POTENTIEL D'UTILISATION DES ISOTOPES STABLES DANS LES CERNES DE CROISSANCE POUR RECONSTITUER LES ÉPIDÉMIES DE LA TORDEUSE DES BOURGEONS DE L'ÉPINETTE AU COURS DES DERNIERS MILLÉNAIRES

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

PAR
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SEPTEMBRE 2010

REMERCIEMENTS

On m'a déjà parlé du doctorat comme d'un marathon. Je n'aurais pu trouver meilleure comparaison! C'est en effet une épreuve d'endurance que l'on traverse non sans douleur mais qui rapporte son lot de satisfactions. L'accomplissement d'un tel projet ne se fait pas seul. Beaucoup de gens ont contribué, au fil du temps, de près ou de loin, à la réalisation de cette thèse. Je suis infiniment reconnaissante envers Hubert Morin et Cornelia Krause, directeur et co-directrice de thèse, pour leur ouverture, leur support et leur patience. Leur soutient, dans mes mille et un projets plus ambitieux les uns que les autres, fut constant. Ils ont su me diriger tout en me laissant une très grande liberté. J'ai pu, grâce à eux, profiter d'une multitude d'opportunités qui m'auront permis de me dépasser sur le plan professionnel mais aussi personnel.

Je remercie aussi tout le personnel du laboratoire d'écologie végétale et du pavillon de la recherche forestière, particulièrement Germain Savard, Jacques Allaire et Daniel Gagnon qui m'ont assuré un support constant et prodigué de judicieux conseils tout au long de cette aventure. Un merci tout spécial aussi à Jean-Guy Girard, mon opérateur de mini-pelle et scie mécaniques préféré, le meilleur!, sans qui je serais certainement encore dans le fond d'une tourbière à creuser pour trouver de vieux bouts de bois! Sans lui, et l'aide de plusieurs aides de terrains dont Jean-Pierre Lebeuf, je n'y serais jamais arrivé. J'en profite pour remercier tous ceux, beaucoup trop nombreux pour être nommé, qui m'ont aidé soit sur le terrain, au laboratoire, mais surtout lors des séances surréalistes et interminables de défoliation où nous nous sommes tous pris pour des tordeuses du bourgeon de l'épinette!

Je remercie personnellement Jean-François Boucher de m'avoir permis de découvrir le monde des isotopes au tout début du projet alors que tout était encore nébuleux et imprécis. Merci de m'avoir reçu à chacune de mes visites et

d'avoir répondu si patiemment à mes innombrables questions. Un très grand merci à toi et Denis Walsh d'avoir fait en sorte, à force de répétitions, que les statistiques deviennent une petite bête un peu moins noire!

Je remercie les organismes qui m'ont supporté financièrement : le Conseil de recherche en sciences naturelles et en génie du Canada (CRSNG), le Consortium de Recherche sur la Forêt Boréale Commerciale et la Fondation de l'Université du Québec à Chicoutimi (FUQAC).

D'un côté plus personnel, je remercie de tout cœur ma famille d'avoir été là pour moi dans mes moments forts comme dans les moins bons. Vous m'avez écouté, épaulé, et entouré. Mes ami(e)s ont aussi été importants et essentiels pour me sortir de la thèse et me soutenir tout au long de ce processus! Les amours passent mais les amitiés restent!

À tous ceux qui ont croisé ma route mais que je n'ai pas nommé, merci. Vous m'avez souvent aidé à continuer et à aller plus loin, que vous en ayez été conscient ou non.

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RÉSUMÉ GÉNÉRAL

La forêt boréale constitue l'un des plus vastes écosystèmes forestiers de la planète, correspondant presqu'au tiers de toutes les forêts du monde et occupant plus de la moitié du Canada. Au Québec, elle constitue le principal domaine de végétation, recouvrant près du tiers de la superficie, soit quelque 560 000 kilomètres carrés dont plus de 75% est occupé par des espèces résineuses telles l'épinette noire et blanche, le sapin baumier, le pin gris. Les régimes de perturbations naturelles font partie intégrante des processus écologiques régissant la dynamique forestière en milieu boréal. Outre les feux de forêt qui affectent chaque année des superficies importantes de forêt, les épidémies d'insectes perturbent périodiquement de larges massifs forestiers agissant également sur la création de mosaïques forestières complexes en termes d'âge, de composition et de structure. Dans la partie sud-est de la forêt boréale, la tordeuse des bourgeons de l'épinette (TBE) est le principal insecte ravageur s'attaquant, de façon récurrente, aux peuplements de sapin et d'épinette. Les épidémies n'ont commencé à être documentées qu'à partir du 20^{e} siècle au Québec et c'est aussi ce siècle qui est le mieux représenté par les données dendrochronologiques. L'historique des épidémies du 19^{e} siècle est basé sur des données de moins en moins nombreuses, et au-delà du 19^{e} siècle, les sources d'informations s'estompent rapidement.

Afin de bien comprendre et caractériser le régime de perturbation lié aux épidémies de TBE, il est essentiel de déterminer la variabilité naturelle associée à ces épidémies sur le plan spatial et temporel. Cette connaissance est d'autant plus importante dans un contexte où l'aménagement forestier écosystémique basé sur l'émulation des processus naturels est suggéré comme une approche alternative à la foresterie actuelle. L'objectif général de cette thèse est d'évaluer le potentiel d'utilisation des isotopes stables dans les cernes de croissance pour reconstituer les épidémies de la TBE au cours des derniers millénaires. L'utilisation des isotopes stables pourrait permettre de reconstituer la dynamique des épidémies de tordeuse des bourgeons de l'épinette en forêt boréale au Québec, dans le secteur des Monts-Valin situé environ 100 km au nord de la ville de Chicoutimi, Saguenay. Afin de reconstituer le plus précisément possible les périodes de défoliation liées à la tordeuse, l'approche multi-indicateurs basée sur la largeur des cernes de croissance d'arbres vivants et subfossiles, de même que leur composition isotopique en carbone et en oxygène a été utilisée. L'approche isotopique basée sur la réponse physiologique des arbres face à un changement environnemental a été introduite afin de préciser l'interprétation dendrochronologique des courbes de croissance des arbres subfossiles puisque dans ce contexte, les méthodes traditionnellement utilisées pour identifier les épidémies de TBE, comme la comparaison de courbes d'espèce hôtes et non hôtes, n'étaient pas praticables.

Les isotopes stables étant utilisés pour la première fois dans ce type d'étude, les résultats escomptés sont basés sur des considérations théoriques. La fiabilité de l'utilisation des ratios isotopiques de carbone et d'oxygène des anneaux de croissance comme aide à l'identification d'épidémies connues de TBE a été vérifiée sur des individus matures de deux espèces de conifères hôtes, le sapin baumier et l'épinette noire. Le signal isotopique observé chez ces espèces a été comparé à celui du pin gris, une espèce non affectée par la TBE. Un signal isotopique, sous la forme d'un enrichissement en ^{13}C de la cellulose des anneaux de croissance formés pendant les années épidémiques par rapport à ceux formés lors d'années endémiques, a été observé chez les espèces hôtes de la TBE. Aucune variation du ratio isotopique de carbone n'a été décelée lors de la même période chez le pin gris qui n'est pas affecté par l'insecte. Par contre, aucune variation spécifique aux périodes de défoliation n'a été observée dans les ratios isotopiques d'oxygène chez les espèces hôtes par rapport aux non hôtes.

Les aspects physiologiques liés à la défoliation ont été examinés à l'aide d'un dispositif expérimental incluant différents niveaux de défoliation artificielle sur de jeunes sapins baumiers a été répété durant quatre saisons de croissance. L'objectif visait à déterminer les facteurs qui limitent l'interprétation des variations de la composition isotopique en carbone et en oxygène des cernes de croissance ($\delta^{13}C$ et $\delta^{18}O$, respectivement) sous l'effet de la défoliation. L'effet de la défoliation sur la composition isotopique en carbone et oxygène des cernes de croissance a pu être reproduit artificiellement. Toutefois, aucune relation positive entre le $\delta^{13}C$ de la cellulose du cerne et l'assimilation en CO_2 n'a pu être mise en évidence. L'hypothèse liant l'augmentation du taux d'assimilation du CO_2 lors de la photosynthèse à l'enrichissement en ^{13}C de la cellulose des cernes de croissance a donc été rejetée. Un mécanisme post-carboxylation semble plutôt à envisager. L'utilisation des réserves enrichies en ^{13}C pour la formation des parois cellulaires dans les anneaux de croissance est l'hypothèse retenue mais reste à vérifier. Similairement aux arbres matures, aucun effet significatif de la défoliation sur les ratios isotopiques d'oxygène n'a été observé. La conductance stomatique est aussi demeurée insensible au traitement de défoliation.

L'application aux subfossiles d'arbres de la méthode d'identification des épidémies de tordeuse des bourgeons de l'épinette à l'aide des isotopes stables, de même que la reconstitution des épidémies passées à partir de ce matériel, ont été finalement été abordé. Les résultats isotopiques obtenus n'ont pas permis de confirmer le lien entre les réductions de croissance observées et les épidémies de TBE. Malgré tout, les résultats suggèrent que les périodes épidémiques sévères au cours de l'Holocène ont été des événements rares en comparaison avec le XX^{e} siècle. Malgré un travail intensif d'excavation de plus de 500 pièces de bois dans trois différentes tourbières, très peu d'arbres excavés ont pu être interdatés. Moins de 10% des arbres du genre *Picea* ont été utilisés pour la construction de chronologies, les variations de croissance interannuelles étant trop faibles pour permettre l'interdatation de la plupart des

séries. Une chronologie flottante à partir de cette espèce arborescente couvrant 364 ans a tout de même été établie et datée au radiocarbone vers 5.1 ka BP, une période où la fréquence de feux de forêt réduite aurait permis la mise en place d'une structure forestière propice aux épidémies. Sur la période de 180 ans analysée à l'aide des méthodes dendrochronologiques et isotopiques, seulement deux épisodes épidémiques potentielles auraient eu un impact suffisant pour être enregistré dans les anneaux de croissance des arbres. Les résultats obtenus suggèrent un moins grand impact des dans le passé par rapport au XIX$^{\text{ième}}$ et XX$^{\text{ième}}$ siècles.

Mots clés : tordeuse des bourgeons de l'épinette, épidémie, défoliation, dendrochronologie, isotope stable du carbone, isotope stable de l'oxygène

INTRODUCTION GÉNÉRALE

La notion d'aménagement forestier écosystémique fait son chemin à travers le Canada depuis quelques années, le but de ce type d'aménagement étant de maintenir l'intégrité écologique et la santé des forêts (CFS 1998). Bien qu'il soit difficile de formuler une définition définitive de l'aménagement forestier écosystémique, Gauthier et al. (2008) proposent : une approche en aménagement ayant pour but de maintenir des écosystèmes forestiers sains et résilients en se concentrant sur une réduction des différences entre les paysages aménagés et naturels afin d'assurer le maintient à long terme des fonctions de l'écosystème et ainsi en préserver les bénéfices sociaux et économiques rendus à la société. Ce n'est qu'en 2005 que la loi sur le ministère des Ressources naturelles et de la Faune (L.R.Q., c. M-25.2) fut modifiée afin d'y inclure les principes de l'aménagement écosystémique de la forêt et de la gestion intégrée et régionalisée de l'ensemble des activités s'exerçant en forêt publique (article 11.2). Cet aménagement écosystémique consiste à réaliser des interventions forestières à l'intérieur des limites en termes de variété et d'irrégularité des forêts naturelles (MRNF 2008a). Afin d'atteindre cet objectif, l'émulation des perturbations naturelles a été proposée (Hunter 1993, Bergeron et Harvey 1997, Gauthier et al. 2008). Elle repose sur les prémisses écologiques suivantes : *(i)* les perturbations périodiques sont inhérentes à la dynamique des écosystèmes forestiers; *(ii)* les perturbations naturelles sont des déterminants de la composition des espèces d'un écosystème de même que de sa structure et fonction; *(iii)* les écosystèmes forestiers et leur composition en espèces se sont adaptés aux perturbations (cf. Klenk et al. 2008). Pour parvenir à introduire des pratiques d'aménagement forestier qui se situent à l'intérieur des limites naturelles il faut bien connaître les régimes de perturbations naturelles.

Ces régimes de perturbations naturelles font partis intégrantes des processus écologiques régissant la dynamique forestière en milieu boréal. Avec les feux, les épidémies d'insectes sont actuellement une des plus importantes perturbations de la forêt boréale au nord de l'Amérique et ont des impacts substantiels sur la structure et la composition de larges surfaces forestières. Dans la partie est de la forêt boréale, la tordeuse des bourgeons de l'épinette (*Choristoneura fumiferana* Clem.) (TBE) défolie les sapins baumiers (*Abies balsamea* [L.] Mill.), espèce hôte principale, ainsi que les épinettes blanches et noires respectivement (*Picea glauca* [Moench] Voss et *Picea mariana* [Mill.] B.S.P.) de façon récurrente. Au cours de la dernière épidémie, plus de 55 millions ha de forêt ont été affectés (Boulet et al. 1996).

Grâce aux études paléoécologiques, entre-autres, la dynamique naturelle des feux en forêt boréale au cours de l'Holocène est mieux connue (e.g. De Grandpré et al. 2000, Carcaillet et al. 2001, Bergeron et al. 2004) et des limites de variabilité naturelle ont été proposées pour certains secteurs (Cyr et al. 2009). En comparaison, les connaissances concernant le régime de perturbation lié aux épidémies de la TBE, sur une échelle temporelle longue, sont beaucoup plus limitées. L'étude des épidémies est principalement basée sur l'utilisation des séries dendrochronologiques construites à partir des anneaux de croissance des arbres. De ce fait, les reconstitutions de périodes épidémiques passées sont limitées par l'âge du matériau bois utilisé (arbres, bois de construction de vieux bâtiments). L'intervalle entre les épidémies de la TBE a été établi à environ 30-40 ans, depuis les derniers 450 ans (Blais 1983, Royama 1984, Krause 1997, Jardon et al. 2003, Boulanger and Arsenault 2004). Très peu d'études sur la dynamique à long terme des épidémies de la TBE en forêt boréale ont été effectuées. Simard et al. (2002, 2006), en utilisant des fèces et des capsules céphaliques de la tordeuse comme indicateurs paléoécologiques, ont montré une grande variation de l'abondance des macrofossiles dans le temps, suggérant que les périodes d'activités intenses de la TBE, telles qu'observées au XX^{ème} siècle, ont été rares au cours de l'Holocène. Des résultats similaires ont été obtenus lors d'une étude préliminaire utilisant les subfossiles d'arbres enfouis dans les tourbières (Simard 2003) et dans d'autres études paléoécologiques (Anderson et al. 1986, Bhiry et Filion 1996, Jasinki et Payette 2005, Lavoie et al. 2009).

L'objectif général de la thèse était de reconstituer la dynamique des épidémies de la tordeuse des bourgeons de l'épinette au cours de l'Holocène, en utilisant l'information contenue dans les cernes de croissance d'arbres enfouis dans différentes tourbières, afin de mieux définir dans le temps les limites de variabilité naturelle des épidémies en forêt boréale.

Les méthodes dendrochronologiques traditionnelles sont sensibles aux périodes de défoliation sévères induites par la tordeuse mais ne permettent pas toujours l'identification précise des périodes de défoliation faibles à modérées. Les épidémies sévères de la TBE, de par la réduction importante de la biomasse photosynthétique, induisent une réduction de la croissance radiale des arbres caractéristique, prenant la forme d'un « V » ou d'un « U » dans les courbes de croissance due à une faible variabilité interannuelle de la croissance, qui perdure quelques années (Blais 1962, Swetnam et al. 1985, Jardon et al. 1994). Cet indicateur n'est toutefois pas présent lors des épidémies légères, une augmentation de la croissance suite à un phénomène de compensation ayant même été rapportée par plusieurs auteurs (Reich et al. 1993, Vanderklein et Reich 1999, Chen et al. 2001, Lavigne et al. 2001, Little et al. 2003). Par conséquent, l'estimation de la durée et de la fréquence des défoliations légères peuvent être sous estimées en utilisant la largeur des cernes de croissance comme unique indicateur de périodes épidémiques. La comparaison de la croissance radiale d'espèces hôtes et non hôtes est communément utilisée afin d'éviter de confondre les effets de la TBE avec ceux du climat, par exemple (Blais 1954). La croissance de l'espèce non hôte peut être soustraite de celle de l'espèce hôte afin d'extraire l'effet des autres facteurs environnementaux et ainsi obtenir un portrait plus juste de la défoliation sur la croissance (Swetnam et al. 1985). Toutefois, cette méthode comporte des limites. Entre autres, elle ne permet pas toujours la mise en évidence de périodes épidémiques légères et n'est pas utilisable dans une situation où les espèces non hôtes ne sont pas disponibles.

Dans ce contexte, une approche multi-indicateurs est à préconiser. Nous proposons d'utiliser les isotopes stables du carbone et de l'oxygène comme indicateurs complémentaires aux méthodes dendrochronologiques usuelles pour l'indentification des périodes épidémiques passées.

Les analyses des isotopes stables sont devenues un outil quasi incontournable dans les études écophysiologiques. Il est maintenant bien établi que le ratio $^{13}C/^{12}C$ de la matière organique des plantes de type C_3 dépend du ratio c_i/c_a, (pression partielle intercellulaire de CO_2 (c_i) par rapport à la pression partielle du CO_2 de l'air ambiant), lequel est contrôlé par la conductance stomatique et le taux d'assimilation du CO_2 (Farquhar et al. 1982). Une modification du ratio c_i/c_a se reflètera par une modification de la composition isotopique en carbone ($\delta^{13}C$) de la matière organique. Comme la défoliation peut induire une modification des échanges gazeux telles une augmentation du taux de photosynthèse au niveau des aiguilles restantes ou encore du nouveau feuillage, tant chez le sapin baumier (Lavigne et al. 2001, Little et al. 2003) que chez d'autres conifères (Welter 1989, Reich et al. 1993, Vanderklein and Reich 1999, Chen et al. 2001), une réponse au niveau du $\delta^{13}C$ de la cellulose du cerne de croissance mise en place pendant une épidémie, sous la forme d'un enrichissement isotopique, est attendue. D'autres modifications physiologiques en réponse à la défoliation peuvent potentiellement agir sur le ^{13}C cellulosique, comme l'utilisation des réserves pour la construction du cerne de croissance. Les assimilats mis en réserves sous formes d'amidon peuvent fournir assez de substrat pré-cellulosique pour permettre la croissance pendant les périodes de défoliation. Comme l'amidon est enrichi en ^{13}C en comparaison aux assimilats (Brugnoli et al. 1988, Le Roux et al. 2001, Damesin and Lelarge 2003, Helle and Schleser

2004), l'utilisation de ce substrat pour la fabrication des parois cellulaires du cerne de croissance pendant les périodes de défoliation devrait induire une signature chimique distincte.

Contrairement au ratio isotopique $^{13}C/^{12}C$ de la matière organique, celui des isotopes d'oxygène ($^{18}O/^{16}O$) n'est pas altérée par des variations du taux de photosynthèse. Une analyse conjointe du $\delta^{13}C$ et $\delta^{18}O$ permet ainsi de discriminer entre une variation du $\delta^{13}C$ découlant d'un changement d'activité de la Rubisco (ribulose 1·5-bisphosphate carboxylase/oxygenase) ou d'une modification de la conductance stomatique (Barbour et Farquhar 2000, Scheidegger et al. 2000). Le $\delta^{18}O$ de la matière organique est principalement *déterminé par la composition isotopique de l'eau du sol, l'enrichissement de* l'eau de la feuille dû à la transpiration et l'effet Péclet, et le fractionnement biochimique lors de l'incorporation (Sternberg 1989, Yakir and DeNiro 1990, Roden and Ehleringer 1999a, b, Roden et al. 2000).

Les résultats attendus sont principalement basés sur des considérations théoriques puisque très peu d'études concernant les isotopes stables se sont intéressées à la problématique des épidémies d'insectes défoliateurs. Une étude portant sur l'effet de dommages causés par un insecte suceur-perceur chez une espèce feuillue n'a montré aucun effet sur le $\delta^{13}C$ des feuilles restantes (Ellsworth et al. 1994). Toutefois, Leavitt et Long (1986) ont observé un enrichissement en ^{13}C de la cellulose des cernes de croissance de deux conifères, *Abies concolor* et *Pseudotsuga menziesii*, attaqués par la tordeuse des bourgeons de l'épinette de l'ouest (*Choristoneura occidentalis* Freeman). *Pinus ponderosa*, une espèce non hôte à l'insecte, n'a pas enregistré cet enrichissement lors de la période épidémique.

La fiabilité de l'utilisation des isotopes stables de carbone et d'oxygène des cernes de croissance afin de faciliter l'identification dendrochronologique de périodes épidémiques connues de la tordeuse des bourgeons de l'épinette chez deux espèces hôtes, *Abies balsamea* et *Picea mariana*, a d'abord été examinée dans le chapitre 2. L'objectif de ce chapitre était de comparer la réponse isotopique des cernes de croissance produits pendant les années endémiques et épidémiques entre les années 1940 et 1990. Cette réponse isotopique a aussi été comparée avec celle d'une espèce non hôte, *Pinus banksiana*.

Le chapitre 3 a été orienté vers les aspects physiologiques de la défoliation. Un plan d'expérience incluant différents niveaux de défoliation artificielle sur de jeunes *Abies balsamea*, répétées pendant quatre saisons de croissance, a été développé. L'objectif était de déterminer les facteurs qui limitent l'interprétation du $\delta^{13}C$ et $\delta^{18}O$ pendant les périodes de défoliation.

Afin d'atteindre l'objectif général de la thèse qui consistait en la reconstitution locale de la dynamique à long terme des épidémies de la tordeuse des bourgeons de l'épinette, les subfossiles d'arbres enfouis dans les tourbières ont été désignés comme matériel par excellence. Les cernes de croissance des arbres offrent un potentiel de reconstitution environnementale à l'année près, ce qui en fait des archives du passé d'une haute résolution temporelle. Grâce aux conditions d'enfouissement anaérobiques dans les tourbières, le bois mort peut être préservé et retrouvé tout le long du profil organique, offrant la possibilité de remonter dans le temps et d'obtenir une chronologie de croissance potentiellement continue.

L'application de la méthodologie d'identification des épidémies de la TBE développée à l'aide des isotopes stables de carbone et d'oxygène aux subfossiles d'arbres excavés de trois tourbières, de même que la reconstitution de la dynamique à long terme des épidémies et les difficultés rencontrées sont discutées dans les chapitres 4 et 5 respectivement.

Le dernier chapitre contient une synthèse des conclusions présentées dans les chapitres précédents ainsi qu'une courte discussion sur les points laissés en suspens et les questions soulevées, de même que quelques suggestions pour des recherches similaires futures.

CHAPITRE I

Carbon and oxygen stable isotopes from tree-rings to identify spruce budworm outbreaks in the boreal forest of Québec

Article publié dans Chemical Geology 252 (2008) 80-87.

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1.1 ABSTRACT

The aim of this study was to test the potential of carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) isotopes as indicators of spruce budworm outbreaks. It was hypothesised that defoliation induced by insects would trigger a ^{13}C enrichment of *Abies balsamea* and *Picea mariana* tree-ring *α*-cellulose through higher photosynthetic compensatory rates, while $\delta^{18}O$ would remain constant. The hypothesis was based on observations of increased photosynthetic rate induced by defoliation, as a compensatory mechanism (Little et al. 2003, Lavigne et al. 2001). Comparison of the two host species, *Abies balsamea* and *Picea mariana* with a non-host one, *Pinus banksiana*, revealed carbon isotope enrichments during both the 1950s and 1970s spruce budworm outbreaks which did not occur in the non-host species. Carbon and oxygen isotope values showed high synchronicity not only within species but also between species (*A. balsamea* and *P. mariana*) and sites. *P. banksiana* $\delta^{18}O$ values were also highly synchronous with those of the two other coniferous species. The comparison of host and non-host ring width, $\delta^{13}C$ and $\delta^{18}O$ chronologies confirmed the potential of combining these isotope indicators of spruce budworm outbreaks.

1.2 INTRODUCTION

Spruce budworm (*Choristoneura fumiferana* Clem.) outbreaks are one of the main disturbances of North Eastern boreal forests of North America (Maclean 1984, Morin 1994). Balsam fir [*Abies balsamea* (L.) Mill.] remains the favourite host species for spruce budworms in the North Eastern boreal forest (Martineau 1985). However, different coniferous species such as white spruce [*Picea glauca* (Moench) Voss], black spruce [*Picea mariana* (Mill.) B.S.P] or larch [*Larix laricina* (DuRoi) K. Koch] can also be affected by this insect.

Extended records of spruce budworm outbreaks have been assembled through core of peatland sediment for macrofossil reconstructions (Simard et al. 2002, Simard et al. 2006). While these records provide useful information about past spruce budworm infestations, their coarse temporal resolution (often greater than 100 years) imprecisely portrays the decadal scale frequency (32 to 34 year intervals) of spruce budworm outbreaks (Royama 1984). A more accurate but shorter duration chronology (300 to 400 years) of spruce budworm infestations in the North Eastern boreal forest has been reconstructed from living, historical and sub-fossil wood (Blais 1983, Krause 1997, Boulanger and Arseneault 2004). This accurate identification of past insect outbreaks from tree-rings was accomplished through detailed comparisons between host and non-host tree species growth curves (Blais 1962, Swetnam et al. 1985). As spruce budworms feed on the foliage they effectively reduce the leaf surface area and photosynthetic capacity of the host trees by varying degrees (heavy, moderate, light) (Kozlowski 1969). During heavy defoliation, growth loss observed in ring width series exhibits a typical "V" or "U" shape lasting for five years or more (Swetnam et al. 1985, Jardon et al. 1994). However, it is highly improbable to find numerous non-host subfossil trees in the peat land repositories of the North Eastern Boreal forest to compare with host tree records and therefore difficult to further extend the records of spruce budworm infestations beyond the past several hundred years (Simard 2003). An additional problem arises when attempting to reconstruct past light to moderate spruce budworm infestations, that is, while infestations resulting in only light to moderate defoliations can also induce tree growth loss, the indicative "V" or "U" shaped patterns are usually not observed.

Attempts have been made to confirm other indicators of insect infestations using wood anatomy (Harper 1913, Bailey 1925, Filion and Cournoyer 1995, Krause and Morin 1995, Liang et al. 1997) and tree growth anomalies (Simard and

Payette 2003). None of these methods provide exclusive evidence of defoliation allowing for assured reconstructions of spruce budworm outbreaks on more extensive temporal scales. The difficulties in identifying and establishing precise onsets of past insect outbreaks using traditional dendrochronological methods has prompted this investigation to determine whether carbon isotopic compositions of host tree annual tree rings unequivocally provide spruce budworm outbreak "signatures" during two known 20th century infestations outbreaks (1950s and 1970s). Here, the carbon isotopic compositions are compared to congruent annual ring width and oxygen isotope values in both host and non-host tree species to confirm their utility in tracing spruce budworm outbreaks.

2.1 Background

From a physiological point of view, various responses may be observed in plants affected by leaf-feeding insects. Lavigne et al. (2001) and Little et al. (2003) studied the effect of defoliation on physiological parameters of *A. balsamea* and showed that the photosynthetic rate of the remaining needles increased, while stomatal activity remained constant. Increase of photosynthetic rate per unit area of residual and/or regrowth foliage as a compensatory mechanism has been observed in other coniferous species as well (Welter 1989, Reich et al. 1993, Vanderklein and Reich 1999, Chen et al. 2001). Decreases or no change of photosynthetic rate have also been observed (cf. Welter 1989).

Discriminations against the heavier carbon isotope (^{13}C) occur during stomatal conductance (g_s) of CO_2 into leaves and during subsequent photosynthetic assimilation (A) of carbon (O'Leary 1981, Farquhar et al. 1982). As discussed previously, the physiological responses of conifers to insect related defoliation in plants would significantly impact leaf stomatal conductance (g_s) and the rate of photosynthetic assimilation (A) and, therefore, carbon isotope analysis of host tree-rings represents a potentially promising method of detecting spruce budworm outbreaks. It is hypothesized that the defoliation induced by leaf feeding insects would trigger a ^{13}C enrichment of *A. balsamea* tree-rings *α*-cellulose through a higher compensatory photosynthetic rate. However, rather than reflecting changes in stomatal conductance and photosynthetic capacity, the oxygen isotopic composition ($\delta^{18}O$) of the tree ring cellulose is expected to reflect the isotopic signature of source water (Roden and Ehleringer 1999a, b, Roden et al. 2000), the isotopic signature of leaf water, consequent of evaporation and the Péclet effect, the biochemical fractionation during biosynthesis of photosynthetic sugars (Sternberg 1989, Yakir and DeNiro 1990) and the reequilibrium exchange between the carbohydrate and xylem water during tree-ring xylem cellulose synthesis (Sternberg et al. 1986, Yakir and DeNiro 1990, Luo and Sternberg 1992).

The impact of phytophagous insects on carbon isotope composition of plant materials is very limited (Leavitt and Long 1986, Ellsworth et al. 1994). Ellsworth et al. (1994) observed that feeding damage induced by piercing-sucking insects on deciduous trees did not provoke compensatory gas exchange, rather a reduction of photosynthesis. In addition, damage induced by feeding insects had no impact on water-use efficiency, WUE (the ratio between photosynthesis and transpiration), or the carbon isotopic composition ($\delta^{13}C$) of the remaining leaves. However, Leavitt and Long (1986) observed enriched $\delta^{13}C$ values in *Abies concolor* and *Pseudotsuga menziesii* tree-rings, two host species, during the maximum infestation period of western spruce budworm outbreak. *Pinus ponderosa*, a non-host species, did not record carbon isotope enrichments.

The objective here was to test the potential of annually resolved tree ring carbon isotopes as indicators of spruce budworm outbreaks in the North Eastern boreal forest. To verify the effects of defoliation intensity and host species exclusivity on tree ring carbon isotope compositions, matching carbon isotope analyses were performed on a secondary spruce budworm host species *P. mariana*.

Annual tree ring widths and isotopic analyses (carbon and oxygen) on a control non-host species, *Pinus banksiana* (Lamb.), helped clarify the origin of the carbon and oxygen isotopic variations.

1.3 METHODS

1.3.1 Study area and samples

Five different sites were selected for sampling, three for *A. balsamea*, one for *P. mariana* and one for *P. banksiana*. All sites were located within a 20 km radius, about 100 km north of Chicoutimi (48° 25'N, 71° 4'W), Québec, Canada (fig. 1.1; table 1). They belonged to the east balsam fir – white birch domain of the continuous boreal forest (Saucier et al. 1998). Regional climatic conditions for 1942-1990 (Bagotville meteorological station, 48° 20'N, 71° 0'W, 159 m a.s.l.) are characterized by a mean temperature of 2.2°C and a mean annual precipitation of 930 mm, 37% of which fell as snow during this period (Environnement Canada 1993). Main disturbances in this environment consist of insect outbreaks and fires.

Sampled *A. balsamea* and *P. mariana* were part of *Abies balsamea – Picea mariana* mixed mature stands located on slopes surrounding depressions forming peatlands. The minimal mean age of *A. balsamea* sample stands was 75 years (age at breast height). For the *P. mariana* samples stand, the minimal age was 140 years (age at base height) with a few trees reaching 250 years old (table 1). All *Abies balsamea – Picea mariana* stand were closed canopies with moss and *Sphagnum* sp. ground coverings. *P. banksiana* trees were sampled in *a pure P. banksiana stand with a mean tree age of 71 years (age at breast* height). The landscape was generally flat (null slope) and ground vegetation was primarily composed of ericaceous shrubs (*Kalmia angustifolia* L. and *Ledum groenlandicum* Retzius) growing on a sandy soil.

Ten dominant trees per site were selected for carbon and oxygen stable isotope analyses. Two cores, at 180° from each other, were extracted from each tree at breast height with a 0.5 cm diameter Pressler borer. Sample surfaces were prepared with a razor blade and measured using a sliding-stage incremental micrometer (Henson, California, USA) with a precision 0.01mm (Cook and Kairiukstis 1990). Visual crossdating was verified using COFECHA program (Holmes 1983). Standardisation (20 year cubic smoothing spline with a fixed 50% cutoff) was done with the ARSTAN program (Cook 1985). The five best crossdated trees per site, i.e showing the highest correlation and also large growth reduction during outbreak periods in the early 1950s and 1970s, were chosen for stable isotope analyses. *P. mariana* samples did not reveal a growth reduction during the 1950s outbreak, although *A. balsamea* growth curves from the same provenance showed the impact of outbreaks in the stand during this period (data not shown).

1.3.2 Sample preparation and stable isotope analyses

Since the two most recent outbreak periods in the studied area occurred in the 1950s and 1970s (Blais 1983, Morin and Laprise 1990), annual rings from the 1940-1990 period were separated from each core with a scalpel, under a binocular microscope, at the earlywood-latewood border. This period was chosen in order to obtain representative pre- and post-epidemic sections for both outbreak periods. Within each site, each same year ring (between 1940 and 1990; 51 years) from the five sampled trees were pooled together to create 51 tree ring samples per site. Rings were ground using a steel ball mill (MM200; Retsch, Haan, Germany). Holo-cellulose was isolated by delignification in an acetic-acid-acidified sodium chlorite solution, after first removing oils and resins with toluene-ethanol and ethanol soxhlet extractions (Leavitt and Danzer 1993). Holo-cellulose was then reduced to alpha-cellulose in sodium hydroxide. Approximately 1.0 ± 0.1 mg of α-cellulose was packed in tin capsules for carbon combustion. Samples for carbon isotopic analysis were converted to CO_2 with an elemental analyzer (ECS 4010, Costech Analytical, Valencia, CA) and analyzed with a continuous flow isotope ratio mass spectrometer (Delta PlusXP, Thermofinnigan, Bremen). Carbon isotopic results are reported in per mil (‰) and expressed in relation to VPDB (Vienna Peedee belemnite) standard. For oxygen pyrolysis, approximately 0.30 ± 0.05 mg of α-cellulose was packed in silver cups. Samples for oxygen isotopic analysis were converted to CO with a pyrolysis elemental analyzer (TC/EA, ThermoFinnigan, Bremen) and also analyzed with a continuous flow isotope ratio mass spectrometer (Delta PlusXP, Thermofinnigan, Bremen). Oxygen isotopic results are reported in per mil (‰) relative to VSMOW (Vienna Standard Mean Ocean Water) standard. The isotope composition (δ) of carbon and oxygen is expressed as deviation from the standard:

$$\delta^{xx}E = \frac{R_{sample}}{R_{standard}} - 1$$

where E is the element of interest (carbon or oxygen), ^{xx} represents the heaviest isotope in the element (^{13}C or ^{18}O) and R is the isotope ratio ($^{13}C/^{12}C$ or $^{18}O/^{16}O$).

The precision and accuracy of mass spectrometric analyses were 0.2‰ (Acetanilide) and 0.03‰ (Acetanilide) for carbon and 0.2‰ (IAEA-602) and 0.005‰ (Hekatek Benzoic acid) for oxygen, respectively. Carbon analyses were carried out at the Idaho stable Isotopes Laboratory (University of Idaho, ID, USA) and oxygen analyses at the Stable Isotope Core Laboratory (Washington State University, WA, USA).

1.4 RESULTS

1.4.1 Ring width analysis

The tree ring time series covers two different known spruce budworm outbreaks (1950s and 1970s; Morin 1994). The Figure 1.2 *A. balsamea* tree-ring indices reveal markedly lower ring widths between 1949-1954 (1955 for site a) and 1975-1980, (the years 1951-52 and 1978 represent the minimum ring widths). Full recovery (to average ring width index values: 1.04) occurred between 1954 and 1955, and in 1980 for the most recent outbreak period. The ring index for *P. mariana* suggests that this host species was not affected by the 1950s spruce budworm outbreak (fig. 1.2). However, during the 1970s outbreak, the *P. mariana* ring width index reduced on cue with the host *A. balsamea* although its recovery to normal growth required two additional years (fig. 1.2).

Only the most recent outbreak period was covered by *P. banksiana* data. A light radial growth decrease is observed at the beginning of the mid 1970s epidemic period although this decrease does not appear to be anomalous (fig. 1.2).

1.4.2 Carbon and oxygen isotopes

Year to year variation of tree-ring carbon and oxygen isotope values shows a high degree of synchronicity both within and between species (figs. 1.3b, c). Carbon isotope enrichments commenced for both tree species in 1947-48 and then again in 1976. Maximum enrichment values in 1950-51 and 1977 followed by depletion minimum values in 1954 and 1980, respectively (fig. 1.3b). A comparison between Figure 1.3a and b shows that the most enriched carbon isotopes value for *A. balsamea* (-22.4 in 1950-51 and -21.4 in 1977) occurred one year before the maximal growth reduction occurred (1951-52 and 1978). For *P. mariana* the 1951 carbon isotope enrichment peak did not coincide with any growth reduction whereas the 1977 enrichment peak occurred one year earlier than maximal ring growth reduction (figs. 1.3a, b). Carbon isotope depletion troughs directly coincide with complete growth recovery rings for *A. balsamea* (1954 and 1980) but not for *P. mariana* (fig. 1.3a, b). During the 1950s outbreak, the carbon isotope enrichments seemed to precede ring width index decreases by one (sites a and d) to two (sites b and c) years. However, carbon isotope enrichments commence one year before ring width index reductions, for all sites, during the 1970s spruce budworm outbreak. Additionally, ring width index increases coincide with carbon isotope enrichments, for both species and both outbreak periods, prior to growth reductions. The carbon isotopic composition of *P. banksiana* did not vary during the mid 70's defoliation period in comparison with the distinct carbon isotope enrichments observed for *A. balsamea* and *P. mariana* (fig. 1.3b).

The oxygen isotope time series for *A. balsamea, P. mariana* and *P. banksiana* show regular amplitude variations that are quite consistent through time, revealing no particular trends (fig. 1.3c). During both outbreak periods (1950s and the 1970s), but particularly the 1970s period, an oxygen isotope enrichment was observed that is coincident with the carbon isotope enrichment (figs. 1.3b, c). For the mid 1970's period, the most enriched oxygen isotope value occurred in 1976 followed by an immediate and sharp depletion in 1977 (fig. 1.3c).

1.5 DISCUSSION

The carbon isotopic compositions of both *A. balsamea* and *P. mariana* enrich in response to defoliation during the 1950s and 1970s spruce budworm outbreaks (fig. 1.3b). Similarly, Leavitt and Long (1986) recorded carbon isotopes enrichments in host species affected by western spruce budworm outbreaks. While hypothetical at present, the mechanisms accounting for carbon isotope enrichments coincident with these budworm defoliation periods must be related to one or all of the well known effects on carbon isotope compositions in plants. In general, carbon isotope enrichments in plants can be the result of reduced stomatal conductance, increased photosynthetic capacity or a combined change in both (Francey and Farquhar 1982). Previous studies have shown that the different levels of defoliation on *A. balsamea* (Piene 1980, Lavigne et al. 2001, Little et al. 2003), and also on other coniferous species (Reich et al. 1993, Chen et al. 2001, Vanderklein and Reich 1999), induced an increase in photosynthetic rates. An increase in leaf conductance was also observed by Reich et al. (1993). Lavigne et al. (2001) suggested that the increased photosynthetic rate observed in *A. balsamea* resulted from an increased allocation of mineral nutrients to 1-year old foliage enhancing their amount and/or activity, as well as increasing chlorophyll concentration.

Supporting the hypothesis of increased photosynthetic rate in the early stages of defoliation, carbon isotope enrichment coincides with an increase in ring width for *A. balsamea* at the beginning of the '50s and '70s outbreaks, as well as for *P. mariana* at the onset of the latest outbreak (figs. 1.3a, b). Therefore, despite the loss of foliar biomass, photosynthetic compensation may have surpassed the initial rate of carbohydrate production in these cases. Reich et al. (1993) also observed overcompensation in terms of whole plant growth due to shifts in allocation and enhanced photosynthesis. Figures 1.3a and 1.3b show carbon isotope enrichments occurring for a few years more after tree ring widths started to decrease. For trees to survive heavy defoliation periods, it is necessary for them to make use of their starch reserves (Kozlowski et al. 1991). The carbon isotopic composition of starch is relatively more enriched than tree ring cellulose (Brugnoli et al. 1988, Le Roux et al. 2001, Helle and Schleser 2004) and, the use of these reserves during periods of heavy defoliation may partially explain the enriched carbon isotope values coinciding with the two spruce budworm outbreak periods. Carbon isotope depletions coincided with ring width increases at the end of both outbreaks indicating a return to non-compensatory conditions (figs. 1.3a, b).

During the 1950s outbreak, the ring width measurements for *P. mariana* indicate that they were not or only slightly affected by the spruce budworm defoliation. Nealis and Regnière (2004) showed that *P. mariana*, although vulnerable to spruce budworm attacks during infestation, is generally less defoliated than other host species, such as *A. balsamea*. This was explained by a temporary reduction in susceptibility of the tree species resulting from a lack of synchronism of spring larval emergence and bud burst. In spite of the absence of radial growth reduction signs, the carbon isotope values reacted (enriched) in the same way as in *A. balsamea* (fig. 1.3a). Two explanations for the carbon isotope enrichment in *P. mariana* during the 1950s outbreak are suggested. First, photosynthetic compensation to defoliation (as discussed previously) may be partially responsible for the carbon isotope enrichment. The second hypothesis is related to the tree stand reaction. The 1950s outbreak was not as severe as the 1970s one and the budworm population, in this mixed *P. mariana* - *A. balsamea* stand, could feed mainly on *A. balsamea* (growth reduction similar to *A. balsamea* from sites a, b and c; data not shown), potentially leaving *P. mariana* less affected. A defoliated canopy could have increased light availability. Increased light reception coupled with foliar nutrient concentration has been known to increase the WUE and enrich carbon isotope values in tree rings (Leavitt and Long 1991, Guehl et al. 1995, Yakir and Israeli 1995). Canopy opening and increased radiation might also have contributed to the carbon isotope enrichment of *A. balsamea*.

Simultaneous analyses of tree ring cellulose carbon and oxygen isotopes may help discriminate whether changes observed in the carbon isotope values originated from a modification of photosynthesis or stomatal conductance since the oxygen isotope composition of the congruent tree rings is not expected to reflect changes in photosynthetic capacity (Craig and Gordon 1965, Flanagan et al. 1991). Barbour et al. (2002) indicate that the response of tree ring oxygen isotopes to changes in coniferous tree leaf conductance of water vapour (g_s) is expected to be rather small compared to broad-leaf trees. Furthermore, Scheidegger et al. (2000) report a negative relationship between d18O values and stomatal conductance. Roden and Ehleringer (1999a, b) and Roden et al. (2000) demonstrated that the source water oxygen isotopic composition has influenced tree ring cellulose oxygen compositions. Spruce budworm outbreaks preceded by dryer early summers have previously been observed (Wellington et al. 1950, Pilon and Blais 1961). A change in the precipitation regime and warmer temperature might account for an enriched source water oxygen isotopic composition and also explain the slight negative growth index observed in *P. banksiana* during the 1970s outbreak (figs 3a, c). Dryer conditions (low relative humidity) and consequent higher vapour pressure deficits (VPD) might also have contributed to an enriched oxygen isotopic composition at the leaf water level (Roden and Ehleringer 1999a, b, Roden et al. 2000, Barbour et al. 2002). Without additional details on the environment at the time of defoliation, it is difficult to give confident details on the origin of the isotope composition variations observed. A comparison between the rather synchronous oxygen isotope patterns of host and non-host species (fig. 1.3c) suggests that these variations are most likely related to climatic changes rather than spruce budworm outbreaks.

It is known that defoliation usually starts one to four years before the first year of important growth reduction (Blais 1958, 1962, Krause et al. 2003). Defoliation might in fact have started between 1946-1949 and 1972-1975 for the earlier and later outbreaks, respectively. Based on the results presented here, the carbon isotope composition of balsam fir α-cellulose may be a more immediate indicator of the beginning of defoliation since carbon being incorporated into the photosynthates reflect an immediate response of physiological processes discussed previously. According to these results, the onset for the first epidemic period would be in 1948 (sites b, c and d) and 1949 (site a). The beginning of the second period would be in 1974 (sites a and d) and 1975 (sites b and c). Those dates fall within the large scale aerial observations of defoliation over

Eastern North America (Hardy et al. 1985) and precisely identify the onset of defoliation at the stand level.

1.6 CONCLUSION

In order to test the potential of carbon and oxygen isotopes as indicators of spruce budworm outbreaks, host and non-host coniferous species were studied. Carbon and oxygen isotopic compositions of annual tree ring cellulose showed high synchronicity not only within species but also between species (*A. balsamea* and *P. mariana*) and sites. *P. banksiana* oxygen isotope values were also highly synchronous with those of the two other coniferous species suggesting regional climate variations were primarily responsible for the oxygen isotope compositions.

Comparisons between host and non-host ring width and carbon and oxygen isotope chronologies reveal their potential as indicators of spruce budworm outbreaks. The carbon isotope enrichments observed in *A. balsamea* and *P. mariana* in conjunction with the outbreak periods were not present in *P. banksiana.* However, the oxygen isotope chronologies of the three species varied in the same manner, indicating an influencing factor other than climate for enriched *A. balsamea* and *P. mariana* carbon isotope compositions. Enriched oxygen isotope compositions in direct relation to outbreak period enriched carbon isotope values is thought to reflect dryer climatic conditions prior to and during those outbreak periods. While the oxygen isotope results did not totally negate the possibility of reduced stomatal activity contributing to enriched carbon isotope values during the spruce budworm epidemic periods, comparisons of host and non-host species isotope compositions in direct relation to ring widths strongly suggest that the primary mechanism behind the carbon isotope enrichments observed in host species is defoliation.

Carbon isotope values were observed to enrich one (sites a and d) or two years (sites b and c) before ring widths decreased. Carbon isotope enrichment and ring width decrease synchronicity strongly supports our hypothesis of photosynthetic compensation induced by defoliation. Even though the 1950s outbreak was undetectable with *P. mariana* ring width measurements, probably due to light defoliation, it was evident in carbon isotope measurements. This bodes well for carbon isotope detection of even light spruce budworm defoliation periods in future studies.

At this point, it is difficult to explain with certainty the mechanisms behind carbon and oxygen isotope variations observed during outbreak periods. Further research on physiological aspect of defoliation in conjunction with isotope measurements is required to address these important questions. Despite uncertainties regarding the mechanisms involved, tree-ring carbon and oxygen isotopes analyses enabled the reconstruction of the two last spruce budworm outbreaks in the North Eastern boreal forest.

1.7 ACKNOWLEDGEMENTS

This research was financially supported by the Natural Sciences and Engineering Research Council of Canada (NSERC), The Consortium de Recherche sur la Forêt Boréale Commerciale and the Fondation de l'Université du Québec à Chicoutimi. We thank Dr. J.-F. Boucher, Dr. W. M. Buhay and two anonymous reviewers for helpful comments and suggestions.

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1.9 TABLES AND FIGURES

Table 1.1 Location and description of sampled trees.

Site	Tree species	Location	Mean diameter (cm)	Mean age
Site a	Abies balsamea	48° 37' 28.44"N -70° 33' 55.65"W	23.2	69
Site b	Abies balsamea	48° 40' 28.98"N -70° 36' 49.74"W	17.4	75
Site c	Abies balsamea	48° 37' 26.65"N -70° 32' 04.41"W	17.3	80
Site d	Picea mariana	48° 54' 06.96"N -70° 36' 34.47"W	15.2	140
Site e	Pinus banksiana	48° 34' 29.96"N -70° 58' 47.64"W	14.7	71

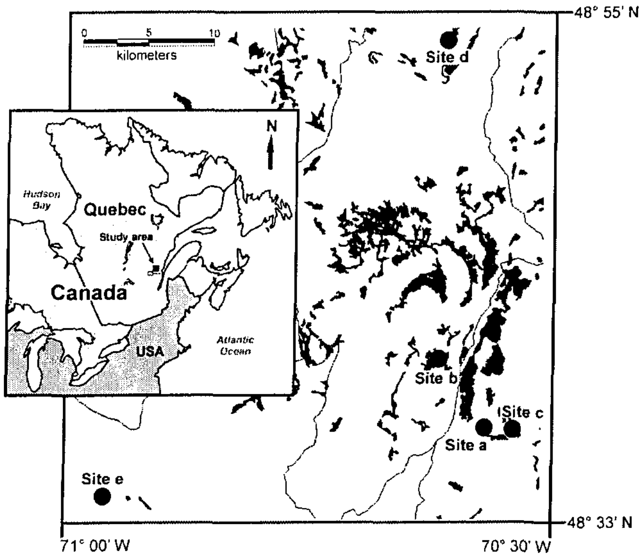

Figure 1.1 Location of the five sample sites.

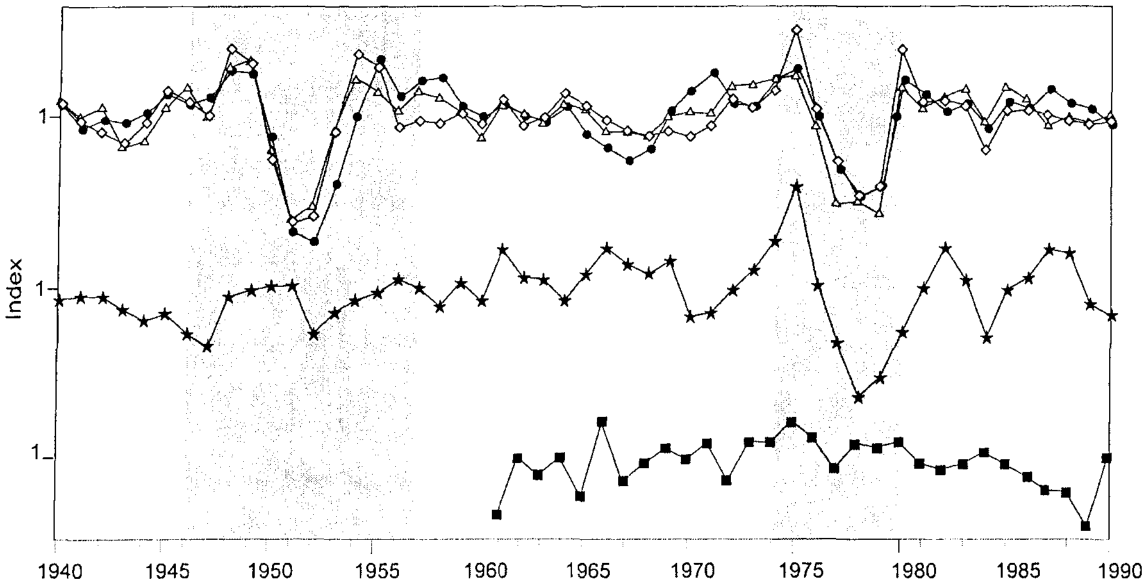

Figure 1.2 Tree-ring indices of *Abies balsamea* (● site a, Δ site b, ◊ site c), *Picea mariana* (★ site d) and *Pinus banksiana* (■ site e). Shaded areas indicate aerial survey observations of defoliation caused by the spruce budworm in the region (Hardy et al. 1985) covering the period 1930-80.

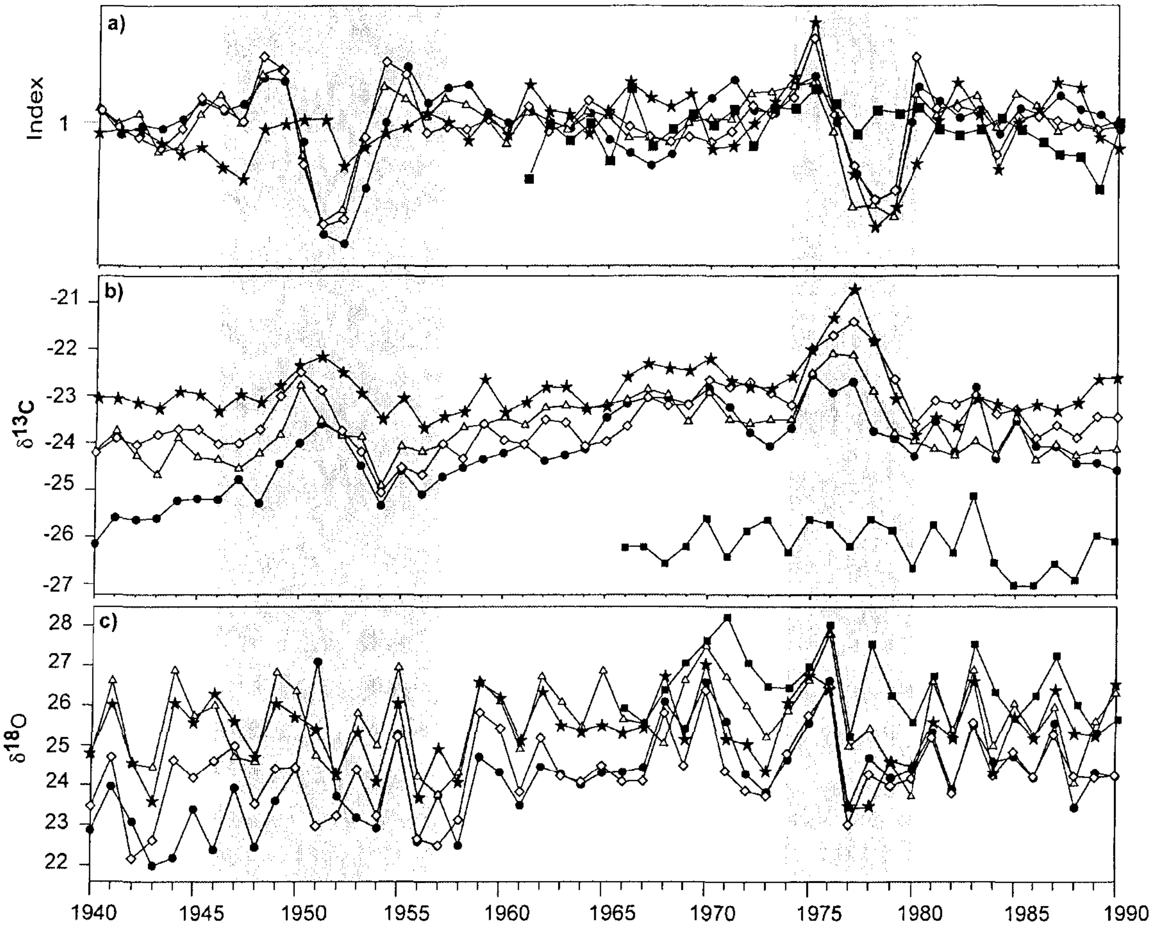

Figure 1.3 *Abies balsamea* (● site a, Δ site b, ◊ site c), *Picea mariana* (★ site d) and *Pinus banksiana* (■ site e) a) tree-ring indices, b) α-cellulose carbon isotope composition and c) α-cellulose oxygen isotope composition. Shaded areas indicate aerial survey observations of defoliation caused by the spruce budworm in the region (Hardy et al. 1985) covering the period 1930-80.

CHAPITRE II

DETECTING PAST SPRUCE BUDWORM INFESTATIONS: RING WIDTHS VS ISOTOPIC COMPOSITION OF ARTIFICIALLY DEFOLIATED BALSAM

2.1 ABSTRACT

During a four-season controlled experiment, *Abies balsamea* seedlings were artificially defoliated to different percentages (levels) to reflect both current (0, 33, 66 and 99%) and past (0, and 50%) year foliage loss. The experiment was designed to clarify the physiological nature of the physical and chemical responses by studying the light-saturated CO_2 assimilation rate (*A)* and stomatal conductance (g_s), tree ring widths (TRW) and the corresponding $\delta^{13}C$ and $\delta^{18}O$. Seedling TRW were observed to be significantly reduced after the second *through fourth growing seasons with increasing levels of defoliation.* Past-year defoliation additionally reduced TRW. An overall positive relationship was detected between tree-ring holo-cellulose $\delta^{13}C$ ($\delta^{13}C_{hc}$) and defoliation magnitudes for the three first seasons. The absence of a significant relationship between the intracellular to ambient CO_2 ratio (c_i/c_a) and defoliation treatments suggests a post-carboxylation mechanism to explain the $\delta^{13}C_{hc}$ enrichment such as the mobilization of ^{13}C-enriched reserves for cell development during the tree ring formation. Early $\delta^{13}C_{hc}$ response to defoliation compared to TRW suggests that carbon isotopes may be a more useful and precise tool in the detection of spruce budworm outbreaks in dendrochronological reconstructions. This study helps bolster the utility of combining both traditional TRW and more recent $\delta^{13}C$ techniques to detect past spruce budworm infestations in the eastern boreal

forest. The study also illustrates the possibility of similarly reconstructing other insect defoliation episodes in other regions.

2.2 INTRODUCTION

Annual rings of trees growing within temperate to boreal forest ecosystems respond both physically and isotopically to a variety of environmental and biophysical influencing and forcing factors. As a result, correlations between the widths and densities of tree rings, their isotopic compositions (carbon, oxygen and hydrogen), and climate parameters such as temperature, precipitation amount, relative air humidity and hydrological growth setting have been extensively reported (Burk & Stuiver, 1981; Feng & Epstein 1994; Buhay & Edwards 1995; Robertson *et al.* 2001; McCarrol & Loader 2004; Verheyden *et al.* 2004). Where significant, these relationships have resulted in many informative reconstructions of past climate and environmental conditions in various regions of the world (Jacoby & D'Arrigo 1995; Brooks, Flanagan & Ehleringer 1998; Gagen, McCarroll & Edouard 2004; Cook, Esper & D'Arrigo 2004; Treydte *et al.* 2006; Kirdyanov *et al.* 2008). However, specific site conditions related to canopy light and nutrient levels or soil and substrate water resources, for example, can sometimes have an effect on these relationships (Dupouey *et al.* 1993; Saurer, Siegenthaler & Schweingruber 1995; Saurer *et al.* 1995, 1997; Kagawa *et al.* 2003; Buhay *et al.* 2008). Site disturbances such as forest fires, selective leaf cuttings or thinning and insect defoliation have also been shown to directly influence plant growth and isotopic compositions (Morrow

& Lamarche 1978; Leavitt & Long 1986; Morin 1994; Trotter, Cobb & Whitham 2002; McDowell *et al.* 2003; Simard *et al.* 2008).

Defoliation of trees by insects is of particular interest in the boreal forest of eastern North America since spruce budworm (*Choristoneura fumiferana* Clem.) outbreaks are one of the main disturbances affecting the forest dynamics and reservoir stocks of balsam fir [*Abies balsamea* (L.) Mill.], white spruce [*Picea glauca* (Moench) Voss] and black spruce [*Picea mariana* (Mill.) B.S.P] (Morin 1994). It would be advantageous both in terms of forest ecology and management strategies to be able to confidently identify past spruce budworm defoliation episodes in order to reconstruct the long-term dynamics of such outbreaks. This information would allow forest managers and ecologists to understand the outbreak dynamics and better predict their behaviour in the context of possible climate change effects on the boreal forest. Moreover, in a context where ecosystem based management is generally considered the most promising approach to the maintenance of healthy and resilient forest ecosystems, it is essential to better assess the long-term variability of insect related disturbances (Gauthier *et al.* 2008). Historical accounts (since the late 1930s) provide adequate information on severe spruce budworm outbreaks in the 1950s and 1970s. However, the real potential in reconstructing longer and more meaningful outbreak records lies in the absolute chronological nature of the annual rings in trees of the northeastern boreal forest (between 200 to 250 years old on average (Viereck & Johnston 1990; Frank 1990; Nienstaedt & Zasada 1990), and the possibility of using historical wood (Krause 1997; Boulanger & Arsenault 2004) and subfossil wood (Simard 2003) to extend reconstructions back in time. It seems reasonable to assume that the residual physical effects of needle defoliations related to spruce budworm outbreaks would be readily identifiable through traditional dendrochronological methods. In fact, this was not the case in a previous reconstruction attempting to extend chronologies back in time using older material such as subfossil wood buried in peatlands (Simard 2003). The standard methodology to identify insect outbreaks in a dendrochronological perspective relies on the comparison of radial growth patterns of host and non-host trees (Blais 1962; Swetnam, Thompson & Sutherland 1985). However, this approach is unlikely to be used in this environment due to the inherent difficulties encountered while building chronologies from subfossil trees, non-host tree species being rarely found in sufficient numbers. Moreover, while traditional dendrochronological methods are sensitive to severe spruce budworm defoliation episodes, they are not reliable indicators of moderate to light defoliation episodes and, consequently, provide inaccurate estimates on the duration of these outbreaks.

A recent and relatively non-traditional dendrochronological stable isotope tree-ring survey of historical spruce budworm outbreaks (1950-1960; 1970-1980), extracted from mature host and non-host conifers from the boreal forest, produced definitive carbon isotope enrichment signatures in both severely and more lightly defoliated trees (Simard *et al.* 2008; chapitre 1). Host-tree enriched carbon isotope signals and reduced ring widths directly corresponded with the two last spruce budworm outbreaks in the northeastern boreal forest while oxygen isotope series showed regular amplitude variations consistent through time similar to the oxygen series of a non-host species used for comparison.

To further investigate the use of ring widths and carbon and oxygen isotope tree-ring compositions to identify spruce budworm outbreaks, a controlled experiment involving different magnitudes of artificially defoliated balsam fir seedlings was conducted. This study was designed to determine the relationships between defoliation magnitudes and annual tree-ring widths, holo-cellulose carbon and oxygen isotopic compositions and plant physiology (CO_2 assimilation rate and stomatal conductance) over four growing seasons. Based on the evidence from Simard *et al.* (2008), reduced carbon isotope discrimination in response to increased levels of defoliation reflected by a corresponding carbon isotopic enrichment of the balsam fir tree-ring cellulose is expected, with no analogous effects on the oxygen isotopic compositions. Tree-ring width reductions are anticipated in rings of the most severely defoliated trees with increasing seasonal impact on radial growth.

2.3 MATERIAL AND METHODS

2.3.1 Plant materials

A growth experiment to test the effects of different degrees of defoliation on tree-ring widths and wood holo-cellulose isotope (carbon and oxygen) composition was conducted in both open field and greenhouse settings at the Université du Québec à Chicoutimi. The defoliation experiment was performed during four growing seasons (natural and simulated) between 2005 and 2006. Two hundred eighty-eight container-grown five-year-old balsam fir (*A. balsamea*) seedlings were obtained from a nursery in the Lac Saint-Jean (Quebec, Canada) area. The uniform height (26.7±4.1 cm) and root collar diameter (7.8±1.2 mm) (mean ±std) seedlings were planted in plastic pots containing peat moss (15.2 cm diameter, ~2.44 litres of peat moss/pot) in the spring of 2004 and allowed to adapt to the new rooting environment during that initial summer.

2.3.2 Experimental design

The experimental design, a three-block fully randomized split-plot design, was subjected to four growing seasons as the main plot and four different percentage levels of current-year needle defoliation (0%, 33%, 66% and 99%) [CYD] and two percentage levels of past-year defoliation (0% and 50%) [PYD], as the subplot. The seedlings were randomly split between three-block plots (96 trees/plot) (Fig. 1b). To expedite this experiment, two growth seasons were simulated per year for a total of four growing seasons between 2005 and 2006.

Specifically, after a 4 to 6 week protected greenhouse cold treatment at/or below 0°C (Dubuc, Y., Canadian Forest Service, Pers. Comm.) seedling dormancy was terminated in the beginning of the winter of 2004-2005. Growing season 1 (winter 2004-2005) was then initiated by elevating the greenhouse temperature and providing irrigation to the seedlings (refer to the specific conditions below). The same cold treatment was carried out before the summer of 2005 open field growing season 2 (after six weeks of initial growth the seedlings were transferred to the open field adjacent to the greenhouse), season 3 (winter 2005-2006 - greenhouse) and season 4 (summer 2006 – open field). To foster synchronized budbreak, each tree in the three blocks was fertilized with 2 g of NPK (20% nitrogen, 8% phosphorous, 20% potassium) dissolved in 500ml of water at the beginning of the growing seasons. Conditions inside the greenhouse were set to 22°C during the day (18 hours) and 17°C during the night (6 hours). An extra daylight photosynthetic photon flux density of 115 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was furnished by 400-W wide-spectrum high-pressure sodium bulbs (Lucalox LU400, General Electric Co., Cleveland, OH). A drip irrigation system supplied the seedlings' daily water to maintain an 80% field capacity hydration level (determined by weighing the seedling pots at full and no hydration).

The factorial design experiment (Quinn & Keough 2002) was devised to *investigate the physical (ring width, height, diameter), isotopic (carbon and* oxygen in tree ring holo-cellulose) and physiological (light-saturated CO_2

assimilation rate (*A*) and stomatal conductance (g_s)) effects of different magnitudes and combinations of both current-year defoliation (CYD) and past-year defoliation (PYD). Eight different combinations of defoliation treatments were applied to the same seedlings during the four growing season conditions described above. The 0% CYD and PYD (CYD0-PYD0) treatment served as the experimental control. Other seedling groups were subjected to a 33% current-year defoliation and a 0% past-year defoliation (CYD33-PYD0) treatment in addition to CYD66-PYD0, CYD99-PYD0, CYD0-PYD50, CYD33-PYD50, CYD66-PYD50 and CYD99-PYD50 treatments (Fig. 1c & d). These levels of defoliation were chosen in order to simulate light to severe outbreaks.

All defoliation treatments started three to four weeks after bud burst to allow for sufficient shoot elongation for needle manipulation. For all treatments, the needles were clipped at their base with scissors. For CYD treatments, a percentage of needles from all currently elongating shoots were removed according to the prescribed level of treatment defoliation (0, 33, 66 or 99%). For PYD treatments, none (0%) or 50% of remaining needles of all other age class verticils were removed (Fig. 1a). These defoliation treatments were repeated during the four growing seasons.

2.3.3 Gas exchange measurements

Approximately two weeks after each of the defoliation treatments, needle gas exchange measurements were taken during all four growing seasons using a LI-

6400 portable photosynthesis system (LI-COR Inc., Lincoln, NE). The two-week period was arbitrarily chosen to give time to seedlings to adapt to the new situation. The isotopic signal measured integrates gas exchange conditions along the season and not only right after the defoliation. The measurements were made in the nursery holding area before midday under supplemental fluorescent lighting to ensure that the seedlings received saturating photosynthetically active radiation (>1 000 $\mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) while needle gas exchange was being evaluated. During the gas exchange evaluations a cylindrical conifer foliage cuvette was alternately placed over the lateral branches of the first and second verticils representing the current year and 1 year-old shoots, respectively (Fig. 1a).

Immediately after the gas exchange measurements, the shoots enclosed in the cuvette were excised and a sub-sample of the needles removed and used to estimate the needle surface area. The method used for the needle surface area estimation was that according to Bernier *et al.* 2001 and Hébert *et al.* 2006. The needle sub-samples used for surface area estimation were dried at 65 °C for 48 hours to obtain leaf dry mass per unit leaf area (LMA). The LMA calculated was then used to find total foliar area in the cuvette; each light-saturated CO_2 assimilation rate (A), stomatal conductance (g_s) and ratios of intercellular to ambient air CO_2 partial pressure (c_i/c_a) measurement was then recalculated with actual total needle surface area.

2.3.4 Sample preparation and stable isotope analyses

One seedling per treatment per block was harvested after the fourth growing season for isotope analysis. Prior to ring separation, ring-widths were measured on eight radii using a high-resolution scanner and image analyser (WinDendro LA1600+; Regent Instruments, Quebec, Canada). Serial transversal cross-sections (90 µm thickness) were removed from the harvested stem sections using a sliding microtome (Leica SM2400, Germany). Annual rings corresponding to growing seasons 1 to 4 were subsequently separated with a scalpel (earlywood-latewood border) under a binocular microscope for all the different defoliation treatments. The separated wood samples were finely chopped using a scalpel and holo-cellulose was isolated by delignification in an acetic-acid-acidified sodium chlorite solution, after first removing oils and resins with toluene-ethanol and ethanol soxhlet extractions (Leavitt & Danzer 1993).

Isotopic analyses of the purified and dried holo-cellulose samples were accomplished by continuous flow isotope ratio mass spectrometry (CF-IRMS) using a VG-Instruments® IsoPrime attached to a peripheral temperature controlled EuroVector® elemental analyzer (EA) (University of Winnipeg Isotope Laboratory, UWIL). Milligram samples (0.2 to 0.3 mg) of prepared holo-cellulose were loaded into tin (carbon) and silver (oxygen) capsules and placed in the EA auto-sampler along with internally calibrated α-cellulose standards (Sigma α-cellulose: $\delta^{13}C$ = -23.5 ‰ VPDB; $\delta^{18}O$ = 27.1‰ VSMOW (see below); Ennadai α-cellulose: $\delta^{18}O$ = 22.2 ‰ VSMOW; Casein: $\delta^{13}C$ = -26.98 ‰ VPDB). A sample-to-standard ratio of 3:1 was employed in each sample run. Carbon and oxygen isotope results are expressed using a standard delta (δ) notation in units of *per mil* (‰). The delta values of carbon and oxygen ($\delta^{13}C_{cellulose}$, $\delta^{18}O_{cellulose}$) represent deviations from a standard, such that

$$\delta_{sample} = [(R_{sample}/R_{standard})-1]*10^3$$

where R is the $^{13}C/^{12}C$ or $^{18}O/^{16}O$ ratio in the sample and the standards (Sigma, Ennadai and Casein) calibrated to Vienna Pee Dee Belemnite (VPDB) and Vienna Standard Mean Ocean Water (VSMOW), respectively. Accuracy was maximized by calibration of laboratory standards (Sigma, Ennadai and Casein) and the reproducibility of natural samples. The $\delta^{13}C_{holo\text{-}cellulose\ (hc)}$ and $\delta^{18}O_{holo\text{-}cellulose\ (hc)}$ values have analytical uncertainties of ± 0.12 and 0.3 ‰, respectively. Holo-cellulose $\delta^{18}O$ was analyzed within a few weeks after it was produced to prevent errors associated with possible oxygen isotope exchanges in stored samples (Wright 2008).

2.3.5 Statistical analyses

2.3.5.1 Height and diameter variables

Differences in height and diameter between seedlings at the end of the last growing season were tested using a completely randomized analysis of variance

(ANOVA). *A priori* contrasts (linear, quadratic and cubic) were conducted when the hypothesis of equal means was rejected.

2.3.5.2 Stable isotopes and tree ring width variables

Stable isotopes ($\delta^{13}C_{hc}$, $\delta^{18}O_{hc}$) and ring width variables were analyzed according to a repeated-measures analysis of variance (ANOVAr) in a randomized block design (Gumpertz & Brownie 1993; von Ende 1993). The Huynh-Feldt corrected probability was used to overcome the sphericity assumption in the case of univariate repeated-measures analysis (von Ende 1993). Intra-season contrasts were done using the IDENTITY command in JMP® 7.0 software (SAS Institute Inc.) when interaction with time was significant. Linear, quadratic or cubic contrasts were tested to identify trends.

2.3.5.3 Gas exchange

Gas exchange variables were analyzed using a fully randomized block split-plot design, with time as the main plot and defoliation treatments (CYD and PYD) as the subplot. *A priori* contrasts (linear, quadratic and cubic) were conducted when the hypothesis of equal means was rejected.

Due to the differing nature of the growing seasons (seasons 1 and 3 indoor, 2 and 4 outdoor) the effect of time alone, when significant but not in interaction with the treatments, was not evaluated.

Homogeneity of variance was verified for all data by visual analysis of residuals (Devore & Peck 1994), and logarithmic transformation performed when necessary to homogenize the variance. Differences were considered significant at $P<0.05$. All statistical analyses were performed using the JMP® 7.0 software (SAS Institute Inc.).

2.4 RESULTS

2.4.1 Morphological variables

For the current-year defoliation treatments seedling heights and diameters were observed to have significantly decreased (linear relationship) with increasing levels of defoliation, by the end of the fourth growing season ($P<0.0001$; Fig. 2.2a & b; Table 2.1), although the quadratic relationship of seedling shoot diameter decreases was also highly significant ($P=0.0005$; Table 2.1). Both seedling heights and diameters reduced by as much as 40% for the CYD99% defoliation treatments. Interestingly, while significant radial growth narrowing was observed for all the PYD treatment seedlings no statistically discernable height effects were observed (Fig. 2.2c; Table 2.1).

2.4.2 Tree-ring stable isotope composition and seedling radial growth

After harvesting, growth rings of seedlings for the four seasons showed significant width variations in time for the different defoliation treatments ($P=0.0006$ and $P=0.043$ for CYD and PYD respectively) (Fig. 2.3a & b; Table 2.2). There were no significant ring width reductions after the first growing season for all CYD and PYD treatments (Fig. 2.3a & b). A linear relationship between increasing levels of current-year defoliation and tree-ring width decreases was observed for the season 2 and 4 seedlings. Both linear and quadratic relationships (similar in significance) were observed for the season 3 seedlings (Fig. 2.3a & b; Table 2.2). Compared to the control seedlings, the ring widths of CYD99 treated ones were reduced in width by 60 % on average after the second growing season and up to 80% after both the third and fourth growing seasons (Fig. 2.3a; Table 2.2). Previous past-year needle defoliation treatments significantly reduced seedling ring widths an extra 30% on average, during seasons 2 and 4, compared to the controls (Fig. 2.3b, Table 2.2). A few seedlings (5) developed incomplete rings in response to the third or fourth year of severe defoliation (CYD99%-PYD0%, CYD99%-PYD50%).

Current and past-year defoliation treatment influences on the seedlings' $\delta^{13}C_{hc}$ varied after the different growing seasons (*P*=0.0239) (Fig. 4; Table 2). In Figure 2.4, the CYD0 carbon isotope values for greenhouse grown season 1 and 3 seedlings seem more depleted in ^{13}C (-28‰ and -29‰, respectively) than the average CYD0 value (-26‰) for the field growing seasons 2 and 4 seedlings. A positive linear trend was observed between all the CYD defoliation treatments (0-99%; PYD0) and $\delta^{13}C_{hc}$, with an overall enrichment of 1.8‰ for seedlings from seasons 1, 2 and 3. Statistically significant carbon isotope enrichments were observed for the CYD-treated season 3 seedlings (P=0.030), season 1 and 2 seedlings being nearly significant with both P=0.06 (Fig. 2.4; Table 2.2). No significant relationship was observed for the season 4 seedlings.

Removal of 50% of remaining needles from previous years did not influence the carbon isotope response of the defoliated seedlings. A statistically significant positive relationship was only observed for the season 2 seedlings (Fig. 2.4).

In contrast to the positive relationship between $\delta^{13}C_{hc}$ and defoliation magnitudes, no discernable general relationship existed for oxygen isotopes (Table 2.2).

2.4.3 Gas exchange

Gas exchange measurements were made once during each growing season, two weeks after the defoliation treatments. Overall, removal of new needles during the CYD treatments altered balsam fir seedling light-saturated CO_2 assimilation rate (*A*) significantly only after the first two growing seasons (*P*=0.001) whereas removing old needles had no significant impact (*P*=0.401) (Fig. 2.5; Table 2.3). Although quadratic significant relationships between the current-year levels of defoliation and *A* dominated the observations (P=0.04 on average, seasons 1 and 2), the negative linear relationship encountered for the season 2 seedlings was highly significant (P=0.001) (Table 2.3). The defoliation treatments had no significant impact on the stomatal conductance of the seedling needles or on c_i/c_a (Table 2.3).

2.5 DISCUSSION

A comparison between defoliation treatments and their effects on the carbon isotopic compositions of balsam fir holo-cellulose supports the findings of Simard et al. (2008) further suggesting that carbon isotope enrichments in tree rings are directly associated with defoliation episodes occurring during spruce budworm outbreaks. Mature balsam fir tree-ring ^{13}C enrichments during known spruce budworm outbreaks have already been observed and linked to related losses of foliage (Simard *et al.* 2008; Leavitt & Long 1986). Increased photosynthetic rates of balsam fir residual and/or newly grown foliage as a compensating response to defoliation was proposed as a possible explanation for the observed carbon isotope enrichments (Simard *et al.* 2008). This compensating response to defoliation has already been observed in other studies on conifers (Welter 1989; Reich *et al.* 1993; Vanderklein & Reich 1999; Chen, Kolb & Clancy 2001; Lavigne, Little & Major 2001; Little, Lavigne & Ostaff 2003). However, the results obtained in this research do not support this hypothesis. Although a significant positive relationship between CYD and *A* was found during the first season of defoliation, as well as significant relationships between ^{13}C enrichment and levels of defoliation for seasons 1 to 3, no significant relationship between net assimilation rates and carbon isotope composition was observed for any of the seasons (p=0.623 for seasons 1 to 3; data not shown).

The carbon isotopic compositions of the tree ring holo-cellulose are influenced by mechanisms other than the ratio of the partial pressure of CO_2 in the leaf intercellular spaces to that of the ambient air (c_i/c_a) as controlled by either or both g_s and A (Farquhar, O'Leary & Berry 1982; Scheidegger *et al.* 2000). For example, the mobilization and use of more ^{13}C enriched stored reserves for radial growth has been shown to contribute to cellulose ^{13}C enrichment in tree rings (Brugnoli *et al.* 1988; Le Roux *et al.* 2001; Damesin & Lelarge 2003; Helle & Schleser 2004). The absence of statistical difference between the c_i/c_a ratios measured on needles from seedlings submitted to the different defoliation treatments support a post-photosynthetic mechanism as a possible explanation for the defoliation related ^{13}C enrichments observed in the seedlings grown during seasons 1, 2 and 3. This remains to be verified.

Despite uncertainties regarding the mechanisms responsible for the carbon isotope response to defoliation, the tree-ring $\delta^{13}C_{hc}$ seems to be a more reliable tool to detect the onset of defoliation periods compared to ring width decreases. While no radial growth decrease occurred in response to defoliation treatments in season 1, tree-ring holo-cellulose values indicate carbon isotope enrichment (see Figure 4). Conifers have been shown to record radial growth reductions in the basal part of the stem up to four years after the onset of a spruce budworm outbreak (Piene 1980, Krause & Morin 1995, Krause *et al.* 2003). It is therefore possible that any season 1 seedling growth reductions, propagated by the defoliation treatments, were proportionally compensated by ^{13}C enriched reserves. Therefore, using tree ring carbon isotope measurements could help to increase the level of confidence in the establishment of the beginning of past spruce budworm outbreak periods.

Other sources of environmental variations related to water availability could lead to a similar $\delta^{13}C_{hc}$ response. Many studies showed the link between soil moisture availability (drier sites) and higher $\delta^{13}C$ (Dupouey *et al.* 1993; Saurer, Siegenthaler & Schweingruber 1995; Saurer, Aellen & Siegwolf 1997; Kagawa *et al.* 2003). $\delta^{18}O_{hc}$ also relates to water conditions as it is expected to reflect the isotopic composition of source water as modified by leaf water transpiration and the Péclet effect, i.e the mixing of enriched water at the sites of evaporation with non-enriched water flowing from the xylem (Farquhar & Lloyd 1993; Roden & Ehleringer 1999a, b; Roden, Lin & Ehleringer 2000; Barbour *et al.* 2004; Barbour 2007). Transpiration rate is either driven by a change in stomatal conductance or evaporative demand, effects that result in different oxygen isotopic modifications of leaf water. While higher evaporative demand tends to enrich the residual leaf water, higher stomatal conductance tends to reduce that enrichment (Farquhar, Cernusak & Barnes 2007). In this study, no significant relationship between defoliation treatments and g_s or $\delta^{18}O_{hc}$ (Table 3) was detected. Similarly, Simard *et al.* (2008) did not find differences in the $\delta^{18}O$ values measured from living mature spruce budworm host and non-host trees again suggesting that oxygen isotopes should not be considered reliable indicators of past spruce budworm defoliation episodes.

Removing old foliage in addition to current-year needles decreased ring widths by an extra 30% on average during seasons 2 and 4. A significant positive relationship between old foliage removal and holo-cellulose carbon isotopic compositions was observed for the season 2 seedlings (Fig. 2.4, Table 2.2) but, in general, old foliage removal appeared not to influence $\delta^{13}C_{hc}$ values (seasons 1, 3 and 4 seedlings). While additional research is required, these initial results suggest that precautions be taken when employing tree ring carbon isotopes to reconstruct outbreaks of defoliators primarily feeding on old foliage.

In order to expedite this study, a total of four growing seasons were facilitated over a two year period (season 1 and 3 seedlings were greenhouse grown in the winter; season 2 and 4 seedlings were both field and greenhouse grown in the summer). On average, the $\delta^{13}C_{hc}$ of season 1 and 3 greenhouse grown seedling is 2.4 ‰ more depleted than the field (season 2 and 4) grown seedlings (Fig. 2.5). While no $\delta^{13}C_{air}$ measurements were made during this study, a reconstruction based on the Farqhuar, O'Leary & Berry's (1982) model [$\delta^{13}C_{plant}= \delta^{13}C_{air}-a-(b-a)*c_i/c_a$], with a=4‰ and b=27‰, using c_i/c_a obtained during gas exchange measurements produced $\delta^{13}C_{air}$ values of -9‰ and -7‰ on average, for the greenhouse and field CO_2, respectively. Additionally, the greenhouse (1 and 3) seasons correspond to the dormant season, a time of year when atmospheric CO_2 is generally higher in concentration and more depleted in isotopic composition in the northern hemisphere (*e.g.* -8.4‰ in winter vs -7.4‰ in summer, in Alaska) (Inoue & Sugimura 1985; Boutton 1991, Bowling, Pataki & Randerson 2008). Further, an anecdotal account of a significantly more depleted greenhouse $\delta^{13}C_{air}$ value (-13‰ Damesin pers. comm.; Université Paris Sud 11, France) also suggests that the more depleted greenhouse seedling (seasons 1 and 3) $\delta^{13}C_{hc}$ compositions are indeed a result of more depleted $\delta^{13}C_{air}$ compositions during their growth. However, the consistent $\delta^{13}C_{hc}$ trends in relation to the defoliation levels suggest that the impact of dual growing seasons was not sufficiently important to override the treatment effects.

2.5.1 Young vs mature trees

Under natural conditions, young trees (seedlings) tend to show a higher resistance to spruce budworm defoliation which subsequently results in greater survival for the same percentage of defoliation compared to more mature trees (Ghent 1958; Morin 1994). Balsam fir seedlings and saplings are recognized for their tremendous tolerance to extreme conditions and can survive many years in heavy shaded closed canopy environments (Ghent 1958; Parent, Morin & Messier 2002). Here, even the most severe CYD99-PYD50 treatments, repeated over four growing seasons, did not result in any seedling mortality.

Mature trees of this species, on the other hand, rarely survive past four to five years of severe defoliation (Blais 1964).

Significant emergence of new foliage from dormant buds along the stem was observed to occur in direct response to the defoliation treatments. Conifers are recognized for their ability to duplicate their basic structure to restore architectural integrity or increase photosynthetic structure in response to increased physiological needs (adaptative reiteration) or environmental stresses (traumatic reiteration) (Bégin & Filion 1999; Ishii, Ford & Kennedy 2007). Traumatic reiteration can occur in the form of epicormic shoots originating from dormant buds in conifers (Bégin & Filion 1999; Hanson & North 2006; O'Hara, York & Heald 2008). In particular it can also occur along balsam fir stems in response to defoliation (Batzer, 1973; Piene 1989; Piene & Little 1990; Piene & Eveleigh 1996). Ishii, Ford & Kennedy (2007) showed that the carbon isotopic composition of current-year epicormic shoot needles were significantly more depleted than current-year needles from their respective parent shoots. However, the influence of this newly grown foliage on the carbon isotopic composition of tree rings is not known.

2.6 CONCLUSION

The main objective of this research was to further validate the use of carbon and possibly oxygen isotopes as efficient and sensitive indicators of past spruce budworm outbreak periods in light of the more traditional tree ring width indicators (Blais 1958, 1962, 1964; Krause *et al.* 2003). Evidence has emerged suggesting that carbon isotopes are in fact sensitive to even minor foliage losses although a combination of physiological responses may be responsible for the observed ^{13}C holo-cellulose enrichments. Oxygen isotopic compositions were not observed to respond to the defoliation treatments.

While improvements to the experimental procedure performed here are necessary in the future, this controlled study was still successful in improving our understanding of the relationships between tree-ring holo-cellulose carbon and oxygen isotope compositions and defoliation magnitudes. Corresponding gas exchange measurements and tree-ring widths did not support the hypothesis that defoliation-induced increases in needle CO_2 assimilation rates may act as the main mechanism responsible for δ^{13}C tree-ring holo-cellulose enrichments during defoliation periods. The absence of relationship between A and $\delta^{13}C_{hc}$, as well as defoliation magnitudes and c_i /c_a suggest that a post-carboxylation mechanism, most likely the mobilization of stored reserves, contributed to the enriched holo-cellulose carbon isotope signatures in response to defoliation although this still needs to be confirmed.

Balsam fir seedlings, compared to mature trees, exhibit a high resilience to defoliation stress. While all seedlings survived even the most severe defoliation treatments over the four growing seasons, mature firs are rarely observed to survive similarly harsh conditions. Therefore, while caution is advised when assessing the response of mature defoliated trees using seedlings, the carbon isotope response of the less resilient mature balsam fir trees may in fact be more obvious in magnitude (Simard *et al.* 2008), a fact that bodes well for carbon isotope detection of even minor spruce budworm infestations in future studies.

2.7 ACKNOWLEDGEMENTS

This research project was funded by the Natural Sciences and Engineering Research Council of Canada (NSERC), The Consortium de Recherche sur la Forêt Boréale Commerciale and the Fondation de l'Université du Québec à *Chicoutimi. Sincere thanks go to D. Gagnon, J. Allaire, J-P. Lebeuf and all those* who helped in the development and implementation of the defoliation experiment. We also thank D. Walsh and Dr. J.-F. Boucher for statistical analysis support and discussion. Anonymous reviewers provided valuable comments on earlier versions of the manuscript.

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2.9 TABLES AND FIGURES

Table 2.1 An ANOVA summary (*F*- and *P*-values) of seedling heights and diameters at the end of growing season 4. Bold numbers indicate greater than 95% significance ($P<0.05$), *ddf* = denominator degrees of freedom, CYD = current-year needle defoliation, PYD = past-years needle defoliation. [a] Ln transformed data.

		Height [a]		***Diameter*** [a]	
Source	***ddf***	***F***	***P***	***F***	***P***
Main plot					
block	2	2.68		2.63	
CYD	3	17.78	**<.0001**	28.65	**<.0001**
Contrasts					
CYD lin	14	47.14	**<.0001**	56.19	**<.0001**
CYD quad	14	4.71	**0.048**	19.99	**0.0005**
CYD cub	28	1.52	0.238	9.76	**0.007**
PYD	1	1.50	0.240	11.30	**0.005**
CYD*PYD	3	0.84	0.493	1.42	0.280

Table 2.2 Summary of a) repeated-measures ANOVA (*F*- and *P*-values) for wood holo-cellulose carbon isotope compositions ($\delta^{13}C_{hc}$), oxygen isotope compositions ($\delta^{18}O_{hc}$) and ring widths; b) within season contrasts for $\delta^{13}C_{hc}$ values and ring widths, during the four growing seasons. *P*-values for repeated-measures ANOVA are presented with Huynh-Feldt corrected probabilities. Bold numbers indicate greater than 95% significance ($P<0.05$). *ndf* = numerator degrees of freedom; *ddf* = denominator degrees of freedom, CYD = current-year needle defoliation, PYD = past-years needle defoliation. [a] Ln transformed data.

		$\delta^{13}C_{hc}$			$\delta^{18}O_{hc}$			Ring width		
Source	*ndf*	*ddf*	*F*	*P*	*ddf*	*F*	*P*	*ddf*	*F*	*P*
a) Between-subjects										
block	2	14	3.50		9	5.99		14	1.39	
CYD	3	14	2.50	0.102	9	1.24	0.352	14	24.87	**<.0001**
PYD	1	14	0.12	0.738	9	1.03	0.337	14	16.06	**0.001**
CYD*PYD	3	14	0.52	0.676	9	0.84	0.507	14	1.44	0.271
Within-subject										
time	3	42	45.10	**<.0001**	27	4.64	**0.009**	42	67.39	**<.0001**
time*CYD	9	42	0.95	0.493	27	1.28	0.290	42	4.25	**0.0006**
time*PYD	3	42	1.44	0.243	27	1.80	0.171	42	2.94	**0.043**
time*CYD*PYD	9	42	2.47	**0.023**	27	1.61	0.162	42	0.59	0.801

b) Within season contrasts		$\delta^{13}C_{hc}$		
	ndf	*ddf*	*F*	*P*
Season 1				
CYD lin (PYD0)	1	14	4.08	0.063
CYD lin (PYD50)	1	14	0.30	0.590
CYD quad (PYD0)	1	14	0.09	0.771
CYD quad (PYD50)	1	14	0.13	0.725
CYD cub (PYD0)	1	14	2.45	0.139
CYD cub (PYD50)	1	14	0.29	0.601
Season 2				
CYD lin (PYD0)	1	14	3.93	0.067
CYD lin (PYD50)	1	14	8.70	**0.010**
CYD quad (PYD0)	1	14	2.37	0.145
CYD quad (PYD50)	1	14	0.00	0.956
CYD cub (PYD0)	1	14	0.50	0.490
CYD cub (PYD50)	1	14	0.45	0.512
Season 3				
CYD lin (PYD0)	1	14	5.82	**0.030**
CYD lin (PYD50)	1	14	1.90	0.189
CYD quad (PYD0)	1	14	2.70	0.122
CYD quad (PYD50)	1	14	1.38	0.260
CYD cub (PYD0)	1	14	1.77	0.204
CYD cub (PYD50)	1	14	2.47	0.138
Season 4				
CYD lin (PYD0)	1	14	1.10	0.312
CYD lin (PYD50)	1	14	0.15	0.700
CYD quad (PYD0)	1	14	0.07	0.798
CYD quad (PYD50)	1	14	0.01	0.921
CYD cub (PYD0)	1	14	0.71	0.412
CYD cub (PYD50)	1	14	2.23	0.157

	Ring width		
	ddf	*F*	*P*
Season 1			
CYD lin	14	0.19	0.666
CYD quad	14	3.02	0.104
CYD cub	14	0.18	0.679
PYD	14	0.01	0.916
Season 2			
CYD lin	14	48.12	**<.0001**
CYD quad	14	7.93	**0.013**
CYD cub	14	3.45	0.084
PYD	14	15.27	**0.001**
Season 3			
CYD lin	14	7.16	**0.018**
CYD quad	14	8.04	**0.013**
CYD cub	14	0.00	0.991
PYD	14	3.03	0.103
Season 4			
CYD lin	14	48.64	**<.0001**
CYD quad	14	7.29	**0.017**
CYD cub	14	0.36	0.556
PYD	14	11.36	**0.004**

Table 2.3 Summary of ANOVA (*F*- and *P*-values) for light-saturated CO_2 assimilation rate (*A*), stomatal conductance (g_s) and ratios of intercellular to ambient air CO_2 partial pressure (c_i/c_a) during four growing seasons. Bold numbers indicate greater than 95% significance ($P<0.05$). *ndf* = numerator degrees of freedom; *ddf* = denominator degrees of freedom, CYD = current-year needle defoliation, PYD = past-years needle defoliation. [a] Ln transformed data.

		A^a			g_s			c_i/c_a		
Source	*ndf*	*ddf*	*F*	*P*	*ddf*	*F*	*P*	*ddf*	*F*	*P*
Main plot										
block	2	5.94	0.51		4.49	0.87		5.99	0.37	
time	3	7.67	15.68	**0.001**	7.61	25.86	**0.0002**	5.99	0.90	0.494
Subplot										
CYD	3	62.05	3.44	**0.022**	64.25	0.40	0.751	55.07	0.12	0.945
PYD	1	84.43	0.71	0.401	74.93	0.02	0.876	55.08	0.14	0.709
CYD*PYD	3	62.22	0.63	0.593	64.28	0.23	0.869	55.07	2.17	0.102
time*CYD	9	62.98	3.53	**0.001**	64.65	1.52	0.157	55.07	1.25	0.283
Contrasts										
Season 1										
CYD lin	1	104.94	3.78	0.054						
CYD quad	1	82.10	13.42	**0.044**						
CYD cub	1	104.94	1.36	0.246						
Season 2										
CYD lin	1	105.48	10.80	**0.001**						
CYD quad	1	80.96	4.31	**0.041**						
CYD cub	1	51.29	8.23	**0.006**						
Season 3										
CYD lin	1	111.72	0.89	0.346						
CYD quad	1	89.29	1.38	0.243						
CYD cub	1	55.35	0.90	0.347						
Season 4										
CYD lin	1	105.00	1.01	0.317						
CYD quad	1	82.96	0.21	0.651						
CYD cub	1	53.64	0.18	0.671						
time*PYD	3	84.36	1.03	0.382	74.93	0.50	0.681	55.07	0.09	0.969
time*CYD*PYD	9	63.12	1.19	0.312	64.62	0.49	0.874	55.07	1.29	0.263

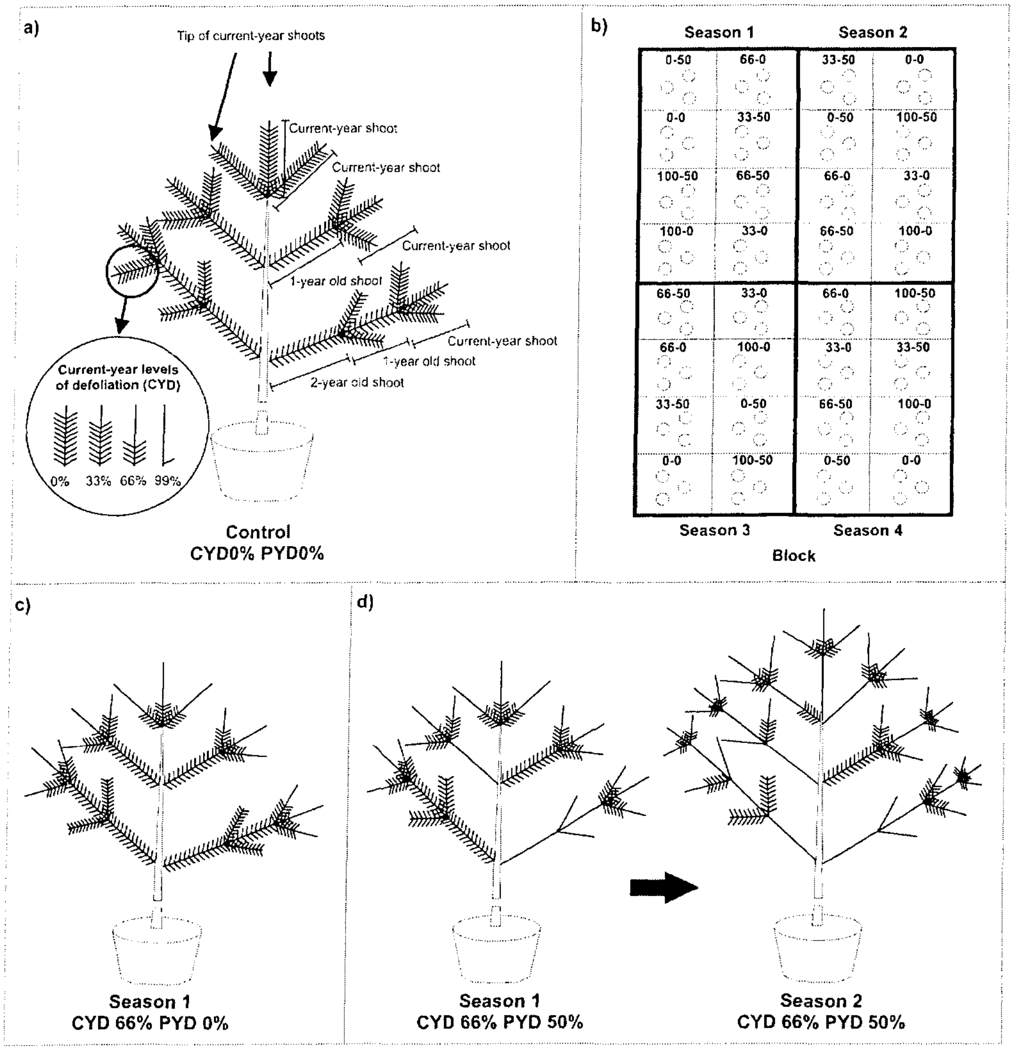

Figure 2.1 a) A schematic balsam fir seedling diagram showing the locations of the current-, first- and second-year shoots (first, second and third verticils, respectively) and the different levels of current-year defoliation (circle inset - CYD 0%, 33%, 66% and 99%) applied to all current-year seedling shoots (on all verticils) during each of the four treatment years, b) a representation of one of the three experimental design blocks , with the eight possible defoliation treatments randomly placed within growing season (1 to 4) main plots, c) a schematic diagram showing an example of current-year defoliation (CYD66%-PYD0%) applied for only one season and, d) a schematic diagram showing the cumulative impact of current- and previous-years defoliation (CYD66%-PYD50%) over two growing seasons.

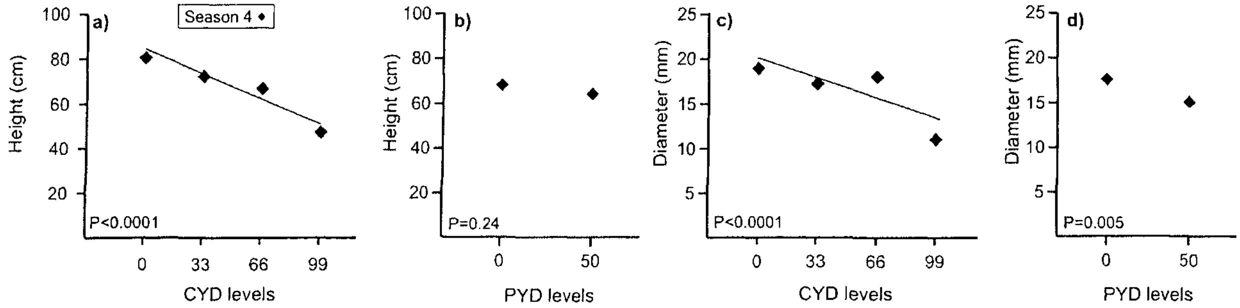

Figure 2.2 Seedling growth heights (a and b) and diameters (c and d) after four seasons of defoliation treatments. Linear relationships are indicated with greater than 95% (P<0.05) significance (see Table 1).

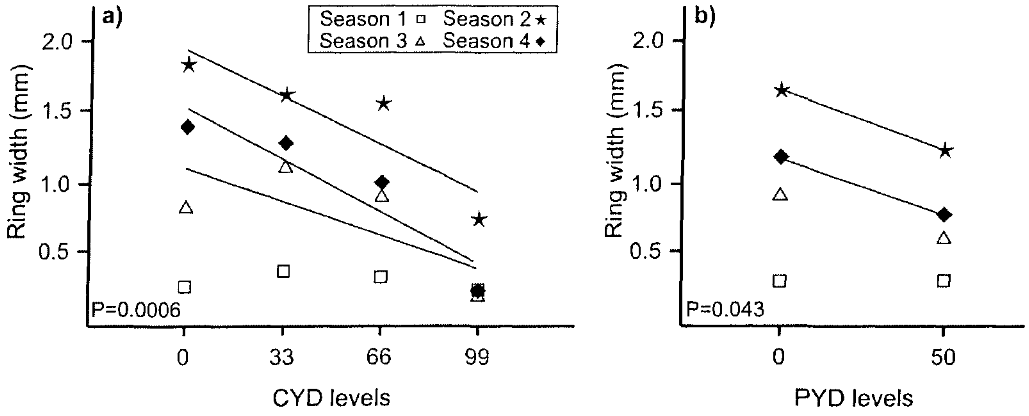

Figure 2.3 Measured effects of a) current-year and b) previous-years artificial defoliation on annual ring widths of the balsam fir seedlings. Linear relationships are indicated with greater than 95% (P<0.05) significance (see Table 2).

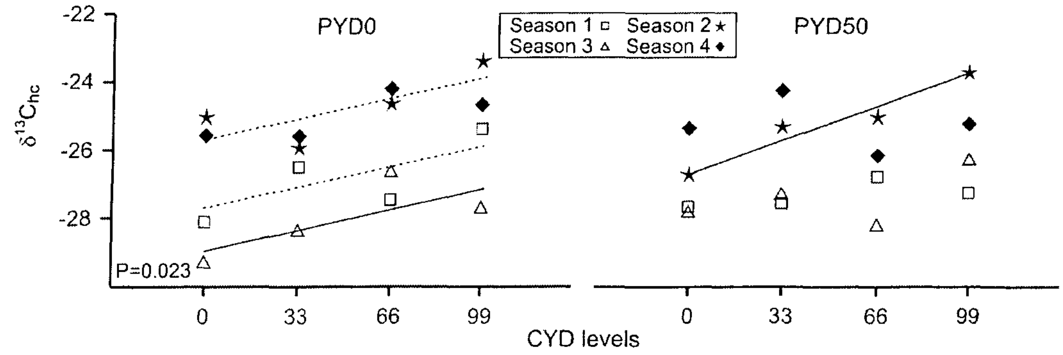

Figure 2.4 Tree-ring holo-cellulose carbon isotope composition ($\delta^{13}C_{hc}$) variations in response to the four growing season current- and past-years defoliation treatments. Solid and dashed lines indicate greater than 95% ($P<0.05$) and 90% ($P<0.10$) significant linear relationships, respectively (*see Table 2*).

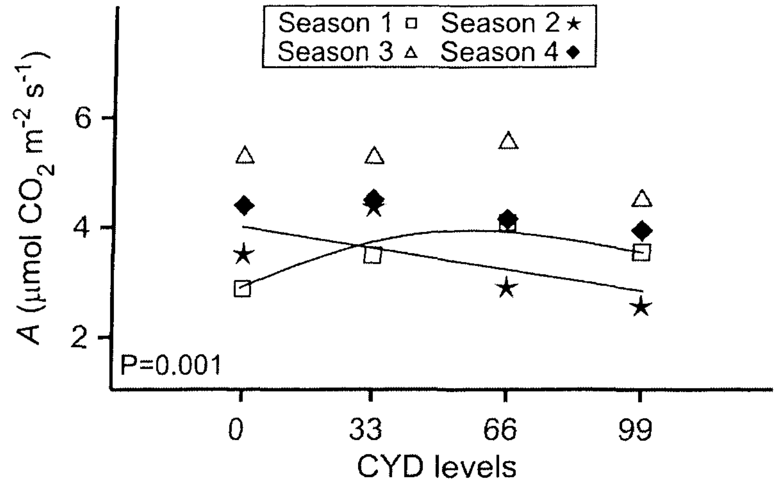

Figure 2.5 Light-saturated CO_2 assimilation rate (*A*) of combined current-year and 1-year old foliage of balsam fir seedlings in response to different levels of current-year defoliation measured during the four growing seasons. Linear and quadratic relationships are indicated with greater than 95% ($P<0.05$) significance (see Table 3).

CHAPITRE III

STABLE ISOTOPES AS PART OF A MULTI-PROXY STRATEGY TO RECONSTRUCT INSECT DEFOLIATION EPISODES USING SUBFOSSIL TREES

3.1 ABSTRACT

To better assess the natural variability of spruce budworm (*Choristoneura fumiferana* Clem.) outbreaks and portray the insect's activity over an extensive time period, reconstruction based on subfossil trees buried in mires was conducted. The aim of the study was to verify and use the variations in stable carbon and oxygen isotopes of tree rings to assist dendrochronological identification of past spruce budworm outbreaks from subfossil wood. Four different criteria to identify defoliation periods were established based on dendrochronological observations of past defoliation episodes on tree growth, as well as on $\delta^{13}C$ and $\delta^{18}O$ variations observed in living hosts (chapters 1 and 2). The criteria are as follow: 1) a minimum of 5 years $\delta^{13}C$ enrichment-depletion cycle, 2) a peak enrichment of a minimum of 0.9‰ during that cycle, 3) a maximal $\delta^{13}C$ value larger than the average carbon isotope signal 5 years before and after the potential outbreak period, and 4) $\delta^{18}O$ that follows the trend in tree-ring width. Despite radial growth reductions in the subfossil chronology that could potentially be associated with spruce budworm outbreaks, the isotopic analysis did not confirm the origin of the growth decrease. Nonetheless, the presented isotopic method demonstrated an interesting potential in identifying spruce budworm outbreaks from living trees and remains the only approach supporting the biological origin of the growth reduction observed in tree rings. Further research on that subject is needed.

3.2 INTRODUCTION

The uniqueness of trees in terms of proxy for environmental reconstruction lies in their highly resolved timescale resolution. Not only can trees give insights into climatic or environmental change at the annual level, but the time frame of analysis can be further broken down to the seasonal level (Deslauriers et al. 2003, Rossi et al. 2006, Cufar et al. 2008, Rossi et al. 2009). Moreover, trees that lived a long time ago but were preserved from degradation either as building material in historical buildings or simply naturally protected from decay in anaerobic conditions, as subfossil trees, provide valuable information pertaining to the environment and climate in which they grew and have been greatly used to reconstruct past environments (e.g. Kullman 1994, Krause 1997, Lageard et al. 2000, Grudd et al. 2002, Boulanger & Arsenault 2004, Sass-Klaassen et al. 2005). The latter offers great potential to reconstruct the long-term history of spruce budworm (*Choristoneura fumiferana* Clem.) outbreaks, a native endemic insect, which causes serious damages to fir and spruce forests in the eastern part of the boreal forest in North America (Blais 1962, MacLean 1980, Morin 1994). Until now, 300 to 400 years of spruce budworm infestations have been reconstructed using the oldest living and historical material suitable for such reconstruction but longer reconstructions are needed to assess the long-term dynamics of outbreaks during the Holocene (Blais 1983, Krause 1997, Boulanger and Arseneault 2004).

The identification of past insect outbreaks from tree-rings is traditionally accomplished through detailed comparisons of growth curves from host and non-host tree species (Blais 1962, Swetnam et al. 1985). The program OUTBREAK has been developed to assist and facilitate the identification of outbreak periods (Holmes and Swetnam 1996). Growth reductions observed in host chronologies only are assumed to originate from insect defoliation rather than being a consequence of climatic or environmental changes that would influence both species. The non-host chronology may also be used to extract the climatic signal embedded in chronologies of both species so as to clarify the outbreak signal (Swetnam et al. 1985). However, building a highly replicated host tree-ring chronology using subfossil wood requires a considerable sampling effort and due to the environment in which trees are recovered from, it is very unlikely that a non-host chronology could be reconstructed as well.

Besides physical parameters such as ring width or density, environmental changes can also affect the stable isotopic composition of tree rings (e.g. Burk and Stuiver 1981, Leavitt and Long 1986, McCarroll and Loader 2004, Gagen et al. 2008). A dendroisotopic approach using $^{13}C/^{12}C$ and $^{18}O/^{16}O$ ratios was used as a tool to reconstruct past spruce budworm epidemic periods from host conifer tree rings (Simard et al. 2008, chapter 1 and 2). A ^{13}C enrichment was observed during outbreak periods and could also be reproduced experimentally with artificial defoliation. No change in the oxygen isotopic composition of tree rings related to defoliation, in natural or experimental conditions, occurred. Using carbon isotope analysis on subfossil material requires particular attention as the different constituents of wood undergo chemical degradation under anaerobic conditions. Hemicellulose degrades faster than cellulose, which in its turn decays more rapidly than lignin, possibly leading to erroneous interpretation if bulk wood is used for analysis as the different moieties have different stable carbon isotope signals. Lignin is depleted by about 5‰ compared to the polysaccharides cellulose and hemicellulose (cf. Bowling et al. 2008). Lignin and cellulose may also experience a certain degree of non-biological chemical alteration during diagenesis, modifying furthermore their isotopic composition (Schleser et al. 1999, van Bergen 2002). Using proper methods, such as working on a single wood component rather than using bulk wood material, stable isotope analysis could greatly improve the identification of past spruce budworm outbreaks using subfossil wood and significantly increase knowledge of the long-term dynamics.

The aim of the study was to use the variations in stable carbon and oxygen isotopes of tree rings to assist dendrochronological identification of past spruce budworm outbreaks from subfossil wood. It was done in order to better assess the natural variability of spruce budworm outbreaks and portray the past insect activity over an extensive time period when no historical data on outbreak events were available and the host - non-host comparison unusable due tu a *lack of non-host chronology for the period studied.*

3.3 METHODS

3.3.1 Study area

The study area is located in the Mont-Valin region, approximately 100 km north of Saguenay, Québec, Canada (approximately 48°N, 70-71°W), within the east balsam fir-white birch bioclimatic domain of the continuous boreal forest (Saucier et al., 1998). Balsam fir and black spruce are the dominant canopy species. Other tree species such as white spruce (*Picea glauca* [Moench] Voss), paper birch (*Betula papyrifera* Marsh.) and trembling aspen (*Populus tremuloides* Michx.) are occasionally found.

The study sites are within the Mont-Valin region characterised by a hilly topography. All sites were located at an elevation of approximately 600 m above sea level (a.s.l.). Regional climatic conditions for *AD* 1942-1990 (Bagotville A meteorological station, 48°20'N, 71°00'W, 159 m. a.s.l.), ~120 km south of the study area, are characterized by a mean annual temperature of 2.3°C and mean annual precipitation of 950.8 mm (36% falling as snow) (Environnement Canada, 2010). Main disturbances affecting the forest in this environment consist of insect outbreaks and fires.

Three sites within the study area were selected based on specific features. Small mires surrounded by forested slopes composed of balsam firs and black spruces with a good potential of buried wood were sought-after. Mires were probed to locate the potential part containing the highest concentration of buried wood. Prior to excavation, the surface of the mire was mapped using a total station. Excavation was done using a mini-excavator and shovels and water pumped out of the excavation site regularly using a gas pump. Each piece of wood was mapped and numbered to assess the depth at which it lay then stored frozen back at the laboratory. Transversal sections were taken at the largest end of the stem for dendrochronological analysis. The surface of each section was either prepared with a razor blade or sanded frozen. Rings on two radii per disc were measured using a sliding-stage incremental micrometer (Henson, California, USA) with a precision 0.01mm (Cook and Kairiukstis 1990).

Each piece of wood was identified using anatomical features (Schweingruber 1982, Hoadley 1990). Although black and white spruces can be anatomically differentiated according to the proportion of uniseriate and biseriate radial tracheids (Marguerie et al. 2000), spruce species were identified at the genus level only. However black spruce is the species most likely to be found being the most common species encountered in the surrounding living forest. Both spruces are affected by the spruce budworm. Spruces sp. were used to build the chronology as they were the most abundant host species recovered from the mires. The percentage of subfossil firs was limited and consequently that species was not considered for the chronologies.

Groups of samples based on their location in the vertical profile of the mire were established to facilitate the cross-dating process. Layers of 50 cm overlapping by 25 cm were selected to test trees against one another to find a possible match. Cross-dating was verified using the COFECHA program (Holmes 1983) and TSAP-Win™ (Rinntech, Germany) software, but also by visual inspection of the growth curves. Samples containing less than 55 rings were not considered. Standardisation (66%n year cubic smoothing spline with a fixed 50% cutoff) was done using ARSTAN program (Cook 1985). Once all possible trees were assembled, radiocarbon dating of material from six trees distributed in different parts of the chronology was carried out by accelerator mass spectrometry (AMS)

(Stuiver et al. 1998). Calibrated dates, expressed in kilo annum before present (ka cal. BP), were used to locate the floating chronology in time.

3.3.2 Cellulose extraction and $\delta^{13}C$ and $\delta^{18}O$ determination

The best replicated segment of the *Picea* sp. subfossil floating chronology including growth reduction periods possibly induced by spruce budworms was chosen for a more detailed analysis. Two growth reduction periods similar to those observed during known outbreak periods of seven overlapping series were selected for carbon and oxygen stable isotope analyses. Individual annually resolved tree rings of the growth reduction in addition to five to ten rings prior and following that growth reduction were scalpel separated (latewood-earlywood border) under a binocular microscope. Each ring was subsequently ground using a steel ball mill for cellulose extraction (MM200; Retsch, Haan, Germany). Holo-cellulose, rather than alpha-cellulose, was isolated by delignification in an acetic-acid-acidified sodium chlorite solution, after first removing oils and resins with toluene-ethanol and ethanol soxhlet extractions (Leavitt and Danzer 1993). This methodological choice was decided in order to keep enough material for subsequent isotope analysis. Consequently, the absolute $\delta^{13}C$ values should be relatively more depleted than $\delta^{13}C$ values from alpha-cellulose but the isotopic offset should however remain similar. All subfossil samples yielded cellulose similar to that extracted from modern wood samples.

Carbon isotopic analyses of the purified and dried holo-cellulose samples were accomplished by combustion and analysis of the analyte gasses via continuous flow isotope ratio mass spectrometry (Delta PlusXP, ThermoFinnigan, Bremen) attached to an elemental analyzer (ECS 4010, Costech Analytical, Valencia, CA). Oxygen isotope analyses were accomplished by pyrolysis at 1400°C and analysis of the analyte gasses via continuous flow isotope ratio mass spectrometry (Delta PlusXP, ThermoFinnigan, Bremen) attached to a thermal conversion elemental analyzer (TC/EA, ThermoFinnigan, Bremen). All analyses were completed at the Stable isotope Core Laboratory, Washington State University. Milligram samples (0.2 to 0.3 mg) of prepared holo-cellulose were loaded into tin (carbon) and silver (oxygen) capsules and placed in the EA auto-sampler along with calibrated running standards (Wheat and corn flour for ^{13}C; IAEA601 and IAEA602 for ^{18}O). An independent, calibrated alpha cellulose sample was repeated in duplicate for every ^{13}C and ^{18}O sequence as a quality control check for the analyses. Precision of the analyses was respectively 0.04‰ and 0.41‰ for carbon and oxygen stable isotopes. The values presented are reported relative to Vienna Pee Dee Belemnite (VPDB) and Vienna Standard Mean Ocean Water (VSMOW), for carbon and oxygen respectively.

Isotope ratios were averaged to present a mean annual time series of carbon and oxygen, respectively. Tree-ring width and carbon and oxygen stable isotope series were smoothed using a 3-year moving average to focus on trends and remove unnecessary noise in the data.

3.3.3 Stable isotopes criteria for spruce budworm outbreak identification

Observations made on 20 mature living spruce budworm host trees were used to define criteria to aid in the identification of defoliation episodes from subfossil wood in an objective way (tab. 1). These criteria were developed from trees sampled in one site that experienced two moderate to severe outbreaks and limits were set according to these observations. Four different criteria were established. The first three criteria concern the carbon isotopic composition of tree-rings while the fourth one refers to their oxygen isotopic composition. The 5 year minimum duration of the δ^{13}C enrichment-depletion cycle of criterion 1 was established based on the knowledge of past defoliation episodes on tree growth and on dendrochronological observations (Swetnam et al. 1985, Jardon et al. 2003) and on δ^{13}C observations in the living host trees (Simard et al. 2008, chapter 1). The 0.9‰ value of criterion 2 was defined based on the lowest $\delta^{13}C$ differential (maximal value – minimal value at the beginning of the cycle) observed during known outbreak periods in the living trees. The third criterion was established to ensure a peak enrichment superior to the average $\delta^{13}C$ variations before and after the potential outbreak period. Criterion 4 for $\delta^{18}O$ corresponds to observations done on living trees (chapter 1).

3.4 RESULTS

A 364-year long chronology, radiocarbon dated between 5.7 and 4.5 ka cal. BP (approx. 5.1 ka cal. BP), was built from 28 subfossil spruce trees recovered from 3 different mires (fig. 3.1). Although spanning a relatively long time period, the sample depth remained low all along the chronology reaching 14 individuals at the most, during a few decades. In the following text, the term "chronology" refers to the detrended dendrochronological average of all individual whereas "series" refers to the individual tree-ring series.

Growth reductions were observed in the tree-ring chronology as well as carbon isotope enrichment. By looking at the isotopic and ring width data, a classification of spruce budworm outbreaks was done using the four criteria presented before. None of the two possible outbreak periods (I and II) from the seven series (a to g) fulfilled all four criteria. Three out of four criteria were satisfied in series c and f for period I and series d for period II. The criterion 2 referring to an enrichment of at least 0.9‰ between the $\delta^{13}C$ minima and maxima of the potential outbreak period I was not satisfied except in the a and d series. Only series e did not meet any of the criteria (tab. 3.1, fig. 3.2c).

Although the carbon isotope pattern of series d–period II is compatible with a response to defoliation, it did not meet the criterion 3 on the average value of the minimal period of 5 years before and after the maximal $\delta^{13}C$ of the potential outbreak in reason of the length of the isotopic series analysed. This observation applies also for series b and c–period II and is valid for all investigated growth reduction episodes. The $\delta^{13}C$ characteristics of series a–period I fulfilled two out of three carbon criteria but did not reach the 5-year minimum condition of duration, lasting only 4 years. The $\delta^{18}O$ condition was not met either.

Isotope ratios were averaged to present their mean annual variations, as for living *Picea mariana* series (fig. 3.2B and A, respectively). The first potential outbreak, period I, met all criteria but criterion 2 (tab. 3.1, fig. 3.2) whereas the second one, period II, fulfilled the first and fourth criteria. Criterion 2 was not met in either of the periods although a carbon enrichment of 0.7 and 0.8‰, respectively, was observed. Criterion 3 is also lacking for the period II due to an insufficient number of years analysed prior to the enrichment.

The carbon isotope analysis of subfossil *Picea* yielded values ranging between -20.81‰ to -24.39‰ with a mean value of -22.32‰.

3.5 DISCUSSION

The presence of spruce budworm populations has been detected in the boreal forest since the early Holocene, recurrently affecting tree growth (Simard et al. 2006, Morin et al. 2007, Lavoie et al. 2009). Carbon and oxygen stable isotopes from tree rings were successfully used to identify the last two spruce budworm outbreaks of the 20th century (Simard et al. 2008; chapter 1) and the relationship between ^{13}C enrichment in tree rings and defoliation demonstrated experimentally (chapter 2).

Subfossil *Picea* sp. yielded carbon isotope values well within the reported $\delta^{13}C$ values of modern C3 plants and trees (-20‰ to -35‰; Dawson et al. 2002). In fact, both subfossil and living trees analysed within this project yielded very similar $\delta^{13}C$ values. However, compared to the signal observed during modern spruce budworm outbreaks, the amplitude of the tree-rings ^{13}C enrichment observed during the presumed outbreak periods between 5.7-4.5 ka BP was visually much lower in spite of a corresponding radial growth reduction similar to that observed during a severe outbreak.

In order to interpret the results in an objective way, criteria were defined based on observations of outbreak events in living *Picea mariana*. These criteria were developed from trees sampled in one site that experienced two moderate to severe outbreaks and limits were set according to these observations. For example, during the two presumed outbreaks, periods I and II, the maximal carbon enrichment reached 0.7‰ and 0.8‰ respectively, 0.1-0.2‰ lower than previously defined. However, the year-to-year δ^{13}C variation calculated in non-epidemic periods for the living trees was 0.30 ± 0.05‰. Although not meeting the limit value of 0.9‰, the variation observed during these two periods are nonetheless closer to the value proposed in the criteria than to the year-to-year variation. Limits need to be set to make objective assertions. However, whether it should be set to 0.9‰ or 0.7‰ is difficult to determine due to the lack of study on different intensity of defoliation in mature trees. Increased sample depth, number of sites, as well as coverage of the whole range of outbreak severity in living trees is essential to improve the precision of those criteria. Longer series are also needed to better assess and interpret the different patterns observed over a tree's growth. The isotopic results obtained from subfossil trees in this study are not convincing enough, compared to that obtained from living trees during known severe spruce budworm outbreaks, to confirm that the growth reductions observed between 5.7-4.5 ka BP were caused by defoliation.

Evidence from an increasing number of palaeoecological investigations suggests that severe outbreaks such as those observed during the 20th century were rare events during the Holocene (Anderson et al. 1986, Bhiry et Filion 1996, Jasinki et Payette 2005, Simard et al. 2006, Lavoie et al. 2009). Although high concentrations of spruce budworm remains were found sporadically along the peat profile in these studies, low amounts were present throughout revealing a continuous presence of the insect during the Holocene. Non optimal environments with low spruce budworm populations give rise to outbreak events where the synchronization is less evident in time and space (Morin et al. 2007, Cooke et al. 2007). Such conditions might explain, at least partly, the apparent low recurrence of severe defoliation periods no matter whether periods I and II from the subfossil chronology can be attributed to spruce budworm outbreaks or not.

Holo-cellulose was used as the material for subfossil isotope analyses. It is composed of the α-cellulose fraction and other wood polysaccharides called hemicelluloses. Diagenetic changes during thousands of years of burial can possibly affect the carbon isotope composition of subfossil wood due to distinct decay rates of wood components (Schleser 1999). Loader et al. (2003) showed that differential decay is likely to lead to changes in the cellulose/lignin ratio that will influence the $\delta^{13}C$ values obtained from whole wood but that the carbon isotopic offset between individual components (lignin, cellulose and whole wood) remains similar in 4350 year-old subfossil oaks.

In a similar way as for carbon, burial might also affect the oxygen isotopic composition of cellulose (Richter 2006, Richter et al. 2008). Environmental water can exchange oxygen atoms with the carbonyl oxygen present in some hemicellulose monomers modifying the original oxygen isotopic composition. Wright (2008) demonstrated clear exchange of oxygen isotopes in holo-cellulose during long-term storage although clear evidence for exchange prior to cellulose extraction was not revealed.

Diagenetic processes are unlikely to have altered the carbon isotopic signal of the subfossil samples but if and to what extent the oxygen isotopic values might have been modified is unknown but could have interfere in the results presented in this study.

3.6 CONCLUSION

Carbon and oxygen stable isotopes used in combination with tree-ring widths have potential to aid the identification of past spruce budworm outbreaks in a multi-proxy approach. They showed promising results in identifying already known insect outbreaks in living mature trees. However, despite radial growth reductions in the subfossil chronology that could potentially be associated to spruce budworm outbreaks, the isotopic analysis did not confirm the origin of these growth decrease. Nevertheless, the presented isotopic method remains so far the only approach supporting the biological origin of the growth reduction observed in tree rings.

Further investigations are needed to confirm the potential of carbon and oxygen stable isotopes to study the impact of insect defoliation with subfossil wood. Additional modern living trees covering a larger spectrum of outbreak severity should be studied to refine the proposed identification criteria. Longer analyses of isotope series from subfossil wood will also be a determinant factor in better assessing $\delta^{13}C$ patterns.

3.7 ACKNOWLEDGEMENT

The authors wish to thank all personnel and research assistants at the Laboratoire d'Écologie Végétale, UQAC, who actively participated in this research, especially J-G Girard and J-P Lebeuf. The project was financially supported by the Natural Sciences and Engineering Research Council of Canada (NSERC), The Consortium de Recherche sur la Forêt Boréale Commerciale and the Fondation de l'Université du Québec à Chicoutimi.

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3.9 TABLES AND FIGURES

Table 3.1 Carbon and oxygen stable isotope criteria to define defoliation episodes and their occurrence in subfossil trees, based on observed isotope patterns during known spruce budworm outbreaks. *TRW=tree-ring width; I and II stand for presumed outbreak periods I and II. a) to g) refer to the individual series analysed.*

	Chronology		a)		b)		c)		d)		e)		f)		g)	
Criteria	**I**	**II**	**I**	**II**	**I**	**II**	**I**	**II**	**I**	**II**	**I**	**II**	**I**	**II**	**I**	**II**
1- ↑ and ↓ $\delta^{13}C$ with an interval between the 2 $\delta^{13}C$ minimums ≥ 5 yrs	X	X				X	X	X		X			X			
2- Δ (enrichment) ≥ 0.9‰			X							X						
3- $\delta^{13}C$ maximum value > than average value 5 yrs before and after minimums	X		X				X						X			
4- $\delta^{18}O$ trend follows TRW	X	X			X	X	X	X		X			X			X

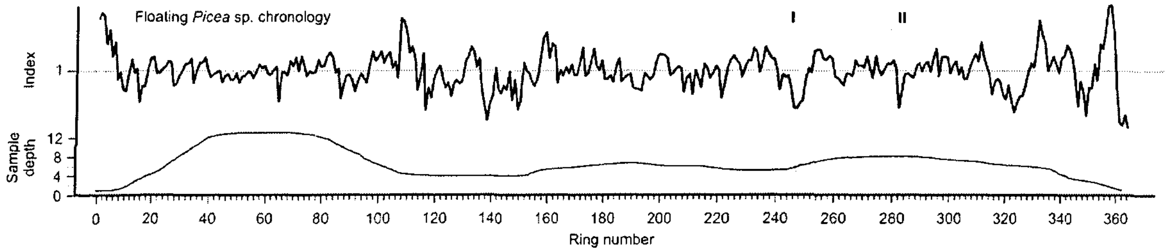
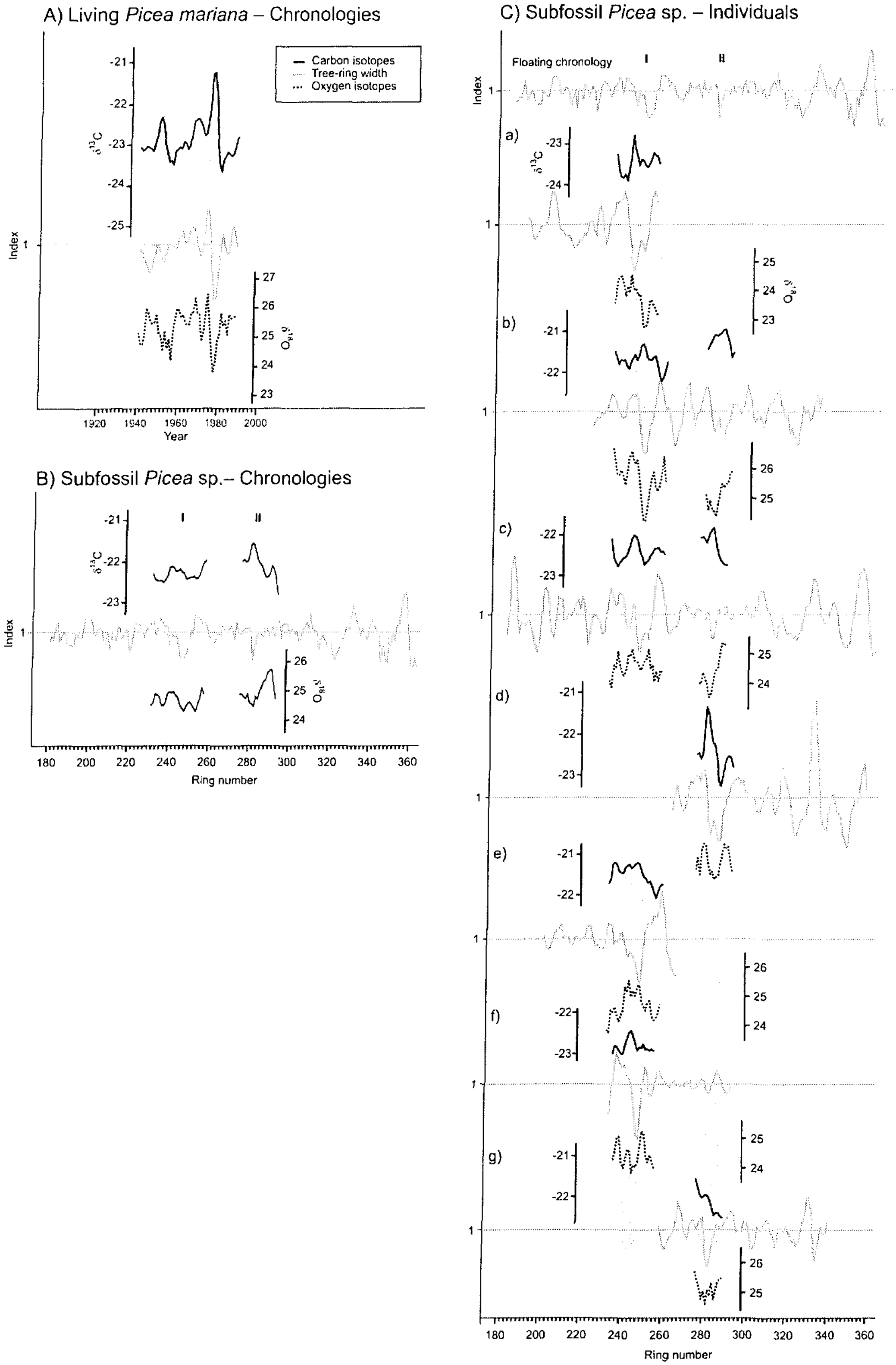


Figure 3.1 Floating subfossil *Picea* sp. chronology. Shaded areas indicate tree-ring width reduction periods chosen for carbon and oxygen stable isotope analyses. The seven series analysed cover the period between ring number 180-360.


A) Living *Picea mariana* – Chronologies

B) Subfossil *Picea* sp.– Chronologies

C) Subfossil *Picea* sp. – Individuals

Figure 3.2 Smoothed (3-year moving average) tree-ring width, carbon and oxygen isotopic composition series of A) living mature *Picea mariana* and *Abies balsamea* from a neighbourhood site, two host species of the spruce budworm (figure modified from Simard et al. 2008) and B) subfossil trees. Individual series of subfossil Picea sp. (a to g) used to build the chronology are presented in C). Grey bands represent actual outbreak periods in living trees and presumed ones in subfossil trees.

CHAPITRE IV

LONG-TERM SPRUCE BUDWORM DYNAMICS IN THE BOREAL FOREST INFERRED FROM SUBFOSSIL TREES AND STABLE ISOTOPES

4.1 ABSTRACT

Subfossil trees were used to reconstruct long-term spruce budworm outbreak dynamics locally in the eastern part of the boreal forest. Although the majority of the excavated wood belonging to the gender *Picea* could potentially reveal the past activity of spruce budworms in the tree-rings, very few could be crossdated due to a lack of marker rings since the narrow ones were produced during outbreak events. All crossdated trees were found at a similar depth and the floating chronology was radiocarbon dated to approximately 5.1 ka cal. BP. Overall, the results obtained in this research agreed with previously published findings stating that severe spruce budworm outbreaks, as observed in the 20th century, were rare events during the Holocene. The trees used to build the chronology grew and recorded events around 5.1 ka cal. BP, a period where the fire frequency was low, leading to susceptible and vulnerable forest stands with regards with the spruce budworm. Different researchers suggested a relationship between fire frequency and spruce budworm outbreaks through its impact on forest composition, fir the main spruce budworm host species increasing with a diminution of the fire frequency. A high natural temporal variability was suggested within this research.

4.2 INTRODUCTION

Forest ecosystem management, a management approach aiming at maintaining healthy and resilient ecosystems by diminishing the gap between natural and managed landscapes, is based on natural disturbance regimes, which are intrinsic ecological processes driving forest dynamics (Gauthier et al. 2008). Besides forest fires, insect outbreaks are one of the most important disturbances in the North American boreal forest and have a substantial impact on the structure and composition of large forest areas. In the eastern part of the boreal forest, the spruce budworm (*Choristoneura fumiferana* Clem.) recurrently defoliates balsam fir (*Abies balsamea* [L.] Mill.), white spruce (*Picea glauca* [Moench] Voss) and also black spruce (*Picea mariana* [Mill.] B.S.P.) (MacLean 1980, Blais 1983, Morin et al. 2007). During the last outbreak (1974-1988), more than 55 million ha of forest were affected (Boulet et al. 1996). The next epidemic period expected for the coming years has indeed already started within the territory of Quebec. The area affected by the budworm population increased drastically in 2004 and continued its progression as up to 2009. The infested area more than doubled in 2009 as compared to 2008 (321 146 vs 133 603 ha respectively) (MRNF 2009).

Direct correlation between mortality and abundance of the host species has been reported in previous studies on past outbreaks at the stand (MacLean 1980, MacLean and Ostaff 1989, Bergeron et al. 1995) and landscape level (Bergeron et al. 1995, Cappuccino et al. 1998). In the eastern Canadian boreal forest, fire is a powerful agent enabling rapid changes in stands composition over large areas. At the regional scale, regions with longer mean fire return intervals (MFRIs) are generally associated with higher abundance of balsam fir whereas regions with shorter MFRIs present a higher abundance of pioneer species not susceptible to spruce budworm defoliation (Bouchard and Pothier 2008 and references therein). The change in fire return intervals hypothesis and associated forest composition to explain the more severe and synchronized outbreaks observed in the 20th century as compared to the 19th century has been supported by many researchers (Bergeron and Leduc 1998, Jardon et al. 2003, Bouchard et al. 2006, Simard et al. 2006).

Among the different components characterizing a disturbance regime, the understanding of a disturbance's natural variability, both spatially and temporally, is essential in a context of forest ecosystem management. Based on dendrochronological studies, spruce budworm outbreaks have displayed a mean return interval of 30-40 years in eastern Canada over the last 450 years (Blais 1983, Royama 1984, Krause 1997, Jardon et al. 2003, Boulanger and Arsenault 2004, Royama et al. 2005). However, the age of living forest limits the length of reconstructions. Other proxies, with a smaller temporal resolution, have been used to extend back in time the history of spruce budworm outbreaks. Spruce budworm faeces and head capsules recovered in a peat profile enabled the reconstruction of the endemic and epidemic presence of the insect in the past (Simard et al., 2006). Variations of the abundance of these macrofossils suggest that intense periods of spruce budworm activity were rare during the Holocene.

The aim of this study was to use widths and stable isotopes of annual tree-rings from subfossil wood buried in mires to extend the existing chronologies back in time and to thus better assess the temporal variability of spruce budworm outbreaks during the last millennia.

4.3 METHODS

4.3.1 Study area

The study area is located in the hilly Mont-Valin region, approximately 100 km north of Saguenay, Québec, Canada (approximately 48°N, 70-71°W), within the eastern balsam fir-white birch bioclimatic domain of the continuous boreal forest (Saucier et al., 1998). Balsam fir and black spruce are the dominant canopy species. Other tree species such as white spruce, paper birch (*Betula papyrifera* Marsh.) and trembling aspen (*Populus tremuloides* Michx.) are occasionally found.

Regional climatic conditions for *AD* 1942-1990 (Bagotville A meteorological station, 48°20'N, 71°00'W, 159 m. a.s.l.), ~120 km south of the study area, are characterized by a mean annual temperature of 2.3°C and mean annual precipitation of 950.8 mm (36% as snow) (Environnement Canada, 2010). Main disturbances affecting the forest in this environment consist of insect outbreaks and fires (Morin 1994).

Three sites within the study area were selected based on specific features. Small mires surrounded by forested slopes composed of balsam firs and black spruces with a good potential of buried wood were sought-after. All trees with a diameter larger than 9 cm at breast height (DBH) within a typical stand 20mX20m plot on the forested slope of each site were sampled using an increment borer for dendrochronological analysis. Two cores per tree were taken. Sample surfaces were sanded and measured using a sliding-stage incremental micrometer (Henson, California, USA) with a precision of 0.01 mm (Cook and Kairukstis, 1990). Visual crossdating was verified using the COFECHA program (Holmes, 1983). Standardisation using a 66%n year cubic smoothing spline with a fixed 50% cutoff was done with the ARSTAN program (Cook and Kairukstis, 1990).

Mires were sounded with a probe so as to delineate the highest concentration of buried wood. Prior to excavation, the surface of the mire was mapped using a total station. Excavation was conducted using a mini-excavator and shovels and water was regularly pumped out of the excavation site using a gas pump. Each piece of wood was mapped and numbered to assess the depth at which it laid. The wood was then taken to the laboratory where it was stored frozen. Transversal sections were taken at the largest end of the stem for dendrochronological analysis. The surface of each section was either prepared with a razor blade or sanded frozen. The same methods as for living trees for measurement and standardisation were applied.

To facilitate the cross-dating process, a first sorting of the samples was conducted based on their depth in the peat profile. Groups of samples were created for each 50 cm of the peat profile with an overlap of 25 cm. Crossdating was verified using the COFECHA program (Holmes, 1983) and TSAP-Win™ (Rinntech, Germany) software, but also by visual inspection of the growth curves. Samples with less than 55 rings were not considered. Only one floating chronology could be built. Radiocarbon dating of wood from six trees in the chronology was carried out by accelerator mass spectrometry (AMS) (Stuiver et al. 1998). Calibrated dates, expressed in kilo annum before present (ka cal. BP), were used to locate the floating chronology in time.

Each piece of wood was identified using anatomical features (Schweingruber 1982, Hoadley 1990). Although black and white spruces can be anatomically differentiated according to the proportion of uniseriate and biseriate radial tracheids (Marguerie et al. 2000), spruce species were identified to the genus level only. Both spruces are affected by the spruce budworm. Spruce sp. were used to build the chronology as they were the most abundant and the longest lived host species recovered from the mires. The percentage of subfossil firs was limited and consequently that species was not considered for the chronologies.

The program OUTBREAK was used to help identify possible outbreak periods (Holmes and Swetnam 1996). It allows the identification of growth reduction periods according to defined criteria. To be considered, a growth reduction had to last a minimum of 5 years and the maximal growth decrease had to be equal or 1.28 times greater than the standard deviation of the chronology (Jardon 2001).

The tree-ring carbon and oxygen stable isotopic composition ($\delta^{13}C$ and $\delta^{18}O$, respectively) of seven subfossil *Picea* sp. series including growth reduction periods possibly induced by the spruce budworm, from the best replicated part of the chronology, was measured to assist the dendrochronological interpretation of spruce budworm outbreaks (chapter 3).

Most of the trees excavated from the mires had either no bark and/or no pith making age estimation difficult. Moreover, parts of stem rather than entire trees were recovered. Consequently, the origin of those tree parts, upper stem versus lower stem for example, was impossible to asses. Moreover, determining the position of these trees in the canopy (dominant, co-dominant, suppressed, etc.) at the time they lived is not possible. In order to evaluate the impact of age and hierarchical status on the likelihood of trees to record an outbreak in this environment, statistical variables of the dendrochronological series from young and old living trees (~75 and 150 years, respectively) experiencing spruce budworm outbreaks in the 20th century were evaluated. The average correlation among trees for the common overlap period among series (Rbar), the standard deviation, signal-to-noise ratio as well as the chronology expressed population signal (EPS) were compared. The same analysis was done with subfossil trees. Four different periods of the chronology were selected. Section 1 (S1) and 4 (S4) were selected according to the number of trees and the length of the common period of analysis whereas section 2 (S2) and 3 (S3) were selected based on isotope analyses. The same statistical parameters as for living trees were compared.

4.4 RESULTS

More than five hundred pieces of wood were excavated from three different mires (tab. 4.1). Among them, the majority (61%; tab. 4.1) belonged to the genus *Picea*. Other species such as *Larix laricina*, *Abies balsamea* and some deciduous trees, most likely *Betula papyrifera*, were also found. Near 30% of the samples were not analysed due to either a low number of rings for crossdating, the minimal amount having been set at 55 rings (10%), their physical status making them unusable (5%) or for other reasons (roots, branches, presence of reaction wood, etc.) (12%).

Despite considerable effort made at the sampling and crossdating steps, and even with relatively well distributed samples across the organic profile of the three mires (fig. 4.1), less than 10% of the *Picea* trees could be put together to form a 364-year floating chronology. These 28 trees were all found at a similar depth in the mires, at 1m depth on average. The chronology was dated to approximately 5.1 ka cal. BP (fig. 4.2A).

The sample number remained low all along the chronology, in average lower than six individuals, reaching at the maximum 14 for a short time period (fig. 4.2A). Within the 364-year floating chronology, two periods of growth reductions (I and II), highlighted by the program OUTBREAK and lasting 18 and 9 years, respectively, could potentially be associated to spruce budworm outbreaks, based on visual criteria such as “V” or “U” shape of growth reduction and low inter annual variability within the growth reduction. These periods were separated by a 25-year interlude of near average growth and chosen for further isotopic analyses based on the fact that they were the two highest replicated segments with growth reductions (5 samples each) (fig. 4.2A). Carbon and oxygen isotopic patterns were associated to these growth reduction periods (chapter 3).

A chronology from living *Picea mariana* sampled in sites surrounding the mires was also built (fig. 4.2B). Nearly 50 trees were included in the chronology with the sample depth decreasing back in time. The chronology spans 1750-2005 and includes all known outbreaks that occurred in the 20th century (1970s-80s, 1940s-50s, 1910s-20s) and the 19th century (1870s-80s, 1835s-60s, 1810s-20s). These outbreaks, particularly from the 20th century, were well recorded in tree-rings and detected with the program OUTBREAK (fig. 4.2B). Around 60% and up to 80% of the trees for the last outbreak simultaneously recorded the growth decrease. Outbreaks from the 19th century apparently had a lower impact as can be observed in the dendrochronological chronology and from results of the OUTBREAK program (fig. 4.2B). Less than 40% of the analysed trees presented simultaneously a maximal growth reduction during the three known outbreak periods of the 19th century. Compared with outbreaks from the 20th century, the results suggest a more diffuse and relatively constant impact of the insect on tree growth rather than clear pulses of defoliation affecting most of the trees at the same time.

In order to assess the effect of age on the likelihood of host trees recording a spruce budworm outbreak, a statistical comparison of dendrochronological series from young and old living black spruces (~75 and 150 years, respectively) was conducted. Young and old spruces experiencing budworm outbreaks in the 20th century showed similar results among the different statistical variables studied (fig. 4.3A). Indeed, the average correlation of trees for the common overlap period among series (Rbar), the standard deviation, signal-to-noise ratio as well as the chronology expressed population signal (EPS) were similar between young and old trees for outbreaks of the 20th century.

The common period of 75 years for the analysis of living trees corresponds approximately to the average number of rings (mean ~77 rings) found in 26 of the 28 subfossil trees used to build the floating chronology. Only 2 trees reached a much higher than average number of rings (185 and 218 rings, respectively). The number of rings from subfossil trees represents a minimal age since the inner and outer part of the stem was not always present. The statistical analysis done in a similar way to living trees showed even higher Rbar values for most parts of the subfossil chronology analysed with however approximately two times lower signal-to-noise ratios (fig. 4.3B). The EPS values, due to low signal-to-noise ratios and number of samples included in the analysis, reached lower values than for living trees.

4.5 DISCUSSION

Despite a relatively consistent vertical distribution of wood in the studied mires, most of the excavated subfossils could not be incorporated into a chronology. Only wood pieces located within a particular layer had distinctive tree-ring patterns that permitted us to confidently crossdate growth series. The difficulties in crossdating most of the trees suggests general environmental conditions leading to complacent tree growth. While severe spruce budworm outbreaks result in a distinct ring-print in terms of radial growth reduction, as observed in living *Picea mariana* from the 20th century, moderate to light defoliation periods are not well captured in tree-ring widths. Moreover, light defoliation, rather than slowing down radial tree growth, has been observed to stimulate it in different coniferous species (Reich et al. 1993, Vanderklein and Reich 1999, Chen et al. 2001, Lavigne et al. 2001, Little et al. 2003).

All subfossil spruces analysed, excluding those having less than 55 rings, had on average 115 rings. Most of those used to build the floating chronology had a minimal average of 77 rings, some of them being potentially immature, although the exact age count was impossible to obtain due to missing bark and piths in most of the samples (Viereck and Johnston 1990). MacLean (1980) showed that mortality in immature fir and spruce stands is lower than in mature ones. However, at the tree level, whether immature individuals are less sensitive than their counterparts is not clear. It has been suggested that spruces and balsam firs living under the canopy, including seedlings, may be less affected by the budworm than larger ones (Baskerville 1975). Fraver et al. (2007) nevertheless found that small diameter slow-growing red spruces clearly expressed the typical budworm signal with proper ring-width data standardization. Similar results were found for other conifer species (Morin & Laprise 1990). The comparison between young and old black spruces living in the 20th century, presented here, strongly suggest that the age factor was not a decisive one in recording outbreaks occurring in that century. The ring pattern of young and old spruces clearly shows the effect of the defoliation periods, especially the severe outbreak that occurred in the end of the 70s. Besides the age factor, site quality might also play a role in the ability of host species to record outbreaks. A number of studies indicated that host species growing on poorly drained sites experienced a greater decrease in the volume and mortality than those growing on well-drained sites (Hix et al. 1987, Dupont et al., 1991, MacKinnon and MacLean 2004). Based on these observations, the weak signal in subfossil tree rings is most likely not an artefact of tree age, their hierarchical status within the canopy or the environment in which they grew but rather a reflection of the environmental conditions prevailing at that moment.

As mentioned previously, fire is a powerful agent inducing rapid changes in the forest composition over large spatial scales. Based on model simulation, Bouchard and Pothier (2008) showed that a change in fire return starting by the end of the little ice age (around 1850) has not been long enough to sufficiently increase the basal area index of balsam fir to explain any radical change in severity from one outbreak to the next, does not mean that the process could not take place earlier during the Holocene. Shifts from shorter to longer MFRIs were observed throughout the Holocene. The early part was characterised by high fire incidence in eastern Canada. A low fire frequency was observed for the period between 8 and 3.2 ka cal. BP (Carcaillet and Richard 2000, Ali et al. 2009, Cyr et al. 2009). Progressively, the fire frequency increased, reaching a maximum ca. 3.4-3.2 ka cal. BP. The period between 8 ka cal. BP and 3.4-3.2 ka cal. BP might have given rise to favourable ecological conditions advantageous to spruce budworm populations. Simard et al. (2006), based on macrofossil and charcoal analyses, observed that the highest peaks of budworm remains coincided with low fire activity between 6.8 and 6.4 ka cal. BP.

Besides forest composition, other plausible explanations might account for the increase in spruce budworm outbreak impact succeeding a change in climatic conditions. Climate can indeed have some effects on balsam fir through changes in foliage characteristics, on the spruce budworm population itself, and/or on its natural enemy complex (Fleming and Volney 1995, Logan et al. 2003, Cooke et al. 2007, Volney and Fleming 2007).

Living balsam firs and spruces from the boreal forest present rather complacent growth. Marker rings in these species, i.e. narrow rings necessary to crossdate trees together, are mostly associated with severe spruce budworm defoliation. It is reasonable to consider that periods in the past in which environmental factors prevented balsam fir from occupying the canopy, most likely due to periods of high fire frequency, possibly led to low impact outbreaks making them difficult to trace in growth rings of trees, thus preventing the construction of complete tree-ring chronologies during the Holocene.

4.6 CONCLUSION

The results presented in this study suggest that the dynamics of spruce budworm outbreaks changed during the Holocene. Severe outbreaks seem to have been exceptional events in the eastern Canadian boreal forest since the end of the last glaciation. These results are in lines with observations of different research (Anderson et al. 1986, Bhiry et Filion 1996, Jasinki et Payette 2005, Simard et al. 2006, Lavoie et al. 2009).

Despite a significant sampling effort, few trees could be crossdated and the number of samples along the floating chronology remained very low. The amount and localization of subfossil wood used to build the chronology, in addition to the possible number of outbreaks recorded in the tree rings, strongly suggest that the budworm population in the study area was rarely high enough during the Holocene to lead to important radial growth reduction. Moreover, stable isotope analysed tree rings produced during reduced growth periods did not confirm that the growth reduction analysed could have been caused by spruce budworm defoliation.

The results presented here, in relation with earlier work from other authors, suggest that the natural variability of the impact of spruce budworm outbreaks during the Holocene was higher than what could be expected when looking at the recent centuries only. In a context where forest management approaches aim at maintaining healthy and resilient ecosystems by diminishing the gap between natural and managed landscapes, this information should be taken into consideration in the forest management strategies. Moreover, the structural composition of actual old growth forests is a direct consequence of spruce budworm outbreaks of the late 19th and 20th centuries. Less severe outbreaks that occurred in the past as suggested by this study probably generated more complex forest structures compared to those that we actually want to reproduce. The obtained results raise some fundamental questions and have serious implications on actual management practices.

4.7 REFERENCES

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4.8 TABLES AND FIGURES

Table 4.1 Summary of excavated wood in terms of species and condition.

			Chronology	
	N	%	N	%
Picea sp.	314	61	28	9
Larix laricina	26	5		
Abies balsamea	19	4		
Deciduous species	21	4		
Less than 55 rings	49	10		
Heavily rotten or destroyed	25	5		
Others	60	12		

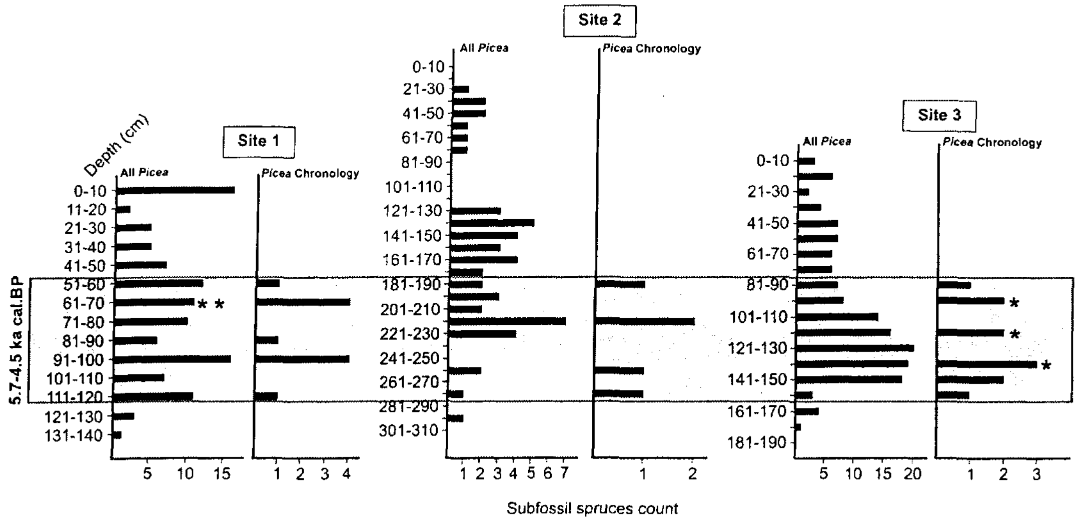


Figure 4.1 Vertical distributions in the three organic profiles of the excavated subfossil *Picea* sp. having more than 55 rings. The shaded area indicates the location where trees crossdated to build the chronology. Stars indicate radiocarbon dates obtained from tree-ring wood.

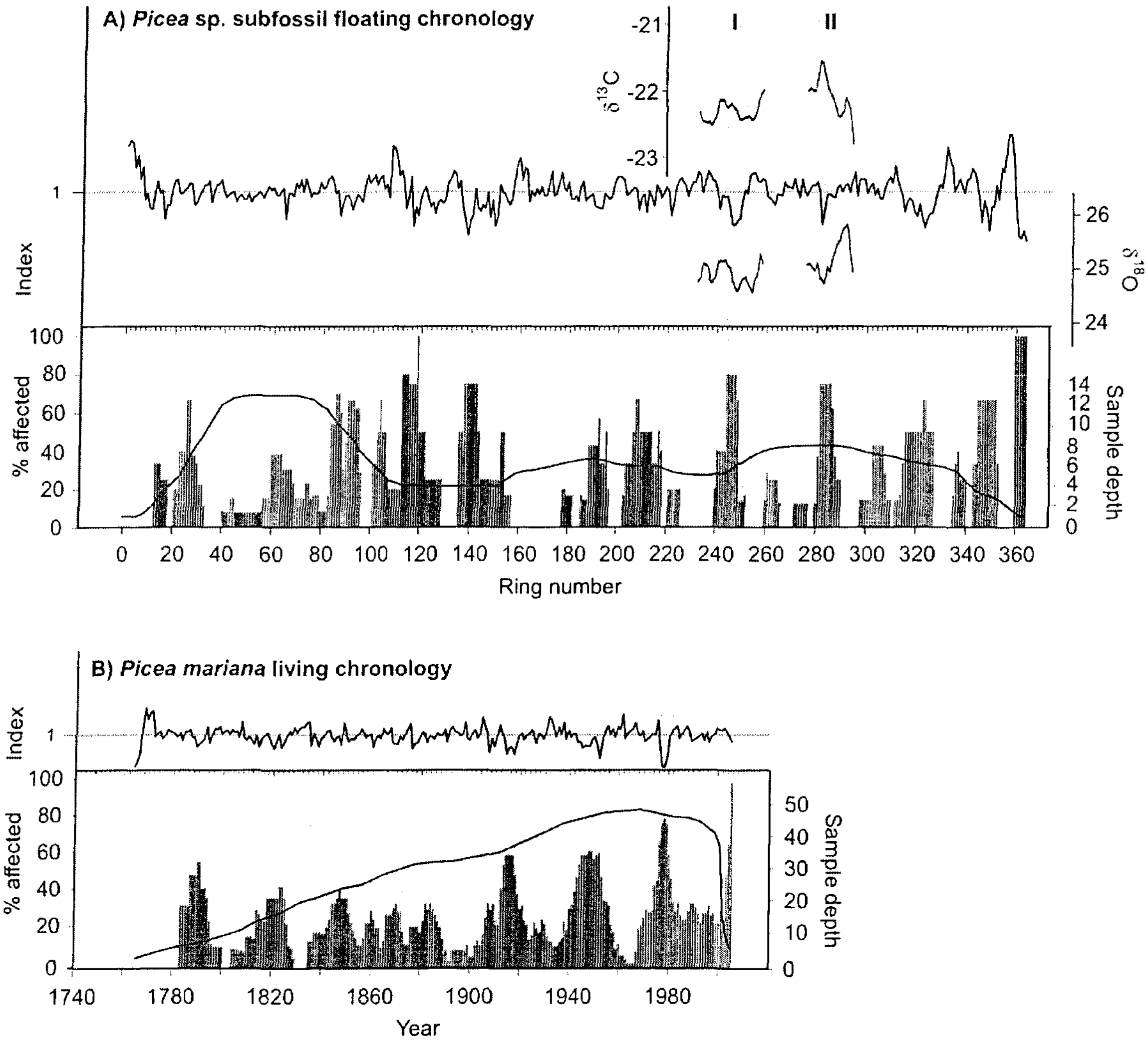
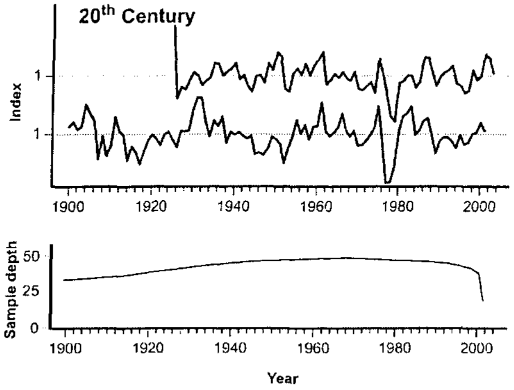


Figure 4.2 Indexed tree-ring chronology and OUTBREAK analysis of growth reductions and sample depth of A) *Picea* sp. subfossil trees and B) living *Picea mariana* trees. Shaded areas indicate possible outbreak periods in the subfossil trees chronology and known ones in the living trees chronology. Carbon and oxygen isotope results of subfossil trees are also presented in A).

A) LIVING TREES

	20th Century	
	Young trees (~100 yrs old)	Old trees (~200 yrs old)
Samples	18	18
Samples common period	18	14
Common period (yrs)	75	75
Rbar	0.24	0.28
Standard deviation	0.15	0.16
Signal-to-noise ratio	5.6	5.5
EPS common period	0.85	0.85



B) SUBFOSSIL TREES

	~4500 yrs BP			
	S 1	S 2	S 3	S 4
Samples	13	11	10	10
Samples common period	5	5	6	5
Common period (yrs)	70	34	35	75
Rbar	0.07	0.31	0.37	0.36
Standard deviation	0.21	0.16	0.15	0.10
Signal-to-noise ratio	0.4	2.3	3.4	2.8
EPS common period	0.29	0.69	0.78	0.74

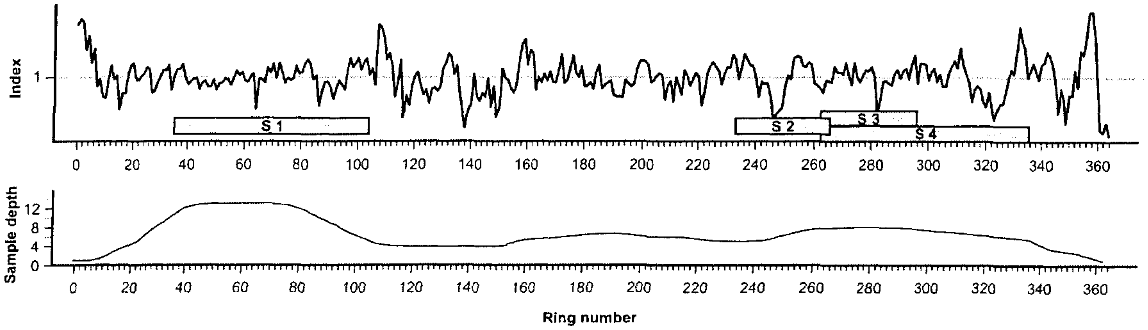


Figure 4.3 Statistical parameters of A) the 19th and 20th century chronologies built from young and old living trees and B) selected sections of the subfossil floating chronology. *S=section.* The grey bands in A) represent known outbreak periods of the 20th century.

CONCLUSION GÉNÉRALE

Plus de 80% des forêts exploitables sur le territoire québécois sont aménageables conformément au principe du rendement soutenu (MRNF 2008b). De ces forêts, 84% est d'ordre public et sur la presque totalité de la superficie, soit 88%, des droits d'exploitation ont été consentis sous forme de CAAF ou CvAF (contrat d'approvisionnement et d'aménagement forestier et convention d'aménagement forestier, respectivement) (MRNF 2008b). Il va sans dire que l'industrie forestière est un secteur d'emploi névralgique au Québec. À lui seul, il génère directement (foresterie et exploitation forestière, fabrication de papier et produits en bois) plus de 15% des emplois manufacturiers de la province et indirectement (fabrication de meubles et produits connexes, impression et activités connexes) plus de 10% de ces emplois, pour un total d'un peu plus de 25% (MRNF 2008b). Avant la crise des dernières années, plus de 75 000 travailleurs québécois trouvaient un emploi directement lié à l'industrie forestière. La pression sur la ressource forestière étant particulièrement forte, il est primordial de connaître les mécanismes et processus gérant la dynamique naturelle de ces écosystèmes. Les plans d'interventions en milieux forestiers doivent respecter la variabilité naturelle des perturbations en vue de ne pas exploiter les forêts au-delà des limites établies.

L'objectif général de cette thèse était d'évaluer le potentiel d'utilisation des isotopes stables dans les cernes de croissance pour reconstituer les épidémies de la TBE au cours des derniers millénaires. Le but était de reconstituer la dynamique à long terme des épidémies à l'aide de subfossiles d'arbres dans la région des Monts-Valin située au nord de Chicoutimi, une des perturbations naturelles majeures en forêt boréale. Suite aux résultats obtenus lors d'une étude préliminaire (Simard 2003), il est apparu clair qu'une méthode permettant d'identifier plus précisément les périodes de défoliation légère à sévère dans les cernes de croissance était nécessaire. Une méthodologie utilisant les isotopes stables du carbone et de l'oxygène de la cellulose des cernes de croissance produits en conditions naturelles lors d'épidémies, et contrôlées suite à de la défoliation manuelle artificielle a ainsi été explorée dans les chapitres 1 et 2 de la thèse, respectivement. L'utilisation de ces indicateurs pour l'identification de périodes épidémiques à partir de cernes de subfossiles d'arbres datant de quelques milliers d'années a été vérifiée dans le chapitre 3 et l'interprétation des résultats obtenus à partir des subfossiles d'arbres, dans un contexte écologique, a été faite au chapitre 4.

L'utilisation des analyses isotopiques s'est avérée intéressante et utile pour mettre en évidence les périodes de défoliation tant chez les arbres hôtes matures en conditions naturelles que chez les jeunes arbres en conditions contrôlées. Dans les deux cas, un enrichissement de la cellulose des cernes de croissance en ^{13}C a été observé lors de périodes de défoliation alors qu'aucun patron particulier de variation de ^{18}O n'a pu y être relié. L'expérimentation en milieu contrôlé a permis de répondre à certaines questions concernant les mécanismes physiologiques induisant une réponse au niveau de la signature isotopique du carbone cellulosique en période de défoliation. Les résultats obtenus n'ont pas permis de soutenir l'hypothèse d'un enrichissement de la cellulose des cernes de croissance en ^{13}C lié à une augmentation de l'assimilation de CO_2. En effet, l'hypothèse de travail reposait sur la réponse isotopique du carbone en termes d'enrichissement des assimilats conséquent à une augmentation de l'assimilation de CO_2 en réponse compensatoire à la perte de biomasse photosynthétique. L'ouverture de la canopée et l'augmentation de la radiation solaire au niveau des aiguilles restantes étaient aussi un mécanisme susceptible de contribuer à l'augmentation du taux de photosynthèse (*A*). Toutefois, aucune relation significative entre *A* et $\delta^{13}C_{hc}$ ou encore c_i/c_a et les différents niveaux de traitement, relations ayant permis de confirmer notre hypothèse, n'a été obtenue. L'enrichissement en carbone isotopique en relation avec la défoliation semble donc survenir lors d'une étape post-photosynthétique. L'utilisation des réserves enrichies en ^{13}C s'avère une hypothèse intéressante pour expliquer la relation observée, qui reste toutefois à vérifier. Afin de bien comprendre les processus physiologiques en jeu, un suivi physiologique poussé s'avèrerait utile. Un suivi de la signature isotopique des assimilats et de l'amidon au niveau des aiguilles serait une avenue intéressante pour une compréhension accrue du fonctionnement des échanges gazeux en période de défoliation. L'existence ou non d'un lien entre le signal isotopique au niveau des aiguilles et celui du cerne de croissance pourrait permettre de vérifier l'origine de l'enrichissement en ^{13}C observé lors des périodes de défoliation. Il pourrait être intéressant d'aller encore plus loin, par exemple, en exposant de jeunes arbres témoins et défoliés à un environnement dont le CO_2 de l'air est enrichi en ^{13}C et suivre l'assimilation ainsi que les flux de carbone au niveau des individus. Une analyse fine de la croissance et de l'utilisation du carbone (réserves versus assimilats récents) permettrait de vérifier l'hypothèse de l'utilisation des réserves pour expliquer l'enrichissement observé lors de périodes de défoliation. Ce suivi pourrait être effectué sur quelques années. Bien que l'étude présente ait requis un certain nombre d'année d'application de traitement dans un contexte temporel défini et limité, il s'avèrerait préférable de ne recourir qu'à des saisons de croissance naturelles et non induites artificiellement afin de minimiser les risques d'effets confondants.

Bien que des résultats intéressants se soient dégagés des analyses isotopiques effectuées chez les arbres matures et les jeunes arbres défoliés, les résultats obtenus à partir des anneaux de croissance provenant des subfossiles d'arbres se sont avérés moins évidents. Les résultats n'ont pas permis de conclure à une réduction de croissance induite suite à une période de défoliation. À la vue des résultats obtenus, de longues séries temporelles plutôt que de courts segments doivent être analysées. Il est essentiel d'avoir la possibilité d'interpréter le patron de variation des isotopes de carbone en comparant les périodes épidémiques et non épidémiques. L'enrichissement en ^{13}C observé dans les cernes de croissance développés lors de périodes épidémiques ne peut prendre son sens que lorsque comparé à ceux ayant été formés lors d'une période de croissance effectuée dans des conditions environnementales où les populations de TBE étaient à des niveaux endémiques. De plus, l'analyse de séries chronologiques complètes favorisera la détection de périodes de défoliation légères à modérés habituellement indétectables sur la base des analyses dendrochronologiques uniquement. La limitation du nombre d'analyses isotopiques étant souvent une question budgétaire, l'avancement des technologies ainsi que la popularité grandissante de l'utilisation des isotopes stables en écophysiologie devrait accroître l'accessibilité à ce type d'analyse avec le temps. Les problèmes de dégradation biologique des composantes chimiques de la paroi cellulaire (cellulose, lignine et hémicellulose) dans le temps n'est pas un problème majeur pour les analyses isotopiques effectuées sur du matériel de type subfossile de bois en autant que ces analyses ne soient effectuées que sur une des composantes, soit la cellulose, et non le bois entier. Par contre, la diagénèse, soit la dégradation chimique d'origine non-biologique de la lignine et de la cellulose, demeure un problème potentiel qui pourrait effectivement modifier la composition isotopique des parois cellulaires du bois subfossile. Ce type de dégradation est toutefois difficile à quantifier. Néanmoins, l'effet de cette dégradation, si présente, devrait hypothétiquement être similaire pour tous les échantillons retrouvés à une profondeur semblable puisqu'ils auront été soumis aux mêmes conditions environnementales pendant une période de temps comparable.

Cette étude sur la reconstitution de la dynamique des épidémies de la tordeuse des bourgeons de l'épinette au cours de l'Holocène s'inscrit dans un contexte où l'idée d'un aménagement forestier écosystémique a progressé dans la communauté scientifique au point d'en faire un large consensus. L'aménagement forestier écosystémique cherche, par une approche écologique appliquée à l'aménagement forestier, à assurer le maintient de la biodiversité et de la viabilité de l'ensemble des écosystèmes forestiers, tout en répondant à des besoins socio-économiques, dans le respect des valeurs sociales liées au milieu forestier (MRNF 2008a). Ce type d'aménagement vise à protéger une large proportion de la biodiversité des milieux exploités en créant une gamme de niches écologiques, autant en quantité qu'en qualité, inspirée de ce que la nature a créé par les perturbations naturelles au fil du temps. Un des buts visés est de réduire l'écart existant entre les paysages naturels et aménagés, en se basant entre autre sur la variabilité naturelle, tant spatiale que temporelle, de ces perturbations. Les résultats obtenus dans cette étude, en accord avec d'autres recherches effectuées dans ce domaine (Anderson et al. 1986, Bhiry et Filion 1996, Jasinki et Payette 2005, Simard et al. 2006, Lavoie et al. 2009), qui suggèrent que l'explosion des populations de la tordeuse des bourgeons de l'épinette menant à des épidémies sévères a vraisemblablement été un phénomène plutôt rare au cours de l'Holocène. Bien que de nombreux arbres aient été extraits des tourbières étudiées, peu possédaient des caractères anatomiques permettant leur interdatation. Seuls les individus datant entre 5.7 et 4.5 ka BP ont permis de construire une chronologie couvrant 364 ans. Très peu de périodes de réductions de croissance relativement sévères ont été potentiellement liées à des épidémies de TBE. Les analyses isotopiques n'ont cependant pas permis d'associer les périodes analysées à des épidémies de la TBE. Les contraintes financières reliées aux analyses isotopiques ont fait en sorte que peu de séries de cernes de croissance des arbres subfossiles et des séries plutôt courtes ont été analysées. Ceci constitue un point faible du projet et devrait être pris en considération. Néanmoins, les résultats dendrochronologiques suggèrent que la variabilité de l'intensité des épidémies de la tordeuse des bourgeons de l'épinette dans le temps, du moins sur le territoire étudié, a été élevée. Sur le plan de l'aménagement écosystémique, les résultats présentés peuvent servir de base pour alimenter une réflexion à ce sujet. Ils soulèvent la question générale à savoir si les régimes de perturbation utilisés comme référence dans le cadre d'aménagement écosystémique sont vraiment représentatif de la dynamique forestière et plus particulièrement à quelle échelle temporelle. La structure des vielles forêts actuelles constitue un legs des épidémies de TBE de la fin du 19$^{\text{ième}}$ et du 20$^{\text{ième}}$ siècle, périodes où les épidémies ont été sévères. Des épidémies moins sévères auront probablement engendré des structures forestières moins complexes que celles que l'on désire reproduire actuellement. Ceci a évidemment des implications énormes sur les pratiques actuelles. Comme le mentionnent Bouchard et al. (2008), il y a peut-être même lieux de se demander, dans ce contexte où les épidémies sévères de la tordeuse des bourgeons de l'épinette semblent avoir été une exception au cours de l'Holocène plutôt que la règle, si l'impact de cette perturbation naturelle devrait tout simplement être émulé. Souplesse et variété dans les méthodes d'aménagement forestier semblent à tout le moins essentielles afin de refléter cette grande variabilité temporelle observée dans l'intensité des épidémies de la tordeuse des bourgeons de l'épinette au cours des derniers millénaires.

Ce qui contrôle précisément la dynamique des populations de TBE est encore sous débat quoi que Volney et Flemming (2007) mentionnent que toutes les théories s'accordent cependant sur le rôle joué par le climat, les ennemis naturels et les conditions forestières. Ce sur quoi les théories divergent sont l'importance et les caractéristiques qu'ils assignent à chacun de ces facteurs, en particulier les conditions forestières. Cooke et al. (2007) démontrent bien avec leur modèle tritrophique comment le déclin des populations d'insecte résulte d'effets émanent des plantes hôtes mais aussi des ennemis naturels, les facteurs stochastiques comme les conditions climatiques pouvant précipiter le déclin d'une épidémie ou au contraire en augmenter l'amplitude. Ces éléments stochastiques, bien qu'affectant l'amplitude et le synchronisme des épidémies ne sont toutefois pas responsables des cycles observés.

La grande variabilité de l'intensité des épidémies au cours de l'Holocène vraisemblablement liée aux conditions changeantes ayant eu cours durant cette période présuppose de modifications de l'intensité des épidémies à venir dans un contexte où les changements climatiques sont devenus indéniables. La réponse de la tordeuse des bourgeons de l'épinette face aux changements climatiques est difficile à évaluer mais les projections futures laissent entrevoir une possible augmentation de l'incidence des épidémies (Volney et Fleming 2000). Depuis la fin du petit âge glacière (environ 1850), il y a un déclin de la fréquence des feux et ce déclin, selon les prévisions des modèles de circulation globale, devrait se poursuivre en raison d'une modification du climat vers des printemps et des hivers beaucoup plus chauds (Flannigan et al. 1998). Associée à la diminution de la fréquence de retour des feux, le vieillissement des forêts en l'absence de feux permettant le remplacement des peuplements forestiers rendra ces derniers d'autant plus vulnérables aux attaques de la TBE. Les changements climatiques n'auront toutefois pas que des impacts au niveau de la composition forestière. De quelle façon la biologie de l'insecte et/ou celle des espèces arborescentes hôtes sera affecté reste encore indéterminée. Un découplage de l'émergence des larves de l'insecte par rapport à l'éclosion des bourgeons des arbres hôtes, ou vice-versa, pourraient avoir des conséquences importante sur la survie de ces dernières. Un tel découplage fut observé sur l'île de Vancouver en Colombie-Britannique où le réchauffement de la température hivernale lié au réchauffement de la température de l'océan dans cette région a favorisé l'émergence de la tordeuse occidentale de l'épinette (*Choristoneure occidentalis* Freeman) plus tôt au début de la saison alors que la phénologie des bourgeons du sapin Douglas (*Pseudotsuga menziesii* [Mirb.] Franco) est demeurée inchangée (Thomson et Benton 2007). Cette désynchronisation phénologique n'a pas eu lieu à l'intérieur du continent où aucun changement de la température hivernal n'a été observé.

Afin de bien comprendre la dynamique des épidémies de la tordeuse, il est impératif de poursuivre les recherches sur l'effet de l'activité des populations de TBE sur la structure et la composition forestière mais aussi les recherches sur l'effet de la structure et de la composition forestière sur les épidémies.

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