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LISTE DES ABREVIATIONS ET SYMBOLES

A _s	Net photosynthesis
AIC	Akaike Information Criterion
°C	degree Celsius
CCA	Canonical correspondence analysis
Cm	Centimeter
CO ₂	Carbon dioxide
CRSNG	Conseil de recherche en sciences naturelles et en génie du Canada
DBH	Diameter at breast height
DF	Degree of freedom
DFD	Degree of freedom denominator
DNA	Deoxyribonucleic acid
EPS	Expressed population signal
FERLD	Forêt d'Enseignement et de Recherche du Lac Duparquet
Fig.	Figure
FRQNT	Fonds de Recherche du Québec – Nature et Technologies
FTC	Forest tent caterpillar
g _s	Stomatal conductance
GML	General model linear
H	Hour
HCL	Hydrochloric acid
HSD	Honest significant difference
Km	Kilometer
L	Liter
LA	Leaf area
M ²	Square Meter
m a.s.l	Meter at sea level

MFFPQ	Ministère des Forêts, de la Faune et des Parcs du Québec
MM	Millimeter
MMOI	Millimole
MPa	Megapascal
NSERC	Natural Sciences and Engineering Research Council of Canada
pH	Power of Hydrogen
PCA	Principal Component Analysis
PI	Paternity index
P/N	ratio size parental root/size new root
PPFD	Photosynthetic photon flux density
r	Response functions coefficient
RDA	Redundancy analysis
RFA	Response function analysis
SE	Standard error
SEM	Standard error of the mean
SLA	Specific leaf area
SFS	Surface foliaire spécifique
SNP	Single-nucleotide polymorphism
SNR	Signal to noise ratio
SVWC	Soil volumetric water content
VPD	Vapor pressure deficit
Ψ :	Leaf water potential
$\delta^{13}\text{C}$	Carbon isotope composition
%	Percent
‰	Per mille

RÉSUMÉ

L'objectif général de cette thèse était d'améliorer nos connaissances sur l'origine des peuplements et l'influence des liens racinaires dans la physiologie, la distribution des produits de la photosynthèse et la croissance des peuplements de peuplier baumier (*Populus balsamifera* L.). Les travaux ont porté sur l'origine des peuplements dans les forêts naturelles, la structure du système racinaire et la présence de liens racinaires (racines parentales et greffes) entre les arbres d'une part, et d'autre part la fonction éco-physiologique des liens racinaires dans la croissance des peuplements forestiers. Le peuplier baumier a la capacité de se régénérer par drageonnement après perturbation anthropique ou naturelle, créant ainsi de vastes réseaux d'arbres interreliés par les racines, pouvant interagir les uns avec les autres. Ce mode de régénération est bien connu et étudié chez le peuplier faux-tremble (*Populus tremuloides* Michx), alors qu'on pensait qu'il était moins répandu chez le peuplier baumier, qui peut également se régénérer par graines ou encore à partir de fragments de branches enfouies.

Pour étudier l'origine des peuplements, six sites (trois au Québec et trois en Alberta) de peuplier baumier matures (82 arbres) ont été excavés à l'aide d'une pompe hydraulique. Les échantillons de tiges et des racines ont été prélevés pour déterminer l'origine des peuplements, et les feuilles ou fragments d'écorces ont été prélevés pour évaluer la diversité clonale de chacun des sites. Les caractéristiques des sites ont été enregistrées (distance entre les arbres, densité, etc.) pour déterminer leur influence sur la présence de racines parentales et sur l'occurrence de greffes racinaires entre les arbres dans les deux provinces. Des analyses dendrochronologiques ont permis de dater les arbres, les racines et les greffes racinaires. Nos résultats ont montré que tous les sites excavés étaient issus du drageonnement puisque des racines plus vieilles (racines parentales) que les arbres ont été identifiées dans le système racinaire de tous les arbres. Au total 22% et 25% des arbres étaient connectés par des racines parentales alors que 53% et 48% des arbres étaient connectés par des greffes racinaires, en Alberta et au Québec, respectivement. Ces résultats ont montré la persistance des racines parentales chez les arbres issus du drageonnement, qui se développent en intégrant l'ensemble du système racinaire. La distance moyenne entre les arbres reliés par des racines parentales était significativement plus faible que celle entre les arbres non connectés ($0,86 \text{ m} \pm 0,18 \text{ SE}$ vs $3,94 \text{ m} \pm 0,10 \text{ SE}$). De la même manière, la distance moyenne entre les arbres connectés par des greffes racinaires était plus petite que celle entre les arbres non connectés par des greffes ($1,14 \text{ m} \pm 0,17 \text{ SE}$ vs $3,98 \text{ m} \pm 0,11 \text{ SE}$). Les peuplements étudiés avaient une faible diversité clonale, où les arbres appartenaient à un seul clone dans quatre sites alors que deux sites (un en Alberta et un au Québec) étaient constitués de cinq et trois clones,

respectivement. Des greffes racinaires s'étaient formées entre les arbres d'un même ou de différents clones. Le maintien d'un système racinaire extensif à travers les greffes racinaires a également permis de maintenir vivantes les racines d'arbres morts, ce qui pourrait contribuer à maintenir la résilience des peuplements face aux perturbations en maintenant la majeure partie du système racinaire en vie.

En conditions contrôlées et après propagation de paires de ramets interconnectés ou non par la racine parentale ($n=32$), la mesure des échanges gazeux, du potentiel hydrique foliaire, la composition en carbone ($\delta^{13}\text{C}$) et la surface foliaire spécifique (SFS) ont permis de déterminer l'influence des liens racinaires sur les fonctions physiologiques et la croissance des ramets interconnectés sous contrainte hydrique. L'application d'un stress hydrique à des ramets reliés par la racine parentale en conditions contrôlées a suggéré le transfert d'eau entre ramets interconnectés, puisque les ramets stressés mais connectés à des ramets arrosés avaient des taux d'échanges gazeux et un potentiel hydrique foliaire similaires à ceux des ramets arrosés. Les résultats du potentiel hydrique foliaire (Ψ) et de la surface foliaire spécifique ont démontré que le lien racinaire était plus bénéfique pour le ramet proximal comparé au ramet situé en position distale sur la racine parentale. Le lien racinaire entre les ramets a également permis aux ramets stressés de discriminer davantage l'utilisation du ^{13}C lors des échanges gazeux, comparé aux ramets non connectés.

Enfin, la mesure de la croissance en diamètre des arbres (mesures des cernes par dendrochronologie) et l'analyse de la relation entre le climat et la croissance radiale des arbres connectés et non connectés par des liens racinaires ont montré l'importance des liens racinaires dans le patron de croissance des arbres face aux variations de températures et lors d'une épidémie sévère de livrée des forêts (*Malacosoma disstria* Hubner). L'analyse de la croissance radiale a montré que la température du mois de mai de l'année en cours a eu une influence positive sur la croissance radiale des arbres connectés et non connectés. Cependant, la croissance des arbres non connectés a été affectée négativement par la température du mois d'août de l'année en cours ($r = -0,3$), tandis que la croissance des arbres connectés ne l'était pas. L'analyse de la variance a montré une différence significative ($p = 0,02$) dans le patron de croissance en diamètre entre les arbres connectés et non connectés lors d'une épidémie sévère de la livrée des forêts. En effet, lors de l'épidémie, les arbres connectés ont eu une croissance radiale supérieure de 16% en moyenne comparativement aux arbres non connectés. Cette réponse des arbres connectés serait due au partage de sucres et d'eau entre les arbres connectés en conditions de stress hydrique et lors de l'épidémie d'insectes.

La présente étude a permis d'approfondir nos connaissances sur l'origine des peuplements de peuplier baumier en forêts naturelles au Québec et en Alberta, sur la persistance des racines parentales dans le système racinaire des arbres issus du drageonnement ainsi que sur la formation de greffes racinaires. Elle a aussi permis de

mieux comprendre l'importance des liens racinaires dans la croissance des peuplements et leur impact éco-physiologique sur les arbres interreliés.

MOTS-CLÉS: Drageonnement, racine parentale, greffes racinaires, liens racinaires, conductance stomatique, photosynthèse nette, échanges gazeux, stress hydrique, transport de l'eau, intégration physiologique, potentiel hydrique foliaire, $\delta^{13}\text{C}$ *Malacosoma disstria*, interactions biotiques, simple nucléotide polymorphique (SNP), facilitation.

CHAPITRE I

INTRODUCTION GÉNÉRALE

1.1 Mise en contexte

Le peuplier baumier (*Populus balsamifera* L.) est l'une des deux espèces de peupliers les plus abondantes au Canada. Cependant son utilisation par l'industrie forestière est très récente de par la qualité de son bois (fibre moins dense), son contenu élevé en eau et la présence de résines rendant la transformation plus difficile. Les réductions des volumes de bois alloués aux usines, l'utilisation de la biomasse forestière pour la production de bioénergie, et la volonté d'aménager la forêt de façon durable font que l'on commence à l'utiliser de plus en plus. De plus, puisque cette espèce se régénère très facilement par bouturage, elle peut aisément être utilisée pour le reboisement à faibles coûts (DesRochers et al. 2004). L'espèce intervient également dans les croisements naturels ou artificiels pour produire des hybrides de peuplier à croissance rapide, de plus en plus utilisés au Canada. Malgré cette contribution essentielle dans la sélection et l'amélioration des hybrides interspécifiques, contrairement au peuplier faux-tremble (*Populus tremuloides* Michx.), le peuplier baumier a été l'objet de très peu de recherches par le passé, particulièrement pour celui poussant en forêt boréale vs en milieu riverain ou dans la tundra.

Pour mettre en œuvre des programmes rationnels d'amélioration et de conservation du peuplier baumier ou encore de sélection des hybrides, il faut impérativement disposer d'informations fiables sur la variabilité génétique de l'espèce et sur sa dynamique naturelle. C'est dans cette optique, que l'amélioration des connaissances sur les mécanismes de régénération de cette espèce se révèle d'une importance fondamentale pour un aménagement durable de la ressource. En plus de reproduction

sexuée par graine, le peuplier baumier est capable se propager végétativement, par drageonnement ou par enracinement de fragments de branches enfouies. Le mode de régénération par drageonnement permet aux arbres d'être interconnectés entre eux par les racines et de partager le même patrimoine génétique. Il est particulièrement important que les programmes de sélection ou de conservation considèrent ces diverses formes de reproduction de l'espèce de façon à respecter la diversité génétique de l'espèce.

1.2 Problématique

Le peuplier baumier est la seule espèce arborescente décidue à atteindre la limite nord de la forêt en Amérique du Nord (Rousseau 1974, Edwards et Dunwiddie 1985). C'est une espèce indigène à croissance rapide, qui s'est bien adaptée à sa zone écologique. Longtemps considéré comme une "mauvaise herbe", le peuplier baumier est très peu utilisé en foresterie. Toutefois, elle apparaît comme une essence appropriée dans la mise en valeur des terres marginales hydromorphes ou les basses plaines d'inondation, là où les espèces exotiques ne s'y prêtent pas. De plus, l'espèce intervient dans les croisements naturels ou dirigés pour produire des hybrides à croissance rapide, de plus en plus utilisés au Canada. Aussi, cette espèce à grande répartition géographique forme souvent avec le peuplier faux-tremble (plus utilisé en industrie) l'essentiel des peuplements mixtes de feuillus exploités au Canada, d'où l'importance de mieux connaître ses mécanismes de régénération dans le contexte de la mise en place de l'aménagement écosystémique.

On dispose de très peu d'informations sur la reproduction de l'espèce en forêt boréale naturelle. En plus de la reproduction par graines, le peuplier baumier est capable d'une propagation clonale dont l'étendue est mal connue (Gom et Rood 1999). Cette régénération végétative se fait par rejets de souches, enracinement de branches

enfouies dans le sol et aussi par drageonnement (rejets de racines) (Zasada et al. 1981). Des études réalisées sur les rives et le long des rivières en Alberta ont montré que les jeunes plants situés à proximité des rivières sont plus fréquemment issus de graines alors que ceux qui se trouvent plus éloignés de la rive sont le plus souvent des drageons (Rood et al. 1994). Les liens racinaires entre les drageons interconnectés par la racine parentale pourront probablement influencer la croissance et la physiologie des peuplements. De même, ces divers mécanismes de reproduction (sexuée et asexuée) de l'espèce après coupe ou passage de perturbations naturelles auront potentiellement des conséquences fortes sur la diversité génétique/clonale de l'espèce. Cette diversité semble nécessaire pour garantir la capacité d'adaptation des forêts dans un environnement changeant (Eriksson et al. 1996).

Actuellement, on ne connaît pas l'importance relative de la régénération clonale, notamment du drageonnement, comparativement à la reproduction sexuée dans les milieux autres que riverains ou le long des cours d'eaux ainsi que la fonction des liens racinaires dans la croissance et physiologie des peuplements. La dynamique de la croissance et la physiologie des arbres seraient possiblement différentes pour l'ouest canadien (Alberta), qui reçoit environ moins que la moitié des précipitations (pluies) de l'est canadien (Québec). On devrait s'attendre à ce qu'il y ait plus de recrutement par semis (plus de génotypes différents) au Québec, ce qui influencerait la structure génétique des peuplements du Québec en comparaison avec ceux de l'Alberta. L'amélioration des connaissances sur ces mécanismes de régénération et sur la fonction des liens racinaires contribuerait à un aménagement durable de l'espèce, dans le cas par exemple de la multiplication et la sélection des hybrides pour le reboisement. La mise en œuvre de programmes rationnels de sélection et d'amélioration des peupliers baumiers et de ses hybrides est limitée par le manque de connaissances sur la variation génétique naturelle. Ainsi, cette thèse vise à mieux comprendre les mécanismes de régénération de l'espèce en forêt naturelle et

l'importance écophysiological des liens racinaires dans la croissance des peuplements de peuplier baumier.

1.3 Etat des connaissances

1.3.1 Répartition géographique du peuplier baumier

Le peuplier baumier, encore connu sous le nom de “*Liard*” ou peuplier noir, est une espèce de peuplier à feuilles caduques de la famille des *Salicaceae*. C'est une espèce indigène de la forêt boréale de l'Amérique du Nord (Zasada et Phipps 1990) qui présente la plus vaste répartition géographique s'étendant de Terre-Neuve-et-Labrador jusqu'à l'Alaska (Fig. 1.1). Le peuplier baumier est une essence à croissance rapide qui se développe principalement dans les sols profonds, humides et sablonneux, des basses-terres fluviales, le long des cours d'eau, dans les zones détrempées, ou encore en forêts naturelles. Elle est également une essence pionnière qui colonise les sites perturbés par la coupe forestière (Gaussiran et Boileau 2007).

1.3.2 Les modes de régénération du peuplier baumier

Les modes de recrutement jouent un rôle fondamental dans la structuration spatiale et temporelle de la diversité génétique au sein et entre les populations d'une même espèce. Déterminant la migration efficace des allèles dans l'espace et dans le temps, ils constituent une des clés majeures pour comprendre la dynamique évolutive de la diversité génétique d'une espèce (Richards 1996, Barrett 2003). Malgré cette importance avérée, nos connaissances sur la régénération du peuplier baumier restent fragmentaires. En effet, des études sur la régénération du peuplier baumier établi après coupe ont montré que les coupes à blanc d'automne favorisaient la régénération

de branches enfouies tandis que, celles d'été et d'hiver favoriseraient la régénération par drageonnement (Zasada et al. 1981).

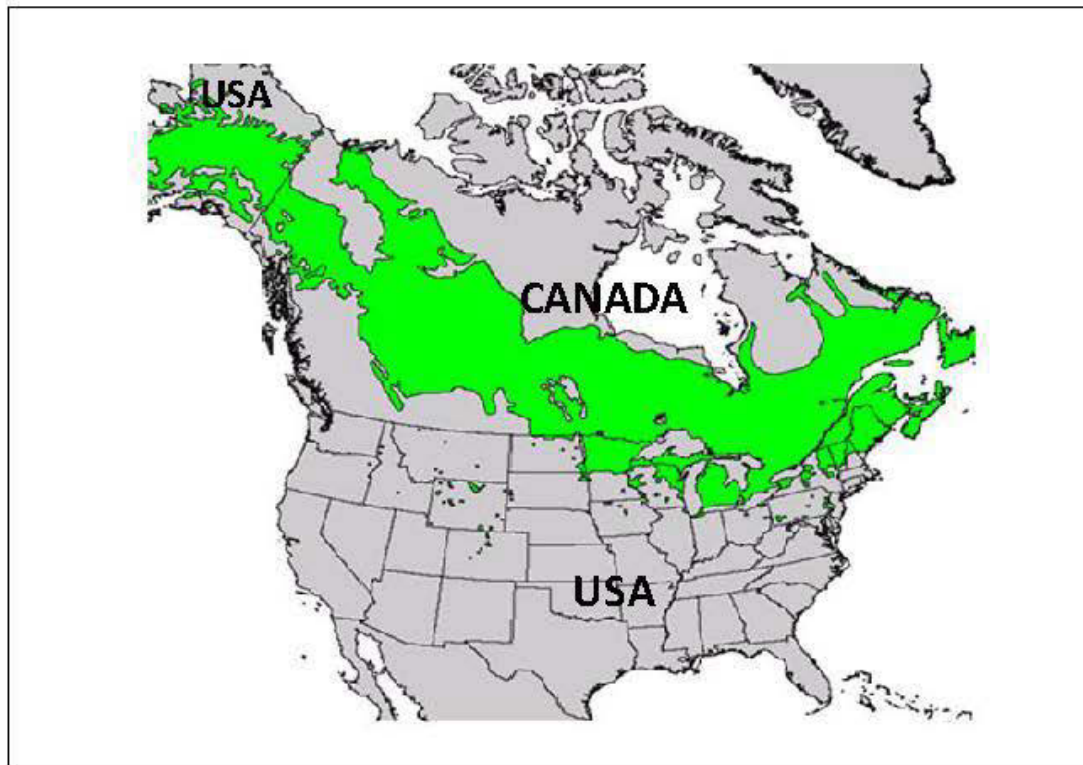


Figure 1.1: Répartition géographique du peuplier baumier en Amérique du Nord (en vert) d'après Little (1971).

Le drageonnement est un mode de propagation végétative qui permet le développement de nouvelles tiges ou drageons (ou encore ramets) à partir d'une racine parentale d'un peuplement résiduel. Le drageonnement est un processus contrôlé par deux hormones: l'auxine et la cytokinine (Hicks 1972). En effet, après une perturbation naturelle ou anthropique réduisant la dominance apicale, il se crée un déséquilibre, caractérisé par une diminution du taux d'auxine par rapport à celui de cytokinine, déclenchant ainsi le drageonnement. Les drageons sont initiés à partir des primordia (ébauche d'organe d'une plante) formés par des cellules méristématiques du cambium racinaire (Schier et Zasada 1973). D'autres études ont aussi rapporté que

l'augmentation de la température du sol après une perturbation (Frey et al. 2003) favorisait la dégradation de l'auxine (Schier et al. 1985) et la production de la cytokinine au niveau des racines (Hungerford 1988) et stimule l'initiation des drageons. La régénération par drageonnement crée des peuplements où des arbres sont interconnectés par leur racine parentale (DesRochers et Lieffers 2001b, Jelínková et al. 2009). Par la suite lorsque des racines des arbres distincts se rencontrent, il est également possible qu'elles fusionnent et forment des greffes racinaires (Graham et Bormann 1966, Basnet et al. 1993, Mudge et al. 2009).

En combinant la reproduction sexuée et la multiplication végétative dans un peuplement naturel, la proportion des arbres issus de la germination par graines *versus* ceux provenant du drageonnement peut être variable selon les sites et les conditions climatiques locales. En effet, l'excavation de petits rejets le long des affluents de la rivière Oldman, a montré que 52 % des rejets étaient des semis, 30 % des rejets de racines, et 18 % des branches enfouies (Rood et al. 1994). La régénération par graines du peuplier baumier peut être limitée par certaines conditions abiotiques sévères (Barnes 1985, Mahoney et Rood 1998). En effet, les graines ne rencontrent pas toujours les conditions idéales, telles qu'un niveau d'humidité suffisant pour une bonne germination. Par ailleurs, l'épaisseur de la litière et la taille des plantes du sous-bois peuvent aussi influencer le recrutement par graine de peuplier baumier. Le développement spatial après feu des populations clonales de peuplier baumier présente une distribution dont la forme dépend de l'âge de la formation (Comtois et Payette 1987, Brodie et al. 1995). En effet, l'un des aspects les plus constants de la structure des formations clonales est leur développement sous forme de zones concentriques d'expansion, caractérisées par la présence des nouveaux drageons à la périphérie du clone, là où la lumière et la chaleur nécessaire au drageonnement est disponible et la compétition avec les tiges les plus âgées est moindre (Brodie et al. 1995).

L'organisation génétique des populations clonales de peuplier baumier du Nouveau Québec déterminée par électrophorèse à partir des caractéristiques morphologiques et enzymatiques foliaires suggèrent la présence d'un flux génique important entre peupleraies (Comtois et al. 1989). En effet, l'analyse isoenzymatique dans les populations de peuplier baumier, révèle très peu de différenciation génétique entre les populations en ce qui concerne les systèmes d'enzymes observés. L'hétérozygoté moyenne variait peu d'une population à une autre (Farmer et al. 1988). Récemment, des études sur la diversité des nucléotides du peuplier baumier ont montré l'existence de deux grands dèmes situées respectivement dans les limites nord et sud de l'aire de répartition de l'espèce en Amérique du Nord (Breen et al. 2009).

1.3.3 Effets physiologiques des liens racinaires entre les arbres

Le mode de régénération par drageonnement permet aux arbres d'être interconnectés entre eux par la racine parentale (DesRochers et Lieffers, 2001b, Jelinkova et al. 2009) ou plus tard par des greffes racinaires, lesquelles résultent de l'union morphologique du cambium, xylème et phloème des racines d'arbres distincts (Graham et Bormann 1966). Ce réseau d'arbres interconnectés peut se partager des ressources telles que l'eau (Stone et Stone 1975), des sucres (Fraser et al. 2006), des minéraux (Horton 1969). Par exemple, il a été démontré qu'en injectant un colorant dans l'aubier d'un tronc par carottage, ce dernier peut passer dans le bois d'arbres greffés par les racines (Bormann et Graham 1959). En s'échangeant des substances, les arbres interconnectés partagent les processus physiologiques liés à leur nutrition, à la constitution des réserves et au transfert des ressources entre les arbres membres du réseau. Ce système racinaire communautaire pourrait être un avantage lorsque les conditions d'alimentation en eau et en minéraux sont difficiles. Ainsi, on s'attend à ce qu'un individu en situation de stress ou de carence puisse, grâce aux liens racinaires, profiter d'une plus grande capacité d'absorption grâce aux individus voisins. Lors d'une sécheresse, par exemple, l'eau pourra être pompée par les arbres en bordure de

rivière et être acheminée via les liens racinaires aux sujets les plus éloignés. Dans d'autres cas, les liens racinaires pourraient aussi être une voie de propagation de maladies, des spores de champignons dans les peuplements (Hessburg et Hansen 1986) ou des phytocides (Eis 1972).

1.3.4 Effets du stress hydrique sur la surface foliaire et les échanges gazeux

Le stress hydrique chez une plante peut être défini comme une baisse de la quantité d'eau nécessaire pour la croissance par rapport à la quantité d'eau disponible. La contrainte hydrique se manifeste au niveau de la plante par la combinaison de la restriction de la disponibilité en eau du sol et l'augmentation de la demande évaporative (Kiani 2007). Le manque d'eau en quantité suffisante et accessible aux racines provoque chez la plante une baisse du potentiel hydrique des cellules. Cette baisse du potentiel hydrique conduit à une réduction de la turgescence (Bouchabke et al. 2006), entraînant la diminution de la vitesse de croissance cellulaire et celle de la taille des cellules, ce qui entraîne par conséquent une réduction de la surface foliaire (Penuelas et al. 1992). Il a été démontré que lors du déficit hydrique, la fermeture des stomates provoque une diminution de la diffusion du CO₂ et une réduction de la photosynthèse (Tardieu et Simoneau 1998, Flexas et Medrano 2002). Toutefois, il est probable qu'en facilitant le transport de l'eau entre les arbres interconnectés, les liens racinaires pourraient influencer les taux d'échanges gazeux (Baret et DesRochers 2011), le potentiel hydrique foliaire et la surface foliaire des arbres stressés mais connectés aux arbres bien arrosés (c'est-à-dire ayant une grande disponibilité en eau). Ainsi, on pourrait s'attendre à ce que les arbres stressés mais connectés à des arbres voisins arrosés aient les taux d'échanges gazeux, *a priori*, similaires à ceux des arbres voisins bien arrosés auxquels ils seraient connectés. Ce transfert d'eau entre arbres connectés contribuerait à atténuer l'effet du stress hydrique chez les arbres

stressés mais connectés à des voisins arrosés comparé aux individus stressés non connectés.

1.3.5 Discrimination isotopique du carbone en situation de stress hydrique

Le carbone possède plusieurs formes d'isotopes stables. Par exemple, le gaz carbonique atmosphérique contient environ 1,11% du carbone sous forme d'isotope lourd ^{13}C et 98,89% de carbone léger ^{12}C (O'Leary 1988, Ciais et al. 1995). La composition isotopique δ (‰) d'un composé est calculée par rapport à un standard prédéfini (Pee Dee Belemnite:PDB) par la relation: $\delta\text{C}(\text{‰}) = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$ (Craig 1957). Certaines plantes ont tendance à discriminer l'un ou l'autre des isotopes. Farquhar et al (1989) ont rapporté que chez les plantes en C3 (comme le peuplier baumier) la discrimination des deux isotopes ^{12}C et ^{13}C dépend des contraintes à la diffusion du CO_2 de l'extérieur vers l'intérieur des feuilles. Ainsi, pour une plante soumise à un stress hydrique, la discrimination isotopique du carbone est la mesure du ratio entre les isotopes stables du carbone ($^{13}\text{C}/^{12}\text{C}$) dans le tissu végétal par rapport au ratio des mêmes isotopes dans l'air qui entoure la plante. Au cours de la photosynthèse, lorsque les stomates sont ouverts, les plantes tendent à utiliser de manière préférentielle l'isotope léger (^{12}C) par rapport à l'isotope lourd du carbone (^{13}C) (O'Leary 1981). Pour une plante bien hydratée, le ratio $^{13}\text{C}/^{12}\text{C}$ est réduit dans la matière sèche de la plante par rapport au ratio présent dans l'air ambiant (O'Leary 1988). En revanche, pour une plante stressée hydriquement, la contrainte à la diffusion du CO_2 résultant de la fermeture des stomates et le maintien de l'activité photosynthétique entraînent un enrichissement de la matière organique du végétal en ^{13}C (Farquhar et al. 1989). Cependant, il est probable, que le transfert d'eau au travers les liens racinaires entre les arbres interconnectés pourrait permettre aux arbres stressés mais connectés à des arbres bien hydratés, d'avoir des stomates ouverts

facilitant la diffusion du CO₂ et de réduire la fixation de l'isotope lourd du carbone (¹³C).

1.3.6 La livrée des forêts et ses impacts sur le peuplier baumier

Originaire de l'Amérique du Nord, la livrée des forêts, (*Malacosoma disstria* Hubner), est un insecte défoliateur qui attaque de manière préférentielle le peuplier faux-tremble (hôte principal) et d'autres essences feuillues comme le peuplier baumier et le bouleau blanc (*Betula papyrifera* Marsh.) (Witter 1979, Ives et Wong 1988). La livrée des forêts est responsable d'importantes perturbations ayant affecté au cours des dernières décennies plus de 81 millions d'hectares au Canada (Simpson et Coy 1999). Caractérisées par une période récurrente de 9 à 13 ans (Cooke et Lorenzetti 2006), les épidémies de la livrée des forêts durent de 1 à 6 années (Hildahl et Campbell 1975, Ives et Wong 1988). Les dégâts peuvent remettre en cause le développement des arbres, voire compromettre l'avenir des peuplements. En effet, les impacts des épidémies de livrée des forêts varient fortement avec l'intensité (sévérité et durée) des défoliations. Les défoliations sévères, qui correspondent à une perte totale de la biomasse aérienne, ont pour conséquence une réduction significative de la croissance radiale ou encore un retard de croissance des arbres affectés (Hildahl et Reeks 1960, Hildahl et Campbell 1975, Hogg et Schwarz 1999) avec la formation de cernes pâles caractéristiques (Hogg et al. 2002a, Sutton et Tardif 2005). Ces défoliations peuvent aussi entraîner une diminution de la croissance en hauteur des arbres (Reichenbacker et al. 1996). Pour certains auteurs, la défoliation, en entraînant un affaiblissement des arbres, peut conduire à la mort des arbres défoliés sous l'effet combiné de divers facteurs prédisposant (gel, dégel, sécheresse, âge, pathogènes ou chablis) (Candau et al. 2002, Hogg et al. 2002b, Brandt et al. 2003). Toutefois, il est probable que le partage et le transfert de ressources (eau, nutriments, sucres) (Bormann 1966, Stone 1974, Fraser et al. 2006) par les racines entre arbres défoliés

connectés à des arbres non défoliés pourrait influencer la réduction de la croissance ou retarder la mortalité des arbres défoliés. Puisque les systèmes racinaires communautaires des espèces clonales constituent des voies de transport de signaux systémiques d'induction de la défense (Gomez et Stuefer 2006, Gomez et al. 2008), les premiers arbres défoliés peuvent aussi partager des signaux chimiques avec les arbres voisins, membres du réseau, pour les avertir de l'imminence d'attaques d'insectes herbivores. Le partage de signaux chimiques par l'intermédiaire des liens racinaires constitue un système d'alerte précoce permettant aux arbres de déclencher la production des composés secondaires de défense (Gomez et Stuefer 2006). Ces composés de défense ont la capacité d'inhiber la croissance et le développement des insectes, ou servent des signaux émis dans l'air pour attirer leurs prédateurs ou parasites (Havill et Raffa 2000, Jelínková et al. 2012) permettant de réduire les populations d'insectes. D'autres études ont aussi montré que l'intensité de la défoliation variait avec les géotypes. En effet, la concentration de glycoside phénolique dans les feuilles de certains clones de peuplier serait un facteur important qui détermine la préférence ou la performance d'insectes défoliateurs (Lindroth et al. 2001, Osier et Lindroth 2001, Jones et DeByle 1985). Ainsi, il est possible que la sensibilité à la défoliation puisse être due à la variation d'origine génétique en phénolique glycoside contenu dans les feuilles.

Devant la diversité des impacts des épidémies de la livrée des forêts sur la dynamique forestière et le rôle des liens racinaires dans le transfert des ressources et les manifestations qu'elles impliquent, on en vient à la question à savoir en quoi les liens racinaires pourraient contribuer à réduire ces effets néfastes dans un environnement forestier changeant affecté par des épidémies d'insectes. L'un des objectifs de cette thèse était d'évaluer l'influence des liens racinaires entre les arbres interconnectés sur la réponse en croissance à une épidémie de livrée des forêts.

1.4 Objectifs et hypothèses de la thèse

L'objectif général de cette thèse était de déterminer l'origine des peuplements forestiers de peuplier baumier, de caractériser leur structure clonale et de mettre en évidence l'influence des liens racinaires entre les arbres dans la croissance et la physiologie des peuplements.

Le deuxième chapitre de cette thèse a permis de caractériser l'architecture du système racinaire et d'identifier la présence de liens racinaires entre les arbres (racines parentales et greffes racinaires) en vue de déterminer l'origine des peuplements de peuplier baumier de l'ouest et de l'est canadien. L'hypothèse posée était qu'en raison d'une pluviométrie plus abondante, l'établissement par graine serait plus fréquent au Québec qu'en Alberta. Nous posons également l'hypothèse que les arbres issus du drageonnement, de façon similaire au peuplier faux-tremble, seraient caractérisés par la présence de racines parentales (i.e. plus vieilles que les arbres) reliant les arbres les uns aux autres. Les ramets matures conserveraient ainsi des liens racinaires avec les ramets voisins par l'intermédiaire des racines parentales ainsi que par la formation de greffes racinaires.

Le troisième chapitre visait à démontrer qu'un arbre pouvait influencer la physiologie d'un autre arbre à l'aide d'un lien racinaire. La réponse des drageons face au stress hydrique a été mesurée selon que leur voisin était connecté ou non et arrosé ou non. Les hypothèses posées étaient que l'application d'un stress hydrique affecterait les échanges gazeux et la croissance des ramets, toutefois les ramets stressés mais reliés à des ramets arrosés par un lien racinaire seraient moins affectés par le stress que les ramets non connectés. Pour tester cette hypothèse, une expérimentation en serre a été conduite sur 32 paires de drageons dont la racine parentale a été conservée ou

sectionnée. Ces drageons ont été soumis à différents niveaux d'arrosage afin d'induire un stress hydrique chez certains drageons.

Le quatrième chapitre avait pour objectif d'évaluer l'impact des liens racinaires sur la croissance radiale du peuplier baumier face aux variations climatiques et aux épidémies d'insectes, en particulier lors de l'infestation par la livrée des forêts de 2000 à 2002 et des sécheresses estivales. Notre hypothèse était que les liens racinaires (racines parentales et greffes) permettraient aux arbres interconnectés de mieux résister aux extrêmes climatiques et à l'épidémie d'insectes. La croissance en diamètre des arbres connectés *vs* non connectés a été étudiée par dendrochronologie en lien avec les précipitations et la température et la dernière épidémie de livrée des forêts.

Enfin, le cinquième chapitre présente les principales conclusions des différentes études réalisées. Après avoir évoqué les résultats marquants obtenus sur les mécanismes de reproduction, le système racinaire et l'influence des liens racinaires, nous aborderons les applications de ces études dans les stratégies d'aménagement, les limites de cette thèse et les perspectives de recherches.

1.5 Matériel et méthodes

1.5.1 Origine des peuplements naturels et système racinaire

Pour caractériser le système racinaire du peuplier baumier, six peuplements naturels (trois au Québec et trois en Alberta,) ont été excavés. Les placettes, d'une superficie d'environ 50 m² chacune (soit 7m x 7m), contenant au moins 10 arbres par site, ont été installées dans des peuplements matures âgés de plus de 40 ans permettant d'avoir suffisamment de cernes de croissance pour la dendrochronologie. Les sites étaient

situés à proximité d'un point d'eau permettant de faire l'excavation des racines à l'eau. L'excavation a été réalisée à l'aide d'une pompe à feu (Wajax) jusqu'à l'exposition du système racinaire (30 à 60 cm de profondeur environ). L'architecture et la disposition du système racinaire de chaque arbre ont été ensuite cartographiées, et des échantillons de racines principales (> 2 cm de diamètre) et des greffes ont été prélevés. Les liens racinaires entre les arbres, lorsqu'ils existaient, ont été identifiés, cartographiés et leur origine (racine parentale ou greffe) a été déterminée au laboratoire grâce à l'interdatation des rondelles prélevées à la base des tiges par analyses dendrochronologiques. Ainsi, dans le cas des arbres issus du drageonnement, on s'attend à avoir un âge racinaire plus grand que celui de la tige. Pour les arbres provenant de graines, on s'attendait à avoir un âge racinaire identique ou plus jeune à celui de la tige. Les greffes racinaires ont été séchées, découpées et sablées afin de vérifier qu'il s'agissait bien d'une vraie greffe (fusion du cambium des deux racines).

1.5.2 Intégration physiologique des ramets connectés

Le matériel végétal utilisé était constitué de paires de ramets de peuplier baumier obtenus par propagation en conditions contrôlées. Des segments de petites racines de diamètre ≤ 2 cm et de longueur d'environ 30-40 cm (Fig. 1.2a) ont été récoltés dans un peuplement naturel près d'Amos (Quebec) Canada ($48^{\circ}36'N$, $78^{\circ}04'W$). Ces segments de racines qui pourraient appartenir ou non à un même clone, ont été plantés en serres dans des bacs contenant du substrat de culture Pro-Mix, un mélange à base de tourbe de sphaigne, sable et vermiculite (1:1:1) (Fig. 1.2b) et régulièrement arrosés 2 à 3 fois par semaine. Après 4 à 6 semaines de drageonnement (Fig. 1.2c), des segments de racines portant 2 drageons distants l'un de l'autre d'environ 25 à 30 cm étaient prélevés et transplantés dans des pots distincts mais encore reliés par la racine parentale (Fig. 1.2d). Après environ 5 mois de développement et lorsque les

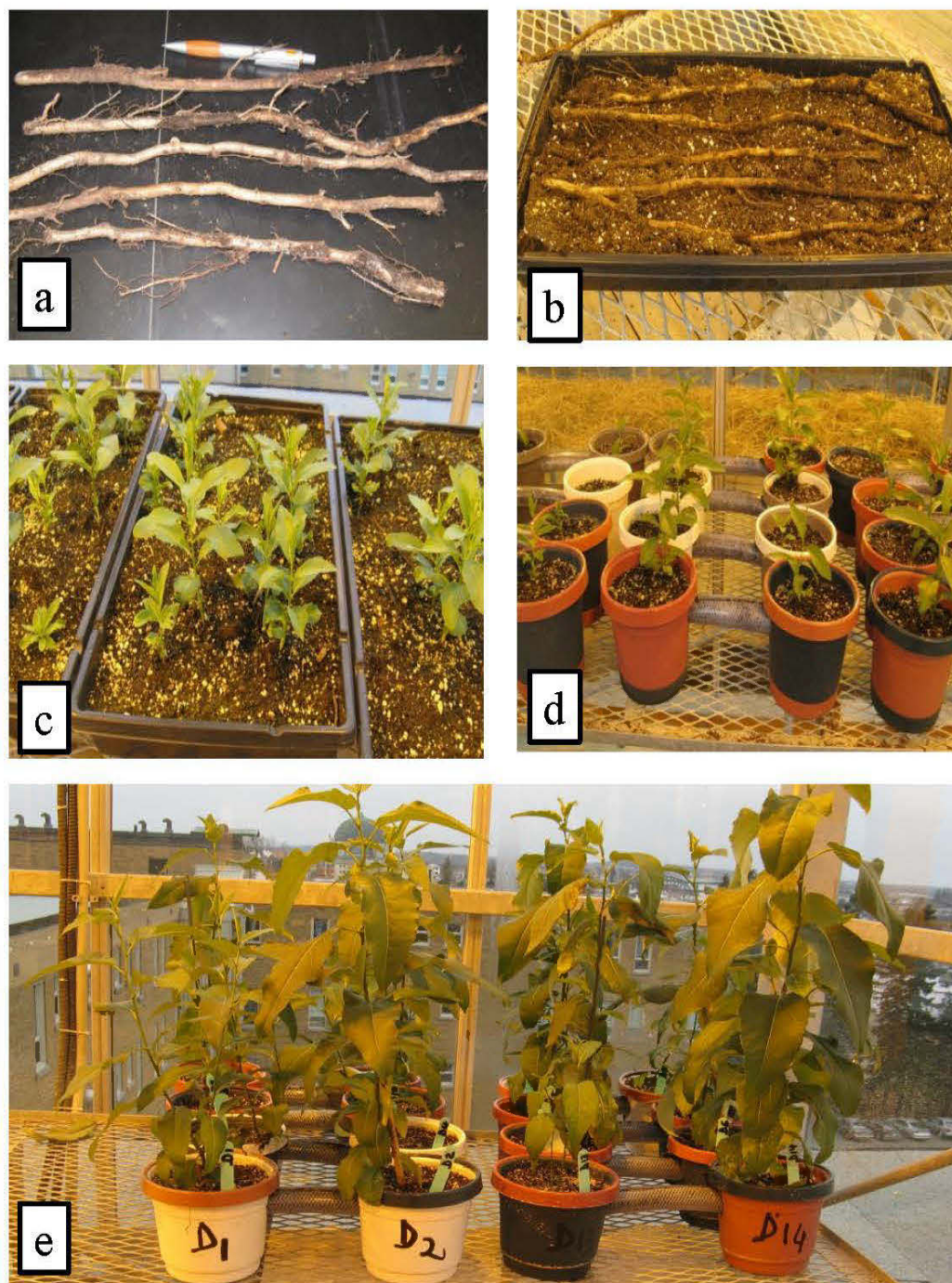


Figure 1.2: Photos des différentes étapes du processus de propagation de ramets interconnectés montrant les racines parentales (a); racines parentales plantées dans un bac contenant du substrat (b); jeunes drageons de 4 à 6 semaines (c); paires de ramets transférés dans 2 pots distincts (d) et paires de ramets d'environ 5 mois (e).

ramets ont atteint au moins 30 cm en hauteur (Fig. 1.2e), des déficits hydriques ont été appliqués aux ramets reliés entre eux ou non par la racine parentale. Les paramètres mesurés étaient les échanges gazeux (conductance stomatique, photosynthèse nette) le potentiel hydrique foliaire, la surface foliaire spécifique, la biomasse et la composition isotopique en carbone des feuilles formées après l'application du stress hydrique.

1.5.3 Impact des liens racinaires sur la croissance radiale

Pour évaluer l'influence des liens racinaires sur la croissance radiale du peuplier baumier, des rondelles de bois prélevées à la base (0 m) et à hauteur de poitrine (1,3 m) de 43 arbres issus des 3 sites excavés au Québec (Canada), ont été séchées et sablées finement pour mesurer la largeur des cernes de croissance des troncs. Ces rondelles ont été scannées (scanneur EPSON GT 15,000) à une résolution de 1200 dpi et la largeur des cernes a été mesurée par le logiciel CDendro et CooRecorder (program package software v7.7 Cybis Elektronik & Data AB in Saltsjöbaden, Sweden). Les chronologies de croissance des arbres connectés ont été comparées avec celles des arbres non connectés afin d'évaluer l'incidence des liens racinaires sur la croissance radiale en portant une attention particulière aux années difficiles ou anormales «pointer years» (années de très faible croissance, années d'épidémie de livrée des forêts).

CHAPITRE II

THE CLONAL ROOT SYSTEM OF BALSAM POPLAR IN UPLAND SITES OF QUEBEC AND ALBERTA¹

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2.1 Abstract

In addition to sexual reproduction, balsam poplar stands can regenerate clonally by root suckering. The origin of stands will in turn affect their genetic structure and root system architecture, which are poorly understood for upland forest stands. Three stands were hydraulically excavated in Quebec (moist) and Alberta (dry) to determine the origin of trees and to characterize root systems with respect to presence of parental roots and root grafts connections. Clones were identified using single nucleotide polymorphism (SNPs), and all stems, roots and root grafts were aged using dendrochronology techniques. All 82 excavated trees were of sucker origin and 4 out of the 6 stands contained a single clone. Parental root connections were found between 22% and 25% of excavated trees, and 53% and 48% of trees were linked with a root graft between trees of the same or different clones, in Alberta and Quebec respectively. Mean distance between trees connected by parental roots was significantly lower than the distance between unconnected trees ($0.47 \text{ m} \pm 0.25 \text{ SE}$ vs $3.14 \text{ m} \pm 0.15 \text{ SE}$ and $1.55 \text{ m} \pm 0.27 \text{ SE}$ vs $4.25 \text{ m} \pm 0.13 \text{ SE}$) in Alberta and in Quebec. The analysis showed that sites AB3 and QC3 were composed of five and three different clones, respectively. In the remaining sites and within each stand, all excavated stems belonged to the same clone. The excavations also revealed many dead stumps with live roots, maintained through root connections with live trees. These results suggest that maintaining an extensive root system through root connections increases the chances of a clone surviving when the above ground tree is dead, and may also enhance the resilience of balsam poplar stands after disturbance.

Keywords: Asexual reproduction; balsam poplar; clonal diversity; communal root system; root connection, root suckering; SNP (single nucleotide polymorphism).

2.2 Résumé

La régénération naturelle du peuplier baumier a été étudiée en Alberta et au Québec, deux provinces du Canada ayant des climats similaires avec différents régimes de précipitations. Six placettes de 50-60 m² (trois au Québec et trois en Alberta) ont été hydrauliquement excavées afin de déterminer l'origine des arbres (semis *vs* drageons), et de caractériser la structure du système racinaire par rapport à la présence de racines parentales et de greffes racinaires. Tous les 82 arbres excavés étaient issus du drageonnement étant donné que des racines plus vieilles (racines parentales) que les arbres ont été identifiées dans le système racinaire de tous les arbres excavés. Au total 22% et 25% d'arbres excavés étaient connectés par des racines parentales, alors que 53% et 48% des arbres étaient connectés par des greffes racinaires, respectivement en Alberta et au Québec. Ces résultats ont montré la persistance des racines parentales chez les arbres issus du drageonnement, lesquelles peuvent se développer et intégrer l'ensemble du système racinaire. La distance moyenne entre les arbres reliés par des racines parentales était significativement plus faible que celle entre les arbres non connectés (0,47 m ± 0,25 SE *vs* 3,14 m ± 0,15 SE et 1,55 m ± 0,27 SE *vs* 4,25 m ± 0,13 SE) en Alberta et au Québec. Les sites AB3 et QC3 étaient composés respectivement de cinq et trois clones. Alors que les quatre autres sites échantillonnés n'étaient formés que d'un seul clone. L'excavation a également révélé la présence de nombreuses souches mortes connectées aux arbres vivants par des liens racinaires. Ces résultats suggèrent que le système racinaire extensif et les liens entre les arbres peuvent augmenter les chances de survie du patrimoine génétique d'un arbre mort, mais dont les racines étaient greffées à des racines d'autres arbres, et améliorer la résilience des peuplements après perturbation.

Mots clés : Drageonnement, peuplier baumier, diversité clonale, système racinaire communal, lien racinaire, simple nucléotide polymorphique (SNP).

2.3 Introduction

Clonal growth directly affects the distribution of phenotypic and genetic variation within and among populations (Eckert 1999). Knowledge of regeneration dynamics of a clonal species is thus essential to understand community structure and ecosystem functioning (Wilsey 2002). Although recruitment from seed is important for many species and may be favored under certain environmental conditions (Kanno and Seiwa 2004), many forest plants are capable of asexual propagation. Balsam poplar (*Populus balsamifera* L.) is distributed throughout North America and is mostly found in riparian mixed wood forests and on moister upland sites (Rood et al 1994). In addition to sexual reproduction, balsam poplar also propagates clonally, the extent of which is poorly understood (Gom and Rood 1999). Vegetative regeneration can occur via root suckering, adventitious shoots emerging from shallow horizontal roots from established, cut or burned trees, stumps and from buried excised branch fragments (Zasada et al. 1981, Shaw 1991, Wijesinghe and Hutchings 1997). However, its regeneration dynamics, including root system architecture, size of parental roots at the time of suckering as well as root grafting occurrence are not documented for upland sites within the boreal forest. Root suckering is generally thought to be less important in balsam poplar following natural disturbance or harvesting when compared to the abundant root suckering observed in aspen (*Populus tremuloides* Michx.; Peterson and Peterson 1992) as reported by Kemperman and Barnes (1976). Several studies along rivers in Alberta (Canada) have shown that saplings in riparian habitat originate more commonly from seedling establishment, whereas those located further away from the river edge are often of sucker origin (Rood et al. 1994, Kalischuk et al. 2000). Colonization by root suckers is thus believed to be more important on dry sites where the probability of seedling recruitment is low (Krasny et al. 1988). Suckering can lead to the formation of extensive clones (Edwards and Dunwiddie 1985) in which suckers may be

interconnected by parental roots (DesRochers and Lieffers 2001b). Such diverse mechanisms of regeneration (sexual and asexual) of the species after disturbance or clear cutting potentially have strong effects on the genetic structure and diversity of the species. This genetic diversity appears necessary to ensure resilience and adaptation of balsam poplar under current and projected climate change (Eriksson et al. 1996).

The extent of root suckering with respect to sexual reproduction in balsam poplar forest settings (non-riparian) is currently unknown. Moreover, the occurrence of root grafts or the persistence of parental root connections between trees is unknown in balsam poplar. It has been shown in aspen that the original parental root connections remain alive throughout the life of the ramets, and that the level of interconnection between trees can even increase with the formation of many root grafts (DesRochers and Lieffers 2001b). More recently, Jelinkova et al. (2009) showed how natural root grafting in aspen produced physiologically integrated clones, with many root grafts between trees of the same or different clones. Seed regeneration should normally increase genetic diversity of populations (Soane and Watkinson 1979), which in turn is influenced by seed characteristics and dispersal capacity. Balsam poplar seeds are characterized by wide-range dispersion, short-term viability, moisture and mineral soil exposure dependence for germination and early survival (Moss 1938, Krasny et al. 1988, Hosie 1990). Seed regeneration may therefore be related to precipitation patterns, the more abundant rain favoring seed regeneration over sucker regeneration. Regeneration mechanisms are thus possibly different between western (Alberta) and eastern (Quebec) Canada, with approximately double the amount of precipitations in the east (889 mm), where seedling recruitment would be expected to be greater than in the west (453 mm). An improved understanding of the regeneration mechanisms of the species can help sustainably manage forests, by contributing to selection strategies of parent material for tree improvement programs or guiding government policy on clonal deployment on the landscape in order to mimic natural scenarios.

The goals of this study were to: 1) investigate natural regeneration patterns, by root suckering *versus* seedlings of balsam poplar; 2) characterize the architecture of the root system (presence or absence of root connections between trees) in naturally-established balsam poplar stands on upland sites; and 3) compare results obtained for each of both regions that have similar climates but contrasting precipitation levels. Because balsam poplar seeds do not exhibit dormancy and germination occurs immediately on moist mineral sites (Zasada and Densmore 1980), we hypothesized that: 1) there would be more seedling recruitment in Quebec than in semi-arid Alberta; and 2) that similarly as for aspen, most ramets of a given clone that have regenerated from the same parental root system would remain interconnected through mature stages of stand development.

2.4 Materials and methods

2.4.1 Study areas

Six balsam poplar stands on mesic upland sites were selected in two provinces (regions) of Canada (three in Quebec and three in Alberta). In each region, typical monospecific balsam poplar stands (>75% of stems) of natural origin established after stand-replacing fires were selected adjacent to a gentle slope with a water supply nearby for hydraulic excavation of root systems. All sites within a region were at least 1 km away from each other.

Two of the Quebec sampling sites (QC1:48°29'N, 79°24'W, 287 m a.s.l. and QC2: 48°24'N, 79°24'W 304 m a.s.l.) were located near the Lake Duparquet Research and Teaching Forest (48°28'N, 79°17'W) and a third site (QC3: 48°32'N, 78°08'W, 305 m a.s.l.) was located near Amos (48°36'N, 78°04'W) in the Abitibi region of northwestern Quebec. This region is part of the Northern Clay Belt of Quebec and

Ontario, which resulted from the maximum extension of the postglacial Lakes Barlow and Ojibway (Vincent and Hardy 1977). The nearest meteorological station to two study sites (QC1, QC2) is at La Sarre, approximately 42 km to the north, while that of the third site (QC3) is at Val d'Or around 67 km to the south. The average climate for the three decades 1971-2000 showed a mean annual temperature of 0.7°C and mean total annual precipitation of 889 mm, with 643 mm as rainfall during the growing season (Environment Canada 2012). The mean frost-free period is 64 days but freezing can occur at any time of the year (Environment Canada 2012). The average growing season with temperatures ranges from 13.2°C to 15.9°C, varies between 153 and 177 days. In the lower areas, lacustrine clay soils derived from the postglacial Lakes Barlow and Ojibway are the primary surface deposits; tills and sandy fluvio-glacial soils dominate the higher ground (Vincent and Hardy 1977). This study region is located at the southern limit of the boreal forest, where most stands originate from natural forest fires and are dominated by an association of aspen and balsam poplar, *Abies balsamea* (L) Mill. *Picea mariana* (Mill.) BSP, *Betula papyrifera* (Marsh) and *Picea glauca* (Moench) Voss.

The three sampling sites in Alberta were located near the town of Athabasca (54°49'N, 113°32'W), 152 km north of the city of Edmonton. Sites AB1 (55°11'N, 113°01'W, 590 m a.s.l.), AB2 (55°11'N, 113°02'W, 606 m a.s.l.) and AB3 (55°13'N, 113°03'W, 616 m a.s.l.) were selected based on the same criteria as for the Quebec sites. This region belongs to the central dry mixed wood subregion of Alberta (Beckingham and Archibald 1996). The closest meteorological station of this study area is at Calling Lake approximately 15 km to the north. Mean annual temperature is 0.9°C and mean annual precipitation is 453 mm, with an average of 349 mm occurring as rainfall during the growing season (Environment Canada 2012). The average growing season with temperatures greater than 10°C is approximately 170 days. Typical mineral soils in the areas are dark brown or black Chernozems developed under mixed deciduous vegetation. The three study sites had similar

vegetation and were characterized as aspen and balsam poplar co-dominated on moderately well-drained mesic sites (Beckingham and Archibald 1996).

2.4.2 Sampling

Similar to Tarroux and DesRochers (2011), we used a high-pressure water spray from a forest fire pump (Mark III, WAJAX Lachine, Quebec) to excavate the root systems. Each excavated plot was 50- 60 m² and included at least 10 trees (Table 2.1). We measured tree diameter at breast height (DBH: 1.3 m) and distance between trees before felling all trees and measuring their heights. Mean soil organic matter thickness was 10.4 cm in Quebec and 26.7 cm in Alberta, which was measured on a diagonal for each plot (one in the center and two in opposite corners) and averaged.

Table 2.1. Characteristics of the six excavated sites of balsam poplar stand in Alberta and in Quebec (standard errors of the mean are given in parentheses).

Sites	Alberta			Quebec		
	AB1	AB2	AB3	QC1	QC2	QC3
Number of excavated trees	14	15	10	11	16	16
Mean DBH (cm)	16.31 (7.36)	15.90 (5.42)	32.52 (7.04)	33.69 (6.53)	29.61 (6.62)	20.91 (6.42)
Mean height (m)	16.03 (2.52)	16.54 (0.30)	19.78 (3.49)	23.28 (2.74)	20.86 (2.57)	18.46 (2.16)
Size of excavated area (m ²)	50	50	60	50	50	50
Soil organic matter (cm)	27.85 (0.56)	25.5 (0.91)	26.65 (1.02)	10.26 (0.25)	10.31 (0.16)	10.61 (0.12)

Depth of excavation to expose all main roots varied from 30 cm to 60 cm (Fig. 2.1a, b). Following excavation, we mapped all trees, stumps, roots, and root connections between trees. For growth-ring analysis, we collected cross sectional disks of all stems at breast height and at ground level (0 m), root connections and root grafts between trees and cross-sectional disks from each root (diameter > 2 cm), parental and non-parental roots (hereafter named “new roots” because they were produced after stand establishment and are thus younger than the trees on which they are growing) where they connect to the stump. Roots are larger closer to the stump, have fewer missing rings and annual growth rings are easier to observe (Krause and Morin 1999). In total, we sampled 43 trees in Quebec and 39 trees in Alberta.

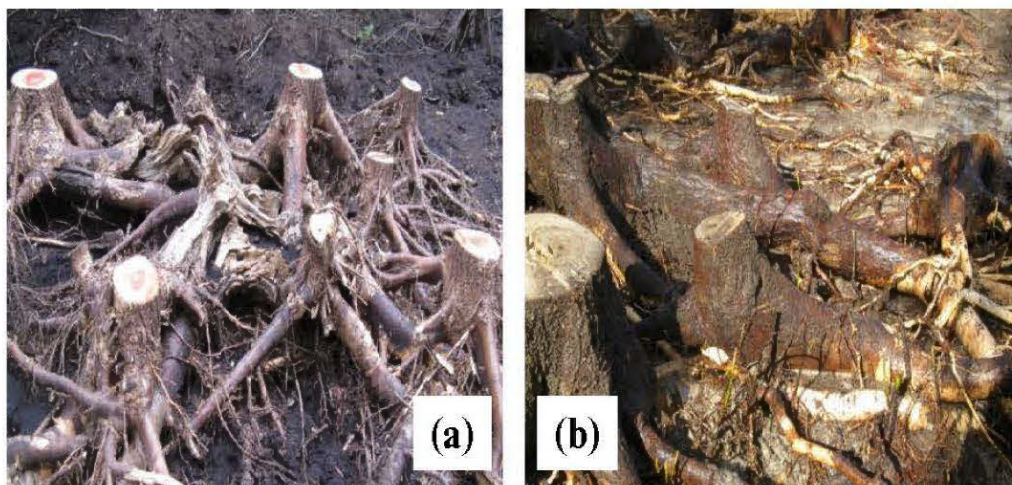


Figure 2.1: Photographs of excavated root systems of balsam poplar showing main root system at site AB1 (a) in Alberta and site QC1 (b) in Quebec, Canada.

2.4.3 Dendrochronological analysis and root measurements

After three months air-drying, all stem and root cross-sectional disks were progressively sanded (80-400 grit paper) to clearly reveal growth rings. We counted growth rings using a binocular microscope. The distinction between root growth rings

was more difficult than in stems because of eccentricity of cross sections and the existence of frequent discontinuous and narrow rings (Fayle 1975, Krause and Eckstein 1994, Tarroux and DesRochers 2011). To improve visibility of small growth rings or to obtain a better ring-to-ring contrast, the surface of root cross-sections were cut with a sharp razor blade and wood cells filled with white chalk (DesRochers and Lieffers 2001b, Tarroux and DesRochers 2011). To accurately determine the age of each individual tree, each ground level cross-section was cross-dated with their corresponding breast height cross-section using pointer years, e.g. narrow or wide rings (Schweingruber 1988). The age of each root cross-section was then compared with the age of its corresponding tree in order to distinguish the origin of each tree, root sucker *vs* seedling. If we found roots older than its corresponding tree, those were labeled as parental roots and trees were determined to be from sucker origin. If all roots were younger than their corresponding tree, we considered those trees to have established from seedling recruitment.

Root grafts were air dried for three months, then cut into cross sectional disks throughout the length of the graft in order to confirm whether or not the grafted roots shared common growth rings (true morphological union). Age of grafts was determined by counting the number of continuous growth rings shared by the grafted roots.

Root diameter (parental and new roots) was measured using a technique similar to that described by Ruel et al. (2003) and Di Iorio et al. (2005). Roots were assumed to be either circular in cross-sectional disks or elliptical with large and short diameters, and diameter of the root disk was calculated as the mean of perpendicular measurements of the longest and the shortest diameters of the root. Root cross-sectional area was calculated as the area of a circle using the corresponding mean diameter. Thus, the ratio size parental root to size new root (P/N) was calculated by

dividing the area of the parental roots by the area of new roots. The ratio of parental to new roots between the two regions (Quebec or Alberta) was tested to assess the importance of the parental root system using root area (size) as a proxy for biomass. In order to characterize the roots from which our sampled trees originated diameter of the parental roots at the time of suckering was determined by measuring its diameter before suckering occurred, i.e. by subtracting from its current size the growth rings that were produced after suckering.

Root diameter as well as distance between trees (connected or not) were tested to better characterize the architecture of the root systems and to verify if trees growing closer to one another had better chances of remaining connected by parental roots or by forming root grafts.

2.4.4 Clone identification

In order to discriminate intraclonal from interclonal root grafting occurrence, leaf samples and cambial tissue from the bark were collected from all trees and living roots from dead stumps. All samples were dried in silica gel and stored at -80 °C. We extracted DNA from dried tissue using the Nucleospin 96 Plant II kit (Macherey-Nagel, Bethlehem, Pennsylvania) and a vacuum manifold according to the manufacturer's protocols with the following modifications: (i) cell lysis using buffers PL2 and PL3 for 2h at 65 °C and (ii) elution with an in-house Tris-HCl 0.01M pH 8.0 buffer. Then, DNA samples were genotyped with an assay of 36 well-characterized SNPs of balsam poplar (average minor allele frequency of 0.26 has been estimated for more than 1000 trees distributed over the entire natural range of the species) (Isabel et al., *submitted*). The SNPs were genotyped with the Sequenom iPlex Gold technology (Agena Bioscience, San Diego, California) at the McGill

University, Génome Québec Innovation Centre using their internal protocols. The variability at these loci was sufficient to assign the 82 trees and 12 dead stumps to clones with high confidence. Pairwise comparisons of the genetic profiles obtained for each individual were manually conducted in order to detect any variations. When no genetic variation was observed among trees from the same stand, they were delineated as a clone.

2.4.5 Statistical analyses

Mixed models linear analyses of variance were performed to investigate size of parent root at the time of suckering, root growth (parental roots and new roots), size ratio of parental roots to new roots (P/N) and distance between trees connected by parental root or by root grafts. All data were tested setting province (Alberta and Quebec) as main factor and sites nested as random variables within province using JMP statistical software (Version 11.2 SAS Institute Inc., Cary, NC, USA). For size of parental roots and root growth analyses, age of trees was used as covariate in order to compensate for age differences between trees across all sites. All data were checked for deviations from normality and for homoscedasticity and logarithm transformations were performed for the diameter of parental roots at the time of suckering, distance between trees connected by parental roots or by root grafts and for ratio (P/N). ANOVAs were followed with post-hoc comparisons, when significant effects occurred and choosing a significance level of $p < 0.05$. Differences in the frequencies of grafted trees from non-grafted and trees connected or not by parental roots were statistically tested using χ^2 analyses.

2.5 Results

2.5.1 Dendrochronological analysis and tree origin

All excavated trees were of sucker origin, since roots older than the stems (parental roots, Fig. 2.1a) were found in the root system of all trees. The age of sampled trees were 86, 87, 44, and 43, 43, 103 in Quebec and Alberta, respectively, and were even-aged i.e. age of trees corresponded to time since fire. The maximum age difference found between parental roots and tree age was 51 and 11 years in Alberta and Quebec, respectively.

At the time of excavation, ratio of the size of parental to new roots (P/N) was significantly greater ($F_{1, 3.3} = 27.8$, $p < 0.001$, Table 2.2) in Alberta than in Quebec (1.15 ± 0.06 vs 0.70 ± 0.06 , Table 2.3). Mean root diameter of parental roots at the time of suckering was also significantly greater ($F_{1, 4.5} = 49.2$, $p < 0.001$, Table 2.2) in Alberta than in Quebec (1.2 vs 0.83 cm, respectively Table 2.3). There was also a significant difference ($F_{1, 1.6} = 54.87$ $p = 0.02$, Table 2.2) in diameter of new roots, with larger roots found in Quebec than in Alberta (17.47 ± 0.4 vs 12.81 ± 0.4).

2.5.2 Clone identification

A total of 94 leaf or bark samples (82 trees and 12 living roots on dead stumps) were successfully genotyped. Intraspecific variation within the study sites was examined with the 36-SNP panel (Isabel et al., *submitted*). Three out of the 36 SNPs were partially informative and one was not informative because they did not vary among the 94 samples, then they were excluded. The clonal structure varied among the 6 sites (Table 2.4). The analysis showed that sites AB3 and QC3 were composed of five and three different clones, respectively (Table 2.4). The minimal number of allelic differences between two clones from the same site were 14 (QC3) and 17 (AB3). In

the remaining sites and within each stand, all excavated stems belonged to the same clone, i.e. with no allelic differences between each other (Table 2.4). All living roots from dead stumps had genotypes corresponding to at least one living tree in the site.

2.5.3 Clonal integration

Parental root connections (Fig. 2.1a) were found between at least two living trees in all stands except in the oldest stand (AB3) where one parental root connection was only found between a living tree and a dead stump. There was a significant difference in the mean distance between trees connected to each other by parental root between Alberta and Quebec ($F_{1, 6.96} = 8.21, p < 0.02$, Table 2.5). The largest root system network observed in an excavated area integrated five living trees in site AB1. In contrast, no parental root connection was found between living trees in site AB3, which had a mean distance between trees of $5.49 \text{ m} \pm 2.27$. In the remaining sites, the mean distance between trees was AB1 = $3.3 \text{ m} \pm 1.6$, AB2 = $3.7 \text{ m} \pm 1.6$ in Alberta and QC1 = $6 \text{ m} \pm 2.8$, QC2 = $5.2 \text{ m} \pm 2.2$ and QC3 = $3.5 \text{ m} \pm 1.7$ in Quebec.

There was a significant interaction between Province x Parental root connections between trees ($F_{1, 545} = 7.37, p < 0.006$, Table 2.5) showing that the distance between trees connected by parental root was significantly lower than the distance between unconnected trees ($0.47 \text{ m} \pm 0.25$ vs $3.14 \text{ m} \pm 0.15$ and $1.55 \text{ m} \pm 0.27$ vs $4.25 \text{ m} \pm 0.13$) in Alberta and in Quebec, respectively. Mean distance between unconnected trees by parental roots was similar in Alberta and Quebec ($3.14 \text{ m} \pm 0.16$ vs $4.25 \text{ m} \pm 0.25$). Overall, 22% and 25% of trees were connected with parental roots respectively in Alberta and in Quebec. There was no significant difference in the frequencies of trees connected by parental root between provinces ($\chi^2 = 0.55, DF = 1, p = 0.4$). Trees were also connected by root grafts (Fig. 2.1b) in all excavated sites and there was no significant difference in mean distance between trees connected by root grafts in

Alberta and in Quebec ($1.38 \text{ m} \pm 0.6$ vs $1.21 \text{ m} \pm 0.4$, $p = 0.51$). Mean distance between trees or between trees and dead stumps with living roots connected by root grafts was significantly lower ($F_{1, 599} = 91.2$, $p < 0.001$, Table 2.5) than the mean distance between trees which were not connected by root grafts ($1.14 \text{ m} \pm 0.17$ vs $3.98 \text{ m} \pm 0.11$).

Table 2.2. Analyses of variance showing sources of variation, degrees of freedom (DF, DFDen), F and Pr values for diameter of parental root, diameter of new root, ratio size of parental root/size of new root (P/N).

Diameter of parental root at the time of suckering				
Source of variation	DF	DFDen	F value	Pr values
Age of tree covariate	1	10.98	19.97	0.001
Provinces (Alberta/Quebec)	1	4.50	49.27	0.001
Diameter of new root at the time of excavation				
Source of variation	DF	DFDen	F value	Pr values
Age of tree covariate	1	2.51	8.50	0.076
Provinces (Alberta/Quebec)	1	1.65	54.87	0.029
Ratio size of parental root/size of new root (P/N)				
Source of variation	DF	DFDen	F value	Pr values
Provinces (Alberta/Quebec)	1	3.38	27.80	0.009

Note: Bold indicates significant effects.

Within the excavated sites, 53% and 48% of trees were linked with a root graft to at least one other living tree or to a dead stump with living roots in Alberta and Quebec, respectively. In site QC3, two trees of the most represented clone in the site (50% of trees belonged to this clone) formed root grafts with trees from different clones (representing 18% interclonal root grafts; Fig.2.1b).

Table 2.3. Mean diameter (cm) of parental and new roots in the excavated balsam poplar stands in Alberta and Quebec (standard errors of the mean are given in parentheses).

Sites	Alberta				Quebec			
	AB1	AB2	AB3	Mean	QC1	QC2	QC3	Mean
Parental roots at the time of suckering (cm)	1.39 (0.53)	1.28 (0.39)	1.02 (0.26)	1.20 (0.03)	0.67 (0.44)	0.7 (0.43)	0.96 (0.55)	0.83 (0.03)
New roots at the time of excavation (cm)	12.85 (0.39)	12.77 (0.39)	12.81 (0.39)	12.81 (0.42)	17.50 (0.41)	17.49 (0.41)	17.41 (0.42)	17.47 (0.44)
Ratio P/N	1.19 (0.05)	1.14 (0.05)	1.11 (0.04)	1.15 (0.06)	0.70 (0.05)	0.68 (0.05)	0.71 (0.05)	0.70 (0.06)

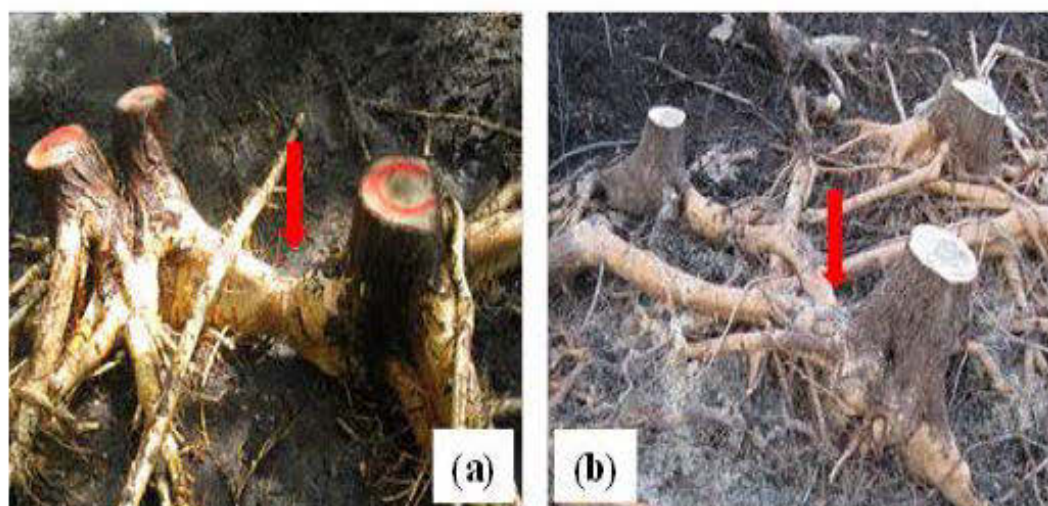


Figure 2.2: Photographs of excavated root systems of balsam poplar showing clonal integration: the arrows indicate parental root connection between trees at site AB2 (a) in Alberta and interclonal root graft between trees at site QC3 (b) in Quebec, Canada.

No interclonal root grafts were found in the multiclonal site of Alberta (AB3). The age of root grafts ranged 7-47 years old. The excavation exposed a total of 12 dead stumps with living roots, which were connected to the roots of living trees, by parental roots or root grafts (Table 2.4). Lastly, 41% and 47% of excavated trees in Alberta and Quebec, respectively, were not connected to other trees within the excavated area by parental roots or root grafts.

Table 2.4. Clones identification, natural root grafting between trees and between trees and living roots from dead stumps in the six sites of balsam poplar in Alberta and Quebec.

Sites	Alberta			Quebec		
	AB1	AB2	AB3	QC1	QC2	QC3
Number of clones (unique genetic profile)	1	1	5	1	1	3
Percentage of interclonal root grafts (%)(Number of grafts)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	18 (2)
Percentage of intraclonal root grafts (%)(Number of grafts)	100 (12)	100 (8)	100 (3)	100 (7)	100 (4)	82 (9)
Percentage of dead stumps uncovered with living roots connected to living trees by root grafts or parental roots (%)/ (Number of dead stumps)	25 (3)	16.6 (2)	8.3 (1)	25 (3)	16.6 (2)	8.3 (1)

Table 2.5. Analyses of variance showing sources of variation, degrees of freedom (DF, DFD), and *F* and *Pr* values for distance between trees connected or not by parental roots and root grafts.

Distance between trees connected or not by parental root				
Source of variation	DF	DFD	<i>F</i> value	<i>Pr</i> values
Provinces (Alberta/Quebec)	1	6.96	8.21	0.024
Parental root connection (PRC)	1	545.2	80.58	<0.001
Provinces*PRC	1	545.2	7.37	0.006
Distance between trees connected or not by root grafts				
Source of variation	DF	DFD	<i>F</i> value	<i>Pr</i> values
Provinces (Alberta/Quebec)	1	6.478	0.48	0.511
Root graft connection (RGC)	1	599.1	91.22	<0.001
Provinces * RGC	1	599.1	0.008	0.924

Note: Bold indicates significant effects

2.6 Discussion

Despite abundant production of airborne seeds by balsam poplar trees (Bessey 1904, Walker et al. 1986, Lee et al. 1991), all sampled trees in this study were from sucker origin, in contrast to subarctic Quebec where a number of balsam poplar stands were found to have originated from seeds (Comtois et al. 1989). This result was unexpected, since although root suckering was a known reproduction mechanism for this species, it was thought to be of lesser importance than for aspen under non-arctic conditions (Peterson and Peterson 1992). Our hypothesis that there would be more

regeneration by seed in Quebec due to more precipitation is thus not verified, at least for these upland sites under boreal forest conditions. Perhaps seed regeneration serves the species more for colonizing new areas, such as exposed mineral soil along road sides (Zasada et al. 1981, Fortin 2008, Laquerre et al. 2009), riparian areas (Rood et al. 1994) or in intensively burnt coniferous forests. Indeed, balsam poplar stands and associated understories do not readily support fire because of their higher moisture content, less flammable leaf chemical content and the fact that they provide less fuel during the fire season (Moretti et al. 2002, Keyser et al. 2005, Proença et al. 2010). Consequently, unburned accumulated organic matter does not create favorable seedbeds for germination (Facelli and Pickett 1991). Therefore, it seems that balsam poplar stands are more likely to regenerate by root suckering after fire while other forest types that burn more intensively may be invaded by seed regeneration. One could also argue that the preponderance of sucker regeneration found in this study is an indication of less intense fires and that greater fire intensity would have favored regeneration by seed. Extensive root suckering is however consistent with previous studies that reported abundant root suckering of the species in upland sites of Alaska following harvesting (Zasada et al. 1981, Edwards and Dunwiddie 1985), and in areas relatively far away from riparian areas (Shaw 1991, Rood et al. 1994). Reports of vegetative regeneration by root suckering by its close relative *Populus trichocarpa* are scarce but have been reported to occur (Roe 1958).

The use of 36 SNP markers allowed us to confirm that 4 out of the 6 studied stands were of clonal origin, since all trees within each site had the same clonal identity. The two other sites (AB3 and QC3), were also of clonal origin but their relatively high level of genotypic diversity is an indication that seed regeneration must have occurred in the past, and that the different genotypes were maintained and expanded vegetatively after stand-replacing disturbances such as forest fires. Similar genotypic diversity has been found in aspen stands of eastern Canada (Namroud et al. 2005). In

the later study, many unique genotypes were found (clones represented by single ramets), and unless the presence of parental roots was verified in the root system of these trees, it is impossible to certify that they were of seedling or sucker origin. We argue that the fact that trees can maintain root systems of dead trees by root grafting between different genotypes (Jelínková et al. 2009, this study) and that they are able to vegetatively regenerate these genotypes after a major disturbance can explain how clonal diversity is maintained over time in the absence of sexual reproduction. Our excavations uncovered many dead stumps with live roots, but these belonged to genotypes already present in the living trees. It does not however exclude the possibility of maintaining genotypes that have disappeared from the canopy and resprouting them after the next stand-replacing disturbance, since root grafting was observed between roots of trees from different genotypes (Table 2.4).

This study provides further evidence that parental roots of trees regenerated from root suckering persist and integrate into the root system of the next generation of trees (DesRochers and Lieffers 2001b). These results somewhat contradict earlier aspen literature which reported that either distal or proximal ends of the parental roots eventually rot away and make trees independent from each other (Gifford 1966). The fact that these old parental roots can maintain roots of dead trees is also evidence that they remain functional over multiple disturbance events. Connected trees by parental root were significantly closer to one another in Alberta than in Quebec. We assume that after suckering, parental roots were enlarged in both acropetal and basipetal directions by the ramets which arose close to each other (Brown 1935), creating parental roots with large diameters. The shorter mean distance between trees in Alberta could thus explain why parental roots on these sites were larger than the new roots on average, while they were slightly smaller at the Quebec sites where trees were further apart (Table 2.3). The relative longer mean distance between trees at the AB3 site (> 5 m) as well as the age of the trees of in semi-arid environments (Gatsuk

et al.1980) could also explain why no parental root connections between living trees were found at this site. However trees were found connected to the roots of dead stumps, suggesting that natural self-thinning in this site reduced the number of living connected trees by increasing distance between residual trees. We presume that large parental root connections have greater probability of dying, due to their large biomass and energy required to maintain them (DesRochers and Lieffers 2001a). The obtained ratio P/N in Alberta indicates that parental roots were larger than the new roots as a whole, which is in accordance with DesRochers and Lieffers (2001b), who reported significant radial growth of parental roots following suckering in aspen from the same region. P/N ratios were smaller in Quebec and the difference could be related to the fact that size of parental roots at the time of suckering was smaller in Quebec compared to Alberta; less energy was drained into maintaining this large parental root network, and trees invested more energy into new roots in Quebec.

Trees were also connected by root grafts in all excavated sites (Table 2.4), indicating that root grafting is a common occurrence in balsam poplar, similarly to what was found in aspen (DesRochers and Lieffers 2001b, Jelínková et al. 2009), in *Pseudotsuga menziesii* (Eis 1972), and in *Pinus banksiana* (Tarroux and DesRochers 2010). It is generally accepted that root graft formation requires firm physical contact and pressure developed as roots increased in size, where intervening bark is broken down and vascular continuity is established (Kozłowski and Cooley 1961, Bormann 1966, Eis 1972). Root grafting therefore, may be facilitated between roots of adjacent trees compared with trees which were more distant from one another. Spatial distribution of trees also explained the frequency of root grafting incidence in other species, with more root grafting occurring between closely located trees (Reynolds and Bloomberg 1982, Tarroux et al. 2010). The percentages of grafted trees in this study (53% in Alberta and 48% in Quebec) were however somewhat lower than in declining aspen stands (60%) (DesRochers and Lieffers 2001b) or in *Pinus strobus* stands (75%) (Bormann 1966) reputed by their capacity to form many root grafts, but

somewhat concurred with observations in aspen stands in Quebec that reported similar levels (48%) of root grafting between trees of the same *vs* different clones (Jelínková et al. 2009). These results however contrast with studies in northern prickly ash (*Xanthoxylum americanum* Mill.), for example, that reported that because of the diffusion of chemical inhibitors, roots from adjacent clones either stopped growth or turned away as soon as they approached roots of different genotypes making interclonal grafting virtually impossible (Reinartz and Popp 1987). The small frequency of interclonal grafts found in this study can easily be explained by the low clonal diversity found in the excavated sites.

Root grafts were initiated relatively late in stand development, when trees were 28 to 41 years old. The fact that root grafts did not form earlier could be explained by little contact between roots of small trees (Tarroux et al. 2010, Tarroux and DesRochers 2011), or by the heavy self-thinning phase in sucker-regenerated stands (Schier 1982) making grafts disappear early if they had occurred between close ramets. Self-thinning as stands mature could also explain the lack of parental connections between trees. As trees grow older and stand mature, natural thinning tends to make trees further apart, and when the parental roots are so large, the energy needed to support them may not be available by the remainder of the clone and portions of the parental root system (including connecting roots) lost with time. Finally, the frequencies of independent trees in this study were probably overestimated, because trees may have been connected to trees outside the excavated plots, with parental roots or grafts.

2.7 Conclusion

This study constitutes the first large-scale excavations done in naturally established upland balsam poplar stands in eastern and western Canada. All excavated trees in

this study were of sucker origin. Most trees were interconnected through root grafts and parental roots connections, except for the oldest site in Alberta where trees were further apart than in the other sites. Root connections were also found to support roots of dead trees. This finding that trees grow on communal root systems should be considered in silvicultural scenarios, rather than consider balsam poplar trees as distinct individuals.

2.8 Acknowledgements

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CHAPITRE III

PHYSIOLOGICAL INTEGRATION OF CONNECTED BALSAM POPLAR RAMETS²

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3.1 Abstract

Clonal integration between ramets can be an ecological advantage of clonal plant species in environments where resources are patchily distributed. We investigated physiological integration among *Populus balsamifera* L. ramets under drought stress in order to demonstrate water sharing between connected ramets. Pairs of connected ramets were grown in separate pots in the greenhouse where half of ramets had the parental root connection severed and half were left intact. Drought stress was applied to one ramet and growth, specific leaf area (SLA), net photosynthesis, stomatal conductance, leaf water potential and carbon isotopic composition ($\delta^{13}\text{C}$) measured after an eight week growing period. Droughted ramets connected to watered ramets were able to maintain high gas exchange activity and water potential, similar to watered ramets. Leaf water potential (Ψ) and SLA results showed that the root connection was more beneficial for proximal compared to distal ramets. The parental root connection also allowed droughted ramets to discriminate more against ^{13}C compared to severed ramets. In conclusion, this study shows compelling evidence of physiological integration of connected *Populus balsamifera* ramets through water sharing.

Keywords: stomatal conductance, net photosynthesis, leaf water potential, $\delta^{13}\text{C}$, drought stress, water translocation, clonal integration.

3.2 Résumé

L'intégration clonale entre ramets peut être un avantage écologique pour les plantes d'espèces clonales dans un environnement où les ressources sont inégalement réparties. Nous avons étudié l'intégration physiologique entre les ramets de peuplier baumier soumis à un stress hydrique afin de démontrer qu'il y avait partage d'eau entre ramets connectés. En conditions contrôlées, des paires de ramets connectés ont été cultivés dans des pots séparés. Après une période de développement, une moitié des paires de ramets avait la racine parentale sectionnée et l'autre moitié avait la racine parentale laissée intacte entre les ramets. Un stress hydrique a été appliqué à l'un des ramets et la photosynthèse nette, la conductance stomatique, le potentiel hydrique foliaire, la croissance, la surface foliaire spécifique (SFS), ainsi que la composition isotopique en carbone ($\delta^{13}\text{C}$) ont été mesurés après huit semaines. Les ramets stressés mais connectés à des ramets arrosés avaient des taux d'échanges gazeux et un potentiel hydrique foliaire similaires à ceux des ramets arrosés. Les résultats du potentiel hydrique foliaire (Ψ) et la SFS ont montré que le lien racinaire était plus bénéfique pour le ramet proximal comparé au ramet situé en position distale sur la racine parentale. La connexion par la racine parentale a également permis aux ramets stressés de discriminer davantage contre le ^{13}C par rapport aux ramets séparés. En conclusion, cette étude a démontré l'intégration physiologique des ramets connectés de peuplier baumier par des échanges d'eau à travers les liens racinaires.

Mots clés: Conductance stomatique, photosynthèse nette, potentiel hydrique foliaire, $\delta^{13}\text{C}$, stress hydrique, transport de l'eau, intégration clonale.

3.3 Introduction

Many clonal plant species can spread or reproduce vegetatively, developing large numbers of interconnected ramets (Alpert and Mooney 1986, Evans 1992, Zhang and He 2009). Ramets can stay interconnected for variable periods of time or even throughout their life span (DesRochers and Lieffers 2001). These extensive connecting structures allow translocation of water, nutrients and carbohydrates through stolons, rhizomes or lateral roots between source and sink ramets (Alpert and Mooney 1986, Stueffer et al. 1996, Wijesinghe and Hutchings 1997). A number of studies have shown that physiological integration was advantageous for clonal plants to exploit patchy resources or to decrease the effects of competition, and therefore improve survival and growth of individual ramets growing in environments where resources were limited (Wijesinghe and Handel 1994, Hutchings 1999, Lötscher 2006). On the other hand, clonal integration may also involve costs such as the maintenance of large connecting structures (DesRochers et al. 2002) or the transmission of pathogens that may efficiently spread over the whole clone (Wennstrom and Ericson 1992, D'Hertefeldt and Putten 1998). The balance between costs and benefits of clonal integration likely depends on the level of integration between ramets and on environmental conditions. Some studies reported that when the costs incurred by supporting a biomass/organ exceeded the benefits under severe stressful habitats, integration could result in lower fitness and clonal plants may cease to support dependent ramets (Hartnett and Bazzaz 1983, Jonsdottir and Callaghan 1990). Other studies showed that clones continue to support dependent ramets despite prolonged stress (Salzman and Parker 1985, Slade and Hutchings 1987). This would be particularly true for large organisms such as trees, where the connections between ramets are large and long-lasting (DesRochers and Lieffers 2001).

While clonal integration has been extensively studied in plant species that can be easily experimentally manipulated (i.e. herbaceous, rhizomatous and stoloniferous) (Lau and Young 1988, Chapman et al. 1992, de Kroon et al. 1996), the extent of resource sharing between connected ramets is not well understood for tree species, which are traditionally considered as distinct individuals. A previous study using aspen (*Populus tremuloides* Michx.) showed that defoliation of a ramet could trigger physiological responses in connected but untouched ramets (Baret and DesRochers 2011). This physiological response seemed to be highly dependent on the water balance between the communal root system and the transpiring surface area (leaves), suggesting that root connections allowed the sharing of water between ramets.

The paucity of experimental studies regarding clonal integration of forest tree species is related to difficulties in obtaining sufficient woody clonal plant material for testing. For species such as those in the *Populus* genus, however, it is relatively easy to obtain connected individuals by propagating them in the greenhouse by root suckering (Maini and Horton 1966, Gifford 1967, Zasada and Schier 1973). In this study we set up a greenhouse experiment to investigate how root connections can affect tree physiology and in particular how trees can share water and alleviate drought stress through root connections. Connected ramets of balsam poplar (*Populus balsamifera* L.) were planted in separate pots while keeping the root connection intact between two ramets. We hypothesized that root connections would allow water transport between ramets and counteract drought stress of a droughted ramet connected to well-watered ramets.

3.4 Materials and methods

In early July 2011, small root segments of ± 2 cm in diameter and about 30-40 cm in length were collected by gentle manual excavation from a natural population of

balsam poplar located near Amos (Quebec) Canada (48°36'N, 78°04'W). The collected root segments, which might or might not belong to the same clone, were stored in soil in a cold room at 2°C and were transferred on July 11th of 2011 into a greenhouse with natural light supplemented with 400 W lamps (High pressure sodium S51, Kavita Canada Inc., Ont., Canada) providing a photosynthetic photon flux density (PPFD) of approximately 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at leaf level. To induce suckering, root segments were planted in large plastic containers (20 x 10 x 6 cm) containing a mixture of peat, vermiculite and sand (1:1:1 by volume), a commercial potting substrate Pro-Mix (Premier Horticulture Ltd, Dorval, Québec, Canada). Containers were watered 2 to 3 times a week as needed. Artificial lighting was set to provide a 16 h lighting period with temperatures set to 15°C during the night and 25°C during the day. The root segments (hereafter named parental roots) produced many suckers (hereafter named ramets) in a period of 4-6 weeks; ramets were then thinned to leave only two ramets per parental root, 25-30 cm away from one another. Any others ramets that emerged later during the experiment from the same parental root were removed quickly with a sharp knife. The two interconnected ramets were then carefully transplanted into two separate plastic pots (2 L in volume) filled with Pro-Mix substrate, leaving the parental root connection intact through a hole made in each pot and covered with a foam tubing and moist Pro-Mix substrate to prevent desiccation and light damage. Holes were sealed with plastic putty to prevent the movement of water in the tube soil along the parental root. Ramets were left to grow for about 20 weeks until they reached at least 30 cm in height. Thirty-two pairs of ramets were used as plant material. The experiment was set-up as a randomized block design with 4 replicates, 2 water levels (Stressed, Watered), 2 positions (Proximal, Distal, *see below*), 2 neighbor ramets water status (n-Stressed, n-Watered) and 2 connection types (Connected or Severed) for a total of N= 64 ramets.

3.4.1 Water treatments

Ramets were individually watered. Soil volumetric water content (SVWC, the fraction of the total volume of soil sample that is occupied by the water contained in the soil, %) was used to establish the Stressed and Watered treatments; SVWC was maintained between 30 and 50% for the Watered treatment, while it was kept between 0 and 20% for the Stressed treatment. Water taken up by the ramets in the Stressed treatment was not replenished until the SVWC reached 2-5% between waterings. SVWC was monitored daily with a soil moisture probe (Field Scout, model TDR 100 with a 12 cm long probe; Spectrum Technologies, Inc., Plainfield, IL, USA). To confirm that there was no water movement between the pots, a test was done with the same treatments as experimental pots but only containing wooden sticks instead of live plants, which showed that there was no water movement between pots.

3.4.2 Connection treatment

Half of the ramet pairs were randomly assigned to remain connected to each other with the parental root left intact (Connected; *see* Fig. 3.1), whereas the other half were disconnected by cutting the parental root connection midway between the two ramets (Severed). After severing the parental root, some ramets quickly died; both ramets were then excluded from the experiment and rapidly replaced by a new pair. The position of each ramet on the parental root was considered in this experiment; suckers establish vascular continuity more strongly with the distal side of the parental root from which they originated (Brown 1935, Baret and DesRochers 2011). The xylem wood tissue from the ramet is continuous with the distal side of the parental root and becomes more enlarged after the suckering event compared to the proximal side, where the xylem wood tissue undergoes a sudden change in direction at the

point of connection with the parental root (*see* Figs. 1-3 from Brown 1935 and Fig. 1 from Baret and DesRochers 2011). We thus anticipated that the position of ramets on the parental root would affect water relations between the connected pairs, or have a larger effect on proximally placed suckers for which the parental root had been severed (Zahner and DeByle 1965). Half of the suckers thus had a proximal position on the parental root, while the other half «neighbor» suckers were placed distally on the parental root.



Figure 3.1: Photograph of connected balsam poplar ramets at the end of the experiment showing intact parental root between ramets and mass of new roots produced by each ramet.

3.4.3 Leaf measurements

The experiment was carried out for 63 days, from December 12th, 2011 to February 13th, 2012 when all 64 plants were measured. Stomatal conductance to water ($\text{mmol m}^{-2} \text{s}^{-1}$) and net photosynthesis ($\mu\text{mol m}^{-2} \text{s}^{-1}$), were measured 7 times on all 64 plants during the course of the experiment using a portable photosynthesis system (CIRAS-2, PP Systems Inc., Amesbury, MA, USA) equipped with a broadleaf chamber (PLC6, PP Systems, Inc., Amesbury, MA, USA). Weekly, from 09:00 am to 03:00 pm, gas exchange measurements were made on a young fully expanded leaf. Leaf temperature during measurements was stable at around 24 °C (ambient temperature), 60% relative humidity and under a vapor pressure deficit between 0.8 and 1.6 kPa. Lighting during measurements was provided by a halogen lamp set at 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at leaf level. Air was supplied to the leaf at a CO₂ concentration of 350 ppm, and flow rate was set at 250 ml min⁻¹.

On days 20, 40 and 60, water potential (Ψ , MPa) of 2 excised leaves per ramet in each treatment was measured using a pressure chamber PMS Model 600 (PMS Instrument Company, Albany, OR, USA). Ψ was determined by measuring xylem sap pressure of the petiole portion of a leaf that was enclosed in a polyethylene bag just before cutting. After cutting, the leaf was immediately introduced into the chamber and subjected to sufficient pressure from a nitrogen cylinder to the leaf to exude xylem sap. Ψ could not be measured as regularly as gas exchange, to avoid excessive defoliation of ramets.

Before drought stress treatments were initiated, the last fully expanded leaf of each ramet was marked in order to distinguish leaves formed before and after drought

treatment began. At the end of the experiment, leaves formed after the start of the drought treatment application were used for the determination of carbon isotopic composition ($\delta^{13}\text{C}$). Leaves were collected and dried in an oven at 70°C until constant mass and then finely ground using a stainless-steel Brinkmann MM2 ball grinder (Brinkmann Instruments Ltd, Mississauga, ON, Canada). $\delta^{13}\text{C}$ values were determined with a Costech ECS 4010 Elemental Combustion System (Costech Analytical Technologies, Inc., Valencia, CA, USA) joined to a continuous flow Finnigan Delta Plus Advantage IRMS (Thermo Finnigan, Bremen, Germany). The isotopic composition of the sample was calculated as:

$$\delta^{13}\text{C} (\text{‰}) = (R_{\text{sample}} - R_{\text{standard}} / R_{\text{standard}}) \times 1000;$$

where R_{sample} and R_{standard} are the ratio of $^{13}\text{C}/^{12}\text{C}$ in the sample and standard, respectively. Grain (BMO), Corn Stover (CS) and Limestone CaCO_3 (NBS) 1575N were used as calibrating standards and red clover a working standard, with carbon isotope compositions of -23.91 , -12.5 , -26.3 and -27.42‰ relative to Pee Dee Belemnite, respectively.

3.4.4 Growth and biomass measurements

Just before the experiment started, the number of leaves, leaf maximum width (w , cm) perpendicular to the main vein, and leaf maximum length (l , cm) from lamina tip to the point where the blade meets the petiole was measured for each ramet to estimate leaf area. Leaf area (LA, cm^2) of all ramets was obtained using the linear relationship: $\text{LA} = 0.6792 (w \times l)$, $R^2 = 0.99$ established for balsam poplar of the same region with non-destructive width and length measurements (Larchevêque et al. 2011). Stem basal diameter and height (cm) were also measured at the beginning and at the end of the experiment. At the end of the experiment, total leaf area of ramets was measured using a LI-COR LI-3100 area meter (LI-COR Biosciences, Lincoln, Nebr., USA) and specific leaf area (SLA, total leaf area/total leaf mass, $\text{cm}^2 \text{mg}^{-1}$)

was calculated. Parental roots and dead leaves were excluded from total biomass weight data. After 8 weeks, all plants were harvested and separated into: leaves, stem, new adventitious roots and parental root. Parental root and new roots were washed clean of soil particles under running water. Plant parts were then oven-dried at 70° C for 48 h or until constant mass and weighed for dry mass determination.

3.4.5 Statistical analyses

Because trees usually need time to acclimate to the modification of their environmental water resources (Marshall and Sagar 1968, Kozlowski and Pallardy 2002), gas exchange data were analyzed using a repeated measures design (proc ANOVAR). Data of the three leaf water potential measurements were averaged (pseudo-replicates) and analysed in a mixed-model of variance (Proc GML of JMP, Type III sum squares) along with the rest of measured variables to test the main factors (Watering, Connection, Neighbor and Position-all fixed effects) and their interactions, with replicate as a random factor. Data were statistically analysed using JMP statistical software (Version 11.2, SAS Institute Inc., Cary, NC, USA). Data were checked for deviation from normality and for homoscedasticity and logarithm transformations were performed for parametric analysis for stomatal conductance and net photosynthesis rates. Post-hoc multiple comparisons of means were done using Tukey's HSD tests when treatment effects or interactions occurred with significance and choosing a significance level of $p < 0.05$. In order to remove the effect of size variation between ramets that existed at the start of watering treatment application, initial height and diameter were used as covariates for growth variables (diameter and height) analysis and leaf area was used as covariate in gas exchange, leaf water potential, $\delta^{13} \text{C}$, SLA and biomass analyses.

3.5 Results

3.5.1 Gas exchange and water relations

Stomatal conductance and net photosynthesis were significantly reduced by the water stress treatment over the course of the experiment, with significant variations over time giving significant Time x Watering interactions (Table 3.1, Fig. 3.1). Time also interacted with Watering, Connection and Neighbor for stomatal conductance ($p = 0.01$) and with Connection and Neighbor for net photosynthesis ($p = 0.01$). In week 1, stressed severed ramets had lower stomatal conductance than watered ramets, while net photosynthesis was not statistically different among all ramets (Table 3.2, Fig. 3.2). In weeks 2 to 7, stomatal conductance and net photosynthesis of Stressed ramets had decreased below that of Watered ramets, unless they were connected to a watered ramet (Table 3.2, Fig.3.2). If a Stressed ramet was connected to a Watered neighbor, both had similar and high stomatal conductance and net photosynthesis, while if a Watered ramet was connected to a Stressed neighbor, its stomatal conductance and net photosynthesis remained high and similar to Severed Watered ramets (Fig. 3.2). There were slight variations over the course of the experiment sometimes giving non-statistically significant differences but not changing physiological trends (Fig. 3.2). Position of ramets had no effect on gas exchange parameters nor did it interact with the other treatments except at week 7 (Table 3.2) where net photosynthesis was slightly higher for proximal Watered and Severed ramets, while they had lower values than distal ramets, in all other treatment combinations (not shown).

Drought treatment significantly reduced leaf water potential (Ψ) of Stressed ramets ($-1.30 \text{ MPa} \pm 0.02 \text{ SE}$) when compared to Watered ramets ($-1.07 \text{ MPa} \pm 0.02 \text{ SE}$; $p < 0.01$, Table 3.3). There was a significant interaction between P x C ($p < 0.001$) showing that connected proximal ramets had greater Ψ values than when they were severed, while the connection (Connected or Severed) had no effect on leaf water

potential of distal ramets, regardless of Watering treatment (Fig. 3.3). There was no significant Neighbor effect ($p=0.1$) on Ψ values (Table 3.3). Leaf carbon isotope ratios ($\delta^{13}\text{C}$) of Stressed ramets were significantly higher (less negative) than those of Watered ramets ($p<0.001$; Fig. 3.4). Similarly, $\delta^{13}\text{C}$ of ramets that had Stressed neighbor ramets (n-Stressed) were significantly higher than those with Watered neighbor ramets (n-Watered, $p<0.001$, Table 3.3). There was also a significant interaction between $W \times C$, showing that mean $\delta^{13}\text{C}$ values of Stressed but Severed ramets was greater than that of Connected Stressed ramets ($p=0.04$), while the Connection had no effect on $\delta^{13}\text{C}$ values of Watered ramets (Fig 3.4).

3.5.2 Growth and biomass allocation

Specific leaf area (SLA) of ramets was significantly affected by an interaction between Position and Neighbor ($p<0.05$; Table 3.3), showing that SLA decreased for proximal ramets when they were connected to Droughted neighbor ramets (n-Stressed; Fig. 3.5). On the other hand, when the neighbor ramet was Watered, SLA of proximal ramets was high and similar to that of distal ramets, which were not affected by the water status of neighbor ramets (Fig. 3.5).

Leaf, stem and total biomass of Stressed ramets decreased by 22%, 10% and 13% compared to Watered ramets respectively ($p<0.001$; Table 3.3). Overall, Position ($p>0.05$), Connection ($p>0.05$) and Neighbor status ($p>0.05$) had no significant effect on leaf, stem and total biomass. Root biomass showed that Severed ramets, produced 16% more root biomass when compared to Connected ramets ($p=0.03$), the Position ($p=0.1$), Watering ($p=0.1$) and Neighbor status ($p=0.5$) however had no significant effect on root biomass.

Table 3.1. Repeated measures analysis of variance for stomatal conductance, net photosynthesis of balsam poplar

Source of variation	Stomatal conductance (mmol m ⁻² s ⁻¹)		Net photosynthesis (μmol m ⁻² s ⁻¹)	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Between subjects effects				
P	0.42	0.52	2.61	0.12
W	494.32	<0.001	383.61	<0.001
C	12.43	0.003	8.06	0.01
N	33.11	<0.001	35.68	<0.001
W*C	51.40	<0.001	16.21	0.001
W*N	53.17	<0.001	47.23	<0.001
C*N	23.74	0.001	5.83	0.02
W*C*N	26.89	0.001	13.04	0.002
Within subjects effects				
T	26.65	<0.001	13	0.001
T*W	6.47	0.005	3.62	0.03
T*C	1.72	0.21	1.67	0.2
T*N	1.23	0.36	3.26	0.04
T*W*N	1.18	0.38	16.38	0.001
T*P*W	1.31	0.33	4.35	0.02
T*W*C	6.62	0.004	1.31	0.3
T*C*N	1.36	0.3	5.24	0.01
T*W*C*N	5.36	0.01	2.98	0.06

Note: W=watering, P= Position of the ramet on parental root, C= connection between ramets, N= neighbor ramet's watering status and T = time. All non-significant interactions both by (As) and (gs) not shown between subjects (P*W, P*C, P*W*C, P*N, P*W*N, P*C*N, P*W*C*N) and within subjects (T*P, T*P*C, T*P*W*C, T*P*N, T*P*W*N, T*P*C*N, T*P*W*C*N). Bold indicates significant effects.

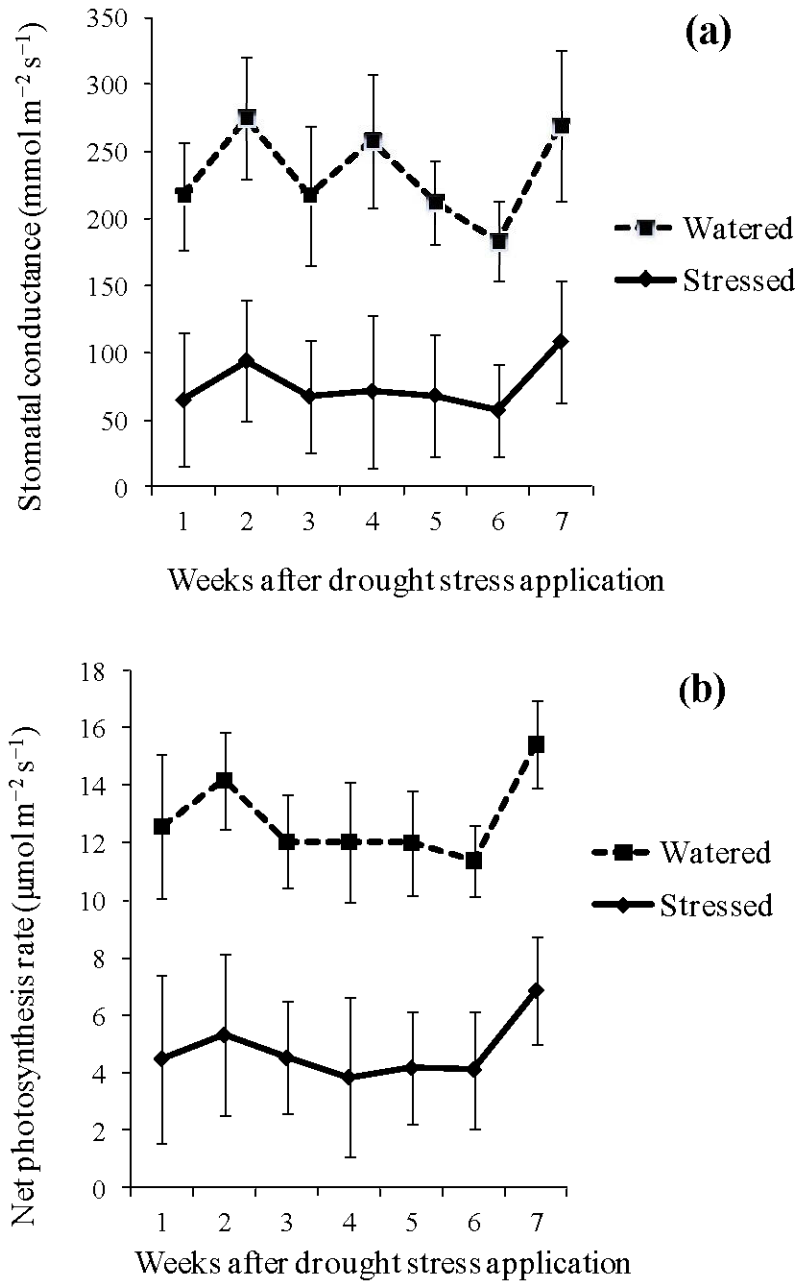


Figure 3.2: Mean stomatal conductance (a) and net photosynthesis (b) of balsam poplar as a function of time and watering treatment. Error bars refer to standard errors of the mean (SEM).

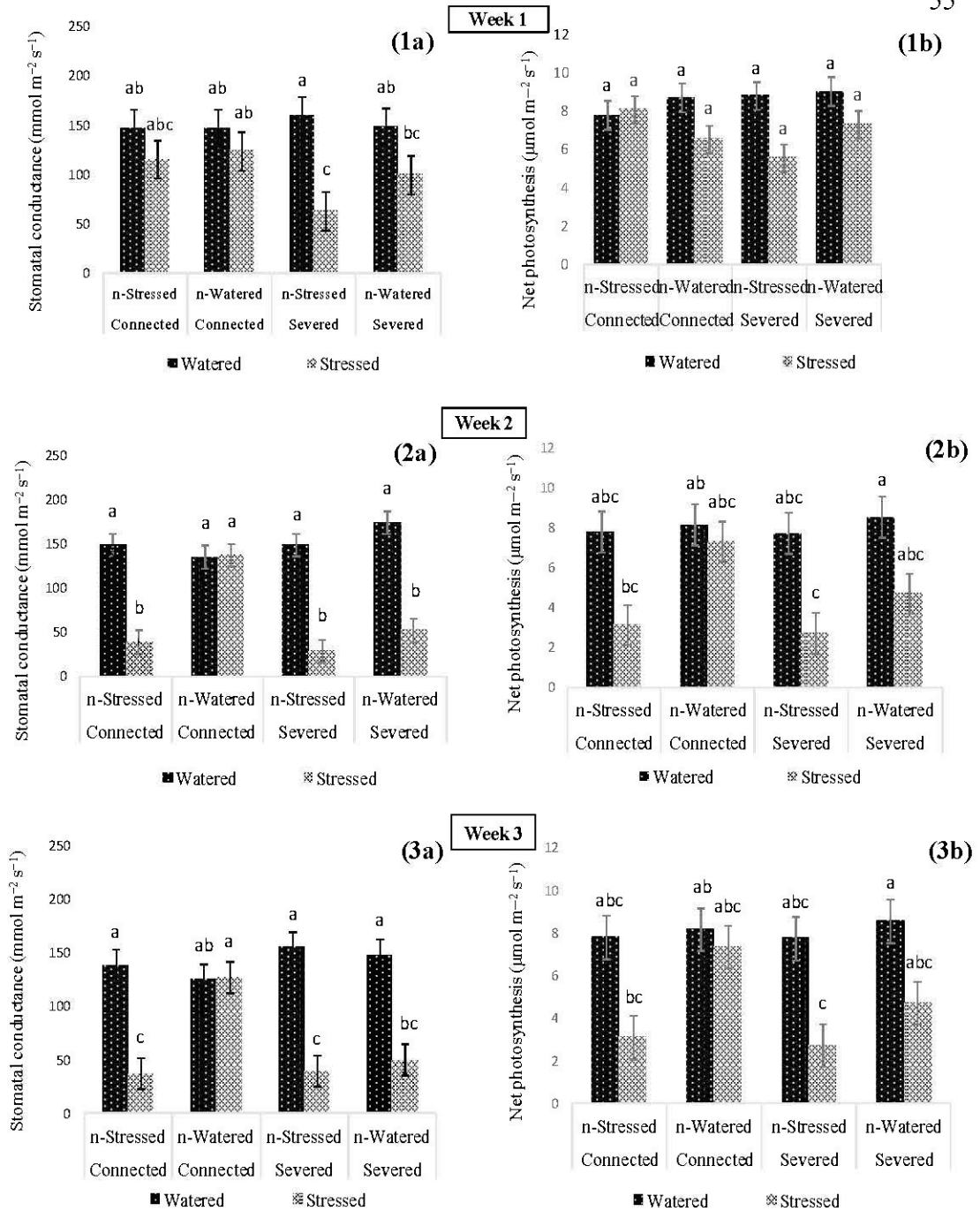


Figure 3.3a: Mean stomatal conductance (a) and net photosynthesis (b) of balsam poplar according to Watering, Connection and Neighbor (n-) status treatments for each week (1 to 3) when measurements were taken. Error bars are standard errors of the mean (SEM). Bars labeled with the same letter within a graph are not significantly different at $p < 0.05$.

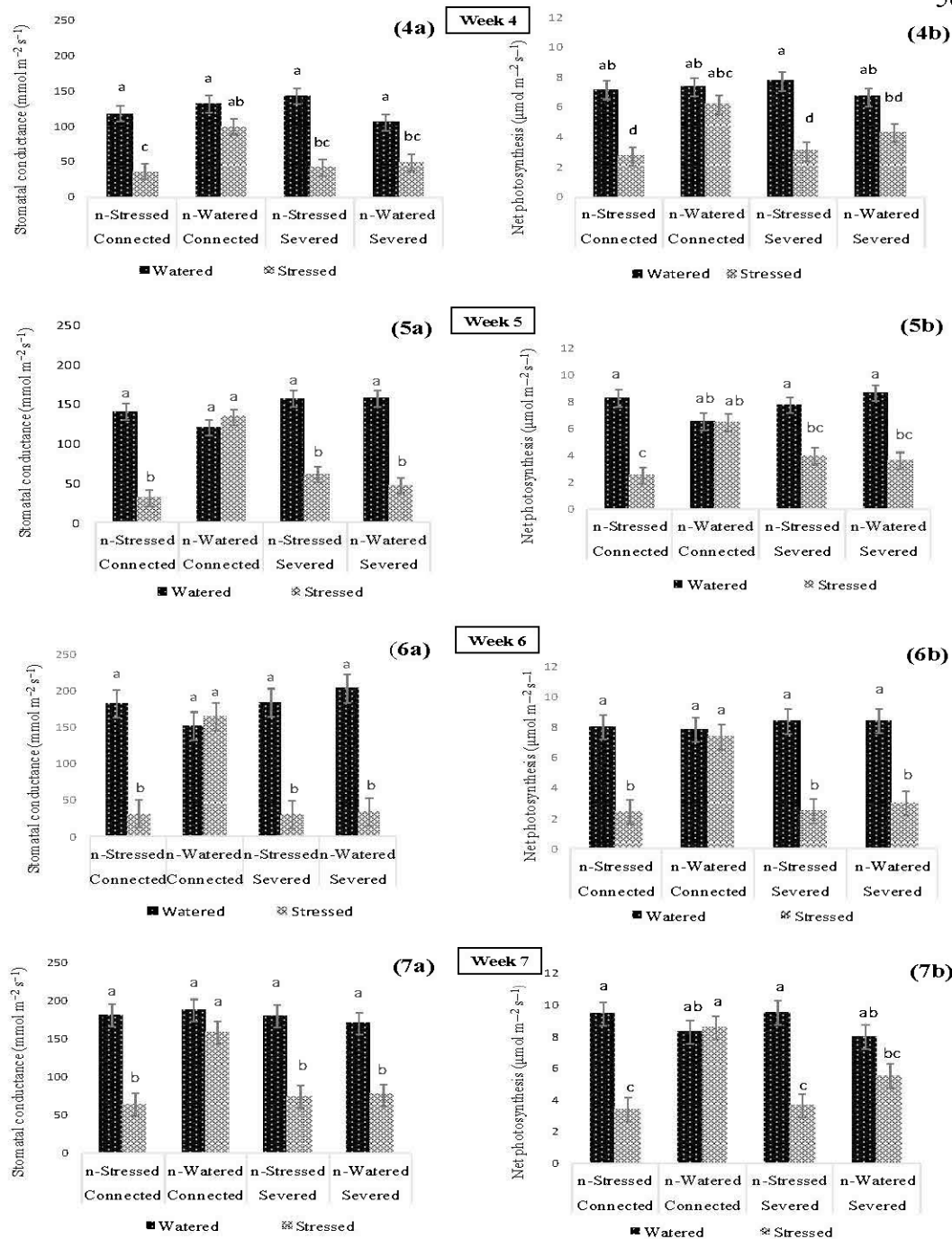


Figure 3.3b: Mean stomatal conductance (a) and net photosynthesis (b) of balsam poplar according to Watering, Connection and Neighbor (n-) status treatments for each week (4 to 7) when measurements were taken. Error bars are standard errors of the mean (SEM). Bars labeled with the same letter within a graph are not significantly different at $p < 0.05$.

Table 3.2. Repeated measures analysis of variance for stomatal conductance and net photosynthesis seven weeks after drought stress application of balsam poplar ramets.

Source of variation	Stomatal conductance (mmol m ⁻² s ⁻¹)							Net photosynthesis (μmol m ⁻² s ⁻¹)							
	No. of weeks after drought	1	2	3	4	5	6	7	1	2	3	4	5	6	7
P		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
W		*	*	*	*	*	*	*	ns	*	*	*	*	*	*
P*W		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
C		ns	ns	ns	ns	ns	*	*	ns	ns	ns	ns	ns	ns	*
P*C		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
W*C		*	*	*	ns	ns	*	*	ns	ns	ns	ns	ns	*	ns
P*W*C		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*
N		ns	*	*	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns
P*N		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
W*N		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns
P*W*N		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
C*N		ns	ns	ns	*	*	ns	ns	ns	ns	ns	ns	ns	ns	ns
P*C*N		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
W*C*N		ns	*	*	ns	*	*	*	*	ns	ns	ns	*	*	*
P*W*C*N		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Note: * $P < 0.05$; ns: not significant ($P > 0.05$). Treatment codes are as follow: W=watering, P= Position of the ramet on parental root, C= connection between ramets on parental root, N= neighbor ramet's watering status.

Table 3.3. Mixed-model analyses of variance showing significance levels for the different traits measures as affected by the treatments and their interactions: watering, position, connection and neighbor effect.

Source of variation	Main effects				Interactions										
	P	W	C	N	P*W	P*C	W*C	P*N	W*N	C*N	P*W*C	P*W*N	P*C*N	W*C*N	P*W*C*N
Leaf $\delta^{13}\text{C}$	ns	**	ns	**	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns
Leaf Ψ	ns	*	ns	ns	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns
Height	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	*	ns	ns	ns	ns
Diameter	**	*	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	*	ns
SLA	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns
T.Biomass	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Leaf mass	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Shoot mass	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Root mass	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Note: * $P < 0.05$; ** $P < 0.01$; ns: not significant ($P > 0.05$). Treatment codes are as follow: W=watering, P= Position of the ramet on parental root, C= connection between ramets on parental root, N= neighbor ramet's watering status. Height growth (cm), diameter (mm). Specific leaf area (SLA), Leaf water potential (Ψ), T.Biomass =Total biomass.

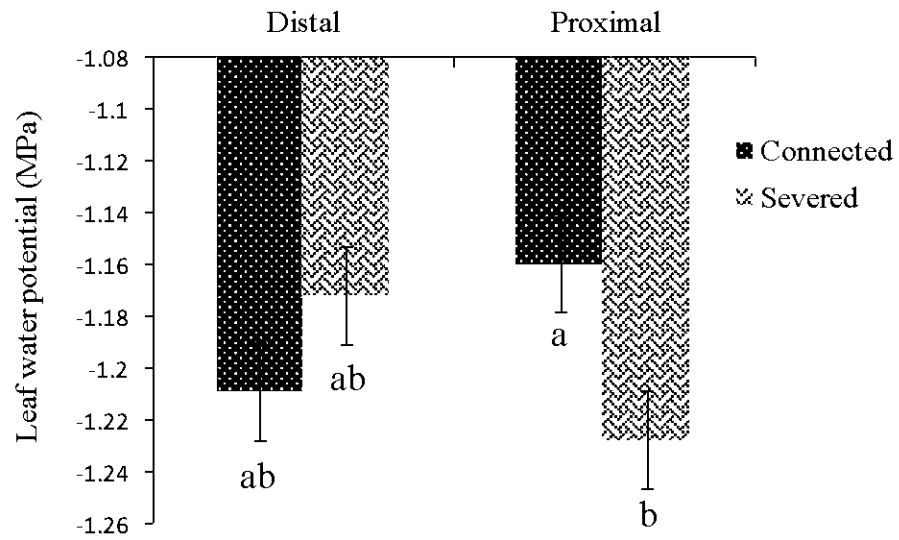


Figure 3.4: Mean leaf water potential of connected or severed distal (N=32) and proximal (N=32) ramets of balsam poplar, all Watering and Neighbor treatments combined. Error bars are standard errors of the mean (SEM). Bars labeled with the same letter were not significantly different at $p < 0.05$.

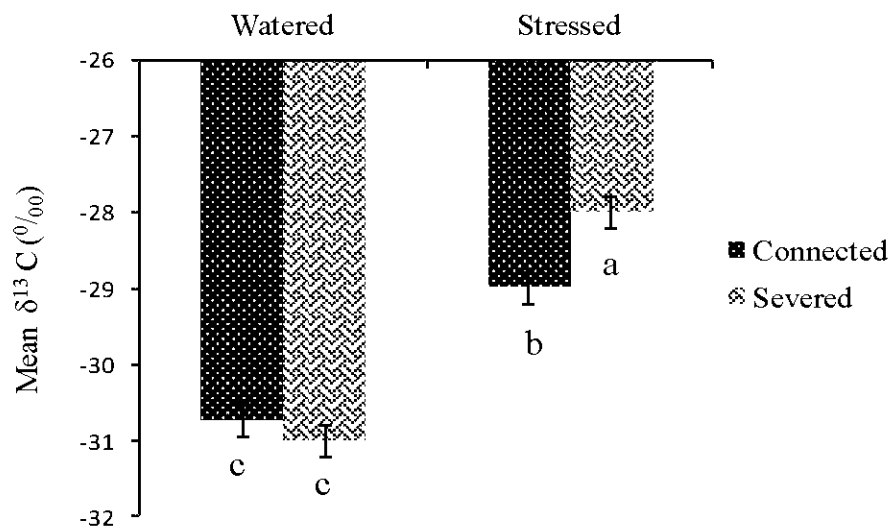


Figure 3.5: Leaf carbon isotope composition ($\delta^{13}\text{C}$) of connected and severed ramets of balsam poplar for each Watering treatment, all Neighbor and Position treatments combined. Error bars are standard errors of the mean (SEM). Bars labeled with the same letter within a graph are not significantly different at $p < 0.05$.

Distal ramets had greater diameter growth ($7.74 \text{ mm} \pm 0.09 \text{ SE}$) than proximal ramets ($7.29 \text{ mm} \pm 0.09 \text{ SE}$; Position $p < 0.01$). Watered ramets also had larger basal diameter growth ($7.91 \text{ mm} \pm 0.13 \text{ SE}$) than Stressed ramets ($7.12 \text{ mm} \pm 0.13 \text{ SE}$). However, basal diameter growth was not affected by the Neighbor status ($p = 0.4$) nor the Connection ($p = 0.5$). Although there was a significant interaction between $W \times C \times N$ ($p < 0.01$) for diameter growth, differences were very small and multiple comparison of means (Tukey) did not reveal significant statistical differences between means. Similarly, height growth was not affected by any of the main treatment effects (Table 3.3) and although there was an interaction between $P \times W \times C$ ($p < 0.05$), differences were so small that Tukey tests showed no statistical difference between means.

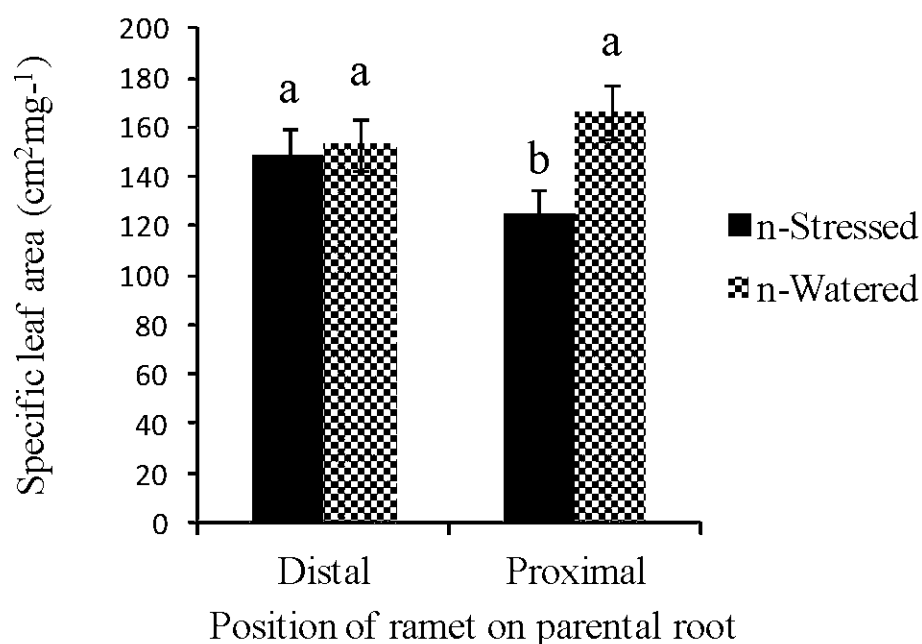


Figure 3.6: Mean specific leaf area of balsam poplar ramets at the end of the experiment for each Neighbor and Position treatment, all Watering and Connection treatments combined. Error bars are standard errors of the mean (SEM). Bars labeled with the same letter within a graph are not significantly different at $p < 0.05$.

3.6 Discussion

This experiment strongly suggests that there was water translocation through the parental root connection between connected balsam poplar ramets growing under contrasting water supply conditions. Stressed ramets connected to Watered neighbor ramets could indeed maintain high levels of gas exchange, similar to those of Watered ramets, while levels significantly dropped if they were Severed or connected to a droughted ramet (n-Stressed; Fig. 3.2). Water translocation between integrated ramets has been explained by a source-sink relationship and physical continuity between ramets (Marshall 1996, Stuefer et al. 2004). Water movement equalizes differences in water supply between connected ramets, and drives water sharing from Watered to their connected droughted ramets. Water transport from one ramet to another results from a high level of physiological integration (Pitelka and Ashmun 1985), and has been observed in several other clonal plant species such as *Carex hirta* L. (de Kroon et al. 1998), *Potentilla anserina* L. (Wang et al. 2011) and *Fragaria orientalis* Los. (Zhang et al. 2012) and for the first time here, in a tree species. Interestingly, Watered ramets connected to Stressed ramets did not have reduced stomatal conductance or net photosynthesis (Fig. 3.2), suggesting that water is shared but not at the expense of the “donor” ramet or that perhaps water availability was above and beyond required levels for the two ramets.

The repeated gas exchange measurements showed that after the first week of water stress treatment, stomatal conductance was more rapidly affected than net photosynthesis (Fig. 3.2), showing that control of water loss through stomatal regulation was an early plant response to drought (Saibo et al 2009). After two weeks, the repeated measures showed no real trend (Fig. 3.2). The significant interactions with Time along the course of the experiment may simply reflect the effects of periodic rewatering of the ramets. It is also likely that the low number of replicates (4

per treatment combinations) failed to detect some of the real differences among treatments.

Water translocation occurred in both directions along the parental root, i.e. acropetally and basipetally; between connected ramets growing in contrasting water supply (Debyle 1964, Alpert and Mooney 1986). Such bi-directional water transfer may have a key implication for water-use characteristics and the management of the species. Water may be translocated through the parental root system from trees growing adjacent to a good water supply, such as a stream or river, to trees growing further away (Tew et al. 1969). Water transfer between trees can be particularly beneficial when riparian trees are cut and their root system remains alive and functional and can transfer water to their neighboring trees (DesRochers and Lieffers 2001). However, leaf water potential (Ψ) measurements only increased for connected proximal ramets and not for distal ramets. Severed proximal ramets attained significantly lower Ψ values than connected ramets, while the parental connection did not affect Ψ of distal ramets. Because ramets establish vascular continuity mostly with the distal side of the parental root from which they originate (Brown 1935), proximal ramets are largely dependent on the distal side of the parental root. It was shown, for *Populus tremuloides*, that survival and growth of proximal ramets were greatly reduced if the parental root was cut off, while there was little effect on distal ramets (Zahner and DeByle 1965). The parental root connection thus allowed proximal suckers to maintain high leaf water potentials despite being droughted. This difference in Ψ between distal and proximal ramets was also reflected in the measured SLA of ramets, which decreased for proximal ramets connected to droughted neighbors (n-Stressed), while SLA of distal ramets was not affected by the water status of their neighbor ramets (Fig. 3.5). The decrease in SLA was probably caused by a more limited ability of proximal ramets to take up water (DeByle 1964). This, in turn, could explain the smaller basal diameter growth of proximal ramets during the course of the experiment.

Our $\delta^{13}\text{C}$ results showed that the parental root connection allowed Stressed ramets to discriminate more against ^{13}C compared to Severed ramets (Fig. 3.4), again a reflection of water sharing between ramets. Under water deficit, Stressed plants close their stomata which restrict CO_2 entry into leaves and increases ^{13}C fixation and incorporation into leaves (Farquhar et al. 1989). The lower $\delta^{13}\text{C}$ values of Watered ramets can thus be explained by greater water availability, therefore less stomatal closure and rapid uptake of atmospheric CO_2 , where light ^{12}C was preferentially fixed (O'Leary 1981).

There was little evidence with this short term experiment that clonal integration could increase biomass production of balsam poplar ramets. Severed ramets, however, produced more root biomass which is indicative that production of new roots was necessary to compensate for the loss of the large parental root and the water transfer it allowed. This result is consistent with other studies using clonal species (Salzman and Parker 1985, Stueffer et al. 1996, Roiloa and Hutchings 2012) that have reported differences in biomass allocation to roots to increase efficiency of resource foraging.

3.7 Conclusion

This study showed that root connections between balsam poplar ramets could directly impact tree physiology by allowing water sharing between ramets. These exchanges of water were significant enough to impact net photosynthesis, stomatal conductance, leaf water potential and ^{13}C discrimination of connected ramets. Severance of the parental root was especially damaging for droughted ramets positioned proximally. Forest management strategies for tree species that are physiologically integrated should consider that a silvicultural treatment done to one ramet may have a direct effect on its neighbors.

3.8 Acknowledgements

This study was funded by a doctoral scholarship Bourse en Milieu Pratique Innovation to K.E. Adonsou provided by Fonds de Recherche Nature et Technologie du Québec (FRQNT), Natural Sciences and Engineering Research Council of Canada (NSERC) and Norbord Industries Inc. Additional financial support was provided through a Discovery grant to A. DesRochers from NSERC

CHAPITRE IV

ROOT CONNECTIONS AFFECT RADIAL GROWTH OF BALSAM POPLAR TREES³

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(Facteur d'impact: 1,70).

4.1 Abstract

Root connections between trees can be an ecological advantage of clonal plant species in environments with unevenly distributed resources. We investigated the effects of root connectivity in stands of balsam poplar in Quebec (Canada). We evaluated differences in growth response between groups of trees with and without root connections through climate-growth analyses, comparison of the growth dynamics, and analysis of growth response to a severe forest tent caterpillar (FTC) outbreak. Current May temperature had a positive influence on radial growth of both connected and non-connected trees. Growth of non-connected trees was negatively affected by August temperatures ($r = -0.3$) while connected trees did not reveal a significant relationship for that month. A mixed effect ANOVA showed a significant difference ($F_{1, 25} = 5.59, p = 0.02$) in growth responses to FTC outbreak between connected and non-connected trees. Connected trees grew on average 16% better than unconnected trees during the outbreak, with bootstrapped 95% confidence range from 2.28 to 31.36%. The study suggests a sharing of resources through root connections, affecting radial growth of connected balsam poplar trees under both average and extreme environmental conditions.

Keywords: *Populus balsamifera*, *Malacosoma disstria*, root grafting, insect outbreak, resource sharing, tree nutrition, biotic interactions, facilitation in plant communities, forest resilience

4.2 Résumé

Les liens racinaires entre les arbres peuvent représenter un avantage écologique chez les espèces capables de propagation clonale dans les habitats où les ressources sont inégalement réparties. Nous avons étudié la relation entre le climat et la croissance du peuplier baumier au Québec (Canada) et comparé les patrons de croissance radiale entre les arbres connectés et non connectés par des liens racinaires. Nous avons également analysé les patrons de croissance radiale des arbres pendant et après une épidémie sévère de livrée des forêts (*Malacosoma disstria* Hubner), dans le but de vérifier si les liens racinaires permettent aux arbres de mieux croître en conditions difficiles. Les résultats ont montré que les températures élevées en mai de l'année courante ont eu une influence positive sur la croissance radiale des arbres connectés et non-connectés. La croissance radiale des arbres non-connectés a été affectée négativement par les températures élevées en août de l'année courante ($r = -0,3$), tandis que les arbres connectés n'ont pas été affectés. L'analyse de variance a montré des différences significatives ($F_{1,25} = 5,59, p = 0,02$) entre les arbres connectés et non connectés dans la réponse en croissance lors de la dernière épidémie de livrée des forêts. La croissance radiale des arbres connectés était supérieure en moyenne de 16% à celle des arbres non connectés lors de l'épidémie, avec un intervalle de confiance de 95% qui se situait entre 2,28 et 31,36%. L'étude indique que le transfert d'eau et de sucres à travers les liens racinaires a positivement influencé la croissance radiale du peuplier baumier face aux conditions environnementales extrêmes et normales.

Mots clés: *Populus balsamifera*, *Malacosoma disstria*, greffes racinaires, épidémie d'insectes, transfert de ressources, interactions biotiques, facilitation, résilience forestière.

4.3 Introduction

Balsam poplar (*Populus balsamifera* L.) regenerates vegetatively and extensively at high densities through root suckering in response to anthropic (e.g. harvest) or natural (e.g. fire) disturbances (Peterson and Peterson 1992). This regeneration strategy creates large networks of ramets interconnected through their original parental root and/or through root grafting (DesRochers and Lieffers 2001, Jelinkova et al. 2009), i.e. morphological unions of previously distinct roots from separate trees (Graham and Bormann 1966, Basnet et al. 1993, Mudge et al. 2009). Connected root systems have been found in many tree species (Graham and Bormann 1966) and there is increasing evidence that biological processes in trees may be directly influenced by other trees via root connections, which allow sharing of water, nutrients, and carbohydrate reserves (Fraser et al. 2006, Tarroux et al. 2010, Baret and DesRochers 2011, Adonsou et al. 2016). For example, snags and stumps of *Pinus* spp, which readily form root grafts, have been shown to grow 2 to 18 years following stem removal, using resources transferred across root grafts from neighboring trees (Stone 1974, Tarroux et al. 2010, Tarroux and DesRochers 2011). Root grafting increases the root system absorptive capacity and may result in greater growth rates (Tarroux and DesRochers 2011), greater efficiency of soil resource use, and reduced mortality in trees under unfavorable conditions (Pitelka and Ashmun 1985, Loehle and Jones 1990).

Root connections may affect tree response to environmental stresses, such as unfavourable climatic conditions or insect attacks. Severe tree defoliation by the forest tent caterpillar (FTC) (*Malacosoma disstria* Hubner), and variations in temperature have caused significant reductions in radial growth and hardwood forest decline in boreal ecosystems in Canada (Brandt 1995, Körner 2003, Jones et al. 2004,

Sutton and Tardif 2005). It remains unclear whether root connections enable trees to share resources or signals under unfavourable conditions, affecting tree resistance to environmental variability. To the best of our knowledge, no previous studies of growth in adult trees have considered the role of tree connectivity by their root systems.

The main objective of this study was to determine whether root connections among trees affect the growth response of balsam poplar to environmental variability. We hypothesized that interconnected trees would have higher radial growth rate under both average and extreme environmental variability, as compared to non-connected trees. We evaluated differences in growth response between groups of trees with and without root connections through climate-growth analyses, comparison of the growth dynamics, and analysis of growth response to a severe forest tent caterpillar (FTC) outbreak.

4.4 Materials and methods

4.4.1 Study area

The study was carried out in three mesic upland sites in Quebec's Abitibi region (Fig. 4.1): QC1 (48°29'N, 79°24'W, 303 m a.s.l.), QC2 (48°24'N, 79°24'W 298 m a.s.l.) and QC3 (48°32'N, 78°08'W, 315 m a.s.l.). The region is part of the Northern Clay Belt of Quebec and Ontario, which resulted from the maximum extension of the postglacial Lakes Barlow and Ojibway (Vincent and Hardy 1977). The nearest meteorological station to two study sites (QC1, QC2) is at La Sarre, approximately 42 km to the north, while that of the third site (QC3) is at Val-d'Or around 67 km to the south. The average climate for 1971-2000 showed a mean annual temperature of 0.7

°C and mean total annual precipitation of 889 mm, with 643 mm as rainfall during the growing season (Environment Canada 2012). The mean frost-free period is 64 days but freezing temperature can occur at any time of the year (Environment Canada 2012). The study region is located at the southern limit of the boreal forest, where most stands originate from natural forest fires and are dominated by an association of aspen (*Populus tremuloides* Michx.) and balsam poplar, *Abies balsamea* (L) Mill., *Picea mariana* (Mill.) BSP, *Betula papyrifera* Marsh., and *Picea glauca* (Moench) Voss.

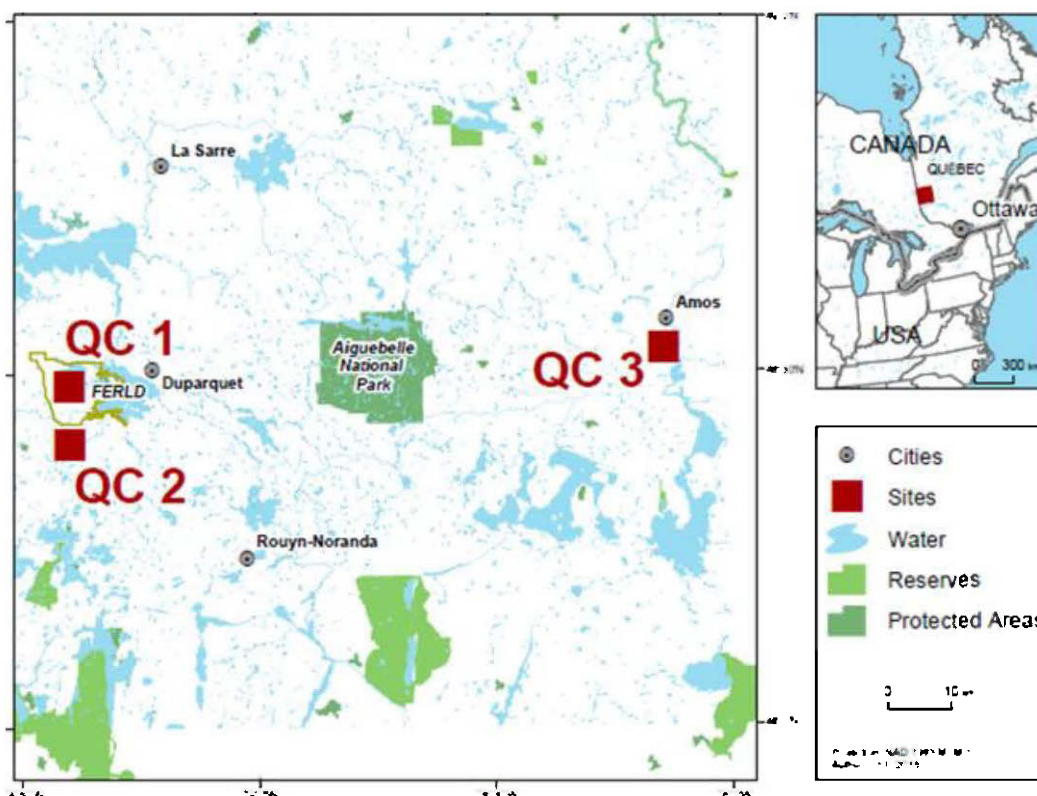


Figure 4.1: Location of the sampling sites QC1, QC2, and QC3 in the Abitibi region of northwestern Quebec, Canada.

4.4.2 Field sample collection and tree-ring data

Pure balsam poplar stands (>75% of stems), naturally regenerated by root-suckering or seeding after fire, were selected adjacent to a gentle slope with a water supply nearby to facilitate the hydraulic excavation (Tarrowx and DesRochers 2011). We used a high-pressure water spray from a forest fire pump (Mark III, WAJAX Lachine, Quebec) to uncover the whole root system of three balsam poplar stands. The area of the excavated plots ranged from approximately 50 to 60 m², depending on spatial distribution of the trees, so that at least 10 trees were included at each site. To age trees and to obtain their growth patterns, we felled 43 trees and collected stem cross-sections at the ground level (0 m) and at breast height (1.3 m). To expose all main roots the depth of excavation varied from 30 cm to 60 cm. Each coarse root was traced until its diameter decreased to less than 2 cm. In each excavated area, all trees, roots, and root connections between trees and the stumps of dead trees were mapped. Parental root connections and root grafts (Fig. 4.2 a, b) between trees were identified, collected and brought to the lab for further analysis. In the field, all suspected root grafts were checked by removing bark and by partial dissection to confirm a common wood layer between the two roots (Tarrowx et al 2010).

4.4.3 Statistical analysis

Dendrochronological methods

In the laboratory, all stem cross-sectional disks were air-dried and progressively sanded with finer sand paper (80-400 grit) to clearly highlight tree-ring patterns. Images of cross-sectional disks were acquired with an EPSON GT 15000 scanner at 1200 dpi resolution. Radial growth was measured on cross-sectional disks taken from the stem base (0 m) when possible or at breast height using CDendro and

CooRecorder program v. 7.7 (Cybis Elektronik & Data AB, Saltsjöbaden, Sweden). Ring-widths were measured with 0.01 mm precision along two radii per disk. The two curves were averaged into single-tree chronologies which were subsequently cross-dated and verified by using signature years (Stokes and Smiley 1968) and COFECHA program (Grissino-Mayer 2001). The number of sampled trees finally included in the analyses varied between 8 and 12 per site.

Expressed population signal (EPS) and the signal-to-noise-ratio (SNR), both the measures of common signals in chronologies (Wigley et al. 1984), were also calculated. To examine the temporal consistency of climate-growth relationships, we calculated moving correlation between tree-ring growth and mean monthly temperature and precipitation using bootres function (Zang and Biondi 2013).

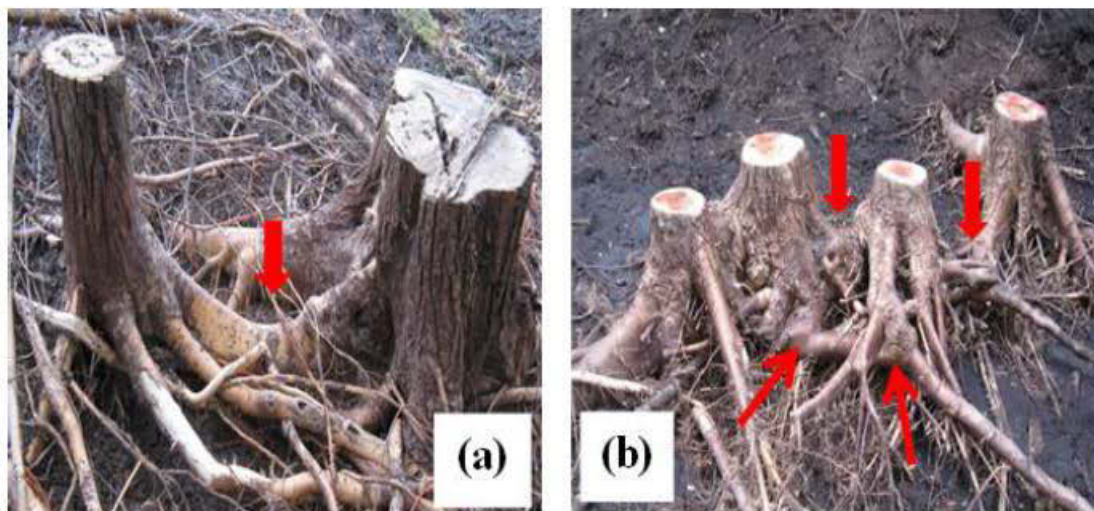


Figure 4.2: Photographs of excavated root systems of balsam poplar showing root connections: the arrows indicated parental root connection (a) and roots grafts (b) between trees in Quebec, Canada.

Residual chronologies resulting from autoregressive modelling of the site chronologies were used to evaluate climate-growth relationships of connected and non-connected trees in response function analysis (RFA) (Cook and Kairiukstis 1990). We limited the analyzed period to 1980-2009. The time frame was a compromise between uncertainty associated with status of the trees (connected vs. non-connected) in the earlier periods of their lifespans and requirements of RFA. Due to a limited length of the chronology we had to limit the number of predictors in RFA to June through August of the year of growth. Prior to RFA we removed low frequency trends in tree-ring data by detrending single tree chronologies by a cubic spline with a 50% frequency response at 32 year frequency band. In addition, we used autoregressive modeling (function *detrend* in the R package *dplR* (Bunn 2010)) on the detrended ring-width series to remove temporal autocorrelation in the chronologies. Autoregressive modeling relied on the values of Akaike Information Criterion (AIC) to select optimal order of autoregressive model. We performed RFA in R package *treeclim* (Zang and Biondi 2015).

Mixed effect models were used to evaluate whether root connectivity differentiated the trees in respect to response to the most significant monthly climate variables identified on the previous step of analysis. We used R package *nlme* (Pinheiro and Bates 2006) to run mixed effects models with tree-specific response coefficient as the dependent variable, site identity as the random factor, and tree rooting status as the independent variable. We bootstrapped results of ANOVA on growth performance during the years with outbreaks with the R package *boot* (Canty 2002), using 1000 iterations and choosing a significance level of $p < 0.05$.

Ordination analyses

We used the ordination package *vegan* in R (Oksanen et al. 2013) to evaluate relationships between climate and growth in trees of different rooting status (connected *vs.* non-connected). Prior to analysis, climate data were normalized to account for the differences in scales among variables. We used progressive selection of explanatory variables (in the R routine *ordiR2step*) to identify RDA axes which significantly contributed to the overall variability in tree growth. Finally, we tested all canonical axes to evaluate statistical significance of their contribution to growth variability in the R routine ANOVA.CCa, using the step number of 1000.

Impact analysis

We tested whether the root connectivity affected tree response during severe FTC defoliation reported in the study area during 2000-2002 (MFFP 2015). To this end we calculated the difference between cumulative increment immediately prior to (1997-1999 inclusive) and during the outbreak (2000-2002 inclusive). The ratio was used as the dependent variable in the mixed model ANOVA with the tree root status (connected *vs.* non-connected) as the fixed factor and the site identity as the random variable.

Climate data

Analyses utilized climate data generated in BioSIM 10.0, a set of spatially-explicit bioclimatic models (Régnière 1996). For each sampling site, the model interpolated data from the five closest weather stations and adjusted for differences in latitude,

longitude, and elevation between the weather stations and the site location. Due to short distances among sites we aggregated climate data by averaging data from respective sites. Climate variables included monthly mean temperature ($^{\circ}\text{C}$), monthly total precipitation (mm), monthly total snowfall (mm), total degree-days ($> 5^{\circ}\text{C}$), and monthly potential evapotranspiration (Thornthwaite method). Preliminary analyses revealed that evapotranspiration was inferior to the other two variables in terms of predictive power, and it was removed from the analyses.

4.5 Results

4.5.1 Dendrochronological analysis and clonal integration

Dendrochronological analysis showed that the ages of sampled sites were 86, 87 and 44 years and were even-aged (age of trees corresponded to age of sites). All excavated trees were of sucker origin, because roots older than the stems were found in the root system of all trees. Parental root connections were found in 25% of trees linking at least two living trees. Within the excavated sites, 48% of trees were linked with a root graft to at least one other living tree and lastly, 47% of excavated trees were not connected to other trees within the excavated area either by parental roots or root grafts.

4.5.2 Pattern of growth response to temperature and precipitations

Over the 1980-2009 period, the single-tree chronologies revealed a strong common signal with the expressed population signal (EPS) and signal-to-noise ratio (SNR) being 0.923 and 12.03, respectively. Current May temperature had positive and significant correlation ($r = 0.43$) with tree-ring chronologies of both connected and

non-connected trees over the studied period of time (Fig. 4.3a). Current growing season precipitation had no significant impact on tree radial growth in either of the groups (Fig. 4.3b).

Growth of non-connected trees displayed a significant positive response to current May temperature ($r = 0.36$) and significant negative impact of August temperature ($r = -0.3$, Fig. 4.3c). The connected trees showed a significant positive impact to current May temperature only (Fig. 4.3e). On the other hand, response function analysis of May ($p = 0.25$) and August ($p = 0.97$) temperatures, which served as our most important monthly climatic predictor variables, did not show any significant difference in radial growth responses between connected and non-connected trees. Moving response function analysis showed generally consistent response of growth to May (positive) and August (negative) temperatures (Fig. 4.4).

4.5.3 Growth-root connections-climate relationships and FTC outbreak

Redundancy analysis revealed that non-connected trees exhibited a greater spread of points across the climatic space, indicating a wider variability in the response to climate, as compared to connected trees (Fig. 4.5 a, b). Indeed, mixed model *ANOVA* on non-detrended chronologies showed significant difference ($F_{1, 25} = 5.59$, $p = 0.02$) in growth responses to FTC outbreak between connected and non-connected trees (Fig. 4.6). PCA conducted on the bootstrapped correlation coefficient of the radial growth showed that on average connected trees performed 16% better than unconnected trees, with a bootstrapped 95% confidence range being 2.28 to 31.36%.

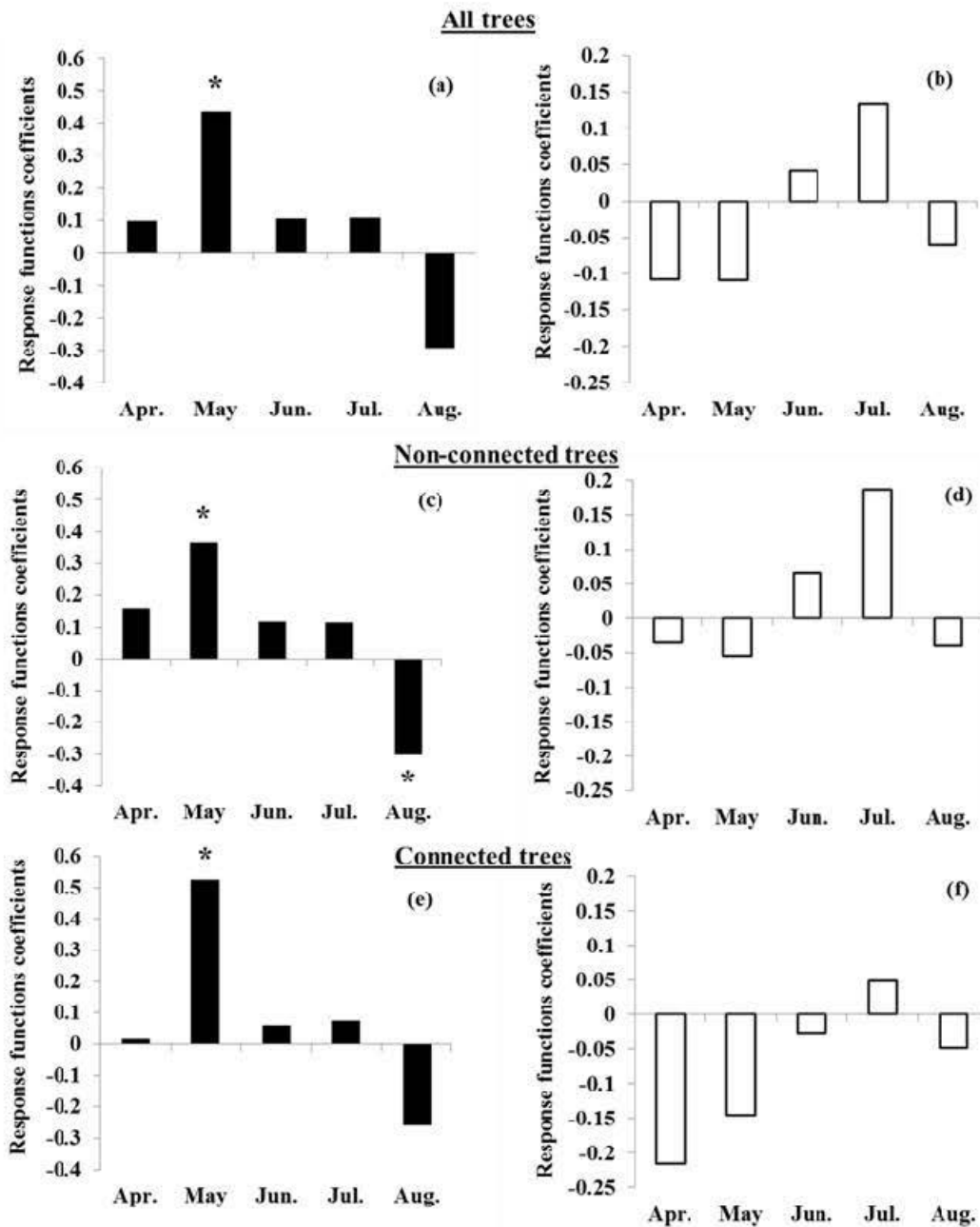


Figure 4.3: Response functions coefficients for balsam poplar radial growth and monthly mean temperature (a) and mean precipitation (b) of all trees, monthly mean temperature (c) and mean precipitation (d) of non-connected trees, and monthly mean temperature (e) and mean precipitation (f) of connected trees. Asterisk showing significant coefficients at 0.05 levels.

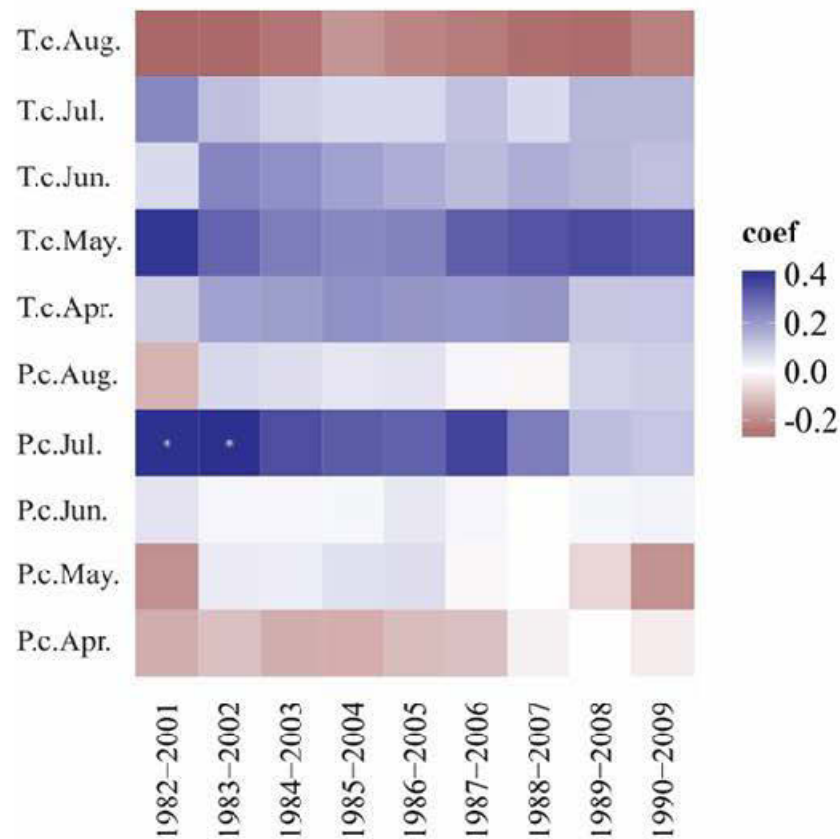


Figure 4.4: Moving response functions coefficients relating tree-ring growth of connected and non-connected balsam poplar to monthly temperature and precipitation. Asterisk shows significant coefficients at 0.05 levels. (Note: P.c. = Precipitation current, T.c. = Temperature current, coef = response functions coefficients).

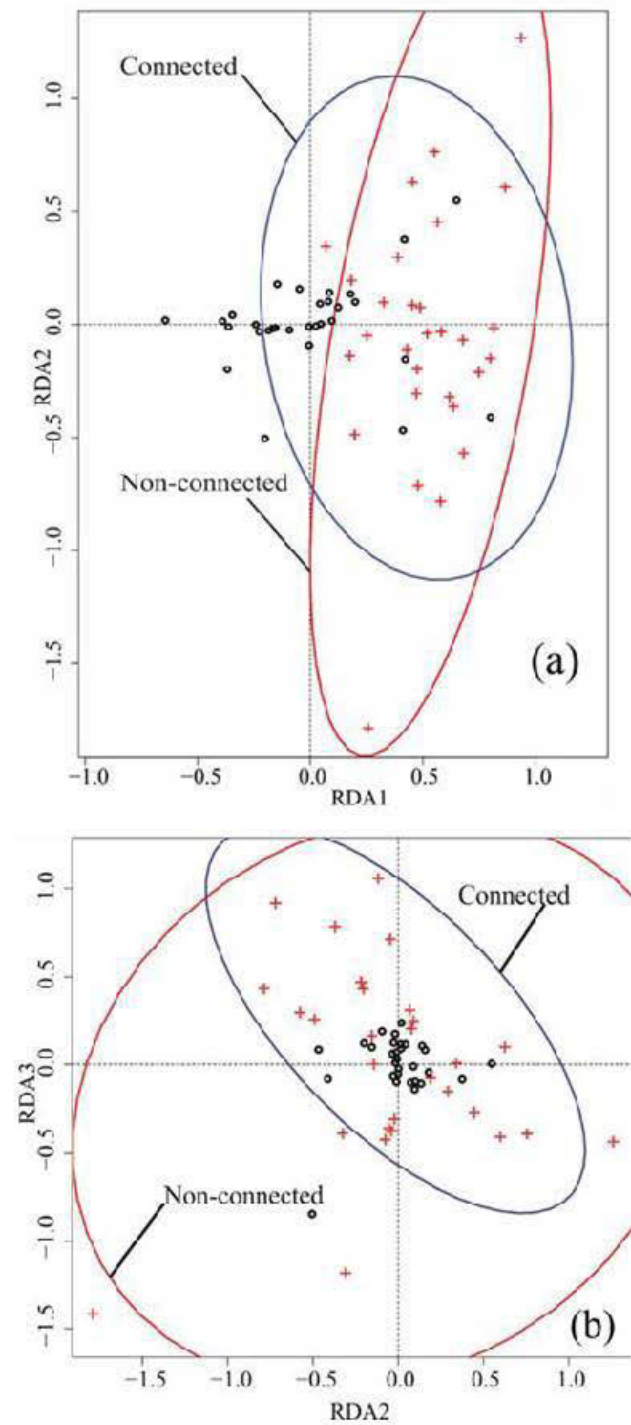


Figure 4.5: Biplots scores of environmental variables for the first and second axes of the constrained RDA (a) and for the second and third axes of the constrained RDA (b) of connected trees (black points) and non-connected trees (red crosses).

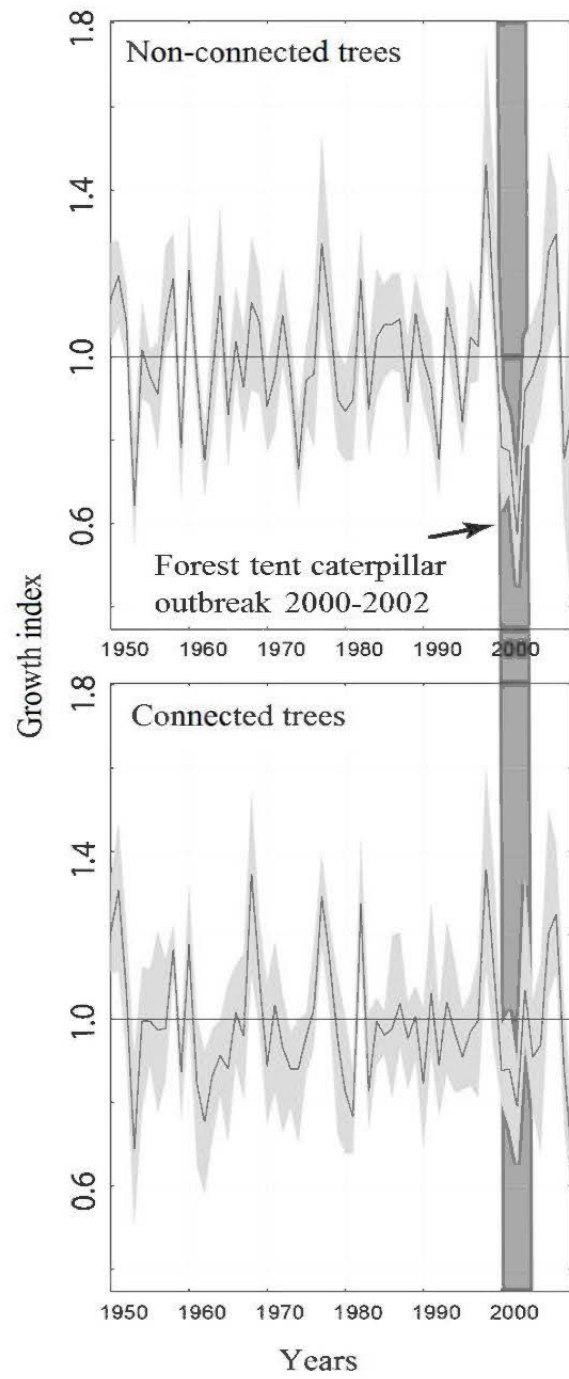


Figure 4.6: Master chronologies for non-connected and connected balsam poplar trees for the period 1950-2009, with the period of FTC outbreak indicated by the dark bar.

4.6 Discussion

Radial growth patterns of studied balsam poplar trees suggested that connected trees were less affected by a severe FTC defoliation as compared to non-connected trees (Fig. 4.6). Moreover, the range of ring width index was smaller for connected trees; this is evidence that they are sharing resources which would attenuate growth response both to temperature, but also during the FTC outbreak. Severe defoliation reduces levels of non-structural carbohydrates in trees (Hogg et al. 2002, Jones et al. 2004), causing significant reductions in diameter growth during defoliation periods (Blais 1962, Morin et al. 1993). Since non-structural carbohydrates can be shared among connected trees (Fraser et al. 2006), it is likely that transfers occurred between less and more affected trees, resulting in an overall reduced impact of the outbreak for trees sharing communal root systems. Perhaps a very severe epidemics affecting all trees for a number of years and killing an increasing number of stems on the root community (Moulier et al. 2014) could also cause the collapse of the whole stand as suggested by Baret and DesRochers (2011). Sharing of root systems has been previously observed among trees of the same or different clones in *Populus spp.* (Jelínková et al. 2009), resulting in physiologically integrated stands.

Root connections may thus help reduce genotype-specific responses in poplar tolerance to FTC outbreaks. *Populus* clones are known to exhibit genotypic variation in tolerance to insect defoliation (Jones and DeByle 1985). Phenolic glycoside concentration in poplar leaves may be an important factor in this context since its variability has been shown to determine the preference or the performance of defoliating insects (Lindroth et al. 2001, Osier and Lindroth 2001). Recent works have shown the occurrence of root grafting in trees belonging to different clones in

trembling aspen (Jelínková et al. 2009) and in balsam poplar (Chapter II). As a result, a more susceptible clone to insect defoliation may better mitigate the outbreak effects by being connected to a more resistant clone.

Root connections apparently did not override effects of main climate controls on growth of balsam poplar trees. Irrespective of root connectivity, poplar trees responded positively to May temperatures (Fig. 4.3a). This result is consistent with previous studies that have emphasized the role of May temperatures as an important factor influencing radial growth, cambial activity and cell differentiation of many tree species in the boreal forest (Körner 2003). Growth of non-connected trees was negatively affected by August temperatures, in contrast to connected trees, although the absolute differences in response appeared low. This negative correlation between August temperatures and balsam poplar radial growth suggested that warm August temperatures might have induced drought stress, which negatively affected tree growth. Similar results have been reported from the same study region in aspen, demonstrating a negative effect of August temperatures or water deficit on tree growth (Drobyshev et al. 2013).

The fact that growth of connected trees was not affected by August temperatures (Fig. 4.3e) suggests an improved absorptive capacity of the communal root system and transfer of water through root connections among connected trees in the stand (Pitelka and Ashmun 1985, Adonsou et al. 2016). Since water has been shown to be shared among connected trees (Adonsou et al. 2016) we had also expected to find a positive growth response to precipitation, particularly in August, a drought-prone period of the growing season in the study region. Root connections might promote the allocation of resources more efficiently within stands, so that connected trees may stay above a threshold in water availability which would result in a visible growth reduction in the master chronology of non-connected trees.

Our results show that root connections may be an important feature of clonal species or grafted tree response to environmental variability. However, the actual mechanisms of resource partitioning, the associated physiological trade-offs, environmental triggers, and the spatiotemporal pattern of this phenomenon remain largely unknown. For example, one might argue that a smaller individual may benefit from being connected to a larger tree, but perhaps large trees could, with their larger crown and leaf area, preferentially draw soil resources from the network during times when they are limited. Available studies have indicated that such interactions probably vary among species (Pitelka and Ashmun 1985) and along gradients in environmental conditions (Debyle 1964). The potential role of root connections may depend upon stand age and its history. Young sucker-regenerated stands initiated by rapid and abundant production of ramets, following a stand-replacing disturbance event may display more functional root connections as compared to older stands. It has been suggested that tree mortality and clonal fragmentation disrupt root connections and lower the effects of root connectivity (Barnes 1966, Gatsuk et al. 1980). However, mature and declining aspen stands do not necessarily exhibit clonal fragmentation and decay of root connections between ramets (DesRochers and Lieffers 2001). Therefore, the impacts of root connections on tree growth may last throughout the life of stands. For instance, root connections could play a significant role in forest stands dynamics, by delaying self-thinning of stands, because survival of suppressed and small weak trees could be enhanced through carbohydrates and water transfers from vigorous and dominant neighbours trees (Fraser et al. 2006). However, general unfavourable conditions affecting all trees could also enhance death of smaller trees (Moulinier et al. 2011), when there are no longer surplus carbohydrates to be transferred to the community and larger trees establish gradients that cause water and nutrients absorbed by the communal root system to move primarily to them (Bormann 1962).

Root connections among trees may represent a physiologically and ecologically important trait in natural tree populations. Forest stands of tree species exhibiting root connections should thus not be considered as groups of individual stems competing with each other for resources, because these connections may allow the sharing of resources such as water or carbohydrates. Likely, the occurrence of root connections between trees should be considered in regards to silvicultural treatments, because treatments applied to stems or stands may also affect neighbouring and intact trees (*see* Tarroux et al. 2010). In the framework of future climate change, where the temperature is expected to increase by an additional 1.5–4.5°C by 2050 (Boer et al. 2000, Plummer et al. 2006), root connections may also play an increasingly important role.

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CHAPITRE V

CONCLUSION GÉNÉRALE

Cette thèse avait pour objectif général de déterminer l'origine des peuplements de peuplier baumier en forêts naturelles autres que riveraines et le long des cours d'eaux, de caractériser leur structure clonale et mettre en évidence l'influence des liens racinaires dans la dynamique des peuplements. Plus spécifiquement, l'étude visait à évaluer, l'importance de la reproduction sexuée (source de diversité génétique) par rapport au drageonnement. De plus, dans le cas du mode de régénération par drageonnement, qui permet aux arbres d'être interconnectés entre eux par les racines, il s'agissait d'évaluer l'influence des liens racinaires sur la croissance et la physiologie des arbres. Les travaux ont été réalisés dans les provinces de l'Alberta et du Québec ayant un climat similaire mais des niveaux des précipitations différents, notamment l'ouest canadien (Alberta), qui reçoit environ moins de la moitié de précipitations (pluies) que l'est canadien (Québec). L'ensemble de ces différentes considérations sous-tendaient nos hypothèses de recherche à savoir:

- En raison d'une pluviométrie plus abondante, il y aurait de la régénération par graine plus fréquente au Québec qu'en Alberta. Les arbres issus du drageonnement seraient caractérisés par la présence de racines parentales (i.e. plus vieilles que les arbres) les reliant les uns aux autres.
- L'application d'un stress hydrique affecterait les échanges gazeux et la croissance des ramets, toutefois, les ramets stressés mais reliés à des ramets arrosés par un lien racinaire seraient moins affectés par le stress hydrique que les ramets non connectés.
- Les liens racinaires (racines parentales, greffes) contribueraient aux échanges d'eau et d'hydrates de carbone, ainsi les arbres interconnectés auraient en général

une meilleure croissance radiale que les arbres non connectés dans les conditions de contraintes environnementales (sécheresses estivales et épidémies d'insectes).

La recherche sur l'origine des peuplements a été menée dans six peuplements naturels, d'une superficie d'environ 50 m² chacun, répartis équitablement au Québec et en Alberta (Chapitre II). Les résultats obtenus, suite à l'excavation et l'analyse dendrochronologique des racines et des tiges, ont montré qu'en dépit d'une pluviométrie plus abondante au Québec, il n'y aurait pas eu de reproduction par graine, ainsi les arbres excavés seraient tous issus du drageonnement et 4 des 6 sites étaient constitués d'un seul clone. Ainsi, malgré la production abondante de graines par l'espèce (Bessey 1904, Walker et al. 1986, Lee et al. 1991), il semble que la reproduction sexuée du peuplier baumier soit assez rare, contrairement à ce qu'on voit sur sites riverains (Rood et al. 1994) ou encore le long des chemins forestiers (Zasada et al. 1981, Fortin 2008, Laquerre et al. 2009). Ces résultats suggèrent que ces sites, probablement issus des forêts feuillues, réputées moins inflammables et incomplètement brûlés, seraient moins favorables à l'établissement par graine. Malgré tout, la présence de plusieurs clones sur certains de nos sites suggère qu'il y a eu par le passé de la régénération par graine. Il est intéressant de constater que des arbres de différents clones pouvaient se greffer entre eux, similairement à ce qui avait été observé chez le peuplier faux-tremble (Jelínková et al. 2009) ou encore chez le pin rouge, une essence réputée pour son uniformité génétique (Stone 1974, Boys et al. 2005). Ces résultats suggèrent une certaine uniformité génétique chez l'espèce. On en déduit qu'il ne semble pas y avoir production d'inhibiteurs chimiques pouvant éviter le contact racinaire et la formation de greffes entre deux clones différents. Ces résultats sont contraires à la production d'inhibiteurs chimiques dans le sol chez certaines essences forestières comme le noyer noir d'Amérique (*Juglans nigra* L.) et le clavalier d'Amérique (*Xanthoxylum americanum*) empêchant tout

contact racinaire et par conséquent, la formation de greffes (Reinartz et Popp 1987). Ainsi, cette aptitude des arbres de clones différents à former des greffes racinaires pourrait participer à la préservation des clones dans le temps, de manière que même si l'arbre meurt, le clone peut survivre dans le peuplement à travers des échanges de sucres produits par un arbre vivant auquel il serait greffé. Dans les conditions environnementales difficiles de croissance, les liens racinaires pourraient contribuer à la survie des arbres malades, dominés ou encore faibles (Fraser et al. 2006) voire stressés (Chapitre III). Ainsi, lorsque les systèmes racinaires des arbres s'unissent en un vaste réseau commun extensif et couvrant une plus grande superficie, les liens racinaires favoriseraient une meilleure redistribution des ressources entre les arbres d'un site (Bormann 1966; Graham et Bormann 1966). En participant à une utilisation efficace des ressources, les liens racinaires contribueraient à une meilleure croissance (Tarrow et DesRochers 2011) de l'ensemble du peuplement, limitant la formation des trouées dans la canopée, ce qui empêcherait l'invasion du peuplement par des racines ou les graines d'autres espèces végétales colonisatrices (Loehle et Jones 1990, Basnet et al. 1993, Tarrow et DesRochers 2011). Toutefois, si ces liens racinaires semblent avoir un effet bénéfique pour la survie et la croissance de l'espèce, leur existence peut être considérée, dans certains cas, comme une forme de parasitisme et pourrait être nuisible pour l'ensemble du peuplement. Par exemple, il a été démontré que les arbres greffés étaient capables de partager des composés antibiotiques, des herbicides et des spores de champignons (Bormann 1966, Fraser et al. 2006, Stone et Stone 1975).

Le diamètre moyen des racines parentales lors du drageonnement était significativement plus important en Alberta qu'au Québec, ainsi que le ratio entre la grosseur des racines parentales et nouvelles (P/N). Ces résultats pourraient suggérer deux hypothèses possibles: 1) la taille des racines parentales étant plus

petite au Québec comparativement à l'Alberta, moins d'énergie aurait été drainée pour le maintien de ce vaste réseau de racines parentales (DesRochers et Lieffers 2001a) et 2) que les arbres auraient investi plus d'énergie dans les nouvelles racines au Québec. Le fait que les racines parentales avaient des diamètres plus grands que les nouvelles racines en Alberta pourrait suggérer une croissance radiale significative des racines parentales après le drageonnement (DesRochers et Lieffers 2001b). La croissance radiale des racines parentales se faisant dans les deux directions (acropétale et basipétale) pour des ramets très proches les uns des autres et se développant sur une même racine parentale aboutissent à des racines parentales de fort diamètre (Brown 1935). Contrairement à une croissance radiale négligeable voire une disparition des racines parentales qui avait été initialement suggéré chez le peuplier faux-tremble (Moss 1938, Sandberg 1951), les connexions racinaires ne semblent pas disparaître avec l'âge (DeByle 1964, DesRochers et Lieffers 2001a). Même après avoir développé leurs propres racines, les ramets restent connectés à la racine parentale et sont toujours interconnectés entre eux (DesRochers et Lieffers, 2001b). Alors que 22% et 25% d'arbres étaient interconnectés par une racine parentale, 53% et 48% étaient connectés par des greffes racinaires respectivement en Alberta et au Québec. Nos résultats ont confirmé la formation des greffes racinaires entre les arbres d'un même ou de différents clones. Dans les deux provinces, la distance moyenne entre les arbres connectés par la racine parentale était plus petite que celle entre les arbres non connectés. Ces résultats pourraient suggérer que plus la distance est grande, plus la connection racinaire entre les arbres a des chances de se briser avec le temps, c'est le cas dans cette étude, pour le plus vieux site (AB3, 103 ans) avec les arbres très espacés les uns des autres (>5 m) suite probablement à de l'auto-éclaircie. Comme un gros système racinaire « coûte cher » à nourrir en hydrates de carbone, il est possible que la dislocation des connections originales dans un milieu semi-aride (comme l'Alberta) serait une réponse à la survie, phénomène qui survient avec l'âge chez les espèces clonales (Gatsuk et al. 1980). Dans les autres sites où

les distances entre les arbres étaient plus petites, les liens racinaires encore existants pourraient influencer la dynamique des peuplements dans un milieu forestier où les ressources sont inégalement réparties

L'influence des liens racinaires a été mise en évidence lors d'une expérimentation en milieu contrôlé sur des ramets de peuplier baumier dans différentes conditions d'alimentation en eau. Les résultats obtenus par l'application d'un stress hydrique sur des couples de ramets (Chapitre III), ont montré qu'un arbre pouvait influencer la physiologie d'un autre arbre à l'aide du lien racinaire qui les unit. En effet, les ramets stressés mais connectés avec des ramets arrosés maintenaient leurs échanges gazeux à des niveaux élevés similaires à ceux des ramets normalement arrosés, alors que les ramets stressés et non connectés présentaient des taux d'échanges gazeux très faibles. En conditions de stress hydrique, la fermeture des stomates, afin de réduire les pertes d'eau au niveau des feuilles, entraîne une diminution de la diffusion du CO₂ et une limitation biochimique du chloroplaste à fixer le CO₂ (Tardieu et Simoneau 1998, Flexas et Medrano 2002). Ceci entraîne une diminution du taux de photosynthèse (par exemple des ramets stressés non connectés) sauf si les ramets stressés étaient connectés à des ramets arrosés. Le maintien des taux des échanges gazeux élevés chez les ramets stressés mais connectés à des ramets arrosés pourrait suggérer un transfert d'eau à travers les liens racinaires permettant à ces ramets d'échapper aux effets du déficit hydrique. De la même manière, les liens racinaires auraient permis aux ramets stressés mais connectés d'être moins affectés par le stress hydrique, et par conséquent, de discriminer davantage l'utilisation du ¹³C lors des échanges gazeux par rapport aux ramets non connectés. L'analyse comparative de la composition isotopique ($\delta^{13}\text{C}$) des feuilles formées après l'application du stress hydrique a montré des valeurs très élevées (-27,99‰) pour des ramets stressés non connectés; tandis que les ramets stressés mais connectés avaient des valeurs

intermédiaires (-28,98‰) et ceux normalement arrosés présentaient des valeurs faibles (-30,85‰). Si l'on admet l'hypothèse que l'activité photosynthétique, sous contrainte hydrique après la fermeture des stomates, augmente le niveau d'incorporation du ^{13}C dans la matière organique de la plante (Farquhar et al. 1989, McNulty et Swank 1995), alors les ramets stressés mais connectés auraient gardé les stomates ouverts leur permettant l'utilisation préférentielle du ^{12}C (O'Leary 1981). Ainsi, la valeur modérée en ^{13}C des ramets stressés mais connectés pourrait suggérer encore un transfert d'eau entre les ramets par l'intermédiaire des liens racinaires qui les unit.

Les ramets connectés situés en position proximale sur la racine parentale avaient des potentiels hydriques foliaires élevés comparés à ceux des ramets proximaux séparés bien que la connexion par la racine parentale ne semblait pas affecter le potentiel hydrique des ramets distaux. Parce que les ramets établissent une continuité vasculaire avec le côté distal de la racine parentale de laquelle ils proviennent (Brown 1935), les ramets proximaux sont largement tributaires de la partie distale de la racine parentale. Des études ont montré que la survie et la croissance des ramets proximaux étaient fortement réduites si la racine parentale était coupée, alors qu'il y avait peu d'impact sur les ramets distaux de peuplier faux-tremble (Zahner et DeByle 1965). Les liens racinaires auraient permis aux ramets proximaux de maintenir des potentiels hydriques foliaires élevés, même lorsqu'ils étaient stressés. Cette différence de potentiel hydrique foliaire entre ramets distaux et proximaux a également été observée avec les résultats sur la surface foliaire spécifique (SFS). En effet, la SFS a diminué pour les ramets proximaux connectés aux voisins stressés, tandis que celle des ramets distaux n'a pas été affectée par le statut hydrique des ramets voisins. La diminution de la SFS des ramets proximaux suggère une capacité limitée des ramets proximaux à prélever de l'eau (DeByle 1964), entraînant la fermeture des stomates. Il a été

démontré que le peuplier baumier est une espèce qui évite le stress hydrique par la fermeture des stomates avant même que le potentiel hydrique foliaire ne soit affecté par la contrainte hydrique (Hack et Sauter 1996). Ainsi, il est également possible que la fermeture des stomates et la chute du potentiel hydrique foliaire soient accompagnées d'une réduction de la croissance. La réduction de croissance est l'un des symptômes caractéristiques du déficit hydrique chez les plantes (Kramer et Boyer 1995, Saab et Sharp 2004).

L'observation de ce partage des ressources entre ramets connectés en milieu contrôlé nous a amené à le confirmer in situ sur des arbres matures dans la troisième étude (Chapitre IV). Nous avons montré que la croissance radiale des arbres connectés a été moins affectée par l'épidémie de la livrée des forêts comparativement aux arbres non connectés. En effet, des études ont montré que la défoliation réduisait les niveaux d'hydrates de carbone non structuraux produits par la canopée (Hogg et al. 2002b, Jones et al. 2004), entraînant des diminutions significatives de la croissance en diamètre pendant les périodes de défoliation (Blais 1962, Morin et al. 1993). Toutefois, comme les hydrates de carbones non structuraux peuvent être partagés entre les arbres connectés (Fraser et al. 2006), il semble que les transferts ont eu lieu entre les arbres moins affectés et plus affectés, entraînant une réduction globale de l'impact de l'épidémie pour les arbres connectés partageant des systèmes racinaires communs. Il est également possible que les premiers arbres affectés par l'épidémie puissent « avertir » en quelque sorte leurs voisins interconnectés de l'arrivée des insectes et ainsi déclencher rapidement la production de composés secondaires de défense (Jelínková et al. 2012); ce qui réduirait l'intensité, la performance de l'insecte et l'impact de la défoliation chez ces arbres membres du réseau du système racinaire. De plus, le greffage a déjà été observé entre arbres de même ou de différents clones de peuplier faux-tremble (Jelínková et al. 2009). Des études ont aussi montré que les

clones de peuplier faux-tremble présentaient des variations génotypiques dans la tolérance à la défoliation (Jones et DeByle 1985) et la concentration en glycoside phénolique dans les feuilles, déterminant la préférence et la performance des insectes défoliateurs (Lindroth et al. 2001, Osier et Lindroth 2001). Ainsi, nos résultats pourraient suggérer qu'un clone plus sensible à la défoliation pourrait atténuer les effets de livrée des forêts en échangeant des hydrates de carbone par l'intermédiaire des liens racinaires avec l'arbre d'un clone plus résistant à la défoliation. En définitive, notre étude sur la croissance radiale a montré que les arbres connectés auraient en moyenne une croissance supérieure (16%) aux arbres non connectés durant la dernière période épidémique de livrée des forêts.

Le patron de croissance radiale des arbres non connectés aurait par ailleurs été négativement affecté par les températures du mois d'août de l'année en cours, contrairement aux arbres connectés. Les températures chaudes en août auraient induit un stress hydrique qui a affecté négativement la croissance des arbres non connectés. Un phénomène similaire a été observé dans la même région chez le peuplier faux-tremble (Drobyshev et al. 2013). Le fait que la croissance radiale des arbres connectés n'ait pas été affectée par les températures du mois d'août de l'année en cours pourrait suggérer une meilleure capacité d'acquisition des ressources par le système racinaire communautaire et le partage d'eau via les liens racinaires entre les arbres (Pitelka et Ashmun 1985). La présence de liens racinaires remet donc en question les notions classiques d'individualité des arbres pour l'accès aux ressources et la compétition, puisqu'ils permettent les échanges d'eau ou d'hydrates de carbone entre arbres connectés même en cas d'épidémies. Dans ces conditions, les arbres connectés réagiraient davantage comme un groupe plutôt que comme des individus distincts.

5.1 Implications dans l'aménagement forestier et perspectives

L'ensemble des résultats a permis de mettre en évidence l'importance du drageonnement par rapport à la reproduction par graines, la persistance et l'impact éco-physiologique des liens racinaires et leur influence potentielle sur les pratiques actuelles d'aménagement sylvicole particulièrement dans un contexte de changement climatique où les liens racinaires pourraient jouer un rôle important dans la résilience des forêts.

Les stratégies d'aménagement des espèces capables de reproduction clonale et/ou physiologiquement intégrées, doivent prendre en considération l'existence de liens racinaires dans la dynamique de tels peuplements. Les traitements sylvicoles tels que la fertilisation ou les herbicides appliqués à certains arbres pourraient se propager à d'autres arbres non traités à travers les liens racinaires. Cet avancement de nos connaissances sur ces diverses réponses éco-physiologiques au niveau des arbres et dans la dynamique des peuplements permettra d'améliorer notre approche sur les pratiques sylvicoles actuelles. Par exemple, l'éclaircie qui consiste à couper dans un peuplement certains arbres (sans grande valeur commerciale ou en mauvaise santé) afin d'augmenter la disponibilité des ressources pour les arbres résiduels et contribuer à augmenter leur croissance. La rentabilité de cette opération pourrait être remise en cause si la biomasse racinaire laissée par les arbres coupés constitue un "puits" de ressources et d'énergie pour les arbres résiduels (Loehle et Jones 1990, Vincent et al. 2009). Des études ont montré chez certaines espèces de pin (*Pinus spp.*) que les racines et les souches des arbres coupés pouvaient survivre longtemps, entre 2 à 18 ans, après la coupe (Stone 1974, Tarroux et al. 2010, Tarroux et DesRochers 2011), en vivant aux dépens des ressources mises en commun avec les arbres résiduels. Ces puits de ressources et d'énergie peuvent limiter la croissance des arbres résiduels. Ainsi, dans les peuplements d'essences forestières susceptibles de présence d'une

forte fréquence de liens racinaires entre les arbres, il semble être plus recommandable de réaliser des éclaircies moins sévères et par le bas (Smith et al 1997) de sorte à laisser une biomasse racinaire moindre que celle des arbres supprimés. Les arbres résiduels dominants et plus vigoureux pourraient ainsi être en mesure de subvenir à la fois à leurs propres besoins de croissance et à celles des souches et des racines laissées après l'éclaircie (Eis 1972).

Vu l'importance des liens racinaires, les résultats ouvrent d'autres perspectives de recherche sur les pratiques sylvicoles en foresterie de courte rotation où les peupliers hybrides sont actuellement les plus utilisés en régions tempérées (Weih 2004). Par exemple, devrait-on planter les arbres plus près les uns des autres pour favoriser le greffage racinaire? Les peupliers hybrides font-ils des greffes racinaires? Les espacements plus réduits, favorisant la formation des greffes racinaires, ne réduiraient-ils pas la croissance et la biomasse aériennes? La connection physiologique est-elle plus importante que la diversité génétique pour la réponse aux changements climatiques? Le transfert d'eau entre ramets connectés est-il aussi précédé de signal hormonal pour avertir le ramet stressé d'une disponibilité en eau?

La principale limite des travaux réalisés au cours de cette thèse réside dans la taille de l'échantillonnage dans le Chapitre II, car il est difficile d'excaver de très grandes superficies. Une autre limite serait le nombre restreint de paires de ramets et l'absence d'analyse génétique des clones utilisés dans le Chapitre III. En effet, lors de l'expérimentation en serre, la difficulté à obtenir un nombre optimal de paires de ramets n'a pas permis de détecter d'éventuelles différences susceptibles d'exister entre les traitements et des variations de comportement d'un clone à un autre. L'analyse globale des liens racinaires en dépit de différences physiologiques qui pourraient exister entre racines parentales et greffes racinaires et l'impossibilité de

connaître la période de désintégration entre arbres initialement connectés sur une même racine parentale dans le chapitre IV pourrait constituer une limite à notre étude.

La problématique de l'importance des liens racinaires dans la croissance et le développement des arbres est une réalité dans la sylviculture et l'avenir des plantations forestières (e.g. peupliers hybrides) dans un contexte de changement climatique. Il serait intéressant d'aller plus loin dans l'étude des liens racinaires, par exemple étudier l'effet des espacements plus réduits dans la formation des greffes racinaires, la croissance et la biomasse aériennes chez les peupliers hybrides. Il serait aussi intéressant d'étudier la formation des greffes racinaires interspécifiques chez les peupliers et évaluer l'impact des greffes interspécifiques sur la défoliation et la croissance radiale en cas d'épidémies d'insectes défoliateurs dans les peuplements mixtes. Ce qui conduit à une nouvelle hypothèse de recherche: en cas d'épidémies d'insectes, l'existence des greffes racinaires interspécifiques dans les peuplements mixtes (peuplier faux-tremble -hôte principale- et peuplier baumier-hôte secondaire) contribuerait à réduire l'impact de la défoliation sur la croissance radiale comparé au peuplement monospécifique.

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