UNIVERSITÉ DU QUÉBEC EN ABITIBI-TÉMISCAMINGUE

LES LIENS RACINAIRES CHEZ LE PIN GRIS

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

PAR
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JANVIER 2011

À toute ma famille et mes amis, je vous aime très fort!!

REMERCIEMENTS

Je souhaiterais remercier en premier ma charmante directrice de thèse, Annie DesRochers, pour sa confiance, son soutien et pour toute l'aide apportée durant mon doctorat. Son rire et sa joie de vivre m'ont permis de travailler dans la gaieté et la bonne humeur. Je voudrais aussi remercier Cornelia Krause pour ses précieux conseils en dendroécologie, Francine Tremblay et Krassimir Naydenov pour leur aide en génétique ainsi que Bill Parsons pour son inestimable contribution en anglais. Toutes ces personnes m'ont non seulement aidée à réaliser un doctorat dans de bonnes conditions mais elles m'ont aussi donné envie de me surpasser afin de devenir un vrai bon chercheur. Pour toutes ces raisons, je suis heureuse que mon chemin ait croisé le leur.

Je tiens à remercier la Chaire Industrielle CRSNG-UQAT-UQAM en Aménagement Forestier Durable, le Conseil de Recherches en Sciences Naturelles et en Génie du Canada (NSERC/CRSNG), le Centre d'étude de la forêt (CEF) et l'Université du Québec en Abitibi-Témiscamingue pour le financement de ce projet. Je souhaiterais aussi remercier le Ministère des Ressources Naturelles et de la Faune du Québec, Matériaux Blanchet Inc., et Forêt et Recherche Harricana pour leur aide dans la localisation des sites. Je tiens aussi à gratifier tous mes aides de terrain sans qui ce projet n'aurait pas été possible : Samuel Boucher (le meilleur technicien au monde), Nadine, Noémie, Dorian Diaz, Benjamin Terrier et Line Blackburn. Un merci tout particulier à Matthieu Baret pour non seulement m'avoir supportée quasiment nuit et jour pendant mes 3 années à Amos mais aussi pour m'avoir donné des conseils et des coups de pouce sur mon terrain. Sans lui, mon doctorat aurai pas été aussi le fun et mes soirées aussi courtes (ou longues ???). Merci aussi à Marc Mazerolle, Xavier Cavard et Julien Moulinier pour leurs conseils en statistique, Noémie Graignic, Manuella Strukelj, Hedi Kebli, Geneviève Tremblay, Julie Arsenault, Sébastien Renard, Dominique Fauteux, Pierre-Antoine St-Amour et tous mes autres amis d'Abitibi pour m'avoir supportée durant mes séances de pleurnichage mais aussi pour leur aide durant mon projet.

Enfin, je remercie ma famille et mes amis de France pour leur soutien général depuis le début de ma thèse. Je sais que je vous manquais beaucoup mais vous m'avez quand même soutenue le plus possible pour que je persévère dans ma lancée, même si c'était parfois

difficile d'être loin de vous. En particulier, un gros merci à ma mère Evelyne Ourdanabia qui m'a mise au monde mais qui m'a surtout fait comprendre dès mon plus jeune âge qu'il était important de faire des études pour bien réussir dans la vie, à ma grand-mère Eliette Ourdanabia pour ses mots de soutien sur facebook ou msn, à ma tante Marilou pour ses réflexions toujours aussi pertinentes, à mon papa Jean-Luc pour sa présence et ses colis remplis de fromage qui m'ont aidée à mieux supporter la malbouffe québécoise, à ma sœur Gawine Romanet pour son saucisson et son amour, à mon bon pote Raphael Beraza pour me remettre si facilement les pieds sur terre, à mon frère Aurelien Thomas et à mon cousin Samy Ourdanabia pour leurs inestimables compliments. Une personne a été présente pour moi du début à la fin de mon doctorat et je souhaiterais la remercier du plus profond de mon cœur : ma meilleure amie, Aude Beraza qui est venu me voir au Québec autant de fois que possible, pour ces nombreux colis, pour ces billets d'avion que tu m'as offerts, l'hébergement que tu m'as fourni lorsque je venais au pays mais surtout pour ton amitié depuis plus de 15 ans; pour toutes ces super belles choses que t'as faites pour moi alors que moi je ne pouvais pas faire grand-chose, je t'en remercie. Je souhaiterais aussi remercier Raphael Hiraboure pour son soutien durant mes longues années d'université à Pau, Anglet et Clermont-Ferrand mais aussi car il m'a permis de trouver au fond de moi-même la rage pour faire un doctorat. Pour finir, je tiens aussi à remercier J.R que j'aime fort et sans qui la fin de ma thèse n'aurait jamais été aussi agréable. À tous, un gros merci d'être dans ma vie !!!!

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RÉSUMÉ

L'objectif principal de cette thèse était d'améliorer nos connaissances sur les liens racinaires chez le pin gris (*Pinus banksiana*). Les travaux ont porté sur les facteurs favorisant la formation de greffes et l'influence des greffes sur la dynamique des peuplements forestiers. Les arbres sont habituellement considérés comme des entités discrètes en compétition pour les ressources. Les précédents travaux ont pourtant démontré que les arbres pouvaient partager leur système racinaire via la formation de greffes et que des arbres ainsi reliés étaient capables de se transférer de l'eau, des sucres ou des nutriments. De cette manière, les arbres seraient directement capables d'interagir les uns avec les autres.

Pour étudier les greffes racinaires, 15 peuplements de pin gris matures (272 arbres) ont été excavés à l'aide d'une pompe hydraulique. Les caractéristiques des sites ont été enregistrées (type de sol, distance entre les arbres, densité, etc.) pour déterminer leur influence sur la fréquence des greffes. Des analyses dendrochronologiques nous ont permis de dater les arbres, les racines et les greffes et la mesure des cernes de croissance des arbres (dendrochronologie) de déterminer l'influence des greffes sur la croissance des arbres inter-reliés. Parmi les 15 sites excavés, 3 étaient des peuplements ayant subi une éclaircie commerciale quelques années auparavant (entre 6 et 9 ans avant). L'analyse de ces sites nous a permis de déterminer l'influence des greffes sur la réponse des peuplements à l'éclaircie.

Nos résultats ont montré que le pin gris était une espèce produisant beaucoup de greffes (entre 20 et 70% des arbres étaient greffés, 55% en moyenne) et que des greffes existaient dans tous les types de peuplements (peuplements naturels et plantations). Contrairement aux idées reçues, les greffes racinaires ne seraient donc pas des phénomènes rares ou anecdotiques, et font partie intégrante de la vie des arbres. La distance entre les arbres était le principal facteur régissant la formation de greffes. Dans les plantations, la fréquence des greffes était moins élevée que dans les peuplements naturels, ce qui est probablement dû à la distribution moins contagieuse des arbres. Le sable étant plus abrasif que l'argile, les greffes se produisaient plus rapidement dans ce type de sol. De plus, le nombre de greffes et le pourcentage d'arbres greffés étaient aussi plus élevés dans le sable. Si les caractéristiques des sites ont eu une forte influence sur la production de greffes, la distance génétique entre les individus a également influencé significativement la présence de greffes. Un peuplement ou une espèce uniforme d'un point de vue génétique devrait donc démontrer une plus grande fréquence de greffes racinaires. Notre étude sur l'effet des greffes sur la croissance diamétrale des arbres a finalement démontré que la croissance des arbres diminuait drastiquement durant la période de formation des greffes. Comme le bois produit pour une greffe est plus complexe que du bois normal, la formation de greffes serait un processus énergétiquement couteux. En dehors des périodes de formation des greffes, il s'avérait cependant que les arbres greffés avaient une croissance égale voire supérieure aux arbres non-greffés. Grâce à la formation d'un système racinaire commun couvrant une plus grande superficie, les greffes racinaires pourraient favoriser l'exploitation des ressources. S'il est difficile de conclure avec exactitude que les greffes ont eu un effet positif sur la croissance des arbres, il s'averait cependant qu'elles aidaient à la survie des individus les plus faibles. Beaucoup de souches greffées avec des arbres vivants survivaient des années grâce au transfert via les greffes. Les greffes racinaires pourraient donc aider à maintenir l'intégrité du

peuplement et la survie de l'espèce, en conservant les ressources d'un site au sein de l'espèce et en empêchant la colonisation du milieu par des racines ou semis d'autres espèces. Comme les greffes avantagent la survie et possiblement la croissance des arbres, une espèce ayant tendance à former des greffes aurait un avantage d'un point de vue évolutif sur les autres espèces. Les espèces ne produisant pas de greffes et/ou les arbres non greffés seraient éliminés du milieu. Cela pourrait expliquer pourquoi les pins gris étaient capables de produire des greffes racinaires peu importe leurs conditions de croissance. Dans les plantations sur argile, des greffes se sont mêmes produites entre des arbres très éloignés, à des distance allant jusqu'à près de 3m. La réponse des individus greffés était différente de celle des individus non greffés suite à une éclaircie commerciale. En effet, à partir de la 4^{ième} année suivant l'éclaircie, les arbres greffés avaient une croissance inférieure aux arbres non-greffés et ce dû au fait que les racines et les souches des arbres coupés survivaient en drainant une partie des ressources mises en commun, limitant ainsi la croissance des arbres laissés debout. De manière générale donc, les arbres greffés semblent réagir à un stress plus comme une communauté que comme des individus à part entière. Les arbres greffés semblent perdre leur individualité et ne plus être en compétition les uns envers les autres.

La présente étude a permis d'approfondir les connaissances liées à la formation de greffes racinaires, sur leur signification écologique et leur influence sur nos pratiques d'aménagement sylvicole. Bien que cette étude s'intéressait particulièrement au pin gris, les connaissances acquises sont probablement applicables à toutes les espèces produisant des greffes racinaires.

Mots-clés : greffes racinaires, pin gris, excavation, dendrochronologie, dynamique des peuplements, éclaircie commerciale, compétition

CHAPITRE I

INTRODUCTION

1.1 Généralités

Les arbres ont toujours été considérés comme des entités discrètes et la compétition pour la lumière, l'eau et les nutriments est vue comme la plus importante force régissant la dynamique des peuplements forestiers. L'idée commune est que la seule manière dont des arbres peuvent agir sur leurs congénères est de manière indirecte, en modifiant leur environnement commun. Il est reconnu que grâce à l'exsudation racinaire, aux mycorhizes, à la mort et à la perte d'organes (sénescence foliaire et racinaire, catastrophes naturelles, abandon des fruits et du pollen…), les arbres peuvent modifier la composition du sol et permettre des transferts intra- et interspécifiques (Woods et Brock 1964). Il a cependant été découvert que les arbres pouvaient partager leur système racinaire et que de cette manière les arbres reliés pouvaient agir directement les uns sur les autres. Les exemples d'arbres reliés par leurs racines sont de plus en plus fréquents (Baric et al. 2008; Fraser et al. 2005, 2006, 2007; Külla et Lõhmus 1999). Les espèces comme le peuplier faux-tremble (*Populus tremuloides* Michx) qui ont des mécanismes de régénération par drageonnement créent des peuplements où tous les arbres sont interconnectés (Jelínková et al. 2009).

Lorsque des racines se rencontrent, il est possible qu'elles fusionnent et forment des "greffes racinaires". Le fait que les racines continuent leur croissance radiale va engendrer une pression mécanique au point de contact. La croissance secondaire est stimulée autour de ce point de pression et une callosité se forme petit à petit (Bormann et Graham 1959). Cette pression peut causer des blessures et l'écorce peut finir par se rompre permettant ainsi que les cambiums des 2 racines (ou plus) soient en contact et fusionnent (Eis 1972). Selon Eis (1972) l'écorce se briserait au bout de la 2^{ième} saison pour des racines de taille normale et au bout de 4 ans pour de plus grosses racines. Ainsi, l'établissement d'une continuité vasculaire est un

mécanisme lent et complexe. Des greffes racinaires ont été observées chez plus de 150 espèces (Bormann 1966). Les greffes inter-spécifiques sont rares mais les greffes intra-arbre (autogreffes) et inter-arbres (greffes intra-spécifiques) sont particulièrement communes dans les espèces de pins de la zone tempérée comme : *Pinus resinosa* Ait., *Pinus strobus* L., et *Pinus radiata* D. Don (Armson et Van den Driessche 1959; Bormann 1966; Dosen et Iyer 1979; Horton 1969; Stone et Stone 1975; Wood et Bachelard 1970), mais généralement plus rares chez *Pinus taeda* L., *Pinus elliotti* Engelm. et *Pinus contorta* Douglas ex Loudon (Fraser et al. 2005, 2006; Miller et Woods 1965; Parsons 1992; Schultz et Woods 1967). À ce jour, aucune étude n'avait relevé la présence de greffes racinaires chez le pin gris.

1.2 Facteurs favorisant les greffes

Du fait du faible nombre d'études dans le domaine, il est difficile de savoir exactement quels sont les facteurs régissant la formation de greffes racinaires. La présence de greffes pourrait être simplement due au hasard de rencontre des racines. Dans ce cas, tous les facteurs favorisant la densité racinaire ou les contacts seraient des facteurs influençant positivement la fréquence des greffes. Dans les sols secs, le ratio racine/tige est plus élevé mais les racines sont plus fines et dispersées dans un large volume (pour augmenter leurs chances de capture d'eau) ainsi les contacts racinaires y sont probablement plus rares, et les greffes devraient l'être aussi. Si le sol est peu profond ou pierreux, les racines auraient une plus forte probabilité de rencontre, favorisant ainsi la formation de greffes (Adams 1940; Armson et Van den Driessche 1959; Bormann et Graham 1959; Eis 1972; Kozlowski et Cooley 1961; Kozlowski et al. 1991; Reynolds et Bloomberg 1982; Schultz et Woods 1967). Les sols sableux et pierreux sont plus abrasifs que les sols argileux; les frottements des racines les unes contre les autres engendrés par le balancement des arbres au vent devraient fragiliser l'écorce et favoriser sa rupture (Cook et Welch 1957; Kozlowski et Cooley 1961; LaRue 1934). De cette manière, les cambiums seraient plus aisément mis en contact et la fusion facilitée. Cependant, les processus d'établissement de la continuité vasculaire sont très délicats et la moindre perturbation pourrait également détruire ce travail (Graham et Bormann 1966; Kozlowski et Cooley 1961).

Si la formation de greffes est simplement due au hasard, il est cependant difficile d'expliquer pourquoi certaines espèces comme le pin rouge forment beaucoup de greffes (Horton 1969) alors que d'autres comme le mélèze laricin (*Larix laricina*), le frêne noir (*Fraxinus nigra*) et le cerisier tardif (*Prunus serotina*) en forment peu ou pas (LaRue 1934). Comme les greffes interspécifiques sont rares, il semblerait aussi nécessaire que les arbres soient proches génétiquement pour pouvoir se greffer (Loehle et Jones 1990). D'ailleurs, le pin rouge est une essence réputée pour son uniformité génétique (Boys et al. 2005; Stone 1974). Eis (1972) a trouvé qu'à l'intérieur d'un groupe de 3 arbres ayant beaucoup de contact au niveau racinaire, seulement 2 de ces arbres était greffés ensemble alors que ni l'un ni l'autre n'était greffé au 3^{ième}. On peut alors supposer que les 2 arbres greffés étaient plus proches génétiquement que le 3^{ième}. Il pourrait aussi exister des facteurs physiologiques favorisant (ou empêchant) la formation de greffes. En effet, certaines essences forestières comme le noyer noir d'Amérique (*Juglans nigra*) et le clavalier d'Amérique (*Xanthoxylum americanum*) ne formeraient pas de greffes car elles produiraient un inhibiteur chimique dans le sol qui ferait en sorte que les racines n'entreraient jamais en contact (Reinartz et Popp 1987).

1.3 Signification écologique des greffes

La signification écologique des greffes racinaires n'est pas encore connue. Dans beaucoup de cas, la théorie du hasard semble très probable mais il est possible que la formation de greffes soit un trait bénéfique pour les arbres afin d'être plus en adéquation avec leur milieu; cela augmenterait leurs chances de survie (Loehle et Jones 1990). D'un point de vue évolutif, cela constituerait un avantage pour la survie de l'espèce. En effet, dans les sites sujets au chablis, la formation de greffes racinaires contribuerait à la stabilité mécanique du peuplement en augmentant la résistance au vent (Basnet et al. 1993; Graham et Bormann 1966). Armsom et Van den Driessche (1959) ainsi que Dosen et Iyer (1979) ont trouvé que les peuplements éclaircis de pin rouge présentaient plus de greffes que les non-éclaircis, ce qui suggère que les greffes se formeraient en réponse à un stress mécanique par le vent.

Il n'existe pas de consensus sur le fait que les greffes aient une influence ou non sur la croissance ou la survie des arbres. Les recherches indiquent que les arbres greffés entre eux

sont capables de partager des ressources comme des composés antibiotiques, de l'auxine, des hydrates de carbone, des herbicides, des minéraux, des spores de champignons et de l'eau (Bormann 1966; Fraser et al. 2006; Kuntz et Riker 1956; Stone et Stone 1975). Ainsi, il est probable que les greffes influencent la croissance des arbres connectés. Via ces liens, les arbres entretiendraient des relations de coopération ; les arbres greffés ne seraient donc pas strictement en compétition les uns avec les autres. Dans certains écosystèmes, il a été démontré que les mécanismes de facilitation entre les plantes importaient plus que les forces compétitrices (Fajardo et McIntire 2007). Les arbres greffés réagiraient plus comme un groupe ou un super-arbre que comme des individus à part entière (Bormann 1966). Une étude comparant la croissance radiale de sapin Douglas (*Pseudotsuga Menziesii*) greffés avec celle d'arbres non greffés (Walters 1963) n'a cependant pas trouvé d'influence des greffes racinaires sur la croissance individuelle des arbres. En revanche, il a été démontré que les peuplements d'arbres greffés avaient tendance à homogénéiser leur taille (Walters 1963). Il est donc probable que les arbres greffés font partie d'une "communauté" et qu'ils ne sont pas seulement en compétition pour les ressources.

1.4 Les greffes racinaires et l'aménagement sylvicole

En sylviculture, des méthodes comme l'éclaircie et l'élagage sont beaucoup pratiquées pour augmenter le rendement et la productivité d'un peuplement. Le principe de ces techniques est qu'en éliminant les arbres (ou les tiges) les moins vigoureux, les individus restant auront de meilleures conditions environnementales (moins de compétition) et qu'ainsi ils pousseront au maximum de leur potentiel. Le problème est que les résultats obtenus ne sont pas toujours ceux escomptés (Cayford et al. 1967; Day et Rudolph 1972; DeBell et al. 2002; Gingras et Favreau 1998; Harrington et Reukema 1983; Staebler 1956; Vincent et al. 2009). Le fait que les arbres fassent des greffes pourrait expliquer les incohérences obtenues. Comme les arbres inter-reliés sont capables de partager des ressources, si l'on coupe un individu greffé à un autre, la souche et le système racinaire de l'arbre coupé pourraient survivre si l'autre arbre fournit l'énergie nécessaire au maintien des cellules vivantes (Fraser et al. 2007). Bormann (1961) a trouvé entre 3 et 44% de souches vivantes dans des peuplements de pin blanc coupés 10 ans auparavant (Bormann 1961). Stone (1974) a reporté que des pins rouges annelés pouvaient survivre jusqu'à au moins 18 ans grâce aux transferts

via les greffes racinaires d'arbres intacts. Ainsi les cas de survie de souches vivantes ne sont pas anecdotiques (Fig. 1.1) et l'influence des greffes racinaires semble de plus en plus évidente (Atwood et al. 2009). Mais le fait de fournir des substances et/ou de l'eau sans rien recevoir en contre partie ne sera pas profitable pour le donneur, car il aura moins de ressources pour ses propres besoins (Eis 1972). Il verra probablement sa croissance diminuer voire stagner.

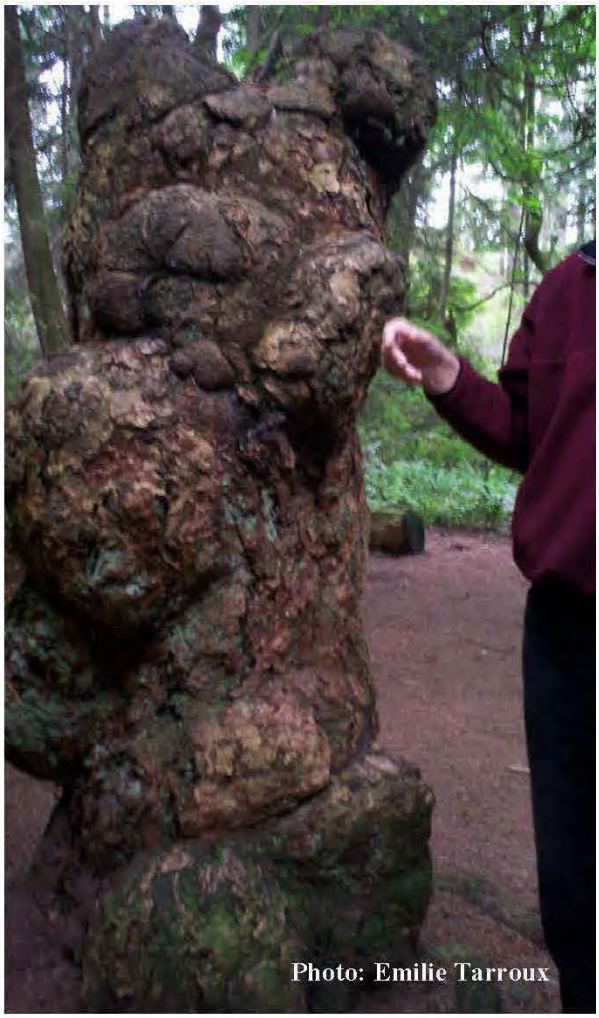

Figure 1.1 Souche qui survit grace à des greffes racinaire. La souche a continué sa croissance après que la tige ait été coupée, mais sous forme irrégulière (bourrelets).

Les greffes racinaires devraient donc être prises en compte dans les pratiques d'aménagement forestier. Si un arbre doit être coupé, il est important de considérer le fait qu'il pourrait être greffé avec un autre. L'arbre vivant auquel il est greffé aura alors à supporter une partie du système racinaire de la souche. Si l'arbre est vigoureux avec une cime bien développée, il pourra supporter les besoins respiratoires supplémentaires et le fait d'acquérir de nouvelles racines déjà fonctionnelles pourrait être un avantage (Bormann 1966). Dans le cas contraire, si l'arbre ne fait pas assez de photosynthèse pour pallier à la respiration des racines nouvellement acquises (rapport racine/tige trop élevé), il verra sa croissance diminuer et une partie du système racinaire récemment acquis mourra. En outre, l'élimination des individus les plus vigoureux au sein d'un groupe d'arbres greffés pourrait ainsi compromettre le développement et la survie des plus faibles qui jusqu'alors poursuivaient leur croissance grâce aux échanges permis par les greffes racinaires. La prise en considération des greffes racinaires pourrait nous permettre d'améliorer nos pratiques actuelles d'aménagement des peuplements forestiers.

1.5 Objectifs et structure de la thèse

Le pin gris est le pin caractéristique des forêts boréales. Il représente la 5^{ième} espèce ayant le plus grand volume marchand (volume de toutes les tiges de diamètre supérieur à 9 cm) et c'est le pin présentant la plus vaste distribution géographique au Canada (Fig. 1.2). Ainsi il apparaît important de mieux connaître la biologie de cette espèce qui peuple nos forêts canadiennes.

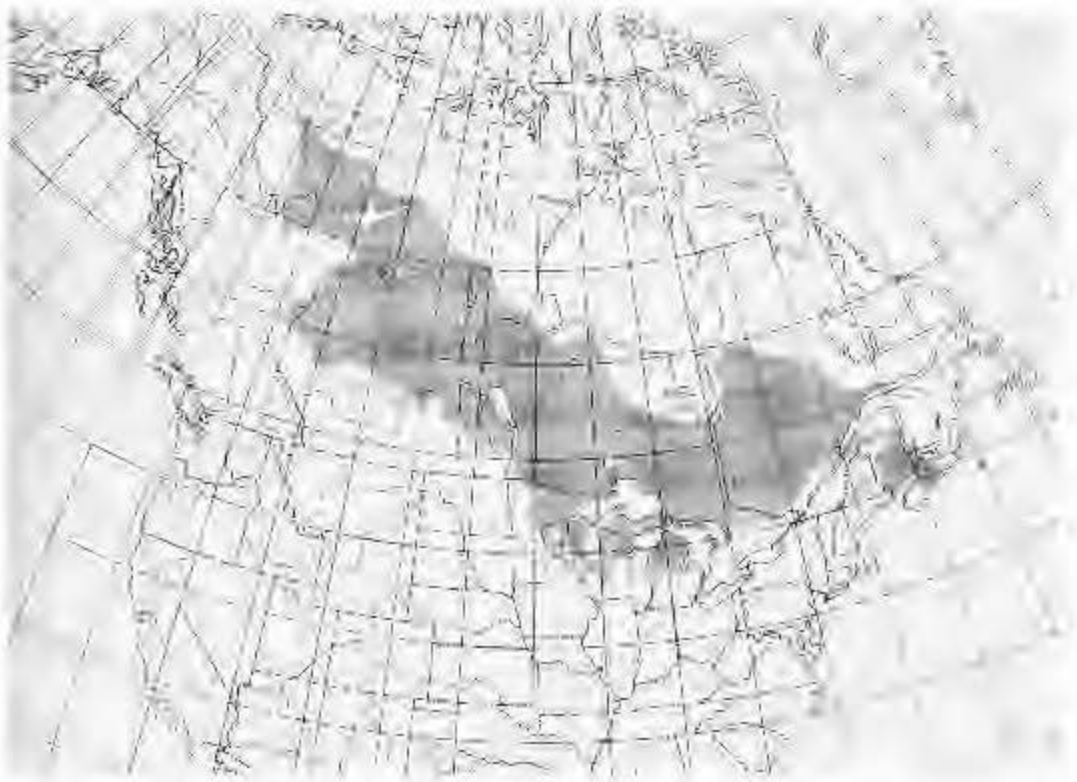

Figure 1.2 Distribution géographique du pin gris (Rudolph et Laidly 1990).

L'objectif général de cette thèse était de déterminer la fréquence des greffes racinaires chez le pin gris, les facteurs favorisant leur formation ainsi que leur signification écologique. Il existe différentes techniques pour détecter la présence de greffes i.e., l'observation de souches vivantes (Bormann 1961; Schultz 1972) ou encore l'injection de traceurs dans un hypothétique donneur (iode, poisons, pathogènes) (Bormann et Graham 1959; De Byle 1964; Graham 1959). Mais il a été prouvé que ces méthodes avaient tendance à sous estimer la présence de greffes (Bormann et Graham 1959). Pour estimer l'intégralité des greffes, il n'existe à notre avis qu'une seule méthode satisfaisante: l'excavation complète. Cette étude est la première à réaliser des excavations à grande échelle de systèmes racinaires de pin gris adultes. Certaines recherches ont réalisé des excavations partielles sur d'autres espèces (Adams 1940; Fraser et al. 2005; Gordon et Roth 1976; Stone 1974), c'est à dire qu'ils n'ont excavé que les souches vivantes ou des couples d'arbres contigus. Le problème avec cette méthode est qu'elle surestime la fréquence des greffes. En effet, les couples

d'arbres excavés ne sont pas choisis aléatoirement mais sont au contraire sélectionnés en présumant que des greffes racinaires existent. Dans cette étude, les sites ont été aléatoirement choisis et tous les arbres ont été excavés afin d'estimer la fréquence réelle des greffes racinaires. Les excavations ont été faites de manière hydraulique, à l'aide d'une pompe à feu (Wajax Mark III, Tyco Suppression systems, Pembroke, Bermuda). La pompe a été placée dans un point d'eau et grâce à la pression la terre a pu être enlevée jusqu'a ce que toutes les racines de surface (horizontales) soient visibles. L'excavation a été conduite jusqu'à que l'on puisse passer la main en dessous des souches des arbres. Enfin, les greffes ont été ramenées au laboratoire afin d'être analysées. Tous les peuplements sectionnés étaient matures (30 à 60 ans) car l'âge des peuplements aurait une influence positive sur le nombre de greffes (Armson et Van den Driessche 1959; Basnet et al. 1993; Bormann et Graham 1959; Fraser et al. 2005; Kozlowski et Cooley 1961). En outre, si les greffes influençaient la croissance des peuplements (ou la réponse des arbres aux traitements sylvicoles), il semblait plus intéressant de déterminer la présence de greffes pour des arbres ayant atteint le diamètre commercial de 9 cm. Tous les peuplements sélectionnés étaient situés en Abitibi-Témiscamingue (Fig. 1.3).

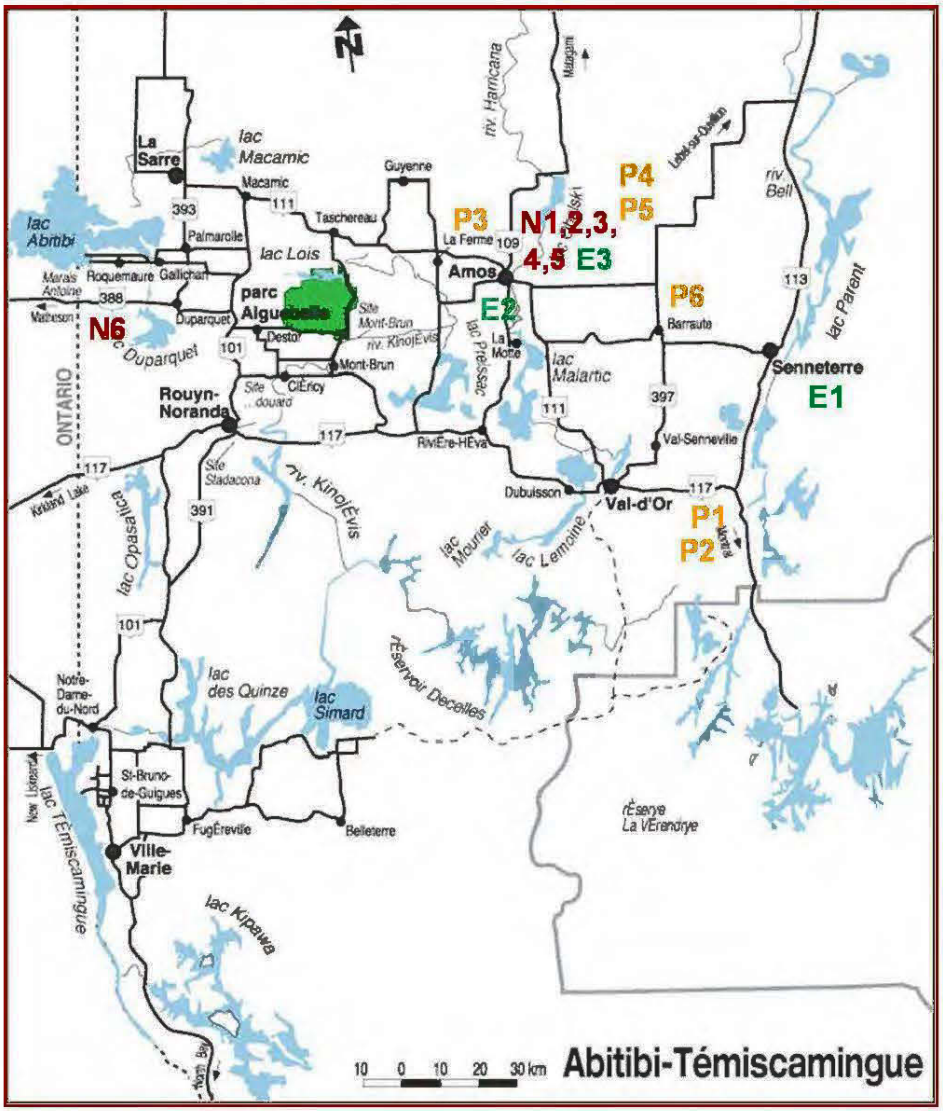

Figure 1.3 Localisation des 15 sites excavés en Abitibi-Témiscamingue, Québec. Les peuplements naturels sont en rouge (N1 à N6), les plantations en orange (P1 à P6) et les peuplements naturels ayant subi une éclaircie commerciale sont en vert (E1 à E3).

Le premier chapitre de cette thèse a consisté à déterminer la fréquence des greffes en plantations *vs* en peuplements naturels, et à caractériser les facteurs qui en favorisent la formation. Les peuplements de pin gris sont habituellement très denses lorsqu'ils s'établissent après feu. Leur distribution est également plus contagieuse dans les peuplements

naturels que dans les plantations, où les arbres sont plantés à distance égale les uns des autres. L'hypothèse posée était que la fréquence des greffes dans les peuplements naturels devait être supérieure à celle des plantations. De plus, étant donné que le sable est plus abrasif que l'argile, les frottements des racines les unes contre les autres causés par le balancement des arbres au vent devraient favoriser la rupture de l'écorce et augmenter la fréquence de formation de greffes. Pour répondre à ces questions, 12 peuplements ont été excavés et les caractéristiques des sites comme la proximité des arbres, la densité, la composition des sols, la texture, la topographie, le drainage et le type de dépôt de surface ont été enregistrés (Tableau 1.1). Des cartes présentant l'emplacement des arbres, des racines et des greffes ont été réalisées. Les facteurs favorisant la formation des greffes ainsi que le diamètre et l'âge des arbres et des racines au commencement de la greffe, l'âge de la greffe ainsi que la date et la durée de formation de la greffe ont été déterminés.

Tableau 1.1 Caractéristiques des sites excavés

Type de peuplement	Type de sol	Emplacement	Nombre d'arbres excavés
naturel	sable	St Maurice	17
naturel	sable	St Maurice	16
naturel	sable	St Maurice	23
naturel	argile	St Maurice	35
naturel	argile	St Maurice	23
naturel	argile	Duparquet	14
plantation	sable	Louvicourt	15
plantation	sable	Louvicourt	16
plantation	sable	Berry	20
plantation	argile	lac Castagnier	12
plantation	argile	lac Castagnier	14
plantation	argile	Bareville	11
Naturel éclaircis	sable	Senneterre	13
Naturel éclaircis	sable	St Mathieu	18
Naturel éclaircis	sable	St Maurice	25

Le second chapitre établit le degré de similitude génétique des arbres reliés et celui des non reliés par des greffes racinaires. L'hypothèse proposée était que les arbres devaient

présenter un certain degré de similitude génétique pour qu'une greffe se forme; les arbres greffés seraient ainsi plus proches génétiquement que les arbres non greffés. Déterminer le degré de similitude génétique entre des individus d'une même population est un travail délicat car les arbres sont tellement proches génétiquement qu'il était nécessaire d'avoir des marqueurs très variables. Pour cela, les microsatellites constituent le choix de marqueurs idéal (Craft et al. 2007; Kirst et al. 2005). L'intérêt de cette technique réside dans le fait que ces séquences sont très polymorphes. Selon le nombre d'unités de répétition, il est possible de voir des différences entre des individus très proches génétiquement. Lors des excavations, du tissu vivant situé sous l'écorce a été prélevé à l'aide d'un scalpel et ce, pour chaque arbre vivant.

Le troisième chapitre concerne l'influence des greffes sur la croissance des arbres inter-reliés dans les peuplements naturels et dans les plantations. L'hypothèse posée était que la formation de greffes represente un coût énergetique important pour les arbres. Cependant, les greffes auraient une influence positive sur la croissance du peuplement. En effet, les ressources d'un site ne seraient pas disponibles juste pour l'arbre situé à coté, car les greffes permettraient de les mettre en commun entre les arbres reliés. Ainsi, les arbres supprimés pourraient continuer à se développer normalement. Pour déterminer l'effet des greffes racinaires sur la croissance des arbres reliés, l'année de formation des greffes a été déterminée et la croissance des arbres greffés et non greffés a été étudiée avant et après ces dates.

Le quatrième et dernier chapitre décrit l'influence des greffes racinaires sur la réponse des peuplements à l'éclaircie commerciale. L'hypothèse proposée était que les arbres greffés à un arbre coupé pendant l'éclaircie répondraient moins bien à l'éclaircie commerciale, en termes d'augmentation de leur croissance, que les arbres non-greffés. En effet, comme les arbres sont inter-reliés par les racines, les racines des arbres coupés survivent et drainent une partie des ressources mises en commun, ce qui affaiblit les arbres laissés debout. Trois peuplements supplémentaires ont été excavés pour répondre à cet objectif. Ils avaient tous subi une éclaircie commerciale en 1998 (Tableau 1.1). Leur croissance en largeur a été mesurée et les variations observées avant et après l'éclaircie ont été comparées avec la croissance enregistrée dans des sites témoins afin de déterminer si les

greffes avaient eu une influence nulle, positive ou négative sur la réponse des arbres à l'éclaircie.

CHAPITRE II

FREQUENCY OF ROOT GRAFTING IN NATURALLY AND ARTIFICIALLY REGENERATED STANDS OF *PINUS BANKSIANA*: INFLUENCE OF SITES CHARACTERISTICS[1]

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[1] Article publié en 2010 dans le Canadian Journal of Forest Research, volume 40: 861-871

2.1 Abstract

We investigated the frequency of root grafting in naturally and artificially regenerated stands of jack pine (*Pinus banksiana* Lamb.) in the western boreal forest of Quebec, Canada. Twelve 30–60 m^2 plots were hydraulically excavated to determine effects of site characteristics on frequency and timing of root grafting. Naturally regenerated stands had grafted tree percentages similar to artificially regenerated stands (21%–71% across plots) but greater numbers of root grafts per tree (naturally regenerated, 0.73 graft·tree^{-1}; artificially regenerated, 0.52 graft·tree^{-1}). Mean percentages of grafted trees, number of grafts per tree, and the speed of graft formation were greater in sandy soils (61%, 0.71 graft·tree^{-1} and 2.43 years, respectively) compared with clay soils (44%, 0.54 graft·tree^{-1} and 2.97 years, respectively). Proximity of trees was a better predictor of root grafting than stand density, despite many root grafts being found with distant trees (>2 m) in artificially regenerated stands. Our results suggested that root grafts form early in stand development. Even if trees are initially separate entities, this relatively high level of root grafting produces stands where trees are extensively interconnected.

Nous avons étudié la fréquence des greffes racinaires dans des peuplements de pin gris (*Pinus banksiana* Lamb.) d'origines naturelle et artificielle dans la forêt boréale de l'ouest du Québec, au Canada. Douze parcelles de 30 à 60 m^2 ont été excavées à l'aide d'un jet d'eau pour déterminer les effets des caractéristiques de la station sur la fréquence et la période de formation des greffes racinaires. Les peuplements régénérés naturellement avaient des pourcentages d'arbres greffés semblables à ceux des peuplements régénérés artificiellement (de 21 % à 71 % dans l'ensemble des placettes), mais avaient un plus grand nombre de greffes par arbre (régénérés naturellement : 0,73 greffe·arbre^{-1}; régénérés artificiellement : 0,52 greffe·arbre^{-1}). Le pourcentage moyen d'arbres greffés, le nombre de greffes par arbre et la vitesse de formation des greffes étaient plus élevés dans les sols sableux (respectivement 61 %, 0,71 greffe·arbre^{-1} et 2,43 années) que dans les sols argileux (respectivement 44 %, 0,54 greffe·arbre^{-1} et 2,97 années). La proximité des arbres était une meilleure variable prédictive des greffes racinaires que la densité des peuplements, même si plusieurs greffes racinaires ont été observées entre des arbres distants de plus de 2 m dans les peuplements régénérés artificiellement. Nos résultats indiquent que les greffes racinaires se forment tôt au cours du développement d'un peuplement. Même si les arbres sont des entités initialement séparées, le nombre relativement élevé de greffes racinaires produit des peuplements dans lesquels les arbres sont abondamment interconnectés.

2.2 Introduction

Trees are traditionally considered as distinct entities that compete with one another for resources within forest stands. However, morphological connections that link the vascular systems of individuals can form between trees via branch or root grafts. Root grafts have been frequently observed within rather than between species of woody perennials, with reports on more than 150 angiosperm and gymnosperm species worldwide (Bormann 1966; Graham and Bormann 1966). Both intratree (self- or autografts) and intertree root grafts are especially common in temperate zone species of pines such as *Pinus resinosa* Ait., *Pinus strobus* L., and *Pinus radiata* D. Don (Armson and Van den Driessche 1959; Bormann 1966; Dosen and Iyer 1979; Horton 1969; Stone 1974; Stone and Stone 1975; Wood and Bachelard 1970), but intraspecific grafts are less commonly encountered in *Pinus taeda* L., *Pinus elliotti* Engelm., and *Pinus contorta* Dougl. ex Loud. (Fraser et al. 2005, 2006; Miller and Woods 1965; Parsons 1992; Schultz and Woods 1967). Since trees can share resources such as water, nutrients, or photosynthates through root grafts (Bormann 1966; Fraser et al. 2006; Stone and Stone 1975), the presence of these connections implies that trees are not independent of one another and that root grafting could likely play a significant role in stand dynamics. For example, survival of suppressed trees could be enhanced through carbohydrate transfers from vigorous neighbours (Fraser et al. 2006). Moreover, the interweaving and grafting of root systems can give individual trees better stability and wind firmness (Basnet et al. 1993; Coutts 1983b; Keeley 1988) but can also constitute pathways for the spread of infections to healthy trees (Epstein 1978a, b; Gordon and Roth 1976; Reynolds and Bloomberg 1982). Consequently, the adaptive significance of this trait is still in dispute (Loehle and Jones 1990).

Due to the relative complexity of studying natural root grafting in trees, little is known on the factors affecting its frequency. The degree to which the woody roots of neighbouring trees intermingle and ultimately form vascular linkages between individuals will likely depend on a variety of factors. First, the spacing of individual boles (Fraser et al. 2005; Külla and Lõhmus 1999) and overall stand density of trees (Basnet et al. 1993; Kozlowski and Cooley 1961) are thought to enhance the incidence of root grafting by locally

increasing root density (Reynolds and Bloomberg 1982). Since trees in naturally regenerated stands usually have more aggregated spatial distributions and higher initial densities than artificially regenerated stands (plantations), natural stands would be expected to contain more root grafts than plantations (Külla and Lõhmus 1999; Schultz 1972). Second, soil texture and slope position can influence root system form and architecture by constraining vertical rooting depth, horizontal root system spread, intermingling, and contact. Consequently, the propensity to form grafts is not only likely influenced by biologically determined properties of root form (Wagg 1967), including genetic relatedness (Keeley 1988; Loehle and Jones 1990; Reinartz and Popp 1987) but also controlled by extrinsic factors such as soil mechanical resistance, shear strength, moisture status, drainage class, temperature, and aeration (Gregory 1987). Third, friction between abutting roots caused by wind swaying of stems possibly enhances root grafting by wearing the root bark and facilitating contact between cambial tissues of the adjacent roots, thereby allowing grafts to be formed (Kozlowski and Cooley 1961; Loehle and Jones 1990). As stones and sand are more abrasive than clay, it is generally thought that graft formation is facilitated in coarse-textured soils, increasing root grafting frequency (Bormann and Graham 1959; Cook and Welch 1957b). However, the processes involved in vascular fusion are very sensitive to disturbance, since grafting is related to wound healing and plant immune responses; instead of leading to root union, abrasion between roots that is exacerbated by wind swaying could also disrupt the delicate processes involved in the establishment of vascular continuity between individuals (Graham and Bormann 1966; Kozlowski and Cooley 1961; Loehle and Jones 1990). Nevertheless, root grafts have been found in all soil types, and no previous study has specifically tested the effects of soil texture on root grafting frequency and processes (Dosen and Iyer 1979; Eis 1972; Stone 1974). Finally, Eis (1972) suggested that root size (as a proxy for age) had an effect on the time needed for a root graft to be formed and that smaller roots with thinner bark would form grafts more quickly than larger (older) ones with well-developed bark. Fraser et al. (2005) effectively found an increase in root grafting frequency with tree age in *P. contorta* stands ranging from 2 to 46 years old, but the majority of grafts had formed by the time the roots were 20 years old.

Until now, most estimates of root grafting frequency were based on observations of stump calluses (Schultz 1972), translocation of dyes, poisons, or radioactive tracers (Bormann and Graham 1959; De Byle 1964; Graham 1959), and partial excavations (Fraser et al. 2005; Gordon 1976; Stone 1974). However, using similar trees from the same site, Bormann and Graham (1959) showed that translocation techniques significantly underestimated root grafting frequency compared with complete excavation of root systems. At the same time, studies based on partial excavations of living stumps or pairs of contiguous trees tend to overestimate root grafting frequency, since individuals are not randomly chosen within a stand. Accurate estimates of natural root grafting frequency are necessary to determine which factors affect root graft formation and their possible influence on stand dynamics. Jack pine (*Pinus banksiana* Lamb.) is the most abundant and harvested pine species in eastern Canada, but there currently is no evidence of root grafting for this species. The main objective of this study was thus to determine root grafting frequency in naturally and artificially regenerated stands of jack pine. Using complete excavation of root systems, we compared root grafting frequency for stands growing on coarse- and fine-textured soils and examined the effects of stand density, distance between trees, and tree and root size. Timing of root grafting, i.e., when grafts occurred during the tree's life history and how long it took for two individual roots to graft, was also analysed using dendrochronology techniques.

2.3 Methods and material

2.3.1 Study sites

The study sites were located in the western balsam fir – paper birch (*Abies balsamea* (L.) Mill. – *Betula papyrifera* Marsh.) bioclimatic domain of the Quebec boreal forest (Grondin 1996). For the last three decades (1971–2000), annual precipitation for the region has averaged 918 mm (rainfall 670 mm, snowfall 248 mm) with an average daily temperature of 1.2 °C and an average 2344 degree-days above 0 °C (Environment Canada 2010). Root systems of jack pine were excavated in six artificially regenerated and six naturally regenerated stands, which were located between 47°58′N and 48°44′N and between 77°24′W and 79°25′W. Naturally regenerated stands were of postfire origin, while artificially

regenerated stands were planted after clearcutting. Stand age ranged from 35 to 90 years with stand densities of 3000–6200 stems·ha^{-1} (Table 2.1).

Table 2.1 Characteristics of the twelve excavated plots.

Plot	Size of excavated area (m^2)	Stand type	Soil type	Stand age (years)	Density (stems ha^{-1})	Mean basal area (cm^2)	Mean height (m)	Number of excavated trees	Number of grafts	Number of grafted trees	Mean number of grafts per tree	Percentage of grafted tree (%)
N1	40	N	S	55	4400	233	15	17	15	12	0.88	70.6
N2	40	N	S	55	4000	136	16	16	11	9	0.69	56.2
N3	50	N	S	90	4600	264	14	23	19	14	0.83	60.9
N4	60	N	C	45	6200	111	12	35	22	20	0.63	57.1
N5	40	N	C	45	6200	163	13	23	20	14	0.87	60.9
N6	45	N	C	75	3100	363	20	14	7	3	0.50	21.4
P1	40	P	S	35	3800	335	16	15	10	9	0.67	60.0
P2	40	P	S	35	4000	265	14	16	10	9	0.63	56.2
P3	40	P	S	35	5000	92	9	20	12	12	0.60	60.0
P4	30	P	C	35	4000	274	12	12	6	5	0.50	41.7
P5	40	P	C	35	3000	123	12	14	4	4	0.29	28.6
P6	30	P	C	35	4000	307	12	11	5	6	0.45	54.5
Mean	**41.3**			**48**	**4358**	**222**	**14**	**18**	**12**	**9.8**	**0.63**	**52.3**

For each stand type (naturally versus artificially regenerated), half were growing on sandy soils, while the other half were on clay soils (Table 2.1). The regional surficial geology is characterized by thick glacial, glaciofluvial, and glaciolacustrine deposits. These deposits represent the retreat of the Laurentide ice sheet (10 100–8000 years BP) during the last glacial cycle and the submergence of the region by proglacial Lake Barlow-Ojibway (Veillette 1994). In this project, sites located on sandy sediments were associated with glaciofluvial deposits (eskers) and sites located on fine-grained sediments in the clay plain were associated with glaciolacustrine deposits. In terms of selection criteria, stands had to contain >90% jack pine stems, have a minimum density of 3000 stems·ha^{-1}, and be mature (>30 years old). Although artificially regenerated stands were younger than naturally regenerated stands, trees in both stand types were of similar size (basal area, P = 0.751; height, P = 0.154) (Table 2.1). All sites were located near a water source (pond, lake, or river) to allow hydraulic excavation. To include a minimum of 10 trees within an excavation plot, the area sampled ranged in size from 30 to 60m^2. Initial tree spacing in the artificially regenerated stands varied from 1.7m × 3m (on sand) to 2m × 2m (on clay).

2.3.2 Laboratory work

After air-drying, all stem and root disks were progressively sanded (80–500 grit). Graft and root samples required particular attention because of eccentric growth and the presence of discontinuous growth rings (Krause and Eckstein 1993). Highly problematic sections (with very narrow or incomplete growth rings) were cut with razorblades and ring-to-ring contrast improved with white chalk. The age of trees and roots was determined by counting growth rings and visually cross-dating using pointer years, such as frost marks, light rings, compression wood, and narrow and wide rings (Schweingruber 1988). To estimate how long it took for a root to reach the location where it grafted with a root from another tree, root age was determined near the stem base and at the graft location. Stem disks at ground level were cross-dated with the breast height sections to alleviate the occurrence of missing rings at ground level (Fortin 2004). The time (year) at which root grafting began and ended was determined; since it was difficult to determine when the bark between two roots in a graft was broken because of callus tissue, the beginning year (t_0) of graft formation was recorded as the year following the last complete growth ring on each root. The last year (t_f) was

recorded as the year when a common growth ring was complete between the grafted roots. Time for graft formation was thus defined as the difference between t_f and t_0, which corresponds to the number of growth rings needed for the initiation (bark deformation and rupture) and completion of a graft.

2.3.3 Statistical analysis

Relationships between the number of root grafts per tree (NUMBER), the percentage of grafted trees per site (PERCENT), and mean stand density, soil type (sandy or clay), and stand type (naturally or artificially regenerated) were analysed with mixed linear models in R (version 2.7.2) (R Development Core Team 2008) (Table 2.2) using the function lme in the nlme library (linear and nonlinear mixed effects models) (Pinheiro et al. 2008). The linear mixed model is a parametric model for longitudinal, clustered, or repeated-measures data that incorporates random effects while quantifying the linear relationship between a continuous dependent variable and various predictor variables (West et al. 2007). Site effects were incorporated as the random effects in the models, making the results more widely applicable to the boreal forest of eastern Canada. Two linear mixed models (lme) were also used to examine relationships between the distance separating grafted trees and stand type, soil type, and stand density together with stand age and tree basal area (Table 2.2). In the first model (DISTGRA), we took the distance between the grafted trees for each graft. Since trees can bear more than one graft, a second model (DISTPAIR) was developed where we considered only grafted pairs of trees without considering each graft separately. As DISTGRA and DISPAIR results were similar, only DISTGRA results are presented.

In parallel analyses, logistic regression was used to examine the relationship between the presence of a root graft and the distance between trees. To avoid "sacrificial pseudoreplication" error that is incurred when data from different experimental units are treated as independent replicates and pooled in the same analysis (Hulbert 1984), a single logistic regression was used for each site. Goodness-of-fit of the model was assessed using the test devised by Le Cessie and van Houwelingen (1991), while omission of important or inclusion of extraneous variables was checked using Cook's distances and hat values (Everitt and Hothorn 2006). Independence of variables and randomness of residuals were also

verified (Everitt and Hothorn 2006). Logistic regressions were not appropriate for sites P2 and P3, given their highly significant goodness-of-fit values, and therefore, these results are not presented.

Table 2.2 Mixed linear models containing all explanatory variables tested with lme function.

	Global model
NUMBER	stand + soil + density + soil × stand
PERCENT	stand + soil + density + soil × stand
DISTGRA	stand + soil + density + surface1 + surface2 + stand age + soil × stand
DISTPAIR	stand + soil + density + surface1 + surface2 + stand age + soil × stand
AGETREE	stand + soil + density + tree surface + stand age + distance + soil × stand
AGEROOT	stand + soil + density + tree surface + root surface + stand age + distance + soil × stand
DIFFAGER	stand + soil+ density + stand age + soil × stand
LENGHT	stand + soil + density + rootsurface1 + rootsurface2 +sum of root surface + stand age + distance + soil × stand
DEAD	stand + soil + density + soil × stand

Notes: Stand is the stand type (naturally vs. artificially regenerated), soil is the soil texture (clay vs. sand); soil × stand is the interaction between soil type and stand type; density is the number of stems ha^{-1}; stand age is the average age of the trees within a site; distance is the distance between grafted trees; surface1 and surface2 correspond to the cross-sectional surface area of the smaller and the larger tree of the grafted pair, respectively; rootsurface1 and rootsurface2 correspond to the surface of the smaller and the larger root of the grafted pair recorded near the stump base, respectively; sum of root surface is the sum of root surface1 and root surface2;tree surface is the basal area of the grafted tree recorded at 0 m and root surface is the surface of the grafted root.

Two other lme examined which site factors affected the ages of trees (AGETREE) and roots (AGEROOT) at the beginning of graft formation, while the time required to complete grafts ($t_f - t_0$) was examined in the model LENGTH (Table 2.2). For the latter model, the Fligner–Killeen test of homogeneity of variances was nearly significant (P = 0.056), and therefore, the data were transformed with a tangent function, which greatly improved homoscedasticity (P = 0.856). The influence of site characteristics on the age difference of roots between the cross section near the base of the stem and the graft location (DIFFAGER; i.e., the time it took for a root to reach the position of the graft) was tested with a mixed linear model (Table 2.2). Finally, the influence of soil/stand types and stand density

on the number of dead stumps was also examined using a linear mixed model (DEAD model, Table 2.2).

Table 2.3 Models selected according to results of the small sample-adjusted Akaike Information Criterion (AICc).

Model	Factors tested	ΔAICc	ω_i
NUMBER	stand + soil + density	0.00	0.47
	stand + soil	1.03	0.28
	soil+ density	2.68	0.12
	stand	4.06	0.06
	stand + density	6.15	0.02
	soil	6.35	0.02
	stand + soil + soil×stand	6.62	0.02
	stand + soil + density + soil×stand	8.77	0.01
PERCENT	soil + density	0.00	0.92
	stand + soil + density	5.57	0.06
	soil	8.29	0.01
	stand + soil + density + soil×stand	9.59	0.01
	stand + soil	12.53	0.00
	stand + density	12.58	0.00
	stand	13.38	0.00
	stand + soil + soil×stand	18.80	0.00

Notes: ΔAICc correspond to the differences in AICc values from the best model, with values < 2 having greatest support. Akaike weights (ω) determine the probability of a model being the best explanatory model, considering the data and the suite of candidate models.

Two model selection techniques were used to determine the most suitable models for NUMBER and PERCENT. First, all plausible models were compared based on the Akaike information criterion corrected for small sample sizes (AICc) (Burnham and Anderson 2004). Differences in AICc values (ΔAICc) were calculated for the respective models relative to the "best" model, i.e., the model with the lowest AICc. Models with ΔAICc < 2.0 and high Akaike weights (ω_i, interpreted as probabilities) were deemed to have the greatest statistical support (Table 2.3) (Burnham and Anderson 2004). Second, traditional backward model selection techniques were used to corroborate the model selected by the Akaike weights (Burnham and Anderson 2004). Since there were too many parameters to test for the other

models (DISTGRA, AGETREE, AGEROOT, DIFFEAGER, LENGTH, and DEAD), simple backward elimination was performed (Table 2.4). Multiple comparisons of means (Tukey's tests) were used when the soil × stand interaction was significant (Table 2.5). Predicted values for the number of grafts and the percentage of grafted trees per site were also compared with the observed data using a simple linear regression to determine the predictive power of the selected models NUMBER and PERCENT. A significance level of $P = 0.05$ was used for all response variables.

Table 2.4 Models chosen using backwards elimination, with significance values for each linear mixed-effects model retained. Statistically significant values ($P < 0.05$) are given in bold.

Model	Selected factors	Estimated Value	Standard Error	DF	P-value
PERCENT	soil (sand)	17.693	4.285	9	**0.003**
	density	0.009	0.002	9	**0.002**
NUMBER	stand (plantation)	-0.150	0.057	8	**0.031**
	soil (sand)	0.184	0.053	8	**0.008**
	density	0.001	0.000	8	**0.029**
DISTGRA	stand (plantation)	0.587	0.256	7	0.056
	soil (sand)	0.159	0.223	7	0.498
	stand age	-0.016	0.007	7	**0.045**
	surface1	0.001	0.000	128	**0.001**
	soil (sand) × stand (plantation)	-1.221	0.319	7	**0.006**
AGETREE	stand (plantation)	-12.453	2.462	9	**0.001**
	soil (sand)	2.469	2.095	297	0.240
	stand age	0.743	0.064	297	**< 0.001**
	tree surface	0.009	0.003	297	**0.005**
	distance	8.164	1.150	297	**< 0.001**
	soil (sand) x stand (plantation)	10.261	3.362	9	**0.014**
AGEROOT	soil (sand)	6.857	1.884	8	**0.007**
	density	0.003	0.001	8	**0.009**
	stand age	0.723	0.048	8	**< 0.001**
	tree surface	0.839	0.409	254	**0.041**
	distance	3.897	1.129	254	**0.001**
DIFFAGER	P > 0.1 for all explanatory variables; therefore, no model was selected				
LENGTH	distance	-0.597	0.271	120	**0.029**
	soil (sand)	-1.011	0.298	10	**0.007**
DEAD	stand (plantation)	-382.568	149.1226	9	**0.0304**
	density	0.166	0.076	9	0.0567

Notes: The stand type or soil type given in brackets corresponds to the type considered by the model. For example, in PERCENT, soil (sand) with an estimated value of 17.693 means that in sandy soils, the percentage increased by 17.693%.

Table 2.5 Tukey multiple comparisons of means for each model where the soil × stand interaction was significant. Statistically significant values ($P < 0.05$) are given in bold.

Model	Interaction	Difference	Lower limit	Upper limit	P-value
DISTGRA	PC-NC	0.933	0.512	1.353	< 0.001
	NS-NC	-0.128	-0.422	0.166	0.668
	PS-NC	-0.185	-0.508	0.139	0.450
	NS-PC	-1.061	-1.486	-0.636	< 0.001
	PS-PC	-1.117	-1.563	-0.672	< 0.001
	PS-NS	-0.056	-0.386	0.273	0.971
AGETREE	PC-NC	-11.417	-17.744	-5.089	< 0.001
	NS-NC	20.882	16.590	25.174	< 0.001
	PS-NC	-8.700	-12.527	-4.873	< 0.001
	NS-PC	32.299	25.575	39.023	< 0.001
	PS-PC	2.717	-3.720	9.153	0.696
	PS-NS	-29.582	-34.033	-25.131	< 0.001

Note: Interaction codes: N corresponds to naturally regenerated stands and P to artificially regenerated stands; S corresponds to sandy soils and C to clayey soils.

2.4 Results

Root grafts were found in all 12 study sites for a total of 141 (Table 2.1). The number of root grafts per site varied from 4 to 22 (Table 2.1). Mean number of root grafts per tree was significantly greater in naturally regenerated stands ($0.73 \cdot tree^{-1}$) compared with artificially regenerated stands ($0.52 \cdot tree^{-1}$) ($P = 0.031$) (Tables 2.1 and 2.4). Trees growing in sandy soils had more root grafts on average ($0.71 \cdot tree^{-1}$) than trees growing in clay soils ($0.54 \cdot tree^{-1}$) ($P = 0.008$) (Tables 2.1 and 2.4). The number of root grafts per tree increased with stand density ($P = 0.029$) (Table 2.4). The following equation ($R^2 = 0.997$, $P < 0.001$) predicted the number of root grafts per tree in relation to stand type, soil type, and stand density (Fig. 2.1a):

$$[1]\ \text{Grafts per tree} = 0.27258613 + (-0.14970216*A) + (0.18390226*B) + (0.00007743*\text{stand density})$$

where $A = 0$ in naturally regenerated stands and $A = 1$ in artificially regenerated stands and $B = 0$ in clay soils and $B = 1$ in sandy soils. Overall, 54% (range 21%–71% across the various plots) of the study trees developed at least one root graft within the excavated areas

(Table 2.1). Mean percentages of grafted trees were higher in sandy soils (61%) than in clayey soils (44%) ($P = 0.003$) (Tables 2.1 and 2.4) and increased with stand density ($P = 0.002$) (Table 2.4). However, the percentage of grafted trees per site was similar ($P = 0.502$) in naturally regenerated stands (55%) and in artificially regenerated stands (50%) (Tables 2.1 and 2.4). The following equation ($R^2 = 0.762$, $P < 0.001$) predicted the percentage of grafted trees according to soil type and stand density (Fig. 2.1b):

$$[2] \% \text{ of grafted trees} = 3.505125 + (17.692874 * B) + (0.009177 * \text{stand density})$$

where B = 0 in clay soils and B = 1 in sandy soils.

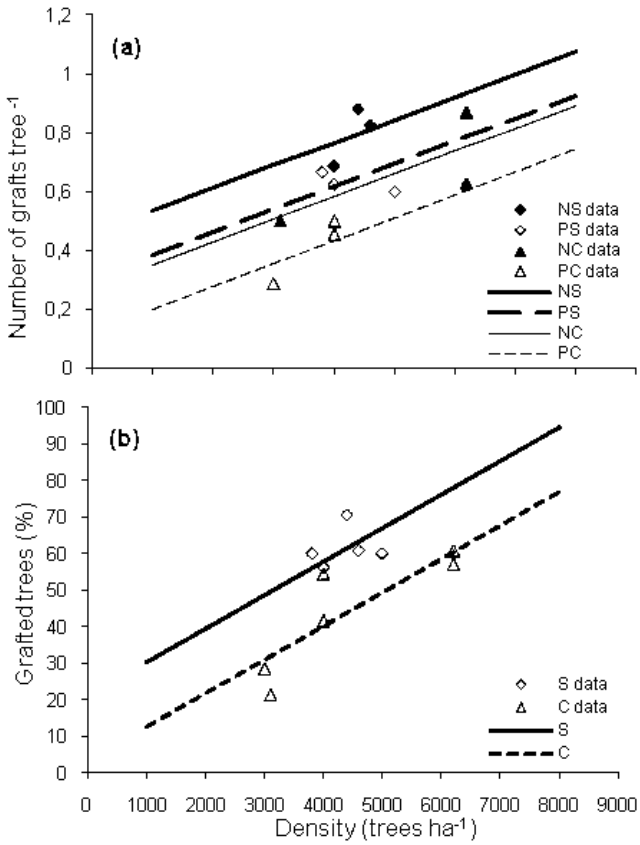

Figure 2.1 Prediction (a) of the number of grafts per tree (equation [1]) in relation to stand type, soil type and stand density, and (b) of the percentage of grafted trees (equation [2]) in relation to soil type and stand density.

In five naturally regenerated stands (N1, N2, N3, N4, and N5) and two artificially regenerated stands (P1 and P6), graft presence was negatively correlated with the distance between trees. Logistic regressions were not significant for sites N6, P4, and P5. Distance between grafted trees decreased with stand age ($P = 0.045$) but increased with basal area of the smallest tree within a grafted pair ($P < 0.001$) (Table 2.4). Average distance between grafted trees was greater in clayey compared with sandy soils in the artificially regenerated stands and with either sand or clay in naturally regenerated stands ($P = 0.006$) (Tables 2.4 and 2.5; Fig. 2.2). Stand density did not affect distance between grafted trees ($P = 0.573$) (Table 2.4).

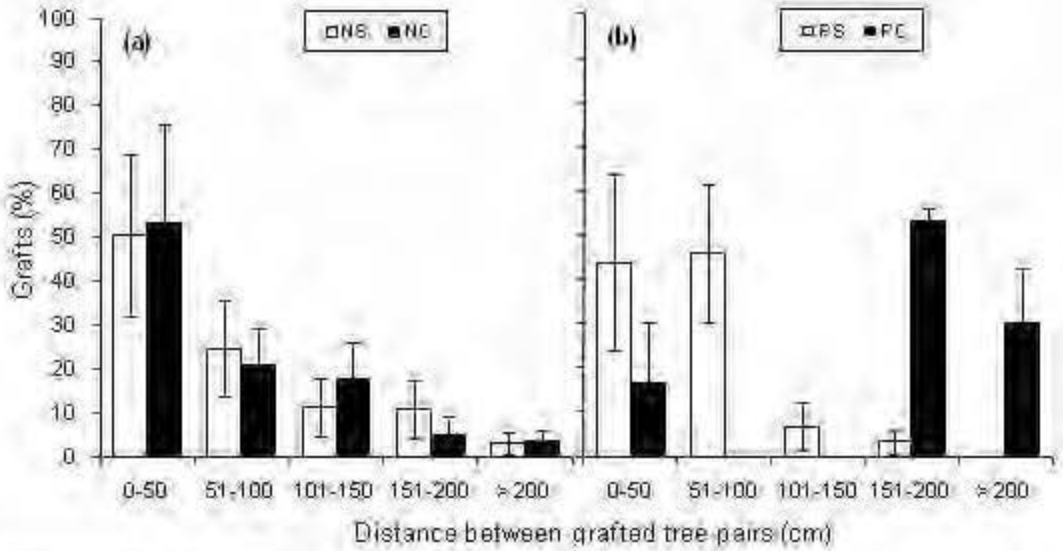

Figure 2.2 Mean percentages of root grafts for (a) naturally (N), and (b) artificially regenerated stands (P) within each distance class between grafted trees according to stand type and soil type (S for sand and C for clay). Error bars are ±1SE.

Age of trees at the time of graft formation ranged from 1 to 90 years across all sites (Fig. 2.3). Stand type, stand age, tree size, distance between trees, and the soil × stand interaction were all significant predictors of tree age at the time of graft formation (Table 2.4). Root grafts established when trees were older in naturally regenerated (Fig. 2.3a; Table 2.4) compared with artificially regenerated stands (Fig. 2.3b, Table 2.4) ($P < 0.001$). In

the oldest naturally regenerated stands (N3 and N6), 92% of root grafts formed when trees were 44 to 90 years old, while in the youngest naturally regenerated stands (N1, N2, N4, and N5), 95% of root grafts formed before the trees were 45 years old. Older ($P < 0.001$) and larger ($P = 0.005$) trees formed root grafts later than did younger and smaller trees (Table 2.4). Root grafts between trees that were located farther apart also formed later (in relation to tree age) than root grafts between trees close to one another ($P < 0.001$) (Table 2.4). Grafts formed at the same time in sandy or clayey artificially regenerated stands ($P = 0.696$) (Fig. 2.3b), while they formed 20 years earlier on average in clayey soils compared with sandy soils for naturally regenerated stands ($P < 0.001$) (Table 2.5; Fig. 2.3a). Root grafts also formed 29 years earlier in sandy artificially regenerated compared with sandy naturally regenerated stands ($P < 0.001$) (Figs. 2.3a and 2.3b), while the difference between naturally and artificially established stands (11 years) was much smaller in clayey soils ($P < 0.001$) (Table 2.5; Fig. 2.3). Stand density did not affect tree age at the time of graft formation ($P = 0.463$), but root grafts formed between older roots in denser stands ($P = 0.009$) and sandy soils ($P = 0.007$) (Table 2.4). There was no difference between the ages of grafted roots in artificially and naturally regenerated stands (Table 2.4). Similar to tree age at graft initiation, age of grafted roots was also affected by the age of trees ($P < 0.001$), by tree size ($P = 0.004$), and by the distance between trees ($P < 0.001$); roots of older, larger, and more widely spaced trees formed root grafts later than roots from younger, smaller, and closer trees (Table 2.4). The difference between the age of roots near the stem base and at the graft location varied from 0 to 24 years (mean ± SD = 3.10 ± 0.85 years) and was not significantly affected by any site characteristic (Table 2.4).

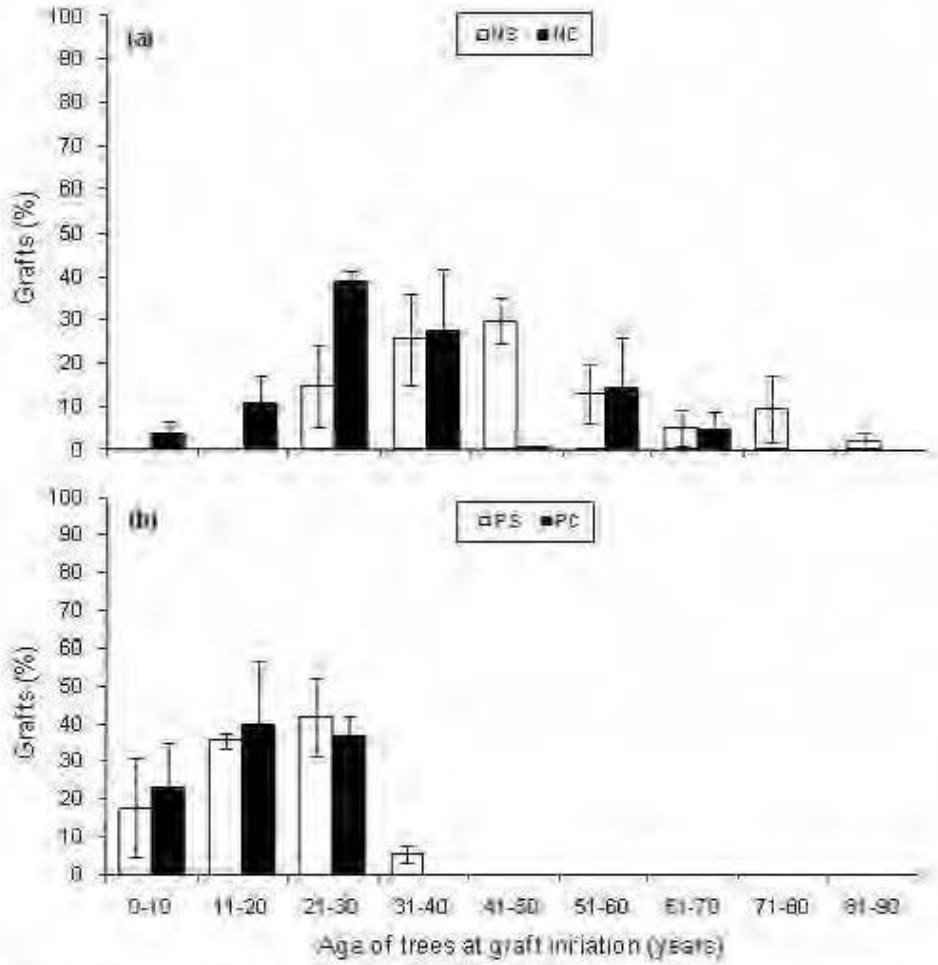

Figure 2.3 Age of trees at graft initiation time for natural (N; a) and artificially regenerated (P; b) stands in each soil type (S for sand and C for clay). Error bars are ± 1SE.

Root grafts required between 1 and 8 years to complete (mean = 2.7 years) (Fig. 2.4). Ninety percent of grafts took less than 4 years to complete (time where a complete and common growth ring was visible between the two roots). Grafts between closely spaced trees needed more time to complete formation than grafts between trees that were farther apart (P = 0.029). Root grafts formed faster in sandy soils (2.43 years) compared with clayey soil (2.97 years) (P = 0.007), while stand density, stand age, stand type, root surface, and the soil × stand interaction did not affect the time required to form a root graft (P > 0.05 for all explanatory variables) (Table 2.4).

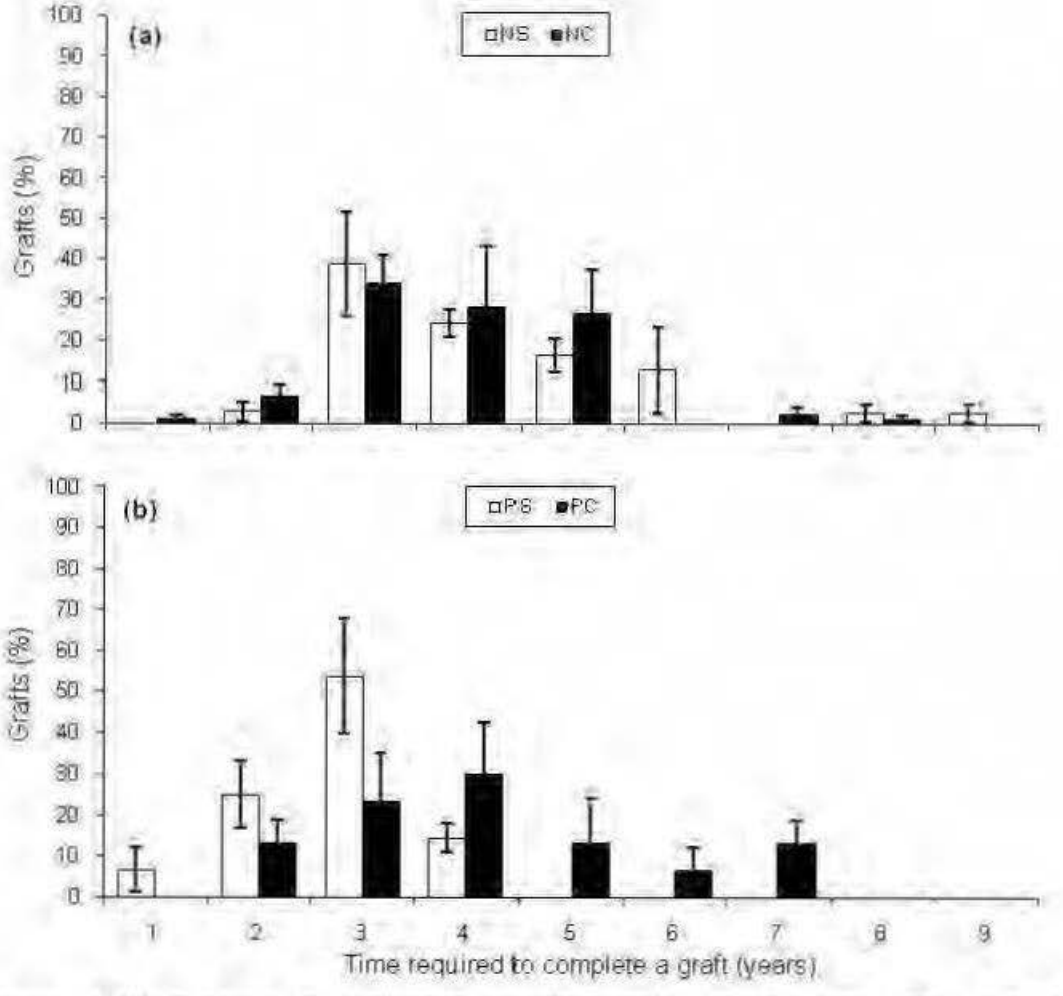

Figure 2.4 Mean percentages of time required for complete graft formation for each stand type ((a) naturally (N) versus (b) artificially (P) regenerated) and soil type (sand (S) versus clay (C)). Error bars are ±1 SE.

Between 250 and 1429 dead trees (snags or stumps of dead trees) per hectare (one to eight dead trees per site) were found during excavation (Table 2.6). The number of dead trees was highest in denser ($P = 0.057$) and naturally regenerated stands (985 dead trees·ha^{-1}) compared with artificially regenerated stands (472 dead trees·ha^{-1}) ($P = 0.03$) (Tables 2.4 and 2.6). The number of dead trees was similar for sandy and clayey sites ($P = 0.52$) (Tables 2.4 and 2.6). In nine of the 12 sites, we found some dead trees grafted with living trees and all of

their grafted root systems (100%) were entirely or partially alive at the time of the excavation (Table 2.6).

Table 2.6 Number of stumps from dead trees found during excavation and percent survival of grafted roots and of total roots from dead trees.

Plot	Number of stumps ha^{-1}	Number of stumps $plot^{-1}$	Number of stumps grafted to live trees	% of grafted roots alive	% of total roots alive
N1	513	2	1	100	67
N2	1250	5	1	100	100
N3	1000	5	1	100	100
N4	1429	8	3	100	100
N5	1053	4	0	0	0
N6	667	3	2	100	80
P1	667	2	2	100	20
P2	500	2	2	100	23
P3	667	2	2	100	100
P4	250	1	0	0	0
P5	250	1	0	0	0
P6	500	2	1	100	20

Note: N corresponds to naturally regenerated stands and P to artificially regenerated stands.

2.5 Discussion

A high level of intraspecific root grafting was found for jack pine, both in postfire naturally regenerated and in artificially regenerated stands. Root grafts were found in all excavated sites (Table 2.1), suggesting that root grafts likely exist in most jack pine stands. Pine species are reputed for their capacity to form root grafts; percentages of grafted trees reached 30% in mature *P. contorta* (Fraser et al. 2005), 50% in *P. strobus* (Bormann 1966), and up to 90% in *P. resinosa* stands (Horton 1969). Various researchers have proposed that the proximity of trees is a more relevant indicator of root grafting frequency than is stand density (Fraser et al. 2005; Gordon 1976; Külla and Lõhmus 1999; Reynolds and Bloomberg 1982), since stands may present the same density but the spatial arrangement of trees could be different. Our results indeed suggest that proximity of trees is a better predictor of root

grafting than stand density; although density increased root grafting frequency (models PERCENT and NUMBER), it did not affect distance between grafted trees (model DISTGRA) (Table 2.4). This indicates that stand density poorly reflected spatial distributions of individuals within stands. Distance between trees was also a better predictor than stand density in explaining the timing of root graft formation and the time required to complete graft formation (models AGETREE, AGEROOT, and LENGHT) (Table 2.4).

Sandy soils increased the occurrence of root grafting (number of grafts per tree, percentage of grafted trees), although many root grafts were also found in clay soils (Table 2.4; Fig. 2.1). More abrasive, coarse-textured soils may indeed be more efficient in breaking root bark away so that cambia contact to form root grafts (Cook and Welch 1957), thereby increasing root grafting frequency. Sandy soils also increased the speed of graft formation, while it took longer to complete grafts between trees that were close to one another (model LENGTH) (Table 2.4).

Carbohydrate transfers decrease with increasing distance between grafted trees and preferentially travel from large to small trees within a graft (Armson and Van den Driessche 1959; Fraser et al. 2006; Stone and Stone 1975). Our results showed that distance between grafted trees was affected by basal area of the smaller tree (Table 2.4), suggesting that root grafts would be preferentially formed with a neighbouring tree if it were smaller. Perhaps dominant and suppressed trees produce secondary metabolites in different proportions, allowing roots to communicate in the same way that chemical inhibitors are produced to prevent root contact (Reinartz and Popp 1987). The fact that distance between grafted trees was only affected by basal area of the smaller tree could also have been a result of root length, since small trees may not have sufficiently long roots to reach the roots of distant trees. Consequently, two small trees could not form root grafts unless they were very close to one another, although the roots of a small tree could be reached by those of a larger one. Factors other than root length are undoubtedly at play, since not all roots that came into contact formed root grafts. Indeed, we observed cases where roots of large trees passed through the root system of two or three close trees before forming a root graft with a more distant individual.

Since trees in artificially regenerated stands are generally distributed more evenly and relatively far apart than individuals in naturally regenerated stands, roots have to travel greater distances to encounter roots extending from other trees. This probably explains why the number of root grafts per tree was smaller in artificially compared with naturally regenerated stands (Tables 2.1 and 2.4; Fig. 2.1a). The fact that the percentage of grafted trees per site was similar in plantations and natural stands (Tables 2.1 and 2.4) was unexpected, however. Interestingly, distance between trees was not as good a predictor of root grafting in artificially regenerated stands (logistic regression results), where many root grafts were found between trees located far from one another (Fig. 2.2b). This result suggests that root grafting constitutes a real adaptative trait for this species, i.e., that root grafts are integral to stand dynamics in jack pine. The fact that average distance between grafted trees was significantly greater for clayey versus sandy artificially regenerated stands is probably due to the greater initial spacing of trees in clayey plantations. It could be argued that the greater number of root grafts per tree found in naturally regenerated stands is due to our artificially regenerated stands being younger than the naturally regenerated stands (Fraser et al. 2006). Yet, our results suggest that grafts formed early in stand development and that they disappeared with natural self-thinning and were replaced by younger root grafts in naturally regenerated stands. When comparing only naturally regenerated stands, we indeed found that root grafts in younger stands formed earlier than in older stands (Table 2.4). For example, no root grafts had formed before 45 years for the 90-year-old stand, while 95% of root grafts found in stands <60 years old were formed before 45 years. It is well known that post fire naturally regenerated jack pine stands usually have very high initial seedling densities (as high as 25 000 seedlings·ha^{-1}: (Gauthier et al. 1993; Lavoie and Sirois 1998; Van Damme and McKee 1990) and that heavy natural self-thinning occurs between 15 and 30 years immediately following crown closure (Smith 1997). It is thus likely that the first root grafts formed in naturally regenerated stands had disappeared with the death of trees during this self-thinning phase. Conversely, initial densities in artificially regenerated stands were much lower, and intertree competition and mortality were also probably low. Consequently, we found more dead stumps in naturally regenerated compared with artificially regenerated stands (Table 2.4); some showed grafts (grafts with other dead trees) that were not tallied because their degree of decay prevented accurate dating. Moreover, postfire seedling

regeneration of jack pine in sandy soils is usually better than in clayey soils (Bell 1991), which suggests that self-thinning rates were higher in sandy, naturally regenerated stands (Morris 2003). It would explain why we found that root grafts formed a little later in sandy compared with clayey, naturally regenerated stands (Fig. 2.3a). It is also plausible that root connections accelerate self-thinning in natural stands (Krasny and Johnson 1992). Due to their larger crown, larger members of a communal root system may be able to establish gradients that cause water to move preferentially towards them, at the expense of less vigorous trees, thereby hastening their death (Graham and Bormann 1966). Vegetatively regenerating species such as *Populus tremuloides* Michx., where most trees are interconnected through their parental roots (DesRochers and Lieffers 2001a, b), are indeed reputed for their rapid natural self-thinning (Bella and Yang 1991; Krasny and Johnson 1992). Forces other than transpiration, however, are involved in the transport of water through a root complex, since dye was also observed to move from living trees to stumps (Greenidge 1955).

Complete excavation of root systems allowed us to uncover many stumps of trees that had died and rotted away (Table 2.6). Most of their roots, however, remained alive when they were grafted with standing trees. This could be seen as an example of cooperative relationship within a species to assure that soil resources on a site remain within the species and prevent roots or seedlings of another species from capturing the space, even after trees previously occupying that space have died. However, some interspecific root grafts have been found, albeit very rarely, and thus were never studied in much detail (Graham and Bormann 1966). Root grafting could also be considered as a case of parasitism, if the biomass of dead or suppressed trees constitutes an excessively large photosynthate sink relative to benefits accrued from having a larger absorbing surface and stronger anchorage (Loehle and Jones 1990).

In conclusion, although site conditions affected root grafting frequency and timing, root grafts were found in all excavated sites. Root grafting frequency and the speed of graft formation were greater in sandy soils, probably caused by greater abrasiveness of sand-sized compared with clay-sized mineral particles. Proximity of trees increased root grafting frequency, although grafts between distant trees were also found, especially in artificially

regenerated stands. Our results suggest that root grafts are formed early in stand development. Thus, even if jack pine trees initially begin life as individual seedlings in naturally and artificially regenerated stands, this relatively high level of root grafting produces stands where adult trees are extensively interconnected with one another. Stands may thus behave more as a functional unit than as a group of individual trees. Since root grafts allow trees to potentially share resources (photosynthates and water) and pathogens, they likely influence tree mortality, stand structure, and forest dynamics. It thus seems important to consider root grafting frequency to anticipate management consequences on tree and stand development.

CHAPITRE III

MOLECULAR ANALYSIS OF NATURAL ROOT GRAFTING IN JACK PINE (*PINUS BANKSIANA*) [2]

Emilie Tarroux, Annie DesRochers, Krassimir Naydenov and Francine Tremblay

[2] Soumis à Molecular Ecology en Octobre 2010.

3.1 Abstract

Natural root grafting has been observed in more than 150 tree species, where up to 90% of trees could be interconnected within a stand. It is not known why root grafting is very frequent in some species and not in others, or why not all roots that cross form root grafts. A high degree of intraspecific root grafting was previously found in jack pine (*Pinus banksiana*) stands, ranging from 21 to 71% of trees grafted with one another (Tarroux and DesRochers 2010). Consequently, we investigated genetic diversity of the grafted and non-grafted trees to determine if root grafting could be explained by the genetic relatedness of trees, i.e., if trees that were more similar genetically were also more susceptible to form root grafts with one another. Seven 40-60 m^2 plots were hydraulically excavated in four natural stands and three plantations of jack pine in the western boreal forest of Quebec, Canada. We selected eight microsatellite markers showing sufficiently high variability to assess genetic differences between individuals (cumulative expected *PI*s = 1.5×10^{-8}). Our analyses revealed that root grafting was influenced by the spatial distance ($P < 0.001$), and less importantly, by genetic distance ($P = 0.0806$) between individuals. Site characteristics (distance between trees, soil type, stand type) influenced root grafting frequency (the mean number of grafts per tree and/or the percentage of grafted trees) more strongly than genetic diversity between individuals, summarized by the fixation index (F_{IS}, $P > 0.05$).

3.2 Introduction

The concept of trees forming discrete physiological entities is still widely accepted among scientists. A number of tree species, however, have been shown to be interconnected through their root systems (Fraser et al. 2005; LaRue 1934; Newins 1916; Tarroux and DesRochers 2010; Tarroux et al. 2010). This phenomenon is especially common in clonal species such as aspen that produce stands where many trees are interconnected through their parental root systems (DesRochers and Lieffers 2001a; Jelínková et al. 2009). Natural root grafting is also observed between non-clonal tree species and has been reported in more than 150 species (Bormann 1966), particularly in pines (*Pinus* spp.) from around the world (Armson and Van den Driessche 1959; De Byle 1964; Eis 1972; Fraser et al. 2005; Horton 1969; LaRue 1934; Tarroux and DesRochers 2010; Thomas et al. 1999). Hence, even if trees are traditionally considered as distinct entities competing with each other for resources, root connections imply that trees are not independent of one another (Loehle and Jones 1990). Interconnected trees can share resources (Bormann 1961; Fraser et al. 2006; Kuntz and Riker 1956; Stone and Stone 1975) and, in turn, enhance the survival of suppressed trees, which are supported by their connected neighbours (Bormann 1966; Fraser et al. 2006; Graham and Bormann 1966). Overlapping and joined root systems can also give trees better wind stability (Basnet et al. 1993; Coutts 1983a; Keeley 1988; Kumar et al. 1985).

The idea that root grafting is coincidental or constitutes an adaptive trait is still controversial (Loehle and Jones 1990). Some species exhibit this trait to such a marked degree that intraspecific competition concepts would need be revised to incorporate frequent non-competitive relationships such as root grafting. Observed differences in competence for root grafting among species has often been explained by the degree of genetic relatedness between trees (Loehle and Jones 1990; Stone 1974). A greater degree of genetic similarity is generally predicted to lead to a greater probability of root grafting, as grafts within trees of the same genotype are reported to be far more common than between trees of the same species (Loehle and Jones 1990). In addition, root grafting is reported to be especially frequent in species known for their genetic uniformity (Stone 1974), such as *Pinus resinosa* Sol ex Aiton (Boys et al. 2005). Yet root grafting also has been frequently encountered in species exhibiting higher levels of genetic diversity. For example, our work in *Pinus*

banksiana Lamb. stands revealed a high level of intraspecific root grafting, ranging from 21 to 71% of trees grafted within each 40-60 m^2 plot (Tarroux and DesRochers 2010). Genetic distance between grafted and non-grafted trees, in fact, has rarely been measured because it requires laborious and costly excavations, as well as suitable molecular markers to determine individual genetic identity. Recently, Jelínková et al. (2009) showed that for a clonal tree species, viz., trembling aspen (*Populus tremuloides* Michaux), between-clone root grafting was just as frequent as within-clone root grafting.

When root grafting occurs, a callosity is produced at the contact point between the two roots (Bormann and Graham 1959; Eis 1972; Graham and Bormann 1966). It is not clear how wood is produced during root graft formation; whether the wood originates from the cambium of one tree or from both is not known. To our knowledge, this is the first attempt to determine the origin of root grafts at the molecular level. In the present work, *P. banksiana* stands were used to test the hypotheses that 1) genetic proximity will be higher for grafted than for non-grafted individuals and 2) tissue around the graft is produced by the two grafted trees, with mosaics occurring at the grafting point. Microsatellite loci were used to produce genetic profiles for grafted and non-grafted *P. banksiana* trees since the level of variability of these markers was sufficiently high to assess genetic differences between individuals. DNA was extracted from trees and from some of the root grafts. For each studied graft, its genotype was compared to those of the two grafted trees.

3.3 Methods and materials

3.3.1 Study area

Sampling was carried out in the western balsam fir-paper birch (*Abies balsamea – Betula papyfera*) bioclimatic domain (Grondin 1996) of the boreal forest of northwestern Quebec, Canada (Fig. 3.1). Sites were located between 47°58'N and 48°44'N, and between 77°6'W and 79°25'W (Fig. 3.1). The climate of the region is cold-continental with an average daily temperature of 1.2°C and average yearly precipitation of 918 mm (rainfall, 670 mm; snowfall, 248 mm (Environment Canada 2010). The region incorporates a large physiographic unit that is characterized by lacustrine deposits from the maximum post-

Wisconsonian extension of proglacial lakes Barlow and Objibway (Veillette 1994). While clay is the dominant deposit throughout the plain (glaciolacustrine deposits), sand (eskers) is also encountered (glaciofluvial deposits). Four sites were stands that had naturally regenerated following fire (STM, DUP, STMO, STMA), while three stands were selected that had been artificially regenerated following clear-cutting (LOU, LOUV, BER; Table 3.1, Fig. 3.1). Spacing in the plantations varied from 1.7 x 3 m (LOU, LOUV) to 2 x 2 m (BER). The study plots ranged in size from 40 to 60 m^2, so that at least 10 trees were included (Table 3.1). The sites were located near a water source (pond, lake, river) so that we could carry hydraulic excavation of the root systems (Tarroux and DesRochers 2010).

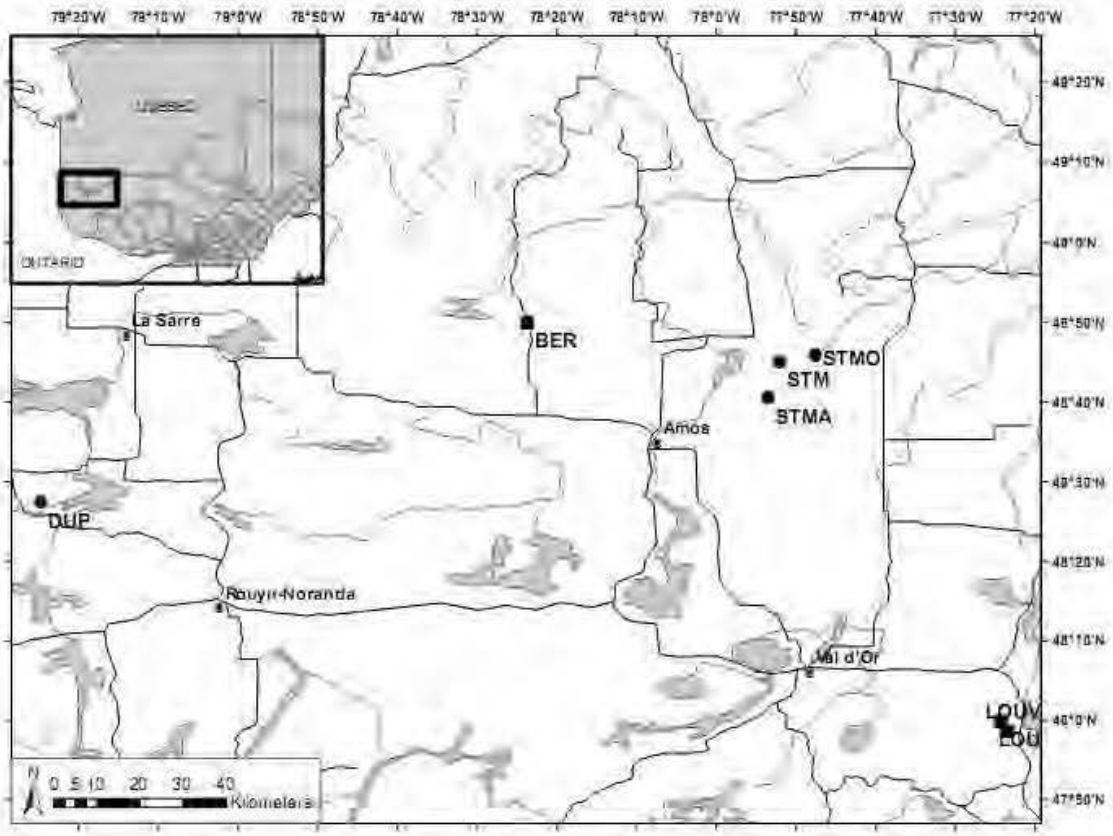

Figure 3.1 Map showing the 7 populations studied in Abitibi-Témiscamingue, Québec. Natural stands are represented by a black circle, while the plantations are represented by a black square.

Table 3.1 Characteristics of the seven excavated plots.

Site	STM	DUP	STMO	STMA	LOU	LOUV	BER
Size of excavated area (m^2)	60	45	50	55	40	40	40
Stand	N	N	N	N	P	P	P
Soil	C	C	S	S	S	S	S
Stand age (years)	45	75	90	65	35	35	35
Density (trees ha^{-1})	6200	3100	4600	4500	3800	4000	5000
Average height (m)	12	20	14	13	16	14	9
Number of excavated trees	35	14	23	25	15	16	20
Number of trees sampled for genetic analysis	24	10	16	13	11	11	19
Number of grafted trees sampled for genetic analysis	15	1	9	7	8	6	8
Number of grafts	22	7	19	18	10	10	10
Number of grafted trees	20	3	14	15	9	9	9
Mean number of grafts per tree	0.63	0.50	0.83	0.72	0.67	0.67	0.63
Percentage of grafted tree (%)	57.14	21.43	60.87	60.00	60.00	60.00	56.25

Note: Stand is for the type of stand (N, natural stands; P, plantations). Soil is for the soil type (S, sand; C, clay).

3.3.2 Field sampling

In summer 2007, trees were felled with a chain saw and cross-sectional disks were cut at ground level (0 m) and at breast height (1.30 m) for age determination. Plots were then excavated with a high pressure water spray using a forestry water pump (Mark III, Wajax, Lachine, QC), to expose the entire root systems and root grafts. Height and diameter at breast height (DBH) of each tree were measured. All trees (alive or stumps), roots and grafts were carefully mapped and distances between all trees in each plot were recorded. A cross-sectional disk was taken from each root with a diameter of at least 2 cm for age determination. All suspected grafts were checked in the field by removing bark and performing a partial dissection to confirm a common wood layer between the two roots.

Diameter of grafted roots was measured. Trees were considered as grafted when there was vascular continuity between their roots. Only true intraspecific grafting that involved the morphological union of cambium, phloem, and xylem (Graham and Bormann 1966) was recorded. Detailed protocols for hydraulic excavation, field sampling, and dendrochronology analysis are detailed in Tarroux and DesRochers (2010). Of the 148 excavated trees, we selected 104 for genetic analysis. Only living and vigorous trees were sampled. We also sampled the wood from callus tissue of six grafts. Using a scalpel, cambial tissue was taken from the graft callus or from the base of tree trunks. Samples were kept chilled on ice and brought to the laboratory where they were conserved at -86°C until DNA extraction.

3.3.3 Genetic analysis

Wood samples were ground and stored at -20°C. GenElute Plant Genomic DNA Miniprep Kit of Sigma-Aldrich (product Code: G2N350; Oakville, ON, Canada) was used to isolate pure DNA from cambial tissue. DNA extraction was done following manufacturer's instructions. We used 12 microsatellite primer pairs, which were labelled with fluorescent dyes (FAM-6, NED and VIC), to genotype individuals. The selected primers were designed specifically for pine species: Pde3, Pde5, and Pde7 of (Lian et al. 2000); and Pde13, PtTX-3118, PtTX-3020, PtTX-3030, PtTX-2123, PtTX-2090, PtTX-3025 and PtTX 4001 PtTX 4009 (Al-Rabab'ah and Williams 2004; Auckland et al. 2002). Despite several attempts, we could not obtain complete and unambiguous amplification for PtTX-3025 PtTX 4001, PtTX 4009, and Pde5. Only microsatellite markers that provided good and reproducible amplification were further used in the analysis. Characteristics of the different microsatellite markers like sequence, dye, observed range and annealing temperature are given in Table 3.2. The standard polymerase chain reaction (PCR) was performed in a Perkin-Elmer 9700 thermocycler (Applied Biosystems, Foster City, CA, USA) using 2.6-3 µL DNA extract and a master mix consisting of 6.6 µL HotMaster buffer (Eppendorf North America, Westbury, NY, USA), 100 µg/µL gelatine, 1% DMSO, 50 µM for each dNTP, 100 nM of each forward and backward primer, 0.325 U HotMaster Taq DNA Polymerase (Eppendorf NA), and 2.5-3 mM $MgCl_2$ in a total volume of10 µL. The standard temperature profile was 9 min at 95°C for Taq activation and 30 cycles of 30 s denaturation at 94°C, 30 s at the annealing temperature (depending on the primers used), and 1 min at the extension temperature at

63.5°C. A final extension of 10 min at 72°C was used. The PCR product (0.6 µL) was mixed with 10 µL Hi-Di Formamide and 0.6 µL Tamra 500 size standard (Applied Biosystems, Westborough, MA, USA) and denatured for 5 min at 95 °C. Tubes were placed on ice and fragments were separated by capillary electrophoresis in 3130 Genetic Analyzer (Applied Biosystems).

Table 3.2 Names, primer sequence, marker dye colour, annealing temperature and observed allele size ranges of the 12 *P. banksiana* microsatellite markers that were used (Al-Rabab'ah and Williams 2004; Lian et al. 2000).

Locus	Primer sequences	Dye	Temp. (°C)	Observed range
PtTX-3118	CACGGCCCTTAGCTTTACCTT TTCTGATGGGGCAACTG	B	61	105-305
PtTX-3020	GTCGGGGAAGTGAAAGTA CTAGGTGCAAGAAAAGAGTAT	Y	69	101-197
PtTX-3030	AATGAAAGGCAAGTGTCG GAGATGCAAGATAAAGGAAGTT	G	61	257-326
PtTX-2123	GAAGAACCCACAAACACAAG GGGCAAGAATTCAATGATAA	G	58	182-202
PtTX-2090	CCCGCCTATTCCACCTA CTACACATTTCACCATAAGTCC	G	58	179-330
Pde3	GTTGATACAATCATTGTTGTAACAC CAAATATTTATATTCCCCTACGTG	G	58	70-145
Pde 7	TTGAGTGAGAGGACTCTAGGC AGGTAGACCCTATGGCGATG	Y	58	118-188
Pde13	AATATTCCTAACGACCCTATC TGTTTCTATATGCATGTATGAGTC	G	58	113-327
PtTX-3025	CACGCTGTATAATAACAATCTA TTCTATATTCGCTTTTAGTTTC	Y		
PtTX-4001	CTATTTGAGTAAGAAGGGAGTC CTGTGGTAGCATCATC	B		
PtTX-4009	ACCTTGACCTTGTAGAGC CTGTGTCCCTTTAGAGATAG	B		*Incomplete amplification*
Pde5	AACGCACCTTTCTCAATGCAC ATAAAGAGGCTACATGGTCCC	B		

Note: Primers are shown in the 5′–3′ orientation. The first primer used is the forward primer, while the second is the reverse primer. Suggested dye labelling B: Blue (FAM-6), Y: Yellow (NED) and G: Green (VIC) for analysis on the Applied Biosystems 3130 Genetic Analyser.

3.3.4 Data analysis

Microsatellite diversity and genetic analysis

Data were analyzed using DataCOLLECTION V3.0 software (Applied Biosystems). Genotypes were inferred from the individual size profiles of the nuclear DNA that we analyzed using GeneMAPPER V.3.7 software (Applied Biosystems). To determine the resolving power of the set of microsatellites that we used, the probability of identity (*PI*) was estimated (Peakall and Smouse 2006; Waits et al. 2001). It corresponds to the average probability that two independent samples will have an identical genotype. Estimates of *PI* assume that the population is randomly mating and that comparisons are between unrelated individuals. We also calculated *PIsibs* estimator as a conservative upper bound of the number of loci necessary to distinguish individuals (Namroud et al. 2005; Waits et al. 2001). Cumulative expected *PI*s corresponds to the probability of identity for multilocus and was calculated as the product of individual locus *PI* and *PIsibs*. The MICRO-CHECKER V.2.2.3 program of van Oosterhout et al. (2004) was used to test the null alleles and scoring errors due to large allele dropout and stutter peaks. The observed heterozygosity (H_o), expected heterozygosity (H_e), unbiased expected heterozygosity (UH_e), the average number of alleles (N), number of different alleles (N_a), number of effective alleles (N_e; Kimura and Crow 1964), Shannon's Allele Information Index (H'; Shannon 1948; Shermin et al. 2006) and *F*-statistics (F_{IS} and F_{ST}; Wright 1965) were calculated using GenAlEx V.6 (Peakall and Smouse 2006). In parallel, an analysis of molecular variance (AMOVA, Excoffier et al. 1992; Huff et al. 1993; Michalakis and Excoffier 1996; Peakall and Beattie 1995) was also done using GenAlEx V.6 (Peakall and Smouse 2006). The AMOVA used Φ_{PT} (a measure of population genetic differentiation analogous to F_{ST}) to describe the partitioning of genetic variation among and within populations.

Individual genetic analysis

To determine the factors influencing root grafting frequency, the relationships between the number of root grafts per tree (NUMBER model), the percentage of grafted trees per site (PERCENT model) and soil type (sand or clay), stand density, stand type (N or P), and the inbreeding coefficient within individuals relative to the subpopulation (F_{IS}) values

were analyzed with a mixed linear model using the `lme` function of the `nlme` library (Linear and Nonlinear Mixed Effects Models, (Pinheiro et al. 2008) in the R statistical environment (v. 2.7.2, (R Development Core Team 2008). F_{IS} was preferred to other F-statistics (as F_{ST}) because it corresponds to a measure of heterozygosity at the individual scale. We had previously found a positive relationship between root grafting frequency (number of grafts per tree and percentage of grafted trees) versus soil type, stand type, and tree density (Tarroux and DesRochers 2010). In the present work, we tested the influence of genetic diversity between individuals on root grafting frequency. To avoid 'sacrificial pseudoreplication' error that is incurred when data from different experimental units are treated as independent replicates and pooled in the same analysis (Hulbert 1984), sites were treated as random effects.

To compare trees, individual by individual genetic distance was calculated using GenAlEx 6 (Peakall and Smouse 2006). The relationship between the presence of a root graft (DISTANCE model) and both spatial and genetic distance between trees were then analyzed with a generalized linear mixed-effects model in R, using a `glmer` function in the in the `lme4` library (Linear mixed-effects models using S4 classes, (Bates and Maechler 2009). This model can implement logistic regression to test presence/absence of root grafts as a binary variable. Sites were considered as random effects. To link the grafts to the corresponding grafted tree, genetic profiles of the six collected grafts were studied: we measured the genetic distance between tissue removed on the grafts callus tissue and compared it to tissue from the trunks of the two grafted trees.

3.4 Results

3.4.1 Genetic diversity of *Pinus banksiana* stands

The average probability that two individuals presented the same multilocus genotype was very low. Cumulative expected *PI*s using the eight loci ranged from 1.6×10^{-3} (*PIsibs*) to 1.5×10^{-8} (*PI*). Among the microsatellites that we tested, loci PtTX-2090 and Pde13 were the most variable primers (*PI* = 0.022 and 0.016, respectively), while PtTX-3020 and Pde7 were the least variable (*PI* = 0.38 and 0.41, respectively). A total of 95 different alleles were

detected in the eight microsatellite loci. The number of alleles per locus (total N) varied from 6 for locus PtTX-2123 and Pde7 to 22 for locus PtTX-2190 (Table 3.3). The average number of alleles per population (N) ranged from 7.429 at locus PtTX-3030 and Pde13 to 15.000 at locus PtTX-2123 and PtTX-2090. The effective number of alleles (N_e) varied from 1.648 to 6.074. Levels of genetic diversity, which were measured in terms of average number of alleles, expected (UH_e), and observed heterozygosities (H_o), were generally high (Table 3.3). F_{IS} were positive, with four loci showing a deficiency of heterozygotes and significant deviations from Hardy–Weinberg equilibrium: Pde7 (F_{IS} = 0.390; P_{HW} = 0.001), Pde13 (F_{IS} = 0.442; P_{HW} < 0.001), PtTX-3030 (F_{IS} = 0.292; P_{HW} = 0.025); PtTX-2090 (F_{IS} = 0.120; P_{HW} = 0.025). Nevertheless, MICRO-CHECKER detected the presence of null alleles at these four loci (Table 3.3) and there was no evidence for large allele dropout or for scoring error due to stuttering. According to MICRO-CHECKER, the populations were in Hardy Weinberg equilibrium with loci Pde7, Pde13, PtTX-3030, and PtTX-3025 showing signs of a null allele. AMOVA showed that 85% of genetic variation was observed within-stands and 15% among stands (Table 3.4).

Table 3.3 Genetic diversity data of the eight microsatellite markers developed for genetic analysis of jack pine.

Site	Total N	N	N_a	N_e	H'	H_o	H_e	UH_e	F_{IS}	F_{ST}	Null alleles	P_{HW}
PtTX-3118	10	13.000 (2.708)	3.714 (0.808)	2.177 (0.255)	0.875 (0.173)	0.650 (0.119)	0.485 (0.085)	0.504 (0.088)	-0.344 (0.087)	0.097	NO	0.894
PtTX-3020	8	13.571 (2.662)	4.143 (0.459)	1.660 (0.070)	0.773 (0.063)	0.463 (0.042)	0.391 (0.024)	0.422 (0.028)	-0.174 (0.057)	0.038	NO	0.978
PtTX-3030	14	7.429 (1.462)	4.000 (1.091)	2.928 (0.710)	1.084 (0.241)	0.378 (0.134)	0.569 (0.104)	0.605 (0.110)	0.292 (0.253)	0.363	YES	0.025
PtTX-2123	9	15.000 (1.964)	5.143 (0.459)	2.968 (0.322)	1.240 (0.109)	0.777 (0.092)	0.631 (0.052)	0.654 (0.052)	-0.210 (0.090)	0.071	NO	0.346
PtTX-2090	23	15.000 (1.964)	9.143 (1.143)	6.074 (0.891)	1.894 (0.181)	0.698 (0.058)	0.797 (0.049)	0.826 (0.049)	0.120 (0.054)	0.100	YES	0.025
Pde3	10	14.571 (1.784)	3.571 (0.369)	2.005 (0.117)	0.839 (0.067)	0.592 (0.089)	0.491 (0.031)	0.510 (0.033)	-0.206 (0.158)	0.163	NO	0.000
Pde 7	10	10.143 (1.580)	3.571 (0.481)	1.648 (0.144)	0.706 (0.112)	0.196 (0.036)	0.364 (0.057)	0.389 (0.064)	0.390 (0.115)	0.070	YES	0.001
Pde13	24	7.429 (1.478)	5.429 (1.066)	3.700 (0.667)	1.390 (0.246)	0.354 (0.115)	0.652 (0.110)	0.695 (0.117)	0.442 (0.162)	0.300	YES	0.000

Note: Total N = total number of alleles; N = mean number of alleles, N_a = number of different alleles; N_e = number of effective alleles; H' = Shannon's information index; H_o = observed heterozygosity; H_e = expected heterozygosity; UH_e = unbiased expected heterozygosity, F_{IS} = inbreeding coefficient within individuals relative to the subpopulation, F_{ST} = inbreeding in subpopulations relative to the total population and P_{HW} is the test for Hardy–Weinberg equilibrium.

Table 3.4 Results of AMOVA estimating the percentage of genetic variation observed among and within populations.

Source	df	SS	Est. Var.	%
Among-Pops	6	133.599	1.091	15%
Within-Pops	98	606.001	6.184	85%

3.4.2 Individual by individual genetic analysis

Mean number of root grafts per tree and the percentage of grafted trees was significantly greater in sandy soils (P = 0.0281 and P = 0.0128, respectively) compared to clayey soils (Table 3.5). Trees growing in natural stands tended to have more root grafts than trees from plantations (P = 0.1063), but the percentage of grafted trees within stands was similar (P = 0.8496, Table 3.5). The percentage of grafted trees increased significantly with tree density (P = 0.0238) but not the mean number of grafts per tree (P = 0.2850, Table 3.5). F_{IS} significantly influenced neither the mean number of grafts per tree (P = 0.7084) nor the percentage of grafted trees (P = 0.6312, Table 3.5). As shown by results of `glmer` model, the presence of root grafts was strongly influenced by the spatial distance between trees (P <0.001) and only marginally affected by the inter-tree genetic distance (P = 0.0806, Table 3.5). The following equations can be used to predict the probability of root grafting presence according to genetic and spatial distances (R^2 =0.341, Fig. 3.2):

$$\hat{y}_i = \exp(\eta_i)/(1+ \exp(\eta_i))$$

where η_i = 2.98040 - 2.53740*(spatial distance) - 0.09569*(genetic distance).

Of the six genotyped grafts, only two matched perfectly with one of the linked trees. For the four other grafts, the smallest recorded genetic distance varied from 2 to 5 and the highest ranged from 3 to 9 (Table 3.6). According to the graft, genetic distance between the two grafted trees varied from 5 to 10.

Table 3.5 Results of the mixed-effects model based on individual scale. NUMBER and PERCENT models (`lme`) were respectively the models relating the number of grafts per tree and the percentage of grafted trees within plots versus the site characteristics and the inbreeding coefficient F_{IS}. DISTANCE (`glmer`) is the logistic regression relating the presence of a root graft against both spatial and genetic distance between trees. The factors, estimated values, standard errors, and P values are given for each model. Statistically significant values ($P < 0.05$) are given in bold.

	Factors	Estimated value	Standard error	P value
NUMBER	(Intercept)	0.4286340	0.14258268	0.0951
	standP	-0.1040475	0.06339364	0.2424
	soilS	0.2098601	0.06801363	0.0909
	density	0.0000298	0.00002870	0.4076
	F_{IS}	-0.0631267	0.14640381	0.7084
	(Intercept)	0.4184329	0.12002197	**0.0399**
	standP	-0.1094027	0.05306438	0.1313
	soilS	0.2131520	0.05768977	**0.0344**
	density	0.0000315	0.00002428	0.2850
	(Intercept)	0.5650000	0.04411160	**< 0.001**
	standP	-0.1183333	0.05694783	0.1063
	soilS	0.2100000	0.06238322	**0.0281**
PERCENT	(Intercept)	-2.755881	17.666062	0.8904
	standP	1.689904	7.854503	0.8496
	soilS	21.636023	8.426921	0.1241
	density	0.009112	0.003556	0.1245
	F_{IS}	-8.589779	18.139502	0.6825
	(Intercept)	-2.431300	14.536951	0.8778
	soilS	22.624898	5.833391	**0.0304**
	density	0.009036	0.002923	0.0536
	F_{IS}	-7.825170	14.690932	0.6312
	(Intercept)	-3.878891	12.939012	0.7793
	density	0.009283	0.002615	**0.0238**
	soilS	22.645290	5.285241	**0.0128**
DISTANCE	(Intercept)	2.98040	0.96893	**0.0021**
	distance	-2.53740	0.36873	**< 0.001**
	GD	-0.09569	0.05477	0.0806

Note: Stand is for the type of stand (N, natural; P, plantation). Soil is for soil type (S, sand; C, clay). Density is the number of stems per hectare. F_{is} is the inbreeding coefficient within individuals relative to the subpopulation. Distance is the spatial distance in meters. GD is the genetic distance measured with GenAlEx 6 computer program (Peakall and Smouse 2006).

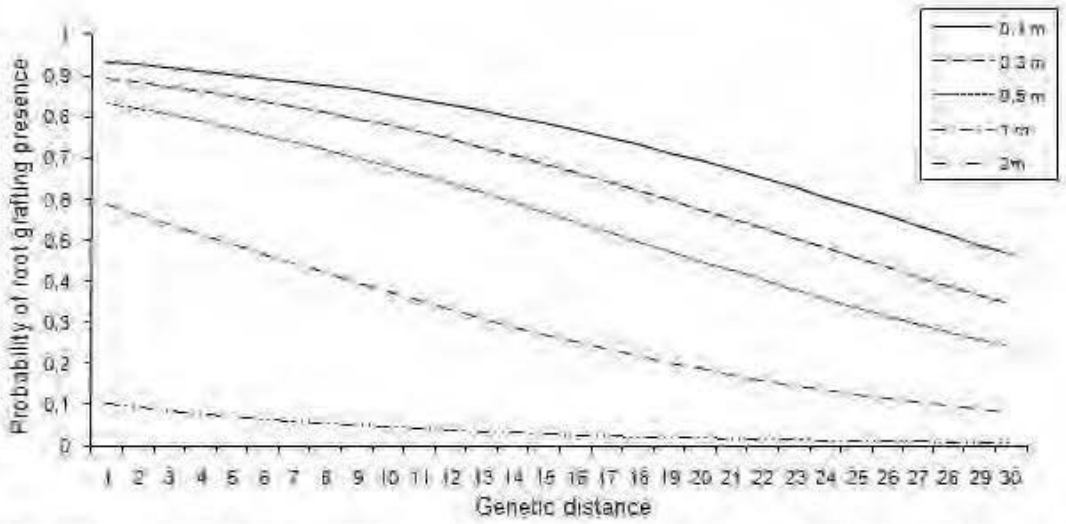

Figure 3.2 Probability of grafting presence according to spatial (m) and genetic distances between trees. The genetic distance was measured with GenAlEx 6 software (Peakall and Smouse 2006), using the "Codom-Genotypic" option.

Table 3.6 Genetic distance (GD) between each graft and the two linked trees.

SITE	GD	GD1	GD2
STMO	8	0	8
	5	0	5
	8	3	7
STM	10	3	6
LOU	6	5	9
DUP	5	1	3

Note: GD-tree is the genetic distance between the two grafted trees measured with GenAlEx 6 computer program (Peakall and Smouse 2006). GD1 and GD2 are the genetic distance between the graft and the two grafted trees. GD1 corresponds to the minimum distance recorded and GD2 to the maximum.

3.5 Discussion

Our study showed that a greater degree of genetic similarity slightly contributed to increasing the probability of root grafting between individuals (Fig. 3.2). This result partially supports the notion that root grafting is more likely to occur between trees having greater genetic similarity (Loehle and Jones 1990, Stone 1974). However, in spite of great genetic distances, trees formed root grafts because spatial distance between trees was a much more

significant factor explaining root grafting (Table 3.5). Moreover, soil type, stand type (plantation or natural), and tree density also greatly affected root grafting frequency (mean number of grafts per tree and/or percentage of grafted trees per plot). Therefore, site characteristics and physical distance between trees had an overall greater influence on root grafting frequency than genetic distance. These results are consistent with those reported by Jelínková *et al.* (2009), where the major contributing factor to root grafting in aspen also was proximity of trees.

The ecological significance of root grafting is still mostly unknown, and it is not clear if root grafting is a beneficial adaptive trait or an accidental event (Loehle and Jones 1990). If root grafting constituted an evolutionary advantage, genotypes that tend to form root grafts would be favoured (Loehle and Jones 1990). Root grafting increases mechanical stability of stands by linking root systems together (Basnet et al. 1993; Coutts 1983b; Graham and Bormann 1966; Keeley 1988; Kumar et al. 1985) and enhances tree growth and chances of stand survival by facilitating resource acquisition (Basnet et al. 1993; Bormann 1966; Loehle and Jones 1990). Although there is no empirical proof that root grafting occurs as a response to wind stress, the results we present here and previously (Tarroux and DesRochers 2010) suggest that root grafting increases in nutrient-poor sites. Root grafting frequencies were greater in sandy soils compared to clayey soils (Table 3.5), which are generally more nutrient-rich. Root grafting could thus be seen as an adaptive response to stressful environments.

In the present study, only two of the six samples collected from the graft tissues corresponded to the same genotype of one of the two grafted trees, while the others were a mixture of both genotypes. This suggests that wood produced around root grafts was synthesized by the two trees. It could be seen as an example of genetic mosaics (Pineda-Krch and Lehtilä 2004; Thomas et al. 1999). The ability of some species (e.g., red algae, fungi) to fuse in the early stages of development and to produce chimeric organisms is not uncommon and raises questions about the validity of the physiological unity and autonomy concept (Santelices 1999, 2004). Intra-organismal genetic heterogeneity (IGH) is often viewed as a biological aberration, but an increasing number of studies have demonstrated that genetically homogenous organisms may rather be rare (Pineda-Krch and Lehtilä 2004). IGH seems less

common in terrestrial plants, but genetic mosaics or chimerism has already been reported in a number of angiosperms and gymnosperms (Korn 2002; Pineda-Krch and Lehtilä 2004; Thomson et al. 1991). As IGH is often associated with cancerous growths (Pineda-Krch and Lehtilä 2004), the formation of callus tissue around the grafts could be a consequence of the fusion of wood of the two (or more) genotypes from individuals that formed a root graft.

Microsatellite markers developed for *Pinus* species have the potential to produce unique DNA profiles in *P. banksiana* trees. The genetic variability found with 4 or 5 microsatellite loci was sufficient to produce unique DNA profiles for trees; with 8 loci, the probability of two individuals in the population having the same genetic profile was extremely low. According to Waits et al. (2001), a *PI* between 0.001 and 0.0001 should be sufficiently low for genetic analysis that require individual identification (Namroud et al. 2005). Genetic analysis using microsatellites represents a more informative tool than do allozymes (Gullberg et al. 1985) or other markers (RAPD, AFLP, RFLP; (Kirst et al. 2005; Rajora and Rahman 2003) to discriminate individuals. Hedrick (1999) has shown that, with modern hypervariable markers characterized by many alleles, F_{ST} values can be considerably lower than for genetic markers with very few alleles. In this study, F_{ST} values was very high (mean value of 0.17) especially for the loci with the highest number of alleles (PtTX-3030, and Pde13). Mean observed heterozygosity (H_o = 0.54) corresponds to values that have been observed for other pine species using nuclear microsatellite markers, including *Pinus radiata* D. Don (H_o = 0.62, (Smith and Devey 1994) or *Pinus strobus* L. (0.51, (Echt et al. 1996). Other studies using nuclear microsatellites also demonstrated the high discriminating power of these kind of markers and their high scientific potential for parentage analysis (Hedrick 1999; Kirst et al. 2005; Kutli and Williams 2001), including applications in forensic botany (Craft et al. 2007).

CHAPITRE IV

DOES NATURAL ROOT GRAFTING AFFECT GROWTH OF JACK PINE (*PINUS BANKSIANA*)? [3]

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[3] Soumis à American Journal of Botany en juillet 2010

4.1 Abstract

- Trees are traditionally considered as distinct entities even though they can share a communal root system through root grafts, which are morphological unions between two or more roots. Little is known regarding the ecological significance of natural root grafting but as grafted trees can share resources and secondary compounds, growth of linked trees can directly be affected by the presence of root grafts. Traditional forest ecology concepts may have to be revised to include direct interactions between connected trees.

- We hydraulically excavated six 30-50 m^2 plots (three natural stands and three plantations). We measured yearly radial growth and determined the influence of root grafting on radial growth of grafted trees.

- During periods of root graft formation, root grafting tended to reduce radial growth of jack pine trees, after which growth generally increased. The influence of root grafting on growth was more significant in natural stands, where root grafting frequency was higher than in plantations.

- These results suggest that root grafting initially is an energetically costly process but that it is afterward non-prejudicial and maybe beneficial to tree growth. The use of a communal root system allows for a maximum use of resources by redistributing them among trees, leading to increased tree growth.

4.2 Introduction

An increasing number of studies suggest that in some environments (stressed or resource-limited), positive interactions or facilitation play a more important role than competition (Bertness and Callaway, 1994; Bruno, Stachowicz, and Bertness, 2003; Fajardo and McIntire, 2010). However, trees are still considered as distinct entities competing with each other for resources. It is nonetheless accepted that trees can indirectly act on their neighbors by modifying their common environment through root exudation, mycorrhizae or altered soil conditions through shedding of plant parts (Woods and Brock, 1964; Brownlee et al., 1983; Pallardy, 2008). Moreover, since many tree species can share a communal root system (Graham and Bormann, 1966), it is likely that they can also directly affect growth of adjacent trees. Some species such as poplars (*Populus* spp.) regenerate by root suckering, thereby producing stands where most trees are interconnected through their parental roots (De Byle, 1964; DesRochers and Lieffers, 2001). Other species where trees are originally distinct (i.e., seedlings) can later form root grafts, which are morphological unions between two or more roots (Eis, 1972; Tarroux and DesRochers, 2010). Although natural root grafts are often viewed as natural curiosities or rare events (Graham and Bormann, 1966), they commonly occur and have been observed in more than 150 species (Bormann, 1966), particularly in pine species (*Pinus* spp.) around the world (LaRue, 1934; Armson and Van den Driessche, 1959; Bormann, 1966; Graham and Bormann, 1966; Horton, 1969; Wood and Bachelard, 1970; Eis, 1972; Stone and Stone, 1975; Fraser, Lieffers, and Landhäusser, 2005, 2006; Tarroux and DesRochers, 2010).

Little is known regarding the ecological significance of natural root grafting, although many agree that it could confer evolutionary advantages to forests stands and that root grafting is a real adaptive trait rather than an accidental consequence of roots crossing one another (Loehle and Jones, 1990; Basnet et al., 1993; Tarroux and DesRochers, 2010). For example, joined root systems can give trees better wind stability (Graham and Bormann, 1966; Coutts, 1983; Kumar, Kulkarni, and Srimathi, 1985; Keeley, 1988; Basnet et al., 1993), preventing weaker trees from blowing down and opening up stands, which could then make stands more susceptible to windthrow (Franklin and Forman, 1987). In this case, root grafting could be seen as an example of cooperative behavior (Kozlowski, Kramer, and Pallardy,

1991). Interconnected trees can also share resources like water, photosynthates or nutrients (Bormann, 1966; Stone and Stone, 1975; Fraser, Lieffers, and Landhäusser, 2006), which could enhance survival of suppressed trees through support by their connected neighbors (Bormann, 1966; Graham and Bormann, 1966; Fraser, Lieffers, and Landhäusser, 2006). The theory that root grafting can increase the absorptive capacity or the area of coverage for nutrition by roots and, thus, lead to maximal exploitation of resources has already been mentioned (Bormann, 1966; Loehle and Jones, 1990; Basnet et al., 1993). According to Basnet et al. (1993), maximal exploitation of resources for grafted trees could even result in faster growth rates. Root grafting also enhances survival of roots, snags, and stumps of dead or cut trees (Fraser, Lieffers, and Landhäusser, 2006; Fraser, Lieffers, and Landhäusser, 2007; Tarroux and DesRochers, 2010; Tarroux, DesRochers, and Krause, 2010) which could in this case constitute a drain on resources for residual living trees (Tarroux, DesRochers, and Krause, 2010). This relationship could be seen as a form of parasitism, if assimilates acquired through root grafts prolong survival of suppressed trees at the expense of dominant trees that appear to be disadvantaged by the union (Stone, 1974; Stone and Stone, 1975; Loehle and Jones, 1990). It could also be seen as an example of cooperation, to ensure that soil resources remain within individuals of a species and prevent roots or seedlings of another species from invading the space.

In previous work, we found a high level of root grafting in jack pine natural stands and plantations (Tarroux and DesRochers 2010). We also found that growth response of trees to commercial thinning was affected by the presence of root grafts, because roots of trees that had died and rotted away were maintained alive by standing residual trees (Tarroux, DesRochers, and Krause, 2010). The effect of root grafting on growth of trees, however, was never evaluated.

The main objective of this study was thus to determine the influence of root grafting on radial growth of jack pine (*Pinus banksiana*. L). Since trees linked by root grafts are able to share resources (Fraser, Lieffers, and Landhäusser, 2006), our hypothesis was that radial growth of grafted trees would be enhanced by the presence of root grafts(Graham, 1959; Bormann, 1966; Graham and Bormann, 1966; Loehle and Jones, 1990).

Three plantations and three natural jack pine stands were excavated between June 2002 and October 2007. Stands were located in the western balsam fir-paper birch (*Abies balsamea – Betula papyrifera*) bioclimatic domain (Grondin, 1996), between 48°26'N and 48°43'N, and between 77°38'W and 77°54'W. Natural stands were of post-fire origin growing on sandy soils (regenerated by seed; sites 1, 2 and 3 from (Tarroux, DesRochers, and Krause, 2010) while plantations were growing on clayey soils (sites P4, P5 and P6 from (Tarroux and DesRochers, 2010). Natural regeneration of jack pine is often deficient on clayey soils after harvesting, resulting in more plantations being established on these sites, compared to sandy sites where natural regeneration is usually abundant, especially after fire (Sims, Kershaw, and Wickware, 1990). Sandy sediments of the region are associated with glaciofluvial deposits (eskers), while fine-grained sediments in the clay plain are associated with glaciolacustrine deposits. These deposits arose from the last glacial cycle and the submergence of the region by proglacial Lake Barlow-Ojibway and represent the retreat of the Laurentide ice sheet (10100-8000 years before present; (Veillette, 1994). The average climate for the last three decades showed that average yearly precipitation was 918 mm (rainfall, 670 mm; snowfall, 248 mm) and daily average temperature was 1.2°C, with an average 2334 degree-days above 0°C (Environment Canada, 2010). Table 1 summarizes the characteristics of the six excavated plots. The three natural stands were commercially thinned in 1998, so we only considered growth of trees until this date, while for the three plantations, radial growth was studied until the excavation date (2004-2005).

Table 4.1 Characteristics of the six excavated plots.

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
	Natural stands			Plantations		
Size of excavated area (m^2)	40	50	50	30	40	30
Stand age (years)	50	55	60	35	35	35
Density (trees ha-1)	4200	3800	5000	4000	3000	4000
Mean DBH (cm)	12.27	15.61	12.80	18.67	12.53	19.78
Mean height (m)	14.38	18.40	12.65	12.36	12.21	12.34
Number of excavated trees	13	18	25	12	14	11
Number of grafts	12	12	18	6	4	5
Number of grafted trees	9	8	15	5	4	6
Mean number of grafts per tree	0.92	0.67	0.72	0.50	0.29	0.45
Percentage of grafted trees (%)	69	44	60	42	29	54

4.2.1 Field and laboratory work

Height and diameter at breast height (DBH) were measured on each tree to determine the status of trees at the time of excavation (dominant, co-dominant, or suppressed). Trees were felled with a chainsaw, and root systems were hydraulically excavated using a high pressure water spray from a Wajax water pump (Mark III, Wajax, Lachine, QC). Cross-sectional disks were taken at ground level (0 m), at breast height (1.30 m) and from each root with a diameter > 2 cm. All suspected root grafts were checked in the field by removing bark and through partial dissection to confirm a common wood layer between the two roots. In the laboratory, age of trees, roots and grafts was determined by counting and cross-dating growth rings (Tarroux and DesRochers, 2010; Tarroux, DesRochers, and Krause, 2010). Radial growth was measured on cross-sections taken from the stem base (0 m) using a Velmex bench interfaced with a computer (Tarroux, DesRochers, and Krause, 2010). The ring-width

series from the pith to the outermost ring of each wood disk was measured on 4 rays (when it was possible; (Tarroux, DesRochers, and Krause, 2010). Curves of radial growth were then crossdated and validated using the computer programs COFECHA (Grissino-Mayer, 2001) and TSAPWin (RINNTECH, Engineering and Distribution, Heidelberg, Germany). To decrease the influence of size differences between trees of different ages and sizes in the ring-width chronologies, series were standardized using ARSTAN program (Grissino-Mayer, 2001). The time (year) at which root grafting began and ended was determined to calculate the time it took for complete graft formation.

4.2.2 Statistical analysis

Statistical analyses were done in R (R statistical program v. 2.7.2, (R Development Core Team, 2008), and a significance level of $P = 0.05$ was used for all response variables. To examine how root grafting affected yearly growth of trees, yearly radial growth (from stand initiation up to 1998 for natural stands and up to 2004-2005 for plantations) of grafted and non-grafted trees was analyzed with a hierarchical mixed linear model (`lme` function; GROWTH model). Contrary to a traditional analysis of variance, linear mixed models include both fixed-effect parameters and random effects for repeated-measures data to estimate the relationship between a continuous dependent variable and the various predictors (West et al., 2007). Years and root grafting status (NG for non-grafted, G for grafted) were fixed as categorical factors, and each ray measurement was nested within its corresponding tree. As yearly growth values are not independent from one another (growth of year t is strongly affected by the value of year t-1), a first-order autoregressive correlation structure was used to reflect the strong correlation existing between successive observations (repeated measures) but that correlation decreases with spacing of the observations in time (Grissino-Mayer, 2001; Pinheiro et al., 2008). Since root grafts did not occur at the same time among the six sites, a different model was used for each site. Root graft formation could take place over several years; consequently, for each graft, we considered the entire period of root graft formation and showed how radial growth was affected during this period and afterwards.

To determine if root grafting affected diameter of trees, basal diameter at the time of excavation (2004-2005 for plantations or in 1998 for natural stands) was compared for

grafted and non-grafted trees with a hierarchical mixed linear model (DIFFAFTER_N for natural stands, DIFFAFTER_P for plantations; Table 2) using the `lme` function. Another model was created to verify if growth of grafted and non-grafted trees was similar *before* root grafting occurred (DIFFBEFORE_N, DIFFBEFORE_P models; Table 2). Sites were treated as random effects. Basal diameters were obtained using diameter values at ground level obtained with ring-width series.

Table 4.2 Results of hierarchical mixed models (`lme`) testing diameter at stem base of grafted (G) and non-grafted trees (NG) before (DIFFBEFORE) and after (DIFFAFTER) root grafting occurred for natural stands (_N) and plantations (_P). The grafting status (in parentheses) corresponds to the type considered by the model and statistically significant values ($P < 0.05$) are given in bold.

	Factors	Estimate	Standard error	*P*-value
DIFFBEFORE_N	graft (NG)	-335.111	145.651	**0.026**
DIFFBEFORE_P	graft (NG)	110.980	64.468	0.0945
DIFFAFTER_N	graft (NG)	-7.394	1263.326	0.995
DIFFAFTER_P	graft (NG)	-116.736	252.234	0.647

Status of trees (dominant, D; co-dominant, C; or suppressed, S) *before* root grafting occurred were determined by comparing basal diameter values. For the plantations, status of trees *after* root grafting occurred were obtained using height and diameter at breast height recorded just before excavation. In natural stands (thinned in 1998), it was not possible to use height and diameter at breast height (some trees had been cut), so we determined status of trees *after* root graft formation using only diameter values at ground level obtained with ring-width series in 1998. The different size classes were determined by comparing size of each tree with the mean stand value using t-tests; suppressed trees were significantly smaller, co-dominant trees were similar and dominant trees were significantly larger than the mean value. An analysis of variance (Anova) was then used to confirm that mean size values of the three

classes were significantly different from each other ($P > 0.05$). To verify if root grafting affected the size distribution structure of stands, the frequency of each size category according to graft presence (grafted, G; non-grafted, NG) was compared using a `glmer` function in the `lme4` library (linear mixed-effects models using S4 classes; (Bates and Maechler, 2009). It is a generalized linear mixed-effects model which allows the application of a Poisson regression. A model was created with frequency of each status obtained *before* root grafting occurred (STATUBEF_P and STATUBEF_N models; Table 3) and another with frequency *after* root grafting occurred (at the time of excavation for STATUAFTER_P model and in 1998 for STATUBEF_N model; Table 3), which allowed us to follow variation in stand structure with time. Multiple comparisons of means (Tukey tests) were used when the interaction between tree status (D or C or S) and root grafting (G or NG) was significant (Table 4).

4.3 Results

4.3.1 Natural stands

Trees of site 1 were established at the beginning of the 1950's (Fig. 1A). Root grafting was initiated twenty years later (1970), and the last root graft was formed in 1992 (Fig. 1A). Eight grafts (67% of grafts) formed between 1970 and 1980 (period b) and four (33% of grafts) between 1986 and 1992 (period d; Fig. 1A). GROWTH models showed that before root grafting started (period a; Fig. 1A), trees that would later form grafts generally had better growth than the other trees (only significant in 1967 and 1968). When trees became grafted (period b), growth of grafted trees decreased and was less than growth of non-grafted trees (Fig. 1A). Between 1980 and 1986 (period c), growth of grafted trees slightly increased but remained significantly less than that of non-grafted trees until 1983 (Fig. 1A). After 1992 (period e), growth of grafted trees increased similarly as during period c, although differences in radial growth between grafted and non-grafted trees were not significant (Fig. 1A).

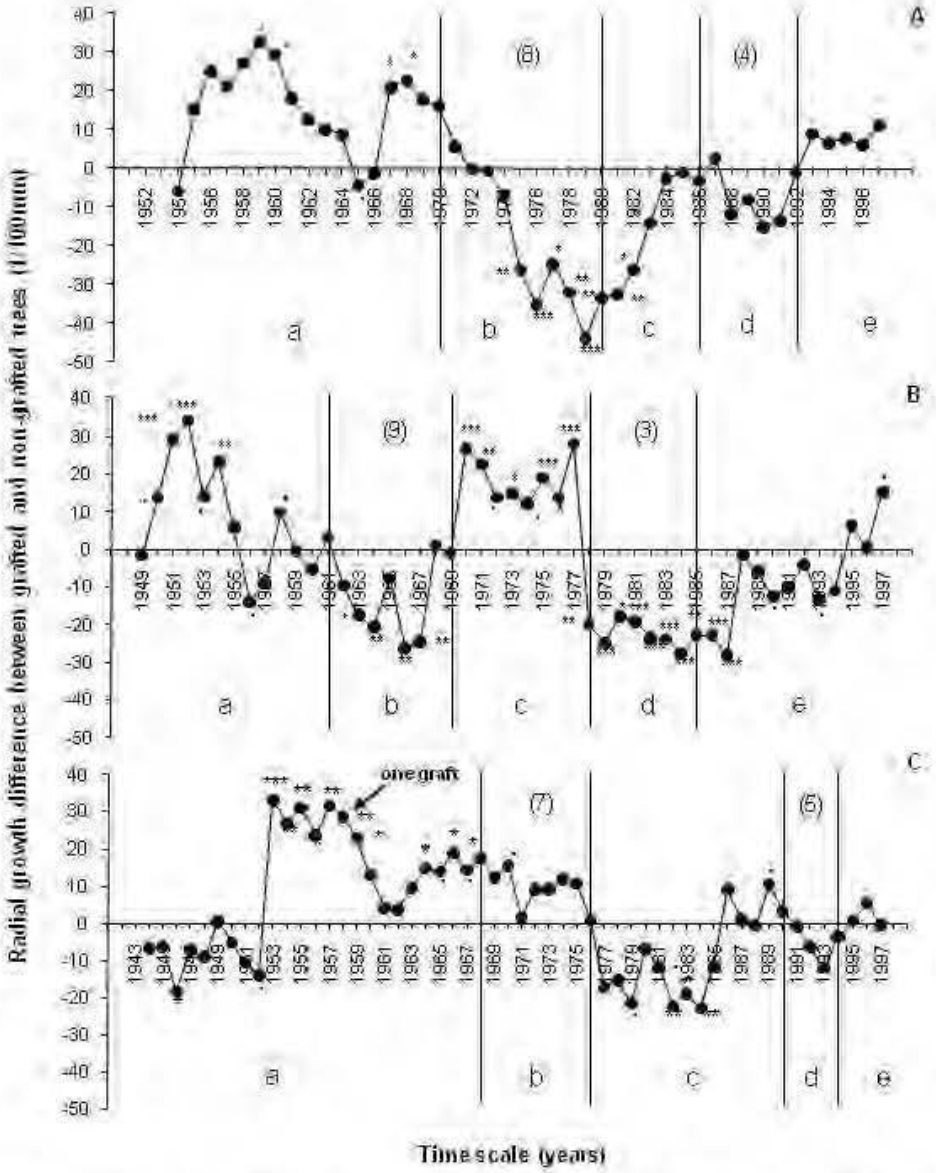

Figure 4.1 Yearly radial growth differences (1/100mm) between grafted and non-grafted trees from natural stands: site 1 (A), site 2 (B) and site 3 (C). 'a' corresponds to the period before root grafting, 'b' and 'd' to periods of root grafting, 'c' and 'e' to periods without graft formation. Significance codes: < 0.0001 = '***'; < 0.001 = '**'; < 0.01 = '*'; < 0.05 = '.'. Numbers in parentheses represent the number of root grafts formed within the corresponding time period.

Trees in site 2 were established at the end of 1940's, and the first and last root grafts were formed in 1961 and 1985, respectively (Fig. 1B). Nine grafts (75 % of grafts) occurred

between 1961 and 1969 (period b) and three grafts (25 % of grafts) formed between 1978 and 1985 (period d; Fig. 1B). Before trees grafted (period a), trees that would later form grafts had greater radial growth than non-grafted trees from 1950 to 1955 and in 1960 (Fig. 1B). When trees began to form root grafts (period b), growth of grafted trees decreased and became less than radial growth of non-grafted trees (Fig. 1B). Between 1970 and 1977 (period c), radial growth of grafted trees was greater than that of non-grafted trees, while it decreased again during the second root grafting period (period d; Fig. 1B). After 1987 (period e), growth of grafted trees gradually increased and was similar to that of non-grafted trees (Fig. 1B). Trees were established at the beginning of the 1940's at site 3 (Fig. 1C). In this site, the first root graft occurred in 1958, which was followed by two periods of root grafting; seven grafts (55% of grafts) formed between 1968 and 1976 (period b), and three grafts (37 % of grafts) formed between 1990 and 1994 (period d; Fig. 1C). Growth of grafted trees decreased after the first graft in 1958, and slowly increased above that of non-grafted trees until 1975 (during period b), when more root grafts were formed (Fig. 1C). Growth of grafted trees decreased rapidly after period b, and slightly increased at the end of period c until the last root grafting period (period d), where it decreased once again (Fig. 1C).

Before root grafting occurred, basal diameter of trees that would later form root grafts in natural stands was greater than that of non-grafted trees (DIFFBEFORE_N model; P = 0.026; Table 2). There was an interaction between root grafting and dominance status before root grafting occurred (STATUBEF_N model; $P < 0.05$; Table 3; Fig. 2A), showing that few suppressed trees made root grafts compared to the number of dominant trees, as observed frequencies of G:S were significantly less than observed frequencies of G:D (P = 0.045). However, the proportion of co-dominant grafted trees was similar to the proportion of dominant and suppressed trees. For the non-grafted trees, observed frequencies of each status were equally distributed ($P > 0.05$; Table 4; Fig. 2A). For trees of the same dominance status, there was no difference between the proportion of grafted and non-grafted individuals (NG:S and G:S, NG:C and G:C, NG:D and G:D, $P > 0.05$; Table 4; Fig. 2A). After the periods of root grafting time, stem basal diameter of grafted and non-grafted trees was similar (DIFFAFTER_N model; P = 0.995) and most trees were in the co-dominant category (STATUAFTER_N model; $P < 0.05$; Table 4; Fig. 2B). Frequency of co-dominant trees for

grafted (G-C) and non-grafted (NG-C) trees was similar (P = 0.997) and was higher than frequency of suppressed and dominant trees (Table 4; Fig. 2B).

Table 4.3 Results of hierarchical mixed models (`glmer` function) testing the frequency of each tree status (dominant, D; co-dominant, C; suppressed, S) according to graft presence/absence (grafted, G; non-grafted, NG) before (STATUBEF) and after (STATUAFTER) grafting occurred for natural stands (_N) and plantations (_P). The status (in parentheses) corresponds to the type considered by the model and statistically significant values ($P < 0.05$) are given in bold

Model	Factors	Estimate	Standard error	*P*-value
STATUBEF_N	(Intercept)	3.664	0.092	**< 0.001**
	Type(NG)	0.281	0.122	**0.022**
	Status(D)	0.338	0.121	**0.005**
	Status(S)	-1.766	0.242	**< 0.001**
	Type(NG):status(D)	-0.999	0.183	**< 0.001**
	Type(NG):(statusS)	0.897	0.284	**0.002**
STATUBEF_P	(Intercept)	3.536	0.099	**< 0.001**
	Type(NG)	0.000	0.120	**< 0.001**
	Status(D)	-0.664	0.169	**< 0.001**
	Status(S)	0.328	0.129	**0.011**
	Type(NG):status(D)	-1.374	0.263	**< 0.001**
	Type(NG):status(S)	-1.656	0.197	**< 0.001**
STATUAFTER_N	(Intercept)	4.374	0.065	**< 0.001**
	Type(NG)	-0.102	0.094	0.280
	Status(D)	-2.253	0.210	**< 0.001**
	Status(S)	-1.835	0.175	**< 0.001**
	Type(NG):status(D)	0.620	0.270	**0.021**
	Type(NG):status(S)	0.225	0.242	0.351
STATUAFTER_P	(Intercept)	4.489	0.061	**< 0.001**
	Type(NG)	-0.078	0.088	0.378
	Status(D)	-2.754	0.250	**< 0.001**
	Status(S)	-2.754	0.250	**< 0.001**
	Type(NG):status(D)	-17.959	2002.176	0.993
	Type(NG):status(S)	1.215	0.292	**< 0.001**

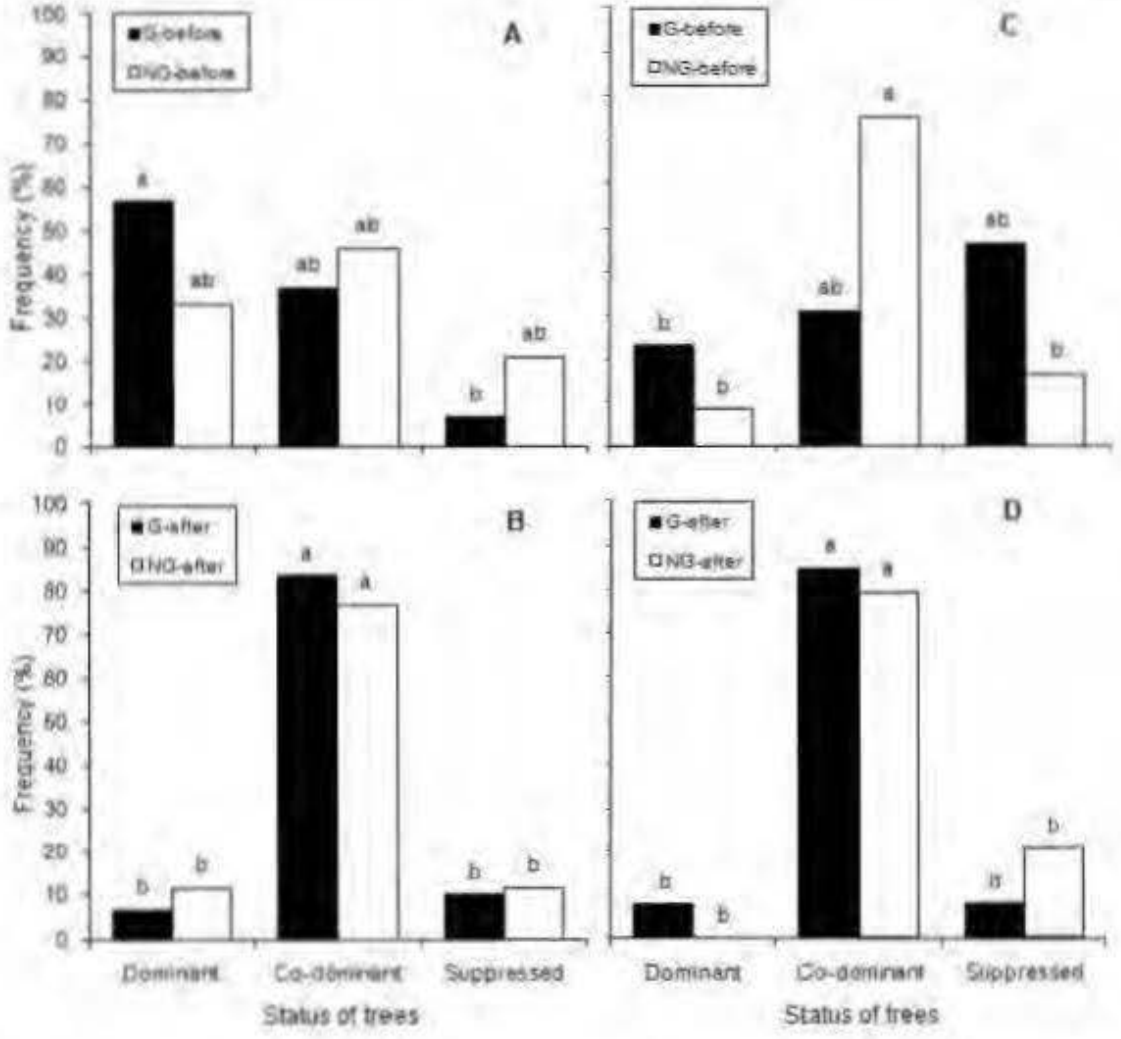

Figure 4.2 Percentages of grafted and non-grafted trees from natural stands before (A) and after (B) root graft formation and from plantations before (C) and after (D) root graft formation, according to their dominance status

Table 4.4 Tukey multiple comparisons of means for each model where the interaction "dominance status (dominant, D; co-dominant, C; suppressed, S) × grafting status (grafted, G; non-grafted, NG)" was significant. Statistically significant values ($P < 0.05$) are given in bold.

Interaction	STATUBEF_N		STATUBEF_P		STATUAFTER_N		STATUAFTER_P	
	diff	*P*-value	diff	*P*-value	diff	*P*-value	diff	*P*-value
NG:C-G:C	12.667	0.938	37.333	0.216	-7.667	0.997	-6.667	0.989
G:D-G:C	15.667	0.865	-16.667	0.876	-71.000	**0.013**	-83.333	**0.000**
NG:D-G:C	-12.333	0.944	-25.000	0.593	-65.333	**0.023**	-89.000	**0.000**
G:S-G:C	-32.333	0.263	13.333	0.946	-66.667	**0.020**	-83.333	**0.000**
NG:S-G:C	-17.333	0.812	-15.333	0.908	-65.000	**0.024**	-71.333	**0.000**
G:D-NG:C	3.000	0.999	-54.000	**0.037**	-63.333	**0.028**	-76.667	**0.000**
NG:D-NG:C	-25.000	0.509	-62.333	**0.015**	-57.667	**0.049**	-82.333	**0.000**
G:S-NG:C	-45.000	0.064	-24.000	0.630	-59.000	**0.043**	-76.667	**0.000**
NG:S-NG:C	-30.000	0.330	-52.667	**0.043**	-57.333	**0.050**	-64.667	**0.001**
NG:D-G:D	-28.000	0.397	-8.333	0.993	5.667	0.999	-5.667	0.995
G:S-G:D	-48.000	**0.045**	30.000	0.414	4.333	1.000	0.000	1.000
NG:S-G:D	-33.000	0.246	1.333	1.000	6.000	0.999	12.000	0.880
G:S-NG:D	-20.000	0.712	38.333	0.196	-1.333	1.000	5.667	0.995
NG:S-NG:D	-5.000	0.999	9.667	0.986	0.333	1.000	17.667	0.618
NG:S-G:S	15.000	0.884	-28.667	0.459	1.667	1.000	12.000	0.880

4.3.1 Plantations

Trees of all plantations were planted at the beginning of the 1970's (Fig. 3A). At site 4, root grafting began in 1989 and ended in 2001 (period b). During root graft formation, growth of grafted trees was slightly better than growth of non-grafted trees, but yearly differences were not significant, except for 1994 where growth differences were marginally significant ($P = 0.056$; Fig. 3A). After this period of root graft formation (2001), growth of

grafted trees decreased and became less than growth of non-grafted trees in 2002 (Fig. 3A). There were only two root grafts at site 5, where the first began to form in 1980 (period b) and the last in 1997 (period d, Fig. 3B). There was no significant difference between radial growth of grafted and non-grafted trees, except in 1979 where growth of trees that would later form root grafts was slightly greater than growth of non-grafted trees (Fig. 3B). Five root grafts were found at site 6, where there were three periods of root grafting formation; one graft began to form in 1982 and one in 1986 (period b), two grafts formed between 1992 and 1996 (period d), while the last graft formed between 1999 and 2002 (period f; Fig. 3C). In 1982 and 1986 (period b), while growth of grafted trees was greater than growth of non-grafted trees, formation of the first two grafts was followed by growth decreases (Fig. 3C). Radial growth was similar between grafted and non-grafted trees between 1986 and 2001 (periods c, d, e and f), and after the formation of the last root graft in 2000, growth of grafted trees was significantly less than that of non-grafted trees (Fig. 3C). Before root grafting occurred (DIFFBEFORE_P model), stem diameter was marginally influenced by root graft status ($P = 0.094$; Table 2), i.e. diameter of future grafted trees was slightly less than that of non-grafted trees. Most non-grafted trees were co-dominant individuals ($P < 0.05$), while size-class distribution of future grafted trees was distributed more evenly among the three classes ($P > 0.05$) with a trend for more trees in the suppressed class (Table 2; Fig. 2C). At the time of excavation (2004-2005; DIFFAFTER_P model), there was no difference between stem basal diameter of grafted and non-grafted trees in plantations ($P= 0.647$; Table 2). Observed frequencies of each status (D, C, S) after root grafting occurred (STATUAFTER_P) showed that most individuals became co-dominants (Tables 3, 4; Fig. 2D). Frequency of co-dominant trees was similar for grafted (G-C) and non-grafted (NG-C) trees ($P = 0.989$; Table 4) and was higher than frequencies of suppressed ($P < 0.05$; G-S and NG-S; Table 4) and dominant (G-D and NG-D; Table 4) trees, regardless of root grafting status (Fig. 2D).

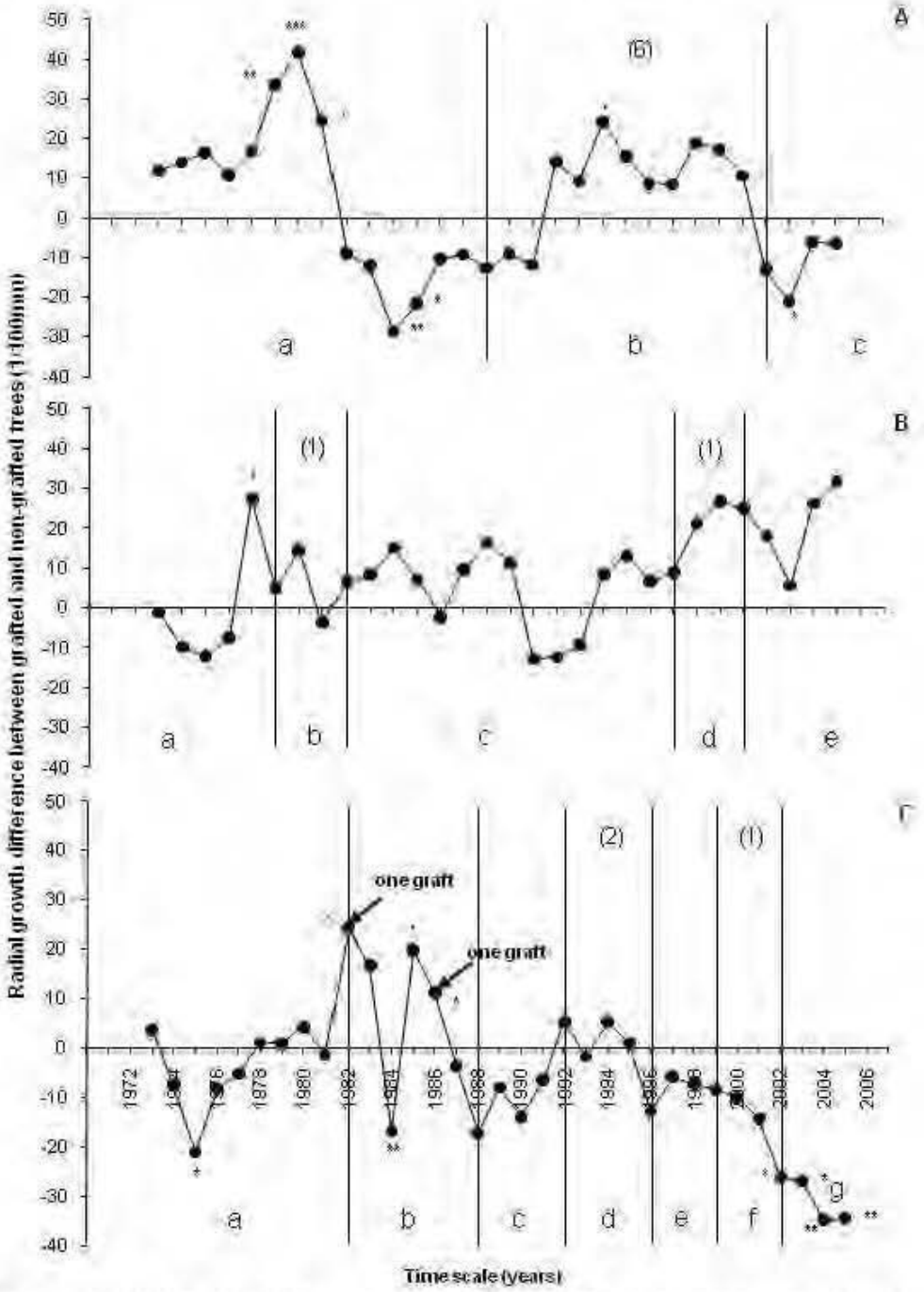

Figure 4.3 Yearly radial growth differences (1/100mm) between grafted and non-grafted trees from plantations: site 4 (A), site 5 (B) and site 6 (C). 'a' corresponds to the period before root grafting, 'b', 'd' and 'f' to periods of root grafting, 'c', 'e' and 'g' to periods without graft formation. Significance codes: ≤ 0.0001 = '***', ≤ 0.001 = '**', ≤ 0.01 = '*', ≤ 0.05 = '.'. Number in parentheses is the number of root grafts formed within the corresponding time period.

4.4 Discussion

There are costs and benefits associated with natural root grafting. This study suggests that it is an energetically costly process but once completed, the grafts seemed to enhance radial growth of trees. Root grafting tended to reduce radial growth of jack pine trees during periods of root graft formation, especially in natural stands that contained higher numbers of root grafts compared with plantations (Fig. 1). It is not completely unambiguous that root grafting caused growth increases in trees, because growth of trees that would later form grafts was generally better than growth of non-grafted trees in natural stands *before* root grafting occurred, until a 'grafting period' occurred and radial growth of grafted trees decreased (Fig. 1). As first suggested by Loehle and Jones (1990), root grafting is undoubtedly an energetically costly process, which could explain why in natural stands, root grafts preferentially formed between larger and dominant trees (Tables 2, 3, 4; Fig. 2A) and why radial growth decreased as root grafts were forming (Fig. 1). Callus tissue is normally produced around root grafts and the structure of this wood is more complex than that of regular roots because tracheids are produced along two axes, allowing transfers between the two grafted roots (Bormann, 1966). Suppressed or weaker trees may lack energy to form root grafts, or perhaps their root systems do not extend far enough to reach roots of other trees. Because they receive less light, smaller suppressed trees in natural forests (initially more dense than plantations, Smith, 1997) have fewer carbohydrate reserves to allocate to root growth (and formation of root grafts) once maintenance respiration needs, i.e., the energy required to keep existing cells and tissues alive, have been satisfied (Kozlowski, Kramer, and Pallardy, 1991). Few root grafts formed before trees were 10-years-old (Figs 1 & 3), which could be a consequence of little contact between roots of small trees (Tarroux and DesRochers, 2010; Tarroux, DesRochers, and Krause, 2010).

In plantations, the influence of root grafting during root graft formation was less apparent; in contrast to natural stands, growth of grafted trees did not significantly decrease compared to growth of non-grafted trees (Fig. 3). The impact of root grafts on radial growth could be less important in plantations because they contained fewer root grafts (Table 1), or because trees were evenly spaced within the stand, giving them access to more or less the same quantity of resources. Furthermore, trees in plantations were more vigorous than in

natural stands, as they were younger but larger in size (Table 1). Trees originating from plantations often come from improved seed stocks, and soil resources in these clayey plantations may have been less limiting than in the sandy natural stands (Wilde et al., 1964; Bell, 1991). When growth is not limited by nutrients, water or light availability, trees can increase their production of photosynthates (Salisbury and Ross, 1992). Carbohydrates formed within tree crowns (sources) travel downward to roots (sinks) according to the principle of source/sinks, and their production can be influenced by sink demand since there is facilitation of photosynthetic reactions when sinks are large (Salisbury and Ross, 1992). Faster growth rates of trees in plantations thus suggest that trees had enough resources to compensate for the cost of root graft formation without negatively affecting their diameter growth.

After each period of root grafting formation, radial growth of grafted trees in natural stands usually resumed comparable or greater levels than non-grafted trees (Fig. 1). In plantations, it did not seems that growth was enhanced by root grafting since stem basal diameter and yearly radial growth was similar between grafted and non-grafted trees at the time of excavation (Table 4; Fig. 3). Nevertheless, the fact that trees that formed grafts tended to be smaller before root grafting occurred (DIFFBEFORE_P; Table 4) and became similar in size to non-grafted trees at the time of excavation (DIFFAFTER_P; Table 4) suggests that root grafting in plantations was also beneficial to tree growth. Bormann (1966), Graham and Bormann (1966), Loehle and Jones (1990) and Basnet el al. (1993) suggested that root grafting should enhance radial growth and stand survival by facilitating acquisition of resources by members of a communal root system that are located further away from such resources (water, for example). If most trees are interconnected, resources of a site become somewhat accessible for all interconnected trees, rather than just for trees with the largest root systems. The use of a communal root system could thus allow for a maximum use of resources by redistributing them among trees and consequently evening tree growth and sizes within a stand (Walters, 1963). However, in our study, most trees were in the co-dominant size category at the time of excavation, whether they were grafted or not (Fig. 2B, D). Nevertheless, prior to root grafting, size distribution of trees that would later form root grafts differed greatly from the size distribution pattern found after root grafting, compared to that

of non-grafted trees, showing that grafted trees further homogenized their size compared to non-grafted trees (Fig. 2). In natural stands, most future grafted trees were dominant trees and only a few were suppressed, while the distribution of non-grafted trees was more regular among status classes (Fig. 2A). In plantations most of non-grafted trees were already co-dominant (Fig. 2C), and remained co-dominant at the end of the study (Fig. 2D).

The fact that root grafting allows the sharing of resources and secondary compounds between trees challenge the classic competition concept in its strict sense (Begon, Harper, and Townsend, 2006). Root grafting could be interpreted as an intraspecific cooperative behavior that maintains stand integrity (Loehle and Jones, 1990; Jelínková, Tremblay, and DesRochers, 2009). Root grafting could promote dominance of a species on a site with bigger trees supplying carbohydrates to suppressed trees within a root complex (Basnet et al., 1993), while circumventing the death of trees that would create gaps in the stand and which would become available for other species to invade.

CHAPITRE V

EFFECT OF NATURAL ROOT GRAFTING ON GROWTH RESPONSE OF JACK PINE (*PINUS BANKSIANA*) AFTER COMMERCIAL THINNING[4]

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[4] Article publié en 2010 par Forest Ecology and Management, volume 260 : 526-535.

5.1 Abstract

Commercial thinning is a silvicultural treatment used to increase the merchantable yield of residual trees. Growth response to thinning, however, is highly variable and discrepancies between studies remain largely unexplained. The objective of this study was to demonstrate the effect of natural root grafting on growth response after thinning. We excavated root systems of jack pine (*Pinus banksiana*) in five naturally regenerated stands, in which three had been commercially thinned 6 and 9 years earlier. Radial growth before and after thinning was examined using dendrochronological techniques. Thinning increased radial growth of trees, however growth increments were significantly less for trees that had root grafts with removed trees, while growth of grafted trees was better in unthinned stands. Furthermore, radial growth response of trees grafted to removed trees was smaller than that of non-grafted trees 4 years and more post-thinning. On average, non-grafted stumps survived less than 1 year (0.4 year), while grafted stumps lived 2.0 years after the stem was removed. Differences in growth response to thinning between grafted and non-grafted trees thus appear to be linked to the support of roots and stumps of removed trees by live residual trees.

5.2 Introduction

Commercial thinning is often prescribed to increase merchantable yield and profitability of forest stands by increasing the diameter growth of residual trees, salvaging potential mortality, increasing product quality, releasing suppressed individuals, and removing undesirable species (Karsh et al. 1994; Pothier and Margolis 1991; Schneider et al. 2008; Smith 1997). Thinning affects tree growth by reducing the number of stems and concentrating resources (light and nutrients) on remaining crop trees (DeBell et al. 2002; Smith and Oerlemans 1988). However, this silvicultural treatment does not always give expected results; in some cases, tree growth stagnates or decreases, and stands often show high mortality rates following treatment (Cayford et al. 1967; Day and Rudolph 1972; DeBell et al. 2002; Gingras and Favreau 1998; Harrington and Reukema 1983; Staebler 1956; Vincent et al. 2009). Mäkinen and Isomäki (2004a, b) compared different thinning intensities with unthinned stands and did not find differences between stem volume increments in Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris* L.). Growth delays after thinning were also observed in tropical species such as *Eucalyptus grandis* (Smith and Brennan 2006). These negative responses, which are referred to as "thinning shock" (DeBell et al. 2002; Harrington and Reukema 1983), have generally been observed immediately following treatment and usually lasted less than 15 years (Harrington and Reukema 1983).

Different explanations have been given to describe "thinning shock," but there is no general consensus in terms of explaining differences in response to thinning. Some authors have shown that residual trees responded to new growing conditions after thinning by increasing root biomass allocation (Liu et al. 2003; Nicoll and Dunn 2000; Nicoll and Ray 1996; Ruel et al. 2003; Urban et al. 1994; Vincent et al. 2009). Wind penetrates more easily into forest stands after thinning or partial cutting, which translates into greater mechanical stress acting on roots (Man and Lieffers 1999; Nicoll and Dunn 2000; Pothier and Margolis 1991; Rizzo and Harrington 1988; Ruel et al. 2003; Vincent et al. 2009). As woody root systems provide anchorage and structural support, trees need to immediately allocate more biomass to parts of the trunk and roots that are subject to higher stresses, while growth of aerial parts may be delayed (Coutts et al. 1999; Rizzo and Harrington 1988; Ruel et al. 2003).

Thinning shock could also be explained by increased water stress following thinning (Bladon et al. 2007; Man and Lieffers 1999; Proe et al. 2001); Although reduced tree density may increase soil moisture availability, greater wind and sunlight penetration into the stand increases evapotranspiration rates (Pothier and Margolis 1991). Furthermore, machinery used for removing trees can cause wounds on residual tree boles and their root systems, which may weaken them or create entry points for diseases (Boddy 2001; Hennon and DeMars 1997). As thinning shock is reduced with nitrogen fertilization (Brix 1993; Crown et al. 1977; DeBell et al. 2002; Devine and Harrington 2009; Winston 1977), it has also been suggested that site quality contributes to differences in stand response to thinning (DeBell et al. 2002; Harrington and Reukema 1983; Mäkinen and Isomäki 2004a, b). In poor sites, thinning may not increase growth of residual trees if nutrients are limiting (Devine and Harrington 2009). Other studies have suggested that thinning shock results from deterioration of leaf photosystems following increased light exposure of the shade tolerant species that remain after thinning (Krause 1988; Leverenz et al. 1990; Marshall et al. 2000; Öquist et al. 1992).

In this study we propose an alternative hypothesis to thinning shock: natural root grafting. Natural root grafts have been found in many tree species (Graham 1959), including natural stands and plantations of jack pine (*Pinus banksiana* Lamb.), where we found a high frequency of natural root grafting (up to 70% or more of the trees; Tarroux and DesRochers 2010). It has been found that the roots and stumps of dead or cut trees could be kept alive through root grafts with living residual trees (DesRochers and Lieffers 2001a; Fraser et al. 2007; Tarroux and DesRochers 2010). This indicates that carbohydrates are transferred from living trees to the roots and stumps of removed or dead trees (Fraser et al. 2006). Consequently, if stands are heavily thinned, grafted residual trees might not benefit from decreased competition for resources that results from stand thinning (Bormann 1966), because the roots and stumps of the removed trees would constitute a sink for carbohydrates, thereby limiting aboveground growth of the residual trees (Bormann 1966; Eis 1972; Graham and Bormann 1966). No previous study has demonstrated that tree response to thinning could be hindered by the presence of root grafts. The objective was to determine if the presence of root grafts affected diameter stem growth response to commercial thinning. We hypothesized

that growth of residual trees grafted to removed trees would be less than growth of non-grafted trees after thinning.

5.3 Methods and material

5.3.1 Study sites

Root systems in five mature (age ≥ 50 years) jack pine stands were excavated. Three of the sites had been commercially thinned (i.e., stems removed had diameter at breast height (DBH) > 10cm) in 1998 (stands T) while the two other sites were control unthinned stands (stands C; Sites N1 and N2 in Tarroux and Desrochers 2010). The stands were located between 48°20'N and 48°41'N, and between 77°16'O and 78°8'O in the western balsam fir-paper birch (*Abies balsamea–Betula papyrifera*) bioclimatic domain of the boreal forest of eastern Canada (Grondin 1996). Precipitation averages 918mm annually (rainfall 671mm, snowfall 248mm) and average (±standard deviation, SD) annual temperature is 1.2±2.2°C (Environment Canada 2010). Soils were sandy sediments associated with glaciofluvial deposits (eskers) originating from the retreat of the Laurentide ice sheet (8000–10,100 years bp) during the last glacial cycle and the submergence of the region by proglacial Lake Barlow-Ojibway (Veillette 1994). Stands were even aged and of post-fire origin, with trees fairly uniform dimensions (mean±standard error (SE): DBH, 14.21±0.85cm; height, 15.34±0.84m; Table 5.1). The stands were constituted of more than 90% jack pine and were located near a water source (pond, lake or river) to allow hydraulic excavation. Prior to thinning, tree density ranged from 3800 to 5000 stems ha^{-1}, which was reduced to 2000, 2200 and 3200 stems ha^{-1} after thinning for sites 1, 2 and 3, respectively (Table 5.1). In control stands, tree density was 4400 and 4000 stems ha^{-1} for sites 4 and 5, respectively (Table 5.1). On all thinned sites, basal diameter of the trees removed in the thinning treatment was similar to that of the residual trees (Table 5.2). This treatment could be labelled as 'crown thinning' (MRNF 2003).

Table 5.1 Characteristics of the five excavated plots.

	Site 1	Site 2	Site 3	Site 4	Site 5
Total size of excavated area (m^2)	40	50	50	40	40
Stand age (years)	1953/1956	1948/1952	1943/1948	1944/1947	1942/1947
Density prior to/after thinning (trees ha^{-1})	3700/2000	3600/1600	5000/3200	4400	4000
Thinning date	1998	1998	1998	unthinned	unthinned
Mean DBH (cm)	12.27	15.61	12.80	17.22	13.17
Mean height (m)	14.38	18.40	12.65	15.40	15.89
Number of excavated trees	13	18	25	17	16
Number of grafts	12	12	18	15	11
Number of grafted trees	9	8	15	12	9
Number of grafted trees thinned	5	5	8	unthinned	unthinned
Number of grafted trees uncut	4	3	7	unthinned	unthinned
Mean number of grafts per tree	0.92	0.67	0.72	0.88	0.69
Mean number of grafts per hectare	3000	2400	3600	3750	2750
Percentage of grafted trees (%)	69	44	60	71	56

Table 5.2 Student t.test results on diameter at stem base of cut and uncut trees at thinning date (1998) for each thinned stand.

	Site 1	Site 2	Site 3
Mean diameter of uncut trees (cm)	8.800	9.419	10.512
Mean diameter of cut trees (cm)	10.230	11.423	9.269
P value	0.598	0.399	0.112

5.3.2 Field work

Sampling was done in June 2005 for sites 1, 2, 4 and 5 and in October 2007 for site 3. Trees were felled with a chain saw, and cross-sectional disks were taken at ground level (0m) and at breast height (1.30m) for age determination. Root systems were excavated using a high pressure water spray from a forestry waterpump (Mark III, Wajax, Lachine, QC). Excavated areas ranged from 40 to 50m^2 in size, depending on the spatial distribution of the trees, so that at least 10 individuals were included per site (Table 5.1). Roots of trees extending outside the excavated area were followed up to a diameter of 2cm to ensure that no root grafts were missed. Maps depicting all trees, stumps, roots, and grafts were carefully drawn by hand. To assess the condition of stumps from thinned trees (dead/alive), we examined the state of decomposition of the wood (Vanderwel et al. 2006). Whenever possible, a stem cross-section was removed and brought to the laboratory for ring counting. When the stump wood was too decomposed for a cross-section to be collected, it was considered as dead immediately after treatment (0 year of survival after thinning). A cross-section was also collected as close as possible to the stem base from each root with a diameter >2cm to allow cross-dating (Krause and Eckstein 1993; Krause and Morin 2005). All grafts were tested (partial dissection) for vascular continuity between the two roots and collected. True grafts involve the morphological union of cambium, phloem, and xylem (Graham and Bormann 1966). Only true intraspecific grafts between trees were considered, while self-grafts (grafts between roots of the same tree) were not considered.

5.3.3 Laboratory work

All cross-sections were air-dried and progressively sanded (80–500 grit paper) to clearly reveal growth rings. Root grafts were dissected with a band saw to examine their anatomy. Age of trees, roots and grafts was determined by counting growth rings and cross-dating using skeleton plots and pointer years (frost marks, light rings, compression wood, narrow and wide rings), to confirm ages and identify missing rings (Schweingruber 1988). To determine the time needed for roots to reach the position where grafting occurred, age of grafted roots were recorded near the stump base (when roots were initiated) and at the graft location (when roots initiated grafting). As graft and root samples often exhibit eccentric

growth and discontinuous rings (Krause and Eckstein 1993; Reynolds and Bloomberg 1982), highly problematic sections (e.g., with very narrow and incomplete growth rings), were cut with razor blades and ring-to-ring contrast was increased with white chalk. During root graft formation, tree rings are blurred due to callus formation and it is difficult to pinpoint exactly when bark was broken and when fusion occurred between the two roots. The year following the last visible ring on each root was thus arbitrarily considered as the year when root grafting begun. The last year (tf) was determined as the year when a first common and complete growth ring appeared. Stump sections were carefully examined to see if any growth rings were formed on the stumps of removed trees after the thinning treatment. As stumps could also be alive without producing growth rings (Bormann 1966), they were considered living if at least a small portion of the bark was living at the time of excavation (6 or 9 years of survival after thinning, according to sites). Stumps that were dead at the time of excavation were considered as dead after thinning, even though they may have survived for a few years without producing any growth rings. Root diameter was measured at the base of trees and at graft initiation. To minimize bias due to root eccentricity, root diameter was determined as the average between the longest and shortest diameters of the root.

Cross-sections that had been taken from the stem base (0m) were used for growth measurements of the trees. Ring-width series from the center pith to the outermost ring of each disk were measured on 4 rays (when possible) using a Velmex unislide measuring table (Velmex Inc., Bloomfield, NY) interfaced with a computer. The first ray was positioned at 22.5° from the longest cross-sectional radius and the three consecutive rays were perpendicular to the first (Zarnovican 1985). Subsequent radial growth curves were then cross-dated with one another and validated with the computer programs COFECHA (Grissino-Mayer 2001) and TSAPWin (RINNTECH, Engineering and Distribution, Heidelberg, Germany). Series were detrended and indexed (standardized) using the program ARSTAN (Grissino-Mayer 2001). Only one detrending method, regression lines, was applied to the data. This method of standardization slightly decreases growth differences between trees of different age and size, without eliminating important growth trends (Grissino-Mayer 2001).

5.3.4 Statistical analysis

Tree age, root age and root diameter at graft initiation of the three thinned sites (T; Figs. 5.1a and 5.2a) and of the two control stands (C; Figs. 5.1b and 5.2b) were tested using χ^2 analyses to determine if observed root grafting frequencies were equally distributed within each age and root diameter class. To examine whether or not a growth response was triggered by thinning and the influence of the grafting status, a hierarchical mixed model analysis was performed in R (v. 2.7.2, R Development Core Team 2008) using the lme function in the nlme library (linear and nonlinear mixed-effects models; Pinheiro et al. 2008). For all sites (T or C), we differentiated grafted trees (G) from non-grafted trees (NG). For the thinned stands, radial growth of grafted trees was radial growth of living trees that were grafted with stumps of removed trees. Radial growth 5 years before (1994–1998; sum of the 5 years of growth prior to thinning; time0) was compared with radial growth 5 years after thinning (1999–2003; sum of the 5 years of growth after thinning; time1). The type of stand (T or C) was incorporated into the model as a categorical actor. The interaction time×stand compared radial growth between the two dates (before and after 1998) and determined if growth differences between thinned and controls stands were statistically significant. Unlike traditional analysis of variance, linear mixed models incorporate both fixed-effect parameters and random effects for longitudinal, clustered or repeated-measures data to estimate the relationship between a continuous dependent variable and various predictor variables (West 2007). Sites were treated as random effects, to avoid sacrificial pseudo-replication error caused when data from different experimental units are used as independent replicates and pooled in the same analysis (Hulbert 1984). Each ray measurement was nested within its corresponding tree and trees were nested within their corresponding site. A first model (THINNING1) was created by pooling grafted and non-grafted trees, while second and third models considered only non-grafted (THINNING2) or grafted (THINNING3) trees (Table 5.3). In THINNING1 and THINNING3, graft presence was integrated as a numerical factor from 0 to 5 grafts tree^{-1}. Backward selection was used to select to most appropriate model explaining growth of trees in response to thinning for THINNING1, THINNING2 and THINNING3 (Burnham and Anderson 2004; Table 5.3). Backward selection tested the model containing all the variables (global model) and removed the least significant variable until all

variables included in the model were significant at $P \leq 0.05$. Tukey's test was used to separate significant mean values.

To further examine how root grafting affected growth response to thinning, radial growth of grafted and non-grafted trees was compared 6 years after the year of thinning (1998), for thinned and control stands (GRAFT TC model, Table 5.3). Unlike THINNING1, this model only focused on growth after 1998 according to tree status (G or NG) which allowed us to concentrate on the difference between grafted and non-grafted trees after commercial thinning. THINNING models allowed us to see if radial growth increased or decreased after 1998 but not to see year-by-year variation. Root grafting presence (G or NG) and the observed years (0, 1, 2, 3, 4, 5 and 6) were fixed as categorical factors (Table 5.3). To fix the observed years as a categorical factor permitted us to reveal yearly radial growth differences. The interaction graft×year compared radial growth of grafted and non-grafted trees for each observed year and the triple interaction stand×graft×year compared radial growth of grafted and non-grafted trees for each observed year between thinned and controls stands. For sites 1, 2, 4 and 5 (excavated in 2004), only 6 years post-thinning could be examined while for site 3 (excavated in 2007), 9 years could be considered. Since data from all sites were pooled, only 6 years post-thinning were considered. Rays, trees and sites were considered nested random effects. As yearly growth values are not independent from each other (growth at year t is affected by growth of year t^{-1}), a first-order autoregressive correlation structure was used to reflect the strong correlation that exists between successive observations (repeated measures), with correlations decreasing with the spacing of observations in time (Grissino-Mayer 2001; Pinheiro et al. 2008). A backward model selection technique was used to validate the most suitable model for GRAFT (Burnham and Anderson 2004) and Tukey's test was used to separate significant mean values. To verify if grafted stumps survived longer than non-grafted stumps, survival in years after stem removal and according to graft status was analyzed with another lme function (DEAD model; Table 5.3).

Table 5.3 Hierarchical mixed models containing all explanatory variables tested with lme functions.

	Global model
THINNING 1	Sum of 5 years of growth ~ stand (T or C) + graft (0 to 5) + period (0 or 1) + graft × period + stand × period
THINNING 2	Sum of 5 years of growth ~ stand (T or C) + period (0 or 1) + stand × period
THINNING 3	Sum of 5 years of growth ~ stand (T or C) + graft (0 to 5) + period (0 or 1) + graft × period + stand × period
GRAFT_TC	Yearly radial growth (1998-2004) ~ stand (T or C) + graft (NG or G) + year (0 to 6) + stand × graft × year
DEAD	Stump survey ~ graft (NG or G)

Notes: Sum of 5 years of growth is the sum of width of 5 growth rings (5 years before thinning and 5 years after thinning). Stand (T or C) is the kind of treatment (T for thinned and C for control stands). graft (0 to 5) is the numerical number of grafts. period (0 or 1) is the date (0 for 1994-1998 or 1 for 1999-2003). graft × period is the interaction between the grafting status and the observed period. stand × period is the interaction between the treatment and the observed period. yearly radial growth (1998-2004) is the width of each growth rings from 1998 to 2004. graft (NG or G) is the root grafting status (NG = non-grafted while G= grafted).year (0 to 6) is the observed years following 1998. stand × graft × year is the interaction between the treatment, the grafting status and the observed years. stump survey is the number of years of survival after stem removal

5.4 Results

Percentages of grafted trees varied from 44 to 69% in the three thinned stands and were 56% and 71% in the two controls stands (Table 5.1). Mean number of grafts per tree ranged 0.67–0.92 in the thinned stands and was 0.69 and 0.88 in the control stands (Table 5.1). The number of grafts per hectare ranged from 2400 to 3600 in the thinned stands (Table 5.1) and was 3750 and 2750 for sites 4 and 5 respectively (Table 5.1). At site 1, there were nine grafted trees that formed three clusters: one group of two trees, one group of three trees and another of four trees. One tree had been thinned in each of the groups of two and three, while three trees were felled in the group of four trees. At site 2, eight trees were grafted into three groups: one group of four trees and two groups of two trees. Three trees were thinned in the group of four trees, while one tree was cut in each group of two trees. At site 3, fifteen trees had root grafts, which clustered into six groups of grafted trees: three groups of two trees, and three groups of three trees. One tree was cut in each group of two trees and in one group of three trees, while two trees were removed in the last two groups of three trees. At site 4, twelve trees had root grafts: one group of two trees, two groups of three trees and one group of four trees. Nine trees had root grafts at site 5: three groups of two trees and one

group of three trees. Trees within a group were usually only grafted to one or two trees of the group.

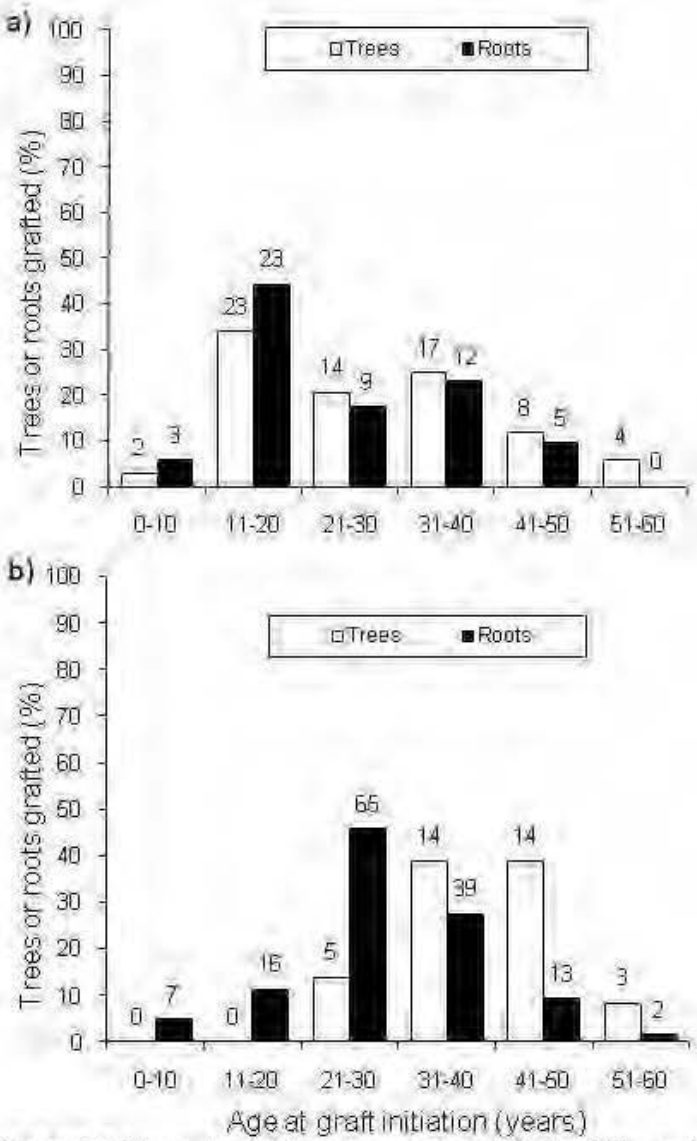

Figure 5.1 Percentage of trees or roots grafted within each age class at graft initiation for thinned (a) and control stands (b). It corresponds to the age of trees/roots when grafting begun. Numbers above the bars indicate the total number of trees/roots measured in each class.

For thinned stands, the age difference of roots between the base of the tree and the graft location, which corresponds to the time it took for roots to reach the location where grafting occurred, varied from 0 to 11 years (mean ± SE = 1.9 ± 0.7 years). For the control stands, the time needed for roots to reach the graft location varied from 0 to 8 years (mean ± SE = 2.4 ± 0.4 years). Root grafting started early in stand development and continued throughout the life of the stands (Fig. 5.1). In the thinned stands, tree age at graft initiation varied from 2 to 58 years and root age varied from 8 to 47 years (Fig. 5.1a), while tree age at graft initiation varied from 21 to 55 years and root age ranged from 18 to 55 years in the control stands (Fig. 5.1b). Although both young and old trees/roots formed root grafts, most grafts in the thinned stands were formed when trees and roots were between 11- and 20-years-old (χ^2 test, $P < 0.001$), and very few grafts were formed when trees and roots were less than 10-years-old (Fig. 5.1a). In the control stands, most grafts occurred when trees were between 31- and 50-years-old, when roots were between 21- and 30-years old (χ^2 test, $P < 0.001$), while few grafts were found in trees/roots less than 20-years old (Fig. 5.1b). In the thinned stands, root diameter at graft initiation was not evenly distributed among size categories (χ^2 test, $P = 0.010$), and most grafts formed between roots that were 21 - 80 mm in diameter (Fig. 5.2a) while in the control stands, root diameter was evenly distributed (χ^2 test, $P = 0.175$; Fig. 5.2b).

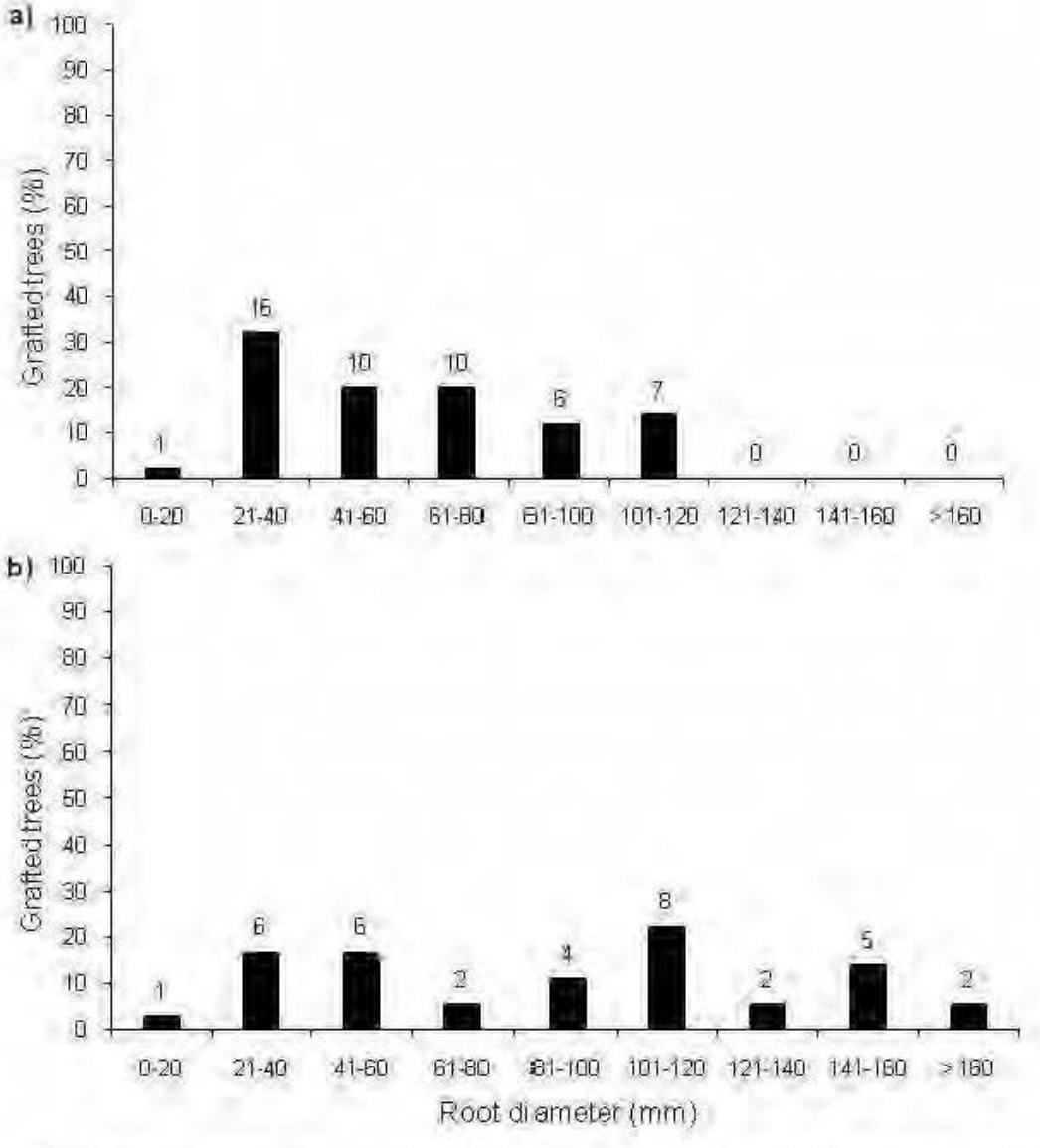

Figure 5.2 Percentage of trees with grafted roots within each diameter class at graft initiation for thinned (a) and control stands (b). Numbers above the bars indicate the total number of trees/roots measured in each class.

The following equations predicted the sum of 5 years of growth in relation to time (before thinning = 0, after thinning = 1), stand type (control = C, thinned = T), and the interaction time × stand (Table 5.4):

[1] For all trees (R^2 = 0.530):

Sum of 5 years of growth = 443.6526+ (67.5400A) + (-102.1530B) + (91.0088AB)

[2] For non grafted trees only (R^2 = 0.517):

Sum of 5 years of growth = 442.6042+ (37.3947A) + (-91.7978*B) + (139.2803AB)

[3] For grafted trees only (R^2 = 0.545):

Sum of 5 years of growth = 428.6718+ (127.8366A) + (-85.4543B)

where A = 0 for the sum of 5 years before 1998 and A = 1 for the sum of 5 years after 1998, B = 0 in thinned stands and B = 1 in control stands.

There was a significant interaction time × stand for THINNING1 and THINNING2 models ($P < 0.001$), indicating that radial growth of trees was significantly greater in control stands compared with thinned stands before 1998, but that this difference disappeared after 1998 (Table 5.4, Fig 5.3a, b). For grafted trees only (THINNING3 model), radial growth of trees was significantly greater after 1998 ($P < 0.001$; Table 5.4; Fig. 5.3c) and tended to be smaller in thinned stands compared with control stands ($P = 0.061$; Table 5.4; Fig. 5.3c). The time × stand interaction was not significant for THINNING3 ($P = 0.324$; Table 5.4, Fig. 5.3c), showing that growth increment for thinned and controls stands was similar before and after 1998. In THINNING1 and THINNING3, there was no effect of the number of root grafts on the growth response to thinning ($P > 0.05$; Table 5.4).

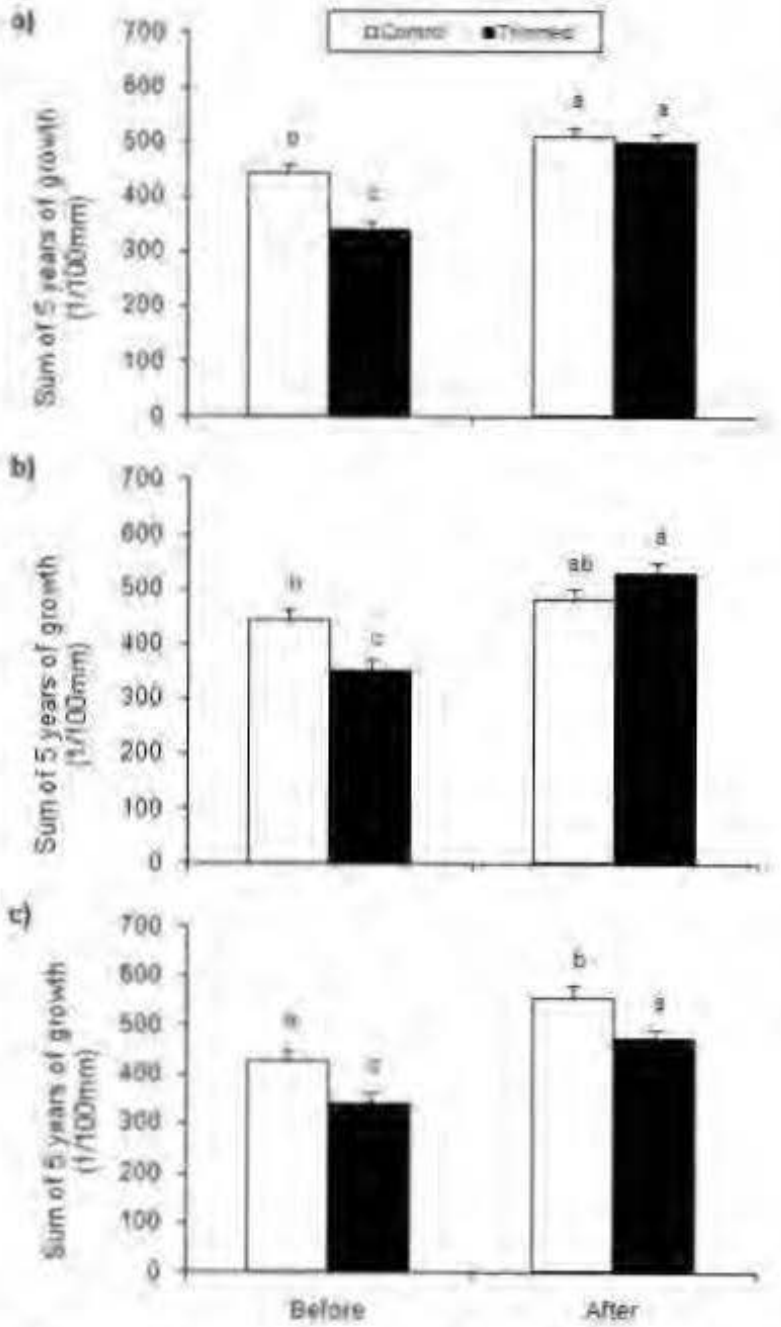

Figure 5.3 a) Sum of five years of growth in thinned and controls stands before and after 1998 (thinning year) for all trees (a), for non-grafted trees only (b) and for grafted trees only (c). Letters above the bars indicate a significant difference within a category (Tukey's test at $P \leq 0.05$).

Tableau 5.4 Selection of mixed-effects models (lme) about the comparison of the radial growth 5 years before the thinning date (1998) and the radial growth 5 years after for the thinned and control stands. THINNING 1 is the model combining all trees while THINNING 2 and THINNING 3 are respectively the models for non-grafted and grafted trees only. The factors, estimates values, standard error and the P values are given for each model. Statistically significant values ($P < 0.05$) are given in bold.

		Factors	Estimate value	Standard error	P value
THINNING 1	Global model	(Intercept)	445.350	20.373	**< 0.001**
		graft	-1.717	9.285	0.854
		period (1)	67.232	17.742	**< 0.001**
		stand (T)	-102.727	24.644	**0.025**
		period (1) × stand	91.052	20.735	**0.000**
		graft × period (1)	0.288	8.388	0.973
		(Intercept)	445.190	19.876	**< 0.001**
		graft	-1.573	8.287	0.850
		period (1)	67.540	15.266	**< 0.001**
		stand (T)	-102.693	24.628	**0.025**
		period (1) × stand	90.991	20.624	**< 0.001**
	Selected model	(Intercept)	443.653	17.387	**< 0.001**
		period (1)	67.540	15.267	**< 0.001**
		stand (T)	-102.153	23.682	**0.023**
		period (1) × stand	91.009	20.625	**< 0.001**
THINNING 2	Global/selected model	(Intercept)	442.604	20.835	**< 0.001**
		period (1)	37.395	20.382	**0.071**
		stand (T)	-91.798	29.592	**0.053**
		period (1) × stand	139.280	28.462	**< 0.001**
THINNING 3	Global model	(Intercept)	434.924	40.055	**< 0.001**
		graft	2.144	14.834	0.887
		period (1)	135.953	35.412	**< 0.001**
		stand (T)	-103.183	34.194	0.057
		period (1) × stand	29.941	30.193	0.324
		graft × period (1)	-14.063	13.036	0.284
		(Intercept)	423.472	38.392	**< 0.001**
		graft	3.787	14.755	0.801
		period (1)	158.924	26.779	**< 0.001**
		stand (T)	-88.296	30.756	0.064
		graft × period (1)	-17.382	12.596	0.171
		(Intercept)	439.016	36.695	**< 0.001**
		graft	-4.899	13.340	0.718
		period (1)	127.814	14.491	**< 0.001**
		stand (T)	-88.271	30.746	0.064
	Selected model	(Intercept)	428.672	23.113	**< 0.001**
		period (1)	127.837	14.491	**< 0.001**
		stand (T)	-85.454	29.162	0.061

Notes: graft is the numerical number of grafts. period is the date (0 for 1994-1998 or 1 for 1999-2003). stand (T or C) is the kind of treatment (T for thinned and C for control stands). period × stand is the interaction between the treatment and the observed period. graft × period is the interaction between the grafting and the date. Letters or numbers in brackets correspond to the type considered. Combined with the values (column estimate) it permits to calculate the predicted values. i.e. in THINNING 3, radial growth at time (1) in stand (T) was 428.671 + 127.837 - 85.454.

Table 5.5 Results of mixed-effects models (lme) about the influence of root grafting on the radial growth in thinned and control stands six years following treatment (GRAFT_TC). DEAD model is the model about the number of year of survival of the stump after stem removal. The factors, estimates values, standard error and the P values are given for each model. Statistically significant values ($P < 0.05$) are given in bold.

Model	Factors	Estimate value	Standard error	P value
GRAFT_TC	(Intercept)	92.798	9.504	**< 0.001**
	stand (T)	-33.627	13.508	0.089
	graft(G)	-0.858	13.050	0.948
	year (1)	7.933	8.173	0.332
	year (2)	2.356	9.597	0.806
	year (3)	5.244	10.084	0.603
	year (4)	-4.267	10.263	0.678
	year (5)	-25.000	10.329	**0.016**
	year (6)	-20.022	10.355	**0.053**
	graft (G) × year (1)	8.867	11.914	0.457
	graft (G) × year (2)	9.019	13.990	0.519
	graft (G) × year (3)	36.456	14.700	**0.013**
	graft (G) × year (4)	22.042	14.960	0.141
	graft (G) × year (5)	33.675	15.057	**0.026**
	graft (G) × year (6)	38.497	15.094	**0.011**
	stand (T) × year (1)	10.339	11.624	0.374
	stand (T) × year (2)	24.417	13.649	0.074
	stand (T) × year (3)	42.256	14.342	**0.003**
	stand (T) × year (4)	67.017	14.596	**< 0.001**
	stand (T) × year (5)	71.182	14.691	**< 0.001**
	stand (T) × year (6)	70.545	14.726	**< 0.001**
	stand (T) × graft (G)	6.130	17.983	0.735
	stand (T) × graft (G) × year (1)	-5.466	16.373	0.739
	stand (T) × graft (G) × year (2)	-10.004	19.225	0.603
	stand (T) × graft (G) × year (3)	-46.071	20.201	**0.023**
	stand (T) × graft (G) × year (4)	-40.099	20.559	**0.051**
	stand (T) × graft (G) × year (5)	-60.818	20.692	**0.003**
	stand (T) × graft (G) × year (6)	-63.231	20.743	**0.002**
DEAD	(Intercept)	0.385	0.552	0.492
	graft (G)	1.615	0.725	**0.034**

Notes: stand (T or C) is the kind of treatment (T for thinned and C for control stands). graft (NG or G) is the root grafting status (NG = non-grafted while G= grafted). year (0 to 6) is the observed years. Stand × graft × year is the interaction between the treatment, the grafting status and the years. Letters or numbers in brackets correspond to the type considered by the model. Combined with the values (column estimate) it permits to calculate the predicted values. i.e. in DEAD model. the predicted values of survey for stumps of non-grafted trees was 0.385 years while for the grafted stumps. it was 0.385 + 1.615 years.

Results of GRAFT_TC model showed a significant interaction stand × graft × year interaction the third ($P = 0.023$), fourth ($P = 0.051$), fifth ($P = 0.003$) and sixth ($P = 0.002$) year after thinning (Table 5.5). This indicated that radial growth of grafted trees remained constant and above that of non grafted trees in control stands while it tended to decline and was below that of non grafted trees in thinned stands (Fig. 5.4).

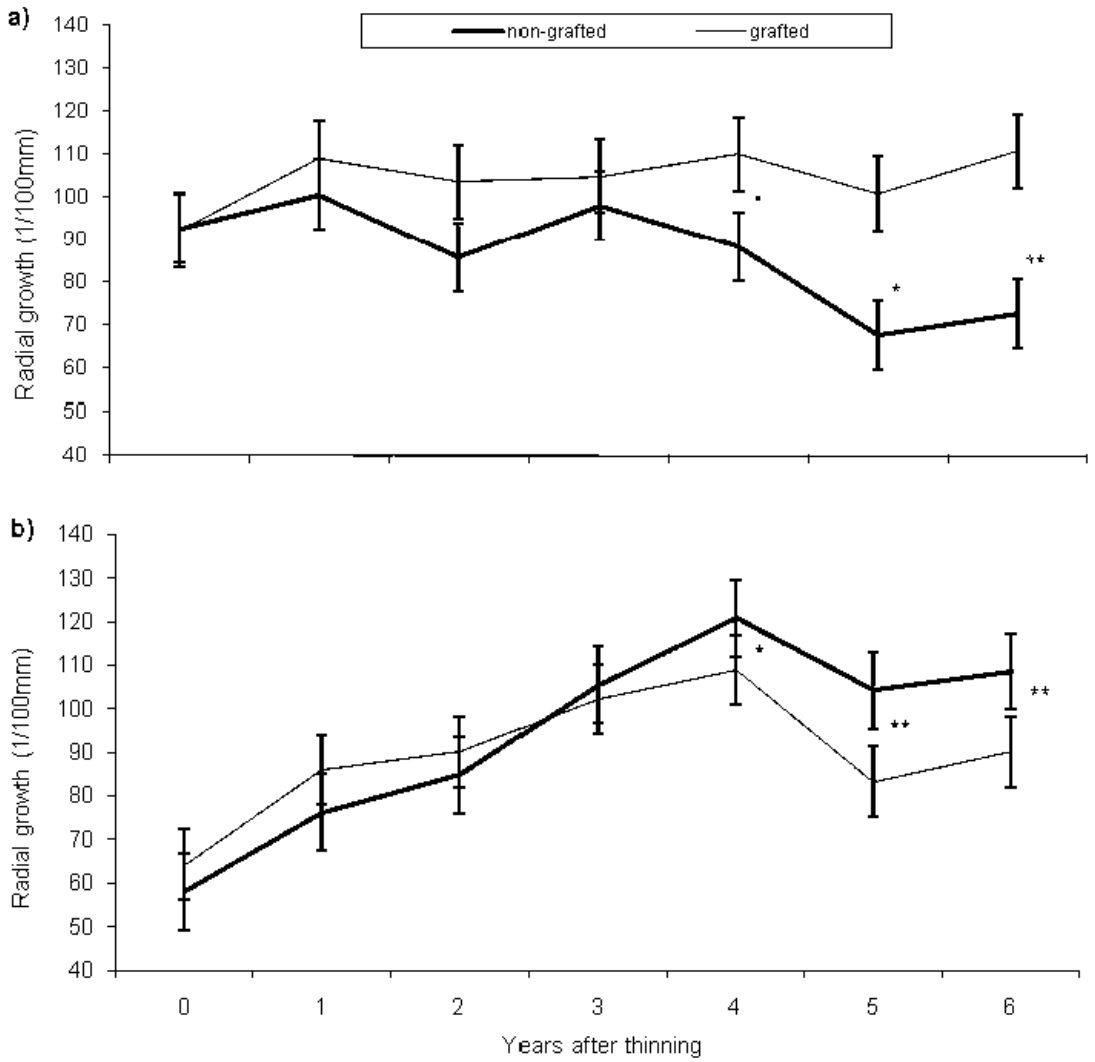

Figure 5.4 Radial growth of grafted and non-grafted trees six years after 1998 (thinning year) in control (a) and thinned stands (b). Symbols indicate a significant difference between grafted and non-grafted trees (Tukey's test): $0.001 \leq P \leq 0.01$ = '**', $0.01 \leq P \leq 0.05$ = '*', $0.05 \leq P \leq 0.1$ = '.'.

In thinned stands, we found thirteen non-grafted stumps and eighteen stumps grafted to living trees. Grafted stumps survived longer than non-grafted stumps ($P = 0.034$; Table 5.5; Fig. 5.5). Ninety percent of stumps from removed trees were dead at the time of excavation, while 3% were still living after 6 years (sites 1 and 2), and 6% after 9 years (site 3). Of these living stumps, 100% had produced partial growth rings near the point where roots were grafted with roots of residual trees. Sixty-nine percent (69%) of non-grafted dead stumps did not produce any growth rings after thinning, while 23% produced one growth ring, and 8% produced two growth rings. Thirty-nine percent (39%) of dead grafted stumps did not form growth rings after their corresponding stems were removed, while a smaller percentage had one (17%), two (17%), three (6%), four (6%), five (6%), six (6%), or nine (6%) growth rings after thinning, respectively. On average, non-grafted stumps survived 0.4 years, while grafted stumps lived 2.0 years after the stem was removed.

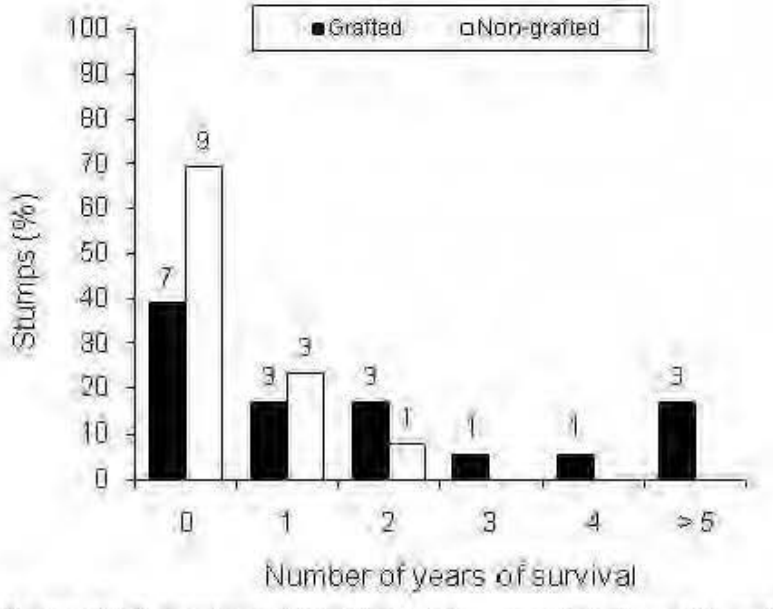

Figure 5.5 Percentage of grafted and non-grafted stumps found in thinned stands according to their number of years of survival after their corresponding stem was thinned. Numbers above bars indicate the total number of stumps measured in each class.

5.5 Discusssion

This study showed that commercial stand thinning increased radial growth of residual jack pine trees starting the first year after thinning and increasingly more for the following three years (Fig. 5.4b). There was thus no stagnation of tree growth after thinning such as observed in other studies (Latham and Tappeiner 2002; Liu et al. 2003; Varmola et al. 2004; Yang 1988; Youngblood 1991). It has been reported that trees generally need time to acclimate to the modification of their environment following thinning and to completely take advantage of higher levels of nutrients and light availability (Bella and DeFranceschi 1974; Juodvalkis et al. 2005; Mitchell 2000). However, after about a 4-year delay, radial growth of grafted trees was generally lower than that of non-grafted trees (Fig. 5.4b), and the overall positive effect of thinning can be mostly attributed to the better growth of non grafted trees (Fig. 5.3b); Although growth was better in the 5 years after 1998 for all three THINNING models, growth of grafted trees in thinned stands (THINNING3 model) tended to be under that of grafted trees in control stands, while it was the opposite for non-grafted trees (Fig 5.3b, c). Furthermore, radial increment from thinned and control stands were similar in THINNING3 while it was greater for thinned stands in the other models that included non grafted trees (Fig. 5.3). These results corroborate the hypothesis that the thinning treatment did not increase growth of grafted trees because photosynthates from living residual trees were diverted via grafts to roots and stems of the removed trees during thinning (Eis 1972; Fraser et al. 2007). This is also supported by the fact that significantly fewer non-grafted stumps survived than grafted stumps and thanks to grafts some of them still alive and forming growth rings 6 or 9 years post-thinning (Fig. 5.5). Since up to 70 % of trees could be linked by their roots, root grafting is an important phenomenon to consider by forest managers. Efforts should be made to detect root grafting frequency and severe thinning treatments should be avoided in potentially heavily grafted stands. In site 3, growth differences between grafted and non-grafted trees were less noticeable (unshown data). Perhaps the weaker responses on this site could be explained by the imposition of a less severe thinning treatment compared to the other sites (36% of trees removed *versus* 46% and 56% for site 1 and site 2, respectively). In consequence, light thinning could be recommended in presumed highly grafted stands.

By looking at yearly growth responses post-thinning between thinned and control stands, it appears that root grafting was beneficial to tree growth in unthinned stands while it negatively affected diameter growth in thinned stands (Fig. 5.4). Perhaps root grafting allows trees to better cope with environmental stresses because of the sharing of water, photosynthates or other substances (Basnet et al. 1993; Bormann 1966; Loehle and Jones 1990). In thinned stands, however, roots and stumps of removed trees grafted to residual living trees probably constitute a large sink for carbohydrates (Fraser et al. 2007), thereby limiting aboveground growth of the residual trees (Bormann 1966; Eis 1972; Graham and Bormann 1966). The observed delay of about four years likely represents the time for the underground biomass of removed trees to exhaust their own carbohydrate reserves, as reported by others (Bormann 1961; Schultz and Woods 1967; Stone 1974). It now seems undeniable that underground biomass left behind after a tree is harvested can acquire carbohydrates and other compounds from residual trees through root grafts with living trees (Fraser et al. 2007). It is well known that a favourable balance between roots and shoots (i.e., root-to-shoot ratio, R/S) is critical for growth of plants (Mata et al. 1996; Mc Cree 1986). Removal of trees from a communal root system by thinning creates a drastic increase in R/S, which may be beneficial for residual trees only if they are able to compensate for respiration costs incurred by the roots and stump biomass left behind by increasing photosynthetic capacity (Eis 1972). The time required for roots and shoots to re-equilibrate after thinning (death of roots and stumps of thinned trees and/or increase in crown size of residual trees) could thus be a valid explanation of 'thinning shock.' It is very unlikely that stumps could still be alive at the time of excavation (up to 9 years later) unless they were supported by living residual trees. Stumps of red pine (Stone 1974) and Douglas-fir (*Pseudotsuga menziesii var. menziesii*; Eis, 1972) have also been reported to survive more than twenty years. Thinning shock has often been linked to site quality; poor sites were found to have greater growth reductions following thinning compared to higher quality sites (DeBell et al. 2002; Mäkinen and Isomäki 2004a, b). We previously showed that root grafting frequency was higher in sandy soils compared to clayey soils, which are generally more nutrient-rich (Tarroux and DesRochers 2010). Hence site quality and root grafting could be linked, thereby in turn affecting responses to thinning.

Secondary growth on stumps after thinning implies that there is mitotic activity, which requires transfer of auxins to the cambial zone (Sundberg et al. 2000; Sundberg and Uggla 1998; Sundberg et al. 1993). Auxins have been shown to circulate only in a single direction (polarized transport), from the apex to the base of trees (Bormann 1966; Sundberg et al. 1993). When living trees are grafted to stumps, it appears that auxins were transferred from the apex to the root system and up toward the cut stump. This suggests that the biomass left behind after thinning (roots and stumps) either becomes part of the residual live tree's root system, independently of its original nature (root or stem), or that auxin transport is not strictly polarized (Jacobs 1979). Since auxins are unstable phytohormones that are mostly synthesized in meristems and young leaves (Epstein et al. 1980; Sundberg et al. 2000; Sundberg and Uggla 1998), there are normally only auxin traces in stumps devoid of leaves and buds (Sundberg et al. 2000; Sundberg and Uggla 1998; Sundberg et al. 1993). It was thus surprising to find that a few non-grafted stumps could 1) generate enough energy to compensate for growth respiration costs, and 2) produce secondary growth without auxin transfers from a grafted tree (Bormann 1961, 1966). Since stands were completely excavated and root systems hydraulically exposed, it is unlikely that some root grafts between stumps and live trees were missed. Perhaps auxins synthesized in apical root meristems, such as found in *Arabidopsis thaliana* (Ljung et al. 2005), could support partial secondary growth of stumps of removed trees. Moreover, auxins in tree stems are not as unstable as have been observed in other systems (Sundberg et al. 2000; Sundberg and Uggla 1998) and it has been shown in girdled Scots pine (*Pinus sylvestris* L.) that cambial activity in the year following treatment was initiated with auxins stored during the whole winter (Egierszdorff 1981). It has also been shown that most of a plant's endogenous auxin is found not as a free and biologically active form, but as conjugates (Bajguz and Piotrowska 2009). Perhaps conjugated compounds could serve as pools of inactive auxins (stable) that can be converted to active forms by de-conjugation reactions, providing a source of free hormones under changeable environmental, developmental or physiological conditions (Bajguz and Piotrowska 2009; Sundberg et al. 2000). Such auxin pools constitute important sources of active hormones in germinating seeds (Epstein et al. 1980), but their importance in secondary growth of trees is unclear (Sundberg et al. 2000).

Armson and van den Driessche (1959), and Dosen and Iyer (1979) found that the percentage of grafted trees was higher in thinned compared to non-thinned red pine stands. This would suggest root grafting as an adaptive response to thinning (Loehle and Jones 1990), for example, in response to increased windthrow susceptibility after stands have been thinned (Ruel et al. 2003). It has indeed been shown that stands with a high proportion of grafted trees were more resistant to windthrow (Basnet et al. 1993; Coutts 1983b; Graham and Bormann 1966). Our results show, however, that most root grafts occurred early in stand development and no increase in root grafting frequency was observed after thinning (Fig. 5.1).

In conclusion, although a 'thinning shock' was not readily observed, root grafting significantly reduced growth of trees in response to thinning. This growth reduction of grafted residual trees appears to be linked to the support of roots and stumps of removed trees that could survive up to six and nine years post-thinning. The fact that standing trees may support grafted stumps and root biomass at the expense of their own growth suggests that stands with communal root systems respond to environmental change as a group rather than as individual trees. Since most silvicultural treatments are based on the fact that trees are discrete entities competing with each other for resources, some forest management concepts may need to be revisited in the light of root grafting.

CHAPITRE VI

DISCUSSION

La signification écologique des greffes racinaires n'avait jamais été démontrée. La présence de greffes avait été reportée chez beaucoup d'espèces de pins mais jamais chez le pin gris, une espèce pourtant très répandue en forêt boréale. Cette thèse avait pour objectifs de déterminer la fréquence des greffes racinaires chez cette espèce et d'identifier les facteurs en favorisant la formation mais surtout d'évaluer leur influence sur l'écologie des peuplements.

6.1 Facteurs influençant la formation de greffes racinaires

Nous avons démontré que les caractéristiques des sites avaient une forte influence sur la fréquence des greffes; le principal facteur étant la distance entre les arbres. Il avait déjà été mentionné que la proximité des arbres importait plus que la densité (Cook et Welch 1957; Eis 1972; Fraser et al. 2005; Gordon 1976; Külla et Lõhmus 1999; Reynolds et Bloomberg 1982; Saunier 1965). Même s'il a été trouvé que la densité du peuplement influençait significativement le nombre de greffes, le pourcentage d'arbres greffés et l'âge des racines au commencement de la greffe, la distance entre les arbres semblait être un meilleur facteur que la densité pour expliquer certaines variables réponses comme l'âge des arbres et des racines au commencement de la greffe ainsi que la durée de formation. En outre, la densité n'a pas montré d'influence sur la distance entre les arbres greffés ce qui suggère que la densité reflète mal la distribution spatiale des arbres à l'intérieur des peuplements. Pour la plupart des sites étudiés, il existait une corrélation entre la distance entre les arbres greffés et la présence de greffes. Les précédents travaux indiquaient que les greffes ne se faisaient pas entre des arbres distants de plus de 3 m (Adams 1940; Eis 1972; Külla et Lõhmus 1999). Dans cette étude, nous avons observé que 95% des greffes se faisaient entre des arbres distants de moins de 1,72m dans les peuplements naturels et de moins de 2,35m dans les plantations. Étant donné

qu'il n'y a pas de bosquets d'arbres dans les plantations, il n'est pas surprenant de voir que les arbres des plantations se greffent plus loin. Par contre, malgré un nombre de greffes légèrement inférieur dans les plantations, le fait que les pourcentages d'arbres greffés soient similaires aux peuplements naturels était par contre plus inattendu étant donné la distance initiale plus grande et la répartition homogène des arbres en plantation. Ceci suggère que la formation de greffes racinaires est un trait important pour les arbres et que peu importe les conditions dans lesquelles les arbres poussent, ils seraient capables de s'adapter et de produire des greffes racinaires dans tous les types de peuplements. Il est intéressant de constater que la distance entre les arbres était une meilleure variable prédictive pour les peuplements naturels que pour les plantations. Non seulement les régressions logistiques étaient plus significatives pour les peuplements naturels, mais dans les plantations sur argile, des greffes entre des arbres distants de près de 3m ont été trouvées. Les arbres se sont donc adaptés et ont quand même produit des greffes peut importe la distance qui les séparaient. Quoiqu'il en soit, dans les peuplements éclaircis, la production de greffes n'a pas augmenté suite au traitement, tel que suggéré par Armsom et Van den Driessche (1959) et Dosen et Iyer (1979) comme étant une adaptation au stress par le vent.

Si les arbres sont parfois capables d'adaptation, il semblerait aussi qu'ils soient capables de communiquer entre eux par des signaux chimiques. Toutes les racines qui entrent en contact ne forment pas des greffes. Tout comme Eis (1972), nous avons observé des cas où les racines passaient sur le système racinaire d'un ou plusieurs arbres voisins pour finalement aller se greffer à un arbre beaucoup plus loin. Comme nous avons démontré une certaine influence de la proximité génétique sur la formation des greffes, cela pourrait s'expliquer par le fait que les arbres qui étaient proches spatialement devaient être plus lointains génétiquement. Cependant, les conditions du milieu et particulièrement la proximité spatiale avaient avoir une plus forte influence sur la formation de greffes racinaires. La génétique permettrait probablement plus d'expliquer la variabilité entre les espèces que la variabilité entre les individus d'une même espèce. Si les caractéristiques des sites (sols, distance entre les arbres) et de l'arbre (âge, taille, génétique) ont une forte influence sur la présence de greffes, d'autres facteurs sont indubitablement en jeu. Nous avons trouvé que la distance entre les arbres greffés était affectée par la taille du plus petit arbre, suggérant

qu'une greffe se formera préférentiellement avec un arbre voisin s'il est plus petit. Cela peut être une conséquence de la longueur des racines mais nous avons démontré que la taille des racines n'influençait pas l'âge auquel les greffes se formaient. Cela suggère bien que les arbres sont capables de communiquer entre eux. Des inhibiteurs chimiques peuvent être produits pour éviter le contact racinaire (Reinartz et Popp 1987). De la même manière, il est probable que les arbres produisent des métabolites secondaires en proportion différente selon leur taille. Par exemple, un gros arbre sera plus exposé à la lumière, permettant la formation de métabolites secondaires en plus grande quantité ou en proportions différentes qu'un arbre supprimé, fournissant une voie de communication entre arbres de différente taille.

Les caractéristiques des arbres avaient aussi une forte influence sur la fréquence des greffes racinaires. D'autres ont observé que l'âge avait une influence positive sur le nombre de greffes (Basnet et al. 1993; Bormann et Graham 1959; Fraser et al. 2005). Nos résultats ont montré que les arbres se greffaient à partir du moment où les arbres étaient suffisamment matures et leurs racines assez longues pour entrer en contact les unes avec les autres. En effet, nous avons trouvé que plus les arbres étaient loin les uns des autres, plus ils étaient vieux lorsqu'ils ont commencé à se greffer. Cependant l'essentiel du système racinaire d'un arbre est produit dès les premières années (Plourde et al. 2009), et des greffes peuvent donc se produire dès le plus jeune âge. Si nous avons aussi observé que des greffes étaient produites dès la phase d'initiation du peuplement, les arbres produisaient en moyenne un maximum de greffes lorsque les arbres étaient suffisamment matures (20 ans pour les plantations, 37 ans pour les peuplements naturels). Cependant, comme nos résultats ont montré que plus les arbres étaient vieux et gros, plus l'âge des arbres et des racines au commencement de la greffe était élevé. Ceci laisse supposer que les premières greffes avaient disparu dans les peuplements naturels lors de leur phase d'auto-éclaircie. Par exemple, pour le peuplement naturel de 90 ans, il n'a pas été possible de retrouver des greffes datant de l'époque où les arbres avaient moins de 45 ans alors que 95% des greffes trouvées dans les peuplements de moins de 60 ans se sont formées avant que les arbres n'aient atteint 45 ans. Cela s'expliquerait par le fait qu'une partie des greffes a disparu en même temps que les arbres morts durant la phase d'auto éclaircie (Smith 1986). Il serait même probable que les greffes accélèreraient la vitesse de dépérissement des arbres durant cette période (Krasny et Johnson

1992). Les plus gros arbres contrôleraient les gradients d'eau et de nutriments, par exemple, par leur plus grand taux de transpiration ce qui pourrait accélérer la mort des arbres les moins vigoureux (Graham et Bormann 1966).

6.2 Les greffes racinaires et l'écologie des peuplements

Le fait que les arbres puissent communiquer entre eux et faire preuve d'adaptation quant à la production de greffes racinaires suggère qu'elles font partie intégrante de la vie et sont un trait bénéfique pour le peuplement. Grâce à la formation d'un système racinaire commun qui couvre une plus grande superficie, les greffes racinaires favorisent l'exploitation des ressources (Bormann 1966; Graham 1959; Graham et Bormann 1966). Dans des conditions de croissance difficiles, les arbres greffés seraient donc mieux adaptés que les arbres non greffés. Comme les sites sableux sont généralement moins riches que les sites argileux, il est possible que la forte présence de greffes dans le sable soit une réponse des arbres au stress. Mais la fréquence élevée de greffes dans le sable pourrait tout aussi bien être due à l'aspect abrasif du sable. Quoiqu'il en soit, notre étude sur la croissance suggère que les arbres greffés avaient généralement une croissance supérieure aux arbres non greffés, du moins en dehors des périodes de formation des greffes. .Non seulement les greffes favorisent la croissance mais les greffes peuvent également aider à la survie des individus les plus faibles (Fraser et al. 2007). Durant les excavations, beaucoup de souches greffées avec des arbres vivant ont survécu des années grâce au transfert via les greffes. Les greffes racinaires pourraient donc aider à maintenir l'intégrité du peuplement et la survie de l'espèce, en conservant les ressources d'un site au sein de l'espèce et en empêchant plus ou moins la colonisation du milieu par des racines ou semis d'autres espèces (Jelínková et al. 2009; Loehle and Jones 1990).

Comme une espèce semble avantagée par la présence de greffes, une espèce ayant tendance à former des greffes aurait un avantage d'un point de vue évolutif sur les autres espèces pour la colonisation du milieu. Dans tous les peuplements excavés, la fréquence de greffes racinaires était relativement élevée. Il est important de rappeler que les sites ont été choisis aléatoirement et non pas en fonction de leur probabilité de trouver des greffes racinaires. Si des greffes ont été découvertes dans tous les sites, il est donc probable que des

greffes existent dans tous les peuplements de pin gris. Parmi les 15 sites et 272 arbres étudiés, 149 (55%) présentaient des greffes racinaires. Cela signifie que plus de la moitié des arbres sont greffés dans les peuplements de pin gris. Selon les peuplements, jusqu'à 70% des arbres pouvaient être reliés entre eux. Le nombre élevé de greffes racinaires dans les peuplements de pin gris pourraient expliquer la croissance initiale rapide et la vitesse de colonisation élevée de cette espèce. Nous avons aussi constaté que les greffes semblaient faciliter la production de peuplements équiens. Or il est souvent remarqué que les peuplements de pin gris sont très équiens. S'il est souvent suggéré que cela est simplement dû au fait que tous les arbres sont issus après feu (même année ou presque), cela pourrait aussi être expliqué par un fort taux de formation de greffes. Le fait que la survie des plus faibles puisse être facilitée par les greffes et que les arbres reliés tendaient à homogénéiser leur taille suggère que le phénomène de compétition dans son sens strict n'est pas observé dans des peuplements où la fréquence des greffes racinaires est très élevée (Basnet et al. 1993; Bormann 1966; Loehle et Jones 1990). Les arbres greffés semblent réagir plus comme un groupe que comme des individus à part entière.

L'étude de Bormann (1966) sur le pin blanc indique qu'entre 2 arbres dominants, les échanges d'eau, de minéraux et de sucres sont équilibrés. Par contre, si un des arbres est dominant et que l'autre est co-dominant ou supprimé, l'eau et les nutriments devraient aller du dominant vers le supprimé. Mc Cree (1986) a décomposé la respiration (ΔR) en deux processus distincts : une respiration de croissance (Rc) et une respiration d'entretien (Rm). La respiration de croissance couvre les besoins énergétiques nécessaires à la production des nouveaux tissus et la respiration de maintenance ceux consécutifs à l'entretien des tissus déjà en place. Si un arbre vigoureux et ayant une partie aérienne bien développée se greffe à un autre arbre plus petit, il pourra supporter les besoins respiratoires supplémentaires d'entretien. Si l'arbre supprimé meurt ou est coupé, l'arbre encore debout va devoir prendre en charge son système racinaire et le fait d'acquérir de nouvelles racines déjà fonctionnelles pourrait être un avantage. En effet, l'arbre pourra augmenter sa capacité d'acquisition des ressources sans avoir besoin de synthétiser de nouvelles racines ce qui constitue un gain d'énergie au niveau de sa respiration de croissance. Mais si les greffes semblent être bénéfiques, cela peut dans certains cas aussi être vu comme du parasitisme si la biomasse racinaire laissée par la

souche constitue un fardeau trop important par rapport au bénéfice rapporté (Loehle et Jones 1990). Dans le cas où la photosynthèse ne suffirait pas pour couvrir tous les besoins, les hydrates de carbone seront alloués préférentiellement à la maintenance (Kozlowski et Cooley 1961; Kozlowski et al. 1991). La croissance serait alors inhibée. Selon la vigueur de l'arbre, le fait d'acquérir des racines déjà fonctionnelles pourrait donc être un avantage ou un inconvénient si l'arbre n'a pas assez de réserves ou de capacité photosynthétique pour les entretenir (Bormann 1966; Horton 1969). De plus, il y a certains inconvénients à la formation de greffes; le principal étant qu'il s'avère être un processus couteux énergétiquement (Bormann 1966; Loehle et Jones 1990). Il semblerait que pour beaucoup de greffes, le coût de la greffe (baisse de croissance radiale) était principalement assumé par le plus gros arbre de l'union. Incidemment, les arbres qui allaient plus tard se greffer dans les peuplements naturels étaient significativement plus gros que les arbres non greffés, probablement dû au fait qu'ils avaient plus de réserves énergétiques (cime plus grande, plus de lumière, plus de photosynthèse…). Dans les plantations, l'effet de la formation des greffes sur la croissance radiale des arbres n'était pas apparent, alors que même les plus petits arbres ont formé des greffes sans que leur croissance n'en soit réduite. Comme la lumière n'est généralement pas limitante en plantation, les arbres avaient possiblement assez de ressources pour augmenter leur capacité photosynthetique (Salisbury et Ross 1992) et compenser la formation des greffes.

6.3 Les greffes racinaires et l'aménagement des peuplements

Nos résultats soulèvent des interrogations sur l'efficacité des traitements sylvicoles actuels, notamment pour ce qui est des éclaircies commerciales. En effet, les résultats montrent que la réponse des individus greffés était différente des individus non greffés. Globalement sur les 5 années qui ont suivi le traitement, seule la croissance radiale des arbres non-greffés a augmenté. En regardant année par année, nous avons constaté que jusqu'à la 3^{ième} année, la croissance des arbres greffés avait augmenté mais à partir de la 4^{ième} année, les arbres greffés avaient une croissance inférieure aux arbres non-greffés. Comme les souches greffées à des arbres non coupés ont survécu plus longtemps que les souches non-greffées et qu'il est improbable qu'une souche ne survive plus de 2-3 ans sur ses propres réserves énergétiques; les greffes racinaires seraient donc à l'origine de leur survie et aussi de la

stagnation de croissance observée chez les arbres greffés résiduels. Les racines et les souches des arbres coupés survivent donc en drainant une partie des ressources mises en commun, ce qui limite la croissance voire affaiblit les arbres laissés debout. Le pin gris est une espèce économiquement importante pour le Québec et le Canada et c'est aussi une espèce chez laquelle beaucoup de traitements d'éclaircie sont effectués. Comme plus de la moitié des arbres d'un peuplement sont greffés, cela signifie que plus de la moitié des arbres risquent de ne pas bénéficier à court terme du traitement d'éclaircie. Ainsi les greffes devraient être considérées lors de la mise en place des pratiques d'aménagement afin de développer des traitements sylvicoles adéquats. Eis (1972) a aussi démontré l'importance de prendre en compte le statut de l'arbre coupé et de penser qu'il est peut être greffé :

- Quand le dominant est coupé d'une union dominant-dominant ou dominant-codominant, la croissance de la souche diminue jusqu'a atteindre environ 20 à 30% de ce qu'elle était avant la coupe. Pour le donneur, l'accroissement du diamètre augmenterait grâce à la baisse de la compétition.

- Quand le co-dominant est coupé d'une union dominant-codominant, la croissance radiale de la souche diminue jusqu'a 20% de ce qu'elle était à la base. Celle du donneur augmente légèrement.

- Quand le dominant est coupé d'une union dominant-supprimé, la souche ne survit que quelques années, produisant des cernes étroits et discontinus. Une baisse de croissance du donneur est enregistrée jusqu'a ce que la souche meurt, ensuite, cela augmente légèrement. Si la souche survit, alors la croissance radiale du donneur reste faible.

Dans une union où l'on souhaite réaliser une éclaircie, Bormann (1966) considère aussi que connaitre le statut et la vigueur des arbres en question est primordial pour comprendre les conséquences sur le développement des arbres. Külla et Lõhmus (1999) ont trouvé chez l'épinette de Norvège que des greffes ne se formaient pas entre 2 arbres supprimés et que dans 86 à 100% des cas, un des arbres était un dominant ou un co-dominant. Ainsi, il serait probablement plus judicieux de réaliser des éclaircies moins sévères et par le bas (Smith 1997) dans les peuplements où la probabilité de présence de greffes est élevée,

afin d'éviter qu'une trop grande quantité d'énergie ne soit gaspillée pour le support énergétique des souches et des racines des arbres coupés. Pour un des sites éclaircis, il semblait que la réponse des arbres greffés était moins drastique que pour les 2 autres sites et ce site avait la particularité d'avoir subi l'éclaircie la moins sévère et beaucoup d'arbres enlevés étaient plus petits.

Cette thèse est la première à établir la signification écologique des greffes et à démontrer que les greffes ont une influence positive sur la croissance et la survie des arbres. La dynamique des peuplements est clairement influencée par la présence de greffes et le fait que les arbres inter-reliés réagissent plus comme un groupe que comme des individus distincts démontre que des relations de type non-compétitrices existent entre des arbres greffés. L'inefficacité des traitements d'éclaircie sur les arbres greffés montre l'importance de ce phénomène. Des millions de dollars sont utilisés chaque année pour la réalisation de traitements sylvicoles dont les résultats ne sont pas garantis. Si les greffes racinaires n'ont pas explication à tout, il est certain que leur prise en considération ne pourrait qu'améliorer l'aménagement des peuplements de pin gris. Dans une optique d'aménagement durable, ce type de relations devrait être pris en compte. Un gros travail a été fait pour mieux comprendre le phénomène des greffes racinaires et si ce projet a permis des avancées, il est cependant important de continuer les recherches. Par exemple, notre étude ne nous permet pas de déterminer l'influence à long terme des greffes racinaires face aux traitements d'éclaircie. Si à court terme, le fait de partager ses ressources avec une souche semble préjudiciable, l'acquisition d'un système racinaire déjà en place pourrait être bénéfique à plus long terme. Selon Bormann (1966), si la croissance de l'arbre greffé à la souche diminue trop drastiquement, un équilibre s'établit et une partie du système racinaire récemment acquis meurt. Si un équilibre peut être atteint, le fait d'être greffé représenterait alors un énorme avantage à long terme pour les arbres greffés à des souches. Une fois l'équilibre obtenu, leur croissance pourrais rattraper voire dépasser celle des arbres non-greffés. Il serait donc intéressant d'étudier des peuplements ayant été éclaircis très longtemps avant et d'étudier des peuplements ayant subi des traitements d'éclaircies pré-commerciales.

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