



Effet combiné de la défoliation et de la température sur l'ouverture des bourgeons et sur l'allocation sous-jacente du carbone chez l'épinette noire et le sapin baumier

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RÉSUMÉ

Dans un contexte d'épidémie de tordeuse des bourgeons de l'épinette et de changements climatiques, la compréhension de la physiologie du débourrement des conifères revêt une grande importance afin de mieux prédire les impacts des changements climatiques et adapter les pratiques d'aménagement forestier en forêt boréale. Actuellement, les modèles de prévision de la date de débourrement se basent uniquement sur les facteurs climatiques. Cependant d'autres facteurs influencent la phénologie des arbres. Les caractéristiques adaptatives génétiques des espèces, des facteurs abiotiques tel que la température, et des facteurs biotiques comme la défoliation par les insectes affectent le débourrement des bourgeons végétatifs au printemps. Ces caractéristiques et ces facteurs influent aussi la physiologie et modifient l'allocation du carbone. La fluctuation du carbone non structural (CNS) dans différents organes de l'arbre, affecte ainsi le débourrement des bourgeons végétatifs et module le moment et la vitesse de leur ouverture. Cette étude vise à évaluer l'impact de l'augmentation de température de 2 °C en combinée à la défoliation sur le débourrement chez deux espèces de conifères (épinette noire et sapin baumier) représentées par les provenances nord et sud. Les hypothèses sont : i) le réchauffement et la défoliation devraient anticiper les phases d'ouverture des bourgeons avec un effet plus prononcé dans les provenances nordiques; ii) la répartition du carbone devrait être modifiée par le réchauffement et la défoliation, avec une diminution des réserves d'amidon et de la consommation de sucre survenant plus tôt dans les arbres lors du débourrement.

Cette étude de deux ans a été réalisée dans un simulateur de réchauffement climatique nommé T-FACE où 24 épinettes noires et 24 sapins baumiers de deux provenances différentes ont été soumis à des combinaisons de température (réchauffement de 2 °C et température ambiante) et de défoliation naturelle (défoliés et non défolié) par la tordeuse des bourgeons de l'épinette. La défoliation a été induite sur la moitié des arbres (24 arbres sur 48) en implantant des larves de tordeuse des bourgeons de l'épinette (stade L2). Les larves de cet insecte se nourrissent principalement pendant le débourrement des bourgeons des essences hôtes. En 2018 et 2019, les différentes phases d'ouverture des bourgeons ont été surveillées sur 48 arbres à l'étude au printemps. Des analyses des concentrations de CNS (sucres solubles et amidon) ont également été effectuées au cours du débourrement. La combinaison de la provenance, de la température et de la défoliation donne un large éventail de dates d'ouverture, mais le groupe d'arbres appartenant au groupe défolié – chauffé – nord ont débourré hâtivement par rapport au groupe non-défolié – ambiant – sud, confirmant notre première hypothèse.

Cette phénologie hâtive des bourgeons a été associée à une diminution de l'amidon mais pas à une diminution des sucres solubles, confirmant ainsi partiellement notre seconde hypothèse. Une température plus élevée est souvent associée à un taux métabolique plus élevé, consommant davantage de carbone, réduisant ainsi la formation de réserves d'amidon. Comme l'accumulation d'amidon dépend également de la photosynthèse actuelle, le bassin de carbone printanier est fortement réduit par la défoliation, ce qui peut influencer sur la croissance et la survie des arbres. Plusieurs facteurs affectent donc la période du débourrement des bourgeons, puis le niveau de synchronisation avec la tordeuse des bourgeons de l'épinette.

TABLE DES MATIÈRES

RÉSUMÉ	ii
LISTE DES FIGURES	vi
LISTE DES SIGLES	vii
REMERCIEMENTS.....	viii
CHAPITRE 1	1
INTRODUCTION GÉNÉRALE	1
1.1 Cycle annuel de la phénologie des bourgeons et influence du climat	3
1.2 Synchronisme entre l'ouverture des bourgeons chez le SAB, l'EPN et la TBE	6
1.3 Physiologie de l'ouverture des bourgeons.....	7
CHAPITRE 2	12
Combined effect of defoliation and temperature on the timing of budburst and underlying carbon allocation in black spruce and balsam fir.....	12
2.1 Authors	12
2.2 Abstract	12
2.3 Introduction	14
2.4 Material and methods	18
2.4.1 EXPERIMENTAL DESIGN.....	18
2.4.2 PHENOLOGY AND GROWTH MEASUREMENTS	20
2.4.3 ANALYSIS OF NON-STRUCTURAL CARBOHYDRATES	21
2.4.4 STATISTICAL ANALYSIS	23
2.5 Results	25
2.5.1 BUD PHENOLOGY	25
2.5.2 SUGAR AND STARCH ALLOCATION DURING BUDBURST	29
2.6 Discussion	35
2.6.1 WARMING AND DEFOLIATION BOTH ADVANCE BUD PHENOLOGY	36
2.6.2 TEMPERATURE AND DEFOLIATION DECREASE STARCH ACCUMULATION DURING BUDBURST BUT NOT SOLUBLE SUGARS	38
2.7 Conclusion.....	40
2.8 Acknowledgement.....	41
CHAPITRE 3	42
CONCLUSION GÉNÉRALE.....	42
3.1 La phénologie.....	42
3.2 Allocation du carbone	43
3.3 L'approche statistique	43
3.4 Les perspectives	44

3.5 Conclusion.....	46
RÉFÉRENCES	47

LISTE DES FIGURES

Figure 1.1 Contrôle de la phénologie chez les arbres par la photopériode et la température.	5
Figure 2.1 Temperature graph and photo of an experimental plot.	19
Figure 2.2 Box plot distribution representing the Day of the Year at which there is 50 % of probability of transitions for each budburst phase varying from 1 to 6.	26
Figure 2.3 Left panel: Factor Analysis for Mixed Data (FAMD analysis) illustrating the correlation between the different factors including year, provenance, heating, and defoliation.	27
Figure 2.4 Explained variance of different factors across different budburst phase in BS and BF found with Permanova analysis.	29
Figure 2.5 Box plot distribution representing the soluble sugar and starch concentration for each budburst phase varying from 1 to 6.	31
Figure 2.6 Left panel: Factor Analysis for Mixed Data (FAMD analysis) illustrating the correlation between the different factors including part, provenance, heating, and defoliation.	32
Figure 2.7 Explained variance of different factors in NSC concentration in BS and BF found with Permanova analysis.	34

LISTE DES SIGLES

BF : Balsam fir
BS : Black spruce
CNS : Carbone non structural
EPN : Épinette noire
NSC : Non structural carbohydrate
SAB : Sapin baumier
SBW : Spruce budworm
TBE : Tordeuse des bourgeons de l'épinette

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CHAPITRE 1

INTRODUCTION GÉNÉRALE

Dans un contexte d'épidémie de tordeuse des bourgeons de l'épinette (TBE, *Choristoneura fumiferana*) et de changements climatiques, la compréhension de la physiologie du débourrement des conifères revêt une grande importance pour mieux prédire l'impact des changements climatiques (CC) sur les relations hôtes-insectes dans la forêt boréale. Actuellement, les modèles de prévision de la date de débourrement se basent sur la température (Hannerz 1999; Peaucelle *et al.* 2018; Svystun *et al.* 2021) ou sur celle-ci combinée à la photopériode (Hunter et Lechowicz 1992; Chuine et Régnière 2017; Peaucelle *et al.* 2018). Cependant d'autres facteurs influencent la phénologie du débourrement des arbres comme l'âge des arbres (Partanen *et al.* 2005), les variations génétiques (Søgaard *et al.* 2008; Yakovlev *et al.* 2008; Rossi et Isabel 2017; Butnor *et al.* 2019), le couvert de neige (Marumo *et al.* 2020), les nutriments (Lumme et Smolander 1996; De Barba *et al.* 2016; Marty *et al.* 2020) et la défoliation (Ren *et al.* 2020). Récemment, Deslauriers *et al.* (2019) ont proposé qu'une réponse induite par l'allocation du carbone dans l'arbre devancerait le débourrement des arbres défoliés. Inclure les effets de l'allocation du carbone sur le débourrement pourrait ainsi augmenter l'efficacité des modèles actuels de lutte contre la TBE qui ne tiennent pas compte du facteur défoliation et se basent uniquement sur le climat pour prédire l'ouverture des bourgeons. Cependant, l'étude de Deslauriers *et al.* (2019) a été réalisée en milieu contrôlé et doit être validée sur le terrain. Ce projet d'étude porte donc sur l'influence d'une élévation de température de 2 °C, d'une défoliation par la TBE et de

l'origine géographique des espèces sur le débourrement du sapin baumier (SAB, *Abies balsamea* (L.) Mill.) et de l'épinette noire (EPN, *Picea mariana* Mill.) afin d'observer leurs effets respectifs et combinés sur l'allocation du carbone et la phénologie des bourgeons. L'augmentation de température est fixée à 2 °C pour représenter la limite maximale de l'accord de Paris sur le climat visant à limiter l'augmentation de la température moyenne mondiale (Rogelj *et al.* 2016; Intergovernmental Panel on Climate Change. 2022). Les résultats de cette étude, couplés aux données climatiques et aux différents traitements des arbres, pourront contribuer à améliorer les modèles de prédictions du débourrement des bourgeons des conifères et servir dans le cadre de la lutte contre la TBE.

En forêt boréale, les conifères sont soumis à des températures froides (≤ 5 °C) pendant les mois d'hiver. Pour survivre à ce froid prolongé, les arbres suspendent leur croissance et protègent les méristèmes primaires de leurs tiges et aiguilles dans des bourgeons dormants (Badeck *et al.* 2004; Delpierre *et al.* 2016; Singh *et al.* 2017). Les bourgeons végétatifs suivent ainsi un cycle de dormance en fonction des saisons et de divers facteurs (Lang *et al.* 1987; Singh *et al.* 2017). Le bourgeon végétatif est un axe très court qui porte une série très dense de primordia foliaires (Vitasse 2009). Par sa morphologie et sa physiologie, le bourgeon est donc la structure qui permet la survie des arbres face aux températures froides et la croissance du nouveau feuillage l'année suivant sa formation (Vitasse 2009; Strimbeck *et al.* 2015). De plus, la formation des bourgeons est sous contrôle génétique modéré et varie entre les populations témoignant ainsi de la présence d'adaptation locale au sein de l'espèce.

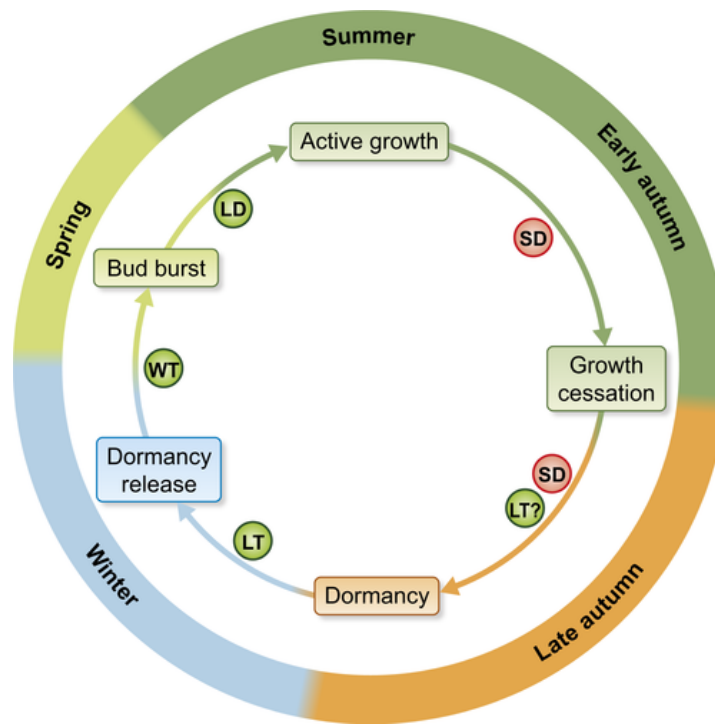
1.1 CYCLE ANNUEL DE LA PHENOLOGIE DES BOURGEONS ET INFLUENCE DU CLIMAT

De sa formation jusqu'au débourrement, le bourgeon évolue selon différents stades de dormance (Lang *et al.* 1987; Dhont *et al.* 2010). Le bourgeon végétatif se forme pendant l'arrêt de croissance de la tige pendant l'été. Chez les conifères, comme l'EPN, la formation des bourgeons végétatifs débute entre la mi-juin et la mi-juillet pour se terminer vers la fin septembre (Dhont *et al.* 2010). Une fois formé, le bourgeon entre en paradormance (dormancy, figure 1.1). Des facteurs tels que la photopériode et la baisse de la température pour déclenchent et maintiennent la paradormance et leur importance varie selon l'espèce (Delpierre *et al.* 2016; Singh *et al.* 2017) (Figure 1.1). Pendant cette phase, des tissus, autres que celui de l'organe en latence, maintiennent la dormance (dormancy, figure 1.1). Par exemple, les bourgeons axillaires sont maintenus en paradormance par le bourgeon apical (Lang *et al.* 1987). Lorsque le bourgeon est exposé aux températures froides (LT, low temperature) et aux jours courts (SD, short day) de la fin de l'automne (late autumn), il entre dans la phase d'endodormance (Singh *et al.* 2017) (Figure 1.1). À ce stade, la croissance demeure généralement impossible même si les conditions redeviennent favorables, car des facteurs endogènes propres aux tissus dormants maintiennent le bourgeon en état de dormance (Lang *et al.* 1987; Singh *et al.* 2017). Les méristèmes apicaux des tiges et les primordia des feuilles deviennent insensibles aux promoteurs de croissance (Singh *et al.* 2017). Pour mettre fin à l'endodormance, le bourgeon doit être exposé au froid (LT) pendant une certaine période minimale (Figure 1.1). Le besoin d'accumulation d'unités de froid, appelé « chilling » en anglais, est spécifique à chaque espèce. Les unités de «chilling» se compte en heure ou en jours où la température ambiante ne dépasse pas un seuil limite selon un modèle de prédiction du débourrement de l'espèce (Cannell et Smith 1983; Man *et al.*

2017). Les arbres boréaux sous exposés au froid présentent des anomalies lors du débourrement et de la croissance (Man *et al.* 2017; Man *et al.* 2021). Une fois que les besoins en unités de froid sont comblés, les activités dans les méristèmes peuvent reprendre (Guzicka 2001; Guzicka *et al.* 2018). Cependant le bourgeon est maintenu dormant par des facteurs externes : c'est l'écodormance ou la quiescence. L'écodormance est une période latente qui cesse lorsque les éléments favorables à la croissance (température, conditions hydriques, etc.) sont réunis. Pendant cette latence les arbres boréaux nécessitent d'accumuler des unités de chaud (forcing units), typiquement calculées à partir d'une température journalière au-dessus de 5 °C, afin d'initier le retour d'activités dans les méristèmes et sortir de la dormance (Figure 1.1, dormancy release) (Delpierre *et al.* 2019b; Baumgarten *et al.* 2021; Vitasse *et al.* 2021). Le besoin d'unités de chaud (forcing units) pour les arbres dont les besoins en froids (chilling) sont comblés varie en fonction de la quantité d'unités de froid accumulées (chilling units) (Harrington et Gould 2015). Un compromis se fait entre les besoins en froid et en chaleur où les arbres exposés à une plus longue période de froid, nécessiteront d'accumuler moins de d'unités de chaud (Baumgarten *et al.* 2021). L'écodormance est suivie par le débourrement (Figure 1.1, bud burst) qui correspond à la période où le bourgeon s'ouvre pour former des rameaux et de nouvelles aiguilles.

Dans la forêt boréale, la température est le principal facteur qui influence la date de débourrement (Antonucci *et al.* 2015; Rossi et Isabel 2017b). Les températures froides (LT, low temperature) du début de l'hiver maintiennent les bourgeons dormants alors que les températures un peu plus chaudes (WT, warm temperature) de la fin de l'hiver mettent fin à la dormance et initient le débourrement des bourgeons (Figure 1.1). Plus tôt la température se réchauffe, plus tôt les bourgeons s'ouvriront (Menzel *et al.* 2006; Schwartz *et al.* 2006;

Bertin 2008) en autant que l'accumulation de température froide (chilling) pendant l'hiver soit atteinte (Man *et al.* 2017). Pour la plupart des modèles de dormance des arbres boréaux, la limite entre les températures chaudes et froides est fixée à 5 °C (Cannell et Smith 1983; Delpierre *et al.* 2019a; Vitasse *et al.* 2021) où les températures sont considérées chaudes lorsqu'elles dépassent cette valeur.



Reproduit avec autorisation de Singh *et al.* 2017

Figure 1.1 Contrôle de la phénologie chez les arbres par la photopériode et la température. SD : jours courts, LD : jours longs, WT : température chaude et LT : température froide.

Généralement chez les conifères, la période d'accumulation d'heures de température froide (chilling units) pour sortir de l'endodormance se termine plus tôt que chez les espèces feuillues (Man *et al.* 2017). Si les besoins en froid hivernal ne sont pas comblés, des retards

de débourrement, des variations dans la population d'arbres et des anomalies dans le développement des bourgeons se produiront (Harrington et Gould 2015; Man *et al.* 2017). D'autres facteurs climatiques telles la fonte des neiges et les précipitations peuvent aussi influencer la phénologie du débourrement (Bertin 2008).

1.2 SYNCHRONISME ENTRE L'OUVERTURE DES BOURGEONS CHEZ LE SAB, L'EPN ET LA TBE

Pendant le stade du débourrement, l'arbre est vulnérable aux gels printaniers tardifs et aux insectes herbivores qui exercent des pressions sélectives. La période du débourrement déterminera d'une part, la croissance l'arbre (indirectement le fitness), et d'autre part, sa la fenêtre d'opportunité pour ses insectes prédateurs (Johnson *et al.* 2010) puisqu'ils ont coévolué depuis des milliers d'années (e.g. Lumley *et al.* 2020). Le début du débourrement est avant tout déterminé par les traits de vie et le patrimoine génétique d'une espèce. Par exemple, le SAB débourre deux semaines plus tôt que l'EPN (Antonucci *et al.* 2015; Rossi et Isabel 2017b; Deslauriers *et al.* 2019). De plus, l'origine géographique des arbres d'une espèce donnée se traduisant en adaptation locale influencera aussi la date de débourrement. Ainsi, dans un jardin commun situé à une latitude intermédiaire, les provenances plus nordiques débourreront plus rapidement que celles du sud (Rossi 2015; Silvestro *et al.* 2019). Les variations génétiques interspécifiques (SAB vs EPN) et la provenance des arbres ont donc un effet important, car tous deux influencent le degré de synchronisation avec les insectes herbivores tel que la TBE (Fuentelba *et al.* 2017; Deslauriers *et al.* 2019).

Aux conditions climatiques actuelles du Québec, le développement des larves de la TBE est synchronisé avec le débourrement des bourgeons chez le SAB (Pureswaran *et al.* 2015). L'EPN débouurant deux semaines plus tard, cette espèce est plutôt asynchrone avec

les stades larvaires de la TBE (Pureswaran *et al.* 2015; Pureswaran *et al.* 2016; Fuentealba *et al.* 2017). Elle subit donc généralement moins de dommages comparativement au SAB (Pureswaran *et al.* 2015). En résumé, la variation génétique intra- et interspécifique, les conditions météorologiques hivernales et printanières (Rossi et Bousquet 2014, Delpierre *et al.* 2016; Rossi et Isabel 2017a; Singh *et al.* 2017) et le degré de la défoliation (Deslauriers *et al.* 2019) influencent le synchronisme entre la TBE et les différentes espèces de conifères hôtes.

Pour l'aménagement forestier en forêt boréale, les patrons de débourrement des bourgeons des différentes essences forestières revêtent une importance particulière. C'est à ce stade que les plans de luttés intégrées contre les insectes sont mis en place (i.e. évaluation de la phénologie pour l'arrosage aérien). Des connaissances plus approfondies sur l'influence de la température et de la défoliation par rapport au débourrement des principaux conifères hôtes de la TBE ainsi que sur le développement larvaire permettront de mieux gérer les plans d'intervention et de réduire les dépenses associées. Une meilleure connaissance des mécanismes physiologiques de l'ouverture des bourgeons permettra aussi de comprendre l'impact des changements climatiques sur la forêt pour en améliorer sa gestion.

1.3 PHYSIOLOGIE DE L'OUVERTURE DES BOURGEONS

Différentes recherches contribuent à comprendre les mécanismes d'entrée en dormance et du débourrement chez les arbres (Badeck *et al.* 2004; Singh *et al.* 2017). Durant l'ouverture des bourgeons, plusieurs mécanismes physiologiques s'activent et ces derniers font intervenir, entre autres, de nombreuses hormones, des sucres solubles et différents

métabolites (Dhuli *et al.* 2014; Guzicka *et al.* 2018). Pris séparément, ces facteurs n'expliquent pas à eux seuls la physiologie du débourrement, ce qui la rend difficile à prévoir.

Plusieurs études récentes ont démontré qu'en plus des hormones telle que l'auxine, les sucres solubles avaient une très grande influence sur le moment et la vitesse d'ouverture des bourgeons (Barbier *et al.* 2015a; Barbier *et al.* 2015b). En effet, ces études ont mis en évidence que les bourgeons de roses dont la concentration en saccharose était plus élevée avaient commencé à se développer trois jours avant ceux qui poussaient à des concentrations plus faibles du même sucre. Chez les conifères tels le SAB et l'EPN, une étude de Deslauriers *et al.* (2019) a permis d'identifier l'allocation des sucres non-structuraux comme une explication physiologique potentielle du mécanisme d'ouverture des bourgeons au printemps. Dans cette étude, réalisée en serre, les concentrations de sucres et d'amidon évoluent différemment entre les arbres témoins et défoliés une fois que les conditions du printemps favorables à la croissance sont réunies. Les arbres témoins accumulent de l'amidon de réserve dans les aiguilles et les branches jusqu'au début du débourrement alors que les arbres défoliés augmentent leur concentration de sucre au détriment de la formation d'amidon. Il en résulte un débourrement hâtif chez ces derniers.

L'allocation du carbone représente la disponibilité des glucides non-structuraux (sucres et amidon) pour la croissance, pour le métabolisme ou d'autres fonctions (reproduction, formation des composés de défense). La quantité des sucres non-structuraux dans les bourgeons varie en fonction des saisons. À l'automne, les arbres de la forêt boréale concentrent une grande quantité de sucres dans les bourgeons (Lipavská *et al.* 2000). Une concentration élevée en sucres tels que le saccharose, le glucose, le fructose et le raffinose, combinée à une diminution de la quantité d'eau, protègent les tissus du gel (Guy 2003; Morin

et al. 2007). Le saccharose et le raffinose agissent comme antigel en empêchant la formation de cristaux de glace susceptibles d'endommager les tissus vivants (Strimbeck *et al.* 2015). Avec la venue des températures croissantes de la fin de l'hiver et de l'arrivée du printemps, cette protection contre le froid diminue et les arbres se désacclimatent au froid. À ce moment, les bourgeons ne sont plus protégés du gel et les sucres stockés pour la protection au froid sont remobilisés. Les sucres remobilisés au printemps ont été peu étudiés, mais Delpierre *et al.* (2019) suggèrent qu'une partie des sucres utilisés pour la protection contre le gel pourrait être retransformée en amidon et entreposée dans les cellules de parenchyme des branches, des racines et des aiguilles, alors qu'une autre partie serait utilisée pour débiter la croissance des méristèmes primaires et secondaires. Dans les aiguilles, la concentration d'amidon augmente lors du débourrement et du haut taux de photosynthèse, alors que la concentration de sucres diminue (Egger *et al.* 1996). La transformation des sucres en amidon dans les tissus des conifères peut débiter dès janvier dans la forêt boréale de l'est du Canada et marque la fin de l'endodormance (Guzicka 2001; Guzicka *et al.* 2018). La reprise de l'activité photosynthétique au printemps contribue à l'augmentation des concentrations en amidon. Ces réserves, en plus des sucres produits par les aiguilles des années précédentes, seront disponibles pour le débourrement, les croissances primaire et secondaire ainsi que la formation des futurs bourgeons.

Lors du débourrement des bourgeons, plusieurs facteurs influencent les niveaux de sucres solubles et d'amidon dans les différents organes des arbres boréaux. Le taux d'accumulation de l'amidon dans les bourgeons est affecté par la température. Les fluctuations de température durant l'hiver et au printemps affectent particulièrement le taux d'amidon qui diminue lors d'un épisode de temps froid peu importe la température moyenne

(Guzicka 2001). Les concentrations de sucres et d'amidon varient aussi en fonction des espèces. Dans les aiguilles et les branches, les concentrations de sucres et d'amidon sont plus élevées chez le SAB que chez l'EPN (Deslauriers *et al.* 2019) correspondant à un écart entre les dates de débourrement des deux espèces. Les arbres défoliés produisent et entreposent moins de sucres pendant l'été (Dietze *et al.* 2014). Au printemps venu, les arbres défoliés contiennent moins d'amidon en réserve et davantage de sucres solubles dans leurs tissus (Deslauriers *et al.* 2019) et présentent un débourrement plus hâtif. La provenance des arbres influence aussi les concentrations de sucres solubles. En effet, dans un même lieu, les individus provenant de populations plus nordiques accumuleraient plus de sucres dans les bourgeons pour être en mesure d'assurer une survie aux conditions de températures plus froides et plus longues, ce qui accélèreraient le débourrement au printemps suivant (Delpierre *et al.* 2019). Dans l'étude de Deslauriers *et al.* (2019) les arbres de la provenance nordique des EPNs et des SABs débourrent plus hâtivement, cependant les concentrations de sucres observées au débourrement n'étaient pas significativement plus élevées. La présence élevée en sucres solubles au printemps correspondant à des débournements hâtifs laisse supposer que l'allocation des sucres expliquerait le moment du débourrement (Deslauriers *et al.* 2019). La combinaison des données climatiques associées à l'allocation des sucres pourrait améliorer la prévision du débourrement des conifères.

Ce projet d'étude porte donc sur l'influence d'une élévation de température de 2 °C, d'une défoliation par la TBE et de la provenance des espèces sur le débourrement du SAB et de l'EPN afin d'observer leurs effets sur l'allocation des sucres et la phénologie des bourgeons. Les résultats de cette étude, couplés aux données climatiques et aux différents

traitements des arbres, pourront contribuer à améliorer les modèles de prédictions du débourrement des bourgeons des conifères et servir dans le cadre de la lutte contre la TBE.

CHAPITRE 2

COMBINED EFFECT OF DEFOLIATION AND TEMPERATURE ON THE TIMING OF BUDBURST AND UNDERLYING CARBON ALLOCATION IN BLACK SPRUCE AND BALSAM FIR

2.1 AUTHORS

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2.2 ABSTRACT

Accelerated climate change already influences forest tree phenology and will likely affect tree-pest interactions. In addition, the timing of budburst, an adaptive trait conditioned by genotype and environmental factors such as temperature, is also influenced by insect defoliation. The timing of budburst is under a small to moderate genetic control while showing a high degree of intraspecific genetic variation. This, in combination with exogenous factors, such as temperature and defoliation, cause the fluctuation of non-structural carbohydrates (NSCs) in different organ of the tree, thereby affecting the timing of budburst.

This study aims at assessing the impacts of warming and defoliation in combination with species-specific genetic variation on the process of bud opening. We evaluated bud

opening in response to a 2 °C warming combined with natural defoliation acting as a change in sink activity to quantify the contribution of NSCs to this process. Warming and defoliation should anticipate the phases of bud opening with a more pronounced effect in northern tree provenances. Carbon allocation should be modified by warming and defoliation with a decrease in starch and sugar concentration.

This two years study was realized in a Temperature-Free-Air Controlled Enhancement system (T-FACE) where 24 black spruce (BS) and 24 balsam fir (BF) saplings from two different provenances were subjected to combinations of temperature (2 °C warming and ambient temperature) and defoliation (defoliated and undefoliated) by the spruce budworm. In 2018 and 2019, the phases of bud opening were monitored on 48 study trees during spring. Defoliation was induced on half of the trees (24 trees of 48) by implanting spruce budworm instars. The larvae of this insect feed mainly during the budburst period of the hosts. Analyses of NSCs (soluble sugars and starch) were also carried out during the process of bud opening. The combination of provenance, temperature and defoliation gives a wide range of opening dates but the group of trees belonging to the defoliated – heated – Northern provenance had earlier phases of bud opening compared with the non-defoliated – ambient and Southern provenance, confirming our first hypothesis. This earlier bud phenology group was associated with a decrease in starch but not with a decrease in soluble sugars, thus partially confirming our second hypothesis. Higher temperature is often associated with higher metabolic rate, consuming carbon, thereby reducing the formation of starch reserves. Because starch accumulation also depends on current photosynthesis, the spring pool is highly reduced with defoliation, which can influence growth and tree survival. Several

factors thus influence the timing of bud opening and then, the level of synchronization with spruce budworm.

2.3 INTRODUCTION

The timing of budburst in boreal conifers is a key adaptive trait that influences the phenological window of susceptibility to herbivory insects. The spruce budworm (SBW, *Choristoneura fumiferana* Clemens), a lepidopteran devastating coniferous tree in eastern North America, has several host species but the degree of defoliation varies depending on the synchronization between the timing of budburst and the early larval instars (Fuentelba *et al.* 2018; Ren *et al.* 2020; Bellemin-Noël *et al.* 2021). Locally, the timing of budburst in balsam fir (*Abies balsamea*, (L.) Mill) is generally synchronized with the development of SBW larvae although large variation is still observed (Pureswaran *et al.* 2015). Within stand, black spruce (*Picea mariana* (Mill.) B.S.P.) generally breakout two weeks later than BF (Antonucci *et al.* 2015), making this species rather out of sync with the critical larvae stages (Pureswaran *et al.* 2015; Pureswaran *et al.* 2016; Fuentelba *et al.* 2017). As a result, BS generally harbours less damage from SBW compared to BF (Pureswaran *et al.* 2015). Depending on the host and prevailing environmental conditions, the degree of host-insect synchronism may decrease or increase with earlier budburst (Ren *et al.* 2020; Bellemin-Noël *et al.* 2021).

The bud meristems are the source of signal perception for dormancy release (Considine *et al.* 2016) responding, not only to temperature and photoperiod but also to endogenous factors, such as phytohormones and soluble sugars of tree physiology and standing genetic variation (Cooke *et al.* 2012). First, winter and spring environmental

conditions are important in determining the date of budburst (Delpierre *et al.* 2016; Rossi et Isabel 2017a; Singh *et al.* 2017). Indeed, for the boreal forest, temperature is the main factor influencing budburst date (Antonucci *et al.* 2015; Rossi et Isabel 2017b). Cold winter temperatures keep buds dormant while the slightly warmer temperatures at the end of the season stimulate dormancy release and initiate budburst (Singh *et al.* 2017). The earlier the spring temperatures warm, the earlier the buds will open (Menzel *et al.* 2006; Schwartz *et al.* 2006; Bertin 2008) provided that the cold temperature accumulation (chilling) during the winter is reached (Man *et al.* 2017). Other climatic factors such as snow cover and precipitation can also influence the phenology of budburst (Bertin 2008). Heavy snow cover particularly delays small tree budburst (Marumo *et al.* 2020).

Since BS has a predetermined growth (Dufour et Morin 2010; Usmani *et al.* 2020), defoliation from previous years also affects current year bud phenology (Quiring and McKinnon, 1999). Studies on the effect of defoliation report variable effects on tree budburst: with the most frequent response being an earlier phenology (Clancy *et al.* 1993; Quiring et McKinnon 1999; Carroll et Quiring 2003) compared to a delay (Quiring et McKinnon 1999). More recently, Deslauriers (2019) and Ren (2020) found that defoliation has the effect of anticipating the period of budburst in the following spring both in BS and BF. In Deslauriers *et al.* (2019) greenhouse study, after one defoliation season by the SBW, bud opening was advanced by 6-7 days for BF and BS compared to control trees. An earlier phenology was also observed in mature fir and spruce trees, but with a magnitude of 2-3 days (Carteni *et al.* 2022).

Intraspecific genetic variability present within and among conifer tree populations (Rossi et Bousquet 2014; Perrin *et al.* 2017) influences the timing of bud opening that is under a small to moderate genetic control (Li *et al.* 1997; Pelgas *et al.* 2011). Indeed, a counter gradient is observed in BS for the timing of budburst meaning that, at the same location (e.g., common garden experiments), bud opening in northern provenances is faster than southern provenances (Rossi 2015; Silvestro *et al.* 2019). Northern provenances developed under colder conditions and reactivated early to maximize the growing season (Rossi et Bousquet 2014). When the temperatures are favorable enough for reactivation in the north, it is already late spring and the photoperiod is longer, which reduces the risk of frost and damage to the plants (Khare *et al.* 2019). The timing of budburst s together with the timing of bud set are phenological traits that determine the duration of the growing season and therefore species' sensitivity to local climate (Perrin *et al.* 2017).

The allocation of non-structural carbohydrates (NSCs) is affected by many abiotic factors such as temperature and water deficit (Rehseh *et al.* 2022). Biotic factors, such as defoliation (Fierravanti *et al.* 2019) in combination with species-specific abiotic life history traits as the dormancy cycle generated by the boreal climate (Bansal et Germino 2009), all affect NSC allocation patterns. Starch accumulation begins at the end of winter with increasing concentration until the beginning of bud opening while NSCs concentration is at its highest level during budburst (Hoch *et al.* 2003; Schadel *et al.* 2009; Schoonmaker *et al.* 2021). In conifers, carbohydrate reserve plays a fundamental role as starch concentration throughout the tree increases prior to the resumption of shoot and stem growth (Little 1970; Hoch *et al.* 2003), with the highest quantities of starch found in the foliage (Deslauriers *et al.*

2019; Fierravanti *et al.* 2019). Defoliated trees produce less and accumulate less non-structural carbon during the growing season (Dietze *et al.* 2014; Fierravanti *et al.* 2019; Barker Plotkin *et al.* 2021). Starch allocation to storage decreases proportionally with increasing defoliation, indicating a gradually lower C-storage in defoliated trees. Starch concentrations, more than soluble sugar concentrations, mainly indicate the state of carbon in defoliated trees (Vanderklein et Reich 1999; Li *et al.* 2002; Deslauriers *et al.* 2015; Puri *et al.* 2015). Warming also affects the concentration of starch in the wood where the reserves decrease at a higher temperature, resulting in a lower density of the wood (Balducci *et al.* 2015). Warmer temperature was also found to decrease the hexose pool in the cambium and xyleme of BS (Way et Sage 2008; Deslauriers *et al.* 2014).

This study aims at assessing the impact of temperature increase in combination with defoliation on the timing of budburst in two conifers species (black spruce and balsam fir) represented by northern and southern provenances. Our hypotheses are: i) warming and defoliation should anticipate the phases of bud opening with a more pronounced effect in northern provenances; ii) carbon allocation should be modified by warming and defoliation with a decrease in starch reserves and sugar consumption occurring earlier in plants whose bud opening is anticipated.

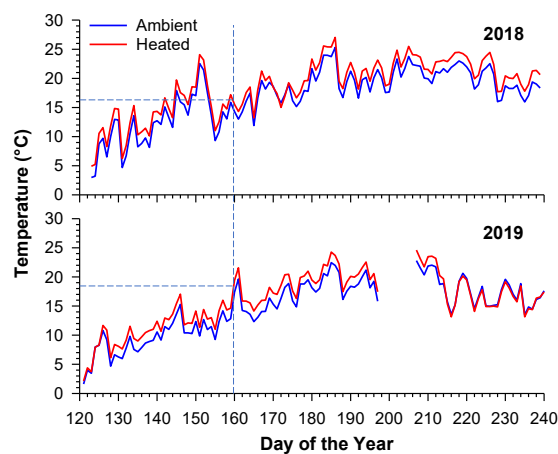
2.4 MATERIAL AND METHODS

2.4.1 EXPERIMENTAL DESIGN

The experimental design was located at the Valcartier Forest Research Station (46°56'59.8"N 71°29'54.3"W) of Natural Resources Canada (Québec, Canada). The average annual temperature at this location is 3.4 °C and the average annual precipitation is 1250 mm (Ressources Naturelles Canada 2019). Snow cover lasts generally from September to May and reaches a maximum depth of up to ~136 cm (Ressources Naturelles Canada 2019).

Our study was conducted as part of a large experimental design established in 2016 in a common garden experiment. Since 2016, twenty-four plots were equipped with a triangle structure like a metal frame surrounding planted trees to sustain six 1000-Watt infrared ceramic radiators at 1.2 m from the ground for heating treatment (Figure 2.1), here called T-FACE (Temperature-Free-Air Controlled Enhancement system) (Kimball *et al.* 2008; Chang *et al.* 2015; Fréchette *et al.* 2020; Bellemin-Noël *et al.* 2021). Twelve T-FACE experimental plots were heated to increase the ambient temperature by ~2 °C from the end of April until the beginning of September each year, and other twelve T-FACE plots were at ambient temperature but equally equipped with non-heating radiators to replicate any disturbance to the tree canopy. The temperature in each heated T-FACE was monitored by a sensor (APOGEE SI-11, Campbell Scientific Corporation, Logan, UT, USA) and compared to the control temperature of T-FACE plots. All data were registered every 30 seconds in Campbell CR1000 (Figure 2.1) (Campbell Scientific Corporation, Logan, UT, USA). In 2016, three years old balsam fir (*Abies balsamea*) and black spruce (*Picea mariana*) saplings were planted into the T-FACE (~40 cm and ~37 cm in height, respectively (Bellemin-Noël *et al.*

2021). Black Spruce originates from two remote ecological regions: Region 39 for southern provenance (Tree code is EPN-Q39-SUD; EPN for black spruce / Q39 for region 39 / SUD for South) and region 35 for northern provenance (Tree code is EPN-Q35-North; EPN for black spruce / Q35 for region 35 / NORD for north) (Saucier *et al.* 2011). The balsam firs come from two ecological regions juxtaposed: region 71 for southern provenance (tree code is SAB-A71-SUD; SAB for balsam fir, A71 for region 71 and SUD for southern provenance) and region 72 for northern provenance (tree code is SAB-A72 North; SAB for balsam fir, A72 for ecological region 72, and NORD for northern provenance) (Saucier *et al.* 2011). The saplings were provided by two different MFFP (Ministère des Forêts, de la Faune et des Parcs du Québec) tree nurseries (Sainte-Luce and Saint-Modeste). In each T-FACE plot, two rows of seedlings surrounded the experimental trees to avoid edge effects.



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Figure 2.1 Temperature trend and photo of an experimental plot. Left panel: Mean daily temperature variation from May to August (expressed in DOY) in Ambient and Heated treatments in 2018 and 2019. Right panel: T-FACE plot disposition installed at the Valcartier Forest Research Station (Natural Resources Canada). The white textile net covered each tree to protect the surrounding environment from spruce budworm (SBW) escape.

Our experimental design consisted of 6 plots, divided into two main groups: ambient treatment, considered as control temperature treatment ($n = 3$), and heating treatment, simulating an increase of 2 °C compared to the control temperature ($n = 3$). In each plot, 8 trees (2 individuals/provenance, 2 provenances/species, 2 species) out of the 32 trees were randomly selected for this study. Therefore, our total number of trees consisted of 48 individuals (2 trees \times 2 provenances \times 2 species \times 6 plots).

Two defoliation treatments were applied: 1) with SBW, named *defoliated*. Defoliation was naturally induced on half of the trees (24 trees of 48) by implanting eastern SBW instars at L2 stage (Deslauriers *et al.* 2019), and 2) without SBW, named *undefoliated*. Larvae came from the Insect Production Services (SPI) laboratory at the Great Lakes Forestry Centre [code standard Glfc:IPQL:Cfum01 to Cfum16, (Roe *et al.* 2018)]. L2 instars were introduced in May 2018 (DOY 144). These instars were randomly deposited on the buds and on the last whorl by group of 10 to 12 larvae, for a total quantity of 100 to 120 larvae per tree. A white fine tulle net (Proteknet 60 grams, mesh size of 1.9 \times 0.95 mm, lets light pass at 93 %) individually covered each tree to protect the surrounding environment (Figure 2.1). In May 2019 (DOY 149), additional larvae L2 (5 groups of 20 larvae) were placed to assure a good effect for defoliation treatment.

2.4.2 PHENOLOGY AND GROWTH MEASUREMENTS

The timing of budburst was monitored on 48 study trees in 2018 and 2019. In 2018, budburst phases were observed once a week from DOY 144 to DOY 205. In 2019, the frequency of bud observations was increased to 3 times per week, from DOY 128 to DOY 168, to increase the accuracy of measurement. Observations of the phases of the apical bud

and terminal bud of two lateral branches of each tree were performed according to Dhont *et al.* (2010) and Rossi et Isabel (2017a) by using a scale from 0 to 6, where phase 0 represents a closed bud; phase 1; an open bud; phase 2, an elongated bud; phase 3, a swollen bud; phase 4, a translucent bud; phase 5, a split bud; and phase 6, exposed shoot.

In 2018 and 2019, the percentage of defoliation was also visually assessed on the apical branch and two lateral branches according to the visual method of Piene *et al.* (1981) and MacLean *et* Lidstone (1982). A visual method classifies the level of defoliation (class 0-7): class 0 for 0-10 % defoliation; class 1 for 11 to 20 % defoliation; class 2 for 21 to 40 % defoliation, class 3 for 41 to 60 % defoliation; class 4 for 61 to 70 % defoliation; class 5 for 81 to 99 % defoliation; class 6 for 100 % defoliation and class 7 for complete destruction of bud and twig.

2.4.3 ANALYSIS OF NON-STRUCTURAL CARBOHYDRATES

Analyses of non-structural carbohydrate were carried out at different dates starting from previous autumn (November 29, 2018), at the end of the cold season (April 27, 2019) and then once a week during the process of bud opening (May 15, May 22, May 29, June 5, and June 12, 2019). At each sampling date, a complete lateral branch section (approximately 25 cm) covering three years of growth was cut from each tree and frozen (-20 °C) to slow enzymatic activity until processing. In the laboratory, buds, twigs, and needles were separated, immersed in liquid nitrogen to stop metabolic and freeze-dried. Afterward, samples were freeze dried for five days then reduced in powder using a ball mill (RETSCH Mixer Mill MM 400).

Soluble sugar concentration was measured using the phenol-sulfuric acid technique with a phenol concentration of 2 % (Chow et Landhüsser 2004). For needle and branch samples (old and current-year tissues blended) 20 mg of powder was weight. Soluble sugar was extract three times with 4 ml of 8:2 ethanol / water solution while starch was analysed from the remaining pellet after sugar extraction according to Bellasio *et al.* (2014). The water used in manipulations and solutions has been distilled and deionized and is referred to in this section as water.

For the analysis, the sugar extractions were chemically treated with phenol (2 %) and sulphuric acid (96 %) to color and quantify the sugars concentration. A UV-VIS spectrophotometer set at 490 nm was used to read the absorbance of each sample to quantify the sugar concentration. A dosage curve allowed to convert de concentration in mg per g of dry weight (mg g⁻¹ dw).

The starch analysis consists in breaking the starch chains with two enzyme, α -amylase from *Bacillus licheniformis* (Megazyme - 3000 U / ml) and amyloglucosydase from *Aspergillus niger* (Megazyme - 3260 U / mL) (Bellasio *et al.* 2014). Before starch analysis, all the remaining pellets were dried for one hour at 65 °C. The first digestion was performed with 3 ml of a buffered α -amylase solution (composed of 850 ml of water, 5.8 ml of glacial acetic acid, 50 ml of 1 M NaOH and 0.74 g of dehydrated CaCl₂) stirred and incubated in a bath at 90-100 °C for 12 minutes. After the mixtures were allowed to cool for 2 minutes, 0.15 ml of amyloglucosidase (3260 U / ml), was added and the samples were incubated for 45 minutes at 50 °C to break down oligosaccharides and dextrans into glucose. To adjust the volume of the tubes to 10 ml, 6.85 ml of water is added, and tubes are centrifuged for 6

minutes (3000 RPM). The solution was diluted at different ratio according to sugar concentration (ratio of 9:1 water / solute for low sugar concentration solute or 19:1 water / solute for high sugar concentration solute). Then, 2 ml of reagent solution (100 ml of water, 1 capsule of PGO enzyme preparation (Sigma #P7119) and 1.6 ml of ortho-dianisidine solution (2.5 mg / ml)) was added in each tube before remaining in dark for 45 minutes (Bellasio *et al.* 2014). After rest, 400 μ L of 75 % H₂SO₄ was added to stop the reaction. Tubes are immediately remained in the dark for 20 minutes before absorbance reading at 530 nm on a UV-VIS spectrophotometer. Starch concentrations obtained were expressed in milligrams per gram of dry weight ($\text{mg}\cdot\text{g}^{-1}\text{dw}$).

2.4.4 STATISTICAL ANALYSIS

Phenological measurements of lateral buds (i.e., the different phases from 0 to 6) were analysed as qualitative variables. Following this equation, ordered probit regressions was used to predict the probability of observing a certain phenological phase (Deslauriers *et al.* 2019):

$$P(E_i, x) = \frac{1}{1 + \exp(x \cdot b_{DOY} + b_{phase})}$$

where P represents the probability of observing a given phenological phase (E_i , from 1 to 6) at day x , expressed in Day of the Year (DOY). b_{DOY} and b_{phase} are the variables for Day Of the Year (DOY) and bud phase that represent the values of the parameters of the ordinal regression models. The relationship between the probabilities of the response variable [$P(E_i, x)$] and the linear predictors (DOY) was determined using with the `polr` function of the R's MASS package (Venables et Ripley 2002). This regression was fit for all sampled trees. We then used the ordered probit regression to determine the DOY at which there is 50 % of probability of transitions for each E_i phases.

To describe the effect of treatments (year, provenance, heating, and defoliation) on species-specific phenology, a FAMD analysis (Factor Analysis for Mixed Data) was performed with the package `FactorMiner` (Lê *et al.* 2008). Then, to explore the correlation between all variables, their contributions have been exported to illustrate their explanatory dimensions, with the functions `fviz_contrib` and `fviz_famd_var` of the `factorextra` package (Kassambara et Mundt 2020). To disentangle the variance explained by the different factors during the budburst process, a permutation multivariate Analysis of Variance (PERMANOVA) was performed with the function `adonis2` of the package `vegan` (Simpson *et al.* 2020). This method used euclidian distance as distance method and with 10000 permutations.

2.5 RESULTS

2.5.1 BUD PHENOLOGY

The mean of 50 % of probability of transitions for each phase according to the time (DOY) was illustrated for each treatment, species, and year (Figure 2.2). In general, the phases representing bud opening had a linear trend over time. The timing of budburst was earlier in year 2019 than in 2018 while being earlier in BF compared to BS. In most of the treatment combinations, defoliated and heated trees budburst earlier (Figure 2.2). To unravel key correlations between time and treatments on budburst process, we explained the main contributions of variables to each principal dimension using Factor Analysis for Mixed Data (Figure 2.3). Dimension 1 explained 28 % and 26 % in BS and BF, respectively, while dimension 2 to 4 explained about 20 % of the variance in the 50 % occurrence of phenology phases for both species. The variables related to bud phenology, such as year, provenance, and heating, contribute significantly to dimension 3, with similar contributions in both species. However, the degree of contribution estimated for each variable varies across the dimensions 1, 2 and 4 according to the species.

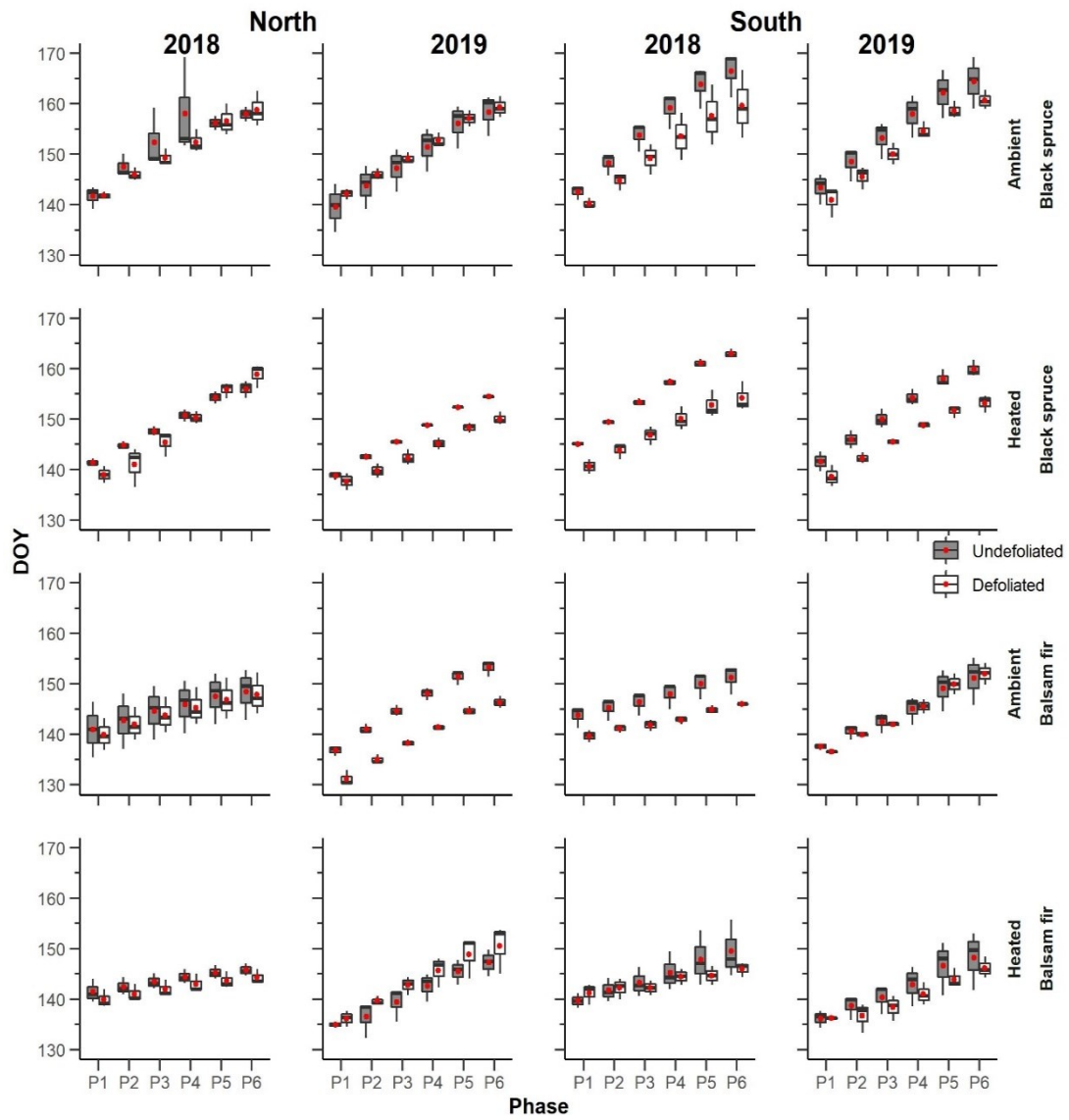


Figure 2.2 Box plot distribution representing the Day of the Year (DOY) at which there is 50 % of probability of transitions for each budburst phase varying from 1 to 6. Results were illustrated for each year, provenance, heating, and defoliation treatments in BS (upper panel) and BF (lower panel).

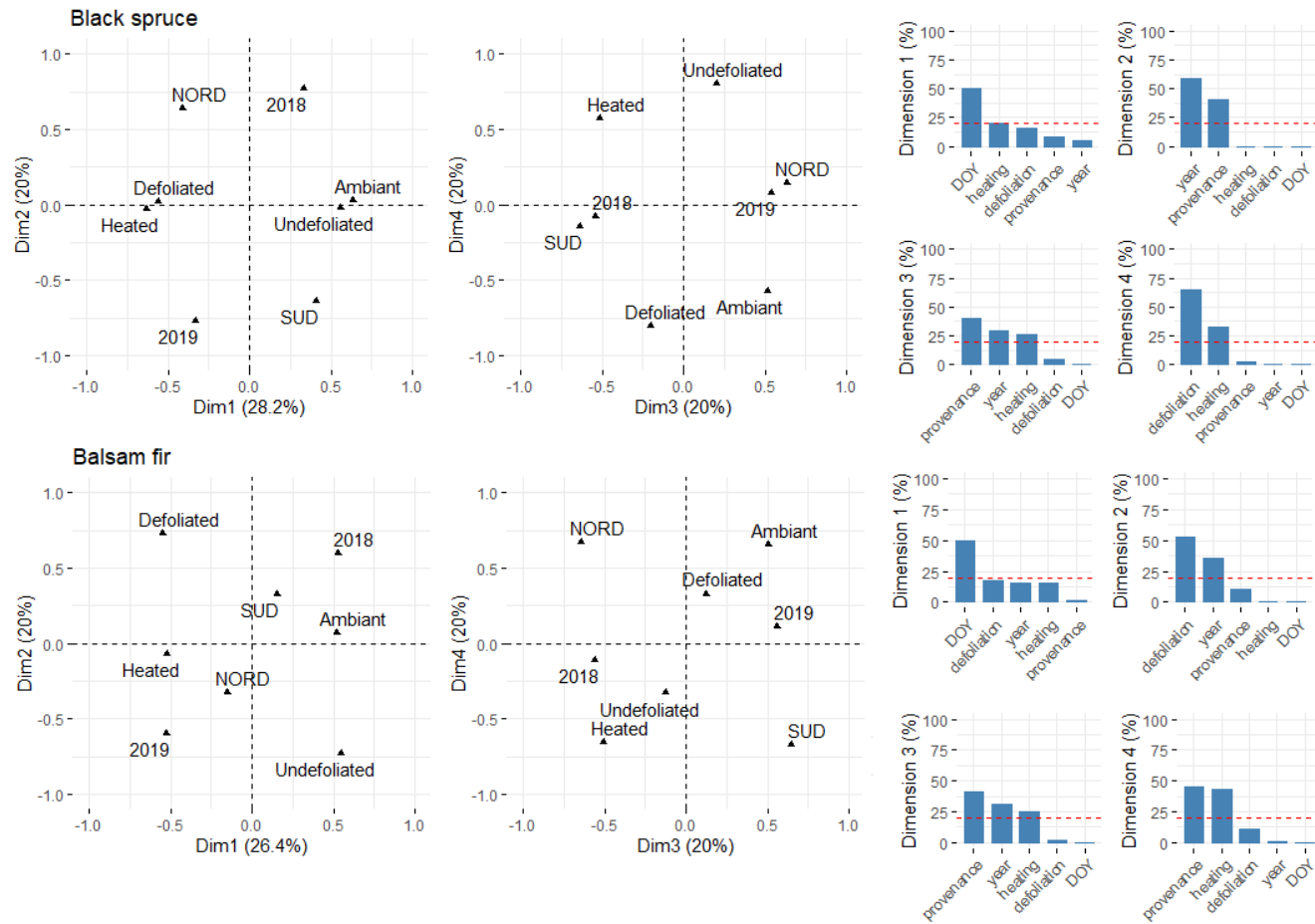


Figure 2.3 Factor Analysis for Mixed Data (FAMD analysis) illustrating the correlation between the different factors including year, provenance, heating, and defoliation. Since DOY, representing a quantitative variable of the progressive phases of phenology, only the contribution of qualitative variables was represented for both species. Right panel: Contribution (in percentage %) of the variables to each of the represent dimension. The red dashed line indicates the expected average value if the contributions were uniform.

In both species, DOY (representing the different phases of phenology) explained ~50 % of variation in dimension 1. A tendency was observed in the combination of « defoliated-heated-north-in 2019 » in the negative part of dimension 1 whereas, « ambient-undefoliated-south-in 2018 » cluster together in the positive dimension 1. In BS, we observed a tendency to cluster nearly heated and defoliated variable on the negative part of the dimension 1, while ambient and undefoliated variable clustered nearly and positively on the dimension 1 (Figure 2.3). In BF, the provenance contributes poorly with dimension 1 and no variables were close correlated.

In BS, year and provenance mostly contributed to the second dimension with year explaining about ~55 % and provenance ~37 %. For BF, year also explains dimension 2 (~30 %) but defoliation was the variable that contribute the most with ~50 %. For dimension 3, both species and provenance variables contribute similarly (~40 %), as year and heating (~25 %). Dimension 4 was mainly represented by defoliation (~63 %) and heating (~30 %) for BS while provenance (~45 %) and heating (~45 %) was the most contributed variable for BF.

Considering species-specific phenology response, PERMANOVA highlighted the biological effects of manipulated factors for each budburst phase (Figure 2.4). In BS, year, provenance, heating, and defoliation, showed significant effect on phases of bud opening, except for provenance in phases 5-6 and year in phase 1 (Figure 2.4). The variance explained by heating and defoliation increased according to the transition of the first budburst phases, although it was more important in BS than BF. Provenance had an opposite effect according to bud development phases between both tree species. In BF, the provenance has a low R^2 of < 0.1 , implying a negligible effect). However, the effect of year was more important during

the three first phases of budburst (corresponding with no visible needles) compared to the following phases. Then the variance explained by defoliation was significant in phase 2 and 5, while the variance explained by heating was significant in phase 2, 5, and 6.

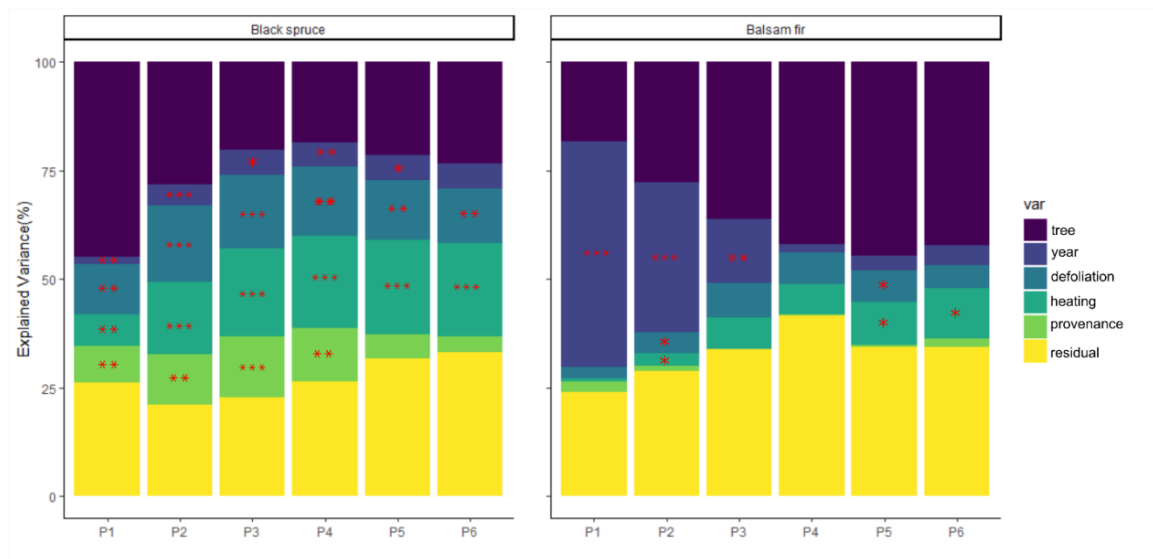


Figure 2.4 Explained variance of different factors across different budburst phase in BS (left) and BF (right) found with Permanova analysis. Level of significance: * <0.05, **<0.01 ***0.001

2.5.2 SUGAR AND STARCH ALLOCATION DURING BUDBURST

The sugar and starch concentration during bud development in 2019 was illustrated for each treatment and species (Figure 2.5). NSC concentrations during bud opening phases showed a similar trend between species : soluble sugars tend to decrease while starch tend to increase. Higher NSC concentrations were observed in needles compared to twigs. In general, defoliated trees had higher concentrations of soluble sugars, especially in needles under heat treatment. On the contrary, starch concentration of defoliated trees was generally lower.

Factor Analysis for Mixed Data (Figure 2.6) highlight differences in NSC concentrations during the bud opening phases. Depending on the species and NSC type, dimension 1 explain between 27 and 32 % while dimension 2 explain between 19 and 22 % (Figure 2.6). The variance explained by dimensions 3 and 4 was low and therefore not represented.

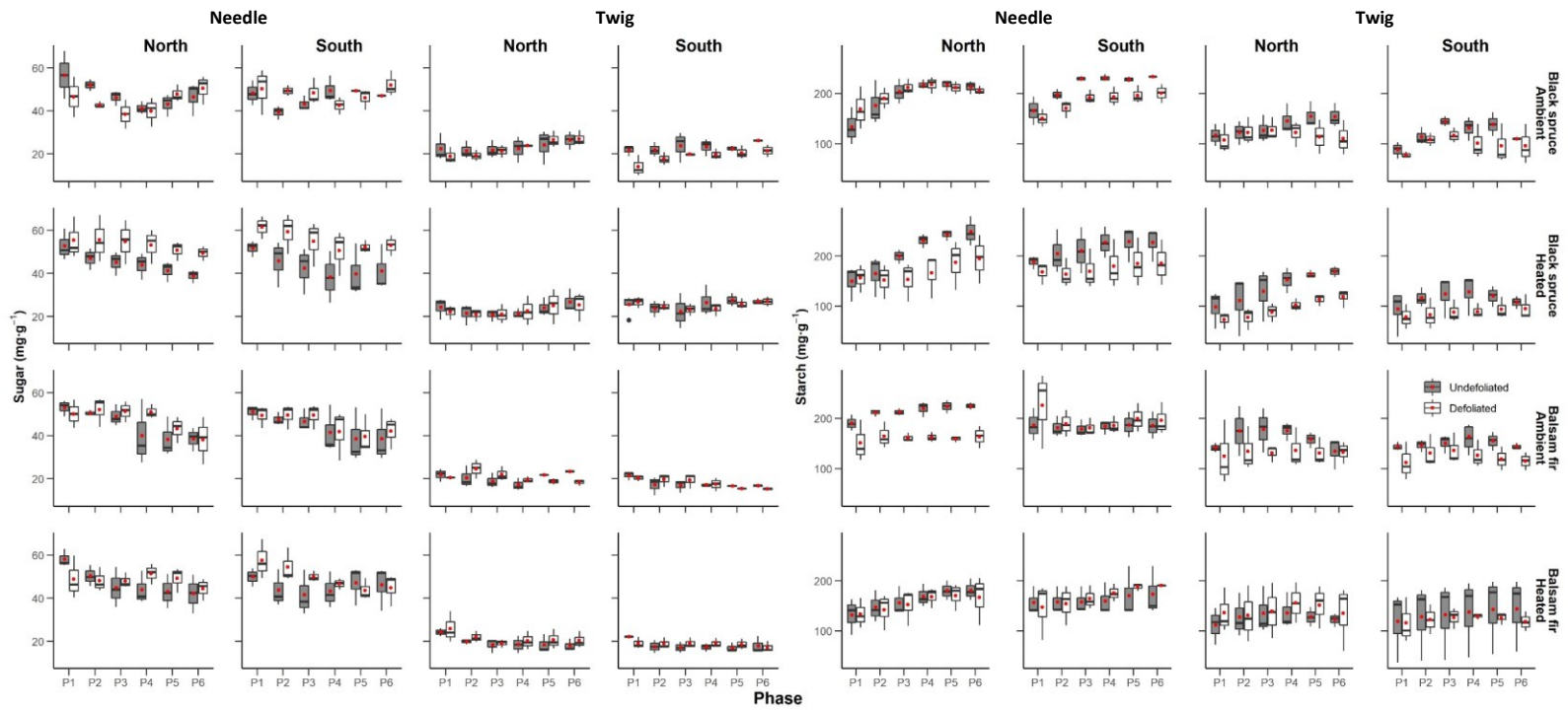


Figure 2.5 Box plot distribution representing the soluble sugar and starch concentration (expressed in mg.g⁻¹ dw) for each budburst phase varying from 1 to 6 in 2019. Results were illustrated for each, provenance, heating and defoliation treatments in BS and BF.

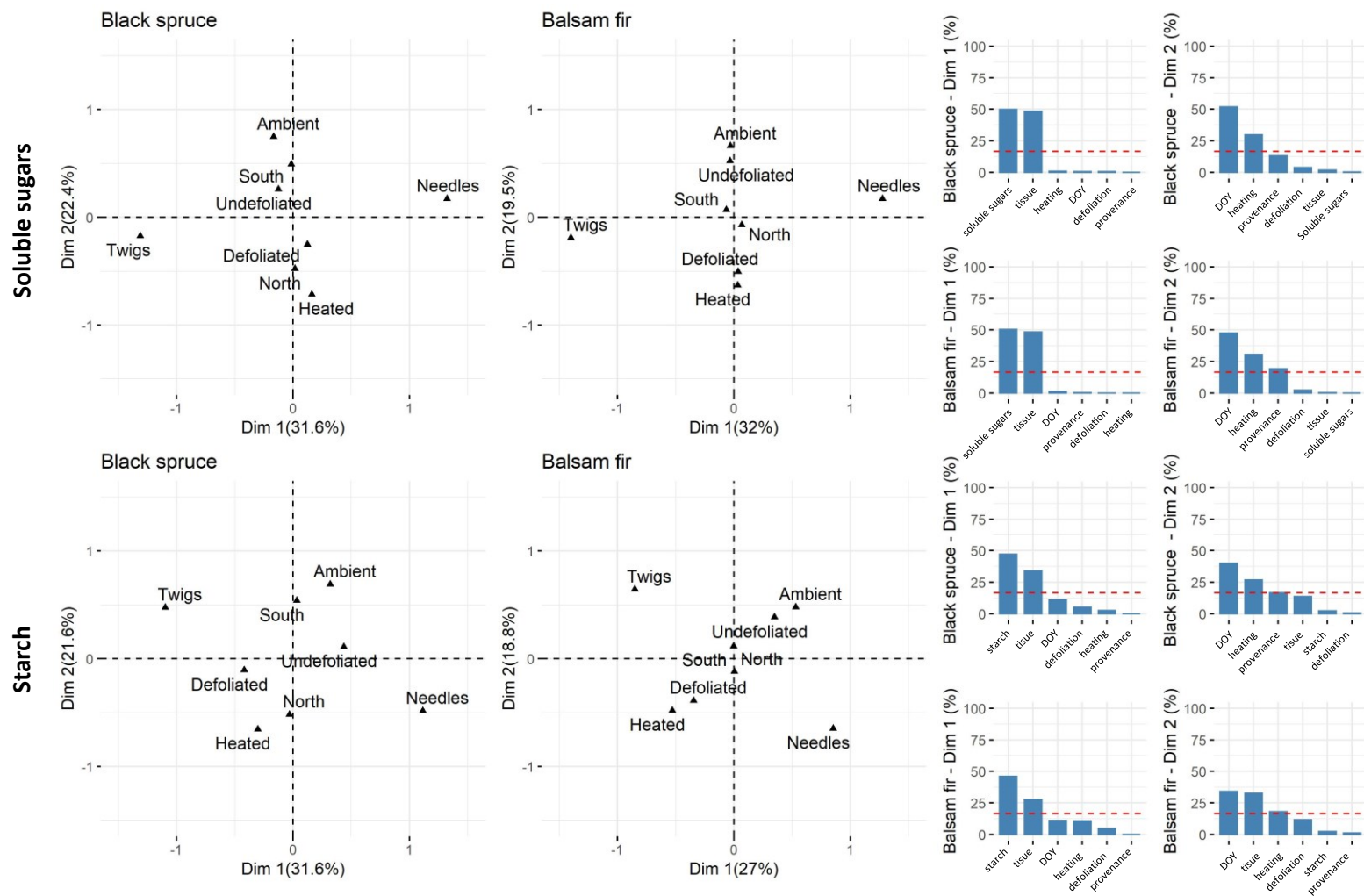


Figure 2.6 Left panel: Factor Analysis for Mixed Data (FAMD analysis) illustrating the correlation between the different factors including part, provenance, heating, and defoliation. Since DOY and PredSS, representing a quantitative variable of the progressive phases of phenology and a concentration, only the contribution of qualitative variables was represented for both species. Right panel: Contribution (in percentage %) of the variables to each of the represent dimension. The red dashed line indicates the expected average value if the contributions were uniform.

For **soluble sugars**, predicted sugar (PredSS 50 %) and tissue (50 %) were the two main explanatory variable representing dimension 1 with twig (negative side) and needle (positive) clearly separate for both species. The predSS factor expresses the difference caused by the concentration measured by DOY and then grouped into different budburst phases using a local polynomial regression (Loess). Therefore, lower concentration of soluble sugars stands on the negative side while higher concentration stands on the positive side on dimension 1. All factors are distributed along dimension2 mostly representing DOY with very similar result to phenology (Figure 2.3). Therefore, provenance and treatments (heating and defoliation) had no effect on soluble sugars. In PERMANOVA analysis for soluble sugar (Figure 2.7), very few factors showed significant effect on NSC concentration during phenological phases of budburst and only scattered effects were detected.

For **starch**, both predicted starch and tissue also represent the main explanatory variable for dimension 1 mostly separating twig (negative side with less starch) and needle (positive side with more starch) (Figure 2.6). Dimension 1 also separate the cluster grouping «defoliated-heating » to the cluster grouping «ambient-undefoliated » meaning that defoliated and heated trees as less starch during the phases of bud opening. In both species, dimension 2 was mostly explained by DOY and heating. In BS, PERMANOVA analysis (Figure 2.7) indicate that defoliation had significant effect on starch concentration in needle and twig during phase 3 to 6, reaching near 35 % of variance explained in phase 4-5. The factor provenance was also important in explaining the starch concentration during phase 4 and 6 in twig of BS. In BF, heating had significant effect on starch concentration in needle during bud opening phase 1 to 4.

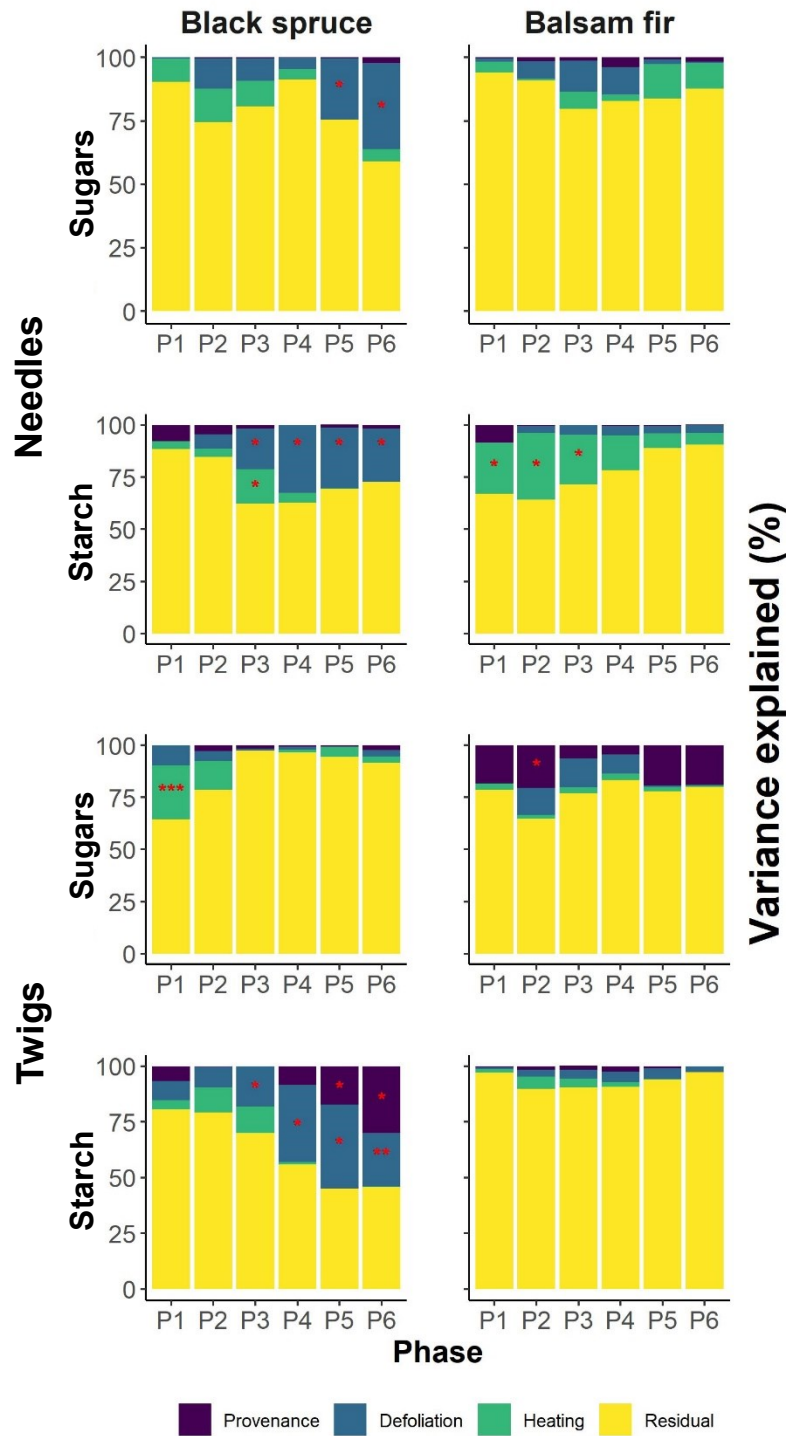


Figure 2.7 Explained variance of different factors in NSC concentration in BS (left) and BF (right) found with Permanova analysis. Level of significance: * <math><0.05</math>, ** <math><0.01</math> *** 0.0

2.6 DISCUSSION

Understanding the impact of species-specific genetic makeup, environmental and biotic factors on the physiology of budburst can help predict how boreal conifers will respond to climate change in terms of bud phenology and therefore on tree-pest interactions (Chang *et al.* 2021). In this study, the combination of provenance, temperature, and defoliation gives a wide range of opening dates. However, it was possible to detect trends in the group of treatments where the opening phases occurred more earlier: it was the case for group of trees belonging to the defoliated – heated – Northern provenance, which had earlier phases of bud opening compared with the non-defoliated – ambient and Southern provenance, confirming our first hypothesis. Key environmental factors, such as temperature, in combination with tree species-specific adaptive variation greatly influence the timing of budburst (Jönsson *et al.* 2010; Cooke *et al.* 2012; De Barba *et al.* 2016; Deslauriers *et al.* 2019; Chang *et al.* 2021; Pan *et al.* 2021). Moreover, as with provenance or increase temperature, defoliation influence the timing of budburst. Even if very few studies examine the impact of herbivory on bud phenology (Tuomi *et al.* 1989; Quiring et McKinnon 1999; Carroll et Quiring 2003), our result confirm that defoliation by SBW advance the date of budburst in boreal conifers.

To better understand the underlying physiological processes involved in the budburst process, we have coupled starch and soluble sugar concentrations measurements with developmental phases of bud in BS and BF organs (needles, and twigs). In needles, starch concentration increases during budburst, from P1 to P6, while sugar concentration decreases (Egger *et al.* 1996), corresponding with our result. The earlier bud phenology for group of trees belonging to the defoliated – heated – Northern provenance was associated with a

decrease in starch but not with a decrease in soluble sugars, partially confirming our second hypothesis.

2.6.1 WARMING AND DEFOLIATION BOTH ADVANCE BUD PHENOLOGY

Among the studied factors explaining the timing of budburst, two combinations of factors similarly influence the timing of budburst in both species: the heated and defoliated conditions, were associated with earlier budburst while the ambient temperature and undefoliated were related with a later budburst. Warming and defoliation were important factors that explained the variance observed in bud opening. However, the 2 °C rise in ambient temperature was the most important factor explaining the degree of variation in timing of budburst. Under the same experiment, Bellemin-Noël *et al.* (2021) observed an advanced in spring phenology of about 2 days in *Abies balsamifera* and *Picea mariana*. In this study, the earlier phenology in heated plots was about 5.7 days in BS and 2.6 days in BF (data not shown). In the boreal forest, heat-accumulation (temperature expressed as the number of degree-day) is the main factor influencing the timing of budburst (Antonucci *et al.* 2015; Rossi et Isabel 2017b). Cold winter temperatures keep buds dormant while warmer spring temperatures end dormancy and initiate budburst (Singh *et al.* 2017). The earlier spring temperatures warm, the sooner buds will open (Menzel *et al.* 2006; Schwartz *et al.* 2006; Bertin 2008) provided that the cold temperature accumulation (chilling) during the winter is reached (Man *et al.* 2017). For the BF tree, the difference between the years in the first three phases is particularly important showing the differences of temperature between years. The difference observed between years highlights the impacts of weather such as earlier spring temperature, snowmelt, and precipitation (Bertin 2008). Early spring in 2019 allowed the trees of both species to open vegetative buds earlier.

For both species, defoliated trees had earlier phenology compared with non-defoliated trees, confirming other study on similar species (Deslauriers *et al.* 2019; Ren *et al.* 2020). In white spruce however, partial defoliation by SBW did not affect the timing of budburst while a complete defoliation resulted in a delayed budburst (Quiring et McKinnon 1999), which is the contrary to what was observe in this study. In our study, the contribution of defoliation, especially in BS, explains the biological variance in a very significant and increasing way during the budburst phases. As expected, the bud phenology of northern provenance was generally earlier compared to southern provenance. However, the difference was less marked and less consistent than what is observed in other conifer ecotype studies (Gould *et al.* 2011; Rossi et Bousquet 2014; Rossi 2015). Indeed, the variance explained by provenance was not significant for BF, possibly because the difference in distance between two juxtaposed areas was rather small. For BS, the provenance significantly explains the phenological variance but only during the first phases, from closed bud (phase 1) to translucent bud (phase 4).

As expected, BF opens their buds earlier than BS, illustrating the species life history trait difference under natural conditions (Antonucci *et al.* 2015; Rossi et Isabel 2017b; Deslauriers *et al.* 2019). The different factor combinations (provenance, temperature, and defoliation) resulted in a wide variety of budburst dates corresponding with the natural landscape variation where stands exhibit spatial and temporal phenological differences (Peaucelle *et al.* 2019) mainly correlated with local conditions (Leinonen et Hänninen 2002; Hänninen *et al.* 2007; Rossi 2015; Perrin *et al.* 2017).

2.6.2 TEMPERATURE AND DEFOLIATION DECREASE STARCH ACCUMULATION DURING BUDBURST BUT NOT SOLUBLE SUGARS

Spring forcing reflects the effect of warmer temperatures where soluble sugars are transformed back into starch at the end of winter (Tixier *et al.* 2019; Deslauriers *et al.* 2021) leading to an increase in starch during the phases of budburst, culminating, in the study, during the last phases of bud opening. This increase in starch concentration was observed mainly in needle where starch accumulate in the chloroplast before the onset of growth (Chabot et Chabot 1975). Starch is then hydrolysed in soluble sugars when carbon consumption for budburst and primary growth increase (Little 1970; Decourteix *et al.* 2008; Klein *et al.* 2016). As already proposed by early researchers, energy to growth the new shoots from buds in conifers is derivate from both reserve (i.e., storage starch) and current photosynthesis of previous year needles (Gordon et Larson 1968; Little 1970). As seen in other studies (Hoch *et al.* 2003; Deslauriers *et al.* 2019; Schiestl-Aalto *et al.* 2019; Schoonmaker *et al.* 2021), the results obtained show higher concentrations of soluble sugars and starch in needles than in twigs during spring for both species. The NSC concentrations in function of the budburst phases of each organ follow the same trend illustrating similar biochemical processes in each organ (leaves and twigs) during budburst (Cooke *et al.* 2012; Singh *et al.* 2017).

Changes in temperature during spring represent a signal that influence the amount of reserves available for growth (Tixier *et al.* 2019). In this study, higher temperature leads to a decrease in reserve in the form of starch, meaning that less starch was accumulated during bud opening under a 2 °C warming. Higher temperature is often associated with higher metabolic rate (Amthor 2000; Turnbull *et al.* 2004) consuming C, thereby reducing the formation of starch reserves. A lower replenishment in starch reserves was observed under

warming in BS at the end of summer (Balducci *et al.* 2015), corresponding with our results in twig and leaf during spring. However, as budburst was earlier under warming, the carbon resources in the form of starch in needles and twigs were also mobilized earlier to sustain bud swelling, possibly explaining the lower amount under higher temperature. But even if conifers allocate carbohydrates in the form of soluble sugar to support budburst (Little 1970; Decourteix *et al.* 2008; Klein *et al.* 2016), this had no impact on the amount found in needles or twigs. In BS, similar concentrations of soluble sugars were also observed in the cambium during a greenhouse warming experiment [+ 6 °C during the day or during the night, (Balducci *et al.* 2015)]. The size of the mobile pool of sugars normally reflects the balance between uptake and the demand for growth and respiration (Li *et al.* 2002). There is limited margin of variation for total soluble sugars, and the C-imbalance is rather observed in the starch pool (Vanderklein et Reich 1999; Fierravanti *et al.* 2019). However, under warming, a decrease in the hexose pool (glucose and fructose) was observed in BS (Way et Sage 2008; Deslauriers *et al.* 2014). In BF, the variance explained by warming in NSC concentration was higher while the defoliation treatment poorly explains the variation in soluble sugars. The results highlight the difference between species in terms of warming and defoliation.

As for under warming, starch concentration was generally lower in the needles and stems of defoliated trees during bud opening. Starch reserves in spring decreases when conifers are subjected to successive years of defoliation (Deslauriers *et al.* 2019). In conifers, the decrease in starch reserves according to the degree of defoliation is very well known (Deslauriers *et al.* 2015; Puri *et al.* 2015; Fierravanti *et al.* 2019). Indeed, highly defoliated BF stores less starch in twigs and needles in May and June which can affect tree survival (Fierravanti *et al.* 2019). Because starch accumulation depends on current photosynthesis,

the spring pool is highly reduced with needle loss (Vanderklein et Reich 1999). A shift in C-allocation in defoliated tree occur, prioritizing primary growth instead of starch accumulation (Deslauriers *et al.* 2019) and explaining the earlier phenology. In our experimental design, defoliation was a factor that contributes the most to explain variance in starch concentration in twigs and needles from the swollen bud to the exposed shoot (phases 3 to 6) with almost no variance explained in soluble sugars. In a complete defoliation experiment, (Li *et al.* 2002) observed a reduction of 53 % in soluble sugars in the current year needle, a level not reached in our experiment. Lower soluble sugars were also observed in defoliated tree, but only at the beginning of the summer (June-July), with inverse pattern toward the end of the summer (Fierravanti *et al.* 2019). It is thus possible that (1) the limited margin of safety for soluble sugars variation was not reach (as for warming) and (2) only one year of moderate defoliation was not enough to cause a decrease in soluble sugars in old needles and twigs.

2.7 CONCLUSION

Our results highlight the need to identify and assess the factors that influence conifer budburst to improve predictions of the impacts of global warming on the boreal forest. Theses factors, such as warming temperatures, defoliation, and genetics of northern species, lead to early budburst. Climate change is expected to increase average spring temperatures and the frequency of potentially damaging freezing temperatures during budburst (Man *et al.* 2009; Lamhamedi *et al.* 2022; Marquis *et al.* 2022). Boreal conifers, considered cold-resistant, are sensitive to spring frosts during budburst. Moreover, biotic effects such as insect defoliation should be considered in predictions. Indeed, a higher frequency and intensity of insect outbreaks are predicted under climate change (Esper *et al.* 2007, Seidl *et al.* 2008, Iyengar *et al.* 2016). And warmer temperature may increase or decrease the synchronism between host

conifers and their consumers (Portalier *et al.* 2022) complicating predictions. The results also confirmed that carbon allocation in tissues can be used to describe the development of budburst and the impacts of early onset factors on it. We observe the effects of temperature and defoliation on the reduction of starch accumulation in needles. However, the level and period of defoliation in this sapling study was not sufficient to observe an impact on sugar concentrations. Further studies on the phenology associated with carbon allocation will be needed, for example on mature forests exposed to higher levels of defoliation, to better predict tree budburst.

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CHAPITRE 3

CONCLUSION GÉNÉRALE

Les changements climatiques exercent des effets directs sur les arbres, tel le changement rapide de leur phénologie. Toutefois, des effets biotiques, comme la défoliation par les insectes doivent aussi être considérés dans les modèles de prédiction de la phénologie. En effet, une fréquence et une intensité plus élevées des éclosions d'insectes (Esper *et al.* 2007, Seidl *et al.* 2008, Iyengar *et al.* 2016) sont prévues en fonction des changements climatiques pouvant modifier les interactions hôtes-ravageurs (Ren *et al.* 2020).

3.1 LA PHÉNOLOGIE

L'étude a permis d'observer comment la période du débourrement des bourgeons végétatifs des EPNs et des SABs est influée par les caractéristiques génétiques des espèces (leur provenance), la température et la défoliation par la TBE. Puisque l'effet des provenances n'était pas toujours significatif, des études sur d'autres provenances représentant une plus grande différence dans la latitude – représentant l'acclimatation à différentes températures – pourraient aider à comprendre comment les adaptations locales des arbres influent sur le débourrement lorsque soumis au réchauffement et à la défoliation; un plus grand différentiel de gradient nord-sud étant associé à des écarts de débourrement plus important (Guo *et al.* 2022). De plus, les mesures de phénologie ont été réalisés sur de jeunes arbres pendant seulement deux années de défoliation sans tenir compte du degré de défoliation (faible à modéré). Des études antérieures semblent indiquer que les arbres très

défoliés ont un débourrement tardif. De plus, la phénologie change avec l'âge des arbres (Li *et al.* 2013; Rossi *et al.* 2008; Vitasse 2013), un facteur qui pourrait modifier les résultats obtenus dans cette étude.

3.2 ALLOCATION DU CARBONE

La phénologie hâtive des bourgeons a été associée à une diminution de l'amidon mais pas à une diminution des sucres solubles. Le taux de défoliation des arbres à l'étude n'était peut-être pas suffisant pour observer son effet sur les sucres solubles. Comme pour la phénologie, il serait intéressant de regarder les concentrations d'amidons des arbres en tenant compte de différents niveaux de défoliation, de plusieurs années de défoliation et sur des arbres matures. Les sucres solubles totaux englobent plusieurs molécules (des monosaccharides comme le glucose et les polysaccharides comme le raffinose) qui ont des fonctions physiologiques différentes (Deslauriers *et al.* 2016). Des analyses de concentrations sur chacun des sucres solubles des échantillons à l'étude préciseraient la physiologie associée à la phénologie du débourrement des bourgeons végétatifs.

3.3 L'APPROCHE STATISTIQUE

L'innovante approche statistique de la phénologie a permis dans un premier temps de mettre en évidence les effets de traitements en associant les jours juliens aux stades phénologiques. Le grand nombre de traitements (16 combinaisons de traitements) et de stades (6 stades) rendent difficile l'observation des effets des traitements sous forme de graphique. Puis, dans un deuxième temps, associer les différentes concentrations de sucres solubles et d'amidon aux des stades phénologiques (et non aux jours juliens) a permis d'observer que les concentrations en CNS suivent la même tendance illustrant des processus biochimiques similaires de chaque organe (aiguilles et tiges). Dans une prochaine étude, il serait intéressant

d'observer les concentrations immédiatement avant et après le débourrement en augmentant le nombre d'échantillonnage hebdomadaire afin d'observer les concentrations de CNS et les associer à d'autres stades de phénologie et de physiologie des conifères boréaux. De cette façon, il serait possible d'observer l'accumulation d'amidon qui commence à la fin de l'hiver avec une concentration croissante jusqu'au début de l'ouverture des bourgeons, et que les CNS sont à leur plus haut niveau pendant le débourrement des bourgeons (Hoch *et al.* 2003; Schadel *et al.* 2009; Schoonmaker *et al.* 2021).

3.4 LES PERSPECTIVES

Nos résultats soulignent la nécessité de déterminer et d'évaluer les facteurs qui influencent le débourrement des conifères afin d'améliorer les prédictions des impacts du réchauffement climatique sur la forêt boréale.

La hausse des températures, la défoliation et la génétique des espèces de provenance plus nordique exposées au réchauffement entraînent un débourrement des bourgeons de plus en plus hâtif chez les jeunes conifères. Dans une perspective de changement climatique, il est important de s'interroger sur comment les forêts matures réagiront. Pour la survie des arbres, deux aspects sont à prédire pendant le débourrement : la sensibilité aux gels printaniers associée au débourrement hâtif et le synchronisme entre les arbres hôtes et les insectes ravageurs.

Dans l'est du Canada, l'augmentation des températures moyennes aux printemps et de la fréquence de températures de congélation potentiellement dommageables pendant le débourrement est à prévoir avec les changements climatiques (Man *et al.* 2009; Lamhamedi *et al.* 2022; Marquis *et al.* 2022). Les conifères boréaux, considérés résistants au froid par

leur débourrement tardif (Sims *et al.* 1990) peuvent devenir vulnérables au gel avec la progression du réchauffement (Hänninen 2006; Man 2009; Guo *et al.* 2022). La génétique des conifères de provenance plus nordique entraîne un débourrement plus hâtif. Ce qui peut augmenter leur vulnérabilité en exposant davantage les jeunes tissus en croissances à des épisodes de gels. Outre la provenance, d'autres facteurs liés à la sensibilité aux gels printaniers sont à considérer. La taille des arbres a une certaine influence sur la résistance aux gels. Les jeunes semis sont plus sensibles aux gels (Man et Lieffers 1999; Man *et al.* 2009; Lamhamedi *et al.* 2022). Selon les particularités et l'étagement des peuplements forestiers, le degré d'exposition au gel varie selon un gradient de conditions microclimatiques (Stathers 1989). Sous un bon couvert forestier, les soubresauts des températures sont moindres et la canopée protège les jeunes arbres (Man et Lieffers 1999). Pour les arbres matures, un gradient de débourrement le long de l'arbre retarde l'exposition au gel d'une partie des bourgeons (Stathers 1989).

Les études portant sur la phénologie du débourrement des arbres sont importantes dans le cadre de la lutte aux insectes tel la tordeuse des bourgeons de l'épinette (TBE). Elles servent, entre autres, à prédire le degré de synchronisme entre l'hôte et l'insecte pendant la phase d'alimentation. De plus, les effets biotiques de la défoliation par les insectes doivent être considérées dans les prévisions du synchronisme puisque qu'elle devance le débourrement des bourgeons des arbres. Avec les changements climatiques, les arbres et leurs ravageurs peuvent réagir différemment à la hausse des températures et par conséquent, modifier leur fenêtre de synchronisme. Selon les modèles de prédiction de Portalier *et al.* (2022), l'augmentation de température pourrait accroître l'inadéquation entre la TBE et le sapin baumier dans les sites méridionaux mais accroître la synchronie dans les sites

nordiques. De plus, l'augmentation de la fréquence et l'intensité des épidémies d'insectes sont prédits avec les changements climatiques (Esper *et al.* 2007, Seidl *et al.* 2008, Iyengar *et al.* 2016).

3.5 CONCLUSION

L'étude des concentrations du CNS associées à la phénologie est prometteuse. Elle contribue à la compréhension et la prédiction des effets des facteurs influant la phénologie et la physiologie des conifères boréaux. Les informations pourront être intégrées dans les modèles de prédiction dans le cadre des plans de lutte contre la TBE et aider à la sélection des arbres mieux résistant à la TBE et aux changements climatiques.

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