the effects of a spruce budworm (Choristoneura fumiferana (Clem.)) outbreak to the combined effects of harvesting and thinning on forest structure. *Forest Ecology and Management*, 246(2-3), 163-174.

Benomar, L., Bousquet, J., Perron, M., Beaulieu, J. & Lamara, M. (2022). Tree maladaptation under mid-latitude early spring warming and late cold spells: implications for assisted migration. *Frontiers in Plant Science,* 13: 920852 (12p.).

Benomar, L., Lamhamedi, M.S., & Rainville, A. (2020). Variation in bud set process among eight genetically improved white spruce seed sources from eastern Canada. *Tree Planter's Note,* 63(1).

Benomar, L., Lamhamedi, M.S., Rainville, A., Beaulieu, J., Bousquet, J., & 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.

Benomar, L., Lamhamedi, M. S., Villeneuve, I., Rainville, A., Beaulieu, J., Bousquet, J., & Margolis, H. A. (2015). Fine-scale geographic variation in photosynthetic-related traits of Picea glauca seedlings indicates local adaptation to climate. *Tree Physiology*, 35(8), 864-878.

Bergeron, Y., Chen, H. Y. H., Kenkel, N. C., Leduc, A. L., & Macdonald, S. E. (2014). Boreal mixedwood stand dynamics: ecological processes underlying multiple pathways. For. Chron. 90, 202–213.

Bigras, F.J., & Colombo, S.J. (Eds.). (2013). Conifer Cold hardiness (Vol. 1). Springer Science

Bigras, F.J., & D'aoust, A.L. (1992). Hardening and dehardening of shoots and roots of containerized black spruce and white spruce seedlings under short and long days. *Canadian Journal of Forest Research*, 22(3), 388-396.

Bronaugh, D. (2014). Climdex.pcic: PCIC implementation of Climdex routines. *Pacific Climate Impacts Consortium, R package version*, 1(1).

Chen, I.-C., Hill, J.K., Ohlemüller, R., Roy, D.B., Thomas, C.D. (2011). Rapid

Range Shifts of Species Associated with High Levels of Climate Warming. *Science*, 333(6045): 1024-1026.

ChenHan, Y. H., KenkelNorman, C., LeducAlbanie, L., & Ellen, M. (2014). Boreal mixedwood stand dynamics: ecological processes underlying multiple pathways. *The Forestry Chronicle*.

Collins, M., Knutti, R., Arblaster, J., Dufresne, J-L., Fichefet, T., et al., (2013). Long-term Climate Change: Projections, Commitments, and Irreversibility. In T. F. Stocker, D. Qin, G-K. Plattner, M.M.B. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex, & P.M. Midgley (Eds.), *Climate Change 2013 - The Physical Science Basis: Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (pp. 1029-1136). (Intergovernmental Panel on Climate Change). Cambridge University Press.

Colombo, S.J., & Raitanen, E.M. (1993). Frost hardening in first year eastern larch (*Larix laricina*) container seedlings. *New Forests*, 7(1), 55-61.

Cozzolino, D., Fassio, A., Restaino, E., Vicente, E. (2015). Instrumental Techniques and Methods: Their Role in Plant Omics. In: Barh, D., Khan, M., Davies, E. (eds) PlantOmics: The Omics of Plant Science. Springer, New Delhi.

D'Orangeville, L., Houle, D., Duchesne, L., Phillips, R. P., Bergeron, Y., & Kneeshaw, D. (2018). Beneficial effects of climate warming on boreal tree growth may be transitory. *Nature Communications*, 9(1), 1-10.

Davis, M.B., & Shaw, R G. (2001). Range shifts and adaptive responses to Quaternary climate change. *Science*, 292(5517), 673-679.

Delpierre, N., Vitasse, Y., Chuine, I., Guillemot, J., Bazot, S., & Rathgeber, C.B. (2016). Temperate and boreal forest tree phenology: from organ-scale processes to terrestrial ecosystem models. *Annals of Forest Science*, 73(1), 5-25.

Depardieu, C., Gérardi, S., Nadeau, S., Parent, G.J., Mackay, J., Lenz, P., ... &

Isabel, N. (2021). Connecting tree-ring phenotypes, genetic associations, and transcriptomics to decipher the genomic architecture of drought adaptation in a widespread conifer. *Molecular Ecology*, 30(16), 3898-3917.

Depardieu, C., Girardin, M.P., Nadeau, S., Lenz, P., Bousquet, J., & Isabel, N. (2020). Adaptive genetic variation to drought in a widely distributed conifer suggests a potential for increasing forest resilience in a drying climate. *New Phytologist*, 227(2), 427-439.

El Kayal, W.E., Allen, C.C., JU, C.J.T., Adams, E.R.I., King-Jones, S, Zaharia, L.I., et al., (2011). Molecular events of apical bud formation in white spruce, *Picea glauca. Plant, Cell & Environment*, 34(3), 480-500.

Erickson, V., Aubry, C., Berrang, P., Blush, T., Bower, A., Crane, B. et al. (2012). Genetic resource management and climate change: genetic options for adapting national forests to climate change. *USDA Forest Service, Forest Management, Washington, DC*, 19.

Fei, S., Desprez, J.M., Potter, K.M., Jo, I., Knott, J.A., & Oswalt, C.M. (2017). Divergence of species responses to climate change. *Science Advances*, 3(5), e1603055.

Field, C.B., & Barros, V.R. (Eds.). (2014). Climate change 2014-Impacts, adaptation, and vulnerability: Regional aspects. Cambridge University Press.

Gallinat, A.S., Primack, R.B. & Wagner, D.L. (2015). Autumn, the neglected season in climate change research. *Trends in Ecology & Evolution*, 30, 169-176.

GIEC, (2014). Changements climatiques 2014 : Rapport de synthèse. Contribution des Groupes de travail I, II et III au cinquième Rapport d'évaluation du Groupe d'experts Intergouvernemental sur l'évolution du climat [Sous la direction de l'équipe de rédaction principale, R.K. Pachauri et L.A. Meyer]. GIEC, Genève, Suisse, 161 p.

Gonzales, L.M., Williams, J.W., & Grimm, E.C. (2009). Expanded response

surfaces: a new method to reconstruct paleoclimates from fossil pollen assemblages that lack modern analogues. *Quaternary Science Reviews*, 28(27-28), 3315-3332.

Greene, D.F., Zasada, J.C., Sirois, L., Kneeshaw, D., Morin, H., Charron, I. et al., (1999). A review of the regeneration dynamics of North American boreal forest tree species. *Canadian Journal of Forest Research*, 29(6), 824-839.

Gu, L., Hanson, P.J., Post, W.M., Kaiser, D.P., Yang, B., Nemani, R., et al., (2008). The 2007 eastern US spring freeze: increased cold damage in a warming world? *BioScience*, 58(3), 253-262.

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)**,** 1-15.

Hamilton, J.A., El Kayal, W., Hart, A.T., Runcie, D.E., Arango-Velez, A., & Cooke, J.E. (2016). The joint influence of photoperiod and temperature during growth cessation and development of dormancy in white spruce (*Picea glauca*). *Tree Physiology*, 36(11), 1432-1448.

Hansen, J., Sato, M., & Ruedy, R. (2012). Perception of climate change. *Proceedings of the National Academy of Sciences*, 109(37), E2415-E2423.

Hoch, G. & Korner, C. (2003). The carbon charging of pines at the climatic treeline: a global comparison. *Oecologia,* 135, 10-21.

Hornoy, B., Pavy, N., Gerardi, S., Beaulieu, J., & Bousquet, J. (2015). Genetic adaptation to climate in white spruce involves small to moderate allele frequency shifts in functionally diverse genes. *Genome Biology and Evolution*, 7(12), 3269-3285.

Howe, G.T., Aitken, S.N., Neale, D.B., Jermstad, K.D., Wheeler, N.C., & Chen, T.H. (2003). From genotype to phenotype: unraveling the complexities of cold adaptation in forest trees. *Canadian Journal of Botany*, 81(12), 1247-1266.

IPCC (2021). Summary for Policymakers. In: Climate Change 2021: The physical

science basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge, CA: Cambridge University Press.

Isaac-Renton, M.G., Roberts, D.R., Hamann, A., & Spiecker, H. (2014). Douglas fir plantations in Europe: a retrospective test of assisted migration to address climate change. *Global Change Biology*, 20(8), 2607-2617.

Jaramillo-Correa, J.P., Beaulieu, J., Khasa, D.P., & Bousquet, J. (2009). Inferring the past from the present phylogeographic structure of North American forest trees: seeing the forest for the genes. *Canadian Journal of Forest Research*, 39(2), 286-307.

Jaramillo-Correa, J.P., Beaulieu, J., & Bousquet, J. (2001). Contrasting evolutionary forces driving population structure at expressed sequence tag polymorphisms, allozymes and quantitative traits in white spruce. *Molecular Ecology*, 10(11), 2729-2740.

Jiang, Y., Zwiazek, J.J., & Macdonald, S.E. (1994). Effects of prolonged cold storage on carbohydrate and protein content and field performance of white spruce bareroot seedlings. *Canadian Journal of Forest Research*, 24(7), 1369-1375.

Johnsen, Ø., & Skrøppa, T. (2000). Provenances and families show different patterns of relationship between bud set and frost hardiness in *Picea abies*. *Canadian Journal of Forest Research*, 30(12), 1858-1866.

Kagawa, A., Sugimoto, A. & Maximov, T.C. (2006). Seasonal course of translocation, storage and remobilization of ^{13}C pulse-labeled photoassimilate in naturally growing *Larix gmelinii* saplings. *New Phytologist,* 171, 793-803.

Kim, J.S., Kug, J.S., Jeong, S.J., Huntzinger, D.N., Michalak, A.M., Schwalm, C.R. et al., (2017). Reduced North American terrestrial primary productivity linked to anomalous Arctic warming. *Nature Geoscience*, 10(8), 572-576.

Kug, J.S., Jeong, J.H., Jang, Y.S., Kim, B.M., Folland, C.K., Min, S.K., & Son, S.W. (2015). Two distinct influences of Arctic warming on cold winters over North America and East Asia. *Nature Geoscience*, 8(10), 759-762.

Lamhamedi, M. S., Rainville, A., Benomar, L., Villeneuve, I., Beaulieu, J.,

Bousquet, J. et al., (2017). L'écophysiologie, un atout pour réussir la migration assistée de sources génétiques d'épinette blanche. *Gouvernement du Québec, ministère des Forêts, de la Faune et des Parcs. Avis de Recherche Forestière*, 89, 1-2.

Lamhamedi, M.S, Renaud, M., & Veilleux, L. (2005). Development of frost tolerance thresholds for 1+0 white spruce seedlings in forest nurseries according to the ecological regions of Quebec. Department of Natural Resources Québec, Wildlife and Parks, Forestry Research Branch.

Lamhamedi, M.S., & Bernier, P.Y. (1994). Ecophysiology and field performance of black spruce (*Picea mariana*): a review. *Annales des Sciences Forestières*, 51(6), 529-551.

Lamhamedi, M.S., Lambert, M.C., & Renaud, M. (2022). Simulation of episodic winter warming on dehardening of boreal forest seedlings in northern forest nurseries. *Forests*, 13(12), 1975.

Lesser, M.R., & Parker, W.H. (2004). Genetic variation in *Picea glauca* for growth and phenological traits from provenance tests in Ontario. *Silvae Genetica*, 53(1-6), 141-148.

Levitt, J. (1980). Responses of plants to environmental stresses. Vol I. Chilling, Freezing, and High Temperature Stresses, 2nd edn. Academic Press, New York. 497 p.

Li, P., & Adams, W.T. (1993). Genetic control of bud phenology in pole-size trees and seedlings of coastal Douglas-fir. *Canadian Journal of Forest Research*, 23(6), 1043-1051.

Li, P., Beaulieu, J., & Bousquet, J. (1997). Genetic structure and patterns of genetic variation among populations in eastern white spruce (*Picea glauca*). *Canadian Journal of Forest Research*, 27(2), 189-198.

Li, P., Beaulieu, J., Corriveau, A., & Bousquet, J. (1993). Genetic variation in juvenile growth and phenology of white spruce provenance-progeny test. *Silvae Genetica*, 42(1), 52-60.

Li, T., Liu, Q.-H., Ohsugi, R., Yamagishi, T., & Sasaki, H. (2006). Effects of high

temperature on sucrose content and sucrose-cleaving enzymes activity in rice during grain filling stage. *Chinese Journal of Rice Science,* 20(6), 626–630.

Li, Y., Suontama, M., Burdon, R.D., & Dungey, H.S. (2017). Genotype by environment interactions in forest tree breeding: review of methodology and perspectives on research and application. *Tree Genetics & Genomes*, 13(3), 60.

Lieffers, V.J., Stadt, K.J., & Navratil, S. (1996). Age structure and growth of understory white spruce under aspen. *Canadian Journal of Forest Research*, 26(6), 1002-1007.

Liepe, K. J., Hamann, A., Smets, P., Fitzpatrick, C.R., & Aitken, S.N. (2016). Adaptation of lodgepole pine and interior spruce to climate: implications for reforestation in a warming world. *Evolutionary Applications*, 9(2), 409-419.

Lloret, F., Escudero, A., Iriondo, J.M., Martínez-Vilalta, J. & Valladares, F. (2012). Extreme climatic events and vegetation: the role of stabilizing processes. *Global Change Biology*, 18, 797-805.

Lu, P., Parker, W.H., Cherry, M., Colombo, S., Parker, W.C., Man, R., & Roubal, N. (2014). Survival and growth patterns of white spruce (*Picea glauca* [M oench] Voss) rangewide provenances and their implications for climate change adaptation. *Ecology & Evolution*, 4(12), 2360-2374.

Lu, W., Cheng, J., Wang, W., Zhang, H., & Zhou, H. (2015). Application of the method of spatial point pattern analysis to the horizontal spatial distribution of preferential flow paths. *Forestry Chronicle*, 91(4), 384-394.

Lüdecke, D. (2018). ggeffects: Tidy data frames of marginal effects from regression models. *Journal of Open-Source Software,* 3(26), 772.

Lüdecke, D., Ben-Shachar, M. S., Patil, I., Waggoner, P., & Makowski, D. (2021). performance: An R package for assessment, comparison and testing of statistical models. *Journal of Open-Source Software*, 6(60).

Man, R., Kayahara, G. J., Dang, Q. L., & Rice, J. A. (2009). A case of severe frost damage prior to budbreak in young conifers in Northeastern Ontario: Consequence of climate change? *The Forestry Chronicle*, 85(3), 453-462.

Man, R., Lu, P., & Dang, Q. L. (2021). Cold tolerance of black spruce, white spruce, jack pine, and lodgepole pine seedlings at different stages of spring dehardening. *New Forests*, 52(2), 317-328.

Margolis, H.A. (2015). Fine-scale geographic variation in photosynthetic-related traits of *Picea glauca* seedlings indicates local adaptation to climate. *Tree Physiology*, 35(8), 864-878.

Marquis, B., Bergeron, Y., Simard, M., & Tremblay, F. (2020). Probability of spring frosts, not growing degree-days, drives onset of spruce bud burst in plantations at the boreal-temperate forest ecotone. *Frontiers in Plant Science*, 11, 103.

Namroud, M-C., Beaulieu, J., Juge, N., Laroche, J. & Bousquet, J. (2008). Scanning the genome for gene single nucleotide polymorphisms involved in adaptive population differentiation in white spruce. *Molecular Ecology,* 17: 3599-3613.

Nienstaedt, H., & Zasada, J.C. (1990). *Picea glauca* (Moench) Voss white spruce. *Silvics of North America*, 1, 204-226.

Otis Prud'homme, G., Lamhamedi, M.S., Benomar, L., Rainville, A., DeBlois, J., Bousquet, J., & Beaulieu, J. (2018). Ecophysiology and growth of white spruce seedlings from various seed sources along a climatic gradient support the need for assisted migration. *Frontiers in Plant Science*, 8, 2214.

Overland, J.E., & Wang, M. (2016). Recent extreme Arctic temperatures are due to a split polar vortex. *Journal of Climate*, 29(15), 5609-5616.

Pedlar, J.H., McKenney, D.W., Aubin, I., Beardmore, T., Beaulieu, J., Iverson, L., O'Neill, G.A., Winder, R.S. & Ste-Marie, C. (2012). Placing forestry in the assisted migration debate. *BioScience*, 62(9), 835-842.

Perrin, M., Rossi, S., & Isabel, N. (2017). Synchronisms between bud and cambium phenology in black spruce: early flushing provenances exhibit early xylem formation. *Tree Physiology*, 37(5), 593-603.

Petit, R. J., Hu, F. S., & Dick, C. W. (2008). Forests of the past: a window to future changes. *Science*, 320(5882), 1450-1452.

Prunier, J., Laroche, J., Beaulieu, J., & Bousquet, J. (2011). Scanning the genome

for gene SNPs related to climate adaptation and estimating selection at the molecular level in boreal black spruce. *Molecular Ecology*, 20(8), 1702-1716.

Prunier, J., Pelgas, B., Gagnon, F., Desponts, M., Isabel, N., Beaulieu, J. & Bousquet, J. (2013). The genomic architecture and association genetics of adaptive characters using a candidate SNP approach in boreal black spruce. *BMC Genomics,* 14(368) : 1-16.

Prunier, J., Tessier, G., Bousquet, J., & MacKay, J. (2015). From genotypes to phenotypes: expression levels of genes encompassing adaptive SNPs in black spruce. *Plant Cell Reports*, 34, 2111-2125.

Purdy, B.G., Dale, M.R.T., & MacDonald, S.E. (2002). The regeneration niche of white spruce following fire in the mixedwood boreal forest. *Silva Fennica*, 36(1), 289-306.

R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URLhttps://www.R-project.org/.

Rainville, A., Beaulieu, J., Langevin, L., Logan, T., & Lambert, M.C. (2014). Prédire l'effet des changements climatiques sur le volume marchand des principales espèces résineuses plantées au Québec, grâce à la génétique forestière. Ministère des Forêts, de la Faune et des Parcs du Québec, Direction de la recherche forestière. . https://www.mffp.gouv.qc.ca/publications/forets/connaissances/recherche/Rainville-Andre/Memoire174.pdf

Régnière, J., Saint-Amant, R., Béchard, A., & Moutaoufik, A. (2014). BioSIM 10: User's manual (p. 74). Québec, QC, Canada: Laurentian Forestry Centre.

Repo, T., Zhang, G., Ryyppö, A., Rikala, R., & Vuorinen, M. (2000). The relation between growth cessation and frost hardening in Scots pines of different origins. *Trees*, 14(8), 456.

Rinne, K. T., Saurer, M., Kirdyanov, A. V., Bryukhanova, M. V., Prokushkin, A. S., Churakova, O. V., & Siegwolf, R. T. (2015). Examining the response of needle carbohydrates from Siberian larch trees to climate using compound-

specific $\delta^{13}C$ and concentration analyses. *Plant, Cell & Environment*, 38(11), 2340-2352.

Rohde, A., & Bhalerao, R. P. (2007). Plant dormancy in the perennial context. *Trends in Plant Science*, 12(5), 217-223.

Ruttink, T., Arend, M., Morreel, K., Storme, V., Rombauts, S., Fromm, J., et al., (2007). A molecular timetable for apical bud formation and dormancy induction in poplar. *Plant Cell*, 19(8), 2370-2390.

Sakai, A. & Larcher, W. (1987). Frost Survival of Plants in Ecological Studies. Springer Verlag, Berlin pp. 321-352.

Schuch, U.K., Duryea, M.L., & Fuchigami., L.H (1989a). Dehardening and budburst of Douglas-fir seedlings raised in three Pacific Northwest nurseries. *Canadian Journal of Forest Research*, 19(2), 198-203.

Schuch, U. K., Duryea, M. L., & Fuchigami, L. H. (1989b). Frost hardiness as acquired by Douglas-fir seedlings in three Pacific Northwest nurseries. *Canadian Journal of Forest Research*, 19(2), 192-197.

Sebastian-Azcona, J., Hacke, U.G., & Hamann, A. (2018). Adaptations of white spruce to climate: strong intraspecific differences in cold hardiness linked to survival. *Ecology & Evolution*, 8(3), 1758-1768.

Simard, S., Giovannelli, A., Treydte, K., Traversi, M.L., King, G.M., Frank, D. & Fonti, P. (2013). Intra-annual dynamics of non-structural carbohydrates in the cambium of mature conifer trees reflects radial growth demands. *Tree Physiology* 33, 913–923.

Singh, R.K., Svystun, T., AlDahmash, B., Jönsson, A.M., & Bhalerao, R.P. (2017). Photoperiod-and temperature-mediated control of phenology in trees–a molecular perspective. *New Phytologist*, 213(2), 511-524.

Soro, A., Lenz, P., Roussel, J-R., Larochelle, F., Bousquet, J. & Achim, A. (2022). The phenotypic and genetic effects of drought-induced stress on apical growth, ring width, wood density and biomass in white spruce seedlings. *New Forests*, 1-23.

Stacklies, W., Redestig, H., Scholz, M., Walther, D., & Selbig, J. (2007).

pcaMethods—a bioconductor package providing PCA methods for incomplete data. *Bioinformatics*, 23(9), 1164-1167.

Ste-Marie, C., A. Nelson, E., Dabros, A., & Bonneau, M.E. (2011). Assisted migration: Introduction to a multifaceted concept. *Forestry. Chronicle*, 87(6), 724-730.

Strimbeck, G. R., Kjellsen, T. D., Schaberg, P. G., & Murakami, P. F. (2008). Dynamics of low-temperature acclimation in temperate and boreal conifer foliage in a mild winter climate. *Tree Physiology*, 28(9), 1365-1374.

Sveinbjörnsson, B. (2000) North American and European treelines: external forces and internal processes controlling position. *Ambio,* 29, 388–395.

Taiyun, W. & Viliam, S. (2021). R package 'corrplot': Visualization of a Correlation Matrix (Version 0.92). Available from https://github.com/taiyun/corrplot.

Thomson, A.M., Riddell, C.L., & Parker, W.H. (2009). Boreal forest provenance tests used to predict optimal growth and response to climate change: 2. Black spruce. *Canadian Journal of Forest Research*, 39(1), 143-153.

Venables, W. N., & Ripley, B. D. (2002). Modern Applied Statistics with S Fourth edition by, World.

Villeneuve, I., Lamhamedi, M.S., Benomar, L., Rainville, A., DeBlois, J., Beaulieu, J., et al., (2016). Morpho-physiological variation of white spruce seedlings from various seed sources and implications for deployment under climate change. *Frontiers in Plant Science*, 7, 1450.

Ward Jr, J. H. (1963). Hierarchical grouping to optimize an objective function. *Journal of the American statistical association*, 58(301), 236-244.

Weiser, C.J. (1970). Cold resistance and injury in woody plants: knowledge of hardy plant adaptations to freezing stress may help us to reduce winter damage. *Science*, 169(3952), 1269-1278.

Wu, Z., Dijkstra, P., Koch, G. W., Peñuelas, J., & Hungate, B. A. (2015). Responses of terrestrial ecosystems to temperature and precipitation change: A meta-analysis of experimental manipulation. *Global Change Biology*, 21(2), 926-935.

Xie, C.Y. (2003). Genotype by environment interaction and its implications for genetic improvement of interior spruce in British Columbia. *Canadian Journal of Forest Research*, 33(9), 1635-1643.