UNIVERSITÉ DU QUÉBEC À CHICOUTIMI

INFLUENCE DES FLUCTUATIONS CLIMATIQUES SUR LA XYLOGÉNÈSE DE *PICEA MARIANA* DES SITES MÉSIQUES DE LA FORÊT BORÉALE CONTINUE

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AVANT-PROPOS

Le projet de thèse original a été déposé il y a 10 ans. À cette époque, les professeurs Hubert Morin et Réjean Gagnon de l'UQAC avaient obtenus des fonds pour l'installation de quatre stations météo permettant de mesurer le climat sur quatre stations d'échantillonnage, non établie. Je fus donc engagé à l'automne 1999 et à l'été 2000, préalablement à mon inscription au doctorat, afin de faire la recherche de sites propices à l'établissement de ces quatre stations, ainsi que pour préparer le terrain et y installer les dispositifs électroniques nécessaires. Mon implication remonte donc à très loin dans le processus et a touché par la suite tous les aspects : tournées hebdomadaires d'échantillonnage (jusqu'au début de la saison 2004), mesures en laboratoire (hiver 2005-2006 et 2006-2007), revue de littérature, analyses des données.

À l'époque de la mise en place du dispositif, nous avions à toute fin pratique les mêmes objectifs généraux que ceux énoncés dans le présent document, mais nous avions des hypothèses bien différentes. Nous savions que l'espèce étudiée, l'épinette noire, se trouvait, dans le contexte étudié, dans sa zone de confort. Cela nous a fait émettre l'hypothèse que sa croissance radiale ne serait pas dominée par un seul facteur limitant, mais par une multitude de liens complexes. Ceci est demeuré, mais ce que nous n'avons pas maintenu est l'hypothèse voulant qu'une très courte période de croissance intra-annuelle explique une très grande partie de la largeur de cerne totale produite, et que la croissance de l'arbre montre une très grande réceptivité au climat durant cette période. Cette dernière est née du fait que la plupart des études climat-croissance réalisée à cette époque, ne mettant en relation que des données mensuelles et la largeur totale du cerne, n'expliquaient que bien peu la variance de croissance observée pour les arbres croissant dans leur « zone de confort ».

En y pensant bien, ces deux hypothèses de départ, sans être contradictoires, comportent leur lot de difficultés. D'abord, pour que l'arbre réagisse favorablement au climat sur une courte période, il faut que les influences climatiques supposément multiples se conjuguent favorablement ensemble au même moment, ce qui rend un tel événement hautment improbable. Mais ce qui a surtout empêché le maintien de l'hypothèse de la courte période est la difficulté technique de la vérifier avec les méthodes de mesure employées. En effet, les micro-carottes extraites répétitivement au cours de la saison souffrent d'une variabilité de croissance d'un point d'échantillonnage à l'autre qui a empêché d'observer des variations intra-annuelles importantes dans le nombre de cellules produites. Nous disposions également de mesures continues prises à l'aide de dendromètres automatiques. Ces mesures permettent d'observer un plus grand accroissement en début de saison, mais cela s'explique aisément par le seul fait que les trachéides produites atteignent un diamètre plus grand, ce qui n'est pas nécessairement synonyme de conditions de croissance meilleures ou d'effort de croissance supérieur de la part de l'arbre.

Nous avons donc plutôt opté pour une hypothèse plus classique voulant que les conditions durant toute la durée de la saison de croissance soient importantes. Les difficultés de faire les liens entre climat et croissance seraient plutôt causées par des mesures de climats incomplètes, l'usage de mesures de croissance intégrantes (largeur de cerne) plutôt que fondamentales (nombre de cellules et diamètre cellulaire), jumelage entre mesures climatiques et d'accroissement déficients (station météo trop éloignées des forêts étudiées) et colinéarité des variables explicatives. Tous ces éléments ont pu être traités à l'aide des méthodes dont nous disposions et c'est ce qui a été fait, entre autres, dans cette thèse.

1. INTRODUCTION

1.1 Mise en contexte

Depuis le milieu des années 90, la foresterie québécoise cherche à appliquer le principe d'aménagement durable de la forêt (ADF), comme en témoigne l'amendement de la Loi sur les forêts de 1996 où les six critères d'ADF ont été introduits en préambule. Cette timide mesure ne fut cependant pas accompagnée de la refonte en profondeur du régime forestier si bien qu'un débat social s'est enclenché au tournant de l'an 2000, ce qui mena ultimement à la mise sur pied de la Commission d'étude sur la gestion de la forêt publique québécoise. Le rapport de cette Commission (Coulombe et al. 2004), jugeant la gestion forestière comme étant trop centrée sur la production ligneuse, sert de base à l'actuelle mise sur pied d'un nouveau régime forestier, chapeauté par la Loi sur l'aménagement durable du territoire forestier, adoptée en 2010. L'esprit de cette loi est la mise en place effective d'une gestion répondant aux mêmes six critères d'ADF qui n'étaient que simplement énoncés en 1996.

Parallèlement, le Bureau du forestier en chef (BFEC) a annoncé des baisses de possibilité forestière pour la période actuellement en vigueur (2008-2013). Les raisons invoquées pour ces diminutions sont la mise en place de superficies affectées à la conservation (Bureau du forestier en chef 2010a, 2010b). Cela démontre bien le défi d'harmonisation posé par l'ADF : la production ligneuse doit laisser de la place aux besoins environnementaux, sociaux et culturels. Cependant, il n'en demeure pas moins que la production ligneuse a toujours sa place dans cette nécessaire recentralisation de la gestion forestière. Pour preuve, au moins deux des six critères d'ADF sont compatibles avec la production ligneuse : le maintient des avantages socio-économiques de la forêt et le maintien de l'apport des écosystèmes forestiers aux grands cycles écologiques planétaires.

En tant que partie intégrante de la grande forêt boréale mondiale, les forêts d'épinette noire participent activement à puiser le carbone de l'atmosphère et à le stocker de façon durable en impliquant, entre autres, le tronc de l'arbre. Ce carbone sous forme ligneuse est la base de toute l'industrie forestière passée, présente et future, établissant ainsi un lien évident entre aménagement forestier et gestion des gaz à effet de serre. Le Groupe d'experts intergouvernemental sur l'évolution du climat (GIEC) stipule qu'une forêt bien aménagée est un moyen efficace pour lutter contre la hausse du CO_2 atmosphérique, un lien qui ne peut qu'être amplifié si le matériau bois qui en découle peut substituer d'autres à plus forte émission de CO_2 tel le béton ou l'acier (Nabuurs, Masera et al. 2007).

Dans un tel contexte, la sylviculture intensive est appelée à jouer un rôle d'importance croissante. Le GIEC affirme que les stratégies d'ADF qui maintiennent ou augmentent les stocks de carbone forestier, tout en produisant un rendement annuel soutenu de bois, de fibre ou d'énergie de la forêt, représentent l'option qui générera le plus de bénéfices d'atténuation (Nabuurs, Masera et al. 2007). On pourrait croire que la foresterie québécoise, sur la base des constats mentionnés ci-haut, va à l'inverse de cette tendance, mais au contraire, la nouvelle loi prévoit l'instauration d'aires d'intensification de la production ligneuse (Gouvernement du Québec 2011) pour contrer les pertes inhérentes à d'autres aspects de l'ADF. Le GIEC recommande d'ailleurs, au niveau mondial, de maintenir ou augmenter la densité de carbone sur pied en favorisant des aménagements forestiers plus intensifs. Cela étant basé sur le postulat voulant qu'une forêt soumise à l'aménagement séquestre plus de carbone dans le temps qu'une forêt non-aménagée.

La sylviculture actuellement applicable aux forêts d'épinette noire ne comporte aucun traitement qui, à la fois, améliore le rendement à l'échelle du peuplement, s'applique à des peuplements naturels d'âge mature et admissible sur un large éventail de territoires incluant ceux ne faisant pas partie des plus productifs. Ainsi, l'étude des facteurs limitant la croissance de l'épinette noire mature, notamment au niveau des facteurs climatiques, peut être la clé de la mise sur pied éventuelle de nouveaux traitements sylvicoles répondant à ces critères.

En plus de la quantité de bois produit, on se préoccupe de plus en plus également de sa qualité, car lorsque cette dernière est élevée, des produits de haute valeur, souvent plus durables, peuvent être produits. Une meilleure connaissance des facteurs limitant les divers aspects de la xylogénèse pourraient, en théorie, permettre également la mise sur pied de nouveaux traitements sylvicoles permettant de contrôler diverses propriétés des tiges. Par exemple, on sait que les traitements d'éclaircie permettent un rehaussement de la croissance radiale de l'épinette noire à l'échelle de l'arbre (mais pas à l'échelle du peuplement) sans affecter ses propriétés mécaniques (Vincent et al. 2009, Vincent et al. 2011). Mais peut-être existe-t-il d'autres moyens de jouer sur les caractéristiques du bois tout en augmentant la croissance à l'échelle du peuplement ?

D'autre part, bien que l'aménagement de la ressource ligneuse fasse partie des moyens de contrer les changements climatiques d'origine anthropique, il n'en demeure pas moins que la relation est en quelque sorte à double sens, puisque la forêt est susceptible aux fluctuations du climat, notamment au niveau de sa croissance. C'est en partie pourquoi le BFEC, responsable de l'évaluation de la croissance de la forêt dans le cadre du calcul de la possibilité forestière, se préoccupe de l'effet des changements climatiques sur la forêt (Bureau du forestier en chef 2010a). Il souligne également la nécessité d'améliorer les nouveaux modèles de croissance (Bureau du forestier en chef 2011). Ici encore, la connaissance des facteurs climatiques limitant la croissance peut jouer un rôle important.

1.2 Cadre de l'étude

L'épinette noire, *Picea mariana* (Mill.) B.S.P., est un conifère arborescent relativement petit et à croissance plutôt lente (Viereck et Johnston 1990), mais il n'en reste pas moins l'arbre le plus répandu de la forêt boréale québécoise. Aux longitudes du Québec, il est présent sur une grande étendue latitudinale, ce qui fait en sorte de le retrouver à partir du nord de la Nouvelle Angleterre (~ 42 °N) jusqu'à la limite des arbres, près de la baie d'Ungava (~58 °N) au-delà de laquelle il présente une forme rabougrie. L'aire d'exploitabilité commerciale est toutefois plus restreinte. Du sud de la sous-zone de la forêt boréale continue (~48 °N) jusqu'à la limite d'attribution des forêts commerciales (~52 °N) il est présent dans une grande majorité des peuplements fermés, étant souvent même en représentation quasi monospécifique. Cette zone sous aménagement se trouve donc en plein cœur de l'aire de répartition de l'espèce et fait l'objet de cette étude. Cependant, même à l'intérieure de cette aire d'étude, les peuplements purs d'épinette noire peuvent présenter diverses conditions écologiques.

Cette étude concerne les conditions les plus représentatives des peuplements d'épinette noire sous aménagement. Si l'épinette noire peut former des peuplements purs sur les trois grands groupes de régime hydrique du sol (xérique, mésique et hydrique), c'est sur les sites mésiques qu'on les retrouve le plus fréquemment dans la plupart des régions écologiques de la sous-zone de la forêt boréale continue (Blouin et Berger 2004). C'est donc sur ces sites mésiques que porte cette étude. Ces peuplements sont caractérisés par une strate muscinale dominée par les mousses hypnacées (*Pleurozium schreberi*, *Ptillium crista-castrensis*, etc.) ainsi qu'une strate arbustive où les éricacées (*Kalmia angustifolia*, *Ledum groenlandicum*, *Vaccinium angustifolium,* etc.) sont présentes.

L'accroissement radial du cerne de croissance de l'arbre est causé essentiellement par deux phénomènes physiologiques fondamentaux : la production de nouvelles trachéides par les divisions cambiales et l'élargissement radial des trachéides en phase de différentiation. Pour une même cellule, la division qui la fait naître et sa différenciation sont séparés dans le temps (Larson 1994) et les différences entre les mécanismes cellulaires impliqués dans la mitose et ceux impliqués dans l'expansion cellulaire sont différents (Taiz et Zeiger 2006). Considérant cela, il est donc naturel de soupçonner une influence du climat sur la production de nouvelles cellules différente de celle sur l'élargissement. Cela justifie donc de faire l'étude en séparant ces deux phénomènes distincts au lieu de faire l'habituelle analyse à l'aide de l'accroissement radial puisque celle-ci n'est pas morphologiquement fondamentale, étant en fait la somme des diamètres de chacune des trachéides qui se juxtaposent radialement.

1.3 Objectifs généraux

Cette étude vise à identifier les facteurs climatiques qui causent les variations dans l'accroissement radial au stade de maturité de l'épinette noire des peuplements fermés sur sites mésiques de la sous-zone de la forêt boréale québécoise. Les paramètres de croissance suivants sont étudiés :

- La dynamique intra-annuelle de production radiale des nouvelles trachéides;
- les facteurs expliquant les variations dans les deux principaux événements phénologiques de cette dynamique, soit l'initiation et l'arrêt de la production cellulaire;
- les facteurs climatiques limitant la production totale des trachéides au cours d'une saison de croissance;
- la dynamique d'élargissement cellulaire au cours de la saison de croissance;
- les facteurs climatiquesqui influencent cette dynamique ainsi que ceux qui limitent le diamètre des trachéides.

1.4 Hypothèses générales

Traditionnellement et encore de nos jours, les études sur les relations climat-croissance n'impliquent que des données mensuelles de température et de précipitation. Selon Fritts (1976), ce choix se justifie par la disponibilité importante de ce type de données et par une soi-disant aisance à interpréter l'effet d'autres facteurs en fonction de la température et des précipitations. Il s'agit donc ici d'un compromis de commodité. Si l'influence de facteurs autres que la température et les précipitations n'est presque jamais démontrée dans la littérature, c'est donc surtout par défaut de l'avoir explicitement étudiée. Pourtant, l'arbre est soumis à bien plus que deux facteurs de types différents, qui forment ensemble ce que Fritts appelle « l'environnement opérationnel » de l'arbre. Suivant cette idée, on peut formuler l'hypothèse générale voulant que la croissance du tronc de l'épinette noire est potentiellement influencée par :

- La température de l'air
- L'humidité de l'air
- La lumière
- La température du sol
- La disponibilité en eau dans le sol

Cela ne sont que des généralités et ces cinq catégories peuvent être complémentées ou modifiées selon le contexte de chaque question étudiée.

La croissance de l'épinette noire dans le cadre de cette étude est présumée être influencée par plusieurs facteurs climatiques agissant de concert. Il existe de nombreux exemples d'études de relations climat-croissance simples ayant données d'excellents résultats chez des arbres croissant en milieux extrêmes, tel les milieux arides ou la limite des arbres (Cook et al. 2004, Wilmking et al. 2004, Esper et al. 2002). En revanche, dans les milieux normaux, c'est-à-dire ceux présentant des conditions auxquelles l'espèce considérée est le mieux adaptée, les études n'ont révélé que très peu de choses (Pensa et al. 2006). On présume donc que, dans de tels cas, les liens entre croissance et climat sont multiples et complexes, ce qui s'applique à cette étude puisqu'elle a lieu au cœur de l'aire de distribution de l'espèce, dans un climat continental humide et à des altitudes relativement faibles (< 700 m), ce qui n'implique donc aucune situation extrême pour l'espèce concernée. Ces relations présumées complexes justifient d'autant plus de considérer l'environnement opérationnel de l'arbre en son entier et d'en prendre une mesure fiable.

Outre la nature de ces facteurs, il est important de considérer également la période de temps effective pendant laquelle ils ont une influence sur la réponse considérée. Cette définition temporelle est donc très variable en fonction de la variable réponse considérée, donc de l'objectif recherché et sera précisé pour chaque cas dans chacun des chapitres concernés. On peut cependant énoncer la règle générale voulant que les événements temporels ponctuels étudiés sont hypothétiquement et principalement influencés par le climat dans la période qui les précède tandis que les variables de magnitude de croissance (comme le nombre de trachéides produites au cours de la saison) sont principalement influencées par le climat pendant le développement du processus concerné. D'autres périodes d'influences complémentaires s'ajoutent selon les différentes questions abordées et les mécanismes impliqués.

1.5 Méthodes d'échantillonnage

Le dispositif établi est de type observationnel, c'est-à-dire qu'il ne comporte aucune manipulation expérimentale et a l'avantage d'éviter ainsi de fausser le réel contexte naturel de l'étude, dont dépendent fortement les objectifs énoncés précédemment. En revanche, cela ne permet pas de faire en sorte que l'effet de chacun des facteurs soit isolé des autres. Ceci a des conséquences importantes sur les analyses à effectuer puisque les relations sont présumées multiples et que les variables climatiques sont très corrélées entre elles (voir plus bas).

Quatre stations d'échantillonnage ont été mises en place au début de l'étude dans le but d'observer l'étendue des conditions possibles sur le territoire étudié. Sur chacune de celles-ci, une station météorologique a été installée afin d'observer les conditions de l'environnement opérationnel de l'arbre. Ce dispositif de mesure se justifie par le manque de paramètres mesurés par les stations météorologiques des services publiques et par la rareté de ces dernières dans l'aire d'étude, ce qui oblige l'utilisation de données climatiques d'un site souvent éloigné du site d'étude. En revanche, en disposant de stations de mesure situées directement sur les sites d'échantillonnage, on s'assure un contrôle sur les paramètres mesurés et un couplage très direct entre les mesures climatiques et les arbres échantillonnés. De plus, cela a permis d'avoir une base de données météorologique disposant d'une définition horaire pour les paramètres suivants : température de l'air à découvert, humidité de l'air à découvert, lumière incidente, température de l'air sous le couvert des arbres, température de l'humus, température du sol minéral et teneur du sol en eau. La période de mesure utilisée s'étend de juin 2001 à fin 2006.

L'échantillonnage du cerne des arbres-sujets se fait de façon répétée et non destructive. Dans tous les chapitres de cette thèse, les objectifs spécifiques recherchés nécessitent une évaluation de la phénologie de la production cellulaire cambiale, c'est-à-dire comment la production de nouvelles trachéides se déroule au cours de la saison de croissance et dans certains cas, il est même nécessaire d'évaluer le temps que chacune des trachéides produites passe en phase d'élargissement (chapitre 5). Pour effectuer ces évaluations, nous avons procéder par échantillonnage intra-annuellement répété du cerne de croissance en utilisant des seringues de ponction osseuse tel qu'utilisées auparavant par Deslauriers *et al.* (2003). Ces appareils permettent d'extraire une carotte de seulement 1 mm de diamètre et d'environ 1,5 cm de long, ce qui permet d'inclure les tissus corticaux, le phloème, le cambium le cerne de croissance en développement ainsi que plusieurs cernes des années antérieures. Ceci a permis d'échantillonner les mêmes arbres au cours des années étudiées de façon hebdomadaire ou bimensuelle de la mi-mai jusqu'au début octobre. Comme ces mesures de croissance ont eu lieu sur les quatre sites pendant cinq saisons, nous disposons de 20 cas d'étude.

Les variables de croissance sont évaluées à l'aide de techniques histologiques et mesurées sous microscope. Ainsi, chaque micro-carotte a été incluse dans la paraffine et découpée en couches minces (7-10 µm) au microtome rotatif. Puis, ces coupes furent colorées et pour certaines montées de façon permanente sur leur lamelle. Les mesures prises vont du dénombrement dans les diverses phases de différenciation cellulaire à la mesure de dimensions cellulaires (largeur, épaisseur des parois). Ces techniques permettent donc une étude plus détaillée du développement du cerne que la seule mesure de la largeur de tout le cerne une fois ce dernier complété.

1.6 Méthodes d'analyse

Comme toutes les variables mesurées sont de nature numérique continue, nous avons utilisé la régression multiple comme méthode de modélisation des différentes réponses de croissance. Rappelons que, selon les hypothèses générales, la croissance est présumée être influencée par de multiples variables climatiques. Or, il est connu que les variables climatiques montrent un haut niveau de multicolinéarité (Briffa 1999). Dans un tel contexte, les modèles que l'on peut construire courent le risque d'inclure des effets confondants s'ils sont sous-paramétrés, comme c'est le cas avec des régressions simples (Whittingham et al. 2006, Burnham et Anderson 2002, MacNally 2000, Draper et Smith 1998).

C'est pourquoi les auteurs tout justes cités recommandent l'usage du modèle global, c'est-à-dire qui inclut, toutes les causes directes pour éviter les effets confondants, mais cela comporte d'autres désavantages. Puisque l'introduction de variables est permissive avec cette approche, on augmente le risque d'introduire des variables non causales. Or, l'introduction trop permissive entraine quant à elle le problème de la sur-paramétrisation du modèle, ce qui signifie que ce dernier inclut des effets trop spécifiques au jeu de donné utilisé, allant même à la modélisation de l'erreur de mesure (Ginzburg et Jensen 2004, Burnham et Anderson 2002), ce qui ne peut que diminuer la capacité d'inférence du modèle.

C'est pourquoi nous avons opté pour une méthode permettant d'éviter les pièges de la sous- et de la sur-paramétrisation. Cette méthode est l'élimination de variables à partir du modèle global. Puisque ce dernier initie la procédure, on s'assure que les coefficients soient jugés en situation de modèle global, sans biais des coefficients (effets confondants), à condition que les hypothèses l'ayant construit soient valides. L'élimination de variables permet ensuite d'obtenir un modèle parcimonieux, qui peut être définit comme l'équilibre entre ce qui est nécessaire pour éviter les effets confondants et ce qui représente une modélisation trop poussée (Burnham et Anderson 2002).

Il reste enfin à préciser la manière d'éliminer les variables. Typiquement, c'est un seuil de probabilité qui est utilisé (test paramétrique), mais l'atteinte d'un seuil donné est très dépendante de la puissance du test (donc de la dispersion observée), de la pureté du signal causal et du niveau de colinéarité propre à chaque variable (Belsley 1991, Neter et al. 1989), ce qui rends le choix d'un seuil très arbitraire (Burnham et Anderson 2002). Une autre approche est celle de la théorie de l'information et se base donc sur un critère d'ajustement, en lien avec la loi du maximum de vraisemblance, pour juger de la parcimonie d'un modèle. Anderson et Burnham (2002) font un éloquent plaidoyer en faveur de l'usage du critère d'information d'Akaike de second ordre, c'est-à-dire corrigé en fonction de la taille du jeu d'observation (AICc) lorsque le jeu de donnée en question est petit (< 40 observations). Puisque c'est notre cas, nous avons décidé d'utiliser ce critère. Ainsi, à partir du modèle global, la procédure élimine la variable qui provoque la baisse la plus marquée de l'AICc lorsque qu'elle est retirée. L'élimination se poursuit et s'arrête dès qu'aucune diminution supplémentaire de l'AICc n'est possible.

1.7 Organisation de la thèse

Cette thèse est constituée de cinq autres chapitres, dont quatre constituent le corps du document. Ceux-ci ont été rédigés de façon à être autonomes puisqu'ils étaient ou sont destinés à être publié en tant qu'articles dans des revues spécialisées avec comité de lecture. Le chapitre deux a déjà été publié dans la revue Dendrochronologia (Dufour et Morin 2007). Il s'agit d'une note technique faisant état d'une nouvelle façon de modéliser le développement cellulaire dans le cambium. Pour les trois chapitres suivants, la méthode statistique décrite ci-haut s'applique. Le chapitre 3 décrit le synchronisme du début et de la fin de la production des trachéides et explique les variations observées en fonction des variations climatiques. Un lien avec la phénologie des bourgeons terminaux y est également établi et le tout est publié dans la revue Tree Physiology en tant qu'article original (Dufour et Morin 2010). Dans le chapitre 4, on établi l'importance du nombre de trachéides produites pour expliquer les variations de la largeur du cerne chez l'épinette noire et on fait la découverte des facteurs climatiques limitant ce nombre. La même chose est effectuée pour le diamètre des trachéides dans le chapitre 5. On y fait également une analyse détaillée de la dynamique de son développement au cours de la saison. La thèse s'achève sur une conclusion générale (chapitre 6).

2. FOCUSING MODELLING ON THE TRACHEID DEVELOPMENT PERIOD - AN ALTERNATIVE METHOD FOR TREATMENT OF XYLOGENESIS INTRA-ANNUAL DATA.

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2.1 Summary

Intra-annual repeated micro-sampling of the developing tree ring is getting more and more applied in xylogenesis studies. Variability in growth magnitude, notably due to different sampling positions on the stem, encouraged application of standardisation and modelling techniques. Among these, methods using Gompertz equation had become widely spread, but tests made with black spruce revealed a frequent occurrence of crossovers between the cumulative number of cells in enlargement and the cumulative number of cells in wall thickening. This was due to a localized problem in the fitting for values close to the asymptote and was a major problem for estimating the timing of each individual cell development phases, which is an interesting application of these data. In this paper, a new method, based on a different approach, has been developed in order to avoid that problem and applied to intra-annual growth curves from 4 sites in Quebec (Canada). Since tracheid development analysis allows discriminating between active and inactive period of a phase, modelling can be restricted on the active period alone. The new method did not cause crossovers between the fitted curves. Therefore, it has been considered appropriate for estimating the timing for each individual cell in the whole range of data. Since resulting functions are polynomials from degree 1 to 3, possible studies concerning general tendency should be easy to lead. Also, the method has been tested with different sampling frequencies. To do this, number of observations from weekly samplings has been halved to simulate a semi-monthly sampling frequency and a comparison of the results from the new method applied on each version of the datasets has been tested. Generally, the simulated semi-monthly sampled dataset did not give significantly different results from the original weekly sampled dataset, in terms of general tendency and predicted intercept time in the extremities of the data range. This is very encouraging for situations when only semi-monthly sampling is available.

Keywords: Micro-sampling, growing season, intra-annual growth modelling, conifers, *Picea mariana*.

2.2 Introduction

Repeated micro-sampling of the developing tree-ring is a good method to study xylogenesis phases throughout the growing season (Antonova and Stasova, 1993, 1997; Deslauriers et al., 2003). However, cell counts for each phase (cell division, radial enlargement, secondary wall thickening and maturity) is not a guarantee of a suitable dataset, since inherent variability due to different sampling positions can hamper analysing efforts. For instance, when variability among samples is higher than cell number increase, an unreal decreasing in cell number in some periods of the growing season can appear (Skene, 1969; Deslauriers and Morin, 2005). Dataset variability can be reduced with different methods of standardization (Whitmore and Zahner, 1966; Skene, 1969; Deslauriers et al., 2003; Rossi et al., 2003), but none seems to completely eliminate decreases. Therefore, authors have used fitted functions to represent the seasonal trend of growth. Rossi et al. (2003) introduced a method using three cumulative curves describing tracheid development (number of tracheids emerged in the enlargement phase, wall thickening phase and mature state). This method is similar to other ones proposed by former authors (Whitmore and Zahner, 1966; Skene, 1969; Denne, 1971; Wodzicki, 1971; Kutscha et al., 1975; Wodzicki and Zajqczkowski, 1983) with the difference that Rossi et al. (2003) used fitted Gompertz functions to represent the cumulative datasets. According to them, modelling cell development phases supports two purposes: it gives a representation of the annual general trend of differentiation phases and allows to calculate the approximate timings of those phases for each individual tracheid. The first purpose seems to be well achieved with the Gompertz function, as mentioned by many authors (Camarero et al., 1998; Mäkinen et al., 2003; Rossi et al., 2006).

However, problems are prone to arise with s-shaped functions concerning the second purpose, which is the evaluation of timing for each individual cell. We observed that crossover between two Gompertz curves (the one for emergence into cell enlargement and the one for emergence into wall thickening) can happen near the asymptote even though the observed values do not show any crossover and the convergence toward the same asymptote has been fixed (Rossi et al. 2003). Other researchers have also observed the same phenomenon (Deslauriers, pers. comm.). When a crossover occurs, the timing of differentiation of the few last cells, which in *Picea mariana* could represent up to 20 % of a ring $^{\text{(unpublished data)}}$, cannot be evaluated. This could become an important issue for studies about timing of cell development (Whitmore and Zahner, 1966; Skene 1968; Wodzicki, 1971; Deslauriers et al., 2003), especially when latewood cells are specifically studied. Therefore, the calculation of the time spent in each phase by all the tracheids of a row requires a good fit in the whole range of data. This is more demanding than evaluating the general tendency for a cell development, for which particular fit in the extremities can be neglected since it does not importantly influence the general rate of the function.

The story of fitting s-shaped curves in biology concerned many kinds of studies, for instance: population dynamics (Frontier and Pichod-Viale, 1998), annual agricultural plants development (Venus and Causton, 1979), over years cumulative tree growth (height, diameter, volume, etc.; Zeide, 1993) and intra-annual cell counts without distinguishing the different tracheid development phases (Camarero et al., 1998). All those situations shared a common point, as they generally implied an unknown time for the end of growth. In such cases, using an s-shaped curve was highly justified: the function allowed for the description of the whole range of the sampled period. When studies involve cell counting for different tracheid development phases, a fundamental difference appears: fairly exact time for the beginning and end of each phase is known, thus the time when growth rate is 0 is fairly well defined. Only the active period pattern remains to be represented. This can become the basis for a new approach of cell development phase modelling, which could be stated as restricting the fit on the observations representing the active period. Developing a new method for fitting a function to tracheid development phases is one of the main objectives of this paper.

Whatever the modelling approach used, an important issue remains, that is precision of the dataset of observations according to sampling frequency. Of course, the more frequent the sampling, the closer to reality the fitted function will theoretically be. Also, since fitting is not very reliable in the extremities of the dataset, precision of the fitted model becomes particularly important in the beginning and end of the development range of a modelled phase. A good way to evaluate the fitting in the extremities according to sampling frequency is to compare observed and predicted values. Usually, sampling on a weekly basis is considered to be good enough by most of the researchers, and is among the highest sampling frequency related in the literature. But when sampling interval cannot be so short, due to logistical or economical constraints, quality of the dataset could be questionable. In this paper, we will test the new proposed method with weekly and semi-monthly sampling frequencies.

2.3 Materials and methods

2.3.1 Study areas

The study took place in the boreal forest of Quebec (Canada). Four permanent plots disposed along a latitudinal transect have been sampled. These are, from south to north, Simoncouche (Sim: 48°13.78' N; 71°15.18' W), Bernatchez (Ber: 48°51.55' N; 70°20.34' W), Mistassibi (Mis: 49°43.92’ N; 71°56.88’ W) and Daniel (Dan: 50°41.78' N; 72°11.03' W). Each plot is installed on even-aged, mature, closed and pure black spruce (*Picea mariana* (Mills.) BSP) stands. The trees, established 120-140 years ago, are growing on gentle slopes (8 to 17 %) and moderately to imperfectly drained glacial tills.

2.3.2 Sampling

In the course of the growing season, sampling has been carried out on five to ten dominant trees per plot. A single micro-core was taken from the stem of each tree at intervals ranging from 3 to 15 days using bone marrow sampling needles, extracting cores about 1 mm in diameter and up to 20 mm long (Deslauriers et al. 2003). Coring points have been disposed along a counter-clockwise elevating spiral centered at breast height (1.3 m). Spacing between points was at least 3 cm horizontally and 2 cm vertically, which have been observed to be enough to avoid prior sampling trauma (resin ducts formation).

Sampling has been made in years 2002 to 2005, from the middle of May until the middle of October.

2.3.3 Sample processing and xylogenesis data collection

Micro-cores have been air-dried or dehydrated in alcohol, then embedded in paraffin and cut with a rotary microtome. Sections (7 to 12 μm thick) have been stained using 0.15% *cresyl violet acetate* filtered solution, and then gently stretched with a needle, pulling on the bark to unfold the cambial zone cells which have been compressed during coring. Observations have been made at oil-immersed 500 X magnification using a microscope disposing of polarised light. Cells in different developmental zones of the tree ring in formation have been counted on three radial files considering the following criteria:

- Cambial zone: thin walled, small and flattened box-shaped cells in the outer side of the developing tree ring (Skene, 1969; Antonova and Stasova, 1993, 1997). That includes cambial initials as well as dividing phloem and xylem cells (Wilson et al., 1966; Larson, 1994).

- Radial enlargement xylem cells: cells situated inward from the cambial zone, visually determined to be roundly shaped and clearly larger than cambial cells (Kutscha et al., 1975; Antonova and Stasova, 1993, 1997).

- Secondary wall thickening zone: cells situated inward from the enlarging ones that show birefringence under polarized light (Kutscha et al., 1975; Riding and Little, 1984; Abe et al., 1997) and violet cell walls (Antonova and Shebeko, 1981).

- Maturity zone: cells situated inward from the wall thickening ones and showing uniform deep blue cell walls (Antonova and Shebeko, 1981).

2.3.4 Standardization

Numbers of cells in each zone have been respectively named n_c, n_e, n_w and n_m. The three last were standardized following the method described in Rossi et al. (2003) to account for the variability due to different sample positions around the stem. However, as a basis for standardization, the tree ring width of the preceding year has been used instead of the previous ring number of cells. The reason behind this change is that correlation between two successive years was generally higher for ring width than for cell number. Then data from every single tree were averaged for each sampling day and site and the following sums were computed:

$$n_{ewm} = n_e + n_w + n_m$$

$$n_{wm} = n_w + n_m$$

2.3.5 Restricting the datasets

Data for n_c, n_e, n_w and n_m have been investigated in order to select active period observations for n_{ewm}, n_{wm} and n_m. The general idea behind the criteria is: a cumulative representation of a development phase is in active period when the actual state for both its corresponding and generating phases (the preceding) shows a non-zero number of cells[1]. The patterns are graphically represented in Figure 2.1. Thus, n_{ewm} starts to increase when n_e increases over 0, and stops as soon as n_c achieves its minimum value. In a same manner, n_{wm} starts to increase when n_w increases over 0, and stops as soon as n_e decreases down to zero. Finally, n_m stops as soon as the actual number of cells in wall thickening decreases down to zero.

2.3.6 Defining the total number of cells

As mentioned before, the proposed fitting method requires that the observed values outside the active period were removed. Since that includes time when the total number of cells (C_{tot}) is achieved, this value cannot be evaluated by the regression. Therefore, the average of the observations of n_{ewm} representing an achieved total number of cells, has been used instead (Fig. 2.1, cross-shaped markers). Since cells are discrete entities, C_{tot} was rounded to the closest unit.

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[1] Except for the number of cells in the cambial zone whose minimum value is not zero (typically 4 for *Picea mariana* in Quebec boreal forest).

2.3.7 Regression

Polynomial functions, from degree 1 to 3, have been fitted on the restricted datasets using Jump In™ software (JMP, release 5.1), which estimates equations by standard least square method. Only the best solution has been kept, according to four selection criteria.

Since fitting process ignores the existence of a total number of cells to achieve, it can happen that polynomials from degree 2 and 3 never reach C_{tot}. In that case, the concerned functions were immediately eliminated.

Fitting in the extremities of the active period range is a major concern of the method and therefore, it is an important criterion evaluated the following way. Day of the first or last observation selected as part of the active period is compared with the intercept day of the function at 0 and C_{tot} respectively. The fit for the intercept day at 0 is considered ideal when it is lying within the sampling interval defined by the days of the first active period point and the preceding one, which is always the last to be 0 cell valued. Similarly, the fit for the intercept day at C_{tot} is ideal when it is lying in the sampling interval defined by the days of the last active period point and the following one, which is always the first to represent an achieved C_{tot}. Then, if the intercept day is lying in a different sampling interval than the ideal one, the goodness of fit is considered decreasing along with the number of intervals separating away the predicted intercept day with the ideal interval. This procedure is efficiently made graphically.

R^2 was high for most of the possible solutions. Therefore, it was not considered a good criterion for selecting the best solution to keep. However, if the preceding criteria do not allow for a selection of one best solution, the one with the highest R^2 can be chosen.

Each set of three functions (n_{ewm}, n_{wm} and n_m), chosen in every single case study, have to be plotted to check for possible crossovers between the three curves. If any crossover occurs, the choice of the function for one or both concerned datasets must be changed, choosing the best compromise regarding the preceding selection criteria, as long as no crossover remains.

2.3.8 Comparison between weekly and semi-monthly sampling

Among the 16 case studies concerned by this paper, 10 implied a weekly sampling and have been used for the test. Each dataset of these 10 case studies has been reduced, holding alternately one sample out of two, creating this way an artificial semi-monthly sampling. Also, number of observations for evaluating C_{tot} has been reduced in order to simulate the effect of sampling frequency on the estimated total number of cells. Then, the resulting dataset has been treated following the new method described above and finally compared with the previously modelled original version of the dataset, using statistical methods. First, each curve for the semi-monthly sampled dataset has been checked for its inclusion inside the 95% confidence interval of the corresponding fitted function for the weekly sampled dataset. Second, the effect of the sampling interval has been evaluated with 3 paired t-tests, one for each three different parameters: C_{tot} value, intercept day at 0 cells and intercept day at C_{tot} cells.

2.4 Results and discussion

2.4.1 Restricting the datasets

Since criteria for selecting the active period observations involve the values of n_c, n_e, n_w and n_m, the standard deviations of these datasets have been investigated. For three of them, namely n_e, n_w and n_m, standard deviation showed a very sharp decrease when averaged cell count is under one. This low dispersion near zero gives a lot of significance to the selection since the criteria demand an evaluation of the day when cell count increases over, or reaches down zero. Active period selection using n_c is not that reliable because standard deviation at the minimum number of cambial cells (about 4) can be as high as 1.5. It means that the definition of the end of the active period for n_{ewm}, which is the day when n_c decreases back to its minimum value, is submitted to high variability and consequently its determination can be deceiving. Figure 2.1 gives a good example for this: the indicated day

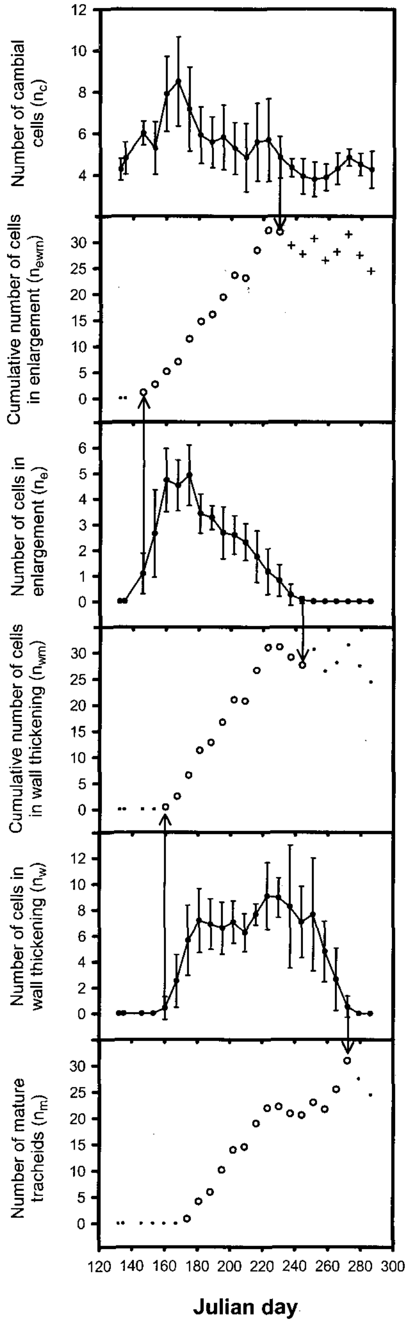

Figure 2.1. Example showing the selection process for the observations of n_{ewm}, n_{wm} and n_m that are to be regressed (open circle markers). Plain circle markers for n_c, n_e and n_w represent observations along with their respective standard deviation (vertical bars). Large open circle markers for n_{ewm}, n_{wm} and n_m represent observations selected as part of the active period of each dataset, and small plain circle markers represent the unselected ones. Cross-shaped markers are unselected observations for n_{ewm} indicating an achieved C_{tot} and serving for the evaluation of this parameter.

for the end of activity for n_c is not determined as clearly as it could be for n_e, n_w and n_m, for which difference between zero and non-zero points is very sharp. However, the following guidelines can help avoid a selection out of any biological sense:

- Since every differentiating tracheid sequentially undertake enlargement and wall thickening, n_c active period last point cannot happen after n_e active period last point.
- To avoid a determined observation for end of cell production that could be earlier than reality, tendency of the number of cells emerged into cell enlargement (n_{ewm}) should be regarded. To do so, fitting an s-shaped curve is very useful. If the chosen observation for a n_c that has reached down the minimum value happens in a day when n_{ewm} still shows a clear increase, then it should be replaced by one occurring later.
- Although this procedure is subjective in the second statement, ambiguity did not concerned more than one sampling interval in the present study. Hence, consequences of this subjectivity was adding or retrieving no more than one point for model fitting and this did not cause important changes in the fitted functions at the end.

2.4.2 Fitting a function

Table 2.1 lists the different case studies and the fitted functions for each dataset along with their corresponding adjusted R^2. Since those are generally very high, it is concluded that the models give a good representation of the active period. The most frequently chosen degree of polynomial was the 1st and 2nd, while the 3rd has been chosen only two times. Of all those different possibilities of function type and rate tendency, the 2nd degree polynomial with a decreasing rate is probably the only one that could match a Gompertz function since this one is mostly represented by its decreasing part (Frontier and Pichod-Viale, 1998). This accounts for only 27 % (13 out of 48) of all the fitted functions, and no n_{ewm} model is included in that proportion. Therefore, systematic use of a Gompertz function to model black spruce tracheid development is not justified by the method

Table 2.1: Fitted functions (t is time in julian days), adjusted R^2 and sampling interval length for the different cases studied.

Case study	Sampling interval (weeks)	Dataset		
		n_{ewm}	n_{wm}	n_m
Sim 2002	1	$-53.6 + 0.351\,t$ $R^2 = 0.960$	$-65.7 + 0.399\,t$ $R^2 = 0.943$	$-117 + 0.901\,t - 0.00137\,t^2$ $R^2 = 0.930$
Ber 2002	1	$-31.5 + 0.198\,t$ $R^2 = 0.929$	$-36.5 + 0.215\,t$ $R^2 = 0.950$	$-35.9 + 0.191\,t$ $R^2 = 0.985$
Mis 2002	1	$-9.39 - 0.0510\,t + 7.21 {*} 10^{-4}\,t^2$ $R^2 = 0.956$	$-47.4 + 0.276\,t$ $R^2 = 0.963$	$-31.1 + 0.174\,t$ $R^2 = 0.887$
Dan 2002	1	$-29.9 + 0.190\,t$ $R^2 = 0.959$	$-38.4 + 0.224\,t$ $R^2 = 0.969$	$-60.1 + 0.402\,t - 4.62 {*} 10^{-4}\,t^2$ $R^2 = 0.979$
Sim 2003	1	$-57.5 + 0.394\,t$ $R^2 = 0.989$	$-201 + 1.82\,t - 0.00355\,t^2$ $R^2 = 0.960$	$-744 + 9.46\,t - 0.0396\,t^2 + 5.6 {*} 10^{-5}\,t^3$ $R^2 = 0.945$
Ber 2003	1	$31.1 - 0.542\,t + 0.00228\,t^2$ $R^2 = 0.973$	$-47.8 + 0.295\,t$ $R^2 = 0.916$	$-35.2 + 0.205\,t$ $R^2 = 0.859$
Mis 2003	1	$-20.1 + 0.0865\,t + 3.81 {*} 10^{-4}\,t^2$ $R^2 = 0.950$	$-127 + 1.13\,t - 0.00223\,t^2$ $R^2 = 0.975$	$-650 + 8.62\,t - 0.0380\,t^2 + 5.65 {*} 10^{-5}\,t^3$ $R^2 = 0.950$
Dan 2003	1	$-2.85 - 0.116\,t + 9.54 {*} 10^{-4}\,t^2$ $R^2 = 0.979$	$-49.0 + 0.300\,t$ $R^2 = 0.964$	$-38.4 + 0.222\,t$ $R^2 = 0.984$
Sim 2004	1	$21.0 - 0.565\,t + 0.00284\,t^2$ $R^2 = 0.982$	$224 + 1.91\,t - 0.00347\,t^2$ $R^2 = 0.965$	$-111 + 0.811\,t - 0.00102\,t^2$ $R^2 = 0.971$
Ber 2004	2	$41.9 - 0.611\,t + 0.00222\,t^2$ $R^2 = 0.949$	$-61.8 + 0.341\,t$ $R^2 = 0.991$	$-45.6 + 0.238\,t$ $R^2 = 0.990$
Mis 2004	2	$-30.8 + 0.200\,t$ $R^2 = 0.973$	$-106 + 0.856\,t - 0.00143\,t^2$ $R^2 = 0.998$	$-74.4 + 0.526\,t - 7.08 {*} 10^{-4}\,t^2$ $R^2 = 0.987$
Dan 2004	2	$-2.17 - 0.0910\,t + 6.97 {*} 10^{-4}\,t^2$ $R^2 = 0.996$	$-48.7 + 0.268\,t$ $R^2 = 0.998$	$-36.9 + 0.189\,t$ $R^2 = 0.989$
Sim 2005	1	$-46.3 + 0.336\,t$ $R^2 = 0.954$	$-135 + 1.19\,t - 0.00208\,t^2$ $R^2 = 0.969$	$-110 + 0.897\,t - 0.00140\,t^2$ $R^2 = 0.963$
Ber 2005	2	$-56.4 + 0.375\,t$ $R^2 = 0.999$	$-106 + 0.851\,t - 0.00120\,t^2$ $R^2 = 0.997$	$-95.8 + 0.711\,t - 9.38 {*} 10^{-4}\,t^2$ $R^2 = 0.992$
Mis 2005	2	$-36.2 + 0.246\,t$ $R^2 = 0.992$	$-47.1 + 0.295\,t$ $R^2 = 0.991$	$-31.2 + 0.190\,t$ $R^2 = 0.899$
Dan 2005	2	$-46.1 + 0.319\,t$ $R^2 = 0.983$	$-136 + 1.23\,t - 0.00239\,t^2$ $R^2 = 0.983$	$-36.1 + 0.221\,t$ $R^2 = 0.951$

presented in this paper, particularly regarding the cumulative sum of cells that undertook enlargement.

Similarly as Rossi et al. (2003) made with Gompertz functions, the resulting polynomial functions can be inversed to express the time in function of the cell rank, which facilitates the calculation of the timing and duration of phases for each individual cell:

- Time for emergence into cell enlargement, $t_e = f(n_{ewm})$
- Time for emergence into secondary wall thickening, $t_w = f(n_{wm})$
- Time for maturity achievement, $t_m = f(n_m)$

An example is presented in figure 2.2, along with the resulting general form of equations from 1st and 2nd degree polynomials. Then, from the inversed forms, duration of enlargement and wall thickening phases for each ith cell can be calculated respectively as:

$$e_i = t_w - t_e$$

$$w_i = t_m - t_w$$

Overall timing of cell enlargement (Δe) or cell wall thickening (Δw) can also be calculated, each being delimited by the day when the first cell emerged into the phase, and the day when the last cell emerged into the following step (figure 2.2).

2.4.3 Comparison between weekly and semi-monthly sampling

Results show that 24 out of 30 curves fitted on the simulated semi-monthly sampled dataset are included inside the 95 % confidence intervals of the corresponding functions fitted on the weekly sampled dataset (Fig. 2.3). For the other 6, the confidence intervals for both the functions for the semi-monthly and the weekly sampled dataset are overlapping, so they are also considered having no significant difference between them.

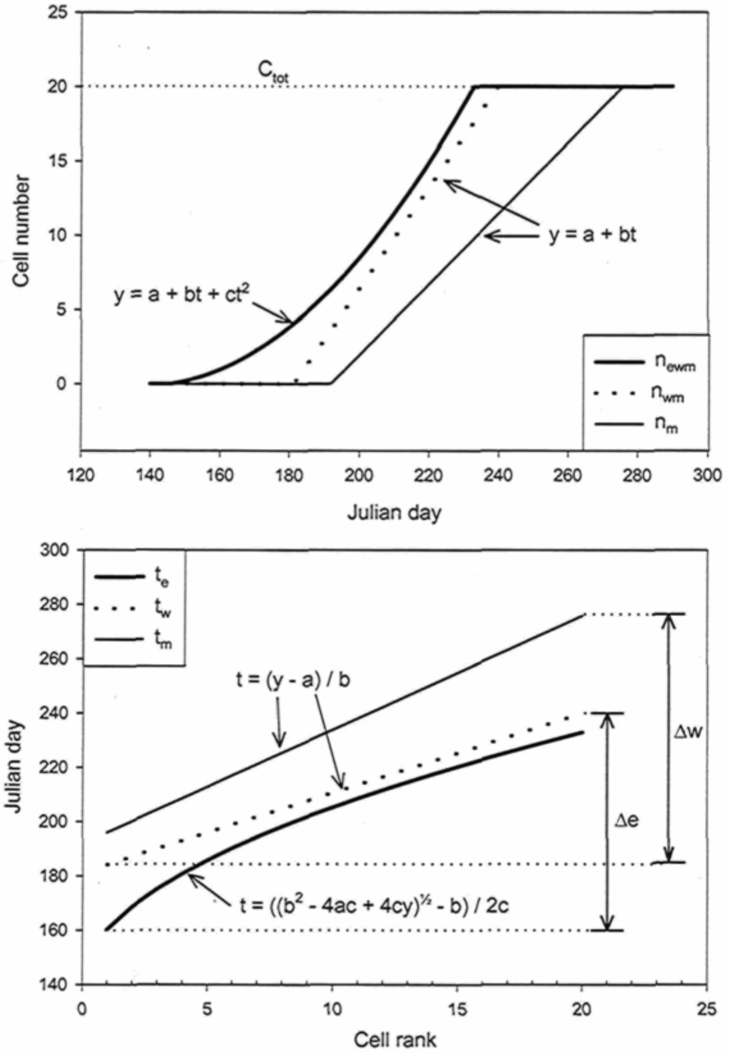

Figure 2.2. Example of the resulting functions for one case study (Ber 2004) in the fitted (n_{ewm}, n_{wm} and n_m) and the inversed (t_e, t_w and t_m) forms. Functions are developed only along the functional range of the case study, which is delimited by 0 and C_{tot}. General equations are given for each function type (1st and 2nd degree polynomials) and the overall phases range are illustrated (Δe) and (Δw). In the functions, y is the cell number and t is the time and a, b and c are function parameters.

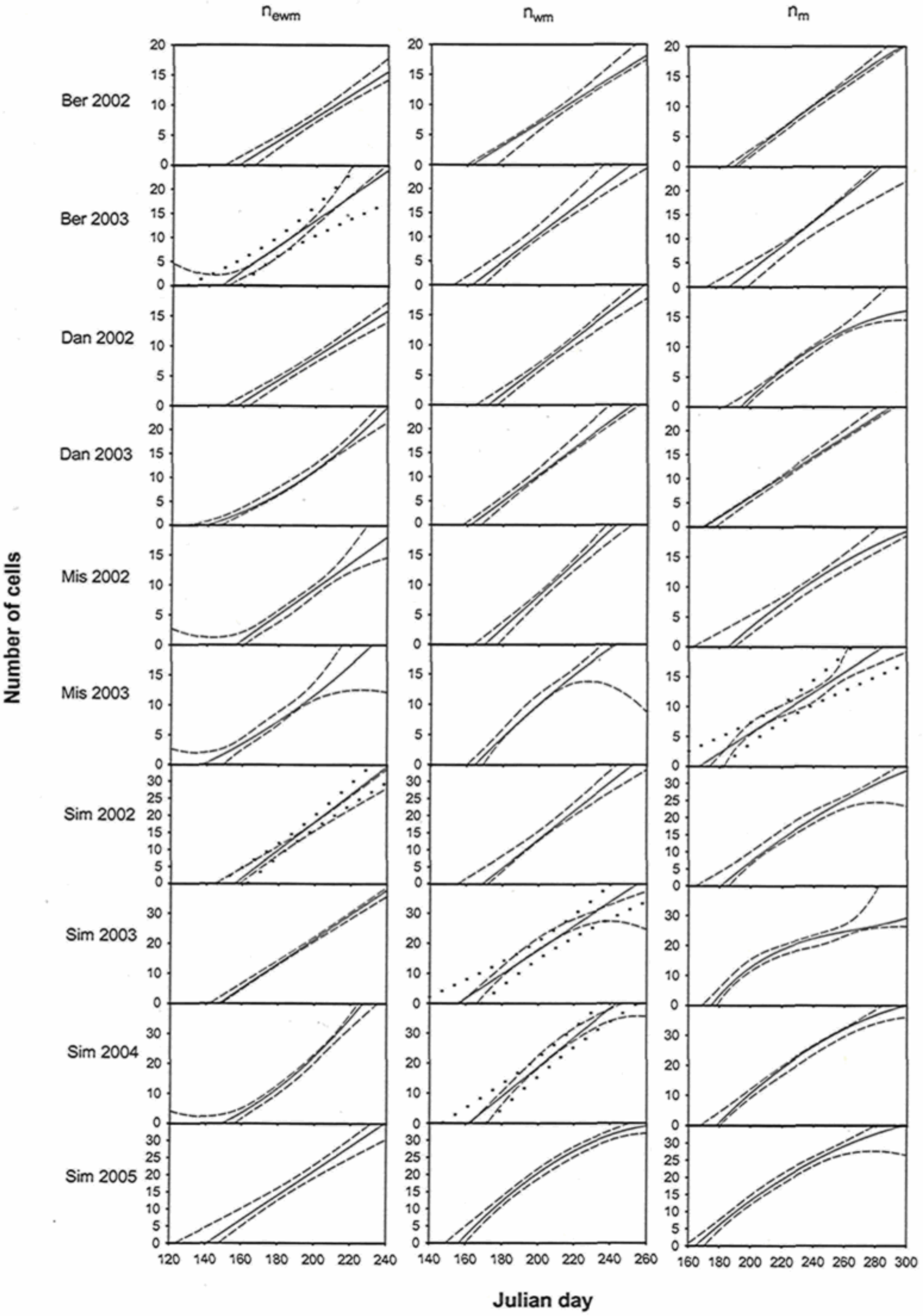

Figure 2.3. Comparison between fitted function for simulated semi-monthly sampled dataset (plain lines) and the corresponding confidence interval (95 %) fitted on weekly sampled dataset (dashed lines). When inclusion is not in the whole range, confidence interval for the semi-monthly fitting is also shown (dotted lines) to check for overlapping with the one for the weekly fitting.

Three paired t-tests have been run. Since there was two versions of 10 different case studies, t-test for C_{tot} implied 10 pairs, and since there was 3 different datasets for each case study, t-test for 0 and C_{tot} intercept day implied each one 30 pairs. Since all the tests are not significant ($P > |t| = 0.69$ for C_{tot}, $P > |t| = 0.36$ for intercept day at 0 and $P > |t| = 0.21$ for intercept day at C_{tot}), it is concluded that weekly samplings do not bring significant improvements compared to semi-monthly samplings in terms of predictive capacity of the functions at the extremities of active period range. Despite that, it should be noticed that for 6 cases out of 60, the predicted dates were different from more than an absolute value of 7 days, most of them being predicted end. This reference of 7 days is arbitrary but corresponds to the length of a sampling interval. Thus, important differences in the predicted dates between the two sampling frequencies are still possible, but only occasional. Furthermore, 1 case out of 10 showed a crossover between n_{ewm} and n_{wm} fitted functions for the simulated semi-monthly dataset, but this was not observed for its corresponding weekly version. That suggests a possible problem that could be occasionally encountered when using a semi-monthly frequency. Despite that, the overall good results for the simulated semi-monthly sampled dataset bring confidence in the results of the real case studies treated in the preceding part.

2.5 Conclusion

This paper describes a new method for xylogenesis phases data treatment. This method shares an important feature with other ones previously developed: it gives a representation of the general tendency for the phase development cumulated data.

The function fitting is restricted to observations representing the active period of each phase. Elimination of the crossover between the curves indicates that the fit is more balanced for all the cells in the ring compared to the former use of the Gompertz function. Thus, the main advantage is that timing of development phases is reliable for every cell of an averaged radial file across a ring. Also, a test using simulated semi-monthly sampling shows that the method is useful in cases when only a reduced number of samples is available.

A disadvantage of the method, compared to fitting an s-shaped non-linear model, is that more data managing is necessary. However, a clever use of modern software functionalities can help a lot to work efficiently.

According to those perspectives, the method gives an alternative to the worker interested by individual cell timing, but it does not bring any clear advantage when only the general tendency is a concern.

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3. TRACHEID PRODUCTION PHENOLOGY OF *PICEA MARIANA* AND ITS RELATIONSHIP WITH CLIMATIC FLUCTUATIONS AND BUD DEVELOPMENT USING MULTIVARIATE ANALYSIS.

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3.1 Summary

Research on cambium phenology in trees and its limiting factors in natural conditions is still at an early stage of development, restricting our capacity to precisely evaluate the effect of growing season length and climate fluctuations on tracheid production. The first objective of this paper was to describe cambial tracheid production phenology of black spruce. Repeated tree ring sampling was performed from 2002 to 2006 on four sites (48°13.78' N, 71°15.18' W; 48°51.92' N, 70°20.57' W; 49°43.92' N, 71°56.88' W and 50°41.78' N, 72°11.03' W) representative of closed black spruce forest in Quebec, Canada. Timing of cambial initiation and cambial cessation in black spruce differs from year to year, the first occurring on the 4th of June on average whereas the second occurs on August 15. During a single year, these events do not vary significantly in space within the study area. The duration of cambial tracheid production does not vary significantly in either time or space. The second objective of this study was to identify the climatic factors that explain variations in initiation and cessation. Air temperature and humidity, soil temperature and water content, rain precipitations, snow cover as well as photosynthetically active radiation were monitored at each studied site. These were then used to create sets of candidate regressors to explain timing of phenological events. Timing of cambial initiation is primarily dependent on mean temperature between mid-March and initiation itself. Vapor pressure during this period is also important, but in a negative way. A significant effect of previous year's August soil and air temperature conditions suggests a link with spring bud activity resumption, an interpretation that is supported by an analysis significantly linking measured timing of bud break to cambial initiation. Cessation of cambial tracheid production is influenced by factors linked to photosynthesis during the period from mid-July to cessation. Those related to water status, namely saturation vapor pressure, soil water content and vapor pressure are particularly influential, but light intensity and soil temperature also have an effect. Also, because mid-July corresponds to the timing of bud set and because the previous late summer's soil temperature has a significant effect, a clear link is established with apical cessation.

Keywords: boreal forest, cambium, climate changes, ecophysiology, multiple regression, repeated tree ring sampling.

3.2 Introduction

Black spruce (*Picea mariana* (Mills.) BSP) is a rather small and slow-growing, but widely-distributed and commercially valuable conifer tree of North America. In Quebec, it dominates in the major part of the commercial boreal forest, hence playing an important ecological and economic role. Climate change will likely alter the timing and length of the black spruce tracheid production season, so consequences on growth can be expected, and thus on the competitive capacity (Murray et al. 1994, Kramer et al. 2000) and also on timber yield and quality. It is therefore relevant to know not only the timing of tracheid production over the years and across the territory but also to determine which factors are limiting and how. Timing could subsequently be included in growth analysis to evaluate its significance, which could help to estimate the consequences of climate change on growth or to improve yield predictions.

Despite the fact that substantial work has been done on bud phenology, there are still few ecophysiological studies aiming to explain or predict cambial growth initiation and cessation. The precise link between bud and cambial phenology and their synchronism at the beginning and end of the growing season still remain unclear. While some authors believe in a rather tight control of cambium by terminal buds (Heide 1974a, Heide 1974b, Kozlowski & Pallardy 1997), others seem to agree with a more or less pronounced independence of these meristems (Schmitt et al. 2004, Rossi et al. 2009). If cambial initiation can be locally and independently stimulated in spruce species (Oribe et al. 2003, Gričar et al. 2006), probably due to cambial over-wintering auxins (Egierszdorff 1981, Little & Wareing 1981), it seems that only buds can provide a sufficient amount of hormones to support a sustained dividing cambium (Barnett & Miller 1994, Kozlowski & Pallardy 1997).

If cambium is controlled by buds, it could be hypothesized that cambium initiation is not only influenced by spring conditions but also by the previous year's late summer conditions since this can influence bud dormancy intensity (Chuine & Cour 1999). Concerning growth cessation, the ecophysiological response depends on the growing mode of the plant material. In trees whose growth season finishes with a free growing phase, photoperiod is commonly considered to be the triggering factor for cessation of terminal (Wareing 1950a, Pollard & Ying 1979) as well as cambial activity (Heide 1974a, O'Reilly & Owens 1989). In this case, for trees growing in their native photoperiod, cessation timing should be influenced only by conditions prevailing during the delay between the triggering signal and the response (Coursolle et al. 1998, Johnsen & Skrøppa 2000). But when a tree exhibits an exclusively determined shoot growing mode, like mature black spruce (Logan & Pollard 1975), an influence of the previous year's late summer conditions should be suspected. This is due to development of the terminal shoot primordium in the bud, which occurs after active shoot elongation and then influences the following year's cessation as this latter cannot happen before a complete predetermined shoot elongation (Wareing 1950b, Heide 1974b, Lanner 1993). Better environmental conditions during black spruce bud maturation, like warm temperature, high soil moisture and good nutrition result in a more developed primordial shoot (Pollard & Logan 1979, Colombo 1986), leading to a longer apical development (Cannell et al. 1976) and a possibly longer cambial activity.

Research on climate influence as a cause of tree phenological fluctuations in natural conditions is still relatively new (Larcher 2003). So far, studies have involved a few different climatic variables or just air temperature alone. However, this kind of assessment does not represent a very realistic evaluation since trees grow in complex ecological contexts that expose them to many fluctuating factors simultaneously (Grossnickle 2000, Larcher 2003). Moreover, climatic variables are prone to inter-correlation (Fritts 1976, Briffa 1999). This means that correlating a growth parameter with a single climatic factor leads to a coefficient that results not only from the single effect of the concerned climatic variable but also from a part of what is due to its collinear links with the other variables (Neter et al. 1990, Draper & Smith 1998, Briffa 1999, MacNally 2000). These considerations are particularly true when studying trees growing in climate conditions far from any adaptive extreme. In these situations, univariate links are difficult to establish and/or account for a small part of growth variations (Fritts 1976, Pensa et al. 2006). It can therefore be supposed that measuring many climatic parameters and analyzing their influence in a multivariate statistical context would be more appropriate.

The first objective of this paper is to characterize the timing of tracheids production initiation and cessation for mature black spruce growing in mesic stand conditions. The second objective is to identify climatic factors explaining the timing of these events and how they relate to known bud phenology influences. We hypothesize that these climatic factors are: soil temperature, water availability, air temperature, air humidity and light intensity. The influence of these factors on spruce growth or other physiological responses has been demonstrated experimentally, as reported in reviews (Lamhamedi & Bernier 1994, Grossnickle 2000), and this is considered sufficient justification to hypothesize an influence on phenology. Also, these factors taken together represent the whole operational environment of the tree (Fritts 1976), which is an important requirement motivated by full modelling, the chosen statistical approach (Burnham & Anderson 2002). Furthermore, conditions occurring immediately before each phenological event (initiation or cessation) are considered as well as those of the period of bud maturation or dormancy development in the previous year to account for the possible influence of apical activity.

3.3 Materials and methods

3.3.1 Study area and sampling plots

Efforts were made to choose sites representative of the mesic conditions prevailing in the middle of Quebec's continuous boreal forest vegetation subzone. Four permanent plots disposed along a latitudinal transect have been sampled in order to account for the climatic variability within this subzone. From south to north, two are situated in the balsam fir-white birch bioclimatic domain: Simoncouche (Sim: 48°13.78' N; 71°15.18' W) and Bernatchez (Ber: 48°51.92' N; 70°20.57' W). The other two are situated in the spruce-moss bioclimatic domain: Mistassibi (Mis: 49°43.92’ N; 71°56.88’ W) and Daniel (Dan: 50°41.78' N; 72°11.03' W). Each plot is installed on even-aged, mature, closed and pure black spruce stands. The trees, established 120-140 years ago, are growing on gentle slopes (8 to 17%) and moderately (3 plots) to imperfectly drained glacial tills (1 plot: Dan). Site index (mean height at 50 years of age), mean current dominant tree height and mean dbh are: 13.6 m, 16.0 m and 20.4 cm at Sim; 6.9 m, 17.6 m and 21.1 cm at Ber; 11.4 m, 18.6 m and 21.4 cm at Mis; 8.7 m, 16.8 m and 20.0 cm at Dan, respectively.

3.3.2 Assessment of in-progress cell production

All the main steps of the methods used are summarized in this section, but emphasis is placed on the differences between these and the more detailed methods given in Dufour & Morin (2007).

From 2002 to 2006, 5 dominant trees at each site were sampled repeatedly throughout the growing season to monitor tree ring formation on a weekly or bi-weekly frequency from mid-May until the initiation of the active cell production period, and thereafter on a weekly (Sim 2002-2006; Ber, Mis and Dan 2002-2003) or fortnightly basis (Ber, Mis and Dan 2004-2006) until mid-October. At each sampling, a single micro-core was taken from the stem of each tree using a bone marrow sampling needle (DBMNI-1501 inter-V medical), extracting cores about 1 mm in diameter and up to 20 mm long (Deslauriers et al. 2003). Coring points were arranged along a counter-clockwise rising spiral centered at breast height (1.3 m). Spacing between points was at least 3 cm horizontally and 2 cm vertically, which has been observed to be enough to avoid resin ducts formation due to prior sampling trauma.

Micro-cores were processed using standard histological methods involving dehydration in alcohol, paraffin embedding and cutting with a rotary microtome. Staining was done with 0.15% *cresyl violet acetate* filtered solution. Observations were made while the sections were gently stretched under a polarized light-equipped transmission microscope to unfold the tissues compressed by coring, making cell types easy to distinguish (for pictures, see Thibeault-Martel et al. 2008). Cells (tracheids) were then counted in each of the following developmental zones: cambial zone, radially enlarging, and enlarged (includes the ones that undergone lignification and mature ones).

The counts for enlarging cells and already enlarged cells were standardized to account for variability due to different sampling positions around the stem (Deslauriers et al. 2003, Rossi et al. 2003). Each count was therefore multiplied by the ratio of the mean ring width of the previous ring of all samples taken on the tree in the same year, divided by the ring width of the previous ring specific to the counted sample.

Mean values from each site and year have been computed by averaging counts from the five trees. The two standardized counts for enlarging and enlarged cells were summed to create datasets of the number of tracheids produced. We fitted a linear and a quadratic model to the summed dataset and kept only the solution showing the best fit. The fit was restricted to the points where tracheid numbers increase (excluding zero and asymptotic observations), so the fit is specifically focused on the period of tracheid production. The first observation of this period is the first when a non-zero number of cells is observed in enlargement, and the last one is the last before the number of cambial cells returns to its base value (4 ± 1 cells).

The timing of the observation points alone can serve directly as estimates of the phenological timing (Thibeault-Martel et al. 2008), but precision and reliability depend on the observation interval. In this study, observations were sometimes two weeks apart and this was judged as being too long to use observation timing directly. Instead, we used extrapolations from the functions fitted as described above. Two pieces of timing information have been extracted from them. First, the day on which the function achieves a value of one cell gives the day of tracheid production initiation (variable TP_{init}). Second, the day on which the function achieves the total number of cells, determined as the mean value of the asymptotic observations excluded from the fit, is the day of tracheid production cessation (variable TP_{cess}). However, the use of this procedure for TP_{cess} is conditional to an extrapolation lying between the two observation points encompassing the period when cell production ceased. In 5 out of 20 cases, TP_{cess} evaluation fell outside the time period defined by the observation points, so for these cases, the timing of the closest of these corresponding observations has been used.

3.3.3 Assessment of bud break

For 3 years at Sim and 2 years at Dan, bud development in spring was monitored on 5 trees per site, which were different from those sampled for cambium phenology. On each tree, a twig was harvested using a clipper fixed at the tip of a 9 m pole for a total sampling height of 10 m, corresponding roughly to one-third to one-half of the crown. Terminal branchlet buds were then observed and bud break was determined as the time when needles were distinguishably free from bud scales (Colombo 1986). Sampling was repeated once or twice weekly. Bud break was revealed to be a fast and clear development stage. Consequently, two patterns have been observed. The first is when two successive samplings showed respectively 0 and 100% broken, within as well as between trees, so the middle day between the sampling dates was determined as the day of bud break. In the second pattern, only one intermediate sampling showed some level of partition between unbroken and broken buds on the sampled twigs either within or between trees. When that was observed, day of bud break was directly determined by the date of this partitioned sample.

3.3.4 Meteorological monitoring and climatic variability

As mentioned earlier, sites were chosen to be representative of the mesic closed black spruce forest, but the spatial layout was also planned to assess a representative climatic variability within the study area. To assess this variability, a weather station was installed in a gap (φ 20 m) on each site. Each system was controlled by a CR10X datalogger (Campbell Scientific®) to which weather sensors were connected (one per parameter) that monitored the following parameters:

- Air temperature, 2 m above ground in the gap

- Relative humidity, 2 m above ground in the gap
- Incident Photosynthetically Active Radiation (PAR) at 8 m high in the gap
- Air temperature, 2 m above ground, under cover
- Humus temperature (~ 5-15 cm below surface)
- Soil upper mineral layer temperature (~ 25-35 cm below surface)
- Soil water content (TDR), upper 30 cm layer
- Precipitations
- Snowpack depth

Measurements were taken every 5 minutes; hourly total (precipitations only), minima/maxima (both air temperature) and means (all measurements except precipitations) were recorded. From this hourly record, daily total, minima/maxima and means have been computed. Compilations made from this daily defined database are described in the next sections.

In all sites during the studied period, the warmest month of the year was July while the coldest was January (Figure 3.1). Yearly mean temperature was, from South to North: 1.8, -0.1, 0.7 and -1.3 °C. Monthly mean temperature was typically above zero from May to October in all sites, but also in April at Sim, the warmest site. During this period, monthly total rain precipitations are, in general, gradually more abundant from south to north. Total precipitations from May to October ranged from 511 mm (Sim) to 655 mm (Dan). The thickest snow accumulations are found in the coldest (Dan) and the highest (Ber) sites, and peaks at 90 (Sim) to 120 (Ber) centimeters in March. Snowpack usually melts almost completely in april at Sim and in May at the other sites.

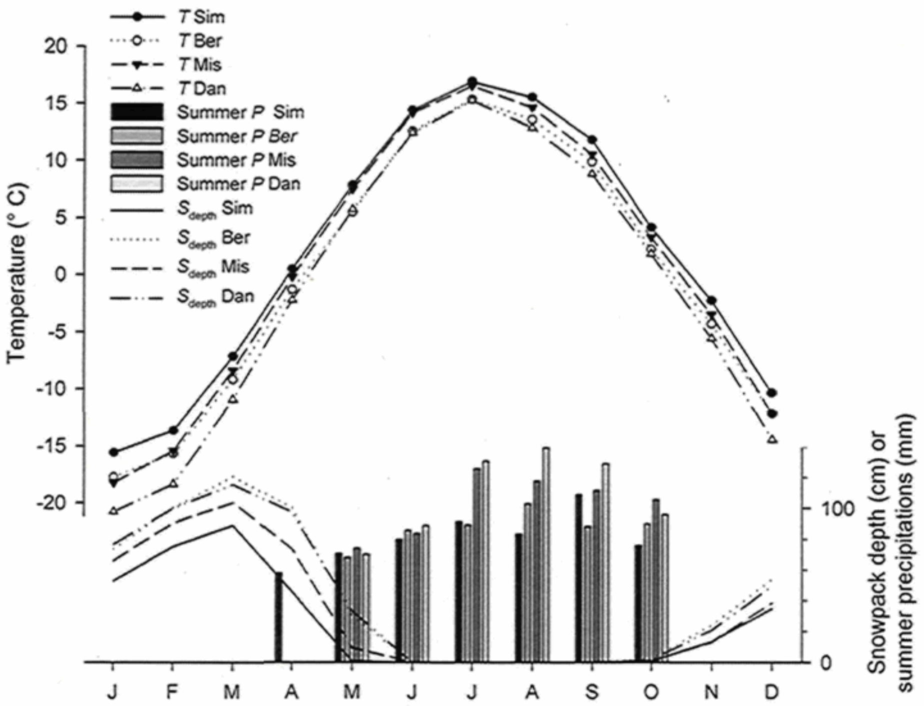

Figure 3.1. Monthly mean Temperature (*T*), total precipitations of months with mean temperature over 0 °C (Summer *P*), and mean snowpack depth (S_{depth}) from 2002 to 2006 for each site.

3.3.5 Modeling method

Since each site has its own weather station, observations can be considered as independent among sites, so all the analyses have been done pooling observations from years and sites.

The statistical approach used is a full model, which means inclusion of all the regressors (factors) that are mechanistically expected to influence the response. These full models are known to be the most effective to decipher the real effect of a factor in a multivariate context, i.e. regression coefficients are not biased (MacNally 2000, Burnham & Anderson 2002, Whittingham et al. 2006). Basically, the factors tested are in accordance with the hypothesis stated in the introduction: soil temperature, water availability, air temperature, air humidity and light intensity. However, the basic 5 factor set is repeated for each time period considered, which are time just preceding the phenological event, and time of shoot primordia development during the preceding season. Once factors had been chosen, we proceeded to an optimization of the full model. Thus candidate variables, often more than one per factor, have been computed (table 3.1). All the possible models including every factor but changing the representing variable for each one were computed and the solution with the lowest AIC has been kept.

Full models are not parsimonious, which means they consider all the variables put in the model to be important. But since we were interested in identification of the most important variable, and because overfit was a concern, a parsimonious final solution was reached. To do this, the full model with k parameters is first compared to all possible models having one factor less. If any of these k-1 parameters models shows an AIC reduction compared to the k parameters one, then the model with the lowest AIC is selected. This procedure is repeated at least until the number of regressors represents no more than one third of the number of observations, and then until no further AIC decrease can be obtained.

Table 3.1. Check list of candidate regressors tested for their influence on various phenological response variables of different nature and period of influence. Regressors are all means computed from daily data and covering periods specific to each model. Response variables abbreviation: BB, bud break; $I_{\mathrm{adv}\%}$, daily percentage of advancement to tracheid production initiation; $C_{\mathrm{adv}\%}$, daily percentage of advancement to cessation of tracheid production.

Regressors			Response variable and climate influence period				
Factor type	Analogous variable (units)	Abbre-viation	BB Current year	$I_{\mathrm{adv}\%}$ Current year	$I_{\mathrm{adv}\%}$ Previous year (August conditions)	$C_{\mathrm{adv}\%}$ Current year	$C_{\mathrm{adv}\%}$ Previous year (Aug-Sept conditions)
Air humidity	Vapor pressure (kPa)	VP		✓	✓	✓	✓
Light	Photosynthetically active radiation (μmol/s.m²)	PAR		✓	✓	✓	✓
Air tempe-rature	Saturated vapor pressure (kPa)	SatP	✓	✓	✓	✓	✓
	Temperature (°C)	T	✓	✓	✓	✓	✓
	Maximum temperature (°C)	T_{max}	✓	✓	✓	✓	✓
	Minimum temperature (°C)	T_{min}	✓	✓	✓	✓	✓
	Degree-days over 0 °C	DD>0	✓	✓			
	Degree-days over 2 °C	DD>2	✓	✓			
	Degree-days over 4 °C	DD>4	✓	✓			
	Degree-days over 6 °C	DD>6	✓	✓			
	Undercover temperature (°C)	T_{uc}	✓	✓	✓	✓	✓
	Maximum undercover temperature (°C)	$T_{\mathrm{uc,max}}$	✓	✓	✓	✓	✓
	Minimum undercover temperature (°C)	$T_{\mathrm{uc,min}}$	✓	✓	✓	✓	✓
Soil tempe-rature	Humus temperature (°C)	T_{hu}		✓	✓	✓	✓
	Mineral soil temperature (°C)	T_{mn}		✓	✓	✓	✓
Water availabi-lity	Soil water content (%)	SWC		✓	✓	✓	✓
	Precipitations (mm)	P			✓	✓	✓
Snow cover	Snowpack depth (m)	S_{depth}		✓			
Timing of initiation	Initiation of tracheid production (day of year)	$\mathrm{TP}_{\mathrm{init}}$	✓			✓	

3.3.6 Regressors compilation and response variables transformation

Climatic variables have been compiled as mean daily values for the period lasting between an arbitrarily fixed starting day, common to all observations, and the day of the phenological event itself (i.e. TP_{init} or TP_{cess}), which is specific to each observation. However, many possibilities for the starting day have been compared and selection of the best was made by choosing the one leading to the best model, in terms of statistical fit (adjusted R^2). The resulting best starting day defines what we called the period of climate influence (of a given phenological stage). Other candidate variables, belonging to the previous year's conditions (table 3.1), are also included in each iteration fitting process but are constant (i.e. not the object of differential period compilation) among the iterations.

Neither TP_{init} nor TP_{cess} have been used directly as the response variable in their respective analyses. The reason for this is that building a model with regressors, compiled according to observation-specific periods, to explain the variable that also makes these periods fluctuate, is a tautology. If it does not inhibit the possibility to judge the most important factors, it clearly prevents any prediction of the model, because knowing the right period for compilation would necessitate having *a priori* knowledge of what has to be predicted (i.e. phenological timing). Since we preferred to also have a predictive utility for our model, response variables have been transformed as follows. Throughout the period of climate influence, which ends with the phenological stage occurrence, the underlying process can be considered to evolve from 0 to 100%. Then, dividing 100 by the duration (in days) gives the mean daily advancement percentage of that process. This transformation of TP_{init} and TP_{cess} results in the response variables 'Initiation Daily Advancement Percentage' ($I_{adv\%}$) and 'Cessation Daily Advancement Percentage' ($C_{adv\%}$) respectively. Having a model that predicts a daily phenological advancement percentage by daily meteorological conditions allows the timing of the phenological event that comes at the end to be predicted, since it happens when the summed daily advancement reaches 100%. This procedure is only valid with strictly linear models. Our models meet this requirement since we model with multiple linear regression and apply no non-linear transformation to regressors or response.

3.3.7 Comparing the influence of each important factor

As mentioned by Quinn & Keough (2002), the partial regression coefficients are differently scaled so they do not allow straightforward comparison of the effect of each factor, nor does their standardized version (standard beta) since these are biased by collinearity. To efficiently assess the relative contribution of each significant variable, a hierarchical partitioning analysis (Chevan & Sutherland 1991) has therefore been done using the hier.part package (MacNally & Walsh 2004) running on $R^{©}$.

3.4 Results

3.4.1 Timing of tracheid production and bud break

Modeling the observed in-progress cell production allowed an evaluation of the times when cell production started and ceased. The fitted functions, showing R^2 values ranging from 0.91 to 0.99 with a mean of 0.97, have been used to extrapolate cambium phenology events (figure 3.2a).These have also all been averaged by sites and by years (figure 3.2b and 3.2c).

The mean dates for TP_{init} and TP_{cess} were day 153 (2nd of June) and day 227 (15th of August) respectively, and the mean length of cell production was 74 days. During the assessed years, variability of TP_{init} was clearly higher between years than between sites as revealed by standard deviations (figure 3.2b); the year-to-year variations at a single site are generally higher than the site-to-site variations during a single year. Indeed, variations among years led to a higher standard error and a significant Kreuskal-Wallis test (P = 0.0097), but a non-significant one for variations among sites (P = 0.3594). Overall variations of cessation were weakly significant among years (P = 0.059), and not significant among sites (P = 0.8231).

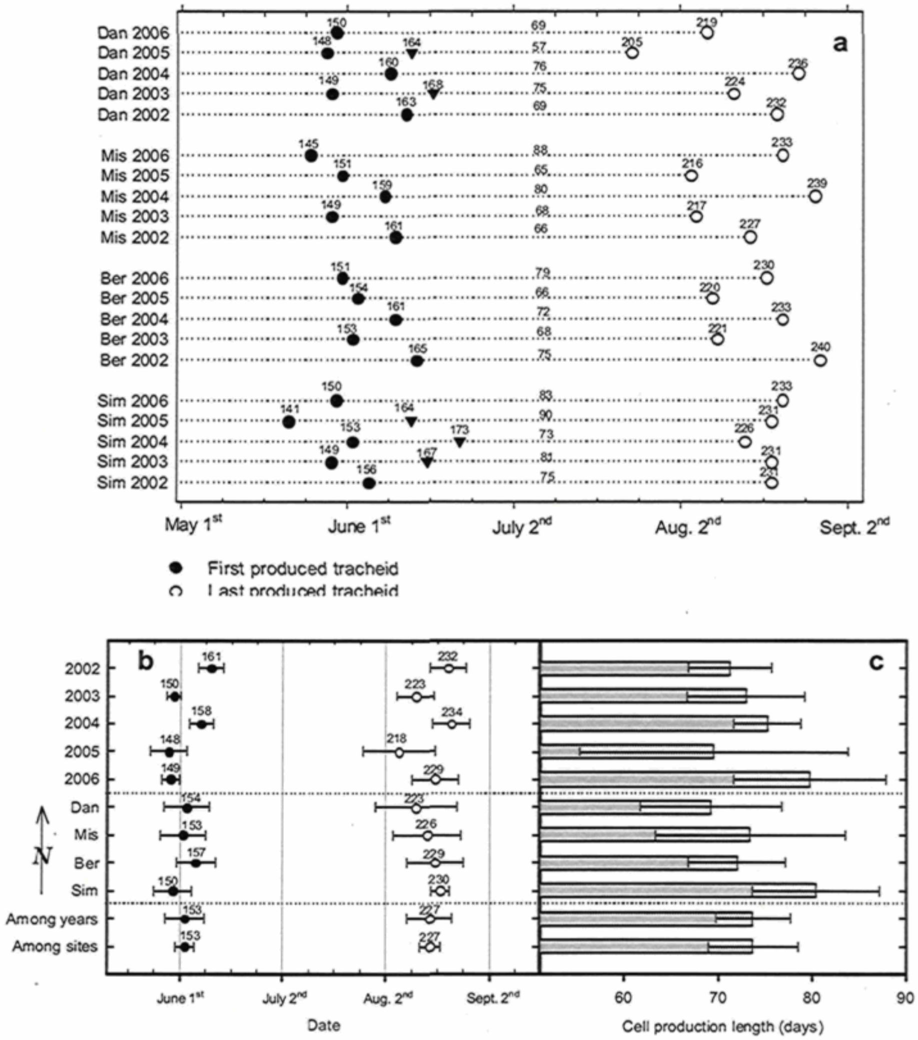

Figure 3.2. Timing of cambial and apical phenology (a) giving: tracheid production initiation, tracheid production cessation, bud break, all three with symbols tagged on day of year. Duration of tracheid production is also given by tags above dotted lines. Cambial initiation and cessation have been compiled for every year (mean of 4 sites), every site (mean of 5 years) and then re-averaged for overall variation across years and sites (b), and the same has been applied to duration (c). Error bars are standard deviation for within years and sites mean but are standard error for 'among' terms.

The pattern for TP_{cess} is similar to TP_{init}: at Ber, Mis and Dan together, an early initiation was followed by an early cessation (figure 3.2a, $P = 0.01$, $r = 0.64$), but Sim diverges from that rule, since it shows a non-significant, negative tendency. Nevertheless, cell production duration (figure 3.2c) was constant among sites ($P = 0.1995$), and among years ($P = 0.3244$).

The timing of bud break has been assessed for 5 cases. We fitted a multiple regression model (table 3.2) to explain bud break variations by $\mathrm{TP}_{\mathrm{init}}$ and mean undercover temperature (T_{uc}) for periods specifically adjusted between $\mathrm{TP}_{\mathrm{init}}$ and bud break. T_{uc} was selected as the best fitting variable from the same air temperature candidates as those used for tracheid production initiation (Table 3.1). The result is a weakly significant test ($\alpha = 0.1$, Table 3.2) with both regressors being significant since the model including both of them has the lowest AIC.

Table 3.2. Multiple regression ANOVA and model coefficients of the multiple regression computed for bud break timing vs. timing of tracheid production initiation ($\mathrm{TP}_{\mathrm{init}}$) and undercover temperature (T_{uc}).

R^2	0.9024	
Observations	5	
F Ratio	9.2519	
Prob. > F	0.0975	
	Coefficient	Coef. Std. Dev.
Intercept	107.4166	46.2655
$\mathrm{TP}_{\mathrm{init}}$	0.5430	0.2861
$T_{\mathrm{uc}}{}^{1}$	-1.6383	0.6530

[1] observation-specific daily mean of the period between $\mathrm{TP}_{\mathrm{init}}$ and bud break.

3.4.2 Influence of climatic variations on timing of tracheid production initiation

Iterations of the modeling process for $I_{\mathrm{adv\%}}$ have been made with a 7 days lag of the starting day, so there was 12 repetitions using starting days ranging from day 55 to day 132. Table 3.1 lists the current year variables, which have been compiled according to the different periods of time, along with variables for the previous late summer, always represented by August conditions.

The best starting date was determined by choosing the one that gave the best fit between the specific selected regressors and $I_{\mathrm{adv\%}}$, using adjusted R^2 as criterion (figure 3.3). Day 76 (17th of March) revealed to be the best estimation of the starting day. The corresponding multivariate regression is detailed in Table 3.3.

Table 3.3. Multiple regression ANOVA, climatic variables' mean and standard deviations, model coefficients and their standard deviation, and percentage of independent effect ($\mathrm{IE}_{\%}$) given by hierarchical partitioning of the best multiple regression found for daily percentage of advancement to tracheid production initiation ($I_{\mathrm{adv\%}}$) vs. daily climatic variables.

R^2	0.8813
Observations	19
F Ratio	25.9849
Prob. > *F*	<.0001

Regressor[1]	Mean value	Intersite Std Dev	Interannual Std Dev	Coefficient	Coef. Std Dev.	$\mathrm{IE}_{\%}$
Intercept	—	—	—	3.0126	0.2738	—
T	1.3413	1.0662	1.3116	0.1281	0.0176	47.7
VP	0.5124	0.0360	0.0360	-3.8575	0.5799	25.2
$T_{\mathrm{uc,min,aug\text{-}1}}$	8.3904	0.7878	0.8014	-0.0384	0.0172	19.9
$T_{\mathrm{hu,aug\text{-}1}}$	11.5078	0.9135	0.3553	0.0522	0.0192	7.2

[1] Regressors are basically observation-specific daily mean compiled from day-of-year 76 to tracheid production initiation. Subscript 'aug-1' indicates previous year August mean daily conditions. For units see table 1.

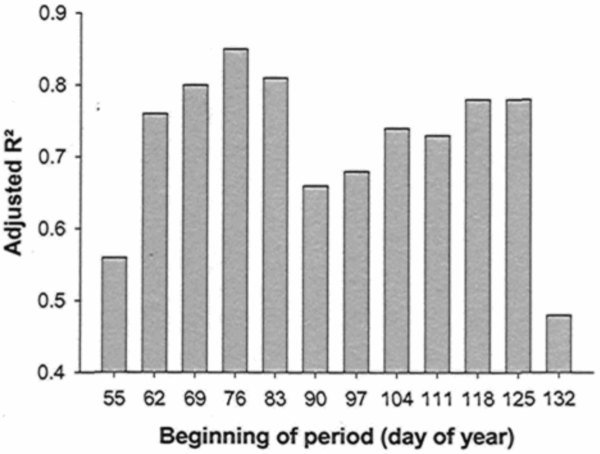

Figure 3.3. Adjusted R^2 for 12 iterations of the modeling process (see methods) for daily percentage of advancement to tracheid production initiation ($I_{\mathrm{adv}\%}$) in function of climatic variables. In each iteration (bar), the length of the considered period for climate influence is changed by modifying its beginning day, in such a way for climate and response variables to be differentially compiled for each of these periods. All models are significant ($\alpha = 0.05$).

Residual plots were checked, but no obvious tendency appeared and Shapiro-Wilk normalcy test was non-significant ($P = 0.9594$), thus residuals are normal and show no obvious heteroscedasticity. No autocorrelation remains once climate effect is removed so the assumption of independence of errors is met (Draper & Smith 1998, Legendre & Legendre 1998, Quinn & Keough 2002).

The relative importance of each isolated effect is expressed by the percentage of independent effect ($\mathrm{IE}_{\%}$) given from hierarchical partitioning, so the parameters are classified in descending order of importance in table 3.3. By far the most influential factor is T, being responsible for almost half of $I_{\mathrm{adv}\%}$ explained variations, while VP accounts for about a quarter. Higher mean daily temperature in spring promotes growth initiation, whereas increasing vapor pressure has a retarding effect. The previous year's late summer conditions account for the last quarter of the explained variance as growth initiation is delayed by higher undercover minimum temperature in August but promoted by warm humus temperature.

The daily probability of reaching a temperature above 0 °C (figure 3.4) indicates that day 76 occurs at the beginning of a period when the probability of reaching non-freezing temperatures shows a regular increase from values close to 0 towards values around 1.

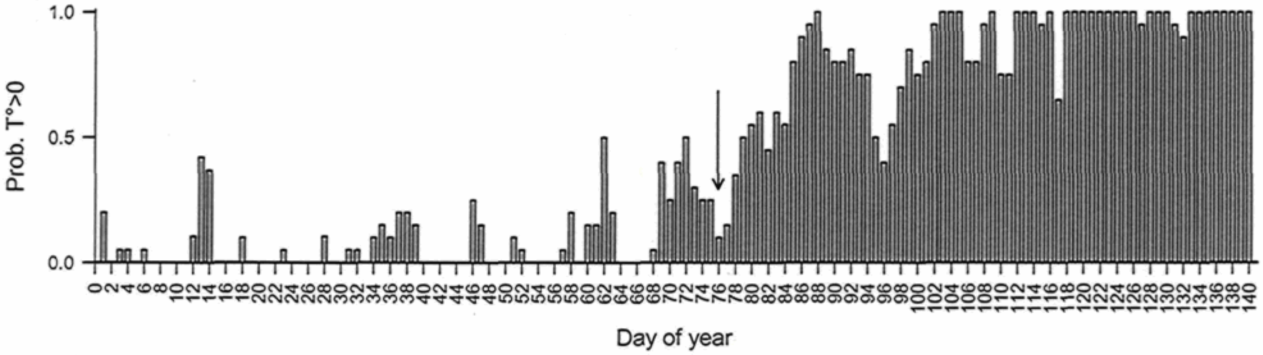

Figure 3.4. Daily probability for daily mean temperatures (T) over 0 °C to occur. These have been calculated by dividing the number of observed occurrence of T > 0 over all observed cases (20), on the four sites from 2002 to 2006. The arrow points to the starting day of the best fit period of climate influence as previously determined (day 76).

3.4.3 Influence of climatic variations on timing of tracheid production cessation

Factor types and their respective analog variables tested for their influence on tracheid production cessation are displayed in table 3.1. Only the end of the period during which the cessation process operates has been measured (corresponding to TP_{cess}). We therefore proceeded once more with progressive duration modeling, varying the starting date. First, we put the starting date back lagging 4 days, in steps ranging from day 202 back to day 174. The lag was then lengthened to 7 days for the period extending back to day 118, this second series extending before the summer solstice (figure 3.5) to investigate for a possible influence of photoperiod on the triggering of TP_{cess}.

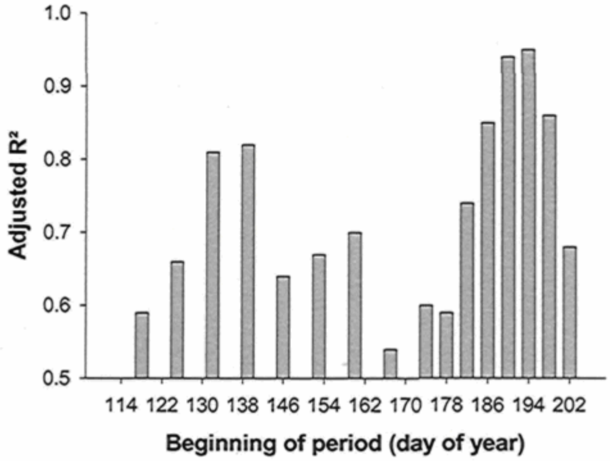

Figure 3.5. Adjusted R² for 16 iterations of the modeling process (see methods) for daily percentage of advancement to tracheid production cessation ($C_{adv\%}$) in function of climatic variables. In each iteration (bar), the length of the considered period for climate influence is changed by modifying its beginning day, in such a way for climate and response variables to be differentially compiled for each of these periods. All models are significant ($\alpha = 0.05$).

The period showing the best fit begins on day 194, considered as a ± 3 days evaluation (consequent to the lag of 4 days) of the signal triggering the growth cessation process. The lag between day 172 (summer solstice) and day 194 corresponds, from the southernmost to the northernmost site, to 21 to 23 minutes diminution of the photoperiod (Lammi 2008).

The best model is shown in table 3.4. Residual plots showed no heteroscedasticity. Spatial autocorrelation of residuals is not significant ($r = -0.06$, $P = 0.8318$), indicating no real spatial autocorrelation once climate is taken into account.

Table 3.4. Multiple regression ANOVA, climatic variables' mean and standard deviations, model coefficients and their standard deviation, and percentage of independent effect ($IE_{\%}$) given by hierarchical partitioning of the best multiple regression found for daily percentage of advancement to tracheid production cessation ($C_{adv\%}$) vs. daily climatic variables.

R^2		0.9684				
Observations		20				
F Ratio		66.3089				
Prob. > F		<.0001				
Regressor[1]	Mean value	Intersite Std Dev	Interannual Std Dev	Model Estimate	Model Std Error	$IE_{\%}$
Intercept	—	—	—	0.2492	1.3981	
SatP	1.8943	0.1228	0.0884	13.9474	1.6004	40.5977
$T_{mn,LS-1}$	9.0865	0.8470	0.2757	-1.8227	0.1606	16.6046
SWC	53.4962	10.3770	7.3330	-4.3187	1.0424	16.4567
PAR	353.0513	43.5584	43.1139	-0.0469	0.0041	14.9993
VP	1.4000	0.0768	0.0675	-13.3251	1.9559	7.4295
T_{hu}	11.6258	0.8848	0.5482	0.6396	0.1485	3.9121

[1] Regressors are basically observation-specific daily mean compiled from day-of-year 194 to tracheid production cessation. Subscript 'LS-1' indicates previous year late summer (August-September) mean daily conditions. For units see table 1.

Mean daily saturation vapor pressure, a function of temperature, is the most influential factor, and has an accelerating effect on the cessation process. Some other significant factors have a retarding effect, reducing the daily advancement of the cessation process: previous late summer's mineral temperature and current year soil water content, PAR and vapor pressure. Humus temperature is the least significant factor and shows a stimulating effect on the process, hence resulting in an earlier growth cessation.

3.5 Discussion

3.5.1 Modeling methods

The motivation for pooling observations from sites and years comes from our aim to draw conclusions that are valuable in time and within the whole study area. The factors included in the models are shared in terms of spatial and temporal dominance of their variability: some are more variable in time, others in space and some are quite even (tables 3.3 and 3.4). Therefore, averaging either sites or years would probably have neglected important variability and resulted in overfitted models, i.e. a too-specific fit to be generalized in space or time (Ginzburg & Jensen 2004, Whittingham et al. 2006). Also, making sure that the whole extent of variation belonging to the study context reduces the risk of coefficient biases, because shortness of data is harmful to coefficient evaluation and this is exacerbated by collinearity (Belsley 1991).

The starting day for climate influence, has been evaluated by comparing many arbitrarily fixed days because we had no data assessing the beginning and its variability in time or space. That this variability is high or low does not inhibit the possibility of using the best compromise given by the best fit as the starting day for climate compilation. But having no variability information means we are unable to draw any conclusion about the constancy of the starting day.

3.5.2 Timing of tracheid production

The general rule concerning TP phenology could be that initiation and cessation are synchronous over the covered territory but variable over years. Despite spatial statistical dependence of phenological responses, initiation and cessation have been significantly related to climate fluctuations, and nothing of this dependence remains in the residuals, so there is no real autocorrelation (Legendre & Legendre 1998).

Duration is not significantly different between sites, nor between years. Constant duration is thus due to a relative synchronism between initiation and cessation. The synchronism is probably not caused by a real dependence of cessation on initiation (see below), but rather by covariance of the factors influencing both events. Indeed, among the 24 possible correlations that could be computed from the 4 and 6 factors influencing initiation and cessation respectively, 10 are significant ($P < 0.05$), including the one between the most influential factor for each, i.e. temperature from day 76 and saturation vapor pressure from day 194 ($P = 0.0136$, $r = 0.56$). Nevertheless, some extreme durations have been observed, as duration ranges from two to three months. Thus, large but occasional variations of duration may occur.

3.5.3 Tracheid production initiation: link with bud initiation and climate.

For the hypothesis of a bud controlled cambium initiation (Rensing & Owens 1994, Kozlowski & Pallardy 1997, Grossnickle 2000) to be proved, it would be best to directly observe if bud mitosis initiation is, at the very least, concomitant with or prior to cambium mitosis initiation. We did not evaluate bud mitosis initiation, but bud break instead. It is consistent to consider that bud break happens 4 to 6 weeks after mitosis initiation (Owens & Simpson 1988, Westin et al. 1999), so that the fact that bud break happens after cambium initiation (Owens & Simpson 1988, O'Reilly & Owens 1989, Westin et al. 1999, Rossi et al. 2009) cannot disprove bud controlled cambium hypothesis. On the contrary, this study demonstrates that cambium initiation can be significantly related to bud break, a direct but delayed consequence of bud mitosis initiation, as long as the modulation effect of air temperature in the days prior to bud break is taken into account.

The link between bud and cambium initiation is also supported by the inclusion of the two factors representing the previous year's August conditions. The most influential of these, undercover minimum temperature, has a retarding effect on cambium initiation in the following spring. Other authors reported similar observations for spruce species bud break, and the explanation seems to be that during dormancy setting up, higher air temperature promotes a deeper rest (Heide 1974b, Granhus et al. 2009). The other previous August factor, soil temperature, has a weaker but opposite effect. High soil temperature is considered to enhance nutrition of the tree (Krause 1991, Domisch et al. 2002a), and during bud development, this produces a more expanded bud structure (Pollard & Logan 1979, Colombo & Smith 1988). Bigger buds (Nienstaedt 1966, Colombo 1986) and high fertilization (Murray et al. 1994, Fløistad & Kohmann 2004) have been associated with an earlier bud flush in the following spring.

Thus, cambium initiation is related to bud activity in spring, bud shaping in the previous year and bud dormancy setting up. Also, cambium reactivation without concomitant natural or artificial bud auxin supply has also been revealed to be either inexistent (Little et al. 1990, Barnett & Miller 1994) or only temporary and unproductive (Little & Bonga 1974, Oribe et al. 2003, Gričar et al. 2006). All these considerations taken together bring the conclusion that cambium initiation depends on shoot initiation.

Climate influence on tracheid production initiation in spring begins on day 76. Whether this reflects a varying triggering in time and space, or a constant one, is a relevant question. The concomitance with the rise of the daily probability of reaching a temperature above 0 °C (figure 3.4) supports the hypothesis of a varying triggering, since temperature is clearly, on an annual basis, a variable factor. The consequence would be that for a better initiation prediction, the time when daily mean temperature reaches 0 °C in spring should be checked instead of systematically computing from a constant day.

So far, cambial initiation has been notably related to a heat-sum approach, using a single base temperature, +5 °C (Schmitt et al. 2004, Seo et al. 2008), in a similar fashion to heat-sum applied to bud phenology (Hänninen 1990, Chuine & Cour 1999, Kozlowski & Pallardy 2002). In this study, we also assumed a heat sum dependency, but many base temperatures have been compared, all in a full model approach, the best suited one to avoid coefficient bias and evaluate the real effect of factors (MacNally 2000, Burnham & Anderson 2002, Whittingham et al. 2006). However, despite the possibility of fitting a significant model including a base temperature heat sum with our data, none has a fit as good as the one including daily mean temperature i.e. using no base temperature.

We suggest that the retarding effect of high air humidity on cambial division initiation could be linked to water conductance dynamics, a hypothesis that is also supported by the no base temperature result. Using a base temperature implicitly means that the modeled process is uninfluenced by temperatures below this base temperature, whereas a process modeled without base temperature experiences backward evolutions in subzero temperatures. The latter is consistent with the conductance dynamics because conifers experience increasing xylem cavitation following freeze-thaw cycles (Sparks et al. 2001, Pittermann & Sperry 2006, Mayr & Charra-Vaskou 2007). Altough the mechanism to recover from cavitation is unknown, it is likely driven by the same factors that create the flowing force through the xylem, i.e. high transpiration demand from the leaf, to which low vapor pressure is closely linked (Nobel 2005). Additional support to this hypothesis are that recovery occurs when the shoot is very dry (Sperry & Robson 2001) and that stomata opening in spring occurs beyond photosynthetic requirements (Day et al. 1990).

Snow cover has been tested but not considered significant, despite the work of Kirdyanov et al. (2003) that reports a positive effect of snowmelt timing on cambial initiation. Snow depth is significantly and negatively correlated to two of the explicative factors for initiation (T and $T_{hu,aug-1}$, $R^2 = 0.67$), and our method allows for a confrontation of snow cover versus these collinear factors. The result is an initiation of radial growth that is much more explained by spring air temperature and the previous year's August humus temperature than by spring snow depth alone, proving that the latter is not necessarily good as a cause.

3.5.4 Influence of climatic variations on timing of tracheid production cessation

Although we do not have any monitoring of bud set, we can draw indirect conclusions about the bud influence on tracheid production cessation. Bud set can be triggered either by photoperiod in free growing trees (Wareing 1950a, Heide 1974a, Pollard & Ying 1979, O'Reilly & Owens 1989) or by complete primordia elongation in determined growth trees (Wareing 1950b, Heide 1974b, Lanner 1993). Mature black spruce is a determined growth tree (Logan & Pollard 1975), and this study reveals that its cambium activity cessation is retarded by high late summer soil temperature in the previous year. Since no preformed, overwintering cambial structure has been observed in our material nor reported in literature, and also because late summer corresponds to the primordial shoot development period (Colombo & Teng 1992), an obvious link with bud is established. The retarding effect likely works as follows: low soil temperature limits nitrogen mineralization from accumulated organic matter (Krause 1991, Domisch et al. 2002a) and the resulting lower nutrition reduces the number of leaf primordia in the bud (Pollard & Logan 1979, Colombo & Smith 1988) which then reduces the time of apical elongation during the following growing season (Cannell et al. 1976), a tendency followed by cambium as well (Heide 1974b, this study).

The determination of day 194 as the starting day for current year climatic influence also supports the hypothesis for cambial phenology to be controlled by bud development, although it first appear to support a photoperiodic triggering since it occurs after summer solstice. In determined growth species like spruces, the first year is the only year in which apical growth cessation is doubtlessly triggered by photoperiod, since there is no possible determined growth (Heide 1974b, Cannell et al. 1976). Because first year cessation effectively happens in mid-September in natural photoperiod at our latitude (Pollard & Ying 1979, Colombo et al. 1989, Coursolle et al. 1998) and considering that the delay between a triggering signal and cessation of apical elongation is shorter than a month (Colombo et al. 1989, Bigras & d'Aoust 1992, Coursolle et al. 1998), the photoperiodic triggering signal cannot happen before mid-August. This implies a difference of at least a month from day 194, so the signal occurring on the latter is unlikely to be photoperiodic by nature. Consequently, a different kind of triggering should be considered. As mid-July corresponds to bud set timing, as observed in mature black spruce (O'Reilly & Parker

1982), the predetermined shoot elongation completion is a more convincing hypothesis to describe the nature of cambial cessation triggering signal.

Cambial division cessation response is delayed in time from the triggering signal initiating the cessation process. During the lag, climatic variations modify the timing of cessation. Air temperature is the most important factor to explain cambial cessation variations: the warmer the late summer, the earlier the occurrence of cessation. Such a hastening effect of temperature is already known for shoot elongation completion (Heide 1974a, Johnsen & Skreppa 2000). The selection of saturation vapor pressure as air temperature analog, instead of any other, reveals that temperature effect is at the very least partly linked to tree water loss. The significance and signs of soil water content and vapor pressure support this idea in a consistent way. Most reviews count water stress among the factors that can trigger shoot dormancy in trees (Kozlowski & Pallardy 2002, Arora et al. 2003). Considering also the retardant effect of higher light intensity, a consistent convergence toward photosynthesis appears. The obvious conclusion is therefore that photosynthesis promoters, i.e. those in favor of a good water status and good illumination, extend the tracheid production season in the bole.

3.5.5 Predictive utility

Altough it was not the most important objective of this study, we built the models to make them usable for predictive purpose. As the response variable predicted by the model is a daily percentage of process advancement, the day of phenological stage occurrence is the one when summed daily predictions from the starting day reaches 100 %. It is important to note, however, that this procedure is made possible only because we assumed linear relationship between climate and phenological responses. From the predicted day of tracheid production initiation, it is also possible to predict the day of bud break using the presented model for it. Making predictions has many useful applications like simulating effects of climate changes on tracheid production duration, but it would also be interesting to compare prediction for different other cases with real measurements of responses to validate the models or evaluate their scopes more precisely.

3.5.6 Black spruce tracheid production season vs climate changes

Possible effect of climate changes on tracheid production duration can be inferred from the models, even tough no simulation has been made so far. Quebec boreal forest is presently experimenting a climatic warming that could reach 2 °C in spring and summer for the period 1990-2020 (Bourque & Simonet 2007). Our model suggests that this leads to an earlier tracheid production initiation since air temperature in spring has a prevailing influence on this phenomenon. Summer temperature influence on tracheid production cessation is also important, but its direct effect for an earlier cessation could be attenuated by the concomitant warming of soil, which has a second order opposite effect. Then, both phenological stages happen earlier with higher temperature, but overall duration of tracheid production should increase because initiation is more hastened than cessation.

3.6 Conclusion

Mature black spruce growing in the heart of its native range and in moderate stand conditions has a tracheid production phenology that is fairly variable in space, but more variable in time and resulting in a rather constant duration of production. Its average initiation takes place in early June, whereas its cessation happens in mid-August.

Tracheid production phenology in the studied context is submitted to complex links with climate that can be well understood by taking into account its relationship with shoot activity. Timing of initiation and cessation is related to conditions during shoot primordia formation (bud maturation) and dormancy setting up, both occurring during the preceding year. Cambium initiation is also controlled by factors influencing bud break and, possibly, xylem conductance dynamics from mid-March until its occurrence. Cessation is hastened by high air and soil temperatures occurring between mid-July and cessation itself, but during the same period it is retarded by photosynthesis promoters, namely light intensity, soil water content and air humidity. These results have major implications for the evaluation of the precise effects of climate change on black spruce phenology. The resulting models are also very interesting tools for cambial and apical phenology predictions.

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4. PHENOLOGY-LINKED MULTIFACTORIAL ANALYSIS OF TRACHEID PRODUCTION IN THE TRUNK OF *PICEA MARIANA*.

Authors : Boris Dufour and Hubert Morin

4.1 Summary

The sum of the radial diameters of all tracheids in the radial rows of a conifer tree ring determines the whole ring width. So variations in the number of tracheids or their mean diameter can lead to ring width variations. This study aims to evaluate the relative importance of variations in the number of tracheids produced to determine ring width variations in the stem of mature black spruce growing in mesic conditions of the boreal closed forest of Quebec, Canada. Another objective is to decipher the factors explaining variations in the number of tracheids over the years and the studied area, assuming that the explanation is climatic by nature. Repeated tree ring micro-coring was performed on four sites representative of closed black spruce forest, from 2002 to 2006 to monitor cambial phenology. In 2007, four more microcores were extracted at each cardinal point of each tree to assess the number of tracheids produced each year and measure their radial diameter. Air temperature and humidity, soil temperature and water content, as well as photosynthetically active radiation were monitored at each studied site. To efficiently avoid spurious effects due to high collinearity between candidate climatic factors, a model selection approach proceeding backwards from a full model was used with AICc as the selective criterion. Results show that variations in the tracheid number are responsible for 88% of the variations in ring width whereas variations in mean tracheid diameter account for the remaining 12%. The number of tracheids produced depends on factors related to photosynthesis during tracheid production, i.e. daily light intensity and maximum temperature between the day of initiation and the day of cessation of tracheid production, plus soil temperature during August of the previous year which is an important period for nutrient uptake. Duration of the period for tracheid production is also important to consider. We conclude that climate warming should increase tracheid production in black spruce.

Keywords: boreal forest, cambium, climate change, ecophysiology, multiple regression, model selection, repeated tree ring sampling, tracheid number.

4.2 Introduction

The boreal forest fixes and stores a large stock of carbon of which tree stems account for an important part (Gaboury et al. 2009, Lambert et al. 2005). Climate change alters the physiological functions of trees and this can also modify the negative feedback that tree radial growth carbon storage has on atmospheric CO_2 (Bigras and Bertrand 2006, Huang et al. 2007). The outcome of climate change obviously varies from case to case since tree species and populations differ in their sensitivity to climate and because climate shows different magnitudes and variations in space. Black spruce (*Picea mariana* (Mills.) BSP) stands, as an important component of the eastern Canada closed boreal forest (MRNF 2009), may play an important part in the carbon cycle, and this could be magnified by its high commercial utility, since the boreal forest yields a large amount of black spruce timber that can sustainably store carbon in many durable wood products.

So far, investigations have attempted to decipher the climatic limiting factors in naturally growing trees, since growth models made from them can likely help to predict climate change effects on forest growth, yield, carbon budget and dynamic. Most of the climate-growth studies in natural conditions concerned radial growth of mature trees, typically using annual radial increment or ring width as the response variable and black spruce is not an exception (Huang et al. 2010, Tardif and Conciatori 2001, Hofgaard et al. 1999). However, conifer ring width is the result of two distinct processes, which are cambial cells radial division and tracheid radial enlargement (Larson 1994). As these two processes are separate in space and time, it is mechanistically justified to treat them separately, since they are likely influenced by different climatic factors. This study focuses on climate growth relationship using the radial number of tracheids produced as the growth response variable.

It is generally admitted that trees growing at the limits of the species range or in extreme conditions are clearly limited by a small number of factors, if not a single one, whereas those growing far from the limits of their range face a complex set of climatic factors (Pensa et al. 2006, Fritts 1976). In the latter situation, investigations of the climate-growth relationships are better supported if many parameters are monitored. Despite this, only temperature and precipitation are commonly used in climate-growth relationships studies. According to Fritts (1976), this is mainly due to the ready availability of temperature and precipitation records, but the same author recognizes that it is advisable to consider 'the entire operational environment of the tree' as factors can influence 'processes occurring at different times in the year, and in different parts of the tree.'

Studying growth response in relation to many different climatic variables should consider another important attribute of the climatic environment of the tree, which is the high collinearity between these climatic variables and its consequent coefficient biases (Burnham and Anderson 2002). To avoid these biases and their confounding effect, the recent biometrical model literature agrees on the use of the full model approach (Whittingham et al. 2006, Burnham & Anderson 2002, MacNally 2000); in other words, multiple regression including all the expected causal factors instead of simple regression.

From that point, an important question arises: what are the candidate variables for the causal factors? Three major attributes define a climate variable: the nature of the factor it represents, the timing of its operational effect and the time stretch during which the variable operates. Determination of those points is closely dependent on the hypothesis for growth explanation. The factors influencing spruce photosynthesis and growth are diversified (Grossnickle 2000, Lamhamedi & Bernier 1994). As mentioned above, black spruce in the studied context are growing far from any extreme so no particular limiting factor set by climate should be assumed. Therefore, the whole operational environment of the tree should be considered.

The timing and duration of climate influences on growth are also likely to be linked to phenology of the tree. In the typical way of assessing climate-growth relationship, monthly climate variables are used (Fritts 1976) and this means that cambial phenology is not used to build the tested hypothesis. As black spruce cambial phenology is known (Dufour & Morin 2010), it can be used to define candidate variables that are compiled to precisely fit the tracheid production period. However, there could be other influences from outside the tracheid production period. Vaganov et al. (2009) found a correlation between the $\delta^{13}C$ signature in the early wood produced in a given year and the latewood produced in the previous year. This indicates a carry-over effect between the two periods. Similarly, Dufour and Morin (2010) found an influence of some late summer factors on the cambial phenology in the following year, since the latter depends on the size of the primordial shoot which is determined during that period and occurring climatic conditions, mainly soil temperature, are influent. As a larger bud means more new needle formed during the following season (Colombo 1986, Pollard and Logan 1979), more photosynthesis and better growth could occur at tree level. Therefore, testing previous late summer conditions on the number of tracheids produced would be relevant.

The objective of this paper is to determine the causal variables explaining variations in the annual tracheid number of black spruce using the approach of full modeling of phenology-related variables.

4.3 Materials and methods

4.3.1 Study area and sampling plots

Efforts were made to choose sites representative of the mesic conditions prevailing in the middle of Quebec's continuous boreal forest vegetation subzone (Blouin and Berger 2004a,b), and to assess representative climate variability within this study area. Four permanent plots disposed along a latitudinal transect have been sampled in order to account for the climate variability within this subzone. From south to north, two are situated in the balsam fir-white birch bioclimatic domain: Simoncouche (Sim: 48°13.78' N; 71°15.18' W) and Bernatchez (Ber: 48°51.92' N; 70°20.57' W). The other two are in the spruce-moss bioclimatic domain: Mistassibi (Mis: 49°43.92’ N; 71°56.88’ W) and Daniel (Dan: 50°41.78' N; 72°11.03' W). Each plot is installed on even-aged, mature, closed and pure black spruce stands. The trees, established 120-140 years ago, are growing on gentle slopes (8 to 17%) and moderately (3 plots) to imperfectly drained glacial tills (1 plot: Dan). Site index (mean height at 50 years of age), mean current dominant tree height, mean dbh and mean commercial stem density are respectively: 13.6 m, 16.0 m, 20.4 cm and 2025 stems/ha at Sim; 6.9 m, 17.6 m, 21.1 cm and 3350 stems/ha at Ber; 11.4 m, 18.6 m, 21.4 cm and 2150 stems/ha at Mis; 8.7 m, 16.8 m, 20.0 cm and 2050 stems/ha at Dan.

4.3.2 Assessment of tracheid production phenology

From 2002 to 2006, 5 dominant trees at each site were sampled repeatedly throughout the growing season to monitor tree ring formation at weekly or fortnightly frequency. At each sampling, a single micro-core was taken from the stem of each tree using a bone marrow sampling needle (DBMNI-1501 inter-V medical), extracting cores about 1 mm in diameter and up to 20 mm long (Deslauriers et al. 2003).

Micro-cores were processed using standard histological methods involving dehydration in alcohol, paraffin embedding and cutting with a rotary microtome. Staining was done with 0.15% *cresyl violet acetate* filtered solution. Observations were made under a polarized light-equipped transmission microscope. Cells (tracheids) were then counted in each of the following developmental zones: cambial zone, radially enlarging, and enlarged (includes the ones with lignificating walls and mature ones).

The counts for enlarging cells and already enlarged cells were standardized to reduce the variability due to different sampling positions around the stem (Dufour & Morin 1997, Dufour & Morin 2010). Each count was therefore multiplied by the ratio of the mean ring width of the previous ring of all samples taken on the tree in the same year, divided by the ring width of the previous ring specific to the counted sample.

Mean values from each site and year have been computed by averaging counts from the five trees. The two standardized counts for enlarging and enlarged cells were summed to create datasets for tracheids production. We fitted a linear and a quadratic model to the summed dataset using only the points where tracheid numbers increase (excluding zero and asymptotic observations), so the fit is specifically focused on the period of tracheid production. The function with the best fit was selected and two pieces of timing information was extracted from it. First, the day on which the function achieves a value of one cell gives the day of tracheid production initiation (variable TP_{init}). Second, the day on which the function achieves the total number of cells, determined as the mean value of the asymptotic observations excluded from the fit, is the day of tracheid production cessation (variable TP_{cess}). More details can be found in Dufour & Morin (2010 and 2007).

4.3.3 Total annual tracheid count and measurements

The radial number of tracheids produced in each year was counted with four microcores per tree, extracted in 2007 from the same trees as the phenological assessment, one year after all the studied rings were completed. All microcores included rings for all the studied years. The same histological techniques as above were applied but sections were stained with Safranin and permanently mounted with Permount®. Number of tracheids along three continuous radial rows per ring was counted. Rows were chosen among the ones producing the widest cells to make the number of tracheids free from the influence of longitudinally overlapping rows (Larson 1994). Tracheids counted in the different rows were averaged for each ring of a core, for each ring of a tree and then for each year on a site.

In the modeling method (see below), radial number of tracheids is the response variable of the multiple regression while climatic factors are the regressors. As the models are made from yearly data from pooled sites, the climatic signal among sites could be impeded by non-climatic influences differentiating sites. That is why we tried to have sites uniform in age, soil, structure, and mean diameter (see above). Stem diameter (circumference) is widely recognized for its influence on radial increment: the wider the stem, the narrower the ring (Fritts 1976). This geometrical constraint is likely to influence radial tracheid number the same way. To reduce the influence of remaining difference in the mean dbh of sampled trees among and within sites, mean tracheid counts of each year on each site have been multiplied by the following site-specific standardization ratio:

$S_{ratio} = dbh_{site} / dbh_{oversite}$

where dbh_{site} is the mean dbh of the sampled trees on the considered site, $dbh_{oversite}$ is the rounded mean dbh of trees of all four sites (21 cm). Since all yearly tracheid counts of a site are multiplied by the same ratio, variation patterns are not changed, only the overall magnitude of the site is modified.

Radial diameter of every tracheid in the chosen cell rows has been measured using WinCELL Pro© 2004a. Tracheid diameter of each cell cannot be directly averaged for the whole sample and tree because cell numbers are alike but nonetheless differ between different rows, cores and trees. Standardization has been applied in order to have the same number of cells, which allows computing a mean diameter series for each year and tree. First, the cell position of each raw diameter series has been converted into relative location, which is given by the ratio between absolute location and the total number of cells in the file (x_i/Ncell). Then, a continuous diameter series has been computed by linearly interpolating diameter values between successive relative locations. The standard series has been created by attributing to each relative location of the standard series (x_i'/$Ncell_{std}$) the diameter value from the continuous series. $Ncell_{std}$ is the mean number of tracheids of the tree as determined above. There is one standard series per row, but since $Ncell_{std}$ is common to all rows from the same tree in a given year, the diameter of each cell in the series can be averaged by core and trees, in this way creating the mean standard diameter series of each tree.

4.3.4 Meteorological monitoring and climate variability

To assess climate variability, a weather station was installed in a gap (φ 20 m) on each site. Each system was controlled by a CR10X datalogger (Campbell Scientific®) to which weather sensors were connected (one per parameter) that monitored the following parameters:

- Air temperature, 2 m above ground in the gap
- Air temperature, 2 m above ground, under cover
- Relative humidity, 2 m above ground in the gap
- Incident Photosynthetically Active Radiation (PAR) at 8 m high in the gap
- Humus temperature (~ 5-15 cm below surface)
- Soil upper mineral layer temperature (~ 25-35 cm below surface)
- Soil water content (TDR), upper 30 cm layer
- Precipitations
- Snowpack thickness

Measurements were taken every 5 minutes; hourly total (precipitations only), minima/maxima (both air temperatures) and means (all measurements except precipitations) were recorded, as well as hourly mean saturation vapor pressure computed from air temperature. The latter has been multiplied by hourly relative humidity to give hourly vapor pressure estimation. Compilations made from this hourly database are described in the modeling method section.

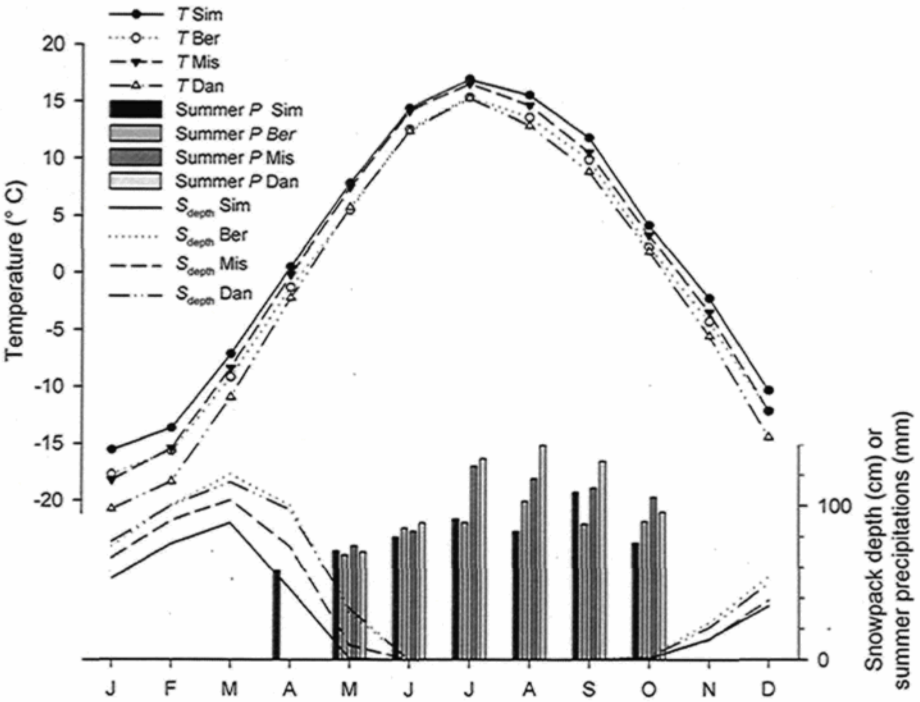

Figure 4.1. Monthly mean temperature (*T*), total precipitations of months with mean temperature over 0 °C (Summer *P*), and mean snowpack thickness (S_{depth}) from 2002 to 2006 for each site.

In all sites during the studied period, the warmest month of the year was July while the coldest was January (Figure 4.1). Yearly mean temperature was, from South to North: 1.8, -0.1, 0.7 and -1.3 °C. Monthly mean temperature was typically above zero from May to October in all sites, but also in April at Sim, the warmest site. During this period, monthly total precipitations are, in general, gradually more abundant from south to north. Total precipitations from May to October ranged from 511 mm (Sim) to 655 mm (Dan). The thickest snow accumulations are found in the coldest (Dan) and the highest (Ber) sites, and peaks at 90 (Sim) to 120 (Ber) centimeters in March. Snowpack usually melts almost completely in April at Sim and in May at the other sites.

4.3.5 Modeling radial increment

From the tree level database, (5 rings on 20 studied trees), the radial increment of the whole ring has been computed by the total of each cell radial diameter along the ring. Then, we built a multiple regression model using the number of tracheid and mean cell radial diameter along the ring to explain the response in the whole ring radial increment.

4.3.6 Modeling the seasonal tracheid production

As the context of the study suggests that growth is influenced by multiple factors, the chosen general approach for modeling is selection of a multiple regression model. However, and since many methods of multiple regression models construction exists, specifications must be given to establish the methodological details that avoid spurious effects related to multicollinearity. Full models, as long as they include all important effects, are the most efficient to avoid coefficient biases (Whittingham et al. 2006, MacNally 2000) but they are likely overfitted and produce imprecise inferences (Burnham and Anderson 2002). On the other hand, too simple ones may give more widely applicable inferences (Ginzburg and Jensen 2004), but since they are underfitted they likely show coefficient biases (Burnham and Anderson 2002, MacNally 2000). Therefore, parsimony of our models has been reached starting from a full model, i.e. the full model has been reduced gradually by removing at each step the variable that allows the best improvement of the AICc criterion (Burnham and Anderson 2002), until no improvement is observed. This approach is analogous to backward elimination of variables but using a fitting criterion instead of coefficient ANOVAs, known to suffer from the inflation of variance due to multicollinearity (Quinn and Keough 2002, Belsley 1991). The fit was performed with SAS 9.2® using the glmselect procedure.

Since each site has its own weather station, observations can be considered as independent among sites, so all the analyses have been done pooling observations from years and sites. Factors that are part of the full model are listed in table 4.1. Most of them represent climatic conditions during the two periods stated in the introduction: previous late summer and tracheid production. For each of these, five types of climatic factors are included in the full model: soil temperature, water availability, air temperature, air humidity and light intensity; i.e. the operational environment of the tree. Duration of the tracheid production period has also been included as a factor.

Each factor of the full model has to be represented by a precise variable. For some factors, more than one variable has been tested (table 4.1). Most variables are daily mean values computed from the hourly database and covering the two previously described periods. The variables for the conditions occurring during tracheid production have been compiled by observation-specific periods spreading from the day of tracheid production initiation to the day of tracheid production cessation. However, some have been compiled in such a way to describe a climate influence on photosynthesis, which is expected to limit radial growth. In the daily mean, these variables consider only the hours of above zero temperature and PAR flux (indicated by the "day" subscript in table 4.1). The variables for conditions during the previous late summer are compiled by observation-constant periods, but three possible ones have been tested. As many analogous variables may account for a single factor, all possible full models showing only one variable per factor have been computed.

Table 4.1. Checklist of candidate regressors of different type and operational period; these have been tested for their influence on the number of tracheids produced. Regressors are all means computed from daily data. TP refers to tracheid production period.

Regressors			Computed period			
Factor type	Analogous variable - standardized	Abbreviation	Previous August	Previous BegAug-EndSep	Previous MidAug-EndSep.	Tracheid production
Air humidity	Daytime vapor pressure	VP_{day}	✓	✓	✓	✓
Light	Daytime photosynthetically active radiation	PAR_{day}	✓	✓	✓	✓
Air temperature	Temperature	T	✓	✓	✓	✓
	Maximum temperature	T_{max}				✓
	Minimum temperature	T_{min}				✓
	Daytime temperature	T_{day}				✓
Soil temperature	Humus temperature	ST_{hu}	✓	✓	✓	✓
	Mineral soil temperature	ST_{mn}				✓
Water availability	Soil water content	SWC	✓	✓	✓	✓
	Precipitations	P				✓
Duration of tracheid production	Duration	D				✓

4.3.7 Weighting the contribution of variables

As mentioned by Quinn & Keough (2002), the partial standard coefficients of a multiple regression are inflated by collinearity. To efficiently evaluate the relative contribution of each variable in the models, a hierarchical partitioning analysis (Chevan & Sutherland 1991) was performed using the hier.part package (MacNally & Walsh 2004) running on R$^{©}$. Hierarchical partitioning measures the improvement in the fit of all models with a given predictor compared to the equivalent model without that predictor and the improvement in fit is averaged across all possible model with that predictor (Quinn and Keough 2002). This has been applied to rank the selected variables of the model explaining the number of tracheids produced, but also to evaluate the contribution of each constituent of ring radial increment i.e. radial number of tracheids and radial tracheid diameter.

4.4 Results

4.4.1 Number of tracheids produced and its importance for cell width

The model based on variations in the number of tracheids and in the mean tracheid diameter explains perfectly ($R^2 = 1$, not shown) the response in ring radial increment. This is not surprising since the latter has been entirely computed from the two regressors. What is interesting here is to evaluate the percentage of ring increment variations explained by each contributor, i.e. tracheids number and mean tracheid radial diameter. To do so, a hierarchical partitioning was performed on data from the 20 studied trees and the 5 studied years. Tracheid number was revealed to be responsible for 88% of ring width variations while tracheid diameter accounted for 12%.

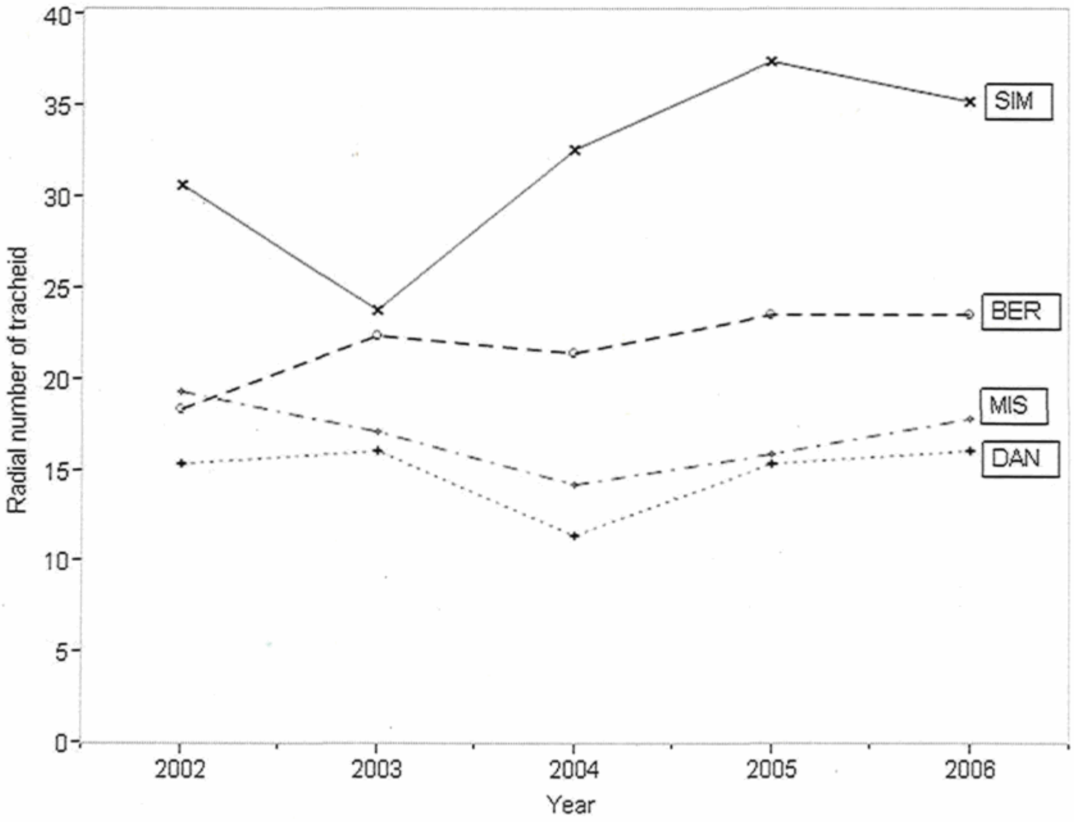

Figure 4.2. Number of tracheids produced at the four studied sites from 2002 to 2006.

Dbh-standardized number of tracheids on each site decreases with latitude (figure 4.2). But this does not perfectly fit the mean annual temperature ranking; while the coldest and the warmest sites (Dan and Sim, figure 4.1) produce the smallest and largest number of tracheids respectively, the second coldest site (Ber) produces more tracheids than the second warmest one (Mis). Figure 4.2 also shows divergences in the year-to-year patterns of tracheid number between the sites. During the five studied years, it was not possible to observe any significant paired correlation between sites (table 4.2).

Table 4.2. Paired-by-site correlations of the number of tracheids produced from 2002 to 2006, along with their significance probability (P).

Pair of sites	Correlation	P
Dan-Ber	0.196	0.752
Mis-Ber	-0.435	0.464
Mis-Dan	0.754	0.141
Sim-Ber	0.322	0.597
Sim-Dan	-0.142	0.820
Sim-Mis	-0.228	0.713

4.4.2 Influence of climate on black spruce tracheid production

The best full model that can be built from the 11 factors stated in table 4.1 reveals a high level of collinearity between these factors. If the 11 chosen variables are taken individually in simple regression for the number of tracheids produced, 8 of them are significant and together explain a total of 261.9% of the variance, this sum being an indicator of information redundancy (Neter et al. 1990). Doing the same with all 26 candidate variables in table 4.1 reveals that 20 of them are significant in simple regression analysis. The high collinearity is also shown when each of the 11 factors of the full model is regressed by all the others. Then the mean coefficient of determination is 0.864.

The full model has been reduced to its parsimonious form, from which conclusions will be drawn (table 4.3). This best model is the result of the comparison of 25920 possibilities that can be formulated from the 26 candidate variables stated in table 4.1. The model reveals that the number of tracheids produced is strongly and positively influenced by the intensity of incident light during the period of tracheid production. An important positive influence of humus temperature in August of the previous year is also shown. Duration of the production period appears in third place with a positive influence.

Table 4.3. Multiple regression ANOVA, model coefficients and their standard deviation, and percentage of independent effect ($IE_{\%}$) given by hierarchical partitioning of the best multiple regression found for the number of tracheids produced vs. duration and climate variables selected in a first run. Mean value is also indicated.

R^2	0.920
Observations	20
F Ratio	43.35
Prob. > *F*	<.0001

Regressor	Coefficient	Coef. Std Dev.	$IE_{\%}$	Mean value
$PAR_{day,TP}$	0.198	0.021	57.0	383.3 $\mu mol\ s^{-1}\ m^{-2}$
$ST_{hu,PrAug}$	2.610	0.673	20.6	11.6 °C
Duration	0.223	0.073	14.4	73.8 days
$T_{max,TP}$	-2.176	0.614	8.0	21.5 °C

Mean maximum daily air temperature during the whole tracheid production period has a negative effect on the number of cells, despite its positive, non-significant simple correlation with the number of tracheids (table 4.4). $T_{max,TP}$ is also positively correlated with the most influent variable, $PAR_{day,TP}$.

Table 4.4. Cross correlations of the variables selected in the multivariate model and the number of tracheids. A single asterisk indicates a significant correlation ($\alpha = 0.05$) while a double asterisk indicates a highly significant correlation ($\alpha=0.01$).

	$PAR_{day,TP}$	$ST_{hu,PrAug}$	Duration	$T_{max,TP}$	Tracheids
$PAR_{day,TP}$	1.000				
$ST_{hu,PrAug}$	0.234	1.000			
Duration	0.025	0.333	1.000		
$T_{max,TP}$	0.661*	0.098	-0.207	1.000	
Tracheids	0.770**	0.573**	0.449*	0.233	1.000

Pooling of sites is supported by an after-analysis of the model. As the mean number of cells produced at each site is significantly different between sites ($P = 9.263 \times 10^{-7}$), no difference remains in the residuals of the model ($P = 0.6798$). Student mean comparison confirms this result and gives a more detailed picture (Figure 4.3).

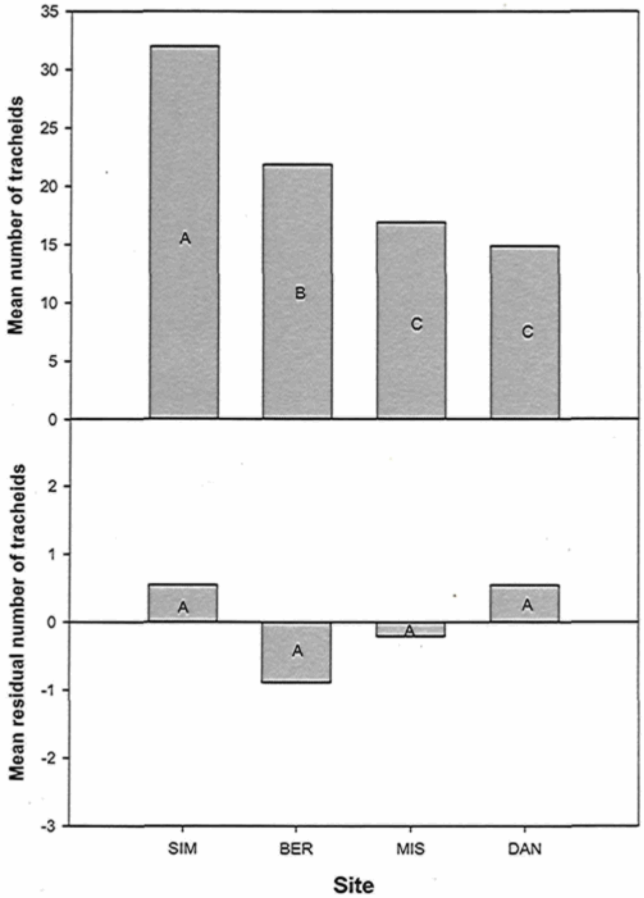

Figure 4.3. Student comparison of the mean number of tracheids produced at each site without taking climate into account (top) and once the effect of climate is removed (bottom).

4.5 Discussion

This study shows that mature black spruce ring width variations are mainly dependent on radial tracheid number variations, as reported for Norway spruce (Mäkinen et al. 2003). It is therefore very relevant to mechanistically study the influences behind the number of tracheids. The number of tracheids has also shown interannual variation patterns that are rather different from site to site. This result is partly due to a low number of observed years that weakens the power of the correlations shown in table 4.2. Despite that, it remains consistent to consider the sites as varying differently from year-to-year, and this study assumes that microclimatic differences between sites are the explanations. Therefore, each site was equipped with climate measurement devices in order to assess these microclimatic differences. As the number of tracheids produced on each site were not perfectly ordered with the mean temperature, other influences were expected and the inclination of climate variables towards multicollinearity justified the use of multiple regression.

The most important factor influencing the number of tracheids is daytime light intensity during the period of tracheid production. Limitation of light in black spruce stands is already known from foresters as indicated by commercial thinning being prescribed in these dense stands (MRNF 2010). Mature trees are known to react positively to this treatment (Vincent et al. 2009). The positive influence of light is obviously related to improved photosynthesis. The mean value of daytime light intensity during tracheid production is 383.3 μmol s^{-1} m^{-2}. This value is lower than light saturation points in field conditions reported in the literature (Goulden et al. 1997, Vowinckel et al. 1975).

Humus temperature from August of the previous year is revealed to be the second important factor. August is known to ecophysiologists as an important moment for spruce growth. At this time, shoot elongation has ceased (O'Reilly and Parker 1982) and the tree accumulates nutrients for the next growing season (Munson et al. 1995). Poor nutrition reduces photosynthesis (Johnsen 1993), but higher soil temperature in podzols are known to improve mineralization of nutrients sequestered in organic matter (Krause 1991, Domisch et al. 2002). Another effect of better nutrition in August is the possible increased bud development (Colombo & Smith 1988, Pollard & Logan 1979), since this also occurs at this time (Colombo & Teng 1992). More leaf primordia in the buds enhances shoot growth in the following year and this has a known positive influence on radial increment (Dufour and Morin 2010, Heide 1974), probably because total photosynthesis of the tree is increased.

Duration of tracheid production period, which is mainly determined by climate (Dufour and Morin 2010), is also importantly linked to the number of tracheids produced. This seems very consistent and in agreement with some authors like O'Reilly and Owens (1989). In black spruce studies, part of it ever been demonstrated using radial increment, as spring temperature revealed to be influent (Huang et al. 2010, Tardif and Conciatori 2001, Hofgaard et al. 1999), which is known to be important for tracheid production initiation (Dufour and Morin 2010, Rossi et al. 2008). It should be made clear that this result concerns the duration of the period of tracheid production, i.e. the formation of new xylem cells with no regard to their period of maturation. Lupi et al. (2010) showed that a higher number of tracheids produced extends the length of the whole xylogenesis period by delaying the cessation of tracheid maturation. Therefore, our study focuses on the number of tracheids and the period that precisely determines it. Another original element in the duration-number relationship is the control of climate influences throughout the tracheid production season. Temperature and light intensity during a part of the tracheid production period are also known to have an influence on its duration, especially on the timing of cessation (Dufour & Morin 2010), and not only directly on the number of tracheids as demonstrated in this study. Therefore this collinearity could bias the coefficient of relationship between duration and number in an underfitted model where climate is not considered.

Among the four important factors, maximum temperature during the tracheid production period is the one with the least influence. Other authors reported such a negative impact of summer temperatures on black spruce radial growth (Huang et al. 2010, Tardif and Conciatori 2001, Hofgaard et al. 1999). Worrall (1973) also reported a low dependence of radial growth on temperature for Norway spruce growing in New Hampshire. As discussed above, the positive influence of light and previous August humus temperature suggests limitation of tracheid production through limitation of photosynthesis; so does the negative influence of maximum temperature. Table 4.4 shows that mean maximum temperature during tracheid production is 21.5 °C, and the 25 % quantile is situated to 18 °C, which means that 75 % of the time, maximum temperature is beyond this value. Field measured temperature optimum for net photosynthesis in black spruce trees or ecosystems is reported to be between 14 and 20 °C (Grant et al. 2009, Goulden et al. 1997, Vowinckel et al. 1975), then it seems that maximum temperature varies mainly beyond the optimum, so its link with photosynthesis is clear. Also, maximum temperature occurred more than 95% of the time between 12:00 and 17:00, so it is clearly a good representation of temperature in the afternoon. High temperature in the afternoon is known to induce stomatal closure and photosynthesis reduction (Grant et al. 2009).

The multiple regression modeling method employed in this study allowed some spurious effects to be avoided that could have occurred if underfitted models like simple regression had been used. In that case 20 variables would have been considered to have an influence on the number of tracheids. The modeling method employed reduces this number to 4, which is a much more parsimonious evaluation. Also, the effect of maximum temperature during tracheid production, not deciphered by simple regression, has been revealed in the final multiple regression. This discrepancy occurs because the real effect of $T_{\mathrm{max,TP}}$ is negative but is overridden by the positive effect of $\mathrm{PAR}_{\mathrm{day,TP}}$ with which it shares a positive relationship (Table 4.4). So when the effect of light intensity is not controlled, like in the simple regression context, maximum temperature shows a positive but non-significant relationship with the number of tracheids. All these considerations suggest that simple regression based on parametric test is not suitable to decipher causality in this observational study concerning a complex multiple effects system, in agreement with the position stated by many authors, including Burnham and Anderson (2002) and also Briffa (1999).

In the same way, the method can be employed to compare the selected factors with other ones deciphered by simple regression or correlation found in black spruce literature. Applying the same model selection procedure of this paper using the four candidate variables described in table 4.5 along with the four variables of the selected model (Table 4.3) lead to the same model shown in this paper (Table 4.3). With the data from this study, only Day of snowmelt is clearly significant in simple correlation, which is the way the original authors analyzed these variables. However, July precipitations can be considered as weakly significant. So these two correlations can be considered as spurious, since they do not hold multiple regression backward elimination when confronted to the causal variables found in this paper. However, Day of snowmelt is a remarkable predictor of the number of cells since it is correlated in such a way that an earlier snowmelt is likely to come with a sunny and long tracheid production season that follows a previous August showing a warm soil, and these three conditions increase the number of cells produced, as shown in this study.

Table 4.5. Description of four newly tested variables. Any of them are selected when confronted to the four variables of the model shown in this paper.

Variable	**Source**	**Correlation with number of tracheids**	**Significantly correlated model variable**
Previous year June precipitations	Huang et al. 2010 Hofgaard et al. 1999	r = 0.13 N.S.	$\mathrm{PAR}_{\mathrm{day,TP}}$(0.45); $T_{\mathrm{max,TP}}$(0.52)
January mean temperature	Huang et al. 2010	r = 0.32 N.S.	
Day of snowmelt	Rossi et al. 2011	r = -0.66 P = 0.0014	$\mathrm{PAR}_{\mathrm{day,TP}}$(-0.59) $\mathrm{ST}_{\mathrm{hu,PrAug}}$(-0.61) Duration(-0.46) $T_{\mathrm{max,TP}}$(-0.55)
July precipitations	Tardif et al. 2001	r =-0.41 P = 0.0732	$\mathrm{PAR}_{\mathrm{day,TP}}$(-0.63)

Analysis of the climate model residuals confirmed the assumption that differences in the number of tracheids among sites are basically created by microclimatic differences. Therefore, other non-climatic growth factors have been either well controlled or maybe fairly influent on the number of tracheids. Stem diameter (dbh), was rather uniform within and among sites as these were all even-aged and of similar ages. But dbh has been used for growth standardization anyway. Stand density, which has been assumed to be uniform in the analysis since all the sites can be considered as dense, was actually uniform among three sites but it was rather higher at Ber (3350 stems/ha instead of ± 2050 stems/ha) and that did not leave significant difference in the residuals. This indicates that radial growth seems to be not sensitive to density variations within the density range of closed black spruce forest. Genetic has also been neglected in this study; we assumed that the study area shows no genotypic differences important enough to be translated into different number of tracheids among sites. Model residual supports this assumption, as well as experiments conducted on white spruce provenances distributed over 5 degrees of latitude in Quebec (Coursolle et al. 1998). The same rationale apply to soil: limiting soil characteristics discrepancy to order (podzol) and broad drainage class (mesic) is enough to control soil characteristics effect on the number of tracheids radially produced. Therefore as long as the conditions of a site shows the same wide context of the study (mature closed black spruce forest, podzol, mesic drainage), the model is applicable. However, quantitative predictions would be better if the number of tracheids is standardized to a dbh of 21 cm.

4.6 Conclusion

In this study, many climatic factors have been precisely monitored in order to more accurately explain the influence of climate fluctuations on the number of tracheids produced in the ring of mature black spruce growing in mesic conditions of the boreal closed forest. We have demonstrated that the number of tracheids is by far the most important parameter comparatively to cell diameter to explain the variation in ring width. Preliminary analysis using climate-growth simple regressions and collinearity diagnostics among regressors revealed the occurrence of many possible spurious effects. To efficiently avoid these, data has been analyzed using a model selection procedure working backwards from a full model starting point.

This analysis revealed that the number of tracheids produced is limited by photosynthesis during tracheid production and duration of this period. Climate warming should increase tracheid production, assuming an unchanged sunlight input, through an earlier tracheid production initiation (Dufour and Morin 2010) and better nutrients availability during early tracheid production due to an increased uptake in the previous August. Higher maximum temperature should only slightly reduce this effect. These results are in agreement with results on black spruce ecosystem net productivity (Grant et al. 2009).

Finally, since climate explained 92 % of the variance in the number of tracheid produced and that model residual unlikely leave place to explain remaining differences among sites, we conclude that climate causes most of the influence on variations in radial increment of black spruce in dense, mature stands growing on mesic podzol in the heart of the boreal forest.

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5. IDENTIFICATION OF A BREAKPOINT IN TRACHEID RADIAL DIAMETER NARROWING: A PHYSIOLOGICALLY-BASED EARLYWOOD TO LATEWOOD TRANSITION.

Authors: Boris Dufour and Hubert Morin

5.1 Summary

The main cell constituents of conifer wood, tracheids, are known to show a generally decreasing radial diameter throughout the tree ring. Despite the importance of tracheid diameter on wood density and hydraulic efficiency of the tree, studies on how this transition operates precisely are sparse. Climate always been expected to influence cell enlargement, but the extent and precise nature of its effect are still unknown. The objectives of this study were to describe the tracheid radial diameter transition throughout the ring, assess the climate influence on this transition and determine the climatic factors limiting tracheid radial diameter in the specific context of closed black spruce boreal forest. Repeated tree ring micro-coring was performed on five trees at four sites representative of closed black spruce forest, from 2002 to 2006 to monitor cambial phenology. In 2007, four additional microcores per tree were extracted from the cardinal points of every tree to assess the number of tracheids produced each year and measure their radial diameter. Air temperature and humidity, soil temperature and water content, as well as photosynthetically active radiation were monitored at each studied site. The occurrence of a breakpoint in the transition from wide to narrow tracheids has been identified. This breakpoint defines two periods: a first one showing a rather variable reduction in diameter and a second showing a constantly decreasing diameter. The time of occurrence of the breakpoint, which is usually in mid-July, depends mostly on the time of tracheid production initiation, suggesting a predetermined duration of the first period, and an important influence of springtime climatic conditions. However, maximum undercover temperature can slightly modify this

duration. The phenological assessment was used to estimate the period in which each tracheid is enlarging so the climate during enlargement has been precisely computed and linked to tracheid diameter. Tracheids enlarging before the breakpoint are strongly influenced by climate despite a general decrease forced by tree physiology. The most important climatic factor during this period is soil temperature, showing a negative effect likely linked to cytokinin production by the roots. Air temperature also has a negative effect while light intensity has a weak positive effect. For tracheids enlarging after the diameter breakpoint, the physiological forcing of diameter decrease operates a slightly weaker influence. Among climate modulations, soil temperature is still the most important, but less so than in the first period. The influence of light intensity increases and air humidity becomes an important factor, suggesting a photosynthetic limitation in this period. Air temperature remains almost as important as in the first period. The most obvious difference between the two periods is the influence of photoperiod which is exclusive to the second period. This is evidence of a fundamental change in the ecophysiological reaction of the tree. The breakpoint therefore represents a physiologically based delimitation of the earlywood to latewood transition.

5.2 Introduction

Ring width variations in black spruce (*Picea mariana* (Mills.) BSP) are mainly determined by variations in the radial number of tracheids (St-Germain & Krause 2008, Wang et al. 2002), while the variations in the radial diameter of these tracheids accounts for only a small part (chapter 4). Despite this mitigated role, cell diameter influences other aspects operating at a different scale. As a determinant of cell lumen, it plays an important role in the hydraulic efficiency of the xylem. In mature trees, limitation in increasing cell diameter limits height growth (Anfodillo et al. 2006) and induces leaf water supply and photosynthesis reduction with height (Koch et al. 2004). Less photosynthesis means less tracheid production in the stem (chapter 4). Although maximum tracheid diameter is genetically determined from a trade-off between hydrological efficiency and risks of cavitation (Pittermann & Sperry 2003, Tyree 2003), some environmental influences remain (Vaganov et al. 2006).

Within a tree ring, from pith side to bark side, tracheid diameter in conifers clearly diminishes, and many classifications have been developed to account for this (Park et al. 2006, Creber & Chalonner 1984), the most common one being Mork's index (Denne 1988) that separates the ring in two zones, earlywood and latewood. The transition between these two zones is also known to be clearly marked in some species, e.g. in larches, while in others it is more gradual, e.g. in spruces (Schoch et al. 2004). Despite these observations, specific studies on how and why tracheid diameter transits from wide to narrow are sparse. Schweingruber (1996) presumed that it is mainly influenced by photoperiod.

Also, assuming a same relative radial position on a ring, it is clear that cell diameter varies from tree to tree and from year to year. Even though information from experimental science exists on how growing conditions influence enlargement of tracheids (Rossi et al. 2009, Denne 1976, 1971), few studies have been performed in natural conditions aiming to decipher the determinants of tracheid diameter variations in specific contexts, and most of these were carried out at the treeline (Vaganov et al. 2006, Wang et al. 2002). Black spruce in the continuous boreal forest sub-zone of eastern Canada does not face strong limitation from a single factor like severe drought or low temperature during the growing season. Consequently, its cambial phenology (Dufour & Morin 2010) as well as its total tracheid production (chapter 4) has been shown to be influenced by multiple factors. Tracheid diameter is therefore also expected to be subjected to multiple environmental influences.

The objectives of this paper are to study tracheid diameter pattern across the ring of mature boreal black spruce and to determine the climatic factor influencing variations in this pattern.

5.3 Materials and methods

5.3.1 Study area and sampling plots

Efforts were made to choose sites representative of the mesic conditions prevailing in the middle of Quebec's continuous boreal forest vegetation subzone (Blouin and Berger 2004a,b), and to assess a representative climate variability within this study area. Four permanent plots lying along a latitudinal transect have been sampled in order to account for the climate variability within this subzone. From south to north, two are situated in the balsam fir-white birch bioclimatic domain: Simoncouche (Sim: 48°13.78' N; 71°15.18' W) and Bernatchez (Ber: 48°51.92' N; 70°20.57' W). The other two are in the spruce-moss bioclimatic domain: Mistassibi (Mis: 49°43.92' N; 71°56.88' W) and Daniel (Dan: 50°41.78' N; 72°11.03' W). Each plot is installed on even-aged, mature, closed and pure black spruce stands. The trees, established 120-140 years ago, are growing on gentle slopes (8 to 17%) and moderately (3 plots) to imperfectly drained glacial tills (1 plot: Dan). Site index (mean height at 50 years of age), mean current dominant tree height and mean dbh are respectively: 13.6 m, 16.0 m and 20.4 cm at Sim; 6.9 m, 17.6 m and 21.1 cm at Ber; 11.4 m, 18.6 m and 21.4 cm at Mis; 8.7 m, 16.8 m and 20.0 cm at Dan.

5.3.2 Assessment of tracheid radial development phenology

From 2002 to 2006, 5 dominant trees in each site were sampled repeatedly throughout the growing season to monitor tree ring formation at weekly or fortnightly frequency. At each sampling, a single micro-core was taken from the stem of each tree using a bone marrow sampling needle (DBMNI-1501 inter-V medical), extracting cores about 1 mm in diameter and up to 20 mm long.

Micro-cores were processed using standard histological methods involving dehydration in alcohol, paraffin embedding and cutting with a rotary microtome. Staining was done with 0.15% *cresyl violet acetate* filtered solution. Observations were made under a polarized light-equipped transmission microscope. Cells (tracheids) were then counted in each of the following developmental zones: cambial zone, radially enlarging, and enlarged (includes those with thickening walls and mature ones). Up to three radial rows per sample were assessed this way.

5.3.3. Total annual tracheid count, dimensions and standardization

The diameter, radial wall thickness and radial number of tracheids produced in each year at each site was measured on four microcores per tree, extracted in 2007 from the same trees as phenological assessment, one year after all the studied rings were completed. All microcores included rings for all the studied years. The same histological techniques as above were applied but sections were stained with Safranin and permanently mounted with Permount®. Tracheids along three continuous radial rows per ring were measured and counted. Rows were chosen from those producing the widest cells to make the number of tracheids free of the influence of longitudinally overlapping rows at cell tips (Larson 1994, Whitmore & Zahner 1966). Radial diameter measurement was performed using WinCELL Pro® (v. 2004a). Tracheid counts from the three rows were averaged for each ring of the sample, then for the whole ring of a tree using the four cores, and for each year using the five trees on a site, which gives the standard number of cells ($Ncell_{std}$).

Tracheid diameter and wall thickness of each cell cannot be directly averaged for the whole sample and tree because cell numbers are alike but nonetheless differ between different rows, cores and trees. Standardization was applied in order to have the same number of cells, which allows computing a mean diameter series for each year and site. First, the cell position of each raw diameter series was converted into relative location, which is given by the ratio between absolute location and the total number of cells in the row (x_i/Ncell). Then, a continuous diameter series was computed by linearly interpolating diameter values between successive relative locations. The standard series was created by attributing the diameter and wall thickness values from the continuous series to each discrete relative location of the standard series (x_i'/Ncell$_{std}$). There is one standard series per row, but since Ncell$_{std}$ is common for all the trees and rows from a same site on a given year, the diameter of each cell in the series was averaged by core and trees, in this way creating the mean standard diameter series of the site.

5.3.4 Modeling the tracheid diameter transition throughout the ring

The site-averaged cell diameters plotted by cell rank from pith to bark appeared to decrease differently according to two distinct zones. To statistically validate this visual indication, a model fitting procedure was created as follows. Each tracheid diameter series was split into two datasets; one for the first cells of the ring, the other for the last cells. All possible combinations of these datasets, with any of the two parts including at least three cells, were tested. A linear and a quadratic function were then fitted to each subset, and the adjusted R^2 for the whole dataset was computed and the best result chosen from all the subset combinations. This best double model solution was compared with a single model solution on the whole dataset, i.e. with no dataset splitting. In other words, using the adjusted R^2 criterion, this confronts the hypothesis of a single tendency for tracheid diameter to decrease with the one where there is a breakpoint that splits the tendency in two. Combining two models increases the number of parameters to consider in the computation of the whole dataset adjusted R^2, and this is prone to decrease its value (Quinn & Keough 2002) compared with the use of a single quadratic function.

5.3.5 Meteorological monitoring and climate variability

To assess climate variability, a weather station was installed in a gap (φ 20 m) on each site. Each system was controlled by a CR10X datalogger (Campbell Scientific®) to which weather sensors were connected (one per parameter) that monitored the following parameters:

- Air temperature, 2 m above ground in the gap
- Air temperature, 2 m above ground, under cover
- Relative humidity, 2 m above ground in the gap
- Incident Photosynthetically Active Radiation (PAR) at 8 m height in the gap
- Humus temperature (~ 5-15 cm below surface)
- Soil upper mineral layer temperature (~ 25-35 cm below surface)
- Soil water content (TDR), upper 30 cm layer
- Precipitations
- Snowpack thickness

Measurements were taken every 5 minutes; hourly total (precipitations only), minima/maxima (both air temperatures) and means (all measurements except precipitations) were recorded, as well as hourly mean saturation vapor pressure computed from air temperature. The latter was multiplied by hourly relative humidity to give hourly vapor pressure estimation. The photoperiod of each day was calculated using a photoperiod calculator (Lammi 2008).

Compilations made from this hourly database are described in the modeling method section.

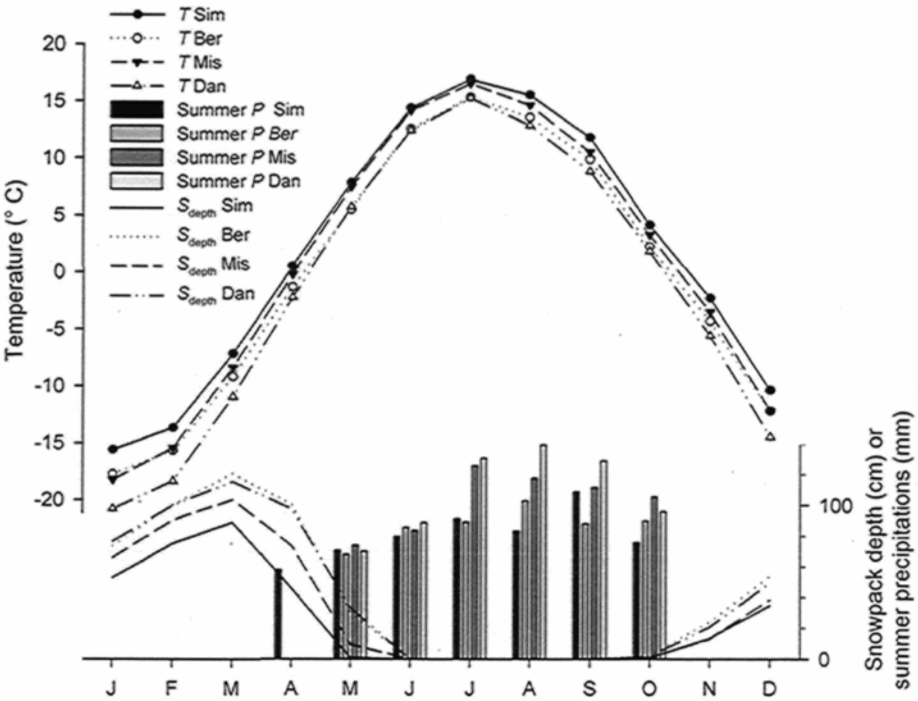

Figure 5.1. Monthly mean temperature (T), total precipitations of months with mean temperature above 0 °C (Summer P), and mean snowpack depth (S_{depth}) from 2002 to 2006 for each site.

In all sites during the studied period, the warmest month of the year was July while the coldest was January (Figure 5.1). Yearly mean temperature was, from south to north: 1.8, -0.1, 0.7 and -1.3 °C. Monthly mean temperature was typically above zero from May to October in all sites, but also in April at Sim, the warmest site. During this period, total monthly rain precipitation was, in general, gradually more abundant from south to north. Total precipitation from May to October ranged from 511 mm (Sim) to 655 mm (Dan). The thickest snow accumulations were found in the coldest (Dan) and the highest (Ber) sites, and peaked at 90 (Sim) to 120 (Ber) centimeters in March. Snowpack usually melted almost completely in April at Sim and in May at the other sites.

5.3.6 Linking tracheid diameter with climate data using tracheid development phenology

This procedure aims to create a final dataset that gives the climatic conditions during the enlargement phase of each individual tracheid, in order to know when each of these tracheids are enlarging and what diameter is finally reached. As mentioned before, we have an assessment for these parameters, but since they are differently sampled and measured in different locations on the tree and measured on different rows, their respective measurements are based on slightly different cell numbers. So standardization was performed, using the same standard cell number as above ($Ncell_{std}$) as the common basis. Tracheid diameter is already standardized with $Ncell_{std}$.

Standardization of the phenological sampling was used to estimate the period of tracheid enlargement specifically for each tracheid. Each intra-annual cell count for enlarging cells and already enlarged cells was standardized to reduce the variability due to different sampling positions around the stem (Rossi et al. 2003, Dufour & Morin 2007, Dufour & Morin 2010) and to obtain $Ncell_{std}$. Each count was therefore multiplied by the ratio of the mean ring width of the previous ring measured on all samples taken on the tree in the same year, divided by the ring width of the previous ring specific to the counted sample. Mean values from each site and year were computed by averaging counts from the five trees. A second standardization followed, consisting of multiplying each count by a ratio computed by dividing $Ncell_{std}$ by the mean value from the counts made after tracheid production ceased (C_{tot} in Dufour & Morin 2007).

Standardized cell counts for enlarging and already enlarged cells were summed to obtain a dataset of tracheid production. A linear and a quadratic model were then fitted on this data but using only the observations showing new tracheid production, and the poorest fit of both was discarded. The same procedure was applied on the enlarged tracheid dataset, using only observations showing new completely enlarged tracheids. Since the resulting functions give the cell number from the time in terms of day of year (DOY), they were inversed to give the DOY from the cell number (Dufour & Morin 2007, Rossi et al. 2003).

The same applied to the enlarged tracheids. From these inversed functions, timing of the onset of cell enlargement and also the timing of its ending can be computed, in this way defining the period of cell enlargement for every tracheid of the ring. Climate data can then be compiled specifically for each of these periods.

The timing of the transition to latewood was also estimated. To do so, the same procedure as above was applied on the phenological assessment of the number of mature cells. But here, the interest is in the wall thickening and lignification phase of each cell, which is defined by the period between the timing of the end of enlargement of the cell and the time it reaches maturity. Cells of the standard series were then classified as being part of either earlywood or latewood using the most latewood-oriented interpretation of Mork's index (Denne 1988). The earlywood to latewood transition was established as the midpoint between the end of wall thickening of the last earlywood cell and the onset of enlargement of the first latewood cell.

5.3.7 Modeling method

Since each site has its own weather station, observations can be considered as independent among sites, so all the analyses have been done pooling observations from years and sites.

The statistical approach used is a full model, which means inclusion of all the regressors (factors) that are mechanistically expected to influence the response. These full models are known to be the most effective to decipher the real effect of a factor in a multivariate context, i.e. regression coefficients are not biased (MacNally 2000, Burnham & Anderson 2002, Whittingham et al. 2006).

Factors representing climatic conditions affecting the tree are basically separated in five factor types aimed to represent the whole operational environment of the tree (Fritts 1976), as used previously in the same study (Dufour & Morin 2010, chapter 4): soil temperature, water availability, air temperature, air humidity and light intensity. Each factor of the full model has to be represented by a precise variable. For some factors, more than one variable has been tested. Most variables are daily mean values computed from the hourly database and covering periods specific to each test. However, some have been compiled in such a way as to describe a climate influence on photosynthesis, which is expected to be an intermediate process limiting radial growth. These variables are assigned as daytime variables and consider only the hours of above zero temperature and PAR flux.

Since identification of the most important variables was a concern despite full models not being parsimonious (Burnham & Anderson 2002), a reduced final solution was reached. The fit was performed with SAS 9.2® using the glmselect procedure. Backward selection method was used and corrected Akaike information criterion (AICc, Burnham & Anderson 2002) was used for step selection and final model choice.

As mentioned by Quinn & Keough (2002), the partial correlation coefficients of a multiple regression are biased by collinearity. To efficiently rank the relative contribution of each variable in the models, a hierarchical partitioning analysis (Chevan & Sutherland 1991) has been performed using the hier.part package (MacNally & Walsh 2004) running on R©.

5.4 Results

5.4.1 Tracheid diameter transition

Evolution of the tracheid diameter follows a general radial decrease from the first to the last tracheid of the ring, as can be seen for the 20 studied cases (Figure 5.2). However, the decrease is not regular as some fluctuations occur. For example, two cases (Sim 2004 and 2006) show a diameter increase in the first 3-4 cells.

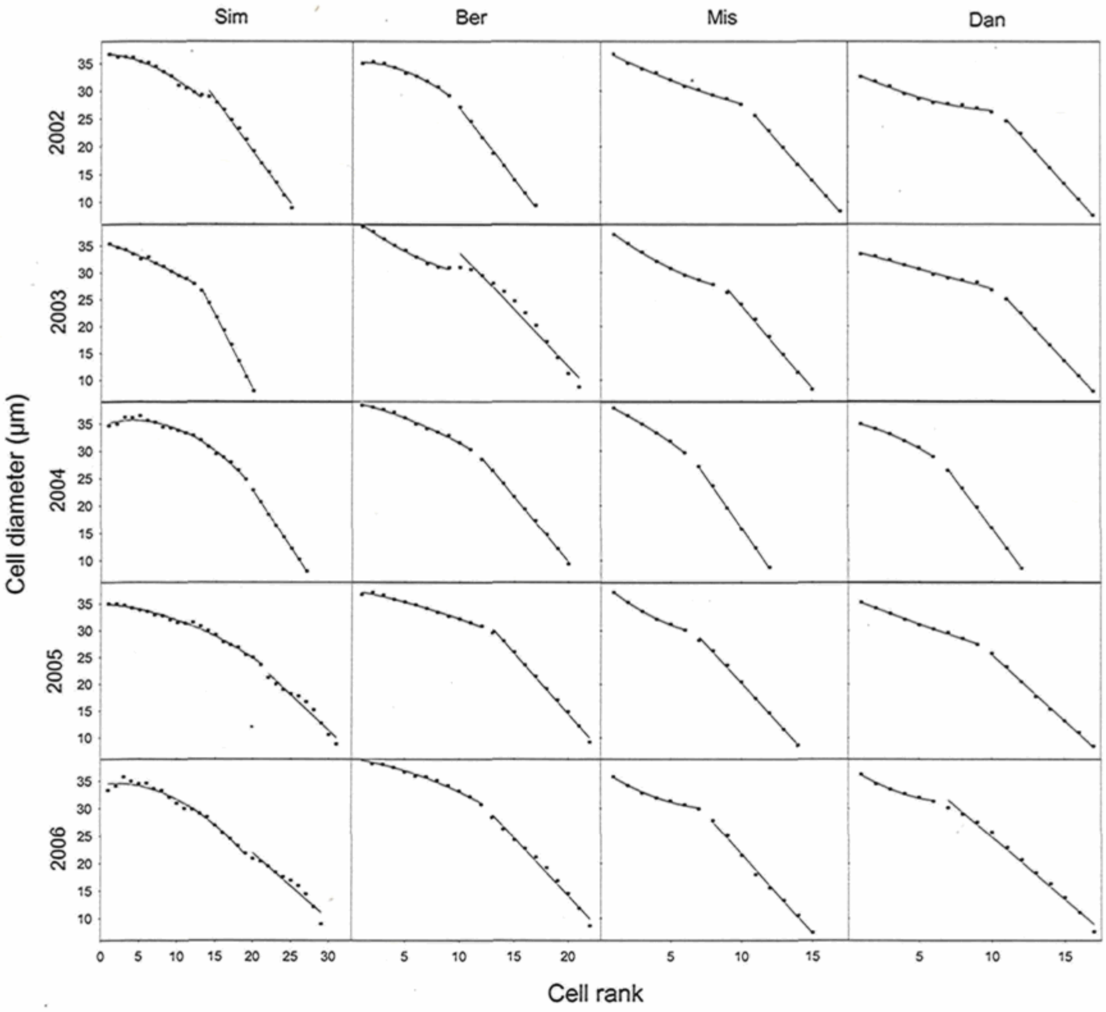

Figure 5.2. Measured (dots) and modeled (lines) tracheid diameter along tree ring radial direction from the first (pith side) to the last (bark side) tracheid.

The cell diameter fitting procedure systematically established two different periods of diameter evolution (Figure 5.2), despite the increased "penalty" on the adjusted R^2 value coming from the use of two models instead of one (i.e. more parameters). According to the model type, the first period evolves along a convex curve, a concave curve or a straight line, while the second always has a linear evolution. So there is clearly a break in the general tendency of tracheid diameter decrease. Breakpoint occurred between July 1st and August 3rd (Figure 5.3a). The overall average breakpoint timing is DOY 200 (July 19th) with a standard deviation of 9.8 days, while Mork's latewood transition happened on DOY 217 (August 5) with a standard deviation of 10.9 days, which is about two weeks later. The correlation between both events is 0.60 (P = 0.005). Compilation by year and by site has been computed. According to the standard deviation, year-to-year variability in a single site (Figure 5.3c) is generally higher than site-to-site variability in a single year (Figure 5.3b).

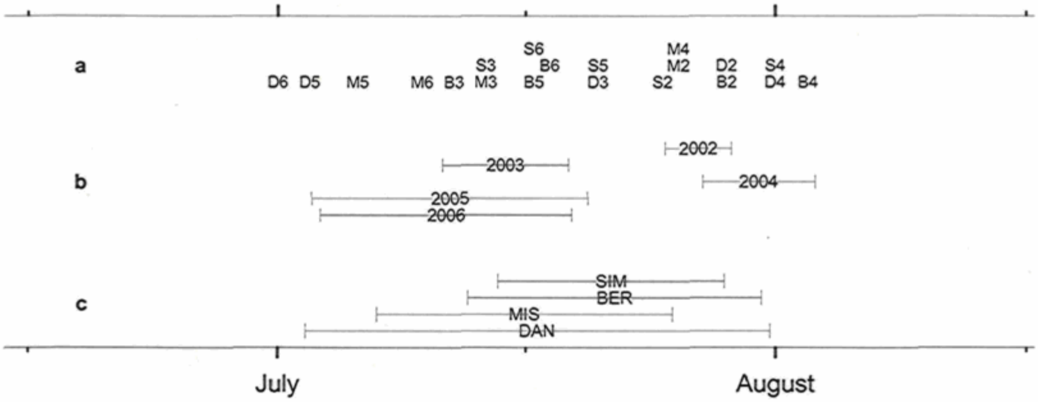

Figure 5.3. a: Timing of the tracheid diameter breakpoint for the 20 combinations of site and year. b: Timing compiled by year. c: Timing compiled by site. Error bars are standard deviation.

The main factors that influence variations in the timing of tracheid diameter reduction breakpoint have been identified by the model selection (table 5.1). The main effect is from the timing of tracheid production initiation which accounts for about 65% of the explained variance, i.e. 45% (64.6% of 0.697) of the total variance. This main effect is modulated by the negative effect of maximum undercover air temperature during the period between tracheid production initiation and tracheid diameter breakpoint: the warmer the undercover, the sooner the breakpoint. This factor explains an additional 25% of the total variance. The by-site mean value of maximum undercover temperature in the studied period shows a site ranking that follows the following increasing order: Sim (17.8 °C), Ber (19.8 °C), Dan (20.4 °C) and Mis (21.0 °C). Therefore, southern sites (Sim and Ber) values show lower undercover temperatures during the considered period than northern sites (Mis and Dan).

Table 5.1. Multiple regression ANOVA, model coefficients and standard deviation, as well as percentage of independent effect ($IE_{\%}$) given by hierarchical partitioning of the best multiple regression found for the timing of tracheid diameter breakpoint vs. climate during the period between tracheid production initiation and the breakpoint. All candidate regressors tested are listed, but only those parts of the selected model have parameters displayed.

R^2	0.697
Observations	20
F Ratio	19.565
Prob. > *F*	< 0.0001

Factor type	Analogous variable (daily basis)	Abbre-viation	Coefficient	Coef. SD	$IE_{\%}$
Air humidity	Vapor pressure	VP			
	Daytime vapor pressure	VP_{day}			
	Relative humidity	RH			
Light	Daytime photosynthetically active radiation	PAR_{day}			
Air temperature	Temperature	T			
	Maximum temperature	T_{max}			
	Minimum temperature	T_{min}			
	Undercover temperature	T_{uc}			
	Maximum undercover temperature	$T_{uc,max}$	-3.228	0.850	35.4
	Minimum undercover temperature	$T_{uc,min}$			
	Daytime temperature	T_{day}			
	Saturation vapor pressure	SVP			
Soil temperature	Humus temperature	ST_{hu}			
	Mineral soil temperature	ST_{mn}			
Water availability	Soil water content (%)	SWC			
	Precipitation (mm)	P			
Xylogenesis initiation	Tracheid production initiation (DOY)	TPI	1.048	0.206	64.6
Intercept			103.514	35.437	

5.4.2 Environmental influence on tracheid diameter.

The breakpoint in the decline of tracheid diameter can be seen as marking the division of two different phenological phases in cell diameter development. These two distinct periods could imply different climate-enlargement responses because of physiological changes in the tree or different climate conditions. To assess this, the model selection approach has been applied to model the effect of climate separately for each period (table 5.2 and 5.3).

In both periods tracheid diameter is mainly influenced by a climate-independent tendency, represented by the autocovariate (table 5.2 and 5.3). Whatever the climate variations during its enlargement, a tracheid is physiologically directed to be radially smaller than its pith side neighbour. This forcing has slightly more influence in the first period (75% of explained variance) than in the second (67%). However, another non-climatic factor, photoperiod, makes the global non-climatic influence more important in the second period (67% + 17.9%) than in the first (75% + 0%). The global importance of climatic influences corresponds to the remaining explained variance of each period, i.e. about 25% and 15% respectively.

Tracheids enlarging before the tracheid diameter breakpoint have their diameter negatively influenced by mineral soil temperature and this constitutes the main climatic influence of the first period (table 5.2). The remaining influences are related to air temperature (negative influences of maximum undercover temperature) and light intensity (positive influence of PAR).

Table 5.2. Multiple regression ANOVA, model coefficients and standard deviation, as well as percentage of independent effect ($IE_{\%}$) given by hierarchical partitioning of the best multiple regression found for tracheid diameter before the tracheid diameter breakpoint vs. climate during cell enlargement. All candidate regressors tested are listed, but only those parts of the selected model have parameters displayed.

R^2	0.982
Observations	184
Prob. > F	2.1×10^{-155}

Factor type	Analogous variable	Abbreviation	Coefficient	Mean value	$IE_{\%}$
Air humidity	Vapor pressure (kPa)	VP			
	Relative humidity (%)	RH			
Light	Photosynthetically active radiation (µmol sec^{-1} m^{-2})	PAR	0.0014	406.4	0.3
Air temperature	Temperature (°C)	T			
	Maximum temperature (°C)	T_{max}			
	Minimum temperature (°C)	T_{min}			
	Undercover temperature (°C)	T_{uc}			
	Maximum undercover temperature (°C)	$T_{uc,max}$	-0.062	19.5	3.8
	Minimum undercover temperature (°C)	$T_{uc,min}$			
	Saturation vapor pressure (kPa)	SVP			
Soil temperature	Humus temperature (°C)	ST_{hu}			
	Mineral soil temperature (°C)	ST_{mn}	-0.097	5.9	20.9
Water availability	Soil water content (%)	SWC			
	Precipitation (mm)	P			
Photoperiod	Day-length (h)	DL			
Autocorrelation	Tracheid diameter autocovariate (µm)	ACov	0.976	32.8	75.0
Intercept			1.242		

The candidate variables in table 5.2 have also been tested for their influence during the second period. The diameter of the cells enlarging after the tracheid diameter breakpoint is also mainly influenced by the physiological forcing that drives the cells to successively decrease their diameter (table 5.3). An important change compared to the first period is the increased influence of light conditions; photoperiod is now influential in the second period and light intensity increases its influence, so together they account for 21.6% of the explained variance. Mineral soil temperature influence decreases to 7.8%, but that of air temperature remains about the same. In addition, air humidity has a positive but weak influence on tracheid diameter after the breakpoint.

Table 5.3. Multiple regression ANOVA, model coefficients and standard deviation, as well as percentage of independent effect ($IE_{\%}$) given by hierarchical partitioning of the best multiple regression found for tracheid diameter vs. climate during cell enlargement after the tracheid diameter breakpoint.

R^2	0.992		
Observations	165		
Prob. > F	1.9×10^{-163}		
Regressor	Coefficient	Mean value	$IE_{\%}$
ACov	1.003	20.5	67.0
DL	0.332	15.2	17.9
ST_{mn}	-0.180	8.8	7.8
PAR	0.0069	355.3	3.7
$T_{uc,max}$	-0.155	19.9	2.9
VP	1.264	1.3	0.7
Intercept	-6.827		

5.5 Discussion

5.5.1 Tracheid diameter transition

Tracheid radial diameter is considered gradually decreasing in spruces, compared to the abrupt decrease shown by larches (Schoch et al. 2004). This study does not contradict this idea, but reveals a common pattern, i.e. a breakpoint in the evolution of tracheid diameter along the tracheid radial rows of the black spruce ring. So there are two distinct periods of tracheid radial diameter reduction. The first shows a variable diameter dynamic, sometimes with short periods of leveling off or even some increases. Consequently, the diameter decrease is not as clear as in the second period. The latter shows a constant and pronounced linear decrease of the tracheid diameter. So the breakpoint represents a sharp switch to a period of strong diameter decrease.

Breakpoint happens about two weeks before the earlywood to latewood transition as computed by Mork's index. There is a significant relationship between both events, indicating a major importance for tracheid radial diameter to determine Mork's latewood transition. The other parameter, cell wall thickness, could only have a secondary importance in black spruce. However, the real question is the relevance of the basis of Mork's index. In fact, as an arbitrary index accounting for cell size and wall thickness (Creber & Chalonner 1984), it is an integrator of the outcome of two biologically distinct processes. This integration is questionable and this paper suggests a new approach based only on cell diameter.

The magnitude of the breakpoint is rather weak; it is difficult to observe graphically when single tree tracheidograms are plotted (not shown). But when cell diameters of trees from the same site have been standardized and averaged, the breakpoint becomes more apparent and this visual indication has been confirmed by the model comparison method used in this study. The standardization of cell rows from all the samples of a tree ring and from all the trees of a site to compute the average that enhances the common signal is therefore necessary for black spruce breakpoint detection.

In the continuous boreal forest subzone, black spruce tracheid diameter breakpoint can occur on any day in July, or occasionally in very early August. Breakpoint timing is more variable across years in a single site, than sites in a single year, a pattern similar to that reported by Dufour & Morin (2010) for the initiation of tracheid production. As breakpoint timing is mainly dependent on the time of tracheid initiation, it is not surprising to observe such a pattern, common to both events.

The relationship between tracheid production initiation and diameter breakpoint means that the latter is indirectly dependent on the conditions of the preceding spring, especially mean daily temperature and vapor pressure, since these are the major influences determining the time of tracheid production initiation (Dufour & Morin 2010). This relationship also means that the tree is programmed to allocate a rather uniform period of time for earlywood production. Whether the cambial growth season begins earlier or later, the switch to constantly decreasing tracheid diameter will follow the same tendency in order to keep the duration of both phenological stages even.

Nevertheless, some modification in the timing of tracheid diameter breakpoint is caused by maximum undercover temperature in the period following tracheid production initiation. Obviously, this effect largely accounts for the tendency of sites to have an earlier breakpoint in the north than in the south, since the site ranking in terms of undercover maximum temperature fits this pattern. At first sight, it seems surprising that this ranking does not follow the expected ranking of an increasing undercover temperature from south to north. It is important to stress that the latitudinal range covered by the sites is about 250 km, so the latitudinal temperature decrease should be weak and easily overridden by local site characteristics. Some of these can explain the inversed undercover temperature gradient. Sim, the southernmost site, occupies a small peninsula jutting out into the Simoncouche lake, so it is almost completely surrounded by water and bogs and therefore there could be an important temperature damping effect. Ber, the other southern site, is situated at a higher elevation and shows a higher stem density than any other site, limiting the radiative warming effect at undercover level during the day. The two northern sites do not show these temperature reduction factors, so normal latitudinal ranking of temperature applies.

5.5.2 Factors influencing tracheid diameter in the first period

In black spruce, as revealed by the strong influence of the autocovariate despite the control of environmental influences, tracheids are physiologically forced to decrease their diameter throughout the growing season. But before the breakpoint happens, i.e. during the first period, there is no influence of photoperiod. An important influence of photoperiod would promote a first step showing an increase in tracheid diameter in the beginning of the season, since photoperiod lengthens for approximately the first three weeks of the growing season before shortening. But that does not happen: only two cases out of twenty show such an increase, but in one of these, it ceases well before the photoperiod begins to shorten.

Tracheid diameter evolves in a more variable fashion in the first period and this indicates a more important role of climate in earlywood development. Results from diameter model selection are consistent with this, assuming that photoperiod is not a climatic factor, as climate variables together are proportionally more influential in the first period than in the second (25% vs. 15% of explained variance).

Climate influence in the first period is mostly driven by soil temperature. The strong negative effect of mineral soil temperature is unexpected. No reported experiment about the influence of soil temperature on tracheid diameter has been found in the literature, although results from the study of Gruber et al. (2010) indicate that a year with warmer soil in spring and early summer produced narrower earlywood tracheids than usual. However, the authors did not analyze their results in that way and so the effect could also be due to other collinear factors. This effect is nevertheless biologically interpretable. It is known that warmer soil enhances nitrogen availability and absorption (Krause 1991, Domisch et al. 2002). This triggers cytokinin production in the roots (Sakakibara et al. 2006), which is then transported acropetally through the xylem (Liu et al. 2009, Aloni et al. 2005) to serve as a signal for meristematic zones. High cytokinin content in the meristematic zones produces smaller differentiated cells, either because it hastens cell differentiation (Moubaydin et al. 2009) or because it enhances the tendency of auxins to diminish enlargement duration (Aloni 2001).

Two other factors influence tracheid diameter in the first period. Maximum undercover temperature, with a negative effect, is the more important of these. The selection of undercover temperature instead of temperature in the open indicates an effect operating at the base of the trunk. The nature of this phenomenon could also be linked to the auxin action. Gray et al. (1998) demonstrated that high air temperature promotes high auxin concentration, which is known to reduce cell diameter (Moubaydin et al. 2009, Aloni 2001). The last important factor in the first period is a weak positive influence of mean daily light intensity. This factor becomes more important in the second period and is discussed below.

5.5.3 Factors influencing tracheid diameter in the second period.

After the breakpoint, physiological forcing of the tracheid diameter decrease is still the most important factor influencing tracheid diameter, despite some of its influence being lost at the expense of day-length. These non-climatic factors together leave little space for real climatic influences. There was a consistent indication of this with the constant selection of a straight line to model the diameter evolution of the cells enlarging during the second period compared with the more variable patterns found for those enlarging during the first period. Influence of photoperiod on tracheid diameter ever been demonstrated in experimental conditions (Denne 1976).

All the climatic factors that have influence in the first period are also influential in the second. Although mineral soil temperature is still the most important climatic factor, its relative importance is lower. This is consistent with the smaller variations of this parameter during this period (not shown). Undercover maximum temperature is almost as influential in the second period as in the first.

In the second period, photosynthesis limits tracheid diameter. This is indicated by the increased positive influence of light intensity and the reappearance of a positive influence of air humidity. It seems that these factors have a widespread influence on growth at the end of the season, since a sunny and humid late summer also helps to delay the end of tracheid production (Dufour & Morin 2010), then for more tracheids to be produced (chapter 4). Following thinning, a silvicultural treatment known to improve light conditions at tree level, black spruce tracheid diameter has been shown to increase in some trees, an effect that seemed to be stronger on latewood (Krause et al. 2011), which is very consistent with our results. Also, low light intensities have been reported to delay photoperiodic responses in spruces grown in experimental conditions (Johnsen & Skrøppa 2000), so the effect of light intensity observed in our studies could also represent a modulation of the photoperiodic forced decrease of tracheid diameter.

5.6 Conclusion

As tracheid radial diameter is commonly considered to decrease gradually in spruces, this study identifies for the first time the occurrence of a sharp breakpoint in this tendency. From this observation, black spruce, despite its gradual tracheid diameter decrease throughout the ring, does not behave in a fundamentally different way than the species showing steep diameter decrease like larches: both share a punctual event triggering a change in tracheid diameter decrease. For black spruce growing in the middle of the species' range, this breakpoint happens generally in mid-July and defines two distinct periods in the intra annual evolution of tracheid enlargement.

The timing of the diameter decrease breakpoint mainly depends on the timing of tracheid production initiation. This leads to uniformity in the duration of earlywood tracheid production and indicates that the amount of earlywood produced does not depend on the timing of onset of cambial activity. Undercover temperature slightly modifies this regulated duration, with a warmer undercover leading to an earlier breakpoint.

Tracheids enlarging before the breakpoint are strongly influenced by climate despite a general decrease forced by tree physiology. The most important climatic factor during this period is soil temperature. It has a negative effect on cell diameter and is likely linked to cytokinin production by the roots. Air temperature also has a negative effect while light intensity has a weak positive effect.

For tracheids enlarging after the diameter breakpoint, the physiological forcing of diameter decrease operates a slightly weaker influence. Among climate modulations, soil temperature is still the most important, but less so than in the first period. Light intensity increases its influence and air humidity becomes an important factor, suggesting a photosynthetic limitation in this period. Air temperature remains almost as important as in the first period.

The most obvious difference between the two periods delimited by the diameter tendency breakpoint is the influence of photoperiod, which is exclusive to the second period. This is evidence of a fundamental change in the ecophysiological reaction of the tree. The breakpoint therefore represents a physiologically based delimitation of the earlywood to latewood transition.

The results also suggest that climate change could have important consequences on black spruce tracheid diameter. As the tracheid production season will begin earlier because of higher temperatures (Dufour & Morin 2010), the breakpoint will do the same, leaving almost the same duration for the first period but extending the second one, when narrow tracheids are produced. Higher soil and air temperature should also reduce tracheid diameter throughout the growing season.

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6. CONCLUSION GÉNÉRALE

6.1 La dynamique d'accroissement radiale chez l'épinette noire.

Les divers chapitres de cette thèse révèlent des informations précieuses sur la dynamique de formation du cerne. Cette section fait un récapitulatif de cette dynamique cellulaire en omettant de décrire en détails les influences climatiques, qui seront traitées à la section suivante.

D'abord, la saison de croissance du xylème dans le tronc commence par l'initiation de la production de nouvelles trachéides par le cambium autour du 2 juin, dépendamment des conditions climatiques depuis la mi-mars, de la profondeur de la dormance et de la taille des bourgeons (chapitre 3, Dufour et Morin 2010). Puis, les premières nouvelles trachéides formées entreprennent tour à tour leur élargissement, influencées surtout par une tendance physiologique de fond les forçant à atteindre un diamètre de moins en moins grand.

À partir de la mi-juillet, le climat commence à exercer une influence sur la date de cessation de la production. À peu près au même moment, un bris dans la tendance à la diminution du diamètre des cellules survient. Il se produit donc, de toute évidence, des changements majeurs dans la physiologie de l'arbre en juillet qui modifient la manière dont le climat influence sa croissance. À partir de ce moment, les conditions climatiques qui prévalent décideront du moment de l'arrêt de production des trachéides mais auront une influence réduite sur le diamètre des cellules formées. C'est également à partir de ce moment que la photopériode impose une diminution rapide et régulière du diamètre des trachéides, définissant ainsi une transition au bois final de nature écophysiologique.

De plus, tel que mentionné au chapitre 3, la mi-juillet correspond également à l'arrêt de croissance apicale chez les épinettes, phénomène bien connu des spécialistes. Cette thèse démontre donc que la mi-juillet représente une période de changements physiologiques généralisés chez l'épinette noire, autant dans son accroissement radial qu'apical. Ceci appui également la théorie répandue voulant que le cambium soit contrôlé par les méristèmes apicaux.

À l'opposé, peu de résultats appuient la théorie d'une croissance cambiale contrôlée par la photopériode. Premièrement, les variations de date d'occurrence des deux événements phénologiques étudiés au chapitre 3 que sont l'initiation et l'arrêt de la production de nouvelles trachéides par le cambium, se sont révélées presqu'entièrement influencées par des variables climatiques (respectivement à 88 % et 97 %). Même chose pour le nombre de trachéides produites (92 %). Ceci laisse peu de place pour croire en l'influence de la photopériode, d'autant plus qu'elle ne varie pas d'année en année pour une même date et très peu sur le territoire étudié. Tout au long de la saison, la production de trachéides peut se révéler généralement constante ou généralement croissante (Chapitre 2, Dufour et Morin 2007). Ce sont les influences climatiques révélées au chapitre 4, qui en détermineront le niveau précis.

Cette thèse ne traite pas du processus d'épaississement et de lignification des parois. On peut donc affirmer qu'elle porte sur les processus qui jouent sur l'accroissement radial (i.e. le nombre de trachéides et leur diamètre) mais seulement partiellement sur ceux qui jouent sur la densité du bois. Des mesures ont été prises sur la phénologie et l'épaisseur des parois et pourront faire l'objet d'une étude ultérieure.

6.2 Influence des facteurs climatiques.

Dans les chapitres 3 à 5, les liens climat-croissance ont été ordonnés en fonction de la variable de croissance étudiée. Nous faisons ici un résumé pour chaque type de facteur de manière à mettre en relief la contribution de chacun.

6.2.1 Luminosité

L'intensité lumineuse incidente a une très grande importance dans la croissance radiale de l'épinette noire dans les peuplements denses tels que ceux étudiés. C'est le facteur ayant le plus d'incidence sur le nombre de trachéides produites, d'autant plus que son effet est double. D'abord, l'ensoleillement général de la saison est très déterminant et constitue le facteur expliquant le plus de variance observée. Il s'agit ici d'un effet indépendant de la durée de la saison. Il découle sans nul doute d'une limitation en lumière disponible pour la photosynthèse totale de l'arbre. Cette limitation vient probablement de la compétition importante que les arbres se font en peuplement dense et qui empêche un éclairement optimal sur une partie de la cime. Ensuite, l'intensité lumineuse a un autre effet sur le nombre de trachéides qui agit par le biais d'un changement sur la durée de la saison. Une luminosité forte à partir de la mi-juillet a tendance à retarder l'arrêt de la période de production de trachéides, ce qui en augmente la durée et permet une production totale supérieure.

La luminosité joue également positivement sur le diamètre des trachéides, particulièrement celles du bois final. La présente étude n'a pas permis de distinguer si la lumière agit sur la durée de l'élargissement d'une trachéide ou sur son taux d'élargissement.

6.2.2 Température de l'air

Dans cette thèse, la température de l'air est étudiée pour son effet sur la croissance, mais contrairement à d'autres études, la colinéarité avec d'autres variables climatiques a été assumée et traitée de manière à faire ressortir l'effet indépendant réel de la température. Il en ressort une série complexe de relations, ce qui à la base n'est pas illogique, car la température est fondamentale dans le fonctionnement de tout système vivant et peut donc intervenir dans de nombreux processus vivants.

Pour l'épinette noire, l'influence de la température se fait fortement sentir par la longue période de dormance annuelle qu'elle impose. C'est d'ailleurs principalement grâce au réchauffement des températures à partir de la mi-mars que l'épinette sortira de sa dormance cambiale au tout début juin. Plus il fait chaud au printemps, plus la production de trachéides recommencera tôt. Cet effet est quelque peu modifié par les températures de la fin de l'été précédent car, tel que mentionné au chapitre 3, de cette dernière dépend la profondeur de la dormance de l'arbre.

Tout au long de la production active de nouvelles trachéides par le cambium qui, rappelons-le, a lieu en plein cœur de l'été, la température de l'air exerce un effet limitant : elle est généralement trop haute pour assurer une croissance optimale du cerne. Le chapitre 4 révèle que la température de l'après-midi a tendance à être au-delà de l'optimum pour la photosynthèse de l'épinette noire et cela se traduit par un effet négatif sur le nombre de trachéides produites. De plus, à partir de la mi-juillet, ces températures, toujours aussi chaudes, vont avoir tendance à devancer l'arrêt de la production des trachéides, phénomène lié à la transpiration plus forte qu'impose des températures plus hautes et qui contribue à la déshydratation de l'arbre. Ceci raccourci donc la saison et peut diminuer le nombre de trachéides produites. Il ne faut toutefois pas oublié que la température de l'air et la température du sol sont indissociables : de hautes températures de l'air font augmenter la température du sol, ce qui a un effet on ne peut plus positif sur le nombre de cellules (section 6.2.3). Il ne faut donc pas croire en un effet globalement négatif d'un réchauffement climatique malgré l'effet négatif des températures estivales. Le réchauffement climatique a un effet sans doutes positif sur ce nombre de trachéides à cause de l'effet printanier, d'autant plus qu'il est généralement reconnu que le réchauffement est quelque peu moins fort en été que dans le reste de l'année (Bourque et Simonet 2007).

Les autres effets de la température de l'air étudiés dans cette thèse concernent le diamètre des trachéides, pour lequel la température a un effet négatif, décliné en deux aspects. Le premier est le devancement du bris de la tendance dans la diminution du diamètre par les températures chaudes. Ces dernières provoquent donc une transition au bois final plus hâtive. De plus, toute trachéide soumise à une température élevée pendant son élargissement verra son diamètre diminué, quoique faiblement.

6.2.3 Température du sol

Ce facteur s'est révélé avoir un effet important sur de nombreux aspects de la croissance radiale. Pour ce qui est de la phénologie et du nombre de trachéides produites, l'effet de la température du sol se fait à retardement : c'est principalement les conditions de la fin de la saison précédente qui agissent sur la saison en cours. De chaudes températures du sol à la fin de l'été précédent ont tout d'abord pour effet d'allonger la durée de la saison de production des trachéides en cours en devançant légèrement son initiation et retardant nettement son arrêt. De plus, de chaudes températures du sol à la fin de la saison précédente augmente beaucoup le nombre de cellules produites et ce, indépendamment de l'effet qu'elles ont sur la durée, puisque l'effet de la durée sur le nombre de trachéides est inclus dans le modèle et est donc par le fait même contrôlé (chapitre 4).

Tous ces effets bénéfiques sur le nombre de trachéides, appliqués à retardement, proviennent d'une dépendance de la croissance cambiale envers la croissance apicale. Un sol chaud à la fin de l'été augmente la minéralisation de la matière organique du sol, ce qui améliore la nutrition de l'arbre, ce qui à son tour augmente le développement des bourgeons apicaux, phénomène démontré expérimentalement depuis longtemps. Les bourgeons plus gros sont plus faciles à sortir de dormance et contiennent plus de primordia foliaires, ce qui prolonge la saison de croissance apicale, retardant ainsi le signal ordonnant l'arrêt cambial (chapitre 3, Dufour et Morin 2010). De plus, les gros bourgeons, puisqu'ils entraînent la formation d'un plus grand nombre de nouvelles aiguilles, entraînent une augmentation de la photosynthèse, révélée limitante pour le nombre de trachéides produites (chapitre 3). Ceci explique donc l'effet indépendant de la durée.

La température du sol s'est révélée très importante également pour le diamètre des trachéides, particulièrement pour celles du bois initial. Un sol plus chaud pendant l'élargissement cellulaire ralentit ce dernier et mène à un diamètre plus petit. Cela est lié également à une meilleure nutrition de l'arbre, ce qui augmente la quantité de cytokinines produites par les racines et a comme effet, reconnue par les spécialistes, d'entraîner une réduction de la taille des cellules produites dans les méristèmes (chapitre 5). Cependant, notons que cet effet, le plus important de la part d'un facteur climatique sur le diamètre des trachéides de l'épinette noire, est bien loin de surpasser le forçage physiologiquement imposé par l'arbre, voulant que les trachéides voient successivement leur diamètre diminué tout au long de la saison.

6.2.4 Humidité de l'air

L'humidité de l'air a un effet négatif sur la durée de la saison de production des trachéides, car un air plus humide au printemps a pour effet de retarder l'initiation. À l'opposé, un air plus humide à partir de la mi-juillet a pour effet de rallonger la durée en retardant l'arrêt de la saison, mais ce second effet est beaucoup moins important que le premier (chapitre 3, Dufour et Morin 2010). L'effet retardant l'arrêt est cependant conjugué à l'effet négatif de la température de l'air, révélé sous sa forme de pression de vapeur saturante. Cela ne fait que confirmer l'effet accélérant des conditions asséchantes sur la mise en dormance.

Un effet de l'humidité de l'air sur le diamètre des trachéides du bois final a également été révélé dans cette thèse (chapitre 5). Des conditions humides en fin de saison pourrait donc augmenter la quantité de bois final, mais ce dernier serait quelque peu moins dense, à moins que l'épaisseur des parois ne soient favorisées, ce qui reste à démontrer.

6.2.5 Disponibilité en eau

La disponibilité en eau ne s'est pas révélé très limitante au niveau de la croissance. Seul un effet au niveau de l'arrêt de la saison de production des trachéides a été trouvé. Un sol trop sec à partir de la mi-juillet favorise un arrêt de production plus hâtif, donc un nombre de trachéides plus faible. Il est en outre, au premier abord, étonnant de constater l'absence d'effet sur le diamètre cellulaire, paramètre pourtant reconnu pour sa sensibilité aux conditions hydriques. Cela s'explique tout de même très bien. Durant l'élargissement des trachéides de bois initial, le contenu en eau du sol est toujours élevé. En moyenne, il est de 62 % avec un écart type de seulement 7,5 % en considérant tous les sites et les années étudiées. Il est donc raisonnable de croire que l'humidité dans le sol dans cette période est toujours suffisante pour les besoins optimaux de l'arbre. Dans la deuxième période, le contenu en eau est quelque peu inférieur (58 %) et plus variable (8,1 %), ce qui laisse présager des conditions parfois assez sèches pour devancer un peu l'arrêt de la saison. Le diamètre des trachéides de bois final n'est toutefois pas affecté et cela est certainement dû aux faibles exigences en pression hydrostatique interne des cellules en élargissement, puisque le diamètre à atteindre est plus faible.

6.3 Théorie émergente: un cerne de croissance qui dépend de la taille des bourgeons et des conditions propices à la photosynthèse concomitante à sa formation.

À la lumière des résultats, il est possible d'élaborer une nouvelle théorie sur la dynamique de production des trachéides chez l'épinette noire mature en forêt fermée. Cette théorie est présentée à la figure 6.1, qui fait également la synthèse des liens climat-croissance établis dans cette thèse. Cette théorie implique les points majeurs suivants :

- C'est fondamentalement la quantité de substrat fourni par la photosynthèse concomitante à la production des trachéides qui détermine le nombre produit. Cette quantité de substrat dépend de la durée de la saison de production (et de sa photosynthèse concomitante) et du taux de photosynthèse.
- Un signal d'induction de la dormance cambiale est donné autour de la mi-juillet. Ce signal regroupe deux événements révélés par cette thèse et synchronisés : le début de la période pendant laquelle le climat influence la date d'arrêt de production et le bris de tendance dans la diminution du diamètre cellulaire, interprété comme la transition au bois final. Ajoutons également le synchronisme avec la fin de l'élongation apicale, si l'on en croit la littérature citée au chapitre 3. C'est d'ailleurs cette dernière qui serait l'élément déclencheur du signal inductif chez l'épinette noire, comme pour toute autre espèce à croissance déterminée.

- La taille des bourgeons produits à la fin de la saison précédente est d'une très grande influence sur cette espèce à croissance déterminée. Elle influence la durée autant par son effet sur la date d'initiation que par celle sur l'induction de la dormance et donc indirectement sur la date d'arrêt. Elle a également un effet sur le taux de photosynthèse générale dans l'arbre puisqu'elle détermine la quantité de nouvelles aiguilles formées au cours de la saison.

- Bien que l'étude ne se soit pas attardée à établir si les variations de la durée de la saison soient dues surtout aux variations dans la date d'initiation ou à celles dans la date d'arrêt, la théorie émergente de cette thèse suggère que la date d'arrêt serait plus influente. Puisque l'induction de la dormance est fortement dépendante de la date d'initiation, on doit s'attendre à ce qu'une initiation plus hâtive ne rallonge pas vraiment la saison car elle provoque en même temps le devancement de l'induction de la dormance. Par contre, les changements importants que les conditions climatiques depuis l'induction peuvent entraîner sur la date d'arrêt rendent cette dernière très influente. Ceci est corroboré par les corrélations : -0,3 entre la date d'initiation et la durée et 0,7 entre la date d'arrêt et la durée.

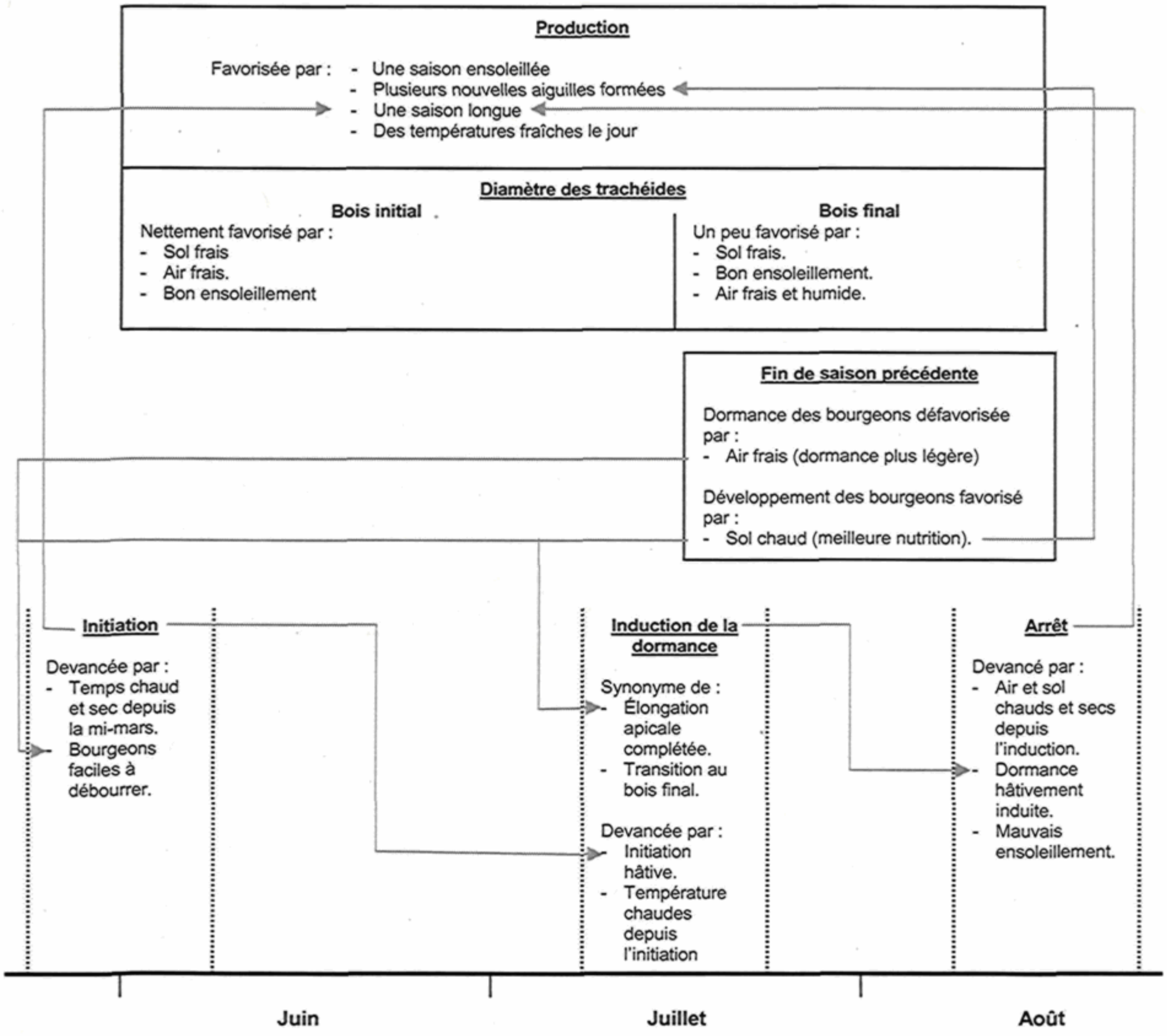

Figure 6.1. Dynamique de croissance radiale conceptualisée à partir des résultats de la thèse.

6.4 Perspectives

6.4.1 Une étude innovante

L'innovation la plus importante de cette étude est d'avoir combiné des éléments méthodologiques déjà connus mais qui forment un tout permettant d'augmenter à la fois la puissance et la fiabilité des analyses climat-croissance : suivi de la phénologie cambiale et apicale (en partie), mesures du cerne à l'échelle cellulaire, mesures *in situ* et complètes de l'environnement climatique des peuplements, ainsi que des analyses prenant en considération la multiplicité et la multicollinéarité des effets. Voilà comment on peut l'exprimer dans sa généralité, mais d'autres éléments plus spécifiques méritent attention.

Les liens entre la phénologie cambiale et le climat ont été étudiés de manière plus poussées que jamais. C'est la seule étude qui à ce jour intègre de façon aussi complète les influences multiples du climat pour étudier la phénologie. Mais de plus, il faut souligner l'originalité de la méthode de modélisation. Cette dernière fait face de manière novatrice au problème suivant : la variable réponse est de nature temporelle et on doit définir des variables explicatives dans le temps pour des périodes qui dépendent de cette variable réponse. La transformation des variables en pourcentage d'avancement journalier introduite au chapitre 3 résout ce problème et apporte aux modèles phénologiques une utilité prédictive : il suffit de faire la somme successive des pourcentages d'avancement journalier pour déterminer le moment de la réponse phénologique, correspondant à un processus terminé, c'est-à-dire ayant atteint un avancement de 100 %.

La méthode de construction des modèles est elle aussi, dans ses détails, exclusive. Elle combine théorie de l'information et parcimonie, deux éléments connus pour leur efficacité face à la multicolinéarité et tout-à-fait en ligne avec les fondements de la méthode par inférence multimodèle (Burnham et Anderson 2002), très utilisée en écologie. Mais l'aspect élimination de variables permet en plus d'éviter de faire un choix arbitraire des modèles parcimonieux à évaluer, étape incontournable de l'inférence multimodèle. Tous les sous-modèles possibles du modèle globale sont admissibles en tant que résultat dans l'élimination de variable. De plus, afin de permettre d'utiliser la meilleure variable possible pour représenter un facteur sans prendre le risque de trop augmenter le niveau de multicolinéarité, nous avons introduit une procédure d'itération de la méthode de modélisation où à chaque itération, le modèle globale de départ est légèrement modifié en ne changeant que la variable représentant un même facteur (par exemple, utiliser la température maximale journalière dans une itération et la température minimale dans une autre). Ceci empêche toutes les itérations d'utiliser en même temps des variables explicatives très semblables (fortement colinéaires), pour représenter le même effet et donc de diminuer dès le départ les inconvénients de la multicolinéarité (Quinn et Keough 2002).

6.4.2 Les limites et les implications de l'étude

Le contexte de l'étude est le principal élément qui dicte les limites de son application. Considérant les caractéristiques communes aux 4 sites d'échantillonnage, on peut affirmer en tout premier lieu que l'étude s'applique aux peuplements d'épinette noire purs (≥ 75 % de la composition arborescente), denses (≥ 40 % de recouvrement des cimes) et de structure régulière. On peut donc considérer, par exemple, que ces forêts montrent un niveau de compétition suffisamment élevé pour que la lumière y soit un facteur limitant. De plus, la portée se restreint aux sols de type podzols sous humus de type mor et de drainage mésique. On doit donc s'attendre à des réponses différentes dans les sites humides ou très secs.

De manière générale, les modèles élaborés dans le cadre de cette étude avaient pour but premier d'identifier des facteurs limitants, ce qui n'est pas en faveur d'une grande portée pratique de ces modèles en tant qu'outil de prévision à des fins de gestion de la ressource ligneuse. Par exemple, afin d'assurer un maximum de degrés de liberté disponibles pour tester les facteurs climatiques, les données du nombre de cellules (chapitre 4) ont été préalablement standardisées pour tenir compte de l'effet des différences dans le diamètre des tiges étudiées. Cela veut donc dire que si l'on veut prédire le nombre de trachéides moyen produit dans un peuplement donné à partir de données climatiques, il serait préférable de transformer la prédiction obtenue en fonction du diamètre réel des arbres, puisque cette prédiction s'applique à un diamètre moyen de 21 cm.

De plus, le fait de décortiquer les mesures de croissance en paramètres anatomiques et de prendre en compte plusieurs facteurs climatiques ne simplifie pas l'utilisation des modèles pour un usage prédictifs. Rappelons que ce n'en était pas le but, mais que les connaissances acquises envers les facteurs limitants pourront permettre de formuler les hypothèse de base d'un modèle prédictif plus simple et basé sur des causalités, donc plus précis et plus fiable que les modèles actuellement en usage. Par exemple un nouveau modèle d'accroissement radial pourrait être construit avec à la fois, comme variables candidates, celles limitant le nombre de trachéides et celles limitant leur diamètre.

Cependant la révélation la plus marquante est l'importance du climat révélée par cette étude mais aussi l'incontournable prise en compte d'un environnement climatique plus complet. Si, dans leur forme actuelle, les modèles expliquant les variations dans le nombre de trachéides et leur diamètre ne sont pas des plus pratiques, leur mise en application seraient de toute manière empêchée par un manque au niveau des mesures prises par les stations météo des services publiques. Pensons notamment à la rareté au niveau des mesures d'ensoleillement et la quasi-absence de celles de température du sol. Ainsi, afin de pouvoir faire des prédictions de croissance basées sur les causes d'origine climatique il serait important de compléter le plus tôt possible le jeu d'instrumentation des stations déjà existantes.

Les facteurs limitants révélés par cette étude peuvent également permettre de soumettre des hypothèses pour l'effet de certains traitements sylvicoles existants ou possibles. Par exemple, on comprend bien pourquoi une éclaircie augmente la croissance à l'échelle de l'arbre, car son environnement lumineux et la température du sol s'en trouvent améliorés. Ceci augmente les taux de photosynthèse et la longueur de la saison. Également, on pourrait imaginer de nouveaux traitements applicables aux peuplements d'épinette noire. Comme la lumière semble un facteur de premier ordre, il serait intéressant de tester un traitement où des dispositifs permettant la réflexion de la lumière sous couvert vers le feuillage seraient installés. Cela pourrait compromettre en partie le réchauffement du sol, mais on peut s'attendre à un effet moindre que l'augmentation due au surplus de lumière capté par le feuillage. Si cela s'avère vrai, le traitement en question peut devenir une alternative intéressante pour l'éclaircie commerciale, car c'est à une augmentation de la croissance de tout le peuplement qui pourrait être envisagé car il n'impliquerait pas le retrait d'une partie des arbres.

Il est donc clair que cette étude suggère une panoplie d'améliorations et de nouvelles applications pour notre foresterie. Reste à voir si les chercheurs, les aménagistes et les décideurs veulent poursuivre dans cette voie. C'est ce que l'avenir nous dira.

7. RÉFÉRENCES DES CHAPITRES 1 ET 6

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