UQAC
Université du Québec
à Chicoutimi

Surviving the winter: tree phenology and frost hardiness at the boundary between temperate and boreal forest in Eastern Canada

par Claudio Mura

Thèse présentée à l'Université du Québec à Chicoutimi en vue de l'obtention du grade de Philosophiae Doctor (Ph. D.) en sciences de l'environnement

Québec, Canada

RÉSUMÉ

Dans les écosystèmes tempérés et boréaux, les arbres alternent entre phases de croissance et de dormance afin d'éviter le gel durant la saison froide. Ce cycle comprend des changements dans la résistance au gel, qui est minimale pendant la saison de croissance et atteint son maximum durant la dormance. À mesure que les changements climatiques s'intensifient, le réchauffement des températures et l'augmentation de la variabilité climatique peuvent créer un décalage entre la phénologie des populations adaptées aux conditions locales et les conditions environnementales, entraînant un risque accru de dégâts causés par le gel. Malgré l'importance fondamentale de ces traits pour la survie des arbres dans les régions froides, il y a encore peu d'études empiriques sur le terrain qui explorent le lien entre la phénologie et la résistance au gel pour de nombreuses espèces d'arbres. Une source particulière d'incertitude est le rôle de la différenciation intraspécifique, car différentes provenances peuvent présenter des réponses divergentes en ce qui a trait à la phénologie et la résistance au gel pouvant engendrer des risques différents d'exposition au gel.

Dans ce travail, j'ai utilisé une combinaison d'observations sur le terrain et d'analyses en laboratoire pour relier la phénologie et la résistance au gel dans deux espèces clés des forêts de l'Est du Canada, l'épinette noire (*Picea mariana* Mill. BSP) et l'érable à sucre (*Acer saccharum* Marsh.). Pour les deux espèces, différentes provenances ont été comparées pour étudier la variation intraspécifique. Les objectifs généraux étaient i) d'évaluer la variabilité intraspécifique de la résistance au gel et de la phénologie du débourrement et de la dormance, et ii) de relier ces deux traits pour comprendre les variations intraspécifiques du risque de dégâts dus au gel. L'étude est articulée en trois chapitres, chacun se concentrant sur un objectif et des hypothèses spécifiques.

Le chapitre 1 a étudié les impacts d'un événement naturel de gel tardif survenant dans un jardin commun de *P. mariana* en analysant les dégâts dus au gel selon les différences intraspécifiques dans les dates de débourrement. Le jardin commun étant au sud de l'aire de répartition de l'épinette noire, la plupart des provenances se trouvait dans des conditions plus chaudes par rapport à leur origine. L'hypothèse était que dans les mêmes conditions, les provenances de climats plus froids étaient plus endommagées par le gel tardif en raison d'un débourrement plus précoce, exposant les jeunes pousses au gel. Les résultats ont confirmé l'hypothèse. Les provenances nordiques de *P. mariana* ont eu un débourrement plus précoce de manière cohérente sur plusieurs années d'observations, entraînant davantage de dégâts lors de l'événement de gel tardif de 2021. Mon étude a démontré comment les différences intraspécifiques dans la phénologie du débourrement peuvent déterminer des risques d'exposition au gel au sein de la même espèce. J'ai pu ainsi mettre en évidence qu'il serait possible de gérer le risque de gel tardif avec la gestion forestière en sélectionnant des provenances méridionales à phénologie printanière plus tardive.

Le chapitre 2 a analysé la résistance au gel chez les jeunes plants de sept provenances *d'A. saccharum* poussant sur deux sites proches de la limite nord de l'aire de répartition de l'espèce. J'ai appliqué des prélèvements destructifs à répétition pour mesurer la résistance au gel durant une saison hivernale afin d'en comparer les contrôles endogènes et environnementaux. Les hypothèses étaient que : 1) les conditions météorologiques locales influencent la résistance au gel, avec les semis du site plus froid montrant une acclimatation plus précoce et plus rapide en automne, une résistance au gel maximale plus élevée en hiver et une déacclimatation plus tardive et plus lente au printemps, par rapport au site plus au sud ; et 2) les conditions climatiques à la provenance influencent la dynamique de résistance au gel, avec les semis des provenances plus froides montrant une acclimatation plus précoce et plus rapide en automne, une résistance au gel plus élevée et une déacclimatation plus tardive et plus lente au printemps par rapport aux provenances plus chaudes. Mes résultats ont partiellement confirmé l'hypothèse 1, car les plants du site plus chaud ont montré une déacclimatation plus précoce et plus rapide, démontrant l'effet des températures chaudes sur la réactivation printanière. J'ai toutefois dû rejeter l'hypothèse 2, car je n'ai observée aucune différence en ce qui a trait à l'acclimatation en automne ni à la résistance maximale au gel pendant l'hiver, ni entre les provenances. La variable LT_{50}, la température létale pour 50 % des

cellules, a varié entre -4 °C en été (juillet) et -68 °C en hiver (février). Toutes les provenances ont atteint des LT_{50} de -55 °C ou moins, bien en dessous des températures minimales hivernales habituelles à la limite nord de la distribution de l'espèce.

Dans le chapitre 3, je me suis concentré sur le rôle des températures sous le point de congélation dans l'accumulation du froid et la levée de l'endodormance. Des semis d'*A. saccharum* appartenant à sept provenances et poussant près de la limite nord de l'aire de répartition de l'espèce ont été exposés à des conditions de refroidissement naturelles ou artificielles (4°C) à partir de la fin de l'automne. Les échantillons ont été transférés à des conditions de forçage à intervalles réguliers pendant l'hiver. J'ai mesuré la résistance au gel au moment du transfert et observé le temps de débourrement sous des conditions de forçage. Les hypothèses étaient que : 1) la levée d'endodormance est plus facile à détecter dans les traitements de refroidissement artificiels, où l'effet de la résistance au gel est limité; 2) un modèle de calcul des unités de refroidissement prenant en compte les températures inférieures à 0 °C est plus efficace pour une espèce de climat tempéré froid où les températures hivernales restent en bas de 0°C pendant plusieurs mois; 3) les échantillons avec une résistance au gel plus élevée ont besoin de plus de temps pour effectuer le débourrement, car ils ont besoin de plus de temps pour la déacclimatation. Les résultats ont confirmé mes hypothèses, mettant en évidence comment une température de 4°C peut à la fois satisfaire le besoin de froid et induire la déacclimatation, menant au développement ontogénétique du bourgeon. À l'inverse, les échantillons soumis au froid naturel ont conservé une plus grande résistance au gel jusqu'à la fin de la saison, ce qui était corrélé avec un débourrement plus tardif et une difficulté accrue d'identification de la levée d'endodormance. La provenance n'a pas eu d'effet significatif sur le temps nécessaire au débourrement sous conditions de forçage. Mes résultats indiquent que l'inclusion des températures inférieures à 0°C peut améliorer les calculs d'accumulation d'unités de refroidissement durant la période de dormance dans les climats froids. Mes travaux montrent que le fait de mesurer la résistance au gel durant les expériences de refroidissement et forçage peut clarifier son rôle dans le processus d'accumulation du froid et la dynamique de la dormance.

Les trois chapitres de ma thèse ont ajouté des données originales à la littérature existante en clarifiant la connection entre résistance au gel et phénologie. Les résultats montrent comment chaque espèce peut posséder différents degrés de plasticité et de différenciation intraspécifique. Les provenances de *P. mariana* transferées au sud ont montré des différences de débourrement entre les provenances, une tendance observée de manière cohérente sur plusieurs années d'observations. Par contre, *A. saccharum* a montré une plus grande plasticité puisque les provenances de la zone d'étude, toutes situées dans la partie nord de l'aire de répartition, ont eu des réponses similaires aux conditions environnementales tant en ce qui a trait à la résistance au gel qu'au débourrement. Ces différences de plasticité entre espèces ont des implications importantes pour la gestion du risque de gel. Les provenances nordiques de *P. mariana* pourraient être plus exposées au gel tardif à l'avenir en raison de leurs besoins de chaleur plus faible, mais la sélection de provenances pourrait être un outil efficace pour réduire les risques, du moins en contexte de forêts aménagées. En revanche, *A. saccharum* serait probablement déjà capable de survivre aux hivers au nord de son aire de répartition actuelle grâce à sa forte résistance au gel. Sa grande plasticité physiologique signifie toutefois aussi que la sélection des provenances serait moins utile pour réduire le risque de gel que pour *P. mariana*. Enfin, mes travaux montrent l'importance d'étudier la résistance au gel et la régulation de la dormance, en particulier en contexte de réchauffement climatique. Ces traits sont des composantes fondamentales du cycle de vie des arbres. Une meilleure compréhension de ces mécanismes est nécessaire pour améliorer les prédictions des impacts des changements climatiques et pour développer des mesures d'adaptation en gestion forestière.

ABSTRACT

In temperate and boreal ecosystems, trees shift between the phenological phases of growth and dormancy in order to avoid frost during the cold season. This cycle includes changes in frost hardiness, which is at a minimum during the growing season and reaches its maximum during dormancy. As climate change intensifies, warming temperatures and increased weather variability may create a mismatch between the phenology of locally adapted populations and their surrounding environmental conditions, leading to increased risk of frost damage. Field studies exploring the link between phenology and frost hardiness are still missing for many tree species, despite the fundamental importance of these traits for tree survival in cold regions. One particular source of uncertainty is the role of intraspecific differentiation, as different provenances may exhibit diverging responses in their phenology and frost hardiness, resulting in different risks of frost exposure.

In this work, I used a combination of field observations and lab analyses to link phenology and frost hardiness in two key species of Eastern Canada's forests, black spruce (*Picea mariana* Mill. BSP) and sugar maple (*Acer saccharum* Marsh.). For each species, different provenances were compared to account for intraspecific variation. The general objectives were i) assessing intraspecific variability in frost hardiness and phenology of budbreak and dormancy, and ii) linking these two traits to understand intraspecific variations in risk of frost damage. The study is articulated in three chapters, each focusing on a specific objective and hypothesis.

Chapter 1 focused on the impacts of a natural late frost event occurring in a *P. mariana* common garden, analyzing frost damage through the lens of intraspecific differences in budbreak timings. Most of the provenances were in warmer conditions compared to their climates of origin, since the common garden was situated at the southern limit of the species' range. The hypothesis was that under the same conditions, provenances from colder climates were more damaged by late frost because of an earlier budbreak, which exposed growing shoots to freezing temperatures. The results confirmed the hypothesis, with northern *P. mariana* provenances performing earlier budbreak consistently over several years of observations, resulting in higher damages during the late frost event of 2021. The study demonstrated how intraspecific differences in budbreak phenology can determine different risk of frost exposure within the same species, and highlighted how forest management can decrease the risk of late frost by selecting provenances with later spring phenology.

Chapter 2 analysed frost hardiness in seedlings belonging to seven *A. saccharum* provenances growing in two sites near the northern limit of the species' range, using repeated destructive sampling to measure frost hardiness during the winter season and compare its endogenous and environmental controls. The hypotheses were that 1) local weather influences frost hardiness, with seedlings in the colder site showing earlier and faster acclimation in autumn, higher maximum frost hardiness during winter, and later and slower deacclimation in spring, compared with the southern site; and 2) climatic conditions at the provenance origin influence frost hardiness, with seedlings from colder provenances showing earlier and faster acclimation in autumn, higher frost hardiness, and later, slower deacclimation in spring compared with warmer provenances. The results partially confirmed hypothesis 1, as the saplings in the warmer site showed earlier and faster deacclimation confirming the strong effect of warm temperatures on spring reactivation. Hypothesis 2 was rejected since no difference was found during acclimation in the autumn or maximum frost hardiness during winter, nor between provenances. LT50, i.e. the lethal temperature for 50% of the cells, varied between -4 °C in summer (July) and -68 °C in winter (February). All provenances attained LT50 of -55°C or lower, far below the common minimum winter temperatures at the northern limit of the species' distribution.

Chapter 3 focused on the role of freezing temperatures on chilling accumulation and endodormancy break. *A. saccharum* saplings belonging to 7 provenances and growing near the northern range limit of the species were exposed to either natural or artificial chilling conditions (4°C) starting at the end of autumn. Samples were transferred to forcing conditions

at regular intervals throughout the winter. I measured frost hardiness at the time of transfer and observed time to budbreak under forcing conditions. The predictions were that 1) endodormancy break would be easier to detect in artificial chilling treatments, where the confounding effect of frost hardiness would be limited; 2) a chilling model considering freezing temperatures would be more effective in a cold climate experiencing temperature below 0°C for several months; 3) samples with higher frost hardiness would take more time to perform budbreak, as they need more time to deacclimate. The results confirmed our hypotheses, highlighting how 4°C can both fulfill the chilling requirement and promote deacclimation, leading to ontogenetic development in the bud. Conversely, samples under natural chilling retained higher frost hardiness until late in the season, correlating with longer time to budbreak and a more difficult identification of an endodormancy break point. Provenance did not have a significant effect in the time to budbreak under forcing conditions. My results indicate that including freezing temperatures can improve chilling calculations in cold climates, where temperatures remain below 0°C during most of the winter. Moreover, measuring frost hardiness during chilling-forcing experiments can clarify its role in chilling accumulation and dormancy dynamics.

The three chapters of this work added original data to the literature, providing insight into the interconnected traits of frost hardiness and phenology. The results show how tree species can have different degrees of plasticity and intraspecific differentiation. *P. mariana* transferred to warmer conditions had different timings of budbreak between provenances, a trend consistently observed over several years of observation. On the other hand, *A. saccharum* had higher plasticity, with provenances from the study area (the northern portion of the species' range) showing similar responses to environmental conditions in both frost hardiness and budbreak. These intra-specific differences in plasticity have important implications for frost risk. Northern *P. mariana* provenances may be more exposed to late frost in the future because of their lower forcing requirements, but provenance selection may be an effective tool to reduce the risks, at least in commercial forestry. *A. saccharum* may already be able to survive winters north of its current range of distribution thanks to its high frost hardiness. Its increased plasticity in frost hardiness and budbreak means that provenance selection would have little effect on frost risk reduction. This work shows the importance of studying frost hardiness and dormancy regulation, especially under global warming. These traits are fundamental components of the life cycle of trees, and a more thorough comprehension is needed to improve predictions of climate change impacts and to develop adaptive forest management practices.

TABLE DES MATIÈRES

LISTE DES TABLEAUX

LISTE DES FIGURES

DÉDICACE

Dedicato a Francesco Mura

Explicit, expliceat,

Bibere scriptor eat.

REMERCIEMENTS

Huge thanks to my supervisors Sergio Rossi, Annie Deslauriers and Patricia Raymond, easily among the hardest workers I ever met. During the 4 years (and something) of the PhD, they have been always present and available to provide feedback and allow me to succesfully complete this first big step in academia. Special thanks to Sergio for spurring and supporting me to overcome my doubts and to always try new approaches and experiments; to Annie for the stimulating discussions and precious insight into all things physiology; to Patricia for the support and the opportunity to collaborate on assisted migration projects, both greatly motivated me during this experience.

I am grateful to my evaluation committee, Guillaume Charrier and Sylvain Delagrange. Their insightful feedback, constructive criticism, and guidance helped refine my research project and greatly improve the quality of my work. Special additional thanks to Guillaume, who was there since the start to teach me the ways of tree frost hardiness and helped me develop my protocol, analyses and interpretation.

Heartfelt thanks to Valentina Buttò for being an invaluable help for the majority of my PhD, and providing both me and my project with solid foundations to build upon.

Big thanks to Al Kovaleski, who was beyond helpful and patient, and greatly helped me improve my 3rd chapter with his insight on the link between frost hardiness and chilling accumulation.

Grateful acknowledgments are due to Denise Alano, who was an invaluable help in the field and the lab. You worked really hard since the start and I have no doubt you will go far.

Desidero ringraziare di cuore tutta la mia famiglia, tutte e due le metà. Mia madre e Luca per il supporto costante e incrollabile, che mi ha permesso di arrivare fin qua. Alessia, Guido e Flavio, per le difficoltà superate insieme e la forza che mi date. Cosima, Sabina e Jacopo per esserci sempre, tappa fisse nelle mie peregrinazioni. Gli zii neozelandesi Claudio e Lise, spero di venire a trovarvi presto. Pumba per tutto il buonumore, anche a distanza.

Un ringraziamento speciale anche a tutti i miei amici. Non riesco a credere alla fortuna che ho avuto nel conoscere così tante persone speciali. Grazie a Gullo, fratello per sempre e compagno di avventure. Passano mesi o anni e ogni volta che ci ritroviamo siamo vicini uguale. A Max, Tundra e Gina per le sessioni di studio, le bivaccate, i viaggi, i festival, l’Interfob. A tutta la Plaza milanese compatta, diversa e stravagante. A tutti i figgeu e figgie del Tigullio per i momenti condivisi nell’infinita ricerca di un dottorato.

A gigantic thanks to Zea for all the adventures past and future, and for encouraging me to try this research thing despite all my self doubt.

Thanks to Aslak for being a great friend and the best guide to Norwegian beer, culture, food, outdoors, and beer.

Un gros merci à la gang du Sag pour le support et le bonheur à travers le doctorat.

Un gros merci également à Mlo pour les beaux mois passés ensemble et les expériences partagées.

INTRODUCTION

In temperate and cold climates, trees alternate between phases of active growth and periods of reduced metabolism (i.e. dormancy) in order to survive unfavorable winter conditions (Hänninen & Kramer, 2007; Junttila, 2007). Transitions between growth and dormancy are accompanied by physiological adjustments, such as inhibition of growth and increased frost hardiness overwinter (Charrier et al., 2015; Sakai & Larcher, 1987). This cycle is marked by key events such as growth initiation and budbreak (the emergence of live tissues from the dormant bud) in the spring and growth cessation and bud set (the formation of the bud) towards the autumn (Hänninen, 2016). Phenology is the study of such recurring, cyclic events, and of the biotic and abiotic factors that influence them (Delpierre et al., 2016).

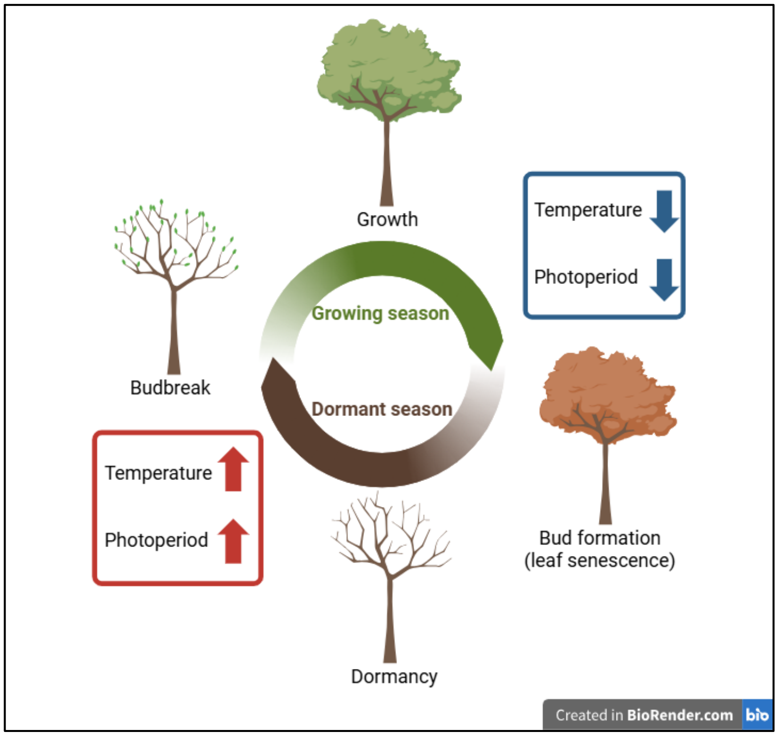


Figure 1 : Example of yearly phenological cycle in temperate and boreal tree species. Trees enter dormancy at the end of the growing season in response to decreasing temperature and photoperiod, a transition accompanied by the phenological events of bud formation, growth cessation, and in deciduous species leaf senescence. After spending the winter in a dormant state, increasing temperatures and photoperiod in the spring induce budbreak and growth resumption, marking the beginning of a new growing season.

The synchronization of the annual phenological cycle with environmental conditions is essential to ensure optimal tree survival and growth (Hänninen, 2016; Vitasse, Lenz, & Körner, 2014). However, climate change can disrupt this balance through rising temperatures and increasing weather variability (IPCC, 2023). Mismatches between phenology and environment can result in exposure to damaging weather events, a typical example being an early budbreak in the spring leaving growing tissues exposed to late frosts (e.g. Hufkens et al., 2012; Kriebel, 1957; Vitasse & Rebetez, 2018). Moreover, the frequency of freezing days can have diverging effects on tree growth and carbon sequestration depending on species- and population-specific traits (Girardin et al., 2022). To address this problematic there is a need for a better understanding of tree phenology, frost hardiness and the effect of environmental cues on these traits (Chuine et al., 2016; Fadón et al., 2020). In particular, winter dormancy, frost hardiness and their environmental and endogenous regulation are still poorly understood, and more scientific studies combining field observations and controlled-conditions experiments are needed (Hänninen et al., 2019; North & Kovaleski, 2024; Wang et al., 2020).

Tree Phenology and Frost Hardiness

Freezing temperatures exceeding the tree's frost hardiness can cause intra-cellular ice formation, rupturing the cell membrane and causing cell death (Sakai & Larcher, 1987; Uemura et al., 2006). Trees adopt two main strategies to survive cold weather: avoidance and tolerance (Charrier et al., 2015). Avoidance is achieved through phenological shifts, by synchronizing dormancy with the unfavorable season, thus preventing the exposure of vulnerable growing tissues to cold temperatures (Vitasse, Lenz, & Körner, 2014). Tolerance is achieved by increasing frost hardiness (i.e., the ability of the tree to withstand cold temperatures) in the overwintering organs (in deciduous species, branches and buds) during the dormant season (Charrier et al., 2018; Sakai & Larcher, 1987). These two strategies are complementary rather than exclusive, as both are employed by trees during their annual cycle.

Frost avoidance

Avoidance is strictly connected to tree phenology, and in particular to dormancy dynamics. Traditionally, dormancy is divided in three different phases: paradormancy, endodormancy and ecodormancy (Lang et al., 1987). Paradormancy is the transition between the growing season and proper dormancy. During paradormancy, bud set and lignification take place in response to decreasing photoperiod and cold temperatures in the autumn, but dormancy is still reversible and may be released in response to warm temperatures (Camargo Alvarez et al., 2018). An increase in the depth of dormancy marks the start of the endodormancy phase (Baffoin, 2021). Endodormancy is characterized by internal growth inhibitors which prevent growth resumption in response to favorable environmental conditions, such as abnormal off-season warm temperatures (Charrier et al., 2011). Endodormancy is released by exposure to a certain amount of low temperatures, named chilling requirement, after which trees enter the ecodormancy phase (Chuine et al., 2016; Sakai & Larcher, 1987). Ecodormancy depends on external temperatures, and is mainly regulated by warm temperatures in the spring inducing budbreak and growth resumption (Charrier et al., 2015; Kalberer et al., 2006). In some species, photoperiod also plays a role in releasing ecodormancy (Basler & Körner, 2012; Heide, 1993). The accumulation of warm temperatures necessary to induce budbreak is called forcing requirement.

Frost tolerance

The dynamics of frost tolerance, i.e. the increase and decrease in frost hardiness in the overwintering organs, closely match the dormancy succession (Figure 2). During the growing season most resources are allocated to growth and reproduction, while frost hardiness is at a minimum (Hänninen, 2016; Welling & Palva, 2006). As the tree enters dormancy, frost hardiness gradually increases in response to cold temperatures and short photoperiod until reaching its maximum levels (Charrier & Améglio, 2011). This process, known as ‘cold acclimation’, is induced by environmental factors (mainly temperature and photoperiod), which trigger endogenous responses linked to frost hardiness at the cellular level, such as osmotic and sugar content adjustments (Baffoin, 2021; Welling & Palva, 2006). Frost hardiness remains high during the winter, then gradually decreases in response to warming temperatures leading to spring, budbreak and growth onset in a process called deacclimation (Charrier et al., 2011; Kovaleski et al., 2018).

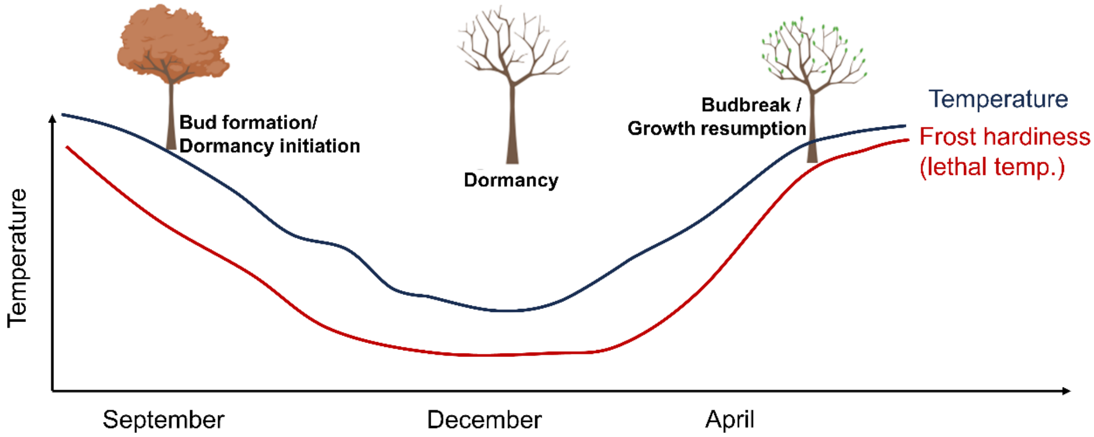


Figure 2: Conceptualization of frost avoidance and tolerance. Trees enter dormancy during the winter (frost avoidance) and at the same time increase frost hardiness in overwintering organs, becoming capable of tolerating lower temperatures during dormancy (frost tolerance).

The physiology of frost hardiness

At the physiological level, reducing cell water content (i.e. dehydration) and increasing the concentration of soluble sugars in the citosol appears to have a primary role in cold acclimation, although several mechanisms are involved (Baffoin, 2021; Deslauriers et al., 2021; Xin & Browse, 2000). During autumn, starch reserves accumulated during the growing season are converted to soluble carbohydrates such as glucose, raffinose, sucrose and fructose, which in turn increase frost hardiness by stabilizing cell membranes, preventing frost-induced dehydration and acting as cryoprotectants (Charrier et al., 2013; Deslauriers et al., 2021). Dehydration, which occurs naturally during winter because of transpiration (evergreens) and root exudation (evergreen and deciduous), increases frost resistance by lowering the freezing point of the cytosol and enhancing the synthesis of soluble sugars (Charrier & Améglio, 2011). Supercooling, i.e. the property of some liquids to avoid freezing at temperatures below their melting point, is also a relevant mechanism in limiting intracellular ice formation and frost damages (Bozonnet et al., 2024).

Overall, dormancy and frost hardiness dynamics are largely driven by environmental cues such as temperature and photoperiod (Hänninen, 2016; Junttila, 2007; Vitasse, Lenz, & Körner, 2014). Therefore, frost avoidance and tolerance closely depend on the phenological cycle to minimize frost risk. Climate change raises the question of whether the phenology of tree species and populations will still match rapidly shifting environmental conditions.

Climate change

The ongoing anthropogenic changes in the earth's climate are quickly modifying environmental conditions (IPCC, 2023). Global mean temperature has been 1.1° C higher during 2011-2020 than in 1850-1900, with additional increases expected for the next future. The warming is stronger and quicker over land masses and northern regions of the boreal hemisphere (IPCC, 2023). Extreme events such as off-season frosts, droughts and floods are projected to increase, with significant impacts on human societies and forest ecosystems alike (IPCC, 2023).

Such drastic changes affect tree phenology, as well as growth and survival, and affect forest structure, composition and response to disturbances (Hogg et al., 2017; Morin et al., 2009; Ruiz-Pérez & Vico, 2020). One main effect of rising temperatures is an earlier ecodormancy release, which advances the timing of budbreak in the spring (Hänninen & Tanino, 2011). The combination of earlier budbreak and increased variability in temperature can increase the risk of a late spring frost, i.e. freezing temperatures occurring after leaf-out and damaging the young shoots (Chamberlain et al., 2019). Indeed, late frosts have received increasing attention in the past few years, as more events and increasing risks are studied (Chamberlain et al., 2021; Liu et al., 2018; Marquis et al., 2022; Vitasse & Rebetez, 2018; Zohner et al., 2020). Direct measurements of the impact of this kind of discrete event are difficult to plan and carry out, calling for more field studies that can provide direct evidence and improve frost damage predictions under climate change (Atucha Zamkova et al., 2021; Jentsch et al., 2007).

At a larger scale, climate change is inducing a poleward shift of tree habitats (McKenney et al. 2007, Boisvert-Marsh and Périé 2014). As the rate of habitat shift is often faster than trees ability to migrate, artificial plantings of tree species and populations at increasing latitudes and elevations beyond their current range (i.e. assisted migration) are proposed to allow tree species to keep up with the pace of climate change (Palik et al., 2022; Pedlar et al., 2012; Ste-Marie et al., 2011). However, as trees migrate or are transferred to colder conditions winter frost damage could be a significant constraint, negatively impacting growth and performance of trees (Kreyling et al., 2015; Winder et al., 2011). For example, Montwé et al. (2018) found that a frost event taking place 20 years after planting induced lasting growth reduction in a *Pinus contorta* (Dougl. ex. Loud) common garden. The common garden was located in the center of the species' range, with the southern provenances being more affected by the frost event. This example highlights how frost hardiness is a relevant trait to determine species' potential for migration into colder areas, and to select provenances or populations at the intraspecific level (Aubin et al., 2016). Quantifying frost hardiness can therefore both improve predictions of tree migration under climate change and the selection of species and provenances in assisted migration projects.

Intraspecific differences

Species with a wide distribution can develop adaptations to local environmental conditions, forming distinct populations or ecotypes. These adaptations can produce differences in functional traits including phenology and frost hardiness, making a population slightly distinct from other populations of the species it belongs to (Westerband et al., 2021). Such intraspecific differences are of primary importance under climate change, as they can produce diverging responses to environmental cues in populations or provenances of the same species (Des Roches et al., 2018). Studies investigating intraspecific variations in functional traits can therefore provide valuable information to scientists, land managers and decision-makers (Hänninen, 2006; Rammig et al., 2010).

The existence of intraspecific gradients in budbreak phenology is well known in the literature. For instance, northern populations of some species can have a lower forcing requirement for growth reactivation in the spring, resulting in earlier budbreak when growing in a common garden with southern populations (Barsoum, 2015; Ren et al., 2020; Risk et al., 2021; Rossi & Bousquet, 2014). Conversely, higher chilling requirements of some populations may remain unfulfilled during warmer winters, causing a delay in budbreak and a shortening of the growing season (Chuine et al., 2016; Kriebel & Wang, 1962). These differences can have important implication for frost risk exposure. For example, populations with earlier budbreak may have a higher risk of exposure to late frost. Indeed, late frost avoidance seems to be a primary driver of intra- and inter-specific differences in budbreak timings for many tree species (Lenz et al., 2016; Vitasse, Lenz, & Körner, 2014).

Intraspecific differences can also be more directly related to frost hardiness levels. For instance, populations adapted to colder locations may have higher frost tolerance and winter survival rates (Vitasse, Lenz, & Körner, 2014). Interestingly, these differences may be specific to one phase of dormancy. Beuker et al. (1998) found that provenances of the boreal species *Pinus sylvestris* (L.) and *Picea abies* ([L.] Karst) had different levels of frost hardiness only during acclimation in the autumn, but not during midwinter and deacclimation. Conversely, Charrier et al. (2011) found that *Juglans regia* genotypes differed during deacclimation, but not during acclimation or midwinter.

Intraspecific differences can be stronger in some tree species than others. Generally, there is a trade-off between phenotypic plasticity (i.e. potential to acclimate to a wider range of conditions) and genetic adaptation (i.e. specializing to local conditions). While the degree of specialization is species-specific, it appears to be more common in conifers, with broadleaves showing greater plasticity (Leites & Benito Garzón, 2023). Moreover, within many species there is a tendency towards lower phenotypic plasticity in populations adapted to the coldest climates (Rehfeldt et al., 2018). Given the lack of knowledge and direct measurements of frost hardiness for many tree species, it is necessary to conduct more experimental research on this subject to elucidate intraspecific patterns in order to improve climate change impact predictions and provenance selection.

Novelty and experimental design

In this work, I evaluated the intraspecific differences in phenology and frost hardiness in two key species of Eastern Canada's forests, black spruce (*Picea mariana* Mill. BSP) and sugar maple (*Acer saccharum* Marsh.). For both species, provenances from different geographic areas were compared. In this work, I chose to use the term "provenance" rather than "population" or "ecotype" as these last two assume a genetic difference. Evaluating genotypic differences is beyond the scope of this work, so I used the term "provenance" to indicate trees originated from a specific geographical area.

Black spruce is an emblematic species of Canada's boreal forest, expanding up to the northern edge of the forest-tundra biome border. The ability of the species to grow in extremely cold climates where temperatures below -40°C can occur is well known, but late and early frosts can significantly damage young shoots even at temperatures as mild as -4°C and represent a common problem in forest nurseries (Dang et al., 1992; Glerum, 1973; Lamhamedi & Bernier, 1994). Using a common garden experiment, established in 2014 and counting five provenances of black spruce from a wide latitudinal gradient (48°13' to 53°47'), I evaluated whether different timings of budbreak in black spruce provenances translate to different risks of exposure to late frost in the spring. Specifically, I collected data of frost damage in the common garden after a naturally occurring late frost event and compared the amount of frost damage with the stage of budbreak measured before the event. Such direct measurements of the effect of late frost are scarce, because of the difficulty in predicting such discrete events.

Sugar maple spans a wide distribution in eastern North-America (Godman et al., 1990) and holds great ecological and economic importance because of maple syrup production, particularly in Canada (Agriculture and Agri-Food Canada, 2019). Despite its importance, studies on sugar maples' genetic variation and intraspecific differences in functional traits are underrepresented in provenance trial experiments compared to other species (Eskelin et al., 2011; Risk et al., 2021). Past and recent studies point to the existence of intraspecific differences in sugar maple (Guo et al., 2020a; Kriebel & Wang, 1962; McCarragher et al., 2011; Ren et al., 2020), but the relationship between phenology and frost hardiness remains to be explored. In this work, I used seven provenances of sugar maple issued from the northern portion of the species' range. I quantified frost hardiness monthly over the course of the 2021/2022 winter in all provenances. Further, during the 2022/23 and 2023/24 winters I carried out chilling-forcing experiments to compare the effect of artificial and natural chilling conditions during the winter on both budbreak timing and frost hardiness acclimation/deacclimation. These experiments yielded valuable data, previously missing from the literature on this species, with valuable implications for forest managers under climate change.

Objectives and hypotheses

The general objectives are i) assessing intraspecific variability in frost hardiness and phenology of budbreak and dormancy, and ii) linking these two traits to understand intraspecific variations in risk of frost damage.

The dissertation is formed by three chapters, each following the structure of a scientific paper, which has either been published or submitted to a peer-reviewed journal at the time of writing. The formatting of the texts has been standardized to enhance the document's readability. A reference to the published versions of the chapter is provided on the title page of each chapter.

Chapter 1 focuses on the impacts of a natural late frost event occurring in a common garden of black spruce, analyzing frost damage through the lens of intraspecific differences in budbreak timings. The hypothesis was that under the same conditions, provenances from colder climates are more damaged by the frost because of an earlier budbreak, which exposes growing shoots to freezing temperatures.

Chapter 2 analyses frost hardiness in seven sugar maple provenances growing in two sites near the northern limit of the species' range, using repeated destructive sampling to compare the endogenous (i.e. determined by seed provenance) and environmental (i.e. induced by conditions in the site of growth) controls of frost hardiness throughout a winter season. The hypotheses were that 1) local weather influences frost hardiness, with seedlings in the colder site showing earlier and faster acclimation in autumn, higher maximum frost hardiness during winter, and later and slower deacclimation in spring, compared with the southern site; and 2) climatic conditions at the provenance origin influence frost dynamics, with seedlings from colder provenances showing earlier and faster acclimation in autumn, higher frost hardiness, and later, slower deacclimation in spring compared with warmer provenances.

Chapter 3 compares the effect of exposure to both natural and artificial chilling conditions on budbreak in sugar maple. The effect of provenance is considered, as well as the contribution of freezing temperatures to chilling during the winter. The hypotheses were that 1) endodormancy break would be easier to detect in artificial chilling treatments, where the confounding effect of frost hardiness would be limited; 2) a chilling model considering freezing temperatures would be more effective for a cold temperate species experiencing below zero temperature for several months; and 3) sugar maple samples with higher frost hardiness would take more time to perform budbreak.

CHAPITRE 1

The early bud gets the cold : Diverging spring phenology drives exposure to late frost in a *Picea mariana* [(Mill.) BSP] common garden.

Published in Physiologia Plantarum

Mura, C., Buttò, V., Silvestro, R., Deslauriers, A., Charrier, G., Raymond, P., & Rossi, S. (2022). The early bud gets the cold : Diverging spring phenology drives exposure to late frost in a Picea mariana [(Mill.) BSP] common garden. Physiologia Plantarum, 174(6), e13798. https://doi.org/10.1111/ppl.13798

1.1 ABSTRACT

Under climate change, the increasing occurrence of late frost combined with advancing spring phenology can increase the risk of frost damage in trees. In this study, we tested the link between intraspecific variability in bud phenology and frost exposure and damages. We analysed the effects of the 2021 late frost event in a black spruce (*Picea mariana* (Mill.) BSP) common garden in Québec, Canada. We hypothesized that the timing of budbreak drives the exposure of vulnerable tissues and explains differences in frost damages. Budbreak was monitored from 2015 to 2021 on 371 trees from five provenances originating between 48° and 53° N and planted in a common garden at 48° N. Frost damages were assessed on the same trees through the proportion of damaged buds per tree and related to the phenological phases by ordinal regressions. After an unusually warm spring, minimum temperatures fell to -1.9°C on 28 and 29 May 2021. At this moment, trees from northern provenances were more advanced in their phenology and showed more frost damages. Provenances with earlier budbreak had higher probability of damage occurrence according to ordinal regression. Our study highlights the importance of intraspecific variability of phenological traits on the risk of frost exposure. We provide evidence that the timings of bud phenology affect sensitivity to frost, leading to damages at temperatures of -1.9°C. Under the same conditions, the earlier growth

reactivation observed in the northern provenances increases the risks of late frost damage on the developing buds.

1.2 INTRODUCTION

In cold-exposed areas, woody plants alternate phases of intense activity, growth and reproduction with dormancy (i.e. low metabolic activity) to survive under unfavourable conditions (Junttila, 2007). In temperate and boreal ecosystems, temperature is a limiting factor, mainly during winter. When temperatures drop below zero, ice is formed within the living tissues, leading to cell death by frost desiccation and disruption of the plasma membrane (Dowgert & Steponkus, 1984; Uemura et al., 2006). Frost damage happens when the exposure to cold temperatures (environmental factor) exceeds plant sensitivity (endogenous factor) (Burke et al., 1976; Charrier et al., 2015, 2018).

Trees adopt two strategies to reduce the risk of frost damage: avoidance and tolerance (Charrier et al., 2011). Avoidance is achieved through the synchronization of phenological processes (dormancy) with the unfavourable season, thus preventing the exposure of sensitive growing tissues to damaging temperatures (Chamberlain et al., 2019). Tolerance is achieved by increasing frost hardiness (i.e., the ability of tissues to withstand cold temperatures by preventing ice formation inside the cells) in the overwintering organs during the dormant season (Atucha Zamkova et al., 2021). Therefore, frost hardiness of trees changes according to the organ (variation in space) and phenological phase (variation in time) (Charrier et al., 2013; Sakai & Larcher, 1987). Current scientific knowledge suggests that trees at high latitudes withstand temperatures lower than -40°C during dormancy, but their developing shoots can be damaged at -4°C (Dang et al., 1992; Glerum, 1973).

The two strategies of frost tolerance and frost avoidance are closely linked and depend on a good synchronization with the seasonal variations between favourable and unfavourable temperatures. Frost hardiness is generally at a minimum during the growing season, when most resources are allocated to growth (Lang et al., 1987). Growing tissues have a high water content to achieve the cell turgor necessary for cell enlargement (Steppe et al., 2015) and are therefore more likely to freeze intracellularly, resulting in frost damage (Charrier et al., 2013). As days get shorter and temperatures decrease, secondary growth stops and the primary meristems become protected from cold temperatures by the formation of winter bud scales, entering into dormancy (i.e. frost avoidance; Charrier, 2022). This phase of dormancy, endodormancy, is internally regulated by growth inhibitors, and released by exposure to accumulated cold temperatures (i.e. chilling requirement) (Chuine et al., 2016). As temperatures get colder, the frost hardiness of overwintering organs (i.e. frost tolerance) increases through physiological mechanisms aimed at lowering the freezing point of the living cells and favouring the formation of extra-cellular ice (Baffoin, 2021; Charrier et al., 2013; Deslauriers et al., 2021). Once the chilling requirements are fulfilled, the trees enter the ecodormancy phase. In this phase, frost hardiness decreases in response to warm temperature (Charrier et al., 2011, 2015; Kovaleski, 2022; Kovaleski et al., 2018; North et al., 2022). After being exposed to a certain amount of warm temperatures (i.e. forcing requirement) trees resume growth through the phenological event of budbreak. The forcing requirement can be measured as heat units accumulation, e.g. with the commonly used growing degree-days (GDD) calculated by subtracting daily mean temperatures from a pre-determined base threshold (Snyder et al., 1999).

The ongoing warming conditions produced by climate change are shifting the timing of phenological events by fulfilling forcing requirements earlier (i.e. accelerating ecodormancy release), potentially exposing plants to an increasing risk of frost damages due to late frost events (Gu et al., 2008; Liu et al., 2018; Vitasse et al., 2018; Zohner et al., 2020). Late frost events are characterized by freezing temperatures taking place after plant leaf-out in the spring, often causing significant damages to crops and forests (Chamberlain et al., 2019; Rigby & Porporato, 2008). Under climate change, the combination of warmer spring conditions and increased variability in temperature values make late frosts a serious threat, which has received increasing attention in the scientific literature (Chamberlain et al., 2021; Marquis et al., 2022). However, late frosts are discrete weather events, more difficult to predict compared to long-term climatic trends and mean values (Jentsch et al., 2007). This makes field measurements of such events challenging to design in advance, limiting empirical evidence about this topic and generating uncertainty in predicting future consequences (Atucha Zamkova et al., 2021; Chuine et al., 2016; Warrington & Rook, 1980).

A relevant source of uncertainty is that species with a wide distribution can exhibit ecotypes with different responses to environmental drivers, resulting in variations in phenological traits. For example, when growing in the same conditions, northern provenances of some species show earlier budbreak than southern provenances (Ren et al., 2020; Rossi & Bousquet, 2014). These diverging responses point to differences in plant sensitivity to environmental cues, e.g. lower forcing requirements in a provenance can lead to earlier budbreak. Since growing tissues are more vulnerable to frost damage, different timings of budbreak entail a different sensitivity to frost within the same species (Charrier et al., 2013). Understanding the link between these intraspecific differences in phenology and the risk of exposure to late frosts can both improve predictions of climate change impacts on forests (both natural and managed) and help adopt management strategies to minimize the risk of frost damage in the spring, e.g. by provenance selection. Given its wide distribution across the boreal region of North America, and its ecological and economic importance, black spruce [*Picea mariana* (Mill.) B.S.P.] is a relevant model species to study this problematic. The current scenario of increasing risk of late frost would entail negative impacts on growth, carbon uptake, and survival of boreal species by loss of photosynthetic and reproductive tissue (Hufkens et al., 2012b) and potentially increase the susceptibility to other stressors, such as drought (Charrier et al., 2021).

This study investigated the impact of a late frost event occurring in spring 2021 in a common garden hosting five *P. mariana* provenances originating from a temperature gradient in the boreal forest of Quebec, Canada. The common garden is located in the southernmost margin of the closed-canopy boreal forest, representing a warmer condition for most provenances. The aim of our work was to understand the relationship between phenological patterns in tree provenances and the potential exposure to frost damage. We tested the hypothesis that under the same conditions, provenances from colder climates are more damaged by the frost because of an earlier budbreak, which exposes growing shoots to freezing temperatures.

1.3 MATERIALS AND METHODS

1.3.1 Common garden and phenological observations

Black spruce [*Picea mariana* (Mill.) B.S.P.] seeds from natural stands were collected from five provenances located along a latitudinal and climatic gradient between 48° and 53° N in the boreal forest of Quebec, Canada (Figure 3, Table 1). The term "provenance" is used in this work to indicate the site of origin, i.e. the natural *P. mariana* stand in which the seeds were collected. Simoncouche (SIM) and Bernatchez (BER) are located in the A. balsamea - B. papyrifera (balsam fir-white birch) bioclimatic domain. Mistassibi (MIS) and Camp Daniel (DAN) belong to the *P. mariana* - moss bioclimatic domain. Mirage (MIR) is located in the *P. mariana* - lichen domain characterized by lower density and growth rates (Morneau, 2021). All sites are natural forests, i.e. no forestry operations or other anthropogenic disturbances are recorded. We therefore consider these stands to be representative of locally adapted and naturally occurring *P. mariana*.

Table 1 : Characteristics of the five sites where black spruce provenances were collected.

Site	Total count of trees	Latitude (N)	Longitude (W)	Altitude (m a.s.l.)	Annual T (°C) 1970-2000	Min T Coldest month (°C) 1970-2000
SIM	89	48°13'	71°15'	338	1.2	-21.6
MIS	56	49°43'	71°56'	342	0.1	-25.2
BER	86	48°51'	70°20'	611	-0.6	-23.3
DAN	71	50°41'	72°11'	487	-0.9	-26.6
MIR	69	53°47'	72°52'	384	-3.4	-28.8

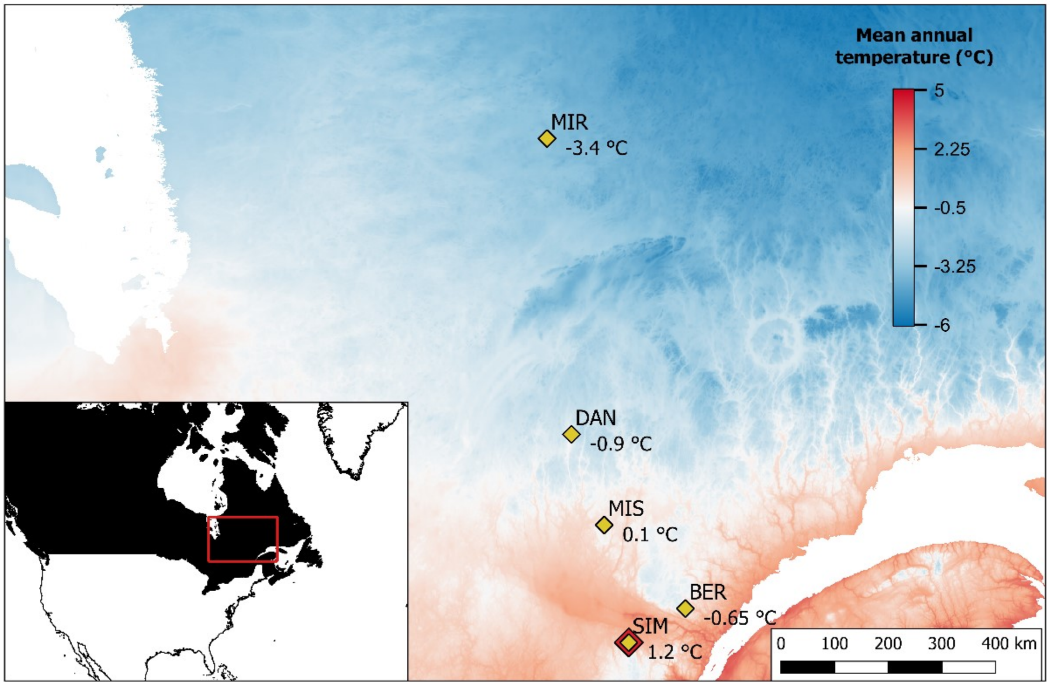


Figure 3 : Geographical location of the five *P. mariana* provenances in Québec, Canada. Provenance origins (yellow diamonds) are shown with their mean annual temperature. All provenances were planted in a common garden located in the southernmost provenance site, SIM (diamond with red border)

The climate of the area is typically boreal, with long, cold winters, and short, cool and wet summers. Temperatures decrease with increasing latitude and elevation, with mean annual temperatures ranging from 1.2 to -3.4°C (Table 1). In this work, we use mean annual temperature at the site of origin to define the climatic gradient in order to account for the effect of elevation. For example, site BER is further south than MIS, but being at a higher elevation the annual temperature is lower (Figure 3, Table 1). Using mean annual temperature to define the gradient allows us to take into account the effective environmental conditions in the provenance's site of origin, instead of only considering the geographical position.

A common garden was established in July 2014 in Simoncouche (SIM), the southernmost site, using seeds collected from the five provenances (Silvestro et al., 2019). Apart from SIM, all the other provenances were transferred to warmer conditions. Seedlings were planted in a 0.5 ha clearcut forest patch, following a grid design with a random distribution of provenances and distances of 2 m × 2 m. On each side of the common garden, two rows of non-experimental spruces were planted to prevent the edge effect of the nearby forest.

Bud phenology was observed once per week from May to October in 2015, 2017-2021 (Guo et al., 2021). Measurements in 2016 were not performed due to logistical constraints. A total of 371 trees were measured for budbreak phenology (Table 1). During weekly visits, the apical bud of each tree was observed to assess the phenological stage. Seven ordinal phases of budbreak were identified: 0) closed bud; 1) open bud with a lightly coloured tip; 2) elongated bud, with scales stretching; 3) swollen bud with smooth scales; 4) translucent bud with needles visible through the scales; 5) bud broken, with needles exposed but not yet spread; 6) exposed shoot and spread needles (Dhont et al., 2010).

1.3.2 Environmental conditions

Environmental conditions for the spring of 2021 were compared with historical averages in order to understand and contextualize the weather leading to the late frost. A weather station located in Simoncouche (500 m from the study area) recorded the temperatures for 2021. The nearest weather station providing long-term data (1990-2020) of temperature and snow cover was in Bagotville, QC, 23 km away from the study site (Environment Canada, 2023). Daily temperature data were recorded at two stations (Simoncouche and Bagotville) from 2010 to 2015. The relationship between the temperatures recorded at the two sites was modelled using linear regressions (Table S1). Slope and intercept of the regression were used to calculate the long-term temperature in the study site by imputation, allowing long-term (1990 - 2020) averages to be computed for the study area in Simoncouche. Growing degree days (GDD) were used as a proxy to represent warming conditions leading to spring and allow for a quantitative comparison between 2021 and the historical average (Gilmore & Rogers, 1958; Snyder et al., 1999). GDD were also used to assess differences in forcing requirements between provenances. We defined the forcing requirement as the mean GDD accumulation necessary to complete budbreak (i.e. reach phase 6), based on our 6 years of phenological observations. In order to select the base threshold for GDD calculation, we tested different base temperatures to compute the GDD necessary for budbreak, and selected the one which produced less variability between years (Snyder et al., 1999). Variability was calculated as the coefficient of variation of the yearly mean GDD value necessary to reach budbreak. Four provenances out of five had a best base threshold (i.e. producing the lowest coefficient of variation) of -3°C (Figure S1). The warmest provenance (i.e. SIM) had very similar variability values using both -2 and -3°C, i.e. 0.0676 and 0.0677 respectively. For consistency, we used -3°C as the base threshold for all GDD calculations in this work.

1.3.3 Frost damage measurement

After the occurrence of damages in the field, a pilot survey was conducted with the objectives of establishing a protocol of frost damage assessment and training the observer to perform the measurement. We based our protocol on the browning method used to visually assess frost damages in conifers by observing tissue browning after frost treatments (Burr et al., 2001; Glerum, 1985). During the pilot survey, the repartition of brown buds on individual trees was also visually assessed to exclude spatial variations in frost damages (e.g. more damage on one side of the tree). Based on the proportion of damaged brown buds on each tree, we defined four frost damage levels: 0) no damaged buds; 1) low, <5% of buds damaged; 2) medium, 5-15% of buds damaged; 3) high, >15% of buds damaged. Observations of frost damages were performed on 11 June 2021, i.e. two weeks after the late frost event. Each tree in the plantation was assigned a score for frost damage, for a total of 371 trees.

1.3.4 Statistical analyses

To test for differences in the occurrence of frost damage between provenances (i.e., presence or absence of damage), we applied a chi-squared (χ^2) test followed by a post-hoc pairwise comparison using Fisher's exact test. We applied an ordinal regression to model the relationship between phenology and frost damage. We set the frost damage level (i.e., 0-3) as an ordinal response variable and the phenological phase at the time of frost occurrence (i.e., 0-6) as ordinal explanatory variable. The Lipsitz goodness of fit test was applied to evaluate the model, with non-significant p-values indicating that the model is reliable (Fagerland & Hosmer, 2016; Lipsitz et al., 1996). Finally, we used the ordinal model to predict the probability of observing each frost damage level based on a given phenological phase. All statistical analyses were performed in R (R Core Team, 2020) using chisq.posthoc.test (Ebbert, 2019), MASS (Venables & Ripley, 2002), generalhoslem (Jay, 2019) and pollen (Nowosad, 2019).

1.4 RESULTS

1.4.1 Phenology across *P. mariana* provenances

During 2015-2020, budbreak varied across years, in duration and timing. In general, it lasted from mid-May until the end of June. The bud phases succession was longer in 2015 (35 days), and shorter in 2019 (55 days). Regardless of the lower threshold temperature used to calculate GDD, provenances from colder climates had lower forcing requirements (Figure S1). The mean value of GDD necessary to complete budbreak (i.e. reach phase 6) varied between provenances along the temperature gradient, with a minimum of 741 ± 47.9 (st. dev.) degree-days for the coldest provenance (i.e. MIR) and a maximum of 892 ± 60.5 (st. dev.) degree-days for the warmest provenance (i.e. SIM). Across years, and despite the inter-annual variation, colder provenances required less GDD to perform budbreak (Figure 4). Having lower GDD requirements (i.e., forcing requirements), provenances from colder sites showed a consistently earlier budbreak compared with provenances from warmer sites (Figure S2). Averaging six years of observations, the coldest provenance (i.e. MIR) completed budbreak (i.e., reached phase 6) on DOY 161 (early May), eight days earlier than the warmest provenance (i.e. SIM).

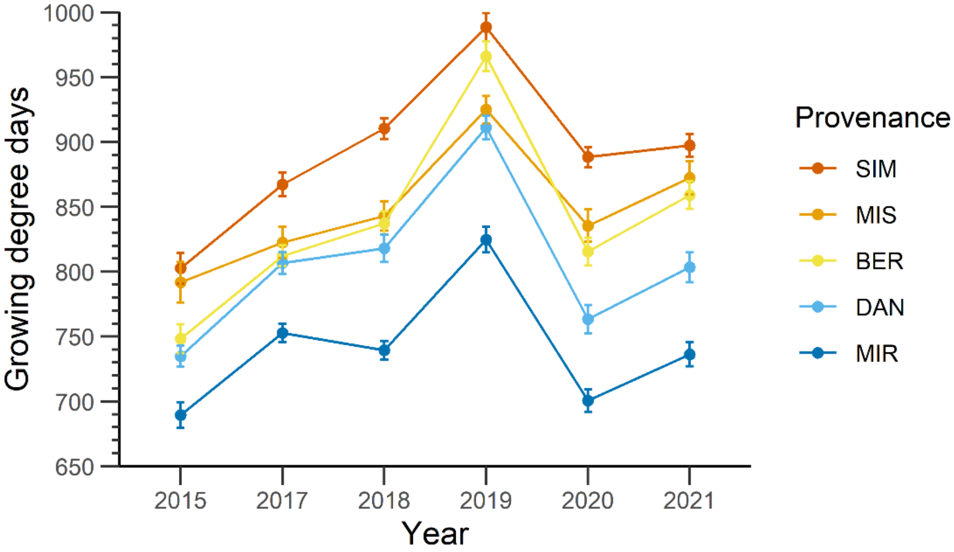


Figure 4 : Mean growing degree days (GDD) necessary to complete budbreak for each year of observation. Colours indicate the five *P. mariana* provenances, points and whiskers the mean ± SE.

1.4.2 The late frost event of 2021

Snow cover in the 2020/2021 winter started on 27 October 2020 (DOY 301), similar to the 30-year average (DOY 304 ± 10 st. dev.). Snow effectively disappeared on April 8 2021 (DOY 98), with only a minor accumulation happening afterwards (Figure 5). Conversely, on the long-term average the snowpack lasts 20 more days, up to DOY 118 (± 10 st. dev.). The snowpack was thin throughout the winter, reaching a maximum snowpack height of 45 cm on 9 March (DOY 68), 15 cm lower than the 30-year average for the same day. GDD (base threshold -3°C) accumulated faster compared to the historical average, reaching 753 degree-days on 27 May (DOY 147), the day before the start of the frost events. This GDD sum was 187 degree-days higher than the 30-year historical average for the same day, indicating the particularly warm conditions of this year (+33% GDD). Maximum temperatures in the late winter peaked twice, on 24 march (DOY 83) and 9 April (DOY 99). In both occasions temperatures reached 17.5°C, which is respectively 17.8 and 13.8°C higher than the long term average. This indicates two warm periods early in the season (Figure 5). Minimum temperatures showed a marked increase after 15 May, culminating at a value of 16.4°C, on 22 May (DOY 142). This value is 11.7°C higher than the average, marking another warm period. The late frost event occurred on 28 and 29 May 2021 (DOY 148-149), when minimum temperatures measured at the study site (Simoncouche) fell abruptly, reaching minimum values of -1.1 and -1.9°C respectively on the two days. Sub-zero temperatures lasted 6 hours on 28 May (2 to 8 AM) and 4 hours on 29 May (3 to 7 AM). Wind conditions during the event were calm, averaging 0.55 and 0.02 m s-1 during the freeze hours of 28 and 29 May, respectively.

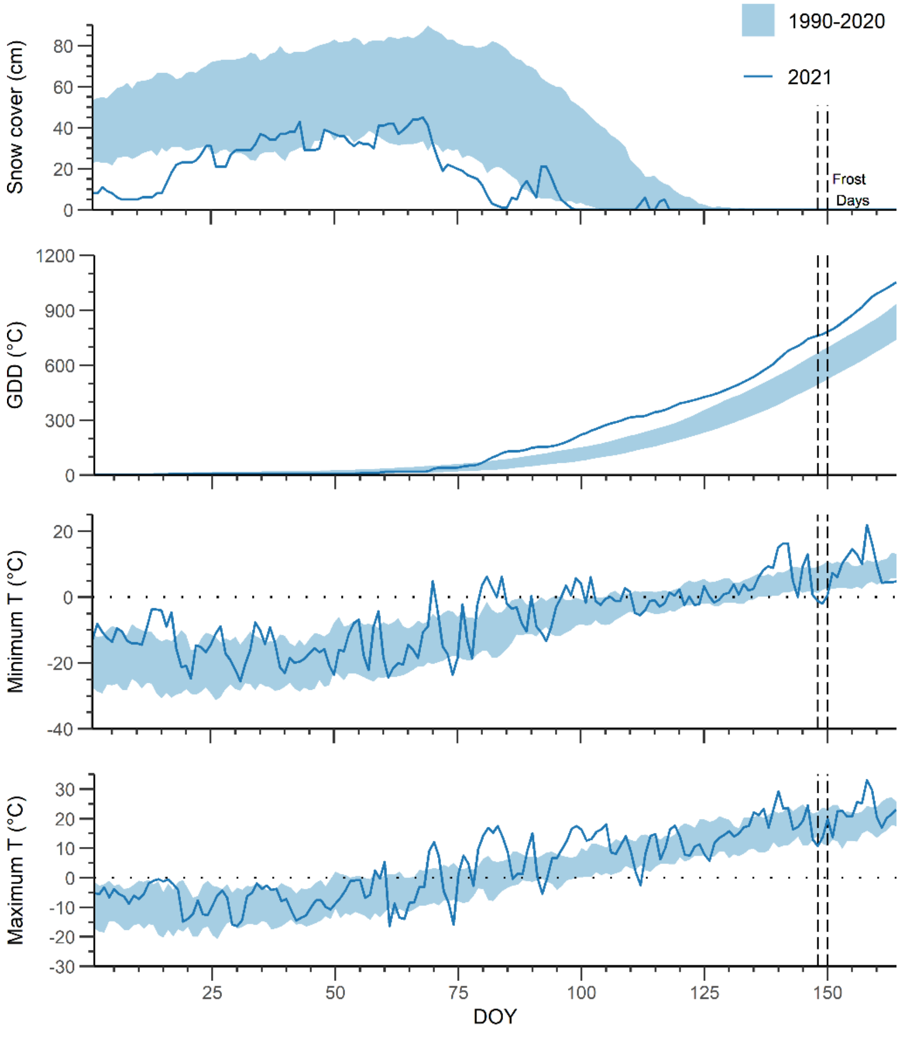


Figure 5 : Climatic conditions of 2021 (blue line) compared to the average for the 30-year period 1990-2020 (mean ± standard deviation, light-blue shaded area). The vertical dashed lines indicate when the frost days occurred. The horizontal dotted lines indicate the reference of 0°C. Temperature data and GDD for 2021 was measured at a weather station located 500 m from the study area. Temperature data and GDD for the 30-year period was estimated for the study area by imputation, from the weather station of Bagotville (23 km from study area). Snow cover data was measured at the weather station of Bagotville.

1.4.3 Timings of spring phenology in 2021

Budbreak was already advanced at the time of frost occurrence, because of the warm conditions of the 2021 spring. On 28 May (DOY 148), the first day on which temperatures dropped below 0°C, 61.5% of the observed trees were at phases 5 or 6 (i.e., the final phases of bud phenology). Provenances originating from colder climates exhibited more advanced phases of budbreak, as observed in 2015-2020 (Figure 6). Indeed, 58.7% of all trees at phase 6 (i.e., the final phenological phase for budbreak) before the frost event were those from MIR, the coldest provenance. In contrast, no tree from SIM, the warmest provenance, had reached phase 6 at the time.

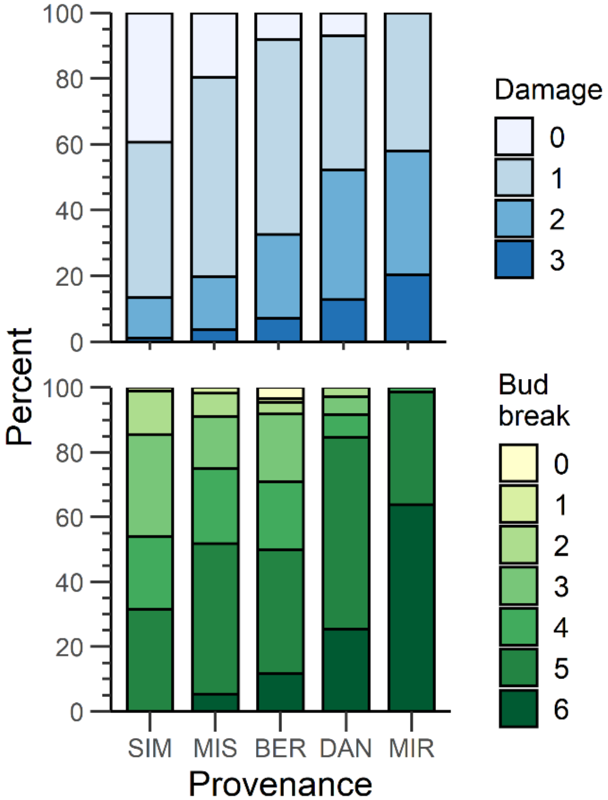


Figure 6 : Distribution of frost damage levels (upper) and budbreak phenological phases at the time of frost occurrence (lower) between trees, by site of provenance. Darker colours indicate a higher proportion of damaged buds and a more advanced budbreak phase, respectively.

1.4.4 Frost damage

Frost damages occurred in the form of collapsed, dead brown buds (Figure 7). The survey confirmed that frost was most likely the cause of the bud mortality, as no sign of other causes (e.g. insects, parasite) was observed. Visual assessment of the repartition of frost damages on individual trees did not identify any spatial variation (i.e. damages appeared well distributed on the tree). Bud mortality rate did not exceed 30%.

Figure 7 : Appearance of the developing buds after the late frost occurred on 28 and 29 May. The brown buds killed by the frost are also visible.

The percentage of damaged trees (i.e. frost damage level > 0) followed the provenance temperature gradient, varying between 60.7 to 100% for the warmest (i.e. SIM) and coldest (i.e. MIR), respectively (Table 2). The chi-square test confirmed differences among the provenances ($\chi 2$ = 58.98, $p < 0.0001$). The post-hoc test indicated that provenances from warmer sites were less damaged than provenances from colder sites, with differences being more marked for the provenances at the extremes of the temperature gradient.

Table 2 : Frost damage occurrence for each provenance of the common garden. Trees are considered damaged if the ordinal level of frost damage is 1 or higher (see section 2.3). The total number of measured trees in the common garden is 371. Letters in brackets indicate significantly different groups, according to posthoc pairwise comparison using Fisher's exact test.

Provenance	N. damaged trees / n. trees	Damaged trees (%)
SIM	54/89	60.7 (a)
MIS	45/56	80.4 (b)
BER	79/86	91.9 (b)
DAN	66/71	93 (bc)
MIR	69/69	100 (c)

Intermediate levels of damage (i.e., level 1 and 2) were more frequent, accounting for 75.7% of the observations. Only 15.6% of the trees had no damage (i.e., level 0); of these, 79.3% belonged to the two warmer provenances (i.e., SIM and MIS). On the other hand, the two coldest provenances (i.e. MIR and DAN) accounted for 71.8% of all observed higher-level damages (i.e., level 3).

The observed distribution of damages matched the phenological phase observed at the time of the frost event, i.e. coldest provenances were more advanced in their timing of budbreak and exhibited more severe damages on their buds (Figure 6). Consistently, the ordinal model predicted higher probabilities of observing damage on more advanced budbreak phases (Figure 8). Goodness of fit indicated that the model is reliable (LR = 3.63, df = 9, p = 0.9339).

1.5 DISCUSSION

This study quantified the effects of a late frost event on *P. mariana* provenances growing in a common garden. We used six years of phenological observations to understand budbreak dynamics in five provenances originating from a thermal gradient along the latitudinal distribution of the boreal forest in Quebec, Canada. We compared the progression of budbreak in 2021 with the resulting levels of frost damage. Provenances from colder sites had an earlier and faster budbreak, and showed more frost damages. We provided field-based evidence that, despite its well-known winter frost hardiness, *P. mariana* can experience damages at air temperatures as mild as -1.9°C in the case of a failure in frost avoidance.

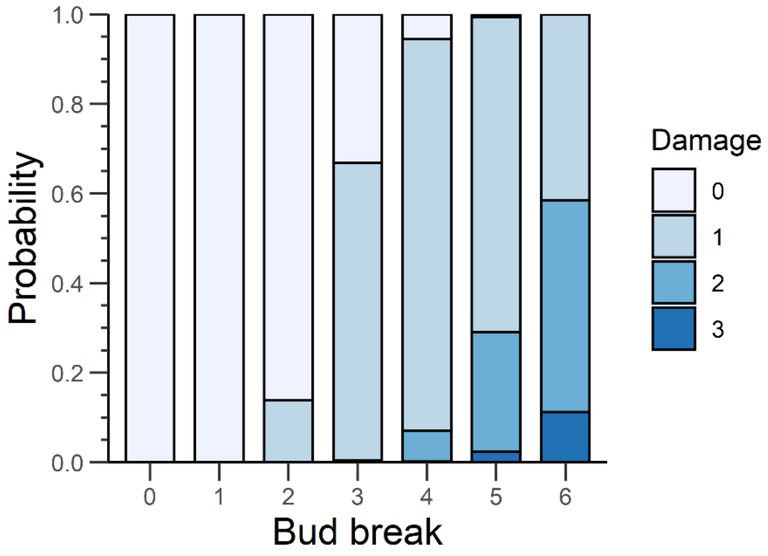


Figure 8 : Probability of frost damage occurring at different phases of budbreak, as predicted by an ordinal regression model.

1.5.1 The late frost event of 2021

Overall, the spring of 2021 showed the typical characteristics of a false spring event, i.e. early warm conditions preceding a late cold spell (Gu et al., 2008). This is evident by looking at GDD accumulation, which started earlier and progressed faster in 2021 compared to the 30-year average. Moreover, snow cover during winter was scarce and melted 20 days earlier than usual (Figure 5). Temperatures reveal three warm spells, with temperatures higher than the norm contributing to hasten budbreak in the trees. Afterwards, there was a return of cold weather, with freezing minimum temperatures recorded on 27 and 28 May in calm air conditions (wind speed < 6 m s-1), indicating a radiative-type frost event (Kalma et al., 1992).

Frost damages were observed after mild temperatures (minimum recorded hourly temperature -1.9°C). While *P. mariana* can withstand temperatures below -40°C during the winter (Glerum, 1973), the growing shoots can be damaged by air temperatures of -4°C (Dang et al., 1992). In the present case, the frost happened during budbreak and we found the highest damages on more advanced buds (i.e. with exposed shoots; Figure 6). In this phase, the cells are highly hydrated to reach the turgor pressure necessary for cell enlargement, have a low concentration of solutes, which act as cryoprotectant in winter, and thinner cell walls (Charrier et al., 2013; Pantin et al., 2012) all factors that increase the likelihood of intracellular ice formation, which is lethal for the cell in natural conditions (Charrier et al., 2015; Wolfe & Bryant, 2001). Moreover, the restoration of water fluxes between the stem and bud favours ice propagation between organs (Neuner & Beikircher, 2010; Villouta et al., 2022). The less densely packed configuration of elongating buds may also contribute to increase bud vulnerability to frost. For these reasons, shoots in the first phase of elongation have lower frost hardiness than both closed buds and fully formed shoots (Lenz et al., 2013). This shows the importance of matching budbreak with favourable environmental conditions. To our knowledge, this is the first time that damages on immature *P. mariana* shoots have been quantified at air temperatures above -2°C. The literature found similar thresholds for other species, indicating that spring temperatures of -2°C are able to damage growing tissues of trees in temperate areas of North America and Europe (Chamberlain et al., 2019).

Frost damages were more frequent and severe in provenances originating from colder sites, which also showed an advanced progression in the timing of budbreak at the time of the frost (Figure 6). Although most trees presented some degree of damage, in most cases only 5 to 15% of buds were damaged. This is in line with results from Charrier et al. (2018), who used modelling approaches to determine that greater frost damages are more related to the intensity of the frost event rather than tree frost hardiness (i.e. tree vulnerability, *sensu* Charrier et al., 2015). In other words, the main variable influencing frost damage is the minimum temperature reached by the frost event: lower minimum temperatures can overwhelm frost hardiness thresholds and cause more severe damages to the tree. Beside the relatively mild temperatures reached, the overall low percentage of damaged buds may be due to the fact that budbreak was still ongoing at the time of the frost. It is likely that only buds in the most vulnerable phases of budbreak were damaged, since closed buds are more resistant to frost and fully developed shoots are not damaged even at -3°C (Dang et al., 1992). It is also possible that microclimatic variations played a role in determining differences in frost damage, e.g. lower temperatures near the soil, in higher parts of the canopy or on bud surfaces (Charrier et al., 2015; Peaucelle et al., 2022; Winkel et al., 2009). However, in the present case the spatial distribution of damaged buds on individual trees appeared homogeneous. This would suggest that phenological phases of the buds (and underlying physiological traits influencing frost hardiness) played an important role in determining bud sensitivity and therefore frost damage occurrence. This hypothesis is supported by our field observations, which found healthy and well-developing buds close to browned buds (Figure 7), suggesting that differences in development among buds at the time of frost occurrence determined differences in frost sensitivity and ultimately in frost damage. Hypothetically, if the frost event studied in this work happened only a few weeks later, buds from northern provenances would have been advanced enough in their cell formation and development to be more frost hardy (Charrier et al., 2015). In this scenario, northern provenances could have been able to better resist the frost. Conversely, southern provenances still in the middle of budbreak would have been more damaged because of their low frost hardiness (Charrier et al., 2013). Further studies quantifying bud frost hardiness levels at different stages of budbreak could validate this hypothesis and shed light on tree sensitivity to frost during this crucial phenological event.

1.5.2 Spring phenology in *P. mariana* provenances

Our results show that provenances originating from colder sites have a lower forcing requirement for spring reactivation and therefore show an earlier budbreak than provenances originating from warmer sites when growing in the same conditions. (Figure 4). The lower threshold for GDD calculation is close to -3°C for all provenances, indicating that there is no difference in sensitivity to warm temperatures (Figure S1). In other words, provenances from colder climates perform an earlier budbreak because they need less heat accumulation, and not because they start accumulating heat at lower temperatures. When growing in the same conditions, i.e. in the common garden, northern provenances fulfil their forcing requirements faster than southern provenances, resulting in an earlier budbreak.

These findings are consistent with existing knowledge of *P. mariana* provenances. Earlier field studies in the same common garden (Guo et al., 2021; Usmani et al., 2020) as well as controlled-conditions experiments (Rossi & Bousquet, 2014) observed that provenances from colder places have lower forcing requirements, leading to faster budbreak under the same conditions. Similar findings have been reported for other conifers (Blum, 1988; Worrall, 1983; Zeltiņš et al., 2021) as well as broad-leaved species living in similar environments, such as sugar maple (*Acer saccharum* Marsh.) (Guo et al., 2020b; Ren et al., 2020). In environments where cold is the main limitation, the timings of budbreak are driven by a trade-off between late frost avoidance and maximization of growth and reproduction during the short growing season (Chuine, 2010). Late frost avoidance appears to be a major evolutionary constraint driving budbreak timings for several species (Lenz et al., 2016; Marquis et al., 2020). In this perspective our findings show that northern provenances have lower forcing requirements that allow them to perform a faster dormancy release and maximize the shorter growing season in cold environments. Future studies should focus on quantifying the risk of late frost damage in northern *P. mariana* forests. Because of lower forcing requirements, trees in these regions could be more affected by the advance in budbreak induced by warmer spring conditions. This, in turn, could leave the trees exposed to the risk of late frost, which may become more frequent as spring temperatures variability increases (Chamberlain et al., 2021; Marquis et al., 2022).

However, it is not clear how far these results can be generalized to other species or ecosystems. Other studies have found opposite results, i.e. that when growing in the same conditions northern provenances perform budbreak later than southern. This was observed in common gardens for *Pseudotsuga menziesii* [(Mirb.) Franco] and *Juglans nigra* (L.) (Bey et al., 1971; Campbell & Sugano, 1979) and confirmed by experimental treatments in *Juglans regia* and *Juglans regia x nigra* (Charrier et al., 2011). This points to factors other than late frost influencing population-specific spring phenology timings. For example, drought-induced growth limitations during the summer can exert selection pressure and drive local provenances to perform an earlier budbreak, in order to maximize growth early in the season (Campbell & Sugano, 1979). Some species may be more sensitive to long photoperiod in the spring, reducing the advancement in budbreak induced by warmer temperatures (Basler & Körner, 2012; Hänninen & Tanino, 2011). These differences highlight the complex interactions between intraspecific traits and environmental factors, and the need for more field observations and experimental studies to better understand intraspecific variations in tree phenology.

Photoperiodic control over budbreak phenology could play an important role in mitigating advances in spring phenology (Basler & Körner, 2012). The role of photoperiod is often difficult to predict due to species-specific differences (Basler & Körner, 2014) and the absence of clear patterns among species (Flynn & Wolkovich, 2018; Way & Montgomery, 2015). In the case of *P. mariana*, photoperiod appears to have a marginal control over budbreak with respect to temperature (Rossi, 2015). However, photoperiod seems to play a more important role when chilling requirements for budbreak are not met (Malyshev et al., 2018; Ren et al., 2020). Unfulfilled chilling requirement can also directly delay budbreak (Chuine et al., 2016). Therefore, warmer winters in the future could mitigate or even offset the advances in spring phenology and reduce the risk of late frost damage in these regions. Further studies on the dormancy dynamics could help quantify these diverging drivers and improve predictions of climate change impacts in these regions.

In a context of provenance selection, the higher GDD requirement of southern provenances would delay budbreak under warming conditions. Assisted migration could therefore be a useful tool to decrease the risk of damage from late frost events. Northward movement of provenances has already been suggested, also because southern provenances have higher potential for growth and timber production (Prud'homme et al., 2018). However, in the present study we moved provenances southward (Figure 3). This allowed us to simulate an increase in temperatures for the northern provenances but leaves open questions regarding the vulnerability to autumn and winter frosts in southern provenances moved north. Working on the same common garden plantation that we analysed in this work, Silvestro et al. (2019) found that a later budbreak correlates with a later bud set in *P. mariana* provenances. More specifically, bud burst occurred 1.2 days later and bud set 1.8 days later for each degree Celsius of increase in mean annual temperature at the provenance origin. This suggests a trade-off between reducing the risk of late and early frost. Therefore, southern provenances may be more vulnerable to early frosts in the autumn (Montwé et al., 2018). Moreover, it is possible that southern populations, adapted to milder winters, have lower maximum frost hardiness and may not survive prolonged harsh winter temperatures. There is evidence that different provenances of conifers have different levels of frost hardiness (Repo et al., 2001; Sakai, 1983), although not all species exhibit the same trend (Charrier et al., 2011; Hawkins et al., 1994; Larcher & Mair, 1968; Morin et al., 2007). In boreal environments, where winter temperatures can reach values lower than -40°C, frost tolerance in overwintering organs is an essential aspect to be considered (Sebastian-Azcona et al., 2019).

A relevant aspect that remains to be investigated is the role of physiological regulators of budbreak and their interaction with environmental factors. While temperature is the main environmental cue inducing budbreak (Hänninen, 1997; Junttila, 2007; Sarvas, 1972), biochemical mechanisms are also involved. In particular, bud reactivation in the spring must be sustained by carbohydrates previously stored in other organs (Rinne et al., 1994; Tixier et al., 2017). During winter dormancy, these carbohydrates have a key function in increasing the tree's frost hardiness (Kasuga et al., 2007). For this reason, carbohydrates ultimately exert a strong regulation over deacclimation dynamics leading to budbreak (Baffoin, 2021; Deslauriers et al., 2021). This suggests a positive correlation between maximum frost hardiness in the winter and the quantity of carbohydrates available in the spring to promote a fast reactivation (Delpierre et al., 2019). In this perspective, higher maximum frost hardiness in northern *P. mariana* provenances driven by harsher local winter conditions could provide a mechanistic explanation for faster budbreak. Investigating the link between differences in frost hardiness, carbohydrate content and rates of growth resumption could provide valuable insight in the drivers of phenotypic differences in *P. mariana* populations.

1.5.3 Conclusions and recommendations

Our results confirm the hypothesis that provenances originating from colder climates have a higher risk of exposure to late frost because of an earlier budbreak. In a scenario where climate warming advances budbreak and increases variability in spring temperatures, northern provenances can be expected to be more sensitive to local increases in late frost risk (Chamberlain et al., 2019; Gu et al., 2008). Our study highlights the importance of investigating intraspecific differences when studying ecophysiological traits of trees, especially in the face of climate change. Diverging responses to spring temperatures entail different sensitivity to late frost in tree provenances, with important implications for studying ecological impacts of climate change and implementing adaptive management actions.

Our results support the idea of northward assisted migration of *P. mariana* provenances to minimize the risk of late frost damage. However, more research is necessary to address unanswered questions and fill in the gaps in knowledge. Further studies should measure frost hardiness in *P. mariana* provenances throughout the dormant period in order to clarify whether southern provenances can survive northern winters. Understanding species-specific dynamics of frost avoidance and frost resistance can improve predictions of climate change impacts and allow for informed choices in assisted migration and provenance selection.

1.6 ANNEXES

Table S1. Linear equations used to calculate temperature minimum (T min), mean (T mean) and maximum (T max) values for the site of Simoncouche based on temperatures measured at the weather station of Bagotville, Saguenay, QC. The equations were calculated based on daily mean values recorded at the two sites for the period 2010-2015. The $\varkappa$ variable is the temperature measured at Bagotville.

Variable	Equation	R^2
Minimum daily temperature (°C)	$-0.24+0.98\varkappa$	0.97
Mean daily temperature (°C)	$-0.65+0.97\varkappa$	0.98
Maximum daily temperature (°C)	$-0.82+0.98\varkappa$	0.98

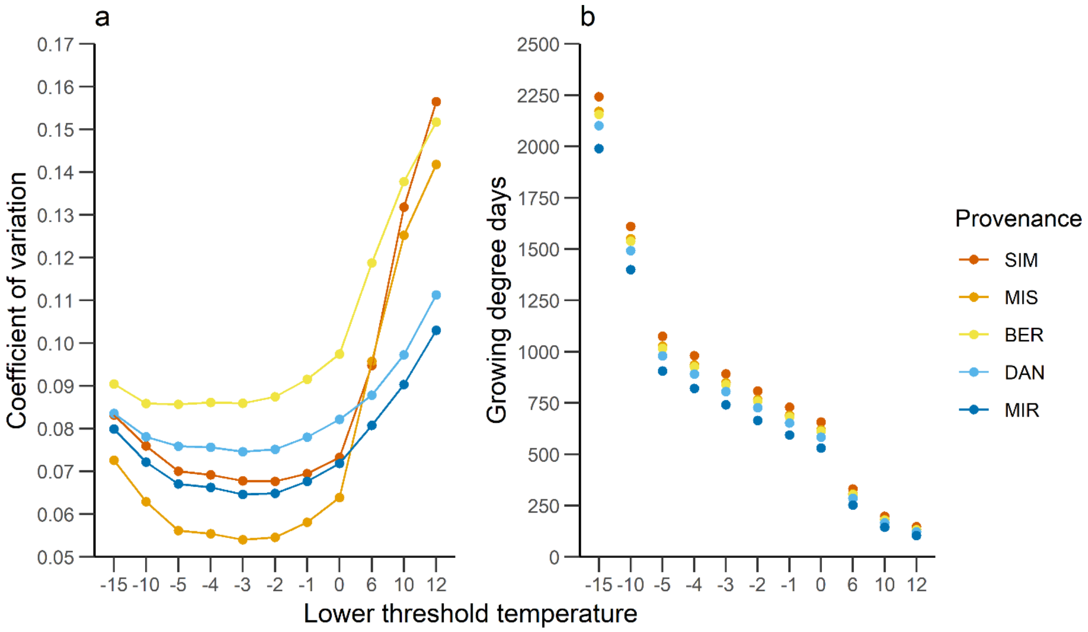


Figure S1: a) coefficient of variation (CV) of the mean growing degree days (GDD) necessary to complete budbreak and b) mean growing degree days (GDD) across years of observation in five *P. mariana* provenances. GDD have been computed and averaged for six years of phenological observations (2015,2017:2021). CV represents the variability of the mean GDD value between years.

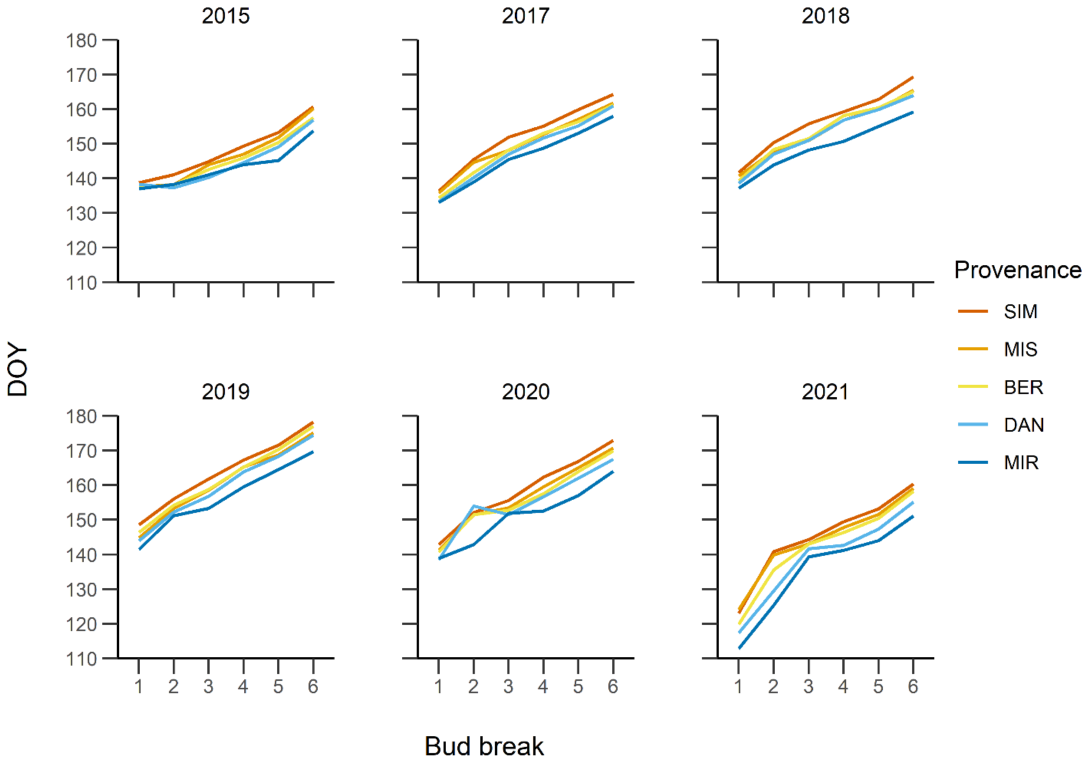


Figure S2: Results from 6 years of phenological observation in a *P. mariana* common garden. Lines indicate the mean day of the year (DOY) values for each budbreak phase. Results are grouped by site of provenance, identified by lines of different colours (warm to cold temperature gradient: SIM, MIS, BER, DAN, MIR). Measurements were taken in a common garden at the southernmost site, SIM

CHAPITRE 2

Local conditions have greater influence than provenance on sugar maple (*Acer saccharum* Marsh.) frost hardiness at its northern range limit

Published in Tree Physiology

Mura, C., Charrier, G., Buttò, V., Delagrange, S., Surget-Groba, Y., Raymond, P., Rossi, S., & Deslauriers, A. (2024). Local conditions have greater influence than provenance on sugar maple (*Acer saccharum* Marsh.) frost hardiness at its northern range limit. *Tree Physiology*, tpae167. https://doi.org/10.1093/treephys/tpae167

2.1 ABSTRACT

In temperate and boreal ecosystems, trees undergo dormancy to avoid cold temperatures during the unfavorable season. This phase includes changes in frost hardiness, which is minimal during the growing season and reaches its maximum in winter. Quantifying frost hardiness is important to assess the frost risk and shifts of species distribution under a changing climate. We investigate the effect of local conditions and intraspecific variation on frost hardiness in sugar maple (*Acer saccharum* Marsh.). Seedlings belonging to seven provenances from the northern area of the species' range were planted at two sites in Quebec, Canada. LT_{50}, i.e. the lethal temperature for 50% of the cells, was measured monthly with the Relative Electrolyte Leakage (REL) method on branches and buds from September 2021 to July 2022. LT_{50} varied between -4 °C in summer (July) and -68 °C in winter (February). Autumnal acclimation rates (September to early December) and mid-winter frost hardiness (December to early March) were similar in both sites. Samples in the southern site deacclimated faster than in the northern site between March and July, because of a warmer and earlier spring. No difference in frost hardiness was detected between provenances. Our results suggest that the frost hardiness trait is similar within the northern part of the sugar maple distribution, with local weather conditions having a greater influence than provenance. We demonstrate that LT_{50} in sugar maple can exceed -55 °C, far below the minimum temperatures

occurring in winter at the northern limit of the species. In order to minimize the risk of damage from extreme frost events exceeding tree frost hardiness, a careful evaluation of site characteristics is more important than provenance selection. Other factors should also be considered within the context of changing climate, in particular the phenology of maple and avoidance of late frost in spring.

2.2 INTRODUCTION

In cold environments, trees have developed the ability to increase their frost hardiness to endure winter conditions. Temperatures exceeding the plant's vulnerability to frost can cause severe tissue damage and death (Sakai & Larcher, 1987). Frost damage occurs when cold temperatures induce ice formation within plant tissues, which can lead to rupture of the plasma membrane and cell death (Uemura et al., 2006). Plants reduce the risk of frost damage by synchronising their growing season with the warmer period of the year through phenological adjustments (frost avoidance), and increasing the frost hardiness of overwintering organs through physiological adjustments (frost tolerance) (Charrier et al., 2015). However, questions remain regarding the intraspecific differences in frost hardiness resulting from local populations, and their implications for forest adaptation to climate change.

At the cellular level, trees can increase their frost hardiness by lowering the freezing point of the cytosol and inducing ice formation outside of the cell to avoid intracellular ice formation and membrane rupturing. This is partially accomplished by physiological adjustments, for example reducing the water content and increasing the concentration of soluble sugars in the cells (Baffoin et al., 2021; Deslauriers et al., 2021). Frost hardiness therefore changes during the year, being at a minimum during the growing season and at a maximum in mid-winter (Lang et al., 1987; Sakai & Larcher, 1987). For example, a study on boreal *Vaccinium* spp. found that frost resistance, quantified as the temperature inducing lethal damage to 50% of the cells, varied between -5 °C during the growing season and -67 °C during winter (Deslauriers et al., 2021).

Frost hardiness dynamics are closely linked with the phenological cycle of growth and dormancy (Leinonen, 1996; Vitasse, Lenz, & Körner, 2014). In mid-summer, trees stop radial growth and form buds to protect the meristems (Buttò et al., 2021; Rohde & Bhalerao, 2007). In autumn, colder temperatures and shortening photoperiod induce dormancy (Hamilton et al., 2016; Way, 2011). Initially, trees enter the endodormancy phase, which is internally regulated by growth inhibitors and requires exposure to cold temperatures (*i.e.* chilling) to be released (Chuine et al., 2016; Lang et al., 1987). During endodormancy, frost hardiness increases in overwintering organs (i.e. acclimation) until reaching a peak in winter (Sakai & Larcher, 1987; Vitasse, Lenz, & Körner, 2014). During autumn and winter, exposure to cold temperatures releases endodormancy and the tree enters the ecodormancy phase, which is mainly controlled by temperature (Charrier et al., 2015; Delpierre et al., 2016; Junttila, 2007) with a minor effect of photoperiod in some species (Flynn & Wolkovich, 2018; Malyshev et al., 2018; Way & Montgomery, 2015). The gradual rise in temperature during spring, defined as forcing units, releases ecodormancy and triggers a decrease in frost hardiness (deacclimation) until budbreak and the resumption of growth (Charrier et al., 2011; Hänninen, 1990a; Kovaleski et al., 2018).

Frost hardiness is a critical trait for tree survival in cold climates and is considered one of the main factors limiting poleward expansion of species range (Inouye, 2000; Sakai & Larcher, 1987). Accordingly, the hardiness zones commonly used to define climate suitability for plant species in North America are based on absolute minimum temperatures (Daly et al., 2012). In a study on 27 native and exotic species growing in a common garden in central Europe, Kreyling et al. (2015) found that tree frost hardiness correlated to the climate at the species' origin, particularly the minimum temperature of the coldest month. Some studies have pointed out the importance of off-season late frosts as a major driver of natural selection and range expansion (Körner et al., 2016; Lenz et al., 2013). However, analyses of frost hardiness are often time-consuming and labour-intensive, which limits their application to special or localized cases (Burr et al., 2001). For this reason, field data quantifying tree frost hardiness during acclimation and deacclimation are still missing for many species.

Under climate change, the habitats of many species are expected to shift polewards or upwards, towards sites submitted to colder conditions (Boisvert-Marsh et al., 2014; McKenney et al., 2007). While winters will generally become warmer, the weather is becoming more variable and less predictable, making frost events likely even under global warming scenarios (Marquis et al., 2022; Screen & Simmonds, 2013). Accordingly, warming events during winter or spring can induce deacclimation and lead to frost damage when the temperatures again fall below 0 °C (Augspurger, 2013; Vitasse, Lenz, & Körner, 2014; Zohner et al., 2020). Even if not lethal, frost events can have critical impacts on tree growth and competitiveness. Montwé et al. (2018) showed that a frost event happening 20 years after planting significantly affected the growth in *Pinus contorta* (Dougl. ex. Loud) common garden, which remained low in subsequent years. Reduced growth and performance of trees and saplings can also hinder the establishment and long-term survival of new cohorts, thus limiting poleward or upward expansion of tree species range (Kreyling et al., 2015; Winder et al., 2011).Frost hardiness is therefore an important aspect to predict the frequency of frost damages, the potential range expansion of trees, and the consequent evolution of forest structure.

Different populations of the same species can differ in their frost hardiness and phenology. A study by Charrier et al. (2011) found that *Juglans regia* (L.) genotypes artificially selected for fruit production reached lower frost hardiness (lethal temperature of -28 °C) than *J. regia x nigra* hybrids selected for wood production (lethal temperature of -35 °C). Intraspecific differences can also occur naturally across latitudinal ranges, where populations from colder climates can exhibit greater frost tolerance and winter survival (Vitasse, Lenz, & Körner, 2014). This trend has been observed both in conifers (Kreyling et al., 2012; Viveros-Viveros et al., 2009) and angiosperms (Kreyling et al., 2014; Li et al., 2003). However, other studies suggested that intraspecific differences in frost hardiness are limited to one phase of the frost hardiness cycle. Beuker et al. (1998) found differences in frost hardiness during the autumnal acclimation, but not during winter and deacclimation, in *Pinus sylvestris* (L.) and *Picea abies* ([L.] Karst). Instead, Charrier et al. (2011) found differences between *Juglans regia* genotypes in deacclimation, but not during acclimation or midwinter. Understanding species-specific drivers of variations in frost hardiness can provide relevant information to guide tree provenance selection under climate change (Hänninen, 2006; Rammig et al., 2010). This is particularly true in cases where tree species and provenances are artificially transferred poleward, i.e. in assisted migration (Ste-Marie et al., 2011). Assisted migration is a tool to promote forest resilience in the face of a rapidly changing climate (Twardek et al., 2023). However, transferring trees poleward begs the question of whether they will be able to survive in colder climates, especially at the sapling stage (Aubin et al., 2016; Pedlar et al., 2011). Quantifying intraspecific differences in frost hardiness can therefore help the selection of provenances for assisted migration, increasing the chances of success. In this study, we measured frost hardiness in sugar maple (*Acer saccharum* Marsh.) seedlings from seven provenances located in the northern portion of the species' distribution and growing in two sites in the Quebec province, Canada. We aimed to quantify intraspecific variability in frost hardiness by comparing the effect of provenance and local environmental conditions. We raised the hypothesis that local weather influences frost hardiness, with seedlings in the colder site showing earlier and faster acclimation in autumn, higher maximum frost hardiness during winter, and later and slower deacclimation in spring, compared to the southern site (**Figure 9**). We also expect that climatic conditions at the provenance origin influence frost dynamics, with seedlings from colder provenances showing earlier and faster acclimation in autumn, higher frost hardiness and later, slower deacclimation in spring compared to warmer provenances (Figure 1).

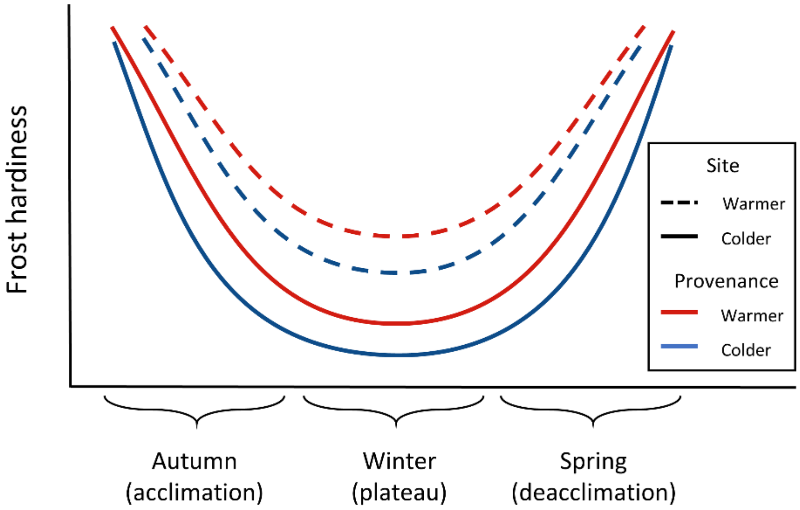


Figure 9. Hypotheses tested for the effect of provenance and site on frost hardiness in sugar maple. We expect samples in the warmer site (dashed lines) to reach lower frost hardiness than in the northern site (solid lines). Within each site, we expect that provenances from colder areas (blue lines) will show faster acclimation but slower deacclimation than provenances from warmer areas (red lines). In this study, we use the temperature causing lethal damage to the tree (LT_{50}) as an indicator of frost hardiness at a given time.

2.3 MATERIALS AND METHODS

2.3.1 Provenance selection

The plant material for this study consisted of 2-year-old seedlings of sugar maple (*Acer saccharum* Marsh.) belonging to seven commercial provenances from Eastern Canada (Table 3, Figure 10). We focused on this study area, which corresponds to the northern portion of sugar maple's range, because a comparison of frost hardiness for different provenances in this zone is currently missing from the literature. Seeds were collected on single mother trees for Duchesnay, Coy Brook and First Eel Lake provenances (abbreviated as DUC, COB, and FEL, respectively) by the National Tree Seed Center (Natural Resources Canada, Fredericton, Canada). The provenances Shawinigan, Lapocatière, Cantley and Sherbrooke (abbreviated as SHW, LAP, CAN, and SHR, respectively) originated from seeds collected at stand level by the Ministère des Ressources Naturelles et des Forêts du Québec, Canada. All stands of seed collection are natural (*i.e.* no artificial selection or tree breeding) and are considered representative of the area of provenance.

Table 3. Characteristics of the seven sugar maple provenances studied in this work. Mother tree indicates whether the seeds were collected on a single tree or on multiple trees for each seed lot. Climate data is averaged for the 1980-2010 period (source: BioSIM).

Name	ID	Lat.	Long.	Elevation (m a.s.l.)	Mother tree	Annual T (°C)	Absolute minimum T of the coldest month (°C)	Annual prec. (mm)
Duchesnay	DUC	46.87	-71.67	250	Single	3.6	-34	1361.9
Lapocatière	LAP	47.36	-70.03	22	Multiple	4.5	-28.5	937.1
Shawinigan	SHW	46.53	-72.65	124	Multiple	4.7	-32.6	1057.7
First Eel Lake	FEL	45.83	-67.62	177	Single	5.1	-30.8	1158.7
Coy Brook	COB	46.27	-65.53	89	Single	5.5	-29.7	1124
Sherbrooke	SHR	45.38	-71.92	301	Multiple	5.5	-31.9	1136.7
Cantley	CAN	45.57	-75.78	154	Multiple	5.7	-31.7	999

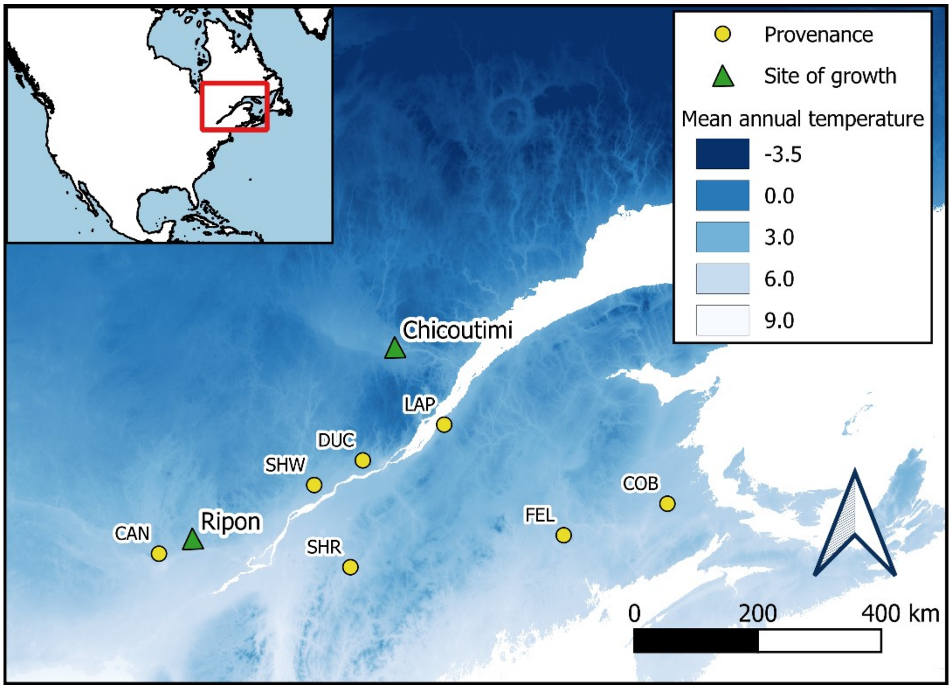


Figure 10. Locations of the seven sugar maple provenances studied (points) and the two sites where seedlings were grown and sampled (triangles).

A total of 1008 seedlings were grown in the forest nursery in Berthierville, QC, Canada. After germination in 2020, seedlings grew in a 85% peat, 7.5% vermiculite, 7.5% perlite substrate with added lime (9 kg lime per 3.1 m^3 peat). Seedlings stayed in tunnels covered by transparent plastic until reaching 25 cm in height and were then transferred outdoors under a shading net. After entering dormancy, the seedlings spent the winter in a cold room at -3 °C. In May 2021, the seedlings were transplanted in trays containing 15 cavities of 320 cm^3 and placed outdoors in two open sites, Chicoutimi and Ripon (QC, Canada, Figure 10). We chose the two sites for their location with respect to maple distribution. Chicoutimi (48°25’ N, 71°02’ W) and the surrounding region represent the northern limit of maple’s range (Godman et al., 1990). Ripon (45°46’ N, 75°06’ W) is a warmer site, 420 km south-west of Chicoutimi, located within the natural range of sugar maple (Table 4).

Table 4. Characteristics of the two sites where the seedlings were grown before sampling for the frost hardiness assessment. Climate data is relative to the 1980-2010 period (source: BioSIM).

Name	Lat.	Long.	Elevation (m a.s.l.)	Annual T (°C)	Average minimum T of the coldest month (°C)	Extreme minimum T (°C)	Annual precipitation (mm)
Ripon	45.78	-75.10	180	4.9	-18.7	-43.3	1091
Chicoutimi	48.42	-71.05	82	3.1	-21.3	-43.3	931

2.3.2 Climate and weather data

Climate and weather data were used to compare the provenances and understand the drivers of frost hardiness. We obtained historical (1980-2010) climate averages for the two sites of sampling and seven geographic provenances using BioSIM (Régnière et al., 2014). We also used BioSIM to obtain weather data for the period of measurement (winter 2021/2022). We obtained snow cover and historical daily temperatures from the nearest available weather station, i.e. Chénéville (13 km from Ripon) and Bagotville (9 km from Chicoutimi) (Environment Canada, 2023). Growing degree days (GDD) were calculated from January 2022 onwards with a base threshold of 0 °C to track above-zero temperature accumulation during the spring. Cumulative chilling days were calculated for the acclimation period (September to January) to quantify cold accumulation in the two sites. Chilling units (CU) were calculated daily as CU = max $(7 - T_m; 0)$, where T_m is the mean daily temperature. We used cumulative daily CU instead of the more common chilling hours because hourly temperatures were missing for Ripon. The threshold of 7 °C was used as suggested in the literature for sugar maple (Raulier & Bernier, 2000; Weinberger, 1967).

2.3.3 Frost hardiness tests

We performed monthly frost hardiness measurements from September 2021 to July 2022. Seedlings were two-years-old at this time, having spent their second growing season outdoors in each site. We defined frost hardiness as the temperature inducing 50% of cellular damage (LT_{50}), measured with the Relative Electrolyte Leakage (REL) method (Repo & Lappi, 1989). All provenances were sampled monthly except COB, which was sampled every two months because of a smaller number of seedlings. Overall, we sampled 154 seedlings per provenance, and 84 seedlings for COB. All samples were analysed in Chicoutimi. Samples from Ripon were delivered to the lab within 24 hours from collection and immediately started the REL analysis. To minimize the impact of the 24-hour lag between sampling and analyses, samples were placed in plastic bags with wet paper towels to maintain humidity and prevent dehydration, and were shipped in a styrofoam box for insulation from external temperatures during transport.

On each sampling date, seven seedlings per provenance were collected in each site. Different samples were collected on each date. Seedlings were separated into three samples, each at least 5 cm long, and distributed randomly between seven target temperatures for the frost treatment. In total, each target temperature had 3 samples per provenance, wrapped in tin foil and placed in a thermal container. Two thermocouples per thermal container were used to keep track of temperature during the tests, one attached to a random sample and one measuring air temperature inside the container.

During each test, we exposed the samples to seven different treatment temperatures ranging from +5 to -80 °C. One thermal container was stored in a cold chamber at +5 °C (control treatment). A second thermal container was stored in a freezer at -80 °C for four hours (lethal treatment) before being transferred to +5 °C. The remaining five thermal containers were exposed to five different temperatures ranging from -7 °C to -60 °C in a controlled-temperature freezer (EH40–2–3, Envirotronics, Grand Rapids, MI). The target temperatures changed during the sampling dates to better quantify the expected frost hardiness, mostly with regular intervals of -10 to -15 °C between target temperatures (i.e. we tested colder temperatures during winter, see Figure S3). The temperature in the freezer was manually adjusted to attain a cooling rate of -10 K h-1. This rate is higher than the cooling rate of -5 K h-1 normally used in the literature, and faster than freezing rates normally observed in nature (Atucha Zamkova et al., 2021;

Cannell & Sheppard, 1982). However, in our case the temperature of the freezer needed to be continuously and manually adjusted in small temperature increments, since the freezer model did not have a function for controlled temperature descent. This made a cooling rate of -5 K h-1 logistically unattainable, requiring 17 hours of continuous presence and input to control the descent rate. Upon reaching one of the target temperatures, one random container was taken out of the freezer and stored in a cold chamber at +5 °C. All containers were then left at +5 °C overnight.

On the second day, the samples were prepared for conductivity measurements. Each seedling was separated in branches (cut in slices 0.5 mm thick) and buds (split in two along the longitudinal axis), then stored in vials with 10 ml of demineralised water. Because of the small number of buds available, buds from the same provenance and within the same target temperature were placed in the same vial. Vials were left to agitate on a multi-platform orbital shaker (Thermo Fisher Scientific, Waltham, MA) at 5 °C overnight.

The third day, conductivity in each tube was measured as an indicator of electrolyte leakage from cells damaged by the frost (C1). Samples were then put in an autoclave at 120 °C, 1.2 bar, for 30 minutes. A second conductivity measurement was performed after the autoclave treatment, corresponding to the maximum cellular damage (C2). REL was then calculated as C1/C2, i.e. the ratio of electrolyte leakage caused by frost to the leakage caused by maximum damage. We calculated the relationship between REL and temperature using the logistic function (Repo and Lappi 1989):

$$REL = \frac{a}{(1 + e^{b(c-T)})} + d \tag{1}$$

where T is the test temperature, d is the higher asymptote, a+d is the lower asymptote and b is the slope at the inflection point c. Frost hardiness (LT_{50}) was calculated for each provenance as the temperature at the inflection point c, i.e. the temperature causing 50% of cellular damage. Temperatures inducing 10% cellular damage (LT10) were also calculated from the logistic curve, to estimate a lower threshold for frost damage occurrence and for comparison with LT_{50}. The logistic function was fitted with the nlsLM function of the minpack.lm package (Elzhov et al., 2022). An example of the end result of monthly REL analyses illustrating the logistic fittings used to estimate LT_{50} can be found in Figure 11.

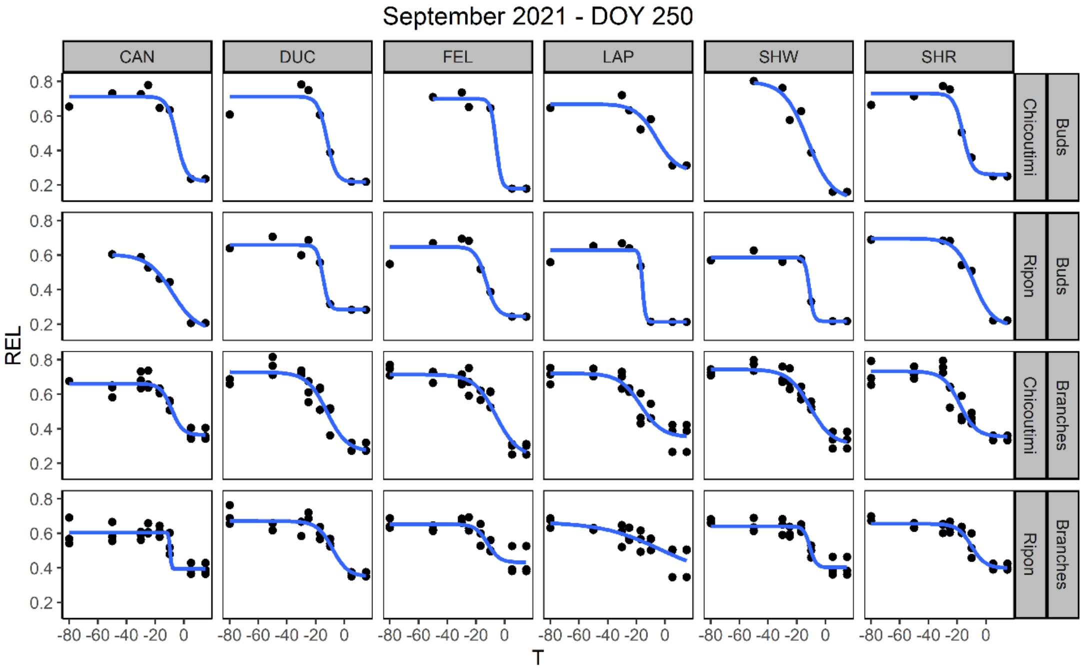


Figure 11. Example of the results of the REL analysis, divided by provenance (columns), organs and site of sampling (rows). Black dots are REL measurements at different test temperatures for each provenance and organ (branches, buds). Logistic curves (in blue) were fitted to estimate LT_{50} based on Relative Electrolyte Leakage (REL) measurements. L50 was estimated as the inflection point of the logistic curve.

2.3.4 Statistical analyses

We applied Wilcoxon rank sum exact tests to compare the differences in frost hardiness between sites on specific sampling dates. Based on the observed pattern of frost hardiness, we identified three periods: (1) acclimation, corresponding to the increase in frost hardiness during autumn (between September and December); (2) maximum hardiness, corresponding to the peak in frost hardiness during midwinter (between December and March); and (3) deacclimation, corresponding to the decrease in frost hardiness during spring (between March and June). For each period, the differences in frost hardiness between sites and provenances were tested using ANCOVA. We set LT_{50} as the response variable, circadian days since the start of the experiment (September 7, 2021) as quantitative covariate, and site and provenance as categorical variables. We fitted ANCOVA with the *Anova* function of the rstatix package (Kassambara, 2023). We tested for normality and homoscedasticity with the Shapiro-Wilks and Levene's tests, respectively. Model goodness-of-fit was evaluated by adjusted R^2 values, distribution of standardised residuals and visual assessment of residuals plots.

We fitted a circular regression model to investigate differences between site and provenance during the whole frost hardiness cycle. Circular regressions allow recurrent biological events to be described by transforming the independent variable, i.e. time, into a circular variable expressed in radians (Batschelet, 1981). We used circular regression in order to test for site (environment) and provenance effects, with a model that could account for frost hardiness variation throughout the year. We expressed the time as circadian days since the start of the experiment, transformed into radians (trad). The sine and cosine functions of trad represent the seasonal pattern of frost hardiness in the model. We fitted a circular model with LT_{50} as the response variable, the sine and cosine functions of trad as quantitative explanatory variables, in addition to the factors site (two levels) and provenance (seven levels). We used delta-AIC (Akaike Information Criterion) to compare models with and without the variables for site, provenance and their interaction in order to select the best model, both for ANCOVA and circular regression. Delta-AIC comparison was carried out with the *aictab* function of the AICcmodavg package (Mazerolle, 2023). All statistical analyses were performed in R (R Core Team, 2020).

2.4 RESULTS

2.4.1 Weather conditions

From September 2021 to July 2022, the northern site (Chicoutimi) and southern site (Ripon) experienced a mean temperature of 0.4 and 3.1 °C, respectively. On average, the temperatures differed from 1 to 1.2 °C between sites during the autumn (September to

November), with Ripon being the warmer site. Chilling units' accumulation, i.e. the days with mean daily temperature < 7 °C, started on day of the year (DOY) 295 (October 22) in both sites and reached 16 and 18 degree-days in Ripon and Chicoutimi, respectively on DOY 305 (November 1). The difference in chilling units between sites remained below 50 until DOY 341 (December 7), then increased to 144 on DOY 365 (December 31), with Chicoutimi being the coldest site (Figure 12).

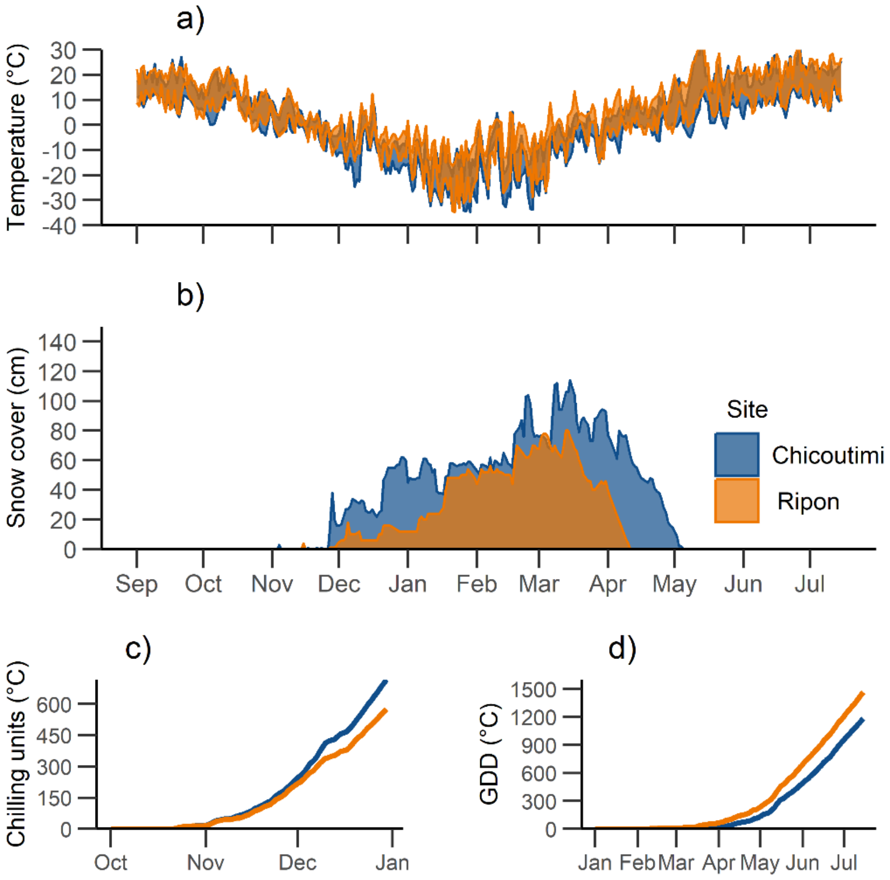


Figure 12. Trends of a) air temperature (°C), b) snow cover (cm), c) daily chilling units (°C) and d) growing degree-days (GDD °C) during the study period in winter 2021/2022. Air temperature is shown as a shaded area between daily maximum and minimum values. Data obtained from the nearest weather stations (Environment Canada, 2023).

In winter (December to February) Chicoutimi was colder by an average of 4.7, 4.3 and 3.9 °C for minimum, mean and maximum temperatures, respectively. The lowest daily minimum temperature recorded during winter was -34.7 °C on both sites, which occurred on

DOY 22 in the southern site (Ripon) and on DOY 29 in the northern site (Chicoutimi). In spring (March to June), Chicoutimi was 2.5 to 2.8 °C colder than Ripon. On DOY 121 (May 1), the growing degree days (GDD) above 0 °C reached 233 in Ripon and 134 in Chicoutimi. Snow cover appeared on the same date in the two sites, on DOY 331 (November 27) (Figure 12, lower panel), reaching a height of 114 cm in Chicoutimi, 34 cm more than in Ripon. The snow on the soil disappeared on DOY 100 (April 10) in Ripon, 24 days earlier than in Chicoutimi (Figure 12).

2.4.2 Acclimation

On average, LT_{50} of branches in early September was -12 and -9 °C in Chicoutimi and Ripon, respectively (Figure 13). At the same time, bud LT_{50} was -9 and -12 °C in Chicoutimi and Ripon, respectively. From September to December, LT_{50} decreased by an average of 15 °C per month in both organs. In early December, LT_{50} reached -58 °C in branches, and -62 °C in buds in Chicoutimi. At the same time, LT_{50} in Ripon were -55 °C and -62 °C for branches and buds, respectively (Figure 13). The overall lowest frost hardiness for branches was assessed in early September, -6 °C in Chicoutimi (provenance FEL) and -3.5 °C in Ripon (provenance LAP). LT_{50} in buds was significantly higher in Chicoutimi (-35.9 ± 5 °C) than in Ripon (-49 ± 4.7 °C) only in November according to the Wilcoxon test ($W = 36$, $p < 0.01$, Table 5). LT_{50} in branches was not significantly different between sites. The ANCOVA for acclimation was significant, showing with an effect of site on LT_{50} in buds ($p < 0.05$), but not in branches. The effect of provenance during acclimation was not significant (Table 6).

Table 5. Results of the Wilcoxon bilateral rank sum exact test comparing differences in LT_{50} between sites for different organs and sampling dates. One, two and three asterisks correspond to $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively.

Organ	Date	W
Buds	2021-09-07	24
	2021-10-04	24
	2021-11-01	36 **
	2021-12-01	24
	2022-01-10	24
	2022-02-07	20
	2022-03-07	22

	2022-04-04	22
	2022-05-02	3 *
	2022-05-23	20
Branches	2021-09-07	11
	2021-10-04	24
	2021-11-01	16
	2021-12-01	19
	2022-01-10	27
	2022-02-07	37
	2022-03-07	12
	2022-04-04	26
	2022-05-02	0 **
	2022-05-23	0 ***
	2022-07-11	39

Table 6. ANCOVA results testing the effect of site and provenance (prov.) during the periods of acclimation (September to December), maximum hardiness (December to February) and deacclimation (March to July). The variable time indicates days since the start of the experiment. The adjusted R^2 for goodness of fit is shown with F values and significance levels for the whole model and terms. One, two and three asterisks correspond to $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively.

		Model		**Terms**			
		R^2	**F**	**(Intercept)**	**time**	**site**	**prov.**
Branches	Acclimation	0.93	88.95***	63.69***	702.96***	1.95	0.19
	Maximum hardiness	0.02	1.113	230***	0.01	0.81	1.34
	Deacclimation	0.85	35.78***	425.97***	254.57***	9.12***	0.91
Buds	Acclimation	0.93	89.04***	29.39***	699.77***	5.65*	0.37
	Maximum hardiness	-0.05	0.698	300.75***	1.77	0.05	0.63
	Deacclimation	0.88	33.64***	358.78***	259.00***	0.68	0.38

2.4.3 Maximum hardiness

From December to early March, LT_{50} reached a plateau and fluctuated around low temperatures (Figure 13). During this period, the variation in LT_{50} between monthly sampling dates was on average below 4 °C in branches and below 5 °C in buds. Between December and March, the lowest LT_{50} attained in Chicoutimi were -58 °C in branches and -64 °C in buds), which were measured in December and February, respectively. In Ripon, the lowest LT_{50} were -60 °C in branches and -63 °C in buds, measured in February and December, respectively (Figure 13). Among provenances, the provenance SHW reached the lowest values of LT_{50} in Chicoutimi, -68 °C for branches in December and -67 °C for buds in February. In Ripon, the lowest LT_{50} was measured on SHW for buds (-68 °C in January) and on COB for branches (-67 °C in February). According to the Wilcoxon test, there were no significant differences between sites during this period (Table 5). ANCOVA for this period was not significant, and with low R^2 (Table 6). This was due to the lack of variability in LT_{50}, which made the inclusion of explanatory variables in the model redundant.

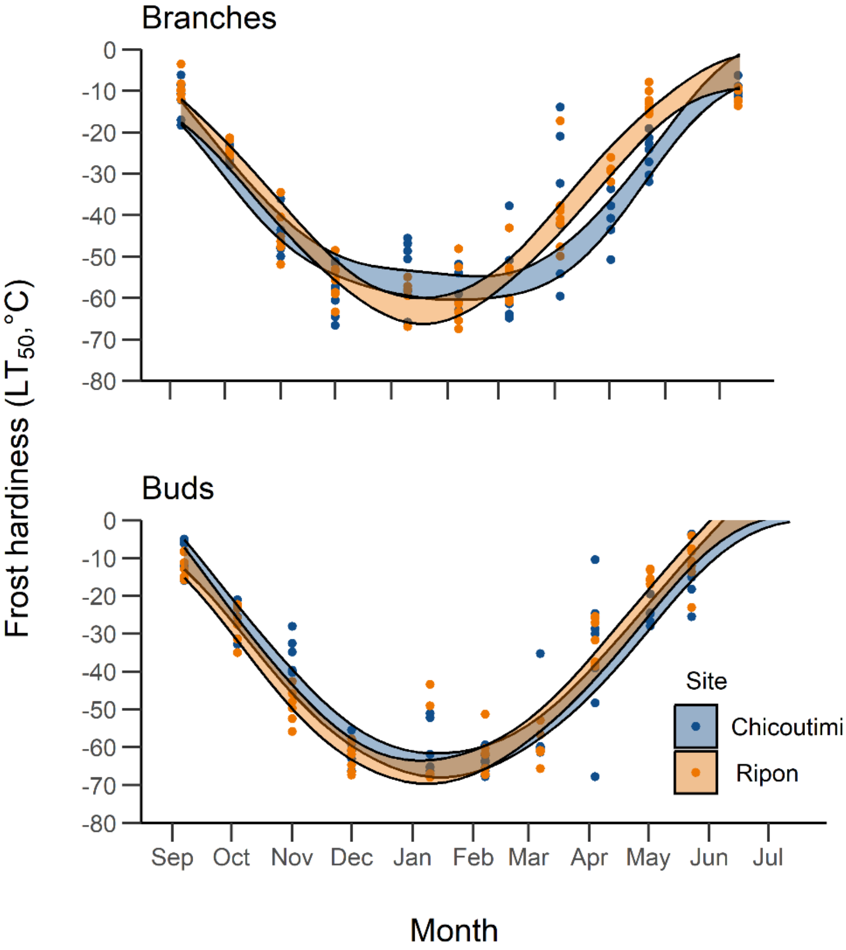


Figure 13. Frost hardiness (LT_{50}) of seven sugar maple provenances sampled in two study sites for branches (above) and buds (below). Points indicate estimated values of LT_{50}. Shaded areas indicate the 95% confidence interval of the circular model's prediction. Ripon is the southern site and Chicoutimi the northern one.

2.4.4 Deacclimation

From March to May, LT_{50} for branches in Ripon rose from -55 °C to -13 °C, with an average increase of 14 °C per month, declining to 1 °C per month between late May and July. LT_{50} for buds in Ripon increased from -59 °C to -15 °C between March and early May, a rate of 16 °C per month, which decreased to 4 °C per month between early and late May. In Chicoutimi, LT_{50} for branches increased from -57 °C to -9 °C between March and July (11 °C per month). Between March and May, LT_{50} for buds in Chicoutimi rose from -56 °C to -13 °C, with an average increase of 14 °C per month (Figure 13). LT_{50} in April showed the highest variability amongst sampling dates. This was likely due to the high conductivity of the sugar-rich sap that maples produce during spring reactivation (Perkins & van den Berg, 2009), which altered the REL. For this reason, LT_{50} from April was considered unreliable and excluded from the successive analyses (i.e. ANCOVA and circular regression analyses).

The overall lowest frost hardiness in buds was measured in late May, -3.6 °C in Chicoutimi (provenance DUC) and -4 °C in Ripon (provenance SHR). Frost hardiness was significantly lower in Ripon in early May according to Wilcoxon test ($W = 3$, $p < 0.05$, Table 5). Frost hardiness in branches was significantly lower in Ripon on both sampling dates of May ($W = 0$, $p < 0.01$), with Ripon showing the lower frost hardiness (Figure 13). ANCOVA indicated a significant effect of site on LT_{50} in branches ($p < 0.01$), but not in buds. Provenance had no significant effect on LT_{50} for the two organs (Table 6).

2.4.5 Circular model

Delta AIC-based model selection indicated that the best circular model for LT_{50} included the terms cos and sin, which defines the time, site as a fixed effect, which represented two different environments, and their interaction. The provenance was discarded during model selection for both branches and buds.

In the final circular model for branches, LT_{50} changed mainly as a function of time (cos and sin variables and their interaction, $p < 0.001$, Table 7). This indicates the strong seasonality of frost hardiness, with an increase in autumn (acclimation) and a decrease in spring (deacclimation), as described earlier. The site also had a significant effect on LT_{50} in branches ($p<0.01$, Table 7). The interactions between sin and cos function, as well as their interaction with site, were significant ($p<0.05$, Table 7), indicating that the site influences the shape of the circular regression curve, i.e. the rate of acclimation and deacclimation. In the final circular model for buds, cos, sin and their interaction were significant ($p < 0.05$, Table 7), indicating the seasonal pattern of LT_{50} following acclimation and deacclimation. Site was not significant but had a significant interaction with the sin function ($p < 0.1$, Table 7).

Table 7. Results of circular modelling of frost hardiness (LT_{50}) in sugar maple branches and buds. LT_{50} is modelled as a function of time (cos and sin variables), sampling site and provenance. The operator "×" is used to indicate interactions between variables. The adjusted R^2 for goodness of fit is shown with the significance level of the linear regression. T values are shown with the significance level of single terms. Points correspond to $P < 0.1$. One, two and three asterisks correspond to $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively.

		Branches			
R^2	**F**	**Terms**	**estimate**	**st. error**	**t**
0.90	171.1***	(Intercept)	-35.88	0.78	-46.07***
		cos	20.63	1.16	17.86***
		sin	-17.15	1.09	-15.81***
		site	2.18	1.10	1.98*
		cos×sin	-11.19	2.25	-4.98***
		cos×site	-1.65	1.63	-1.01
		sin×site	-4.70	1.53	-3.06**
		cos×sin×site	12.23	3.18	3.84***
		Buds			
R^2	**F**	**Terms**	**estimate**	**st. error**	**t**
0.91	195.9***	(Intercept)	-31.59	1.00	-31.50***
		cos	22.55	1.44	15.69***
		sin	-27.30	1.46	-18.75***
		site	-0.11	1.30	-0.09
		cos×sin	-4.52	2.51	-1.80 .
		sin×site	-3.39	1.87	-1.81 .
		cos×site	-2.03	1.77	-1.15

2.5 DISCUSSION

Frost hardiness ranged between -4 °C during the growing season and -68 °C in the coldest period of winter, showing the typical pattern of acclimation, lower plateau and deacclimation reported in the literature for other species. This range of values confirms the capacity of sugar maple to maximize its frost resistance during the dormant period (Charrier et al., 2015; Sakai & Larcher, 1987). Our results show that acclimation (i.e., increasing frost hardiness levels) took place between September and December. LT_{50} reached a plateau between December and March, followed by deacclimation (i.e. decreasing frost hardiness) until July.

Differences between sites were observed mainly during the deacclimation in spring, with the southern site showing a faster decrease in frost hardiness (Figure 13), corresponding with the earlier warming recorded at that site (Figure 12). No difference in LT_{50} was detected between provenances. Our results suggest that frost hardiness adaptations are similar across the sampled area, located in the northern portion of sugar maple's range. Local environment had greater importance than geographic provenance in determining the dynamics of frost hardiness, in agreement with previous studies reported in the literature (Charrier et al., 2011, 2018).

2.5.1 Acclimation

Overall, acclimation in branches was similar between sites, contradicting our hypothesis that seedlings in the colder site would acclimate faster. This lack of difference is likely due to the similar weather and photoperiod in the two sites. Chilling accumulation was similar between October and early December 2021, with daily temperatures differing on average by 1 °C between sites. Moreover, the two sites have similar photoperiods, with differences during the acclimation period ranging between 10 minutes on September 1 and 20 minutes on December 21. Temperature and photoperiod are the main environmental signals inducing growth cessation, dormancy and cold acclimation (Charrier et al., 2015; Vitasse, Lenz, & Körner, 2014;

Weiser, 1970). Therefore, similar weather conditions can explain the converging acclimation patterns between the two sites.

Buds showed more differences between sites during acclimation, as shown by the significant effect of site on frost hardiness (Table 6). Although LT_{50} was similar on most sampling dates, differences in LT_{50} were detected in November, with the southern site (Ripon) showing higher frost hardiness. The mean temperatures during the week before sampling were warmer in the southern site (7.3 °C) than the northern site (6.2 °C), which is counterintuitive since we expected a higher frost hardiness in the colder site. The observed difference in LT_{50} may be due to the sampling day (see section 2.3.3). In the northern site, samples were collected on November 1, after a short warm event with minimum temperatures reaching 3.6 °C. In the southern site, samples were collected on November 2, after a cold night with minimum temperatures falling to 1 °C. The frost could have stimulated a rapid adjustment of frost hardiness in the southern site, which would explain the differences observed. Several other studies documented quick fluctuations of tree frost hardiness in response to temperature changes during dormancy (Neuner et al., 1999; Sakai & Larcher, 1987; Vitasse, Lenz, & Körner, 2014), which confirm our hypothesis.

2.5.2 Maximum hardiness

During the coldest part of the winter (between January and February) frost hardiness reached stable and maximum values, with LT_{50} varying between -43 and -68 °C. All provenances attained LT_{50} below -55 °C in both sites. The low R^2 of ANCOVA indicated a lack of trend in LT_{50}, which varied around maximum values without a distinct pattern (Table 6). Our LT_{50} values are much lower compared to those recorded in other temperate deciduous species, which in most cases reach maximum values of -40 °C (Charrier et al., 2011; Vitra et al., 2017). This could reflect the harsher conditions of our sampling sites, which lie close to the border between the temperate and boreal forest. Indeed, a study on wild blueberry (Vaccinium spp.) conducted in the same geographical area as our study found LT_{50} of -68 °C during the winter, which are similar to our findings in maple (Deslauriers et al., 2021). Cold hardiness below -60

°C, and occasionally below -70 °C, is known for several boreal woody species (Sakai, 1983; Sakai & Larcher, 1987).

In our experiment, provenances growing in the colder site (Chicoutimi), were effectively moved to the northern limit of the species (Godman et al., 1990). LT_{50} in Chicoutimi exceeded both the lowest daily minimum temperature recorded during our study (-34.7 °C), the average minimum winter temperature of the last 30 years (from 1990 to 2020) (-33.4 ± 2.7 °C) and the lowest minimum temperature recorded since 1981 (-43.3 °C; Environment Canada, 2023). Even when considering the more conservative LT10 (i.e. temperature inducing 10% of cellular damage), all provenances attained values below -40 °C, indicating a good ability to minimize frost damages in harsh winter conditions. Our results demonstrate that Canadian sugar maple provenances transferred to northern areas at the limit of the species range can endure the minimum temperatures expected under winter conditions. This is consistent with a three-year common garden study on sugar maple seedlings, which found that northern populations are able to survive and grow well beyond the species' northern distribution limit (Putnam & Reich, 2017). Several other studies have found that deciduous species generally exhibit a frost hardiness higher than the local minimum temperatures of winter (Charrier et al., 2018; Kollas et al., 2014; Lenz et al., 2013).

Extreme frost events could limit the expansion and establishment of sugar maple. Extreme events are difficult to predict (Jentsch et al., 2007) and can be particularly relevant when considering trees, which have a long life span, particularly in the northern regions. An extreme frost could exceed the frost hardiness of maple, cause widespread mortality, and have long-lasting effects on a developing stand (Montwé et al., 2018). For example, a single extreme frost event occurring more than 30 years after the date of planting induced a widespread mortality in southern provenances of *Pinus pinaster* (Ait.) in southern France, highlighting a maladaptation that was previously not evident (Benito-Garzón et al., 2013).

In our experimental design, the exposure of samples to target temperatures did not last more than a few minutes. This means that our LT_{50} corresponded to the temperature that would cause instantaneous lethal damage. It is possible that longer exposures (e.g. several hours during a nighttime frost) to low temperatures above the observed LT_{50} could cause severe or lethal damage to maple. Because of the inherent differences between controlled experiments and natural conditions, the link between LT_{50} or different levels of damage and actual mortality still remains to be quantified (Burr et al., 2001). In our protocol, we used 10 K h-1 as a freezing rate, while the thawing rates (i.e. the rate of warming of the samples after the frost treatments) were not controlled. In the literature, most studies adopt freeze rates of 5 K h-1, which seems to be closer to what observed during freezing events in the field (Atucha Zamkova et al., 2021). Fast freeze or thaw cycles can increase frost damages; therefore, caution should be applied when comparing our results with LT_{50} obtained with different protocols.

2.5.3 Deacclimation

Deacclimation generally lasted from March to late May for all organs and sites, with the exception of branches in the northern site (Chicoutimi), which continued to lose frost hardiness between the end of May and beginning of July. Differences between sites were evident in May, with the warmer southern site showing an earlier and faster deacclimation. These results point to a strong environmental control over deacclimation, consistently with our hypothesis. It is well known that environmental signals, such as temperature and photoperiod, have a strong influence on ecodormancy release and budbreak (Hänninen, 1997; Kovaleski et al., 2018). Temperature is regarded as the main factor inducing ecodormancy release and budbreak (Charrier et al., 2015; Junttila, 2007), while the effect of photoperiod is less linear than temperature and is often species-specific (Flynn & Wolkovich, 2018; Hänninen & Tanino, 2011). In our study, warmer temperatures in the southern site led to faster snowmelt and growing degree days accumulation (Figure 12). This suggests a strong effect of temperature, a likely explanation for the observed differences in LT_{50} between sites. Photoperiod, on the other hand, was longer in the northern site after the equinox (March 20, DOY 79). A longer photoperiod would have induced a faster deacclimation in the northern site, contrary to what was observed in our case. It is also possible that differences in photoperiod between sites (30 min at maximum) were too marginal to affect the experiment. We conclude that deacclimation in sugar maple is mainly driven by temperature in our study region, which is consistent with other studies on spring reactivation and budbreak in this species (Guo et al., 2020b; Ren et al., 2020).

Further research should focus specifically on investigating intraspecific variations in spring late frost risk. Our results indicate that sugar maple is sensitive to warm spring temperatures, which could lead to early budbreak and frost damage in the case of false spring events (Chamberlain et al., 2019). Moreover, there is evidence of intraspecific differences in budbreak phenology for sugar maple in the literature (Guo et al., 2020b; Zeng et al., 2022). This is an important aspect to consider since the risk of desynchronization between spring phenology and favourable environmental conditions is increased under climate change (Augspurger, 2013).

2.5.4 Intraspecific differences

Our experimental results found no significant differences in frost hardiness between provenances. The provenance factor variable was not significant in ANCOVA when performed separately for acclimation and deacclimation. Moreover, model selection for the circular regression considering the whole frost hardiness cycle discarded the provenance factor. Most of the variation in LT_{50} is explained by the time of sampling, with site having a minor effect. This lack of intraspecific differentiation may stem from the limited geographic gradient considered in our study. We selected seven provenances from the same geographic area, i.e. southern Quebec and New Brunswick. This corresponds to the northern distribution of sugar maples, which extends southwards to Tennessee (U.S.) and westwards to the states of Missouri and Minnesota (Godman et al. 1990). Recent unpublished data on sugar maple genetic diversity showed no clear genetic structure within this study area, with the larger variation occurring between individuals than between provenances, thus demonstrating a high level of gene flow (Yann Surget-Groba, unpublished data). Kriebel (1957) studied the intraspecific variation of ecophysiological traits in sugar maple across a wider range and proposed three broad ecotypes: southern, central and northern. Similarly, a study of maple spring phenology by Buttò et al. (2023) found intraspecific differences only when considering the whole species range. It is therefore likely that the provenances considered here are genetically homogeneous and have similar adaptations, belonging to the northern ecotype. Other studies considering a wider geographical gradient could find higher intraspecific variability.

In the current study, we do not assess the risk of off-season frost, i.e. frost events occurring before acclimation in autumn (early frost) or after deacclimation in spring (late frost). However, within their distribution range, there is evidence that sugar maple provenances differ in their spring phenology, likely because of local adaptations to avoid late frost risk (Guo et al., 2020b; Zeng et al., 2022). To study these critical stages with more accuracy, the meteorological gradient should be enlarged to include warmer conditions simulating the expected future temperatures in the northern range of the distribution. Moreover, future investigations should consider observations at higher temporal resolutions (weekly), in order to include differences in phenology between the provenances. Such an approach might test whether spring phenology and deacclimation dynamics are more important drivers of tree species range limits than winter frost hardiness, as suggested in previous studies (Kollas et al., 2014; Körner et al., 2016). Further studies are needed to quantify the chilling and forcing requirements regulating dormancy dynamics in sugar maple provenances (Chuine et al., 2016). This aspect is particularly important for forest management and provenance selection, since late frost damage can affect tree establishment and growth during the first years after planting (Hufkens et al. 2012; Vitasse et al. 2014).

2.5.5 Conclusion

This study assessed frost hardiness in sugar maple seedlings belonging to seven Canadian provenances and growing in two sites in Quebec, at the northern portion of the species' range. Over the 2021/2022 dormant season, frost hardiness was similar between sites during the periods of acclimation and maximum hardiness. This contradicted our hypothesis that seedlings in the northern sites would have faster acclimation and higher frost hardiness during winter. Deacclimation was faster and earlier in the southern and warmer site, partially confirming our hypothesis and underlining the importance of temperature in determining the timings of budbreak.

We did not find any significant difference between provenances in either acclimation, maximum hardiness period, or deacclimation. LT_{50} was far lower than the long-term minimum temperatures occurring at the northern border of the species range. This suggests that winter conditions are not a limiting factor for the northward expansion of sugar maple, consistently with existing knowledge in the literature (Charrier et al., 2018; Kollas et al., 2014; Lenz et al., 2013; Putnam & Reich, 2017). In order to minimize the risks of frost for maple, the climatic characteristics of the planting site should have priority in respect to provenance selection. Provenance selection should be guided by other factors, such as phenological avoidance of late frosts, growth performance, and enhancing biodiversity and gene flows between populations (Aitken & Bemmels, 2016). This information is particularly relevant in forest management projects considering the northward transfer of provenances, i.e. assisted migration (Pedlar et al., 2011).

2.6 ANNEXES

Figure S3: Target temperatures tested in Relative Electrolyte Leakage (REL) analysis by date of sampling.

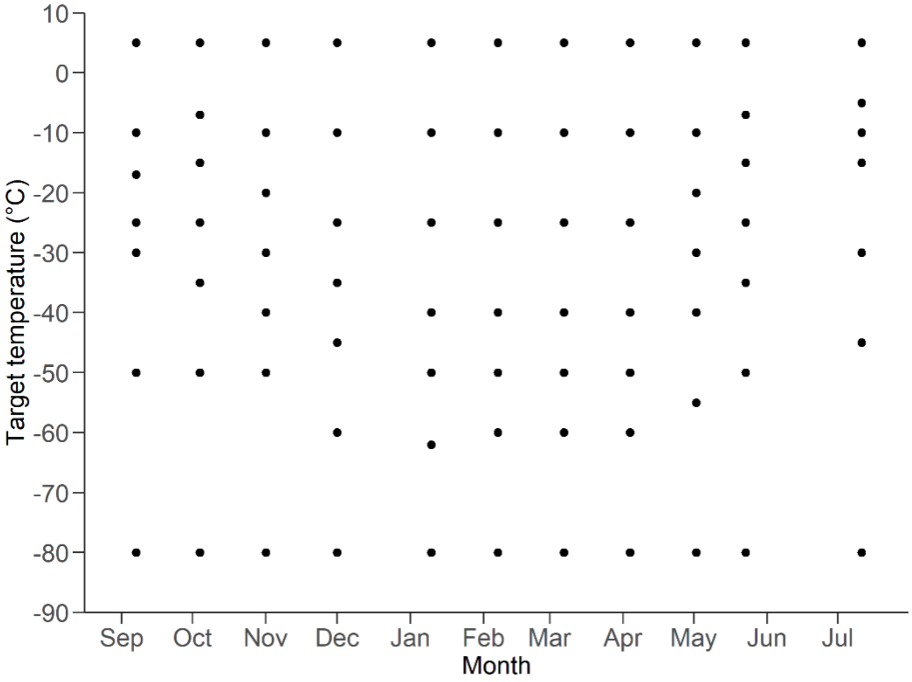
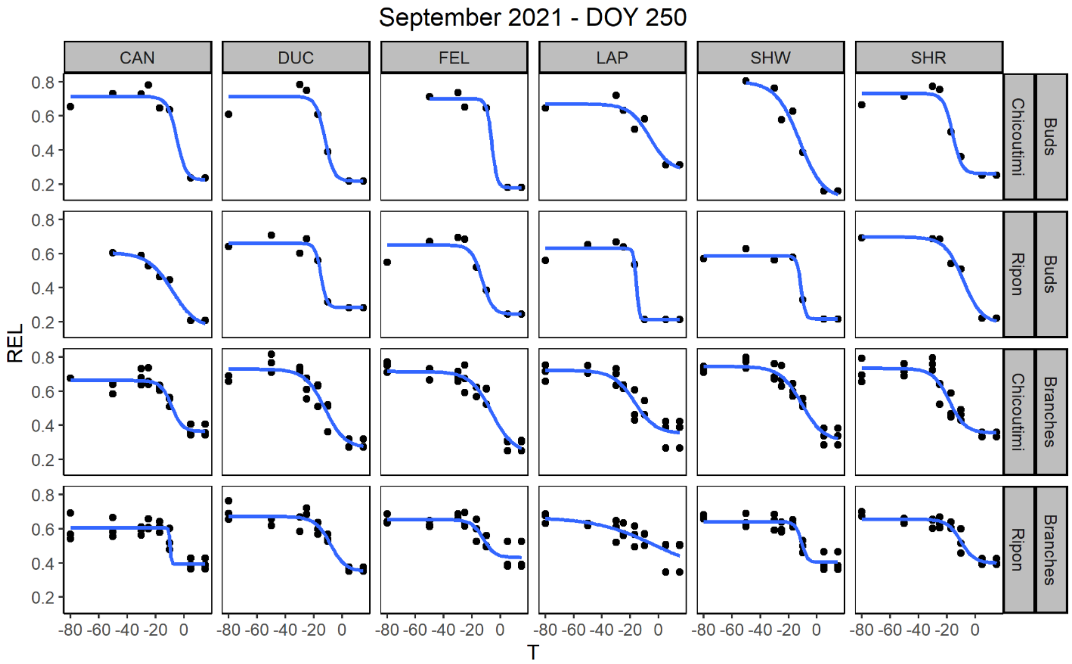


Figure S4 (continued): Logistic curves (in blue) fitted to estimate LT50 based on Relative Electrolyte Leakage (REL) measurements. Black dots are REL measurements at different test temperatures of sampling for each provenance and organ (branches, buds). L50 was estimated as the inflection point of the logistic curve.



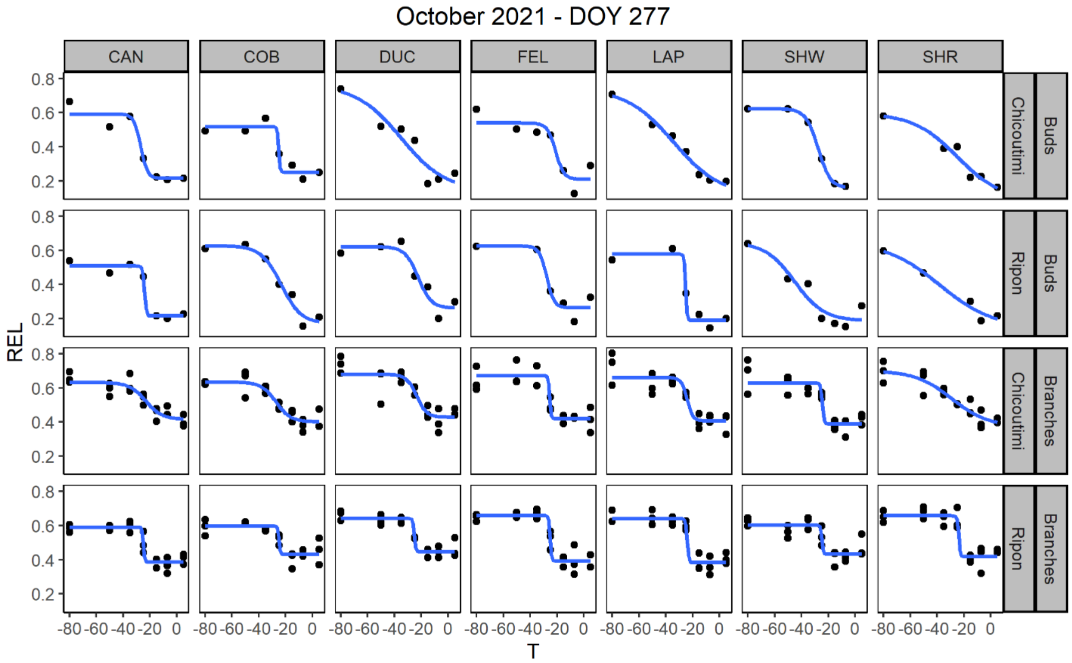
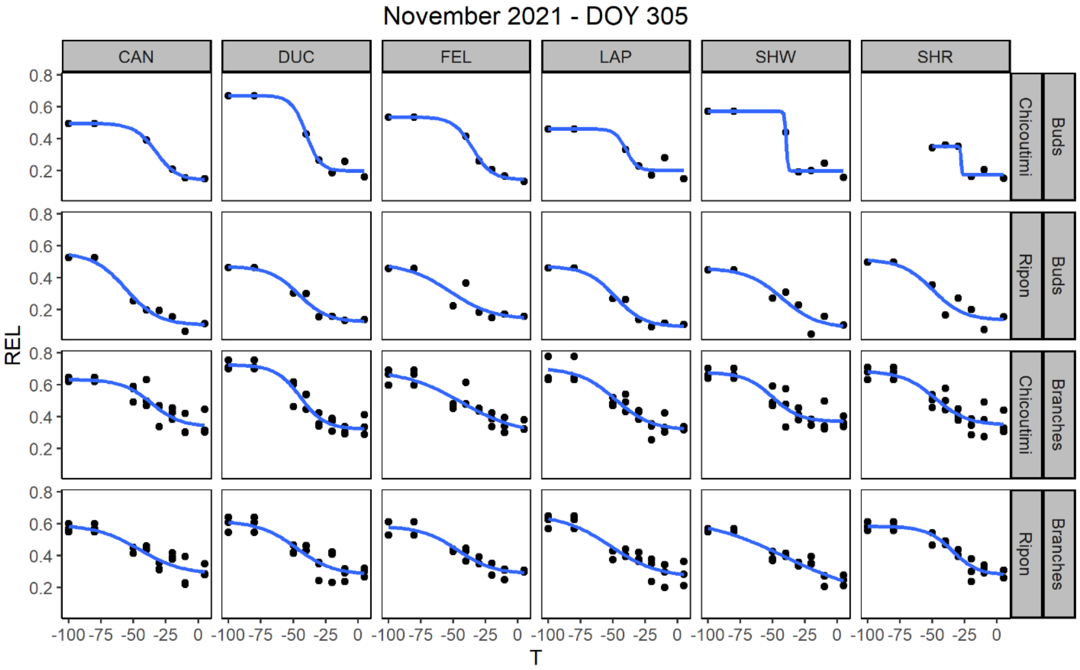


Figure S 2 (continued)



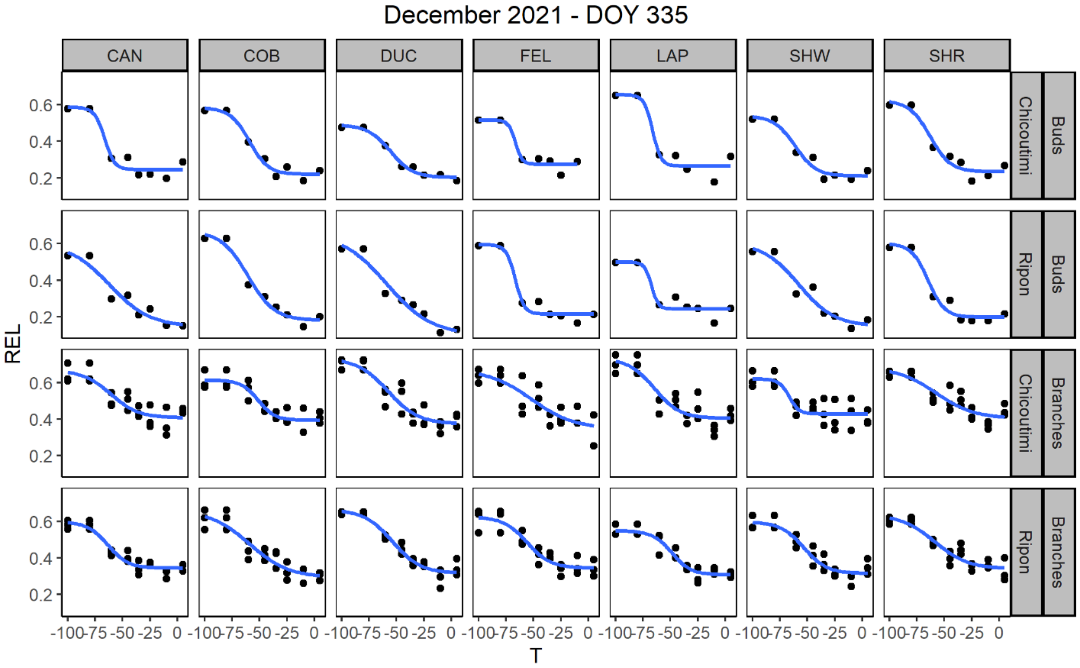
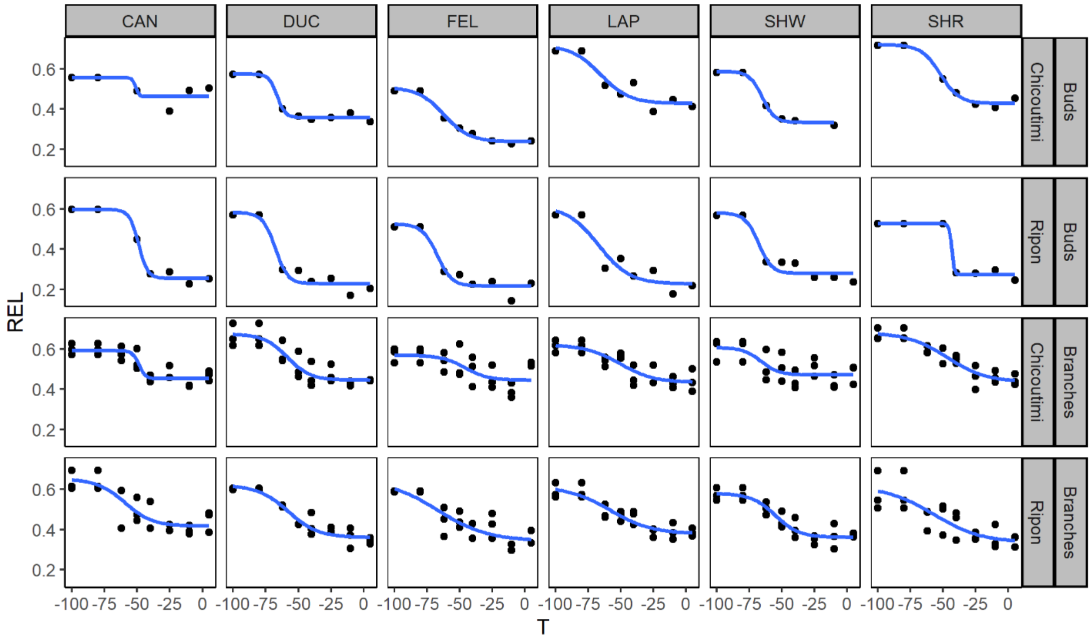


Figure S 2 (continued)

January 2022 - DOY 10



February 2022 - DOY 38

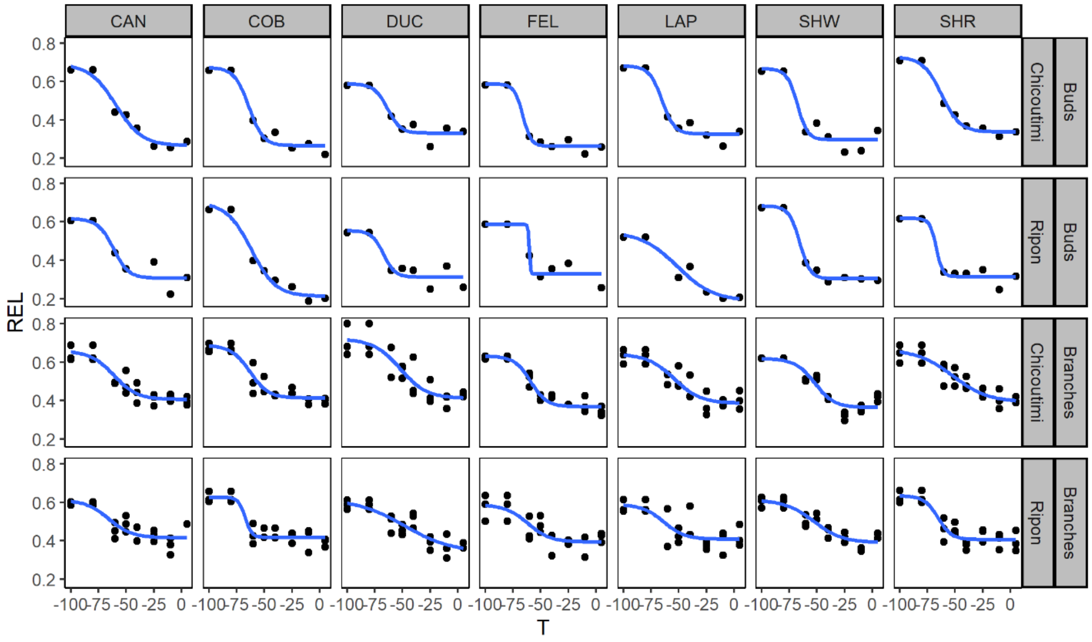
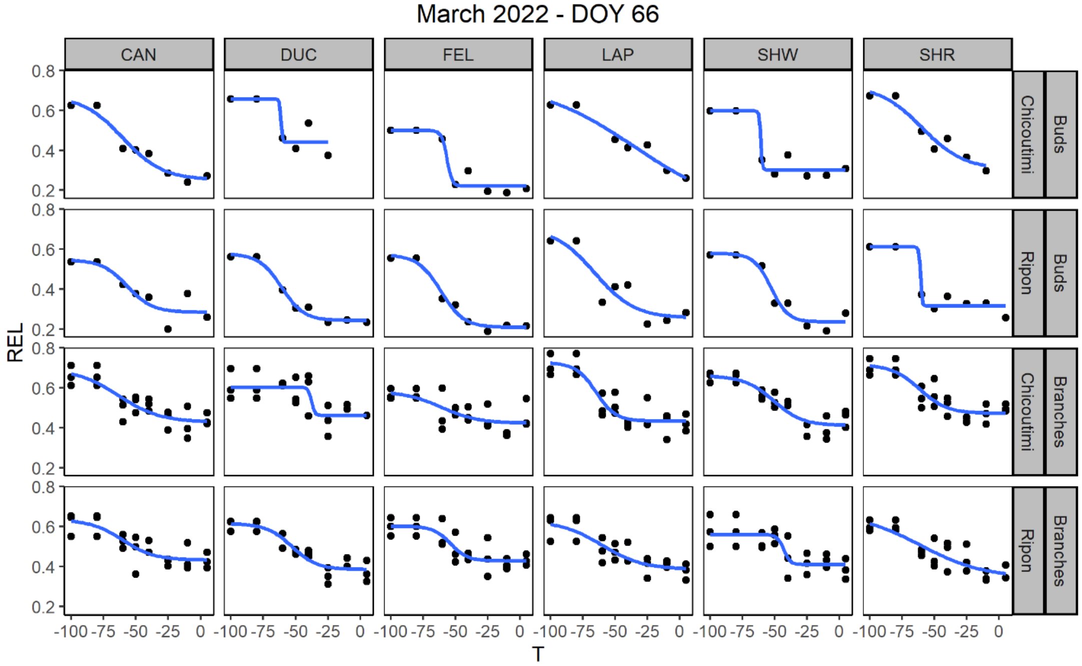


Figure S 2 (continued)


March 2022 - DOY 66

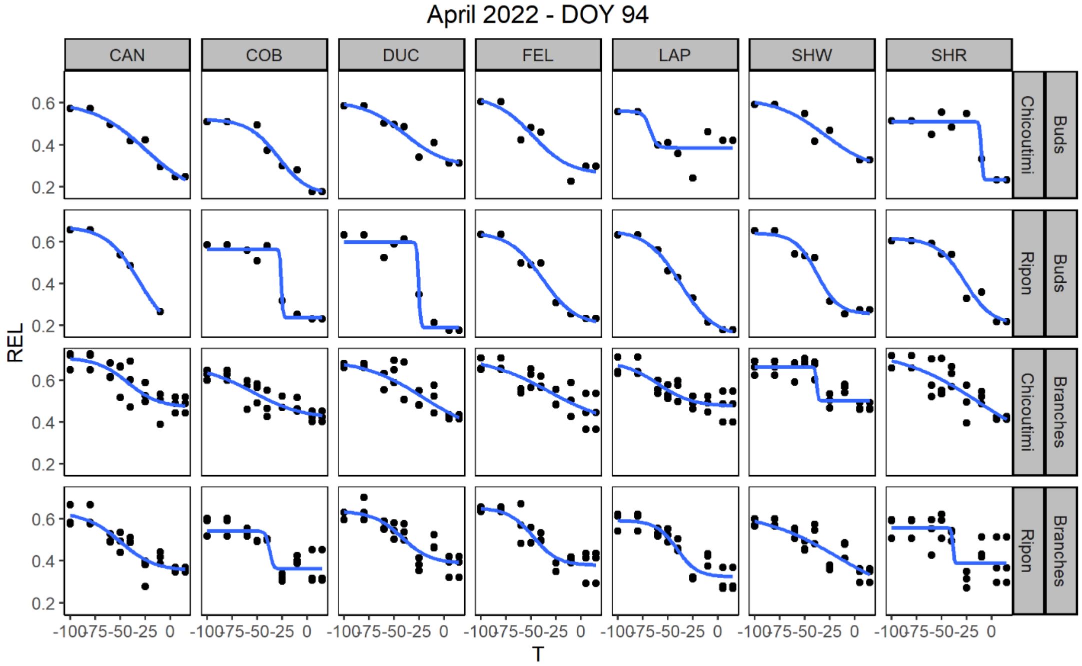
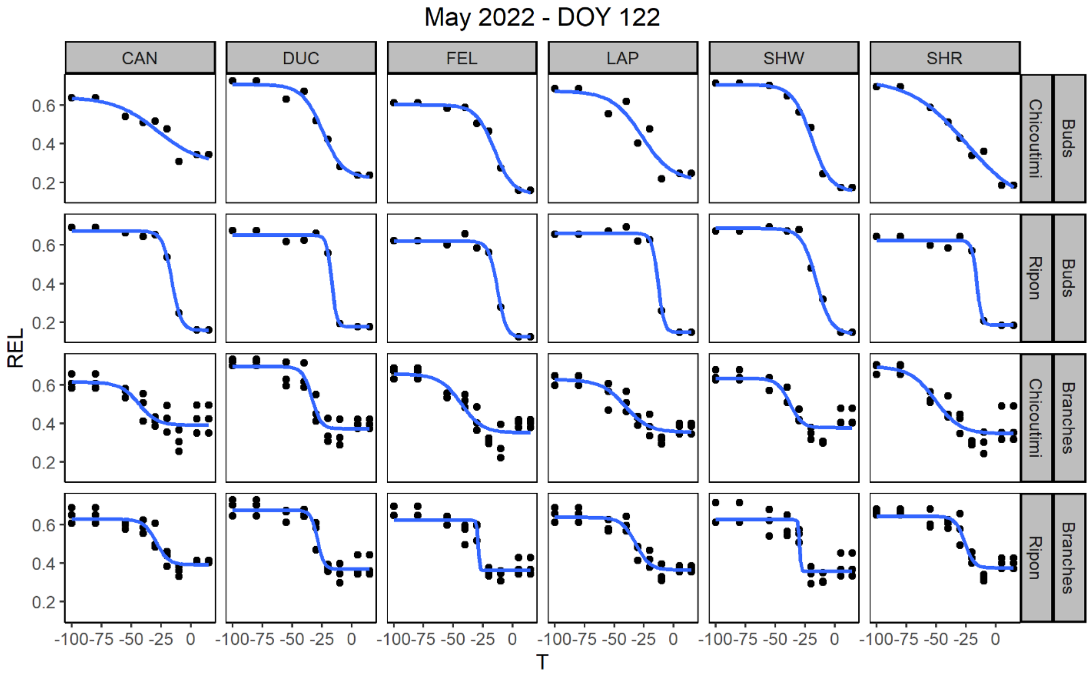

April 2022 - DOY 94

Figure S 2 (continued)


May 2022 - DOY 122

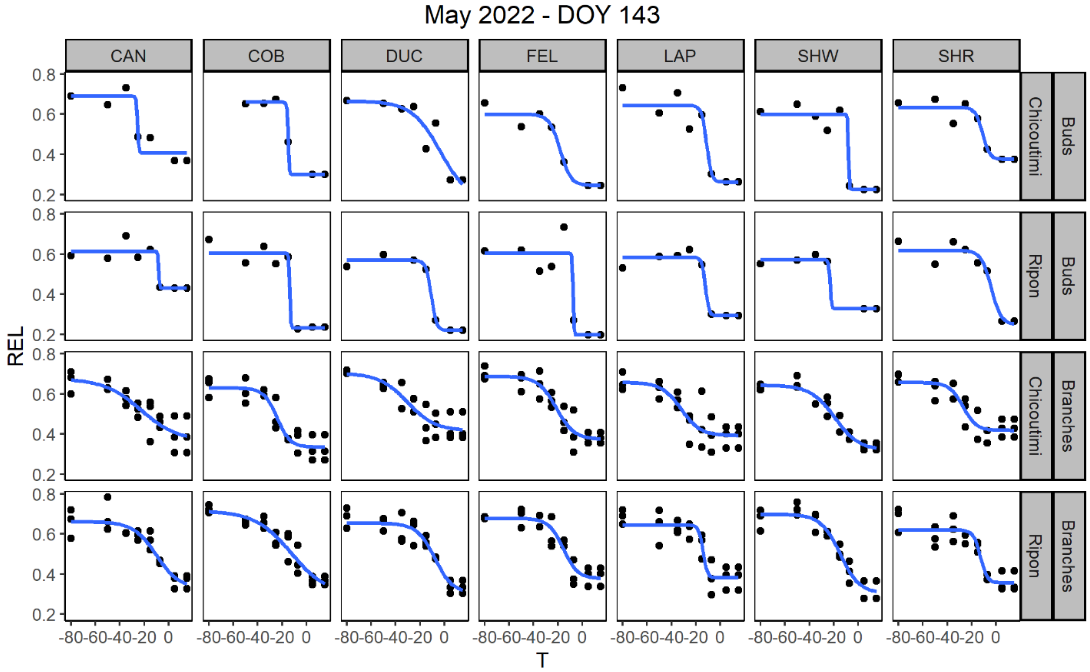
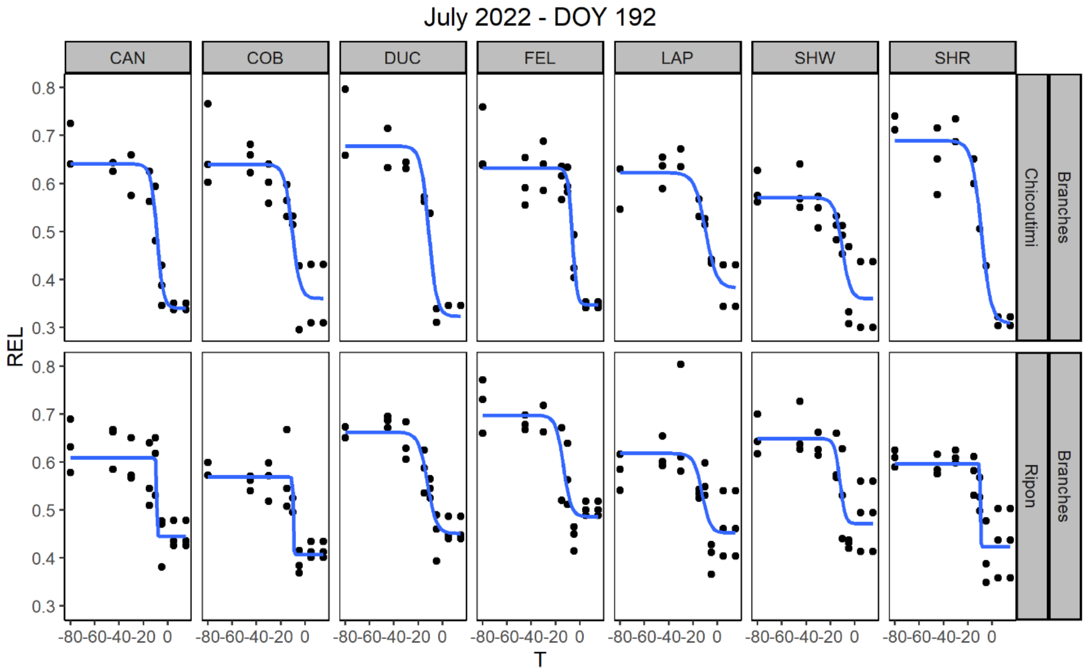

May 2022 - DOY 143

Figure S 2 (continued)



CHAPITRE 3

Is it cold enough? Effect of artificial and natural chilling on budbreak and frost hardiness in *Acer saccharum* (Marsh.)

Manuscript submitted to Physiologia Plantarum

Mura, C., Charrier, G., Kovaleski, A. P., Delagrange, S., Raymond, P., Deslauriers, A. & Rossi, S.

3.1 ABSTRACT

In cold climates, trees need to adjust their phenology and frost hardiness to environmental conditions. A crucial part of this cycle is the exposure to chilling temperatures during dormancy, which release endogenous growth inhibitors allowing the tree to react to external cues. Chilling requirements are studied by performing chilling-forcing experiments, commonly under mild, above-freezing artificial chilling conditions. In this study, we compare the effect of artificial chilling and natural freezing chilling on sugar maple (*Acer saccharum* Marsh.) seedlings.

Samples were either placed in growing chambers under controlled chilling conditions (4 °C) or outdoors in Chicoutimi, Canada. Starting in late autumn, we performed regular transfers to forcing conditions, quantified the frost hardiness (LT_{50}) at the time of transfer, and observed the time necessary to perform budbreak. We measured chilling accumulation both with classic models considering only temperatures above 0°C and with a simple model accounting for temperatures down to -5°C.

We estimated endodormancy break point for sugar maple between 2715 and 3075 hours at 4°C. Samples in artificial chilling until late April initiated the budbreak process, indicating that 4°C can both fulfill the chilling requirement and initiate ontogenetic development. Moreover, 4°C induced progressive loss of frost hardiness in maples, suggesting deacclimation. Conversely, samples under natural chilling retained higher frost hardiness until late in the season, correlating with longer time to budbreak and a more difficult identification of an

endodormancy break point. The chilling model accounting for freezing temperatures outperformed classic chilling models. Provenance did not have a significant effect.

Our results indicate that including freezing temperatures can improve chilling calculations in cold climates or boreal species, where temperatures remain below 0°C during most of the winter. Moreover, measuring frost hardiness during chilling-forcing experiments can clarify how acclimation and deacclimation influence tree dormancy dynamics.

3.2 INTRODUCTION

In temperate and boreal ecosystems, trees need to synchronize their annual cycle with external conditions to avoid frost damage. When freezing temperatures exceed the tree's frost hardiness, ice formation inside the cytosol can lead to cell death, tissue damage and death (Charrier et al., 2018; Uemura et al., 2006). There is a trade-off between growth and stress resistance, and trees improve their frost resistance by concentrating their activity in the favorable growing season, and spending the winter in a dormant state (Hänninen & Tanino, 2011; Volaire et al., 2023). This phenological cycle of growth and dormancy is associated to physiological adjustments, e.g. increased frost hardiness overwinter (Charrier et al., 2015).

The main signals inducing dormancy are shortening photoperiod and decreasing temperatures in the late summer and autumn (Hamilton et al., 2016; Rohde & Bhalerao, 2007). Low temperatures can activate dormancy-associated Mads-box (DAM) genes, which are responsible for dormancy induction (Lloret et al., 2018; Wu et al., 2017). Shortening photoperiod interacts with photoreceptors such as phytocrome A (PHYA) to initiate growth cessation (Lloret et al., 2018; Olsen, 2010). The paradormancy, the initial stage of dormancy, is a transition phase in which growth resumption is still possible under favorable external conditions (Lang et al., 1987). As temperatures get colder and photoperiod shortens, trees enter the endodormancy phase. Endodormancy is characterized by endogenous inhibition of growth in the bud, which prevents loss of frost hardiness (i.e. deacclimation) and growth resumption in response to favorable conditions, e.g. late warm spells during autumn (Charrier et al., 2015; Kovaleski, 2024). During endodormancy, trees also increase their frost hardiness in a process called cold acclimation (Charrier et al., 2011; Vitasse, Lenz, & Körner, 2014). Exposure to cold temperatures, i.e. chilling, is necessary to break endodormancy (Chuine et al., 2016). Additionally, extreme stress can also induce endodormancy release (Mohamed et al., 2014). After the endodormancy break, trees enter the ecodormancy phase, which is externally regulated by environmental signals (Charrier et al., 2015; Lang et al., 1987). At this stage, warm temperatures and increasing photoperiod during spring induce cold deacclimation, budbreak and growth resumption (Delpierre et al., 2016; Flynn & Wolkovich, 2018; Junttila, 2007). The endodormancy, and the chilling required to break it, are therefore key components of the phenological cycle of the tree (Charrier, 2022).

One major concern of climate change is that warmer winter conditions could be insufficient to fulfill the chilling requirement, thus preventing an endodormancy break, which could delay budbreak and lead to phenological maladaptations (Chuine et al., 2016). Assessing the chilling requirements of forest species is therefore necessary to better predict the phenological responses of dormancy and growth to future conditions (Laube et al., 2014). In most cases, chilling requirements are assessed by chilling-forcing experiments, in which samples are transferred at regular intervals from chilling conditions to growing conditions (i.e. forcing) during the autumn and winter (Hänninen et al., 2019). This allows to identify the endodormancy break, which is defined as the point where time to budbreak stops decreasing significantly or the percentage of successful budbreak is maximized (Hänninen, 2016). Chilling-forcing experiments are most often conducted in controlled conditions, whereas the derived models are tested on budbreak occurring in natural conditions (Hänninen et al., 2019). However, to our knowledge few studies have compared time to budbreak and endodormancy break under both natural and artificial chilling conditions.

Several models are proposed to calculate chilling accumulation, including the classic Chilling Hours model (Weinberger, 1950), the Dynamic Model (Fishman et al., 1987) and the Utah Model (Richardson et al., 1974). These models have different approaches to calculate cumulative chilling units, and consider different temperature ranges to be effective for chilling.

Chilling Hours accounts for temperatures between 0 and 7.2°C, with no contribution outside this range. The Utah model considers temperatures between 1.4°C and 12.4°C, with different weights at set temperature ranges and a negative effect (chilling negation) at temperatures warmer than 12.4°C. The Dynamic Model is a two-step model in which warm temperatures have a negative effect on chilling accumulation only in the first step. For chilling computation, the Dynamic Model assumes a bell-shaped curve for chilling accumulation, between 0°C and 12.6°C. A shared feature of these models is the assumption that temperatures below 0°C do not release endodormancy. However, this assumption is not based on mechanistic understanding of the physiological processes underlying chilling requirements, which is still lacking nowadays (Fadón et al., 2020; North et al., 2024; Wang et al., 2020). In colder environments where temperatures remain below 0°C for several months (i.e. cold temperate, high latitude or elevation environments), the above-mentioned chilling models could therefore lead to underestimating the chilling accumulation. Indeed, several authors support the addition of freezing temperatures in chilling models for a diversity of both conifer and deciduous tree species (Baumgarten et al., 2021; Hänninen, 2016; Sarvas, 1974), fruit crops (Guak & Neilsen, 2013; Mahmood et al., 2000) and vine cultivars (North et al., 2024).

Another confounding aspect of chilling units' computation is the influence of frost hardiness on dormancy depth. Most chilling-forcing experiments evaluate time to budbreak without accounting for the plant frost hardiness at the time of transfer to forcing conditions. However, recent studies have highlighted that trees under deeper frost hardiness take more time to deacclimate and perform budbreak (Kovaleski et al., 2018; North & Kovaleski, 2024). For example, a study by Kovaleski (2022) found that frost hardiness at the time of transfer to forcing conditions explained differences in time to budbreak in 15 woody perennial species spanning the seed plant phylogeny. Compared to fluctuating natural conditions, stable chilling temperatures in controlled environments can decrease plant frost hardiness, leading to faster budbreak under forcing conditions (North & Kovaleski, 2024). These studies highlight the importance of investigating the link between frost hardiness and time to budbreak in chilling-forcing experiments.

In this study, we performed chilling-forcing experiments in saplings belonging to seven sugar maple (*Acer saccharum* Marsh.) provenances in Eastern Canada. Our aim was to quantify time to budbreak after exposition to artificial and natural chilling treatments, testing for different chilling models and the influence of frost hardiness on budbreak. We predicted that: 1) endodormancy break would be easier to detect (i.e. lower standard deviation on the estimation of endodormancy break date) in artificial chilling treatments, where the confounding effect of frost hardiness would be limited; 2) a chilling model considering freezing temperatures would perform better in a cold climate experiencing temperature below 0°C for several months; 3) samples with higher frost hardiness would take more time to perform budbreak, as they need more time to deacclimate.

3.3 MATERIALS AND METHODS

3.3.1 Plant material

This study used sugar maple (*Acer saccharum* Marsh.) seedlings of seven provenances produced by a forest nursery in Berthierville, QC, Canada (Table 8, Figure 14). Provenances Duchesnay, Coy Brook and First Eel Lake (DUC, COB, and FEL, respectively) were collected on single mother trees by the National Tree Seed Center (Natural Resources Canada, Fredericton, Canada). Seeds for the Shawinigan, Lapocatière, Cantley and Sherbrooke provenances (abbreviated as SHW, LAP, CAN, and SHR, respectively) were collected at stand level by the Ministère des Ressources Naturelles et des Forêts du Québec, Canada. All sites of seed collection are natural (*i.e.* no artificial selection or tree breeding) and are thus considered representative of the provenance area.

Seedlings germinated in 2020 at the forest nursery and grew in transparent plastic tunnels until they reached approximately 25 cm in height, at which point they were transferred outdoors and kept under a shading net. After the end of the growing season, seedlings were transferred into a cold room at -3°C for the rest of the winter. In May 2021, the seedlings were transplanted into trays containing 15 cavities of 320 cm^3 and placed outdoors in Chicoutimi, Canada (named sampling site, Figure 14). Chicoutimi is located at the northern limit of the sugar maple range, with an average annual temperature of 2.8°C and an average minimum temperature of -22.1°C in the coldest month (Environment Canada, 2023; Godman et al., 1990). Seedlings spent the following growing seasons outdoors under a shading net until the experiments, which started in late 2022.

Table 8. Characteristics of the seven sugar maple provenances examined in this study. Climate data is relative to the 1970-2000 period (source: WorldClim).

Provenance	ID	Elevation (m a.s.l.)	Annual temperature (°C)	Average minimum temperature of the coldest month (°C)	Annual precipitation (mm)
Duchesnay	DUC	250	3.4	-18.9	1364
Shawinigan	SHW	124	3.95	-18.8	1063
La Pocatière	LAP	22	4.21	-16.3	939
Coy Brook	COB	89	4.83	-15.2	1119
Cantley	CAN	154	4.88	-17.2	994
First Eel Lake	FEL	177	4.88	-16.7	1100
Sherbrooke	SHR	301	5.38	-15.9	1077

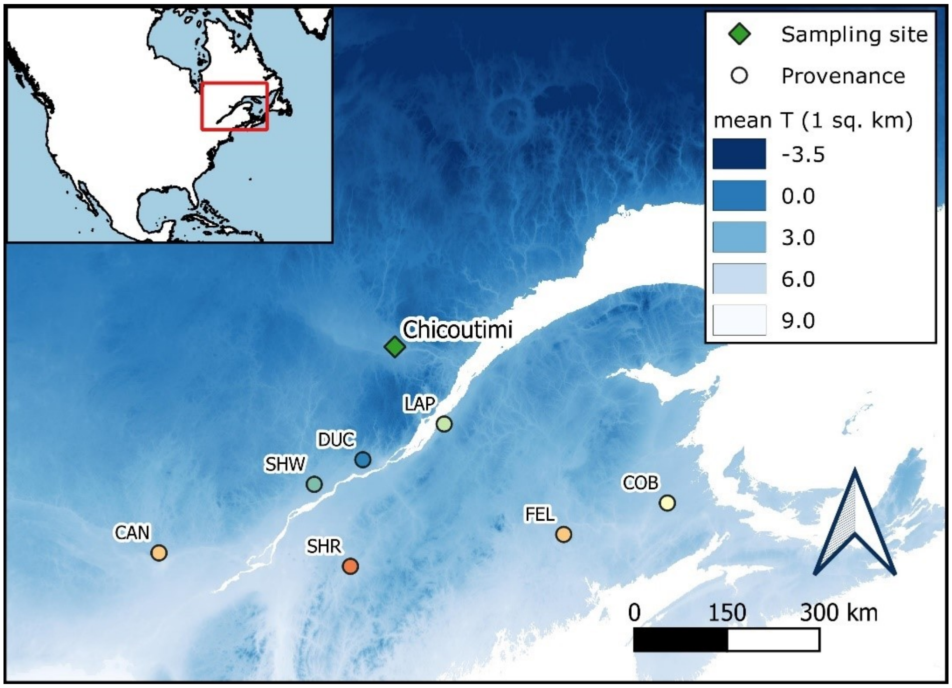


Figure 14. Sampling site (green diamond) and provenances (points) for sugar maple seedlings used in this study.

3.3.2 Experimental design

We performed two experiments to assess chilling and forcing requirements in sugar maple seedlings, which took place in the winter 2022/2023 (hereafter experiment 1) and 2023/2024 (experiment 2) (Figure 15). Following leaf fall (12 December in experiment 1, 9 November in experiment 2), seedlings were either left outdoors in natural chilling treatment or placed in growth chambers (Conviron models CMP6050 and CMP6060) for artificial chilling (4°C, 8h photoperiod). Over the winter, saplings were sampled to obtain twig sections, named cuttings, of at least 5 cm, including the apical bud. Samplings were destructive, i.e. different individuals were sampled on different dates (no repeated measurements). Cuttings were placed in trays with the bottom tips immersed in water and transferred to growth chambers at forcing treatment for budbreak observations.

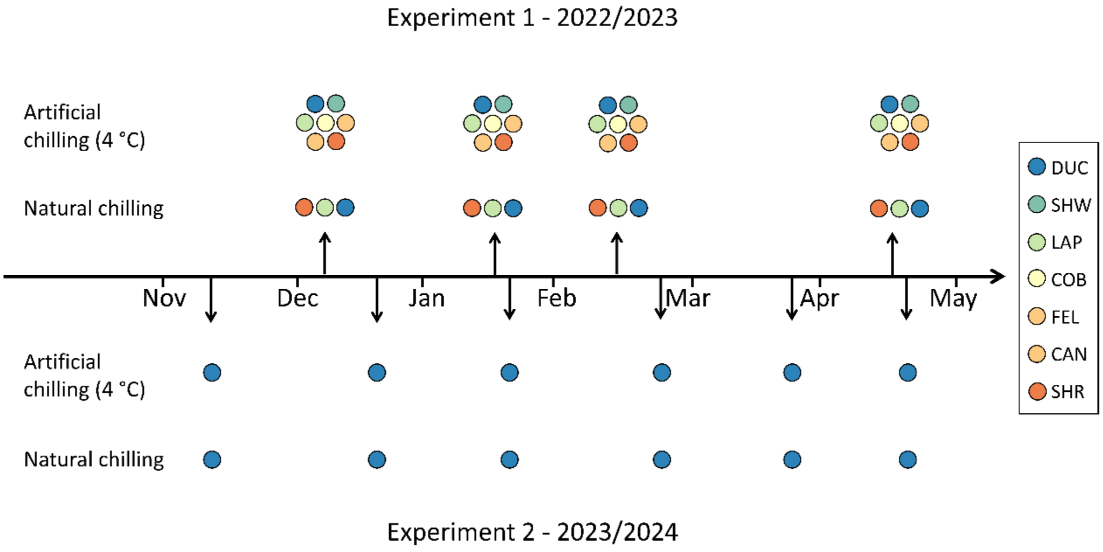


Figure 15. Experimental design adopted in this study and divided in two experiments during winter 2022-2023 (experiment 1, top panel) and winter 2023-2024 (experiment 2, lower panel). Arrows indicates dates of transfer to forcing treatment. Each point symbol indicates a different sugar maple provenance. Note that in experiment 2 only one provenance (DUC) was analyzed.

Experiment 1 used all seven provenances of sugar maple. DUC, LAP, and SHR provenances were placed in both artificial and natural chilling treatments, while the other four provenances were only artificially chilled due to seedling availability. Over the course of chilling, we performed four transfers to forcing treatments (20°C, 16h photoperiod, 75% RH) (Figure 15) at different times. Transfer dates during the winter 2022/2023 were 12 December (DOY 346), 16 January (DOY 16), 13 February (DOY 44), and 17 April (DOY 107). On each transfer date, 10 seedlings from each provenance and each chilling treatment were sampled for observations.

During experiment 2, we only used seedlings from the DUC provenance. We performed six transfers to forcing treatment (18°C, 16h photoperiod, 75% RH) during the 2023/2024 winter. Transfer dates were: 9 November (DOY 313), 18 December (DOY 352), 22 January (DOY 22), 28 February (DOY 59), 26 March (DOY 86) and 22 April (DOY 113). On each transfer date, 15 seedlings per chilling treatment were sampled for budbreak and frost hardiness assessment. A lower forcing temperature was used in experiment 2 due to desiccation observed during experiment 1. It should be noted that DUC is one of the provenances collected at tree level, which can be a limiting factor for the genetic diversity between samples. We chose this specific provenance because of the higher number of available samples.

3.3.3 Budbreak observation

Once transferred to forcing treatment, buds were observed twice a week to assess budbreak. We used a phenological scale adapted from Skinner & Parker (1994) shown here with the corresponding BBCH stage (Meier, 2001): 0) dormant bud, no sign of swelling (BBCH stage 00); 1) bud elongation, yellowish color visible between the scales (BBCH 01); 2) budbreak, with leaves visible between the scales (BBCH 09); 3) leaf emergence from the bud, leaves still not fully expanded (BBCH 10) and 4) complete leaf expansion (BBCH 11). Time to budbreak (TBB) was defined as the number of days necessary to reach stage 2 after transferring the cuttings to forcing treatment.

3.3.4 Frost Hardiness measurements

Frost hardiness was measured for buds and branches using the REL (Relative Electrolyte Leakage) technique (Repo & Lappi, 1989). On each transfer date, seven seedlings per chilling treatment (artificial *vs* natural) were sampled for frost hardiness measurement. Three cuttings at least 5 cm long were obtained from each seedling, wrapped in tin foil, and randomly distributed between seven thermal containers. Thermal containers were then placed in a controlled-temperature freezer (CryoMed controlled rate freezer, Thermo Fisher Scientific) and exposed to freezing temperatures. On each transfer date, we tested seven target temperatures between +5 and -80°C. The cooling rate was set at 6 K h^{-1}, the minimum reached by the controlled-temperature freezer. After exposure to freezing temperatures, samples were placed in vials with 10 mL demineralized water. Conductivity in the vials was measured a first time after the target temperature treatment (C1) and a second time after an autoclave treatment to damage all the cells (C2, 120°C for 30 min). The ratio between C1 and C2, named REL, is a proxy of cellular damage caused by the frost. We modelled REL values by temperature treatments as a logistic curve in order to identify LT_{50}, *i.e.* the temperature inflicting 50% of cellular damage (Repo & Lappi, 1989). More detailed information on the REL technique is provided in supplementary materials.

3.3.5 Weather and climate data

We obtained long-term (1980-2010) climate averages for the sampling site (Chicoutimi) and the seven provenances with WorldClim (Fick & Hijmans, 2017). For the winters of experiments 1 and 2, temperature data was measured hourly with an on-site temperature weather station (Priva North America Inc., Vineland Station, Ontario, Canada). Missing data were replaced by data from the nearest available weather station, Bagotville (9 km from Chicoutimi, Environment Canada, 2023).

We calculated hourly chilling units accumulation using four chilling metrics: Chilling Hours as suggested by Weinberger (1950), Freezing Hours calculated using the same formula as Chilling Hours, but customized in R to include temperatures below 0°C (higher and lower temperature limits at 7.2 °C and -5°C, respectively), Chill Units according to the Utah Model (Richardson et al., 1974) and Chill Portions according to the Dynamic Model (Fishman et al., 1987). We used the *chilling_hourtable* function in the R package *chillR* (Luedeling et al., 2023) to calculate Chilling Hours, Chill Units and Chill Portions.

Table 9 : Chilling models used in this study, including corresponding chilling metrics, lower and upper threshold for the effective range of chilling temperatures, and reference.

Chilling model	Chilling metric	Lower chilling threshold (°C)	Upper chilling threshold (°C)	Reference
Chilling Hours	Chilling hours	0	7.2	Weinberger, 1950
Freezing Hours	Freezing hours	-5	7.2	Modified from Weinberger 1950
Dynamic Model	Chill Portions	0	12.6	Fishman et al., 1987
Utah Model	Chill Units	1.4	12.4	Richardson et al., 1974

3.3.6 Statistical analyses

We tested normality of budbreak dates for each treatment using the Shapiro-Wilks test and the homogeneity of variance using Bartlett's test. We used Pearson's Chi-squared test to compare the proportion of buds performing budbreak in artificial and natural chilling treatments. P-value of reference for statistical significance was 0.05.

We used ANCOVA to test for significant effects of several variables on the time tu budbreak under forcing conditions (TBB). We compared several ANCOVA models, each using a different quantitative covariate (either time of transfer since start of the experiment in days, Chilling Hours, Chill Units, Chill Portions or Freezing Hours) with chilling treatment (artificial *vs* natural) as a qualitative covariate. For experiment 1, provenance was also included as qualitative covariate. For each quantitative variable, we performed model selection using delta-AIC (Akaike Information Criterion, Akaike 1974). We also used delta-AIC to compare models with different quantitative variables to identify the best one. Delta-AIC comparison was carried out with the *aictab* function of the AICcmodavg package (Mazerolle, 2023). Model goodness-of-fit was evaluated by adjusted R^2 values, distribution of standardized residuals and visual assessment of diagnostics plots.

We fitted an exponential curve to test for the relationship between time to budbreak and frost hardiness:

$$TBB = a * e^{(b*LT_{50})}$$

Where *TBB* is the time to budbreak (days) after transfer to forcing conditions, LT_{50} is frost hardiness at the time of transfer, and *a* and *b* are the terms of the function.

In order to identify the date of endodormancy break, *i.e.* the moment in which further chilling accumulation does not reduce TBB, we applied segmented regression with TBB as the response variable and the days since start of the experiment as explanatory variable. We used the *segmented* package in R (Muggeo, 2008). All statistical analyses were performed in R version 4.3.1 (R Development Core Team, 2023).

3.4 RESULTS

During experiment 1, temperatures below 0°C began occurring under natural conditions on 14 November. Mean daily temperatures remained below 0 for most of the winter, from 31 December 2022 until 14 March 2023. As a result, the accumulation of classic chilling metrics (*i.e.* not accounting for freezing temperatures) during this period was very low, amounting to 117 for Chilling Hours, 22 for Chill Portions and 49 for Chill Units. By contrast, the Freezing Hours model, accounting for freezing temperatures, accumulated a total of 4254 hours over the same period. The weather during experiment 2 showed a similar pattern, with temperatures remaining mostly below 0°C between 18 December 2023 and 28 February 2024, resulting in low chilling accumulation for classic chilling models, while the Freezing Hours model kept accumulating (Figure 16).

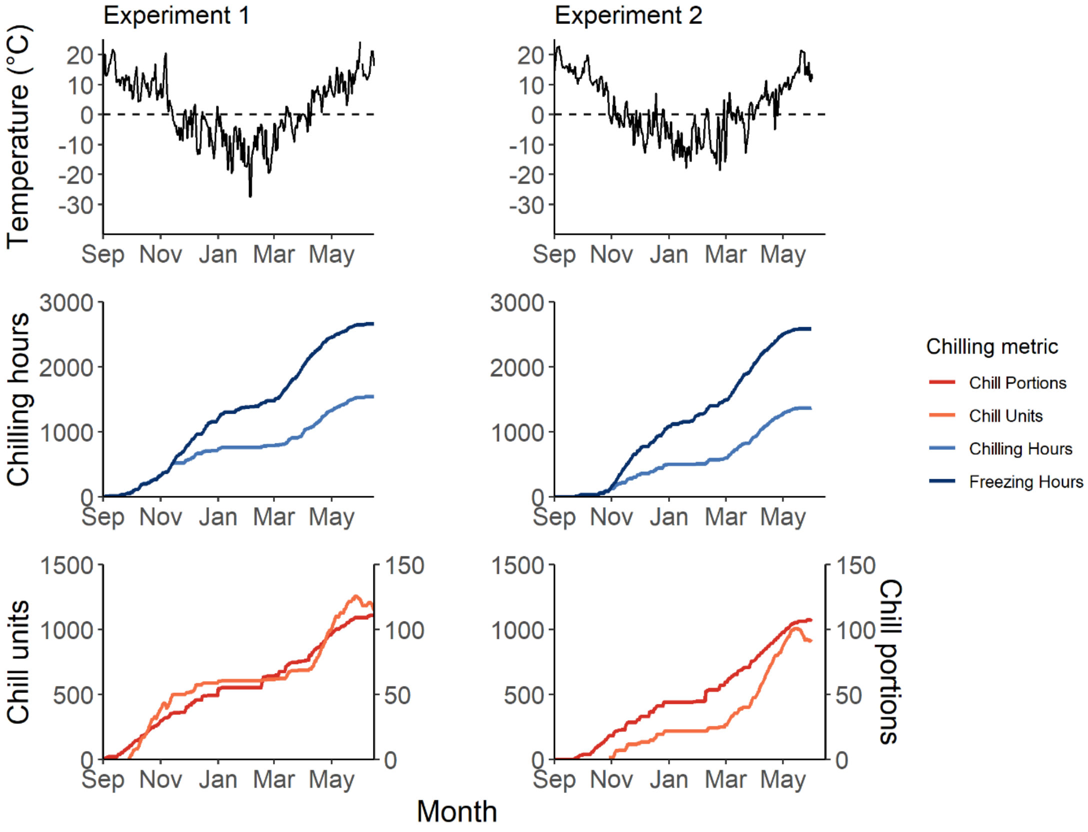


Figure 16. Daily air temperatures (top), accumulation of chilling metrics based on Weinberger, 1950 (middle) and accumulation of chilling metrics from the Dynamic and Utah models (bottom). Freezing Hours is the only chilling metric accounting for the chilling effect of freezing temperatures. Chill Portions, Chill Units and Chilling Hours are classic chilling metrics which only consider above-freezing temperatures.

Time to budbreak (TBB) decreased over time in both experiments (Figure 17). During experiment 1, TBB decreased from 70 ± 20 days on the first transfer date (12 December 2022) to 12 ± 7 days on the last transfer date (17 April 2023). During experiment 2, TBB decreased from 77 ± 16 days to 6 ± 3 days. During both experiments, some samples attained stage 1 of the budbreak process (bud elongation) under artificial chilling treatments in April. Model selection indicated that the best ANCOVA model for experiment 1 included the variables time (days since start of the experiment), chilling treatment (natural *vs.* artificial), provenance and the interaction between time and chilling treatment. Both time and chilling treatment had a significant effect on TBB, while provenance and the interaction between time and chilling were not significant (Table 10). The best model for experiment 2 included time and chilling treatment, which were both significant (Table 10). In both years, samples in artificial treatments showed a faster decrease of TBB over time (Figure 17). Delta-AIC and R^2 indicated that time was the best quantitative covariate for TBB, closely followed by freezing hours. Classic chilling metrics not accounting for temperatures below 0°C had lower performance in both experiments (Table 10).

Table 10. Results of ANCOVA models testing the effect of quantitative covariates, chilling treatment (artificial vs natural) and provenance (only in experiment 1) on the time to budbreak (TBB) under forcing treatment. Quantitative variables are either Time (days since the start of the experiment) or a chilling accumulation metric (Chill Units, Chill Portions, Chilling Hours or Freezing Hours). For the whole model, the adjusted R^2 for goodness of fit and F values and significance levels are shown. For the variables, F values and significance levels are shown. One, two and three asterisks correspond to $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively. NA indicates that the corresponding variable was discarded during model selection.

	Time	**Chill Units**	**Chill Portions**	**Chilling Hours**	**Freezing Hours**
Experiment 1					
R^2	0.7	0.69	0.69	0.69	0.7
F	74.57***	68.63***	71.23***	69.99***	73.9***
AIC	2272.6	2281.7	2285.2	2289	2274.4
Quantitative variable	472.7***	138.85***	282.66***	175.79***	293.192***
Chilling treatment	8.19**	48.16***	5.2*	22.77***	8.98**
Provenance	1.89	1.74	1.8	1.78	1.87
Quantitative×chilling	3.36	90.61***	19.64***	70.96***	26.45***
Experiment 2					
R^2	0.82	0.7	0.79	0.76	0.81
F	187.6***	64.14***	100.5***	84.13***	113.5***
AIC	611.8	627.5	638.6	654.7	619.6
Quantitative variable	334.49***	130.44***	265.13***	188.5***	294.13***
Chilling treatment	15.26***	3.71	0.98	0.045	4.32*
Quantitative×chilling	NA	45.71***	17.83***	56.96***	37.12***

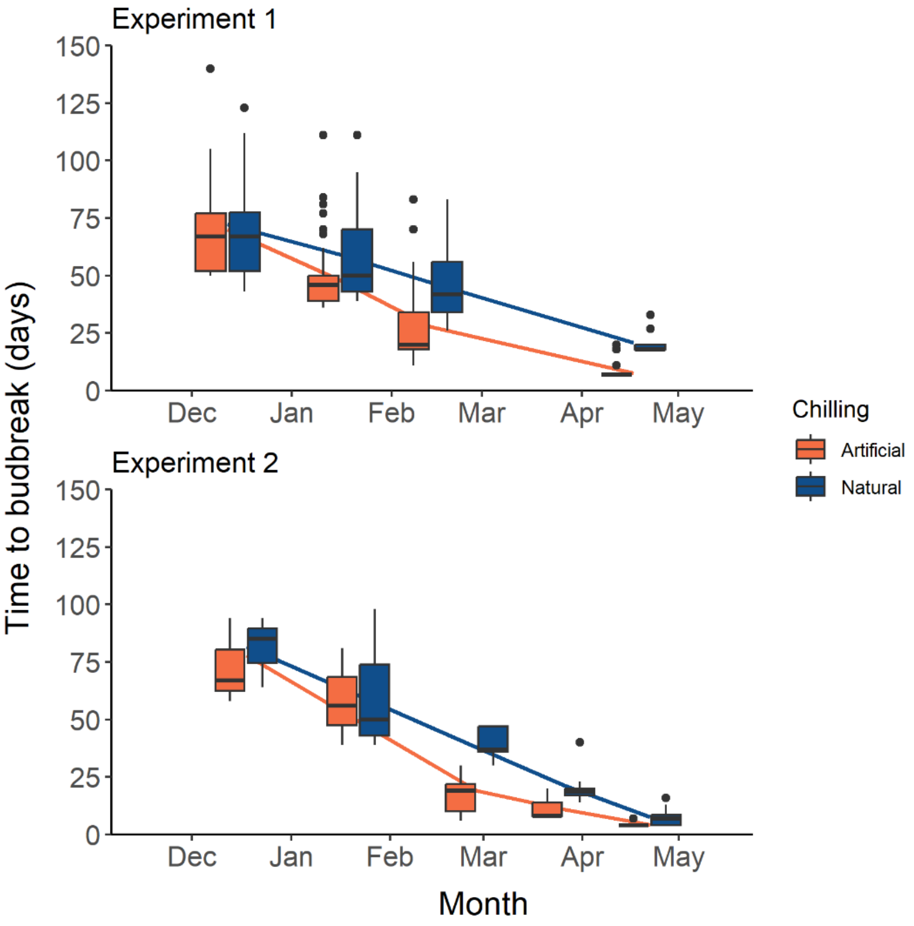


Figure 17. Boxplots of time to budbreak (TBB) in sugar maple seedlings transferred to warm treatments during experiments 1 (winter 2022-2023) and 2 (winter 2023-2024). Different colors indicate whether the samples were exposed to artificial (orange) or natural (dark blue) chilling treatments prior to sampling. Lines indicate the segmented regression model used to identify endodormancy break.

During both experiments, the budbreak percentage of samples increased with duration of chilling (Figure S5). Percentage of successful budbreak varied from a minimum of 52% (12 December 2022, natural chilling) to a maximum of 100% (17 April 2023, artificial chilling) in experiment 1, and from a minimum of 6% (9 November 2023, natural chilling) to a maximum of 100% (22 April 2024, artificial chilling) in experiment 2. Samples in artificial chilling had higher budbreak percentages than samples in natural chilling in most cases. During experiment 1, samples in artificial chilling treatment had higher percentage of budbreak than those in natural chilling treatment on both 13 February (χ^2= 6.4, p =0.01) and 17 April (χ^2 = 14.2; p <0.001). Conversely, during experiment 2, samples in natural chilling had a higher budbreak percentage than samples in artificial chilling on 28 February (χ^2 = 3.9; p = 0.049), with no significant differences in other dates.

During experiment 2, frost hardiness, based on LT50, varied between -53°C and -12°C in buds, measured on 18 December 2023 and 22 April 2024 respectively (Figure 18). LT50 in branches was slightly lower and varied between -61°C and -15°C, measured on 26 March and 27 May 2024, respectively (Figure 18). LT50 in buds reached its lowest values between December and mid-January, and consistently increased afterwards in both natural and artificial chilling treatments. LT50 in branches in artificial chilling followed a similar pattern to buds, increasing from January onwards. Conversely, LT50 in branches under natural treatment showed a less linear pattern, with a marked decrease between 28 February and 26 March. A negative exponential relationship was observed between bud LT50 and time to budbreak, where higher bud frost hardiness (i.e lower LT50 values) corresponded to longer TBB, for both natural and artificial chilling (Figure 19). A similar relationship was found for branch LT50 and TBB under artificial conditions, but not under natural chilling (Figure 19, Table 11).

.

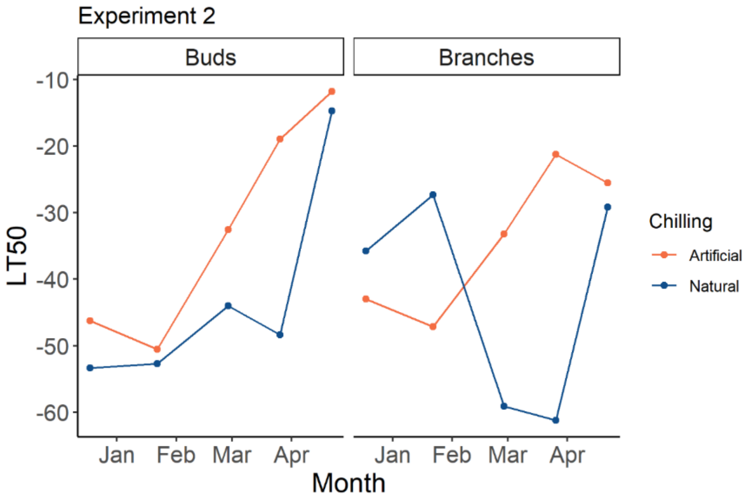


Figure 18. LT$_{50}$ values in sugar maple buds and branches under two different artificial (orange) and natural (dark blue) chilling treatments during experiment 2 (winter 2023-2024).

Table 11. Results of exponential curve fitting for the relationship between time to budbreak and frost hardiness. Residual sum of squares (RSS), root mean square error (RMSE) and Pseudo R^2 values are shown for the overall fit, as well as estimate, standard error and p-value estimation for the model terms. One, two and three asterisks correspond to *P* < 0.05, *P* < 0.01 and *P* < 0.001 respectively.

Organ	Chilling	RSS	RMSE	Pseudo R^2	Term	Estimate	Std. err.	P-value
Buds	Artificial	4232	10.04	0.79	a	2.578	0.80	0.003**
					b	-0.065	0.01	< 0.001***
	Natural	10848	16.27	0.63	a	0.111	0.15	0.462
					b	-0.120	0.03	< 0.001***
Branches	Artificial	5388	11.33	0.73	a	0.927	0.41	0.029*
					b	-0.092	0.01	< 0.001***
	Natural	27331	25.82	0.02	a	43.543	15.4	0.007**
					b	0.007	0.01	0.445

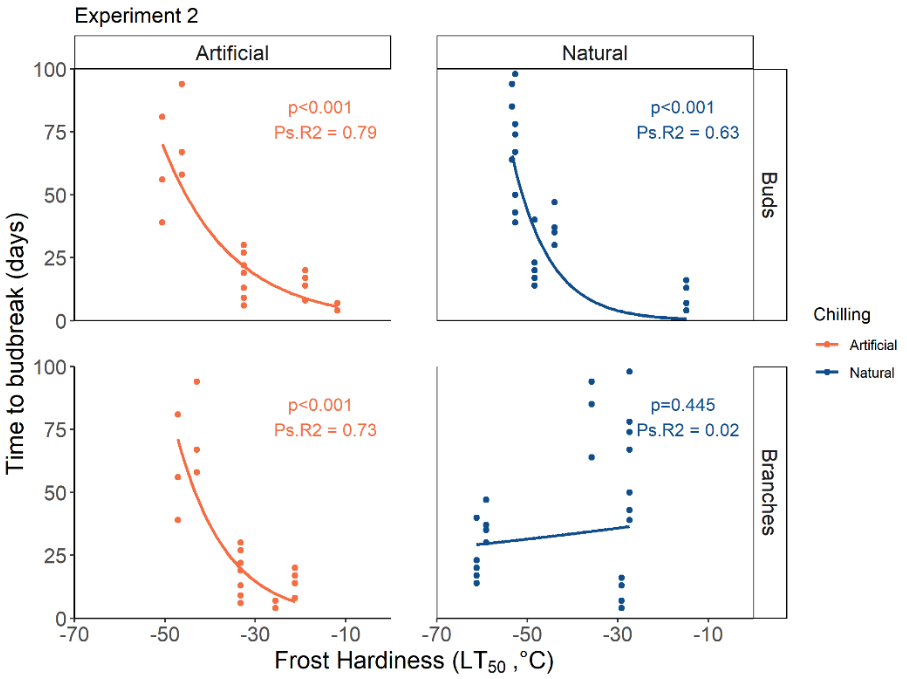


Figure 19. Relationship between time to budbreak (TBB) under forcing treatment and frost hardiness for sugar maple under artificial (orange) and natural (dark blue) chilling treatments. Points show LT_{50} (°C) estimation using the REL method. Lines refer to exponential curve fit of the data.

Segmented regression suggested that sugar maple broke endodormancy (minimized TBB) on 13 February on experiment 1 (Table 12). The same date was estimated for both artificial and natural chilling treatments. For experiment 2, endodormancy break date was estimated on 2 March and 26 March in artificial and natural chilling, respectively. For both experiments, the estimation for natural chilling had a much higher standard deviation due to the linear pattern of TBB decrease, making the estimation unreliable (Figure 17). The Chill Portions units, associated to the Dynamic model (Fishman et al., 1987), produced the smallest difference between dates of endodormancy across years for artificial chilling. Coefficient of variations of the date of endodormancy break across years ranged between 0.02 and 0.04 for all chilling metrics.

Table 12. Timings of endodormancy break identified by segmented regression. Standard deviation of the estimated date of dormancy break is shown. Cumulative values for several chilling metrics are also reported for each dormancy break date: Chilling Hours as suggested by Bennet et al. (1947), Chill Portions according to the Dynamic Model (Erez et al., 1990) and Chill Units according to the Utah Model (Richardson et al., 1974).

	Experiment 1		**Experiment 2**	
	Artificial	**Natural**	**Artificial**	**Natural**
Dormancy break	2023-02-13	2023-02-13	2024-03-02	2024-03-26
St. Dev. (days)	12	741	10	71
Chilling Hours	2715	762	2968	834
Chill Portions	103.9	55.3	108.5	71.2
Chill Units	2711.5	604.5	2830.5	405
Freezing Hours	2716	1413	3075	1924

3.5 DISCUSSION

Time to budbreak (TBB) under forcing conditions decreased with increasing exposure to chilling temperatures during the winter. The rate of decrease was faster under artificial chilling compared to the natural chilling. A cumulative chilling metric accounting for mild temperatures below 0°C (Freezing Hours) was a better predictor than classic metrics (Chilling Hours, Chill

Units and Chill Portions, only considering temperatures above 0°C). This suggests that accounting for freezing temperatures improves the measurement of chilling accumulation in cold temperate species such as sugar maple.

Endodormancy break was only reliably detectable in samples under artificial chilling conditions. We observed that the artificial chilling at 4°C induced earlier deacclimation, initiating ontogenetic development in the bud in the first half of April (before transfer to forcing conditions). Conversely, samples in natural conditions retained higher frost hardiness levels until late spring. Samples were able to reach LT_{50} below -50°C until the end of March, a temperature well below the average minimum winter temperatures common in the study area (−33.4 ± 2.7 °C for the 1990-2020 period, Environment Canada, 2023). Higher frost hardiness correlated with longer time to budbreak, possibly suggesting a confounding effect of frost hardiness on TBB. These results highlight the importance of measuring frost hardiness when performing chilling-forcing experiments, especially in cold climates where frost damage is a risk until late in the growing season.

3.5.1 Chilling temperatures

The timing of transfer from chilling to forcing conditions was the best explanatory variable for the time necessary to perform budbreak (TBB), closely followed by Freezing Hours (Table 10). Interestingly, Freezing Hours (i.e., the chilling model including freezing temperatures down to -5°C) outperformed classic chilling metrics (i.e. Chilling Hours, Chill Units and Chill Portions). This suggests that accounting for temperatures below 0°C in chilling accumulation improved prediction of TBB, confirming our hypothesis. It should be noted that in our study classic chilling units (ignoring temperatures < 0°C) still had reasonably high R^2 values, indicating a good overall performance. However, Chilling Hours and Chill Units, in particular, seem to underestimate chilling accumulation in natural conditions (Figure S6).

This could be explained by the rigid winter temperatures, remaining below 0°C during several months. As a result, classic chilling models (Chilling Hours, Chill Units and Chill

Portions) calculated virtually no chilling accumulation over much of the winter (Figure 16). Indeed, classic chilling models were first developed to predict fruit tree phenology in temperate climates (Fishman et al., 1987; Richardson et al., 1974; Weinberger, 1950), with no consideration of chilling accumulation below 0°C. This assumption (i.e. that chilling accumulation happens only above 0°C) therefore does not hold in temperate and cold boreal climates and can lead to an underestimation of chilling requirements, negatively affecting endodormancy break calculation and thus predictions under future climate conditions. Other studies have shown the importance of including freezing temperatures in chilling accumulation (Baumgarten et al., 2021; Guak & Neilsen, 2013; Hänninen, 1990b; Mahmood et al., 2000; North et al., 2024). A recent study evaluated the effectiveness of different chilling models using a vast phenological databases, and found that models accounting for freezing temperatures performed better than those limited to temperatures above freezing (Wang et al., 2020).

The conviction that there is no chilling accumulation below 0°C probably stems from the assumption that molecular processes, involving the mechanisms of endodormancy release, cannot take place at freezing temperatures. However, trees can avoid intracellular freezing by dehydration, soluble sugar concentration and supercooling (Bozonnet et al., 2024; Neuner et al., 1999), which could allow the molecular processes involved in endodormancy break to function below 0°C. The main problem comes from the fact that the underlying physiological regulation of the endodormancy break is still largely unknown (Cooke et al., 2012; Hänninen et al., 2019). Until we have a more mechanistic understanding of the physiological and molecular processes underlying the chilling requirement and the endodormancy break, temperature ranges for chilling accumulation models remain based on experimental evidence and assumptions. Exploring other approaches to model tree phenology and budbreak, for example focusing more on the physiological effects of chilling on carbohydrate metabolism, can provide new interesting perspectives to study the internal regulation of plant phenology (Cartenì et al., 2023).

Our results show that between 2715 and 3075 hours (i.e. 113 and 128 days) at 4°C are necessary to break endodormancy and minimize TBB. While these results do not reflect natural conditions, they are still relevant for comparison with other studies of chilling at mild temperatures and to clarify endodormancy break in sugar maple under artificial conditions. In a chilling-forcing experiment on several provenances of sugar maple, Kriebel & Wang (1962) found that the earlier budbreak under forcing conditions took place after 1235 to 1637 chilling hours below 7.2°C. The different results could be explained by ecotypic differentiation. In our study, we considered maples from the northern part of the species' range. Conversely, Kriebel & Wang (1962) used samples from a wider gradient, and their northern provenance remained south of the U.S.-Canada border. Kriebel & Wang (1962) did find higher chilling requirements in northern maple provenances, which reinforces this hypothesis.

Little variation in chilling and forcing requirements was found between provenances. This lack of differences could be explained by the limited geographic gradient considered. The provenances used in this study were all located in eastern Canada, an area corresponding to the northern portion of sugar maple's range (Godman et al., 1990). In a previous study, we compared frost hardiness between the same provenances and found no differences, suggesting an overall similarity between maples in this area (Mura et al., 2024). Other studies have found lower genetic diversity in sugar maple populations closer to the range edge, further corroborating this interpretation (Perry & Knowles, 1989; Young et al., 1993). Studies considering provenances over a wider geographic gradient may find more pronounced differences (Ren et al., 2020). For example, Kriebel (1957) compared budbreak in sugar maple provenances from a wider area ranging from Tennessee (US) to Québec (Canada) and found three main ecotypes, namely "southern", "central" and "northern". It is likely that the samples used in our study belong to the northern ecotype, which would explain the observed lack of differences. The lack of differences in budbreak timings suggests that provenance selection in forestry has a limited role to play in reducing the risk of late frost damage. Other tools may be more effective, such as choosing a plantation site sheltered from extreme frosts or adopting silvicultural techniques such as a shelterwood system to create a more favorable microclimate (Charrier et al., 2015; Dumais et al., 2024). This is particularly relevant for sugar maple, as the species is shade-tolerant and can grow well in a sheltered site where an upper canopy is present (Boulet, 2013; Godman et al., 1990).

3.5.2 Budbreak and frost hardiness

The time to reach budbreak (TBB) decreased as transfers to forcing conditions became closer to spring (usually late April / early May in the study area). As chilling exposure accumulates, trees enter ecodormancy and become more sensitive to external conditions, decreasing the time necessary to perform budbreak (Charrier et al., 2015; Chuine et al., 2016; Lang et al., 1987). Phenology advanced faster under artificial chilling treatment (4°C). In most cases, artificial chilling also led to higher percentages of budbreak. At first glance, this could suggest that mild and stable chilling conditions (at 4°C) were more effective than fluctuating natural temperatures in fulfilling chilling requirements. However, we argue that bringing frost hardiness into the picture may offer an alternative explanation, as mild artificial chilling may have both fulfilled the chilling requirement and contributed to forcing, initiated ontogenetic development in the bud. Conversely, low freezing temperatures in natural conditions could have fulfilled the chilling requirement but also induced high levels of frost hardiness and prevented deacclimation until late in spring.

Indeed, while temperature of 4°C were cold enough to fulfill chilling requirements in sugar maple, artificial chilling promoted deacclimation to frost, which could potentially be detrimental for trees. Bud frost hardiness decreased earlier under artificial 4°C chilling: in late March, bud LT_{50} under artificial and natural conditions was -18°C and - 48°C, respectively, leading to a 30°C of difference. Temperatures lower than -18°C are not uncommon in the study area in April, and highlight the potential risk of frost damage following deacclimation under warming winter conditions. The earlier deacclimation under artificial chilling was also confirmed by visual observation of buds initiating the budbreak process inside the growth chambers at 4°C in April, before the transfer to forcing conditions. Other studies report that above-freezing chilling temperatures can induce ontogenetic development once chilling requirements are fulfilled (Hänninen, 1990b). Kovaleski (2022) found similar results in red maple (*Acer rubrum* L.), which lost around 0.2 °C in LT_{50} per day at 4°C after fulfilling the chilling requirement. Both sugar maple and red maple are species with wide distribution, which spans northwards until the limit of the boreal forest, and these species may be particularly sensitive to mild temperatures above 0°C in the spring. Conversely, species accustomed to warmer climates may be less sensitive to such mild cool temperatures. For example, a study by Charrier et al. (2011) on European walnut (*Juglans regia* L.) found some signs of budbreak in samples at 5°C artificial chilling conditions, but at very low rates.

Longer time to budbreak was correlated with stronger frost hardiness in buds (i.e. lower LT_{50}). This relationship was observed in both artificial and natural chilling, although stronger in natural conditions. In branches, we found a correlation between frost hardiness and time to budbreak only under artificial chilling. Although correlation does not necessarily entail causation, frost hardiness could explain the observed differences in TBB between samples in artificial and natural chilling. Samples in natural chilling treatment were exposed to freezing temperatures until late March, inducing lower LT_{50} and thus preventing deacclimation (Neuner et al., 1999; Vitasse, Lenz, & Körner, 2014) and potential cell damage. Kovaleski (2022) has recently demonstrated that both the level of frost hardiness at the time of transfer and the rate of deacclimation can explain the time required to reach budbreak in several tree species, including sugar maple. This correlation between frost hardiness and budbreak may be caused by the physiological mechanisms involved in increasing frost hardiness. These changes involve dehydration in the cells and the formation of a barrier between bud and stem (Bozonnet et al., 2024; Neuner et al., 2019). These adjustments are not compatible with growth resumption, budburst and leaf formation, which require highly hydrated tissues connected to the stem (Hänninen, 2016; Xie et al., 2018). Plants with higher levels of cold hardiness need to adjust physiologically before performing their budbreak, i.e. by rehydrating their tissues and restoring connectivity between bud and stem, resulting in a longer TBB after transfer to forcing conditions as observed by Kovaleski (2022).

The identification of an endodormancy break point was only reliable in samples under artificial conditions. Indeed, TBB in samples under natural chilling showed a linear decrease without a break point (Figure 17). By contrast, samples in artificial chilling treatment started deacclimating earlier, leading to a faster decrease of TBB and allowing to easily identify an endodormancy break point. One possible explanation is that freezing air temperatures in natural chilling could have kept the samples at high frost hardiness levels, inducing a series of physiological adjustments (dehydrated cells, increased sugar content, frost barrier between bud and stem). This would cause a confounding effect, where plants in natural chilling have longer TBB than plants in artificial chilling because of the need to revert the physiological changes required to increase frost hardiness, independently of the actual fulfillment of chilling requirements. However, a concurrent explanation could also be that very low freezing temperatures (e.g. below -5°C) prevented or delayed the fulfillment of chilling requirements by stopping biogeochemical reactions in the cells.

3.5.3 Conclusion

We performed chilling-forcing experiments on sugar maple saplings for two consecutive winters. We tested the effect of natural and artificial chilling treatments, and measured frost hardiness on each transfer to forcing conditions. Fulfillment of chilling requirements was easier to detect in artificial chilling treatment, while cold temperatures in natural conditions induced strong acclimation until spring and masked the effect of endodormancy break. As a result, budbreak advanced faster under artificial chilling conditions. Accounting for freezing temperatures in chilling accumulation worked better than classic models. Provenance did not have a significant effect on time to budbreak.

This study provides quantitative measurements of the chilling requirements of sugar maple, an important factor to account for in a changing climate scenario. Moreover, our results join a growing body of literature highlighting the importance of both measuring frost hardiness and accounting for freezing temperatures in chilling accumulation when performing chilling-forcing experiments (Baumgarten et al., 2021; Kovaleski, 2022; North & Kovaleski, 2024). Taking into account frost acclimation and deacclimation dynamics can greatly improve the understanding and interpretation of chilling-forcing experiments results in the future. Future studies employing chilling-forcing experiments should account for deacclimation dynamics by performing regular frost hardiness (such as LT_{50} tests) measurements after the transfer to forcing conditions (Kovaleski et al., 2018; North & Kovaleski, 2024). Similarly, repeated measurements of LT_{50} under different chilling conditions could help quantify the contribution of mild chilling temperatures to forcing and deacclimation (Flynn & Wolkovich, 2018). These aspects can help clarify the role of above- and below-freezing temperatures in regulating frost hardiness and dormancy, advancing the knowledge on this crucial and yet still partially understood aspect of plant physiology.

3.6 ANNEXES

3.6.1 REL method for experiment 2

On each sampling date, seven seedlings per provenance were collected in each site. Seedlings were separated into three samples, each at least 5 cm long, and distributed randomly between seven target temperatures for the frost treatment. In total, each target temperature had 3 samples per provenance, wrapped in tin foil and placed in a thermal container. Thermocouples (two per container) were used to measure sample temperature during the tests.

During each test, we exposed the samples to seven different treatment temperatures ranging from +5 to -80°C. One thermal container was stored in a cold chamber at +5°C (control treatment). The remaining six thermal containers were exposed to five different temperatures ranging from -7°C to -80°C in a controlled-temperature freezer (CryoMed controlled rate freezer, Thermo Fisher Scientific). The target temperatures changed during the sampling dates to better quantify the expected frost hardiness (i.e. we tested colder temperatures during winter). The temperature in the freezer was manually adjusted to attain a cooling rate of -6°C h^{-1}. Upon reaching one of the target temperatures, one random container was taken out of the freezer and stored in a cold chamber at +5°C. All containers were then left at +5°C overnight.

On the second day, the samples were prepared for conductivity measurements. Samples were separated in branches (cut in slices 0.5 mm thick) and buds (split in two along the longitudinal axis), then stored in vials with 10 ml of demineralized water. Because of the small number of buds available, buds from the same provenance and within the same target temperature were placed in the same vial. Vials were left to agitate on a multi-platform orbital shaker (Thermo Fisher Scientific, Waltham, MA) at 5°C overnight.

The third day, conductivity in each tube was measured as an indicator of electrolyte leakage from cells damaged by the frost (C_1). Samples were then put in an autoclave at 120°C, 1 bar, for 30 minutes. A second conductivity measurement was performed after the autoclave treatment, corresponding to the maximum cellular damage (C_2). REL was then calculated as C1/C2, *i.e.* the ratio of electrolyte leakage caused by frost to the leakage caused by maximum damage. We calculated the relationship between REL and temperature using the logistic function (Repo & Lappi, 1989):

$$REL = \frac{a}{(1 + e^{b(c-T)})} + d \tag{2}$$

where *T* is the test temperature, *d* is the higher asymptote, *a*+*d* is the lower asymptote and *b* is the slope at the inflection point *c*. Frost hardiness (LT_{50}) was calculated for each provenance as the temperature at the inflection point *c*, i.e. the temperature causing 50% of cellular damage. Temperatures inducing 10% cellular damage (LT_{10}) were also calculated from the logistic curve, to estimate a lower threshold for frost damage occurrence and for comparison with LT_{50}. The logistic function was fitted with the nlsLM function of the minpack.lm package (Elzhov et al., 2022).

3.6.2 Supplementary figures

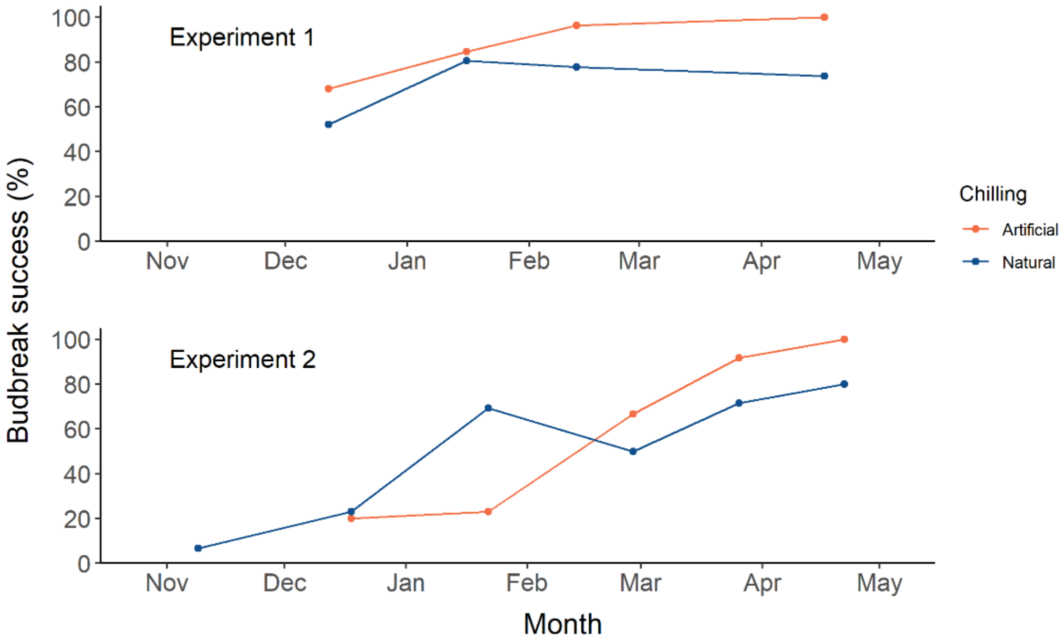


Figure S5. Percentage of buds performing budbreak after transfer to forcing treatment and divided in two experiments during winter 2022-2023 (experiment 1, top panel) and winter 2023-2024 (experiment 2, lower panel). Bar colors indicate artificial (orange) and natural (dark blue) chilling treatment. For experiment 1, all provenances are grouped together because of a non-significant effect according to ANCOVA.

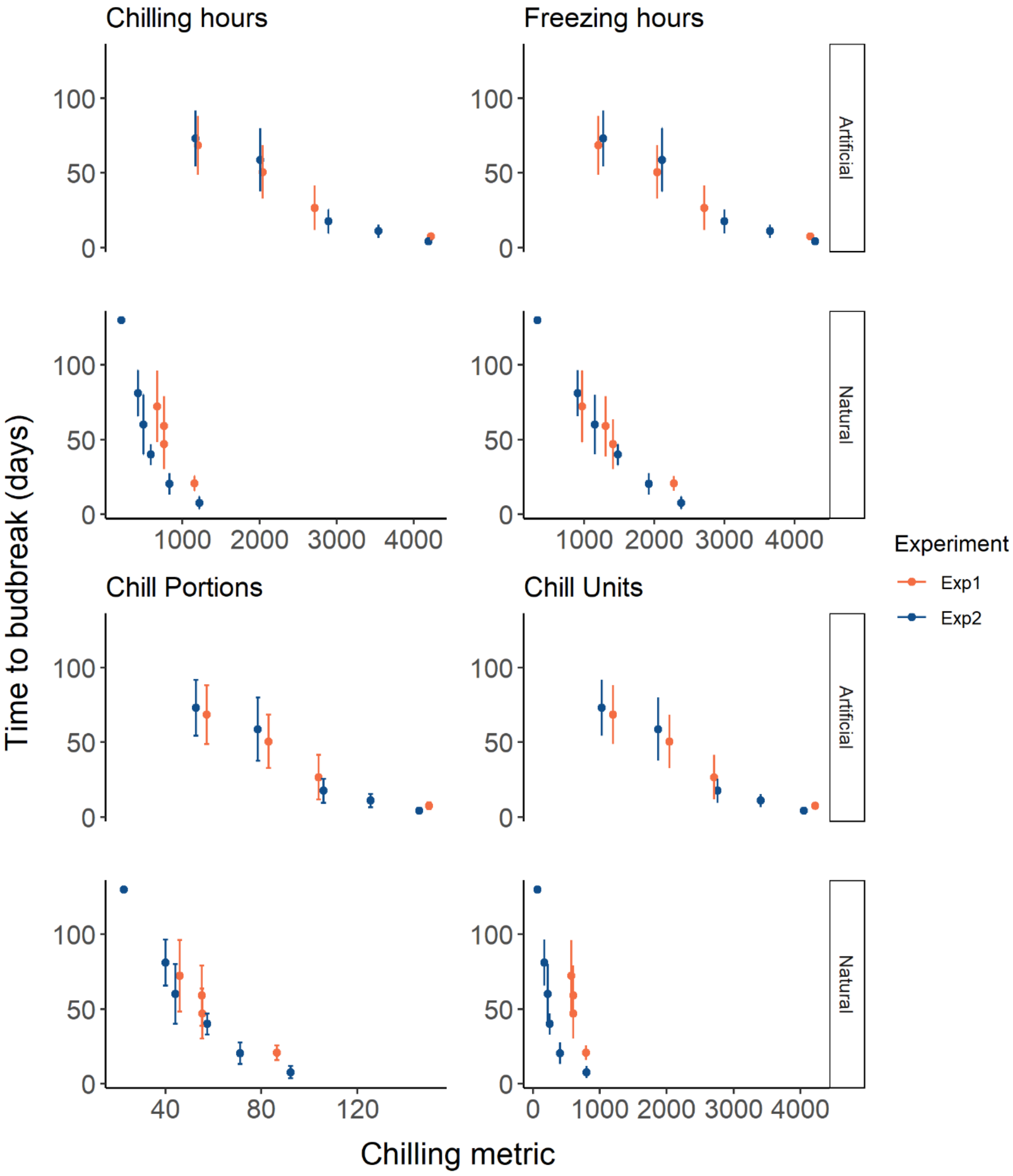


Figure S6. Time to budbreak by the main chilling metrics used in this study, under both natural and artificial chilling conditions.

CONCLUSION GÉNÉRALE

The general objectives of my dissertation were i) assessing intraspecific variability in frost hardiness and phenology of dormancy and budbreak, and ii) linking these two traits to understand intraspecific variations in risk of frost damage. Concerning the general objective i), my results highlighted interesting differences in the intraspecific variations between *Picea mariana* and *Acer saccharum*. *Picea mariana* provenances showed a clear pattern in budbreak timing, with northern provenances performing earlier budbreak consistently over several years of observations. On the other side, *Acer saccharum* appeared to lack intraspecific differentiation between the provenances considered in this work, both in their frost hardiness and sensistivity to temperatures during dormancy.

One possible interpretation for this divergence would be that the gradient for *P. mariana* provenances is wider, leading to higher climatic distance between the provenances. Indeed, the gradient for *P. mariana* provenances covers a 4°C variation in mean annual temperature, while difference between *A. saccharum* provenances is limited to 2°C. By covering a larger climatic gradient, it follows that *P. mariana* provenances would show higher differentiation in their functional traits. Had we selected *P. mariana* provenances exclusively from the northern portion of the species' range, as we did with maple, it is possible that physiological responses would have been more similar. However, in *P. mariana* the intraspecific differences were clear even between provenances that have only 1 to 2 °C difference in mean annual temperature, suggesting that this interpretation may be not sufficient.

An alternative explanation of the results is that the different species have different plasticity and adaptive potential, where *A. saccharum* shows more capacity to acclimate and to produce similar intraspecific responses in the same environmental conditions compared to *P. mariana*. This interpretation is reinforced by a recent review of tree species adaptations by Leites & Benito Garzón (2023), which found that genetic adaptations to local climates are more common in conifers than broadleaves, hinting to different evolutionary strategies. This has relevant implications for climate change: plasticity of functional traits is essential to adjust to climate change, and *A. saccharum* seems to have an advantage in this regard. In order to better understand this point, further studies considering larger climatic gradients for *A. saccharum* should be carried out. Such studies should take into account this work and the research by Kriebel & Wang (1962), who considered intraspecific differences in sugar maples from a larger portion of the species' distribution and identified three major ecotypes (southern, central and northern). Replicating this study, with a higher number of samples and more functional traits analyzed, could greatly clarify patterns of genetic variation and phenotypic plasticity in *A. saccharum.*

These different trends in intraspecific differentiation are also relevant when considering the general objective ii), i.e. the influence of phenology and frost hardiness on the frost risk exposure. The clear intraspecific pattern in *P. mariana* spring phenology showed a clear trend where northern provenances have higher risk of exposure to late frost because of an earlier budbreak. This highlights a possible problematic under climate change, i.e. that northern populations of *P. mariana* may see an increase in late frost damage as spring conditions become both warmer and more variable (Chamberlain et al., 2019). A follow-up study of chapter 1 that I co-authored found that northern *P. mariana* provenances did not change their budbreak timing following the frost event of 2021 (Silvestro et al., 2023). Despite a reduction in apical growth in the year following the event, northern provenances continued to show an earlier budbreak, suggesting limited plasticity and underscoring the problematic.

Chapter 1 highlights how provenance selection in forest management may help mitigate the problem of late frost exposure, e.g. by transferring southern provenances northwards to decrease late frost risk. However, one limitation of the study is that only one common garden was considered, where all provenances were either local or transferred to the south. Realising a second *P. mariana* common garden in the northern portion of the species' range with the same provenances could provide relevant information to better identify intraspecific differences. Realising such a plantation would allow to compare the effect of the local environment versus genetic adaptations on functional traits, similarly to what I did for *A. saccharum* in chapter 2. The main constraint to experimental plantings in the northern portion of *P. mariana'*s range is the remoteness of these territories. For example, reaching the northernmost provenance MIR (Mirage) requires more than 20 hours of driving from the nearest university, making the collection of weekly phenological data a logistical challenge. One way to solve this problem would be to focus on traits that require less frequent surveys, such as yearly apical shoot growth or winter survival.

Compared to *P. mariana,* the lack of intraspecific differences in *A. saccharum* frost hardiness and budbreak suggests that all provenances have similar risk of frost exposure. The results on frost hardiness also indicate that this species is capable of attaining LT_{50} values much lower than actual minimum temperatures at the species' range limit. This suggests that *A. saccharum* can survive winters beyond its current northern range limit, an important pre-requisite for northward transfers in assisted migration projects. The similar phenology and budbreak between provenances suggest that provenance selection has little potential to limit frost risk, and might instead focus on other desired traits such as growth performance or drought tolerance (Dumais et al., 2024). *A. saccharum'*s current range may be determined by factors other than winter survival, such as edaphic conditions (e.g., acid or poorly drained soils typical of boreal ecosystems) or lack of associated mycorrhizal organisms in the soil (Carteron et al., 2020). Such limitations need to be addressed and evaluated in order to maximize the success of assisted migration projects (Park et al., 2018).

Chapter 3 highlighted that more knowledge is necessary to better understand chilling accumulation and endodormancy break, as these are key in predicting tree responses to future climate. In particular, the possible influence of frost hardiness levels on the depth of dormancy needs to be further investigated, as there is a growing body of studies highlighting the interdependency of these factors. This is especially relevant in cold climates such as our area of study, where temperatures can remain below 0°C for most of the winter, making frost hardiness a necessity and influency chilling accumulation. In my work, I measured frost hardiness at the time of transfer from chilling to forcing conditions and found an interesting correlation with the time necessary to perform budbreak. Further studies should perform repeated frost hardiness measurements, in order to track the progressive loss of frost hardiness of samples under forcing conditions. While these analyses are destructive and tipically require a huge number of samples, employing techniques of frost hardiness measurement that require less samples such as the DTA (Differential Thermal Analysis) can help tackle the logistical challenges of these studies. A reference for this kind of research design can be found in Kovaleski (2022).

Results from the three chapters of this work added original data and insight to the existing literature, improving the understanding of these fundamental components of the life cycle of trees. With global temperatures and weather variability increasing as an effect of climate change, frost hardiness and dormancy regulation remain extremely relevant aspects of tree ecophysiology. Although interest in this field is rising, many aspects require clarification. Frost hardiness measurements spanning whole dormant seasons are still missing for many species, and the mechanistic functioning of chilling accumulation needs to be further explored and clarified. Expanding studies on these subjects in the near future, covering new species, provenances and geographical areas, will be key to understanding this important driver of plant adaptation and to improve predictions of climate change impacts. Interdisciplinary studies linking physiological knowledge of trees to the physics of ice formation in plant tissues and the molecular control of dormancy will be necessary to fully elucidate these points.

LISTE DE RÉFÉRENCES

Agriculture and Agri-Food Canada. (2019). *Statistical Overview of the Canadian Maple Industry*. http://multimedia.agr.gc.ca/pack/pdf/maple_erable_2019a-eng.pdf

Aitken, S. N., & Bemmels, J. B. (2016). Time to get moving : Assisted gene flow of forest trees. *Evolutionary Applications*, *9*(1), 271-290. https://doi.org/10.1111/eva.12293

Akaike, H. (1974). A new look at the statistical model identification. *IEEE Transactions on Automatic Control*, *19*(6), 716-723. IEEE Transactions on Automatic Control. https://doi.org/10.1109/TAC.1974.1100705

Atucha Zamkova, A.-A., Steele, K. A., & Smith, A. R. (2021). Methods for measuring frost tolerance of conifers : A systematic map. *Forests*, *12*(8), 1094. https://doi.org/10.3390/f12081094

Aubin, I., Munson, A., Cardou, F., Burton, P., Isabel, N., Pedlar, J., Paquette, A., Taylor, A., Delagrange, S., Kebli, H., Messier, C., Shipley, B., Valladares, F., Kattge, J., Boisvert-Marsh, L., & McKenney, D. (2016). Traits to stay, traits to move : A review of functional traits to assess sensitivity and adaptive capacity of temperate and boreal trees to climate change. *Environmental Reviews*, *24*, 164-186. https://doi.org/10.1139/er-2015-0072

Augspurger, C. K. (2013). Reconstructing patterns of temperature, phenology, and frost damage over 124 years : Spring damage risk is increasing. *Ecology*, *94*(1), 41-50. https://doi.org/10.1890/12-0200.1

Baffoin, R. (2021). *Modélisation de la résistance au gel des arbres : Vers la généricité d'un modèle osmo-hydrique* [PhD Thesis]. Université Clermont-Auvergne.

Baffoin, R., Charrier, G., Bouchardon, A.-E., Bonhomme, M., Améglio, T., & Lacointe, A. (2021). Seasonal changes in carbohydrates and water content predict dynamics of frost hardiness in various temperate tree species. *Tree Physiology*, *41*(9), 1583-1600. https://doi.org/10.1093/treephys/tpab033

Barsoum, N. (2015). Mixed provenance and mixed species trials : Informing the debate on how to prepare native tree species for climate change in England. *Quarterly Journal of Forestry*, *109*(3), 201-207.

Basler, D., & Körner, C. (2012). Photoperiod sensitivity of bud burst in 14 temperate forest tree species. *Agricultural and Forest Meteorology*, *165*, 73-81. https://doi.org/10.1016/j.agrformet.2012.06.001

Basler, D., & Körner, C. (2014). Photoperiod and temperature responses of bud swelling and bud burst in four temperate forest tree species. *Tree Physiology*, *34*(4), 377-388. https://doi.org/10.1093/treephys/tpu021

Batschelet, E. (1981). Chapter 8 : Periodic Regression. In *Circular statistics in biology* (Sibson, p. 159-177). Academic Press.

Baumgarten, F., Zohner, C. M., Gessler, A., & Vitasse, Y. (2021). Chilled to be forced : The best dose to wake up buds from winter dormancy. *New Phytologist*, *230*(4), 1366-1377. https://doi.org/10.1111/nph.17270

Benito-Garzón, M., Ha-Duong, M., Frascaria-Lacoste, N., & Fernández-Manjarrés, J. (2013). Habitat Restoration and Climate Change : Dealing with Climate Variability, Incomplete Data, and Management Decisions with Tree Translocations. *Restoration Ecology*, *21*(5), 530-536. https://doi.org/10.1111/rec.12032

Beuker, E., Valtonen, E., & Repo, T. (1998). Seasonal variation in the frost hardiness of Scots pine and Norway spruce in old provenance experiments in Finland. *Forest Ecology and Management*, *107*(1), 87-98. https://doi.org/10.1016/S0378-1127(97)00344-7

Bey, C. F., Toliver, J. R., & Roth, P. L. (1971). Early Growth of Black Walnut Trees From Twenty Seed Sources. *Research Note NC-105. St. Paul, MN: U.S. Dept. of Agriculture, Forest Service, North Central Forest Experiment Station*, *105*. http://www.fs.usda.gov/treesearch/pubs/11283

Blum, B. M. (1988). Variation in the phenology of bud flushing in white and red spruce. *Canadian Journal of Forest Research*, *18*(3), 315-319.

Boisvert-Marsh, L., Périé, C., & Blois, S. de. (2014). Shifting with climate? Evidence for recent changes in tree species distribution at high latitudes. *Ecosphere*, *5*(7), art83. https://doi.org/10.1890/ES14-00111.1

Boulet, B. (2013). Chapitre 1 : L'autécologie des essences commerciales. Érable à sucre. In *Le guide sylvicole du Québec. Tome I: les fondements biologiques de la sylviculture* (Vol. 1, p. 8,9). Gouvernement du Québec.

Bozonnet, C., Saudreau, M., Badel, E., Amglio, T., & Charrier, G. (2024). Freeze dehydration vs supercooling in tree stems : Physical and physiological modelling. *Tree Physiology*, *44*(1). Scopus. https://doi.org/10.1093/treephys/tpad117

Burke, M. J., Gusta, L. V., Quamme, H. A., Weiser, C. J., & Li, P. H. (1976). Freezing and Injury in Plants. *Annual Review of Plant Physiology*, *27*(1), 507-528. https://doi.org/10.1146/annurev.pp.27.060176.002451

Burr, K. E., Hawkins, C. D. B., L'Hirondelle, S. J., Binder, W. D., George, M. F., & Repo, T. (2001). Methods for Measuring Cold Hardiness of Conifers. In F. J. Bigras & S. J. Colombo (Éds.), *Conifer Cold Hardiness* (p. 369-401). Springer Netherlands. https://doi.org/10.1007/978-94-015-9650-3_14

Buttò, V., Khare, S., Drolet, G., Sylvain, J.-D., Gennaretti, F., Deslauriers, A., Morin, H., & Rossi, S. (2021). Regionwide temporal gradients of carbon allocation allow for shoot growth and latewood formation in boreal black spruce. *Global Ecology and Biogeography*, *30*(8), 1657-1670. https://doi.org/10.1111/geb.13340

Buttò, V., Khare, S., Jain, P., de Lima Santos, G., & Rossi, S. (2023). Spatial patterns and climatic drivers of leaf spring phenology of maple in eastern North America. *Science of The Total Environment*, *857*, 159064. https://doi.org/10.1016/j.scitotenv.2022.159064

Camargo Alvarez, H., Salazar-Gutiérrez, M., Zapata, D., Keller, M., & Hoogenboom, G. (2018). Time-to-event analysis to evaluate dormancy status of single-bud cuttings : An example for grapevines. *Plant Methods*, *14*(1), 94. https://doi.org/10.1186/s13007-018-0361-0

Campbell, R. K., & Sugano, A. I. (1979). Genecology of bud-burst phenology in Douglas-fir : Response to flushing temperature and chilling. *Botanical Gazette*, *140*(2), 223-231. https://doi.org/10.1086/337079

Cannell, M. G. R., & Sheppard, L. J. (1982). Seasonal Changes in the Frost Hardiness of Provenances of Picea sitchensis in Scotland. *Forestry: An International Journal of Forest Research*, *55*(2), 137-153. https://doi.org/10.1093/forestry/55.2.137

Cartenì, F., Balducci, L., Dupont, A., Salucci, E., Néron, V., Mazzoleni, S., & Deslauriers, A. (2023). PhenoCaB : A new phenological model based on carbon balance in boreal conifers. *New Phytologist*, *239*(2), 592-605. https://doi.org/10.1111/nph.18974

Carteron, A., Parasquive, V., Blanchard, F., Guilbeault-Mayers, X., Turner, B. L., Vellend, M., & Laliberté, E. (2020). Soil abiotic and biotic properties constrain the establishment of a dominant temperate tree into boreal forests. *Journal of Ecology*, *108*(3), 931-944. https://doi.org/10.1111/1365-2745.13326

Chamberlain, C. J., Cook, B. I., García de Cortázar-Atauri, I., & Wolkovich, E. M. (2019). Rethinking false spring risk. *Global Change Biology*, *25*(7), 2209-2220. https://doi.org/10.1111/gcb.14642

Chamberlain, C. J., Cook, B. I., Morales-Castilla, I., & Wolkovich, E. M. (2021). Climate change reshapes the drivers of false spring risk across European trees. *New Phytologist*, *229*(1), 323-334. https://doi.org/10.1111/nph.16851

Charrier, G. (2022). Is winter coming? Minor effect of the onset of chilling accumulation on the prediction of endodormancy release and budbreak. *Physiologia Plantarum*, *174*(3), e13699. https://doi.org/10.1111/ppl.13699

Charrier, G., & Améglio, T. (2011). The timing of leaf fall affects cold acclimation by interactions with air temperature through water and carbohydrate contents. *Environmental and Experimental Botany*, *72*(3), 351-357. https://doi.org/10.1016/j.envexpbot.2010.12.019

Charrier, G., Bonhomme, M., Lacointe, A., & Améglio, T. (2011). Are budburst dates, dormancy and cold acclimation in walnut trees (Juglans regia L.) under mainly genotypic or environmental control? *International Journal of Biometeorology*, *55*(6), 763-774. https://doi.org/10.1007/s00484-011-0470-1

Charrier, G., Chuine, I., Bonhomme, M., & Améglio, T. (2018). Assessing frost damages using dynamic models in walnut trees : Exposure rather than vulnerability controls frost risks: Frost risks in walnut trees. *Plant, Cell & Environment*, *41*(5), Article 5. https://doi.org/10.1111/pce.12935

Charrier, G., Martin-StPaul, N., Damesin, C., Delpierre, N., Hänninen, H., Torres-Ruiz, J. M., & Davi, H. (2021). Interaction of drought and frost in tree ecophysiology : Rethinking the timing of risks. *Annals of Forest Science*, *78*(2), Article 2. https://doi.org/10.1007/s13595-021-01052-5

Charrier, G., Ngao, J., Saudreau, M., & Améglio, T. (2015). Effects of environmental factors and management practices on microclimate, winter physiology, and frost resistance in trees. *Frontiers in Plant Science*, *6*. https://doi.org/10.3389/fpls.2015.00259

Charrier, G., Poirier, M., Bonhomme, M., Lacointe, A., & Améglio, T. (2013). Frost hardiness in walnut trees (Juglans regia L.) : How to link physiology and modelling? *Tree Physiology*, *33*(11), 1229-1241. https://doi.org/10.1093/treephys/tpt090

Chuine, I. (2010). Why does phenology drive species distribution? *Philosophical Transactions: Biological Sciences*, *365*(1555), 3149-3160.

Chuine, I., Bonhomme, M., Legave, J.-M., García de Cortázar-Atauri, I., Charrier, G., Lacointe, A., & Améglio, T. (2016). Can phenological models predict tree phenology accurately in the future? The unrevealed hurdle of endodormancy break. *Global Change Biology*, *22*(10), Article 10. https://doi.org/10.1111/gcb.13383

Cooke, J. E. K., Eriksson, M. E., & Junttila, O. (2012). The dynamic nature of bud dormancy in trees : Environmental control and molecular mechanisms. *Plant, Cell & Environment*, *35*(10), 1707-1728. https://doi.org/10.1111/j.1365-3040.2012.02552.x

Daly, C., Widrlechner, M. P., Halbleib, M. D., Smith, J. I., & Gibson, W. P. (2012). Development of a new USDA plant hardiness zone map for the United States.

Journal of Applied Meteorology and Climatology, *51*(2), 242-264. Scopus. https://doi.org/10.1175/2010JAMC2536.1

Dang, Q. L., Lieffers, V. J., & Rothwell, R. L. (1992). Effects of summer frosts and subsequent shade on foliage gas exchange in peatland tamarack and black spruce. *Canadian Journal of Forest Research*, *22*(7), 973-979. https://doi.org/10.1139/x92-130

Delpierre, N., Lireux, S., Hartig, F., Camarero, J. J., Cheaib, A., Čufar, K., Cuny, H., Deslauriers, A., Fonti, P., Gričar, J., Huang, J., Krause, C., Liu, G., de Luis, M., Mäkinen, H., del Castillo, E. M., Morin, H., Nöjd, P., Oberhuber, W., … Rathgeber, C. B. K. (2019). Chilling and forcing temperatures interact to predict the onset of wood formation in Northern Hemisphere conifers. *Global Change Biology*, *25*(3), Article 3. https://doi.org/10.1111/gcb.14539

Delpierre, N., Vitasse, Y., Chuine, I., Guillemot, J., Bazot, S., Rutishauser, T., & Rathgeber, C. B. K. (2016). Temperate and boreal forest tree phenology : From organ-scale processes to terrestrial ecosystem models. *Annals of Forest Science*, *73*(1), 5-25. https://doi.org/10.1007/s13595-015-0477-6

Des Roches, S., Post, D. M., Turley, N. E., Bailey, J. K., Hendry, A. P., Kinnison, M. T., Schweitzer, J. A., & Palkovacs, E. P. (2018). The ecological importance of intraspecific variation. *Nature Ecology & Evolution*, *2*(1), Article 1. https://doi.org/10.1038/s41559-017-0402-5

Deslauriers, A., Garcia, L., Charrier, G., Buttò, V., Pichette, A., & Paré, M. (2021). Cold acclimation and deacclimation in wild blueberry : Direct and indirect influence of environmental factors and non-structural carbohydrates. *Agricultural and Forest Meteorology*, *301-302*, 108349. https://doi.org/10.1016/j.agrformet.2021.108349

Dhont, C., Canada, & Natural Resources Canada. (2010). *Field guide for identifying apical bud break and bud formation stages in white spruce*. Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre.

Dowgert, M. F., & Steponkus, P. L. (1984). Behavior of the Plasma Membrane of Isolated Protoplasts during a Freeze-Thaw Cycle. *Plant Physiology*, *75*(4), 1139-1151.

Dumais, D., Raymond, P., & Champagne, E. (2024). Translocated southern seedlings perform as well as local provenances : Insights from an ecophysiological monitoring under varying cutting modalities. *New Forests*, *56*(1), 20. https://doi.org/10.1007/s11056-024-10089-z

Ebbert, D. (2019). *chisq.posthoc.test : A Post Hoc Analysis for Pearson's Chi-Squared Test for Count Data*. https://cran.r-project.org/web/packages/chisq.posthoc.test/index.html

Elzhov, T. V., Mullen, K. M., Spiess, A.-N., & Bolker, B. (2022). *minpack.lm : R Interface to the Levenberg-Marquardt Nonlinear Least-Squares Algorithm Found in MINPACK, Plus Support for Bounds*. https://CRAN.R-project.org/package=minpack.lm

Environment Canada. (2023). *Historical Climate Data*. https://climat.meteo.gc.ca/climate_data/daily_data_f.html?StationID=5889

Eskelin, N., Parker, W. C., Colombo, S. J., & Lu, P. (2011). *Assessing assisted migration as a climate change adaptation strategy for Ontario's forests : Project overview and bibliography* (p. 55). Ontario Forest Research Institute.

Fadón, E., Fernandez, E., Behn, H., & Luedeling, E. (2020). A Conceptual Framework for Winter Dormancy in Deciduous Trees. *Agronomy*, *10*(2), Article 2. https://doi.org/10.3390/agronomy10020241

Fagerland, M. W., & Hosmer, D. W. (2016). Tests for goodness of fit in ordinal logistic regression models. *Journal of Statistical Computation and Simulation*, *86*(17), 3398-3418. https://doi.org/10.1080/00949655.2016.1156682

Fick, S., & Hijmans, R. (2017). WorldClim 2 : New 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*, *37*. https://doi.org/10.1002/joc.5086

Fishman, S., Erez, A., & Couvillon, G. A. (1987). The temperature dependence of dormancy breaking in plants : Mathematical analysis of a two-step model involving a cooperative transition. *Journal of Theoretical Biology*, *124*(4), 473-483. https://doi.org/10.1016/S0022-5193(87)80221-7

Flynn, D. F. B., & Wolkovich, E. M. (2018). Temperature and photoperiod drive spring phenology across all species in a temperate forest community. *New Phytologist*, *219*(4), 1353-1362. https://doi.org/10.1111/nph.15232

Gilmore, E. C., & Rogers, J. S. (1958). Heat units as a method of measuring maturity in corn. *Agronomy Journal*, *50*(10), 611-615. https://doi.org/10.2134/agronj1958.00021962005000100014x

Girardin, M. P., Guo, X. J., Gervais, D., Metsaranta, J., Campbell, E. M., Arsenault, A., Isaac-Renton, M., & Hogg, E. H. (2022). Cold-season freeze frequency is a pervasive driver of subcontinental forest growth. *Proceedings of the National Academy of Sciences*, *119*(18), e2117464119. https://doi.org/10.1073/pnas.2117464119

Glerum, C. (1973). Annual trends in frost hardiness and electrical impedance for seven coniferous species. *Canadian Journal of Plant Science*, *53*(4), 881-889. https://doi.org/10.4141/cjps73-170

Glerum, C. (1985). *Frost hardiness of coniferous seedlings : Principles and applications*. Forest Research Laboratory. Oregon State University. https://scholar.google.com/scholar_lookup?title=Frost+hardiness+of+coniferous+seedlings%3A+principles+and+applications&author=Glerum%2C+C.&publication_year=1985

Godman, R. M., Yawney, H. W., Tubbs, C. H., Honkala, B. H., & Burns, R. M. (1990). Acer saccharum Marsh. Sugar Maple. In *Silvics of North America : 2. Hardwoods.* (Vol. 2, p. 194:215). USDA Forest Service. https://www.srs.fs.usda.gov/pubs/misc/ag_654/table_of_contents.htm

Gu, L., Hanson, P. J., Post, W. M., Kaiser, D. P., Yang, B., Nemani, R., Pallardy, S. G., & Meyers, T. (2008). The 2007 Eastern US spring freeze : Increased cold damage in a warming world? *BioScience*, *58*(3), 253-262. https://doi.org/10.1641/B580311

Guak, S., & Neilsen, D. (2013). Chill unit models for predicting dormancy completion of floral buds in apple and sweet cherry. *Horticulture, Environment, and Biotechnology*, *54*(1), 29-36. https://doi.org/10.1007/s13580-013-0140-9

Guo, X., Khare, S., Silvestro, R., Huang, J., Sylvain, J.-D., Delagrange, S., & Rossi, S. (2020a). Minimum spring temperatures at the provenance origin drive leaf phenology in sugar maple populations. *Tree Physiology*, tpaa096. https://doi.org/10.1093/treephys/tpaa096

Guo, X., Khare, S., Silvestro, R., Huang, J., Sylvain, J.-D., Delagrange, S., & Rossi, S. (2020b). Minimum spring temperatures at the provenance origin drive leaf phenology in sugar maple populations. *Tree Physiology*, *40*(12), 1639-1647. https://doi.org/10.1093/treephys/tpaa096

Guo, X., Klisz, M., Puchałka, R., Silvestro, R., Faubert, P., Belien, E., Huang, J., & Rossi, S. (2021). Common-garden experiment reveals clinal trends of bud phenology in black spruce populations from a latitudinal gradient in the boreal forest. *Journal of Ecology*, *110*(5), 1043-1053. https://doi.org/10.1111/1365-2745.13582

Hamilton, J. A., El Kayal, W., Hart, A. T., Runcie, D. E., Arango-Velez, A., & Cooke, J. E. K. (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. https://doi.org/10.1093/treephys/tpw061

Hänninen, H. (1990a). Modelling bud dormancy release in trees from cool and temperate regions. *Acta Forestalia Fennica*, *0*(213). https://doi.org/10.14214/aff.7660

Hänninen, H. (1990b). Modelling bud dormancy release in trees from cool and temperate regions. *Acta Forestalia Fennica*, *0*(213). https://www.silvafennica.fi/article/7660

Hänninen, H. (1997). Effects of Climatic Change on Overwintering of Forest Trees in Temperate and Boreal Zones. In G. M. J. Mohren, K. Kramer, & S. Sabaté (Éds.), *Impacts of Global Change on Tree Physiology and Forest Ecosystems : Proceedings of the International Conference on Impacts of Global Change on Tree Physiology and*

Forest Ecosystems, held 26–29 November 1996, Wageningen, The Netherlands (p. 149-158). Springer Netherlands. https://doi.org/10.1007/978-94-015-8949-9_20

Hänninen, H. (2006). Climate warming and the risk of frost damage to boreal forest trees : Identification of critical ecophysiological traits. *Tree Physiology*, *26*(7), 889-898. https://doi.org/10.1093/treephys/26.7.889

Hänninen, H. (2016). The Annual Phenological Cycle. In H. Hänninen (Éd.), *Boreal and Temperate Trees in a Changing Climate : Modelling the Ecophysiology of Seasonality* (p. 35-138). Springer Netherlands. https://doi.org/10.1007/978-94-017-7549-6_3

Hänninen, H., & Kramer, K. (2007). A framework for modelling the annual cycle of trees in boreal and temperate regions. *Silva Fennica*, *41*(1). https://doi.org/10.14214/sf.313

Hänninen, H., Kramer, K., Tanino, K., Zhang, R., Wu, J., & Fu, Y. H. (2019). Experiments Are Necessary in Process-Based Tree Phenology Modelling. *Trends in Plant Science*, *24*(3), 199-209. https://doi.org/10.1016/j.tplants.2018.11.006

Hänninen, H., & Tanino, K. (2011). Tree seasonality in a warming climate. *Trends in Plant Science*, *16*(8), Article 8. https://doi.org/10.1016/j.tplants.2011.05.001

Hawkins, B. J., Russell, J., & Shortt, R. (1994). Effect of population, environment, and maturation on the frost hardiness of yellow-cedar (Chamaecyparisnootkatensis). *Canadian Journal of Forest Research*, *24*(5), 945-953. https://doi.org/10.1139/x94-124

Heide, O. M. (1993). Dormancy release in beech buds (Fagus sylvatica) requires both chilling and long days. *Physiologia Plantarum*, *89*(1), 187-191. https://doi.org/10.1111/j.1399-3054.1993.tb01804.x

Hogg, E. H., Michaelian, M., Hook, T. I., & Undershultz, M. E. (2017). Recent climatic drying leads to age-independent growth reductions of white spruce stands in western Canada. *Global Change Biology*, *23*(12), 5297-5308. https://doi.org/10.1111/gcb.13795

Hufkens, K., Friedl, M. A., Keenan, T. F., Sonnentag, O., Bailey, A., O'Keefe, J., & Richardson, A. D. (2012a). Ecological impacts of a widespread frost event following early spring leaf-out. *Global Change Biology*, *18*(7), 2365-2377. Scopus. https://doi.org/10.1111/j.1365-2486.2012.02712.x

Hufkens, K., Friedl, M. A., Keenan, T. F., Sonnentag, O., Bailey, A., O'Keefe, J., & Richardson, A. D. (2012b). Ecological impacts of a widespread frost event following early spring leaf-out. *Global Change Biology*, *18*(7), 2365-2377. https://doi.org/10.1111/j.1365-2486.2012.02712.x

Inouye, D. w. (2000). The ecological and evolutionary significance of frost in the context of climate change. *Ecology Letters*, *3*(5), 457-463. https://doi.org/10.1046/j.1461-0248.2000.00165.x

IPCC. (2023). *Climate Change 2023 : Synthesis Report. Summary for Policimakers. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, P. Arias, M. Bustamante, I. Elgizouli, G. Flato, M. Howden, C. Méndez (Venezuela), J. Pereira, R. PichsMadruga, S. K. Rose, Y. Saheb, R. Sánchez, D. Ürge-Vorsatz, C. Xiao, N. Yassaa (eds.)].* IPCC. https://report.ipcc.ch/ar6syr/pdf/IPCC_AR6_SYR_SPM.pdf

Jay, M. (2019). *Package « generalhoslem »*. https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=&ved=2ahUKEwikjcDQqND2AhXOjYkEHdoDAW0QFnoECA8QAQ&url=https%3A%2F%2Fcran.r-project.org%2Fweb%2Fpackages%2Fgeneralhoslem%2Fgeneralhoslem.pdf&usg=AOvVaw1ftkaxwj9xjGV4ONKuXouw

Jentsch, A., Kreyling, J., & Beierkuhnlein, C. (2007). A new generation of climate-change experiments : Events, not trends. *Frontiers in Ecology and the Environment*, *5*(7), 365-374. https://doi.org/10.1890/1540-9295(2007)5[365:ANGOCE]2.0.CO;2

Junttila, O. (2007). Regulation of annual shoot growth cycle in northern tree species. In *Physiology of northern plants under changing environment* (p. 177-210). Research Signpost.

Kalberer, S. R., Wisniewski, M., & Arora, R. (2006). Deacclimation and reacclimation of cold-hardy plants : Current understanding and emerging concepts. *Plant Science*, *171*(1), 3-16. https://doi.org/10.1016/j.plantsci.2006.02.013

Kalma, J. D., Laughlin, G. P., Caprio, J. M., & Hamer, P. J. C. (1992). The Occurrence of Frost : Types, Distribution and Prediction. In J. D. Kalma, G. P. Laughlin, J. M. Caprio, & P. J. C. Hamer (Éds.), *The Bioclimatology of Frost : Its Occurrence, Impact and Protection* (p. 5-12). Springer. https://doi.org/10.1007/978-3-642-58132-8_2

Kassambara, A. (2023). *rstatix : Pipe-Friendly Framework for Basic Statistical Tests*. https://CRAN.R-project.org/package=rstatix

Kasuga, J., Arakawa, K., & Fujikawa, S. (2007). High accumulation of soluble sugars in deep supercooling Japanese white birch xylem parenchyma cells. *New Phytologist*, *174*(3), 569-579. https://doi.org/10.1111/j.1469-8137.2007.02025.x

Kollas, C., Körner, C., & Randin, C. F. (2014). Spring frost and growing season length co-control the cold range limits of broad-leaved trees. *Journal of Biogeography*, *41*(4), 773-783. https://doi.org/10.1111/jbi.12238

Körner, C., Basler, D., Hoch, G., Kollas, C., Lenz, A., Randin, C. F., Vitasse, Y., & Zimmermann, N. E. (2016). Where, why and how? Explaining the low-temperature range limits of temperate tree species. *Journal of Ecology*, *104*(4), 1076-1088. https://doi.org/10.1111/1365-2745.12574

Kovaleski, A. P. (2022). Woody species do not differ in dormancy progression : Differences in time to budbreak due to forcing and cold hardiness. *Proceedings of the National Academy of Sciences*, *119*(19), e2112250119. https://doi.org/10.1073/pnas.2112250119

Kovaleski, A. P. (2024). The potential for an increasing threat of unseasonal temperature cycles to dormant plants. *New Phytologist*, *244*(2), 377-383. https://doi.org/10.1111/nph.20052

Kovaleski, A. P., Reisch, B. I., & Londo, J. P. (2018). Deacclimation kinetics as a quantitative phenotype for delineating the dormancy transition and thermal efficiency for budbreak in Vitis species. *AoB PLANTS*, *10*(5), ply066. https://doi.org/10.1093/aobpla/ply066

Kreyling, J., Buhk, C., Backhaus, S., Hallinger, M., Huber, G., Huber, L., Jentsch, A., Konnert, M., Thiel, D., Wilmking, M., & Beierkuhnlein, C. (2014). Local adaptations to frost in marginal and central populations of the dominant forest tree Fagus sylvatica L. as affected by temperature and extreme drought in common garden experiments. *Ecology and Evolution*, *4*(5), 594-605. https://doi.org/10.1002/ece3.971

Kreyling, J., Schmid, S., & Aas, G. (2015). Cold tolerance of tree species is related to the climate of their native ranges. *Journal of Biogeography*, *42*(1), 156-166. https://doi.org/10.1111/jbi.12411

Kreyling, J., Wiesenberg, G. L. B., Thiel, D., Wohlfart, C., Huber, G., Walter, J., Jentsch, A., Konnert, M., & Beierkuhnlein, C. (2012). Cold hardiness of Pinus nigra Arnold as influenced by geographic origin, warming, and extreme summer drought. *Environmental and Experimental Botany*, *78*, 99-108. https://doi.org/10.1016/j.envexpbot.2011.12.026

Kriebel, H. B. (1957). *Patterns of Genetic Variation in Sugar Maple* (791). Ohio agricultural experiment station.

Kriebel, H. B., & Wang, C.-W. (1962). The interaction between provenance and degree of chilling in bud-break of sugar maple. *Silvae Genetica*, *11*.

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. https://doi.org/10.1051/forest:19940601

Lang, G. A., Early, J. D., Martin, G. C., & Darnell, R. L. (1987). Endo-, para-, and ecodormancy : Physiological terminology and classification for dormancy research. *HortScience*, *22*(3), 371-377.

Larcher, W., & Mair, B. (1968). Resistance to cold of Quercus pubescens, Ostrya carpinifolia and Fraxinus ornus in three sites with different temperature regimes. *Oecologia Plantarum*, *3*(4), 255-270.

Laube, J., Sparks, T. H., Estrella, N., Höfler, J., Ankerst, D. P., & Menzel, A. (2014). Chilling outweighs photoperiod in preventing precocious spring development. *Global Change Biology*, *20*(1), 170-182. https://doi.org/10.1111/gcb.12360

Leinonen, I. (1996). A Simulation Model for the Annual Frost Hardiness and Freeze Damage of Scots Pine. *Annals of Botany*, *78*(6), Article 6. https://doi.org/10.1006/anbo.1996.0178

Leites, L., & Benito Garzón, M. (2023). Forest tree species adaptation to climate across biomes : Building on the legacy of ecological genetics to anticipate responses to climate change. *Global Change Biology*, *29*(17), 4711-4730. https://doi.org/10.1111/gcb.16711

Lenz, A., Hoch, G., Körner, C., & Vitasse, Y. (2016). Convergence of leaf-out towards minimum risk of freezing damage in temperate trees. *Functional Ecology*, *30*(9), 1480-1490. https://doi.org/10.1111/1365-2435.12623

Lenz, A., Hoch, G., Vitasse, Y., & Körner, C. (2013). European deciduous trees exhibit similar safety margins against damage by spring freeze events along elevational gradients. *New Phytologist*, *200*(4), 1166-1175. https://doi.org/10.1111/nph.12452

Li, C., Viherä-Aarnio, A., Puhakainen, T., Junttila, O., Heino, P., & Tapio Palva, E. (2003). Ecotype-dependent control of growth, dormancy and freezing tolerance under seasonal changes in Betula pendula Roth. *Trees*, *17*(2), 127-132. https://doi.org/10.1007/s00468-002-0214-2

Lipsitz, S. R., Fitzmaurice, G. M., & Molenberghs, G. (1996). Goodness-of-fit tests for ordinal response regression models. *Journal of the Royal Statistical Society: Series C (Applied Statistics)*, *45*(2), 175-190. https://doi.org/10.2307/2986153

Liu, Q., Piao, S., Janssens, I. A., Fu, Y., Peng, S., Lian, X., Ciais, P., Myneni, R. B., Peñuelas, J., & Wang, T. (2018). Extension of the growing season increases vegetation exposure to frost. *Nature Communications*, *9*(1), Article 1. https://doi.org/10.1038/s41467-017-02690-y

Lloret, A., Badenes, M. L., & Ríos, G. (2018). Modulation of Dormancy and Growth Responses in Reproductive Buds of Temperate Trees. *Frontiers in Plant Science*, *9*. https://doi.org/10.3389/fpls.2018.01368

Luedeling, E., Caspersen, L., & Fernandez, E. (2023). *chillR: Statistical Methods for Phenology Analysis in Temperate Fruit Trees*. https://CRAN.R-project.org/package=chillR

Mahmood, K., Carew, J. G., Hadley, P., & Battey, N. H. (2000). Chill unit models for the sweet cherry cvs Stella, Sunburst and Summit. *The Journal of Horticultural Science and Biotechnology*, *75*(5), 602-606. https://doi.org/10.1080/14620316.2000.11511293

Malyshev, A. V., Henry, H. A. L., Bolte, A., Arfin Khan, M. A. S., & Kreyling, J. (2018). Temporal photoperiod sensitivity and forcing requirements for budburst in temperate tree seedlings. *Agricultural and Forest Meteorology*, *248*, 82-90. https://doi.org/10.1016/j.agrformet.2017.09.011

Marquis, B., Bergeron, Y., Houle, D., Leduc, M., & Rossi, S. (2022). Variability in frost occurrence under climate change and consequent risk of damage to trees of western Quebec, Canada. *Scientific Reports*, *12*(1), Article 1. https://doi.org/10.1038/s41598-022-11105-y

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*. https://www.frontiersin.org/article/10.3389/fpls.2020.01031

Mazerolle, M. J. (2023). *AICcmodavg : Model selection and multimodel inference based on (Q)AIC(c)*. https://cran.r-project.org/package=AICcmodavg

McCarragher, S. R., Goldblum, D., & Rigg, L. S. (2011). Geographic Variation of Germination, Growth, and Mortality in Sugar Maple (*Acer saccharum*) : Common Garden and Reciprocal Dispersal Experiments. *Physical Geography*, *32*(1), Article 1. https://doi.org/10.2747/0272-3646.32.1.1

McKenney, D. W., Pedlar, J. H., Lawrence, K., Campbell, K., & Hutchinson, M. F. (2007). Potential Impacts of Climate Change on the Distribution of North American Trees. *BioScience*, *57*(11), 939-948. https://doi.org/10.1641/B571106

Meier, U. (2001). *Growth stages of mono-and dicotyledonous plants* (BBCH Monograph, p. 158). Federal Biological Research Centre for Agriculture and Forestry.

Mohamed, H. B., Zrig, A., Geuns, J. M. C., & Khemira, H. (2014). Near-lethal heat treatment induced metabolic changes associated with endodormancy release of Superior Seedless grapevine cv. (Vitis vinifera L.) buds. *Australian Journal of Crop Science*, *8*(4), 486-494.

Montwé, D., Isaac-Renton, M., Hamann, A., & Spiecker, H. (2018). Cold adaptation recorded in tree rings highlights risks associated with climate change and assisted migration. *Nature Communications*, *9*(1), 1574. https://doi.org/10.1038/s41467-018-04039-5

Morin, X., Améglio, T., Ahas, R., Kurz-Besson, C., Lanta, V., Lebourgeois, F., Miglietta, F., & Chuine, I. (2007). Variation in cold hardiness and carbohydrate concentration from dormancy induction to bud burst among provenances of three European oak species. *Tree Physiology*, *27*(6), 817-825. https://doi.org/10.1093/treephys/27.6.817

Morin, X., Lechowicz, M. J., Augspurger, C., O'keefe, J., Viner, D., & Chuine, I. (2009). Leaf phenology in 22 North American tree species during the 21st century. *Global Change Biology*, *15*(4), Article 4. https://doi.org/10.1111/j.1365-2486.2008.01735.x

Morneau, C. (2021). *Classification écologique du territoire québécois*. Ministère des Forêts, de la Faune et des Parcs, Gouvernement du Québec. https://mffp.gouv.qc.ca/nos-publications/classification-ecologique-territoire-quebecois/

Muggeo, V. M. R. (2008). segmented : An R Package to Fit Regression Models with Broken-Line Relationships. *R News*, *8*(1), 20-25.

Mura, C., Charrier, G., Buttò, V., Delagrange, S., Surget-Groba, Y., Raymond, P., Rossi, S., & Deslauriers, A. (2024). *Local conditions have greater influence than provenance on sugar maple (Acer saccharum Marsh.) frost hardiness at its northern range limit.* [Submitted for publication].

Neuner, G., Ambach, D., & Buchner, O. (1999). Readiness to frost harden during the dehardening period measured in situ in leaves of Rhododendron ferrugineum L. at the alpine timberline. *Flora*, *194*(3), 289-296. https://doi.org/10.1016/S0367-2530(17)30916-7

Neuner, G., & Beikircher, B. (2010). Critically reduced frost resistance of Picea abies during sprouting could be linked to cytological changes. *Protoplasma*, *243*(1), 145-152. https://doi.org/10.1007/s00709-009-0052-9

Neuner, G., Monitzer, K., Kaplenig, D., & Ingruber, J. (2019). Frost survival mechanism of vegetative buds in temperate trees : Deep supercooling and extraorgan freezing vs. Ice tolerance. *Frontiers in Plant Science*, *10*. Scopus. https://doi.org/10.3389/fpls.2019.00537

North, M. G., & Kovaleski, A. P. (2024). Time to budbreak is not enough : Cold hardiness evaluation is necessary in dormancy and spring phenology studies. *Annals of Botany*, *133*(2), 217-224. https://doi.org/10.1093/aob/mcad182

North, M. G., Workmaster, B. A., & Atucha, A. (2022). Effects of chill unit accumulation and temperature on woody plant deacclimation kinetics. *Physiologia Plantarum*, *174*(3), e13717. https://doi.org/10.1111/ppl.13717

North, M. G., Workmaster, B. A., Atucha, A., & Kovaleski, A. P. (2024). Cold hardiness-informed budbreak reveals role of freezing temperatures and daily fluctuation in a chill accumulation model. *Journal of Experimental Botany*, *75*(19), 6182-6193. https://doi.org/10.1093/jxb/erae287

Nowosad, J. (2019). *pollen : Analysis of Aerobiological Data*. https://CRAN.R-project.org/package=pollen

Olsen, J. E. (2010). Light and temperature sensing and signaling in induction of bud dormancy in woody plants. *Plant Molecular Biology*, *73*(1-2), Article 1-2. https://doi.org/10.1007/s11103-010-9620-9

Palik, B. J., Clark, P. W., D’Amato, A. W., Swanston, C., & Nagel, L. (2022). Operationalizing forest-assisted migration in the context of climate change adaptation : Examples from the eastern USA. *Ecosphere*, *13*(10), e4260. https://doi.org/10.1002/ecs2.4260

Pantin, F., Simonneau, T., & Muller, B. (2012). Coming of leaf age : Control of growth by hydraulics and metabolics during leaf ontogeny. *New Phytologist*, *196*(2), 349-366. https://doi.org/10.1111/j.1469-8137.2012.04273.x

Park, A., Talbot, C., & Smith, R. (2018). Trees for tomorrow : An evaluation framework to assess potential candidates for assisted migration to Manitoba’s forests. *Climatic Change*, *148*(4), 591-606. https://doi.org/10.1007/s10584-018-2201-7

Peaucelle, M., Sabate Gil, C., Penuelas, J., Verbeeck, H., Gisler, J., & Vitasse, Y. (2022, juillet 20). *Uncertainties in estimating budburst heat requirement when using local or gridded temperature compared to bud tissue temperature* [Conference presentation]. Phenology 2022: Phenology at the crossroads, Avignon, France. https://pheno-2022.colloque.inrae.fr/

Pedlar, J. H., McKenney, D., Beaulieu, J., Colombo, S., McLachlan, J., & O’Neill, G. (2011). The implementation of assisted migrationin Canadian forests. *The Forestry Chronicle*, *87*(06), 766-777. https://doi.org/10.5558/tfc2011-093

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. https://doi.org/10.1525/bio.2012.62.9.10

Perkins, T. D., & van den Berg, A. K. (2009). Chapter 4 Maple Syrup—Production, Composition, Chemistry, and Sensory Characteristics. *Advances in Food and Nutrition Research*, *56*, 101-143. https://doi.org/10.1016/S1043-4526(08)00604-9

Perry, D. J., & Knowles, P. (1989). Allozyme variation in sugar maple at the northern limit of its range in Ontario, Canada. *Canadian Journal of Forest Research*, *19*(4), 509-514. https://doi.org/10.1139/x89-078

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*. https://doi.org/10.3389/fpls.2017.02214

Putnam, R. C., & Reich, P. B. (2017). Climate and competition affect growth and survival of transplanted sugar maple seedlings along a 1700-km gradient. *Ecological Monographs*, *87*(1), 130-157. https://doi.org/10.1002/ecm.1237

R Core Team. (2020). *R: A Language and Environment for Statistical Computing* [Logiciel]. R Foundation for Statistical Computing. https://www.R-project.org/

Rammig, A., Jönsson, A. M., Hickler, T., Smith, B., Bärring, L., & Sykes, M. T. (2010). Impacts of changing frost regimes on Swedish forests : Incorporating cold hardiness in a regional ecosystem model. *Ecological Modelling*, *221*(2), 303-313. https://doi.org/10.1016/j.ecolmodel.2009.05.014

Raulier, F., & Bernier, P. Y. (2000). Predicting the date of leaf emergence for sugar maple across its native range. *Canadian Journal of Forest Research*, *30*(9), 1429-1435. https://doi.org/10.1139/x00-064

Régnière, J., Saint-Amant, R., & Béchard, A. (2014). *BioSIM 10 user’s manual*. Natural Resources Canada.

Rehfeldt, G. E., Leites, L. P., Joyce, D. G., & Weiskittel, A. R. (2018). Role of population genetics in guiding ecological responses to climate. *Global Change Biology*, *24*(2), 858-868. https://doi.org/10.1111/gcb.13883

Ren, P., Liang, E., Raymond, P., & Rossi, S. (2020). Phenological differentiation in sugar maple populations and responses of bud break to an experimental warming. *Forests*, *11*(9), Article 9. https://doi.org/10.3390/f11090929

Repo, T., & Lappi, J. (1989). Estimation of standard error of impedance-estimated frost resistance. *Scandinavian Journal of Forest Research*, *4*(1-4), 67-74. https://doi.org/10.1080/02827588909382547

Repo, T., Nilsson, J.-E., Rikala, R., Ryyppö, A., & Sutinen, M.-L. (2001). Cold Hardiness of Scots Pine (Pinus sylvestris L.). In F. J. Bigras & S. J. Colombo (Éds.), *Conifer Cold*

Hardiness (p. 463-493). Springer Netherlands. https://doi.org/10.1007/978-94-015-9650-3_17

Richardson, E. A., Seeley, S., Walker, D., Seeley, S., & Walker, D. I. (1974). A model for estimating the completion of rest for « Redhaven » and « Elberta » peach trees. *Hortscience*. /paper/A-model-for-estimating-the-completion-of-rest-for-Richardson-Seeley/c8563ee343d1071e899c38166fdeb97f10cfa43c

Rigby, J. R., & Porporato, A. (2008). Spring frost risk in a changing climate. *Geophysical Research Letters*, *35*(12). https://doi.org/10.1029/2008GL033955

Rinne, P., Tuominen, H., & Junttila, O. (1994). Seasonal changes in bud dormancy in relation to bud morphology, water and starch content, and abscisic acid concentration in adult trees of Betula pubescens. *Tree Physiology*, *14*(6), 549-561. https://doi.org/10.1093/treephys/14.6.549

Risk, C., McKenney, D. W., Pedlar, J., & Lu, P. (2021). A compilation of North American tree provenance trials and relevant historical climate data for seven species. *Scientific Data*, *8*(1), 29. https://doi.org/10.1038/s41597-021-00820-2

Rohde, A., & Bhalerao, R. P. (2007). Plant dormancy in the perennial context. *Trends in Plant Science*, *12*(5), 217-223. https://doi.org/10.1016/j.tplants.2007.03.012

Rossi, S. (2015). Local adaptations and climate change : Converging sensitivity of bud break in black spruce provenances. *International Journal of Biometeorology*, *59*(7), 827-835. https://doi.org/10.1007/s00484-014-0900-y

Rossi, S., & Bousquet, J. (2014). The bud break process and its variation among local populations of boreal black spruce. *Frontiers in Plant Science*, *5:574*. https://doi.org/10.3389/fpls.2014.00574

Ruiz-Pérez, G., & Vico, G. (2020). Effects of Temperature and Water Availability on Northern European Boreal Forests. *Frontiers in Forests and Global Change*, *3*. https://doi.org/10.3389/ffgc.2020.00034

Sakai, A. (1983). Comparative study on freezing resistance of conifers with special reference to cold adaptation and its evolutive aspects. *Canadian Journal of Botany*, *61*(9), 2323-2332. https://doi.org/10.1139/b83-255

Sakai, A., & Larcher, W. (1987). *Frost Survival of Plants* (Vol. 62). Springer. https://doi.org/10.1007/978-3-642-71745-1

Sarvas, R. (1972). Investigations on the annual cycle of development of forest trees. Active period. *Communicationes Instituti Forestalis Fenniae*, *76*(3), 110.

Sarvas, R. (1974, février 22). *Investigations on the annual cycle of development of forest trees. II. Autumn dormancy and winter dormancy*. https://www.semanticscholar.org/paper/Investigations-on-the-annual-cycle-of-development-Sarvas/5ac837955e432105708d0b21dbdf3f12fbebd617

Screen, J. A., & Simmonds, I. (2013). Exploring links between Arctic amplification and mid-latitude weather. *Geophysical Research Letters*, *40*(5), 959-964. https://doi.org/10.1002/grl.50174

Sebastian-Azcona, J., Hamann, A., Hacke, U. G., & Rweyongeza, D. (2019). Survival, growth and cold hardiness tradeoffs in white spruce populations : Implications for assisted migration. *Forest Ecology and Management*, *433*, 544-552. https://doi.org/10.1016/j.foreco.2018.10.046

Silvestro, R., Mura, C., Alano Bonacini, D., de Lafontaine, G., Faubert, P., Mencuccini, M., & Rossi, S. (2023). Local adaptation shapes functional traits and resource allocation in black spruce. *Scientific Reports*, *13*(1), Article 1. https://doi.org/10.1038/s41598-023-48530-6

Silvestro, R., Rossi, S., Zhang, S., Froment, I., Huang, J. G., & Saracino, A. (2019). From phenology to forest management : Ecotypes selection can avoid early or late frosts, but not both. *Forest Ecology and Management*, *436*, 21-26. https://doi.org/10.1016/j.foreco.2019.01.005

Skinner, M., & Parker, B. L. (1994). *Field Guide for Monitoring Sugar Maple Bud Development*. University of Vermont, Burlington.

Snyder, R. L., Spano, D., Cesaraccio, C., & Duce, P. (1999). Determining degree-day thresholds from field observations. *International Journal of Biometeorology*, *42*(4), 177-182. https://doi.org/10.1007/s004840050102

Ste-Marie, C., A. Nelson, E., Dabros, A., & Bonneau, M.-E. (2011). Assisted migration : Introduction to a multifaceted concept. *The Forestry Chronicle*, *87*(06), Article 06. https://doi.org/10.5558/tfc2011-089

Steppe, K., Sterck, F., & Deslauriers, A. (2015). Diel growth dynamics in tree stems : Linking anatomy and ecophysiology. *Trends in Plant Science*, *20*(6), 335-343. https://doi.org/10.1016/j.tplants.2015.03.015

Tixier, A., Sperling, O., Orozco, J., Lampinen, B., Amico Roxas, A., Saa, S., Earles, J. M., & Zwieniecki, M. A. (2017). Spring bud growth depends on sugar delivery by xylem and water recirculation by phloem Münch flow in Juglans regia. *Planta*, *246*(3), 495-508. https://doi.org/10.1007/s00425-017-2707-7

Twardek, W. M., Taylor, J. J., Rytwinski, T., Aitken, S. N., MacDonald, A., Van Bogaert, R., & Cooke, S. J. (2023). The application of assisted migration as a climate change adaptation tactic : An evidence map and synthesis. *Biological Conservation*, *280*, 109932. https://doi.org/10.1016/j.biocon.2023.109932

Uemura, M., Tominaga, Y., Nakagawara, C., Shigematsu, S., Minami, A., & Kawamura, Y. (2006). Responses of the plasma membrane to low temperatures. *Physiologia Plantarum*, *126*(1), 81-89. https://doi.org/10.1111/j.1399-3054.2005.00594.x

Usmani, A., Silvestro, R., Zhang, S., Huang, J.-G., Saracino, A., & Rossi, S. (2020). Ecotypic differentiation of black spruce populations : Temperature triggers bud burst but not bud set. *Trees*, *34*(5), 1313-1321. https://doi.org/10.1007/s00468-020-01999-4

Venables, W. N., & Ripley, B. D. (2002). *Modern Applied Statistics with S* (4^e éd.). Springer-Verlag. https://doi.org/10.1007/978-0-387-21706-2

Villouta, C., Workmaster, B. A., Livingston, D. P., & Atucha, A. (2022). Acquisition of freezing tolerance in Vaccinium macrocarpon Ait. Is a multi-factor process involving the presence of an ice barrier at the bud base. *Frontiers in Plant Science*, *13:891488*. https://doi.org/10.3389/fpls.2022.891488

Vitasse, Y., Lenz, A., Hoch, G., & Körner, C. (2014). Earlier leaf-out rather than difference in freezing resistance puts juvenile trees at greater risk of damage than adult trees. *Journal of Ecology*, *102*(4), 981-988. https://doi.org/10.1111/1365-2745.12251

Vitasse, Y., Lenz, A., & Körner, C. (2014). The interaction between freezing tolerance and phenology in temperate deciduous trees. *Frontiers in Plant Science*, *5*. https://www.frontiersin.org/articles/10.3389/fpls.2014.00541

Vitasse, Y., & Rebetez, M. (2018). Unprecedented risk of spring frost damage in Switzerland and Germany in 2017. *Climatic Change*, *149*(2), 233-246. https://doi.org/10.1007/s10584-018-2234-y

Vitasse, Y., Schneider, L., Rixen, C., Christen, D., & Rebetez, M. (2018). Increase in the risk of exposure of forest and fruit trees to spring frosts at higher elevations in Switzerland over the last four decades. *Agricultural and Forest Meteorology*, *248*, 60-69. https://doi.org/10.1016/j.agrformet.2017.09.005

Vitra, A., Lenz, A., & Vitasse, Y. (2017). Frost hardening and dehardening potential in temperate trees from winter to budburst. *New Phytologist*, *216*(1), 113-123. https://doi.org/10.1111/nph.14698

Viveros-Viveros, H., Sáenz-Romero, C., Vargas-Hernández, J. J., López-Upton, J., Ramírez-Valverde, G., & Santacruz-Varela, A. (2009). Altitudinal genetic variation in Pinus hartwegii Lindl. I : Height growth, shoot phenology, and frost damage in seedlings. *Forest Ecology and Management*, *257*(3), 836-842. https://doi.org/10.1016/j.foreco.2008.10.021

Volaire, F., Barkaoui, K., Grémillet, D., Charrier, G., Dangles, O., Lamarque, L. J., Martin-StPaul, N., & Chuine, I. (2023). Is a seasonally reduced growth potential a convergent strategy to survive drought and frost in plants? *Annals of Botany*, *131*(2), 245-254. https://doi.org/10.1093/aob/mcac153

Wang, H., Wu, C., Ciais, P., Peñuelas, J., Dai, J., Fu, Y., & Ge, Q. (2020). Overestimation of the effect of climatic warming on spring phenology due to misrepresentation of chilling. *Nature Communications*, *11*(1), 4945. https://doi.org/10.1038/s41467-020-18743-8

Warrington, I. J., & Rook, D. A. (1980). Evaluation of techniques used in determining frost tolerance of forest planting stock : A review. *New Zealand Journal of Forestry Science*, *10*(1), 116-132.

Way, D. A. (2011). Tree phenology responses to warming : Spring forward, fall back? *Tree Physiology*, *31*(5), 469-471. https://doi.org/10.1093/treephys/tpr044

Way, D. A., & Montgomery, R. A. (2015). Photoperiod constraints on tree phenology, performance and migration in a warming world. *Plant, Cell & Environment*, *38*(9), 1725-1736. https://doi.org/10.1111/pce.12431

Weinberger, J. H. (1950). Chilling Requirements of Peach Varieties. *Proceedings of American Society for Horticultural Science*, *56*, 122-128.

Weinberger, J. H. (1967). *Some temperature relations in natural breaking of the rest of peach flower buds in the San Joaquin Valley, California. 91*, 84-89.

Weiser, C. J. (1970). Cold Resistance and Injury in Woody Plants. *Science*, *169*(3952), 1269-1278. https://doi.org/10.1126/science.169.3952.1269

Welling, A., & Palva, E. T. (2006). Molecular control of cold acclimation in trees. *Physiologia Plantarum*, *127*(2), 167-181. https://doi.org/10.1111/j.1399-3054.2006.00672.x

Westerband, A. C., Funk, J. L., & Barton, K. E. (2021). Intraspecific trait variation in plants : A renewed focus on its role in ecological processes. *Annals of Botany*, *127*(4), 397-410. https://doi.org/10.1093/aob/mcab011

Winder, R. S., Nelson, E., & Beardmore, T. (2011). Ecological implications for assisted migration in Canadian forests. *The Forestry Chronicle*, *87*(06), 731-744. https://doi.org/10.5558/tfc2011-090

Winkel, T., Lhomme, J. P., Laura, J. P. N., Alcón, C. M., del Castillo, C., & Rocheteau, A. (2009). Assessing the protective effect of vertically heterogeneous canopies against radiative frost : The case of quinoa on the Andean Altiplano. *Agricultural and Forest Meteorology*, *149*(10), 1759-1768. https://doi.org/10.1016/j.agrformet.2009.06.005

Wolfe, J., & Bryant, G. (2001). Cellular cryobiology : Thermodynamic and mechanical effects. *International Journal of Refrigeration*, *24*(5), 438-450. https://doi.org/10.1016/S0140-7007(00)00027-X

Worrall, J. (1983). Temperature–bud-burst relationships in amabilis and subalpine fir provenance tests replicated at different elevations. *Silvae Genet*, *32*(5/6), 203-209.

Wu, R., Tomes, S., Karunairetnam, S., Tustin, S. D., Hellens, R. P., Allan, A. C., Macknight, R. C., & Varkonyi-Gasic, E. (2017). SVP-like MADS Box Genes Control Dormancy and Budbreak in Apple. *Frontiers in Plant Science*, *8*. https://doi.org/10.3389/fpls.2017.00477

Xie, Z., Forney, C. F., & Bondada, B. (2018). Renewal of vascular connections between grapevine buds and canes during bud break. *Scientia Horticulturae*, *233*, 331-338. https://doi.org/10.1016/j.scienta.2018.02.019

Xin, Z., & Browse, J. (2000). Cold comfort farm : The acclimation of plants to freezing temperatures. *Plant, Cell & Environment*, *23*(9), 893-902. https://doi.org/10.1046/j.1365-3040.2000.00611.x

Young, A. G., Warwick, S. I., & Merriam, H. G. (1993). Genetic variation and structure at three spatial scales for Acersaccharum (sugar maple) in Canada and the implications for conservation. *Canadian Journal of Forest Research*, *23*(12), 2568-2578. https://doi.org/10.1139/x93-318

Zeltiņš, P., Gailis, A., & Zariņa, I. (2021). Long-term performance of Norway spruce in two provenance trials in Latvia. *BALTIC FORESTRY*, *27*(1), Article 1. https://doi.org/10.46490/BF195

Zeng, Q., Marquis, B., Delagrange, S., Zhou, P., Yuan, S., Sylvain, J.-D., Raymond, P., & Rossi, S. (2022). Late frost as a driver of ecotypic differentiation in sugar maple : Implications for assisted migration. *Agricultural and Forest Meteorology*, *323*, 109041. https://doi.org/10.1016/j.agrformet.2022.109041

Zohner, C. M., Mo, L., Renner, S. S., Svenning, J.-C., Vitasse, Y., Benito, B. M., Ordonez, A., Baumgarten, F., Bastin, J.-F., Sebald, V., Reich, P. B., Liang, J., Nabuurs, G.-J., de-Miguel, S., Alberti, G., Antón-Fernández, C., Balazy, R., Brändli, U.-B., Chen, H. Y. H., … Crowther, T. W. (2020). Late-spring frost risk between 1959 and 2017 decreased in North America but increased in Europe and Asia. *Proceedings of the National Academy of Sciences*, *117*(22), 12192-12200. https://doi.org/10.1073/pnas.1920816117