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ÉTUDE DES RELATIONS SOURCE/PUITS DE CARBONE DANS LA SYMBIOSE ENDOMYCORHIZIENNE À ARBUSCULES

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Résumé long

Le carbone (C) est l'un des éléments clé sur lequel repose toute symbiose mycorhizienne. La présente thèse regroupe plusieurs aspects des relations source/puits de C mettant en jeu des champignons mycorhiziens à arbuscules (MA) associés à diverses plantes hôtes. Les composantes essentielles régissant les mouvements de C entre partenaires fongiques et végétaux ont été étudiées à différents niveaux d'intégration.

Un premier volet éco-physiologique a mis en évidence le transfert de C en conditions naturelles entre espèces végétales de phénologie différente connectées par des champignons MA. Au printemps, des érythrones photosynthétiquement actifs fournissaient du C à des juvéniles d'érable à sucre au moment où ceux-ci développaient leurs feuilles de l'année. À l'automne, un transfert de C des juvéniles d'érable vers les érythrones renouvelant leur système racinaire a également été observé.

La force de puits de C des champignons MA a ensuite été étudiée en relation avec la diversité fongique et végétale à l'aide d'un système «split-root». La force de puits représentée par *Gigaspora rosea*, *Glomus intraradices* et *Glomus mosseae* a été comparée chez l'érable à sucre et l'orge. La force d'attraction du C de ces trois champignons variait en fonction de la combinaison d'espèces fongique et végétale. Trois souches de *G. mosseae* ont également été testées sur l'orge. Ces travaux ont révélé que la force de puits de C des champignons MA varie aussi en fonction de la souche fongique.

Finalement, le statut MA d'érables à sucre matures a été étudié dans un contexte de perturbation naturelle (verglas) ayant gravement endommagé leur houppier. Les érables à sucre affichant une mauvaise reprise de croissance l'année suivant le verglas avaient des taux de colonisation mycorhizienne supérieurs à ceux des érables à bonne reprise de croissance. Par contre, les populations de spores MA récoltées autour de ces deux catégories d'arbres ne différaient pas d'un point de vue taxinomique.

L'ensemble de ces travaux montre que les besoins en C des champignons MA dépendent de l'identité des partenaires fongique et végétal impliqués dans la symbiose ainsi que du stade phénologique et de l'état de santé de ce dernier.

Long summary

Carbon (C) is a key element underlying the establishment of all mycorrhizal symbioses. The present thesis investigates several important aspects of C source-sink relationships between arbuscular mycorrhizal (AM) fungi and diverse host plants. The essential components ruling C movements between fungal partners and host plants have been studied at different levels of integration.

In an initial ecophysiological-based study carried under natural conditions, C transfer between plants exhibiting different phenologies but connected by a common network of AM fungal hyphae was documented. Briefly, in spring the photosynthetically active *Erythronium americanum* plants supplied sugar maple saplings with C during leaf expansion of the latter. By contrast, a C transfer from sugar maple saplings towards *E. americanum* roots was observed during the autumn.

In a second series of experiments, the effect of the fungal taxon and the host plant species on C sink strength of AM fungi was studied using a split-root system. The sink strength represented by *Gigaspora rosea*, *Glomus intraradices* and *Glomus mosseae* was compared using sugar maple and barley host plants. The C draining force of the three AM fungi varied with the plant host used. In a further study, three *G. mosseae* strains were tested with barley. This work revealed that C sink strength of AM fungi was also fungal strain dependent.

Finally, the AM status of mature sugar maple trees exhibiting either poor or good regrowth following severe crown damage during the 1998 ice storm was studied. Sugar maple trees exhibiting a poor regrowth in the year following the ice storm had higher root colonization levels than those exhibiting good regrowth. However, AM fungal spore populations collected around trees in both categories did not differ significantly.

In conclusion, the present study shows that the C demand of AM fungi depends on the identity of both the fungal and plant partners involved in the symbiosis, and on the phenological stage and health of the latter.

Résumé court

Les relations source/puits de carbone (C) dans la symbiose mycorhizienne à arbuscules (MA) ont été examinées sous différents aspects. Une première partie éco-physiologique rapporte l'existence d'échanges de C entre espèces végétales en conditions naturelles via des champignons MA répondant à la phénologie différente des plantes impliquées. Deux études physiologiques basées sur le système de «split-root» ont montré que la force de puits de C des champignons MA est dépendante de l'espèce fongique, de la souche fongique, ainsi que de l'hôte végétal. Enfin, une étude écologique se positionnant dans un contexte de perturbation naturelle (verglas) endommageant le houppier d'arbres matures a révélé qu'une mauvaise reprise de croissance post-traumatique peut être associée à des taux de mycorhization racinaire plus élevés que ceux d'arbres à bonne reprise de croissance. En résumé, les besoins en C des champignons MA varient en fonction des espèces impliquées dans la symbiose et du stade phénologique de chacune d'elles.

Short summary

Different aspects of the carbon (C) source-sink relationships in the arbuscular mycorrhizal (AM) symbiosis were investigated. In an initial ecophysiological-based study carried under natural conditions, the existence of AM fungal mediated C exchanges between plant species with different phenology was reported. Two physiological studies based on a split-root system showed the C sink strength of AM fungi to be fungal species, fungal strain and plant species dependent. Finally, an ecological study carried out subsequently to the ice storm of 1998 (natural disturbance) which severely damaged mature tree crowns revealed that poor post-traumatic crown regrowth was associated with higher mycorrhizal colonization levels than in good regrowth trees. In conclusion, the C demand of AM fungi varies with the species involved in the symbiosis and with the phenological stage and health of the phytobiont.

Avant-Propos

Ce n’est qu’au moment de terminer cette thèse que je mesure l’ampleur du chemin parcouru depuis mon entrée à l’université il y a plus de dix ans déjà. Le travail présenté dans cet ouvrage ne compile pas seulement mes quelque trois années et demi passées à l’Université Laval mais représente l’aboutissement ultime du parcours scolaire de toute une vie. Alors bien évidemment, mes premières pensées vont à mes parents et à ma famille qui m’ont encouragé depuis toujours et continuent encore à me soutenir.

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Introduction

Dans la nature, la majorité des végétaux terrestres vit en symbiose avec des champignons. Cette étroite relation entre les plantes supérieures et les micro-organismes que sont les champignons s'élabore au niveau des racines. Les organes résultant de cette association sont appelés mycorhizes, du grec *mukês* pour champignon et *rhiza* pour racine. On estime à 90% la proportion de familles de plantes possédant des représentants pouvant former des associations mycorhiziennes (Harley & Smith 1983). De plus, il a été avancé par Taylor & Osborne (1995) que les végétaux supérieurs n'auraient pu conquérir la terre ferme s'ils n'avaient été associés à des champignons mycorhiziens, soulignant ainsi le rôle crucial qu'ont joué, et jouent encore, les mycorhizes dans l'évolution des plantes terrestres.

La mycorhize est une association de type symbiotique, c'est-à-dire que chacun des partenaires engagés (symbiotes) tire des profits de cette union. Le terme symbiose exclut ainsi toute notion de parasitisme ou de commensalisme où seul l'un des individus recevrait de son hôte, voire sa victime, sans rien donner en retour. Ainsi, l'association mycorhizienne est basée sur des bénéfices réciproques et s'articule autour d'échanges bidirectionnels d'éléments nutritifs entre les symbiotes. Les champignons fournissent à la plante mycorhizée une grande partie des minéraux et de l'eau que celle-ci requiert et qu'elle se procurerait bien plus difficilement seule sur sol naturel non fertilisé. La plupart des minéraux se retrouvent dans le sol en quantités limitantes et, de plus, leur mobilité est souvent limitée (Marschner 1990). La structure mycélienne propre aux champignons fournit aux végétaux un bien meilleur accès à ces minéraux que ne le ferait le système racinaire nu. En contrepartie de ces avantages nutritionnels, les plantes alimentent leurs partenaires fongiques en squelettes carbonés. Les champignons sont dépourvus d'appareil photosynthétique et par conséquent hétérotrophes vis-à-vis du carbone (C). Les processus d'échanges de nutriments entre ces partenaires sont toujours demeurés au cœur de toute symbiose mycorhizienne.

Nous comprenons plus aisément les avantages que de telles associations procurent aux plantes dans l'acquisition des ressources minérales en observant l'agencement structural

des champignons mycorhiziens. Le mycélium extraradical de ces champignons, organisé en réseaux complexes, prospecte son environnement spatial dans les trois dimensions, de sorte qu'il permet une exploration très efficace du sol; ce que ne peuvent faire les plantes dont le système racinaire est plus limité. La longueur racinaire totale d'une plante est souvent relativement faible et son diamètre relativement gros. En revanche, les hyphes des champignons ont un diamètre beaucoup plus fin et peuvent devenir très longs. Pour un même volume de matière fraîche, la surface représentée par les hyphes fongiques est considérablement plus élevée que celle des racines (Rousseau *et al*. 1994).

On distingue plusieurs types d'associations mycorhiziennes basés sur le partenaire fongique impliqué dans la symbiose. Les symbiotes végétaux sont si nombreux et si diversifiés qu'une classification selon leur genre serait trop complexe (Smith & Read 1997). Les endomycorhizes à arbuscules constituent les symbioses végétales les plus communes et l'apparition des champignons mycorhiziens à arbuscules (MA) remonterait à une époque située entre 353 et 462 millions d'années (Simon *et al*. 1993). Une classification récente regroupe les champignons MA sous l'embranchement des Glomeromycota (anciennement Glomales) (Schüssler *et al*. 2001). Ce sont tous des symbiotes obligatoires incapables de compléter leur cycle vital sans hôte végétal. Malgré leur âge d'apparition très reculé et le fait qu'ils prolifèrent de par le monde, on n'en dénombre sur une base taxinomique qu'un peu plus de 150 espèces (Walker & Trappe 1993). Les champignons MA sont capables de s'associer à des familles d'angiospermes, de gymnospermes, de fougères, de lycopodes et de bryophytes (Smith & Read 1997). Ce sont donc des espèces à large spectre d'hôte, mycorhizant de très nombreuses espèces végétales et qui, par conséquent, peuvent se rencontrer dans des écosystèmes très divers (Morton *et al*. 1995).

Le terme «arbuscule» caractérisant les champignons MA décrit la structure typique formée par toutes les espèces de cet ordre. Les arbuscules prennent l'apparence d'un arbuste (Gallaud 1905; Brundrett *et al*. 1984) et on ne les retrouve qu'à l'intérieur des cellules corticales de la racine. Cette particularité permet de caractériser les champignons MA comme endomycorhiziens en opposition aux champignons ectomycorhiziens où les hyphes s'intercalent entre les cellules racinaires de leur hôte sans jamais y pénétrer. Grâce à leur

structure singulière très ramifiée, les arbuscules permettent d'accroître considérablement la surface de contact entre le champignon et la plante. Ceci a largement contribué à reconnaître les arbuscules comme étant le siège privilégié d'échanges de nutriments entre les symbiotes (Cox & Tinker 1976; Gianinazzi *et al*. 1979; Dexheimer *et al*. 1985). D'autres structures, les vésicules, se retrouvent chez quatre des six genres que comptent les champignons MA. À l'instar des arbuscules, les vésicules peuvent se retrouver à l'intérieur des cellules corticales mais également intercalées entre celles-ci. De forme variable, elles renferment d'abondants lipides et de nombreux noyaux (Jabaji-Hare *et al*. 1984; Smith & Read 1997). Il est par conséquent reconnu que les vésicules sont d'importants organes de réserve chez les champignons MA mais peuvent également endosser la fonction de propagule (Biermann & Linderman 1983; Bonfante-Fasolo 1984).

La remarquable amélioration de la nutrition minérale des plantes par les symbiotes fongiques s'accompagne d'un coût pour le partenaire végétal. Ce coût se traduit par la fourniture aux champignons de C dérivant directement des produits de la photo-assimilation (Ho & Trappe 1973). Il semble que le C est transféré de la plante vers le champignon sous forme d'hexoses (Shachar-Hill *et al*. 1995; Solaiman & Saito 1997). Ces squelettes carbonés sont immédiatement convertis en tréhalose, sucre exclusivement et universellement répandu dans le monde fongique, et en glycogène (Shachar-Hill *et al*. 1995; Bago *et al*. 2000b), deux formes de C inexploitables par la plante. Il n'est pas encore certain si l'entrée des hexoses (glucose et fructose) dans les structures fongiques s'accomplit de façon active ou passive (Bago *et al*. 2000a) mais leur disparition rapide a pour effet d'entretenir un gradient de concentration d'hexoses permanent entre les compartiments racinaires et fongiques, de telle sorte que le champignon se montre constamment demandeur de cette forme de C. D'importantes fractions du tréhalose et du glycogène sont mobilisées pour la synthèse de lipides fongiques. Toutes les structures des champignons MA contiennent des lipides (Sancholle *et al*. 2001) mais les vésicules et les spores en sont particulièrement riches (Jabaji-Hare *et al*. 1984; Bécard *et al*. 1991). Il apparaît clairement ici que le développement du champignon MA de même que la multiplication des structures impliquées dans sa prolifération (vésicules et spores) dépendent en exclusivité du C puisé chez l'hôte végétal. D'un point de vue «métabolisme

carboné», le champignon semble être ainsi placé sous la gouverne de la plante. La relation régnant entre les deux partenaires est alors qualifiée de type source/puits: la plante produit et fournit du C, c'est la source; le champignon en demande et en consomme, c'est le puits. Dès lors, le bon déroulement de la symbiose mycorhizienne se révèle intimement régi par ces relations source/puits.

La présente thèse regroupe différents aspects des relations source/puits de C dans la symbiose MA. Elle s'articule principalement autour de l'érable à sucre comme espèce végétale hôte mais d'autres plantes ont également été employées dans nos travaux. L'érable à sucre, *Acer saccharum* Marsh., est un arbre d'intérêt commercial des régions tempérées strictement colonisé par des champignons MA (Yawney & Schultz 1990; Cooke *et al*. 1993). Mais surtout, bien que la culture en serres de plantules d'érable à sucre à partir de semences soit erratique, cette essence permet de conjuguer à la fois expériences physiologiques en laboratoire et études éco(physio)logiques de terrain afin de considérer la physiologie du métabolisme carboné dans la symbiose MA à plusieurs échelles. Les différentes expérimentations qui sont exposées dans cet ouvrage se positionnent aux niveaux du système racinaire de plantules, de juvéniles entiers et d'individus matures d'érables à sucre.

Le travail présenté dans cette thèse a débuté en exploitant une des caractéristiques de la symbiose mycorhizienne. Partant du fait que les champignons mycorhiziens n'ont pas (ou peu) de spécificité d'hôte, il est reconnu que les réseaux mycéliens que ceux-ci développent peuvent être connectés à plusieurs plantes de même ou différente espèce. Les hyphes fongiques constituent alors des «ponts» reliant différentes plantes et deviennent ainsi des voies de passage de nutriments potentielles. En laboratoire, des études ont mis en évidence, grâce à l'utilisation de marqueurs isotopiques, des transferts entre plantes de C (Brownlee *et al*. 1983; Francis & Read 1984; Finlay & Read 1986), d'azote (Arnebrant *et al*. 1993; Bethlenfalvay *et al*. 1991) et de phosphore (Newman & Eason 1993; Wittigham & Read 1982) par l'intermédiaire de mycélium de partenaires fongiques communs. Ces mouvements semblent toujours gouvernés par des relations source/puits et dirigées dans ce sens. Cependant, les quantités d'isotopes détectées chez la plante receveuse restent minimes

(Duddridge *et al*. 1980; Brownlee *et al*. 1983; Francis & Read 1984; Frey & Shüepp 1992). Ainsi, Newman (1988) s'est interrogé sur la signification écologique et nutritionnelle de ces faibles flux de nutriments entre plantes. Mais dans une étude plus récente, Simard *et al*. (1997b) ont mesuré des transferts nets de C marqué en quantités significatives entre deux espèces ligneuses à ectomycorhizes (*Betula papyrifera* et *Pseudotsuga menziesii*), dont l'une était ombragée dans le but d'établir un puits de C. Une troisième espèce, de type MA (*Thuja plicata*), présente dans le même espace expérimental paraissait exclue de ce partage des ressources carbonées et ne recevait que de faibles quantités de C marqué.

Certains auteurs considèrent que dans le cas de transferts de C impliquant des champignons MA la plante dite «receveuse» ne reçoit en réalité pas de C (Robinson & Fitter 1999). Ces derniers s'appuient sur le fait que le C transféré chez la plante receveuse a toujours été détecté au niveau de ses racines. Ce C resterait donc uniquement cantonné aux structures fongiques. De plus, le passage de C à partir de champignons MA vers une plante hôte n'a jamais été mis en évidence. Cependant, Bidartondo *et al*. (2002) ont récemment montré que des plantes épiparasites (plantes non-photosynthétiques qui obtiennent leur C en parasitant des champignons mycorhiziens) étaient mycorhizées par des champignons MA du genre *Glomus*. Cette découverte suggère fortement que les champignons MA sont capables de fournir du C aux plantes vasculaires et relance l'intérêt de l'étude du transfert de C entre plantes connectées par ce type de champignons.

Un **premier chapitre** aborde l'étude de la possibilité de transfert de C entre plantes MA en conditions naturelles. Pour ce faire, il convenait d'exclure tout recours à la création artificielle d'une relation source/puits par l'ombrage de la plante receveuse potentielle. Le moteur du transfert de C se devant d'être naturel et de refléter une certaine réalité écologique, il a été opté d'étudier des espèces de phénologie différente se côtoyant naturellement sur le terrain. Les juvéniles d'érable à sucre passent la très grande majorité de leur saison de croissance dans des conditions lumineuses limitantes. Pourtant, elles sont capables de survivre de nombreuses années à ces conditions d'ombre de sous-bois (Canham 1988; Lei & Lechowicz 1990; Ellsworth & Reich 1992). Notre hypothèse était que les érables à sucre juvéniles pouvaient recevoir une fraction de C provenant de plantes mieux

exposées à la lumière via les champignons MA. L'érythrone d'Amérique est une plante pérenne de type MA commune des érablières qui, au printemps, tire profit des conditions éphémères de luminosité élevée précédant la fermeture de la canopée. C'est une plante qui à chaque automne renouvelle complètement son système racinaire (Brundrett & Kendrick 1990a; Lapointe & Molard 1997). À la condition que ces deux espèces soit connectées par des champignons MA, leurs différences de phénologie pourraient être à l'origine de l'établissement de relations source/puits. Une première relation aurait lieu au printemps lorsque les juvéniles d'érable à sucre développent leurs feuilles de l'année et que les érythrones sont déjà photosynthétiquement actifs. Une seconde relation s'établirait à l'automne au moment où les juvéniles d'érable à sucre profitent des bonnes conditions lumineuses laissées par la disparition de la canopée et que les érythrones développent leurs nouvelles racines.

La possibilité de transfert de C, en conditions naturelles, entre juvéniles d'érable à sucre et érythrones d'Amérique connectés par des champignons MA étant établie dans le premier chapitre, l'étape suivante a été consacrée à étudier plus précisément la force de puits de C des champignons MA. Les coûts en C attribués à la symbiose MA ont été estimés dans de nombreux travaux (Pang & Paul 1980; Kucey & Paul 1982; Snellgrove *et al*. 1982; Koch & Johnson 1984; Douds *et al*. 1988; Wang *et al*. 1989) et il est aujourd'hui considéré par plusieurs auteurs qu'entre 4 et 20% des photosynthétats d'une plante mycorhizée sont utilisés dans l'entretien des champignons MA (Bago *et al*. 2000a; Douds *et al*. 2000; Graham 2000). Les espèces fongiques et végétales utilisées dans les études mentionnées ci-dessus, de même que les méthode de calcul des coûts, divergeaient souvent mais les chiffres avancés (4-20%) témoignent néanmoins d'une grande variabilité. Cela signifie que soit les plantes affichent des capacités de fourniture de C variables entre les espèces, soit les forces de puits des champignons diffèrent d'une espèce (ou d'une souche) à l'autre, soit les deux.

Dans l'état actuel de nos connaissances, aucune étude n'a examiné les forces de puits de C des champignons MA en combinant les facteurs espèce fongique et espèce végétale. Seuls Koch & Johnson (1984) ont étudié les coûts de la mycorhization chez deux espèces

d'agrumes avec une souche de champignon MA. Cet objectif est atteint dans le **deuxième chapitre** où les forces de puits de C de trois espèces de champignons MA (*Gigaspora rosea*, *Glomus intraradices* et *Glomus mosseae*) sur deux espèces d'hôtes végétaux (l'érable à sucre et l'orge) ont été comparées.

Le **troisième chapitre** s'inscrit dans la continuité du chapitre précédent qui avait révélé que, contrairement aux deux autres espèces de champignon MA testées, *G. mosseae* ne se comportait jamais comme un puits fort, ni avec l'érable à sucre, ni avec l'orge. Afin de vérifier si ce caractère est spécifique à *G. mosseae* ou propre à la souche utilisée précédemment, trois souches de ce champignon d'origines géographiques diverses ont été testées sur l'orge.

Enfin, le **quatrième chapitre**, se positionne dans un contexte de perturbation naturelle affectant le houppier d'érables à sucre matures. En janvier 1998, une vague de verglas de forte ampleur frappe le sud du Québec et l'est de l'Ontario dévastant les érablières de ce secteur à un niveau de dommages sans précédent (Irland 1998). La grande majorité des arbres adultes touchés voient ainsi la partie supérieure de leur tronc brisée et leur cime presque totalement détruite. Au lendemain de ce sinistre, le Ministère des Ressources Naturelles du Québec (MRN) a effectué un suivi des érablières atteintes par le verglas dans des parcelles permanentes situées à l'intérieur de la zone dévastée. Des relevés réalisés sur chacun des arbres adultes de ces parcelles ont permis de dresser un «inventaire aérien» des érablières endommagées. Le volume de houppier vivant, exprimé en pourcentage du volume de houppier total théorique, a été noté aux étés 1998 et 1999. En comparant les valeurs de volumes de feuillage obtenues au cours des deux années de croissance qui ont immédiatement suivi le verglas, on a pu caractériser comment les arbres ont réagi au stress induit par cette perturbation. Ainsi, on a pu distinguer des individus ayant eu une bonne reprise de croissance à la suite du verglas (augmentation du volume de houppier) d'autres individus qui en ont eu une mauvaise (pas de changement, ou diminution, du volume de houppier). Curieusement, deux érables vivant côte à côte, ayant subi les mêmes niveaux de dommages et ayant approximativement la même taille (diamètres de troncs semblables) peuvent avoir réagi au verglas de façon totalement différente. L'un peut présenter une très

bonne reprise de croissance alors que son plus proche voisin en affiche une mauvaise (observations personnelles). Pour quelle(s) raison(s) la croissance de certains arbres a bien redémarré après le verglas leur permettant de reconstituer leur cime alors que d'autres ont eu plus de difficulté à faire de même et semblent végéter? Quels sont les facteurs influençant ce phénomène?

Plusieurs raisons expliquant ces différences de reprise de croissance peuvent être avancées: la vigueur des arbres avant le verglas, la présence de carie ou encore des facteurs génétiques. Cependant, une autre cause doit être prise en considération dans l'étude de l'état de santé post-verglas des érables à sucre: le fait que ces derniers vivent en symbiose avec des champignons MA. La destruction du feuillage des érables à sucre touchés par le verglas a entraîné une baisse de la fourniture de C alors que les champignons MA étaient déjà installés dans les racines de ces arbres. Les coûts d'entretien de ces champignons sont alors devenus proportionnellement plus élevés.

Comme il l'a déjà été montré dans les chapitres 2 et 3, les coûts d'entretien de ces champignons MA peuvent varier selon l'espèce. Il se pourrait qu'un arbre adulte n'héberge qu'un nombre limité de champignons mycorhiziens. En effet, une fois qu'un champignon a mycorhizé une plante hôte, celui-ci a tendance à s'y maintenir et à gêner de nouvelles colonisations par d'autres espèces (Pearson *et al.* 1993; Pinior *et al.* 1999; Vierheilig *et al.* 2000a) Ceci aurait pour effet de restreindre le nombre de champignons colonisant une même plante. Il est donc possible qu'un arbre soit mycorhizé en majorité par des espèces moins bénéfiques et plus coûteuses en C. À la suite d'un événement aussi stressant pour un arbre que la perte de sa cime, la composition de ses partenaires fongiques pourrait en influencer la reconstitution. Si un arbre est associé à des champignons coûteux en C et que ceux-ci continuent de représenter un puits de C important après la perte de la cime, cet arbre pourrait avoir moins de ressources à sa disposition pour régénérer son feuillage. Par conséquent, il serait intéressant d'examiner s'il existe une corrélation entre l'abondance et la composition en champignons MA que l'on retrouve dans la rhizosphère des érables endommagés par le verglas de 1998 et leur capacité de reprise de croissance.

L'ensemble de ces différentes études et des résultats qui en ont découlé a mené à l'élaboration d'une synthèse qui est présentée dans la conclusion générale. Quelques perspectives de recherche sont également proposées dans cette discussion.

CHAPITRE I

^{14}C transfer between the spring ephemeral *Erythronium americanum* and sugar maple saplings via arbuscular mycorrhizal fungi in natural stands

1.1 Avant-Propos

Le texte de ce chapitre a été publié dans la revue Oecologia (Lerat, Gauci, Catford, Vierheilig, Piché & Lapointe 2002). Rachel Gauci a participé au marquage au ^{14}C sur le terrain, Jean Guy Catford a collaboré à l'analyse de la radioactivité et Horst Vierheilig a contribué à la rédaction de la discussion. Une partie de ce chapitre a fait l'objet d'une présentation orale au Colloque du Centre de Recherche en Biologie Forestière, Université Laval, (Québec, Canada) qui s'est déroulé les 22 et 23 février 2002 ainsi que d'une affiche présentée au congrès Mycorhize 2001 tenu à Montréal (Canada).

1.2 Résumé

Le transfert de carbone (C) entre des juvéniles d'érable à sucre (*Acer saccharum*) et une éphémère de printemps, l'érythrone d'Amérique (*Erythronium americanum*) via le mycélium de champignons mycorhiziens à arbuscules (MA) a été étudié sur le terrain. Des juvéniles d'érable à sucre et des plants d'érythrone ont été placés ensemble dans des pots placés dans le sol d'une érablière en 1999. Des bouleaux jaunes ectomycorhiziens (*Betula aleghaniensis*) y ont été ajoutés comme plantes témoins. Au printemps 2000, durant l'expansion foliaire des juvéniles d'érable à sucre, les feuilles d'érythrone ont été marquées au $^{14}CO_2$. Sept jours après le marquage, la radioactivité a été détectée dans les feuilles, la tige et les racines des érables à sucre. La radioactivité spécifique chez les érables à sucre

était 13 fois plus élevée que chez les bouleaux jaunes révélant l'existence d'un transfert direct de ^{14}C entre les plantes MA. La quantité de ^{14}C transférée aux juvéniles d'érable à sucre était négativement corrélée au pourcentage de ^{14}C alloué à l'organe de réserve de l'érythrone. Un second marquage a été réalisé à l'automne 2000 sur les feuilles d'érable à sucre durant la croissance annuelle des racines d'érythrone. De la radioactivité a été détectée chez 7 des 22 systèmes racinaires d'érythrone mais était absente chez les bouleaux jaunes. Ces résultats suggèrent que les champignons MA connectant différentes espèces de sous-bois peuvent agir comme des ponts de transfert de C réciproque entre les espèces végétales en relation avec la phénologie des plantes impliquées.

1.3 Abstract

We investigated in the field the carbon (C) transfer between sugar maple (*Acer saccharum*) saplings and the spring ephemeral *Erythronium americanum* via the mycelium of arbuscular mycorrhizal (AM) fungi. Sugar maple saplings and *E. americanum* plants were planted together in pots placed in the ground of a maple forest in 1999. Ectomycorrhizal yellow birches (*Betula alleghaniensis*) were added as control plants. In spring 2000, during leaf expansion of sugar maple saplings, the leaves of *E. americanum* were labelled with $^{14}CO_2$. Seven d after labelling, radioactivity was detected in leaves, stem and roots of sugar maples. Specific radioactivity in sugar maples was 13-fold higher than in yellow birches revealing the occurrence of a direct transfer of ^{14}C between the AM plants. The quantity of ^{14}C transferred to sugar maple saplings was negatively correlated with the percentage of ^{14}C allocated to the storage organ of *E. americanum*. A second labelling was performed in autumn 2000 on sugar maple leaves during annual growth of *E. americanum* roots. Radioactivity was detected in 7 of 22 *E. americanum* root systems and absent in yellow birches. These results suggest that AM fungi connecting different understorey species can act as reciprocal C transfer bridges between plant species in relation with the phenology of the plants involved.

1.4 Introduction

Mycorrhizal fungi are symbiotic partners associated with the great majority of land plant species (Smith & Read 1997). They colonise roots and improve plant nutrition mainly by transferring phosphate (P) from the soil to the plant. The plants provide the fungi with carbohydrates (Smith & Read 1997). Because of low host specificity of mycorrhizal fungi their mycelium can form a network that interconnects host plants of the same (Brownlee *et al*. 1983; Newman & Eason 1993; Graves *et al*. 1997) or different species (Francis & Read 1984; Arnebrant *et al*. 1993). This means fungal hyphae can provide pathways to transfer of compounds between mycorrhizal plants. Isotope tracer studies have documented transfer of water (Duddridge *et al*. 1980) and nutrients such as P (Newman & Eason 1993), N (Arnebrant *et al*. 1993) and C (Francis & Read 1984; Finlay & Read 1986).

The occurrence of direct C transfer from donor to receiver plants through fungal mycelium has been mostly studied under laboratory and greenhouse conditions (Francis & Read 1984; Finlay & Read 1986; Simard *et al*. 1997a) with arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) fungi. On most of these studies receiver plants were artificially shaded demonstrating that C transfer is strongly governed by a source-sink relationship. Nevertheless, physiological importance of such transport in ecosystems has not been proven (Robinson & Fitter 1999). Brownlee *et al*. (1983) hypothesised that newly germinated seedlings of *Pinus sylvestris* could receive photoassimilates from mature trees in their vicinity and overcome forest understorey shade conditions. Recently, Simard *et al*. (1997b) measured net transfer of C in the field between seedlings of two ECM tree species *Betula papyrifera* and *Pseudotsuga menziesii*. There was a much smaller C transfer (18% of that between the ECM species) towards AM tree seedlings of *Thuja plicata* present in the vicinity. They also found that the amount of C exported from the donor plant was positively correlated with the level of shading on the receiver plant. To our knowledge, C transfer between species with different developmental phenology has not been reported within a plant community.

In natural plant communities dominated by endomycorrhizal species, AM fungi rapidly colonise roots of newly germinated seedlings and plants that renew their entire root system

every year, for example Liliaceous spring ephemerals. Spores of AM fungi are an improbable inoculum because of their low viability in the soil (Read *et al*. 1976; Zahka *et al*. 1995). Therefore, the most likely source of inoculation of new roots is hyphae from the roots of neighbouring plants previously colonised by AM fungi.

Erythronium americanum Ker-Gawl (trout lily) is an abundant spring ephemeral in maple forests of North America that is colonised by AM fungi (Brundrett & Kendrick 1990b; Lapointe & Molard 1997). In the region of Québec City, epigeous development of *E. americanum* follows snow melt in late-April when the tree canopy is completely open (Fig. 1.1a, b). *E. americanum* takes advantage of these full light conditions for active carbohydrate assimilation until canopy closure. Carbohydrate storage in the corm (underground reserve organ) is completed three wk after leaf expansion but high levels of photosynthesis (10-14 μmol CO_2 m^{-2} s^{-1}) are maintained until the overstorey canopy starts to close (Taylor & Pearcy 1976; Lapointe, unpublished data). Leaves of *E. americanum* then senesce during canopy closure. Leaf senescence is completed by mid-June followed by root senescence at the beginning of July (data not shown). In early autumn (mid-September) *E. americanum* produces new unbranched roots that are rapidly colonised by AM fungi (Brundrett & Kendrick 1990a; Lapointe & Molard 1997).

Sugar maple (*Acer saccharum* Marsh) is a common North American tree species associated with AM fungi (Yawney & Schultz 1990; Cooke *et al*. 1993). Bud burst of sugar maple saplings occurs about three wk after leaves of *E. americanum* have unfolded and started to photosynthesise whilst bud burst of mature sugar maple trees is delayed by two wk (Fig. 1.1a). Thus, the expansion of the leaves of sugar maple saplings occurs under high light conditions. Leaf development is a costly process in terms of carbohydrates (Poorter & Villar 1997) and sugar maple saplings could receive C during leaf expansion through mycorrhizal hyphae from neighbouring plants already photosynthetically active.

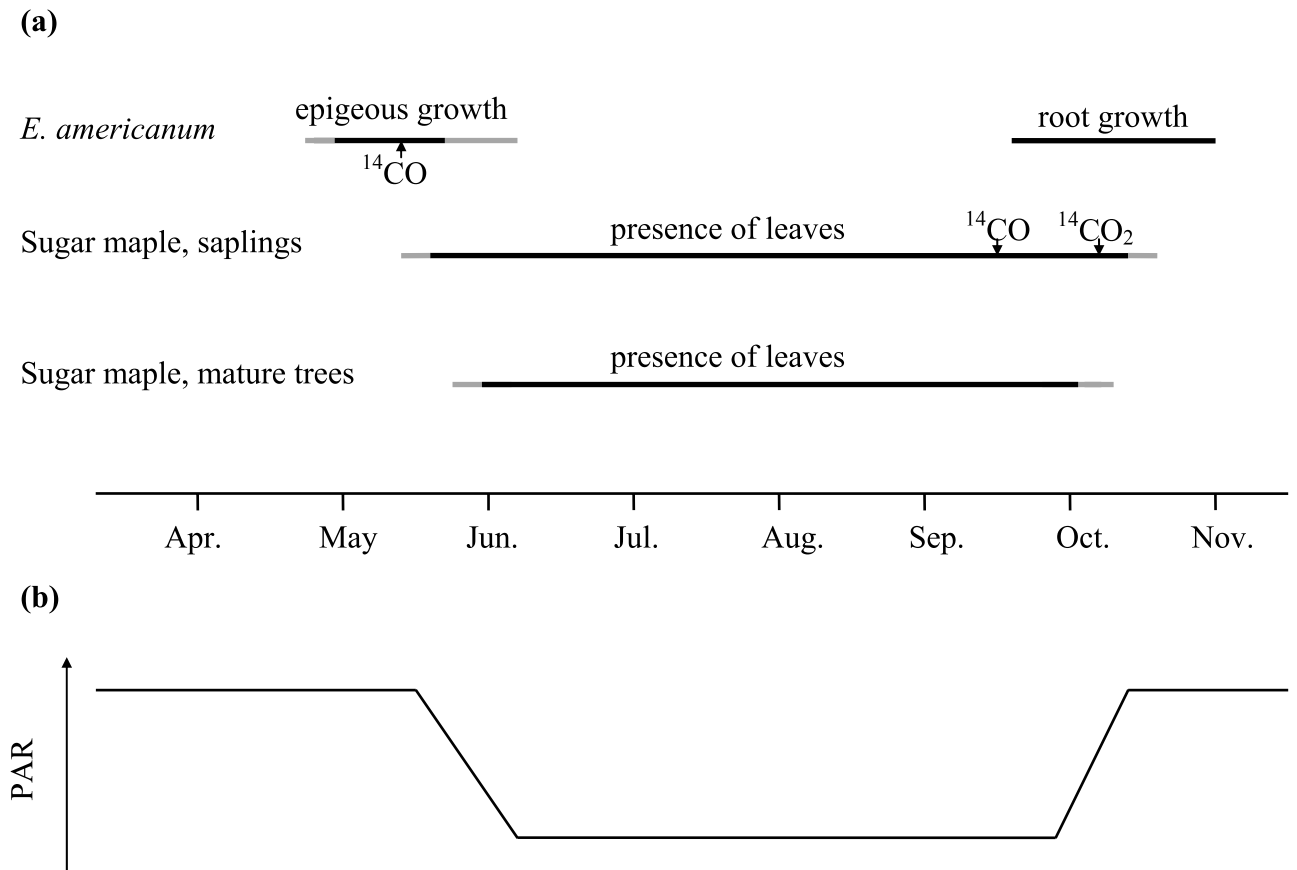

Figure 1.1. Phenology of *Erythronium americanum* and leaves of sugar maple saplings and mature trees (a) and relative PAR (photosynthetically active radiation) on the ground of a maple forest (b) over a growing season in the region of Québec City. Grey bars represent development (beginning of line) or senescence (end of line) of leaves. Dates of labelling with $^{14}CO_2$ are represented by arrows.

In the autumn, mycorrhizal colonisation of *E. americanum* roots occurs during root growth, which represents a significant carbohydrate cost at a time when plants do not have leaves (Lapointe & Molard 1997). C required for root production in *E. americanum* could be partly supplied by photosynthetically active neighbouring sugar maple trees and juveniles via interconnecting mycelium.

The goal of this work was to test for reciprocal and temporal transfers of C between the spring ephemeral *E. americanum* and 1-year old saplings of the woody species sugar maple in a natural plant community. Such net transfers would be restricted in time to the period when one species is photosynthetically active while the other is not.

1.5 Materials and methods

1.5.1 Study site

The experiments were carried out in a sugar maple forest in Saint-Augustin-de-Desmaures (46°48’N, 71°23’W) in the vicinity of Québec City. The site has been previously described in Lapointe & Molard (1997). The canopy is dominated by *Acer saccharum* Marsh with *A. rubrum* L., *Fraxinus americana* L. and *Ulmus rubra* Mühl as companion species. The soil is a clay-loam with a thin moder type humic horizon. The most abundant understorey species are *Erythronium americanum* Ker-Gawl, *Trillium erectum* L. and *Veratrum viride* Ait.

1.5.2 Experimental design

In spring 1999 (mid-May), 60 organic fibre pots (v=3 1, d=16 cm, h=16 cm) were buried into the soil at the study site to a depth allowing the edge to remain 1-2 cm over ground level. Pots were filled with natural soil collected at the site. The soil included fine roots of mature trees as source of mycorrhizal inoculation for sugar maple seedlings. One year-old seedlings of sugar maple were carefully collected near the experimental site and transplanted into the middle of each pot (one seedling per pot). Five wk later, three corms of *E. americanum* were planted 5 cm deep into each pot. At that time, leaves of the

transplanted *E. americanum* had already senesced and corms had entered summer dormancy. In August, yellow birch saplings (*Betula alleghaniensis* Britton) (<12-cm high) were collected from a distant site at l'Île-aux-Grues (47°03'N, 75°28'W). Birches are typically colonised by ECM fungi rather that AM fungi. Presence of ECM features was visually checked and seedlings with the highest mycorrhization were transplanted into the pots (one per pot). Pots were regularly removed from soil and immediately replaced to avoid possible intrusion of roots from surrounding trees.

1.5.3 Labelling experiments with ^{14}C

1.5.3.1 Spring labelling

In mid-May 2000, at bud burst of sugar maple and yellow birch saplings (Fig. 1.1a), 12 of the pots were randomly selected for ^{14}C labelling. The leaf of one of the three *E. americanum* plants in the selected pots was enclosed in a 3.7-l transparent freezer bag (Ziploc®) that was firmly anchored between two wooden stakes. A 12-cm-rubber tube (d=5 mm) was fixed to an upper corner of the bag and tightly closed during the experiment. A sealing compound placed around the stem of the plant insured hermetic closure of the bag during labelling. A 29.5-ml cup containing a basic solution of 185 kBq (5 µCi) $NaH^{14}CO_3$ (Amersham Pharmacia Biotech) was placed inside the plastic bag and the bag tightly closed. Pulse-labelling started when 1 ml lactic acid (85%) was syringe-injected through the bag into the cup to liberate gaseous $^{14}CO_2$ (the release of $^{14}CO_2$ in the bag was estimated to increase the CO_2 concentration by 4-6 ppm). Plants were left exposed to sunlight, >1000 µmol m^{-2} s^{-1} PAR (photosynthetically active radiation), for 1.5 h. At the end of the experiment, excess $^{14}CO_2$ was evacuated through the rubber tube and trapped in a 1 *N* NaOH solution.

After a 7-d chase period, which allowed complete expansion of sugar maple leaves, all plants in the pots were harvested and dried at 65°C for 24 h except for roots of non-labelled *E. americanum* plants, which were collected for determination of mycorrhizal colonisation.

1.5.3.2 Autumn labelling

In the autumn 2000, two consecutive labelling sessions were performed (Fig. 1.1a). In mid-September, 12 pots were chosen for labelling sugar maples. At this time in early autumn, leaves of sugar maple saplings were still green and capable of photosynthesis (assimilation rate: 1.7 ± 0.8 µmol CO_2 m^{-2} s^{-1}) while *E. americanum* corms had no functional roots. This early session insured labelling of the sugar maple saplings before leaf senescence. The technical procedure was identical to that used in spring with the following modifications. Pots were moved to an open field where ambient sunlight reached 1200 µmol m^{-2} s^{-1} PAR and the whole foliage of sugar maple 1-year saplings was pulse-labelled in plastic bags for 2 h. Three wk later, root samples harvested in other pots showed that *E. americanum* corms had formed new roots and these had been colonised by AM fungi. At this time leaves of the previously labelled sugar maples were still green, and the plants were labelled a second time. Pots were harvested two wk after the second labelling session (14-d chase period). Plant material was destructively harvested and dried with exception of all *E. americanum* roots which were stored in a FAA solution.

1.5.4 Determination of radioactivity

1.5.4.1 Spring labelling

Dried sugar maple, non-labelled *E. americanum* (root excepted) and yellow birch were exposed to autoradiography film (Kodak BioMax). The films were developed after six wk of exposure.

Leaves, stem and roots of sugar maple saplings and labelled *E. americanum* and leaves of non-labelled *E. americanum* were separated and ground in liquid nitrogen likewise whole yellow birch saplings. An aliquot of each material type (15-50 mg) was digested as in Clifford *et al*. (1973) and radioactivity determined by liquid scintillation spectrometry. Counts were standardised with a quench curve and radioactivity was expressed in dpm.

1.5.4.2 Autumn labelling

Yellow birch saplings and dried corms of *E. americanum* were exposed to autoradiography film. The films were developed after six wk of exposure.

Roots samples of *E. americanum* were fixed in paraffin and 5-µm thick sections were mounted on microscope slides. Paraffin was then dissolved with toluene and root sections were coated with LM-1 nuclear emulsion (Amersham Pharmacia Biotech; crystal size of 0.2 µm). The emulsion was processed after exposure at 4°C for two to four wk. The rest of *E. americanum* root samples were rinsed several times with distilled water, dried, weighed and assessed for radioactivity as described previously, using liquid scintillation. Radioactivity in yellow birch saplings was also assessed.

1.5.5 Mycorrhizal colonisation levels

Roots of *E. americanum* were cleared in a 10% (w/v) KOH solution for 12 min at 90°C prior to staining with a trypan blue solution (Koske & Gemma 1989). AM colonisation was assessed microscopically at 78× magnification using the method described by Trouvelot *et al*. (1986). The following parameters were calculated: frequency of colonisation, intensity of colonisation and arbuscular content.

1.5.6 Statistical analysis

One-way ANOVA was performed to compare radioactivity data in sugar maple and yellow birch saplings after spring labelling using the GLM procedure of the SAS statistical package.

1.6 Results

Following spring radiolabelling of *E. americanum* plants, the macro-autoradiography revealed traces of ^{14}C in all sugar maple saplings (Fig. 1.2a-c). In contrast, no labelling was observed in yellow birch saplings and non-labelled *E. americanum* growing in the same pots as the labelled *E. americanum* (autoradiographs not shown), indicating absence of

transfer between *E. americanum* plants. Roots, stem or leaves of the sugar maple saplings were labelled with ^{14}C. Five of the saplings were extensively labelled in the leaves. Two of the saplings showed only slight labelling in their roots.

Total radioactivity (dpm) and specific radioactivity (dpm g^{-1} dry matter) were much greater in sugar maple compared to yellow birch saplings (P<0.01). Average total radioactivity in sugar maple saplings was 738 ± 642 dpm (means ± 1 SD; range, 136 to 2233 dpm) and was 15 ± 26 dpm (range, 0 to 82 dpm) in yellow birch saplings. Average specific radioactivity was 3884 ± 3046 dpm g^{-1} dry matter in sugar maple saplings and was 290 ± 450 dpm g^{-1} dry matter in yellow birch saplings. Small amounts of radioactivity (224 ± 318 dpm g^{-1} dry matter) were detected in the leaves of non-labelled *E. americanum* indicating that very few ^{14}C was absorbed by photosynthetically active leaves during the chase period. In sugar maple saplings specific activity was the highest in leaves (Table 1.1). Radioactivity was thus mainly localised in the shoot (leaves and stem), with less radioactivity measured in the roots (Table 1.1). In labelled *E. americanum* the ^{14}C content was $1.12\times10^6 \pm 0.22\times10^6$ dpm (18.6 ± 3.6 kBq) which was equivalent to ca. 10% of the $^{14}CO_2$ used for labelling. On average, 53.9 ± 30.4% of the total was found in the corm of labelled *E. americanum* plants.

The total activity in the sugar maple saplings was 0.064 ± 0.049% of that in *E. americanum* while dry biomass was 195 ± 76 mg for sugar maple saplings and 292 ± 96 mg for *E. americanum*. It appeared that when the percentage of ^{14}C in the corm of labelled *E. americanum* was the lowest, the ^{14}C contents in sugar maple saplings was the highest. Consequently, a negative correlation (r^2=0.61, P=0.0028) was established between the percentage of ^{14}C translocated to the corm of labelled *E. americanum* and the percentage of ^{14}C translocated to sugar maple saplings (Fig. 1.3).

The root systems of non-labelled *E. americanum* plants showed high levels of root colonisation by AM fungi in spring. Frequency of colonisation was 44.7 ± 27.0% (n=28), intensity of colonisation was 13.3 ± 14.7% and arbuscular content was 84.8 ± 19.6%.

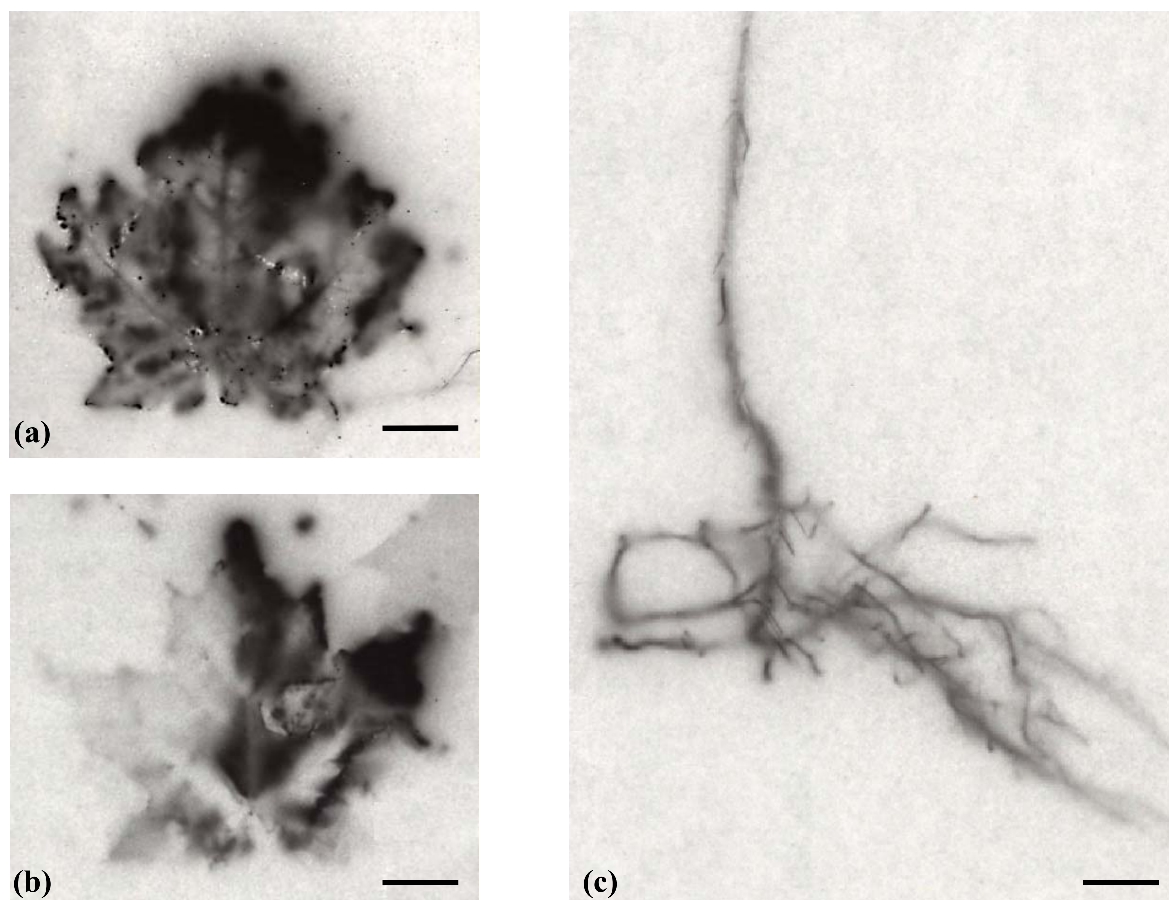

Figure 1.2. Macro-autoradiographs of leaves (a and b) and stem and root system (c) of labelled C in receiver sugar maple saplings. Dry plant material was exposed for six wk on Kodak scientific imaging films. Bars represent 1 cm.

Table 1.1. Specific activity (dpm g^{-1} dry matter), biomass ratio, and distribution of radioactivity in leaves, stem, and roots of sugar maple saplings one wk after spring labelling. Data represent means ± 1 SD.

	Leaves	Stem	Roots
Specific activity	6186 ± 5953	2386 ± 2552	2941 ± 4360
Biomass ratio	30.8 ± 11.4%	36.6 ± 13.4%	32.6 ± 7.4%
% radioactivity	50.4 ± 24.6%	27.6 ± 16.3%	22.0 ± 16.9%

In autumn, after sugar maple labelling, ^{14}C was detected with liquid scintillation in 7 of 22 *E. americanum* root samples. The average total radioactivity was 230 ± 269 dpm (range, 43 to 645 dpm) and the average specific radioactivity was 4244 ± 5288 dpm g^{-1} dry matter for the 7 labelled root systems. No radioactivity was detected in the yellow birch saplings. ^{14}C content in labelled sugar maple saplings was $1.12\times10^6 \pm 0.51\times10^6$ dpm (20.8 ± 8.5 kBq), which was less than 6% of $^{14}CO_2$ used for labelling the plants. The total activity in the seven labelled *E. americanum* root systems was 0.018 ± 0.021% of that in sugar maple saplings.

Radioactivity was not detected on autoradiographs after six wk of exposure in *E. americanum* corms, or in yellow birch saplings. Microscope slides of *E. americanum* roots showed no trace of radioactivity after two to four wk of exposure to LM-1 nuclear emulsion. After four wk, the background was too high to detect ^{14}C in the root and fungal tissues.

1.7 Discussion

This is the first report, to our knowledge, of source-sink relationships that were not governed by shading the receiver plants but by the phenology of receiver and donor plants.

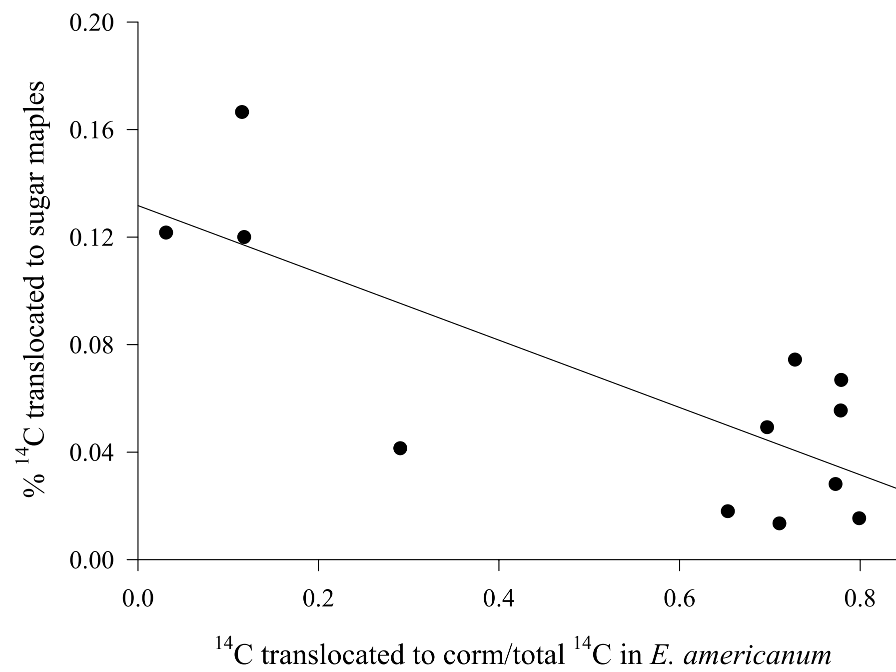

Figure 1.3. Relationship between the percentages of ^{14}C exported to sugar maple saplings following spring labelling of *Erythronium americanum* and the fraction of ^{14}C in the corm of labelled *E. americanum*.

In our experiment autoradiographs and quantitative analyses of radioactivity revealed ^{14}C in all sugar maple saplings. Sugar maple saplings showed labelling not only in roots but also in leaves and stem clearly demonstrating that (i) ^{14}C transfer from the AM fungi to the receiver plants occurred and (ii) the transferred ^{14}C was translocated into the plant and thus available for plant growth. Earlier C transfer studies reported ^{14}C only in the roots of the receiver plants (Francis & Read 1984, Finlay & Read 1986, Wu *et al*. 2001). However, because ^{14}C could be confined to the AM fungal tissues colonising the roots, labelled C in the shoot is clear evidence for C transfer from a donor plant via the mycorrhizal mycelium to a receiver plant.

We found no visible radioactivity in yellow birches and non-labelled *E. americanum* on autoradiographs (data not shown) indicating that absorption of ^{14}C-root exudates by ECM and AM fungi was insignificant. The low levels of ^{14}C detected in the quantitative analyses of ^{14}C of yellow birch showed that passive absorption of radio-labelled material could occur. However, average ^{14}C specific activity in sugar maple saplings was 13-fold higher than in yellow birch saplings while the lowest ^{14}C specific activities in sugar maples were comparable with yellow birches. It is likely that in the low activity sugar maples the ^{14}C detected in those saplings was due to passive absorption of labelled root exudates by associated AM fungi or the saplings roots.

The fraction of ^{14}C fixed (and retained) by the *E. americanum* and delivered to the sugar maples saplings (0.064% ± 0.049) seems tiny. However, we have estimated that over the 7 d of leaf expansion of sugar maple saplings with optimal sunlight conditions, an *E. americanum* plant with photosynthetic rates of 12 μmol CO^2 m^{-2} s^{-1}, 6 h per d, would transfer up to 21 μg C to sugar maples. From results of another study (unpublished data) where starch and sucrose contents were measured in sugar maple saplings in late autumn after bud formation and spring we have assessed the cost of leaf expansion in our sugar maple sapling was about 1.32 mg C per sapling. Thus, the C transferred from one *E. americanum* could cover up to 1.6% of leaf expansion of 1-year sugar maple saplings. It is likely that in undisturbed ecosystems sugar maple saplings would be connected to several *E. americanum* plants, as *E. americanum* is often more abundant than sugar maple saplings

(personal observation). We can thus expect that sugar maple saplings would receive more C than measured in our one-to-one labelling experiment, and that the C contribution of *E. americanum* during sugar maple sapling leaf expansion is physiologically significant.

The distribution of radioactivity in sugar maple saplings was consistent with the concept of source-sink relationships in C transfers between donor and receiver plants. Seven d after the exposure of potential donor plants to ^{14}C, the label had been transferred to the sugar maple saplings and half of it was localised in leaves, which represented less than a third of the total sapling biomass. This labelling pattern would be a consequence of bud burst in sugar maple saplings which occurred at the time the labelling experiment was performed. New leaves development acts as a strong sink for carbohydrates and thus directs high quantities of C towards these growing organs. Our results are consistent with those of Francis & Read (1984), Finlay & Read (1986) and Simard *et al*. (1997b) who underlined the essential role of source-sink relationships between plants connected by common mycelia. C transfer between plants was modified by applying different levels of irradiance to the receivers.

Robinson & Fitter (1999) argued that there was as yet no evidence of C transfer from AM fungi to the shoots of the receiver plant. They suggested that the low levels of ^{14}C detected in the shoots of receiver plants (e.g. Francis & Read 1984) originated from $^{14}CO_2$ respired by roots and microbes and re-fixed during photosynthesis by the receiver plant. In our experiment, labelling was performed at bud burst of the receivers and the chased period (7 d) ended after leaf expansion of sugar maple. Photosynthetic $^{14}CO_2$ fixation would not explain our findings as photosynthetic rates are restricted in developing leaves, as in *Acer campestre* (Küppers 1984). Furthermore, the quasi-absence of radioactivity in non-labelled *E. americanum*, which would have had elevated photosynthetic rates during the chase period (Taylor & Pearcy 1976), argues against the possibility of net $^{14}CO_2$ fixation by photosynthesis in the receiver plants.

The close correlation between C allocated to the corm of *E. americanum* and C translocated to sugar maple saplings suggests that *E. americanum* export C once its own carbohydrate

storage is completed emphasising again the importance of source-sink relationship in C transfer. At the time of labelling, eight of the 12 *E. americanum* had apparently not completely filled up their starch reserve, while three *E. americanum* plants were translocating small amounts of C to the corm, suggesting that the storage process was about to end in the former eight *E. americanum*. We can estimate that if the labelling of *E. americanum* would have occurred 2-3 d later, more *E. americanum* would have then completed their storage of carbohydrates and we would have observed higher ^{14}C movements towards sugar maple saplings. The magnitude of C transfer between *E. americanum* and sugar maple saplings is probably dependent on the climatic conditions prevailing at spring between the unfurling of *E. americanum* leaves and the canopy closure.

In spring, the *E. americanum* plants were highly mycorrhizal. The colonisation levels were, however, less than reported by Brundrett & Kendrick (1990b) and by Lapointe & Molard (1997) who found mycorrhizal levels of 67% and 82.8% respectively. Nevertheless, the presence of AM fungi in every tested plant reflected a sufficient inoculum potential in the experimental pots.

In autumn, the ^{14}C detected in 7 of the 22 *E. americanum* root systems strongly supports the notion that AM fungi living on sugar maple roots can be a source of mycorrhization of *E. americanum* roots. If the source of activity had been sugar maple root exudates then all *E. americanum* would have been equally labelled. Therefore, we concluded that about one third of *E. americanum* plants were connected to the donor sugar maples in autumn 2001.

Notwithstanding, autumn labelling did not demonstrate whether C was transferred from sugar maple saplings to *E. americanum* root cells. Micro-autoradiographs with nuclear emulsion did not show any traces of radioactivity. The quantity of $^{14}CO_2$ we used for labelling donor plants (2×185 kBq per plant) was very low compared to micro-autoradiography of Bücking & Heyser (2001) who used 7.4 MBq (200 μCi) $^{14}CO_2$ per plant. In our work, ^{14}C detection threshold levels were below background. Hence, radioactivity in fungal and root cells was not visually documented.

Possible transfer of C in autumn from sugar maple saplings to *E. americanum* for the root formation was less clear than the spring case of C transfer from *E. americanum* to maple saplings. However, traces of ^{14}C were detected in some of the *E. americanum* plants, suggesting a possible payback of C from sugar maples to *E. americanum* in autumn.

In conclusion, we have documented C transfer between sugar maple saplings and the spring ephemeral *E. americanum* connected by AM mycelium that appears reciprocal. An initial transfer occurs in spring from *E. americanum* to sugar maple saplings during leaf expansion of the saplings. A reciprocal transfer is highly likely in autumn. This second transfer was from the sugar maple saplings to newly developed roots of *E. americanum* plants, but we do not have conclusive proof at this point that imported C was used for the formation of *E. americanum* roots. Reciprocal transfers could be frequent in natural ecosystems where plants with different phenologies share AM fungi.

1.8 Conclusion

Ce chapitre fait pour la première fois la preuve de la possibilité à l'état naturel d'échanges bidirectionnels de C entre plantes de phénologie différente connectées par des champignons MA. Un premier transfert a lieu au printemps à partir d'érythrones d'Amérique photosynthétiques vers des juvéniles d'érable à sucre au moment de leur développement foliaire. L'amplitude de ce transfert de C est corrélée au niveau de stockage en amidon de l'organe de réserve des érythrones (corme). Il apparaît donc que l'érythrone ne délivre du C aux juvéniles d'érable à sucre par le biais de champignons MA qu'une fois le remplissage de son corme complété. Un second transfert semble très probable à l'automne à partir des juvéniles d'érable à sucre vers les racines d'érythrone nouvellement formées. Cependant, il a été impossible de prouver que le C importé a été utilisé dans la construction des racines d'érythrone. De tels transferts de C pourraient être fréquents dans les écosystèmes naturels où des plantes de phénologie différente partagent des champignons MA communs.

CHAPITRE II

Carbon partitioning in a split-root system of arbuscular mycorrhizal plants is fungal and plant species dependent

2.1 Avant-propos

Ce chapitre a été publié par Lerat, Lapointe, Gutjahr, Piché & Vierheilig (2003) dans la revue New Phytologist. Horst Vierheilig a initié et a participé à la phase expérimentale de ce travail. Sylvain Gutjahr a réalisé l'étude de croissance de plants d'orge en serres. Le contenu de ce chapitre a également été présenté oralement au Colloque Canadien sur les Mycorhizes tenu en avril 2002 à Québec (Lerat, Lapointe, Piché & Vierheilig) et une partie a fait l'objet de la présentation d'une affiche au congrès Plant Biology 2001 tenu à Providence, Rhode Island, USA (Lerat, Lapointe, Piché & Vierheilig).

2.2 Résumé

La répartition racinaire de carbone (C) a été étudié chez deux espèces végétales hôtes colonisées par une parmi trois espèces de champignon MA. Des systèmes à racines dédoublées («split-root») d'orge (*Hordeum vulgare*) et d'érable à sucre (*Acer saccharum*) ont été mycorhizés d'un côté avec l'un des trois champignons MA. Trois semaines après inoculation, leurs feuilles ont été marquées à l'aide de $^{14}CO_2$. Les plantes ont été récoltées 24 heures plus tard et le ^{14}C a été analysé séparément dans les systèmes racinaires des côtés mycorhizés (M) et non mycorhizés (NM). La répartition du ^{14}C entre les côtés M et NM variait en fonction de l'espèce fongique et de la plante hôte utilisées. *Gigaspora rosea* était un puits de C de forte capacité avec les deux espèces végétales, *Glomus intraradices* était un puits de forte capacité avec l'orge alors que *Glomus mosseae* ne modifiait la répartition

du ^{14}C chez aucune des deux espèces. La force de puits de C chez les racines d'orge M inoculées avec *Gi. rosea* ou *G. intraradices* était linéairement corrélée au niveau de colonisation fongique. L'utilisation de trois espèces de champignons MA et de deux espèces végétales a permis de conclure que la force de puits de C des champignons MA dépend des deux partenaires impliqués dans la symbiose.

2.3 Summary

Split-root systems of barley (*Hordeum vulgare*) and sugar maple (*Acer saccharum*) were inoculated on one side with one of three AM fungi. Three wk after inoculation, leaves were labelled with $^{14}CO_2$. Plants were harvested 24-h later and the root systems from the mycorrhizal (M) and non-mycorrhizal (NM) sides were analysed separately for ^{14}C. Partitioning of ^{14}C between M and NM sides varied depending on the fungal and host plant species used. *Gigaspora rosea* showed a strong C sink capacity with both plant species, *Glomus intraradices* showed a strong C sink capacity with barley, and *Glomus mosseae* did not affect ^{14}C partitioning. C sink strength of the M barley roots inoculated with *Gi. rosea* or *G. intraradices* was linearly correlated with the degree of colonization. The use of three AM fungal and two plant species allowed us to conclude that C sink strength of AM fungi depends on both partners involved in the symbiosis.

2.4 Introduction

The roots of most terrestrial plants are symbiotically associated with obligate biotrophic fungi in the order Glomales (Zygomycotina) (Hayman 1983). This arbuscular mycorrhizal (AM) association improves plant mineral nutrition (in particular phosphorus (P)), and can influence water uptake and resistance towards root pathogens (Smith & Read 1997). In return, the plant supplies AM fungi with carbohydrates derived from photoassimilation (Ho & Trappe 1973).

It has been demonstrated that photoassimilation and subsequent carbon (C) supply to the root system are closely linked to the development of AM fungi in the roots. Thomson *et al.* (1990) established a positive correlation between mycorrhizal (M) colonization levels and

the concentration of soluble carbohydrates in subterranean clover roots colonized by either *Scutellospora calospora* or *Glomus fasciculatum*. Moreover, Vierheilig *et al*. (2002) recently showed that bean plants inoculated with *Glomus mosseae* and grown in the dark did not become colonized. Wright *et al*. (1998b) demonstrated, using *Trifolium repens*, that M plants show higher photosynthetic rates than their non-mycorrhizal (NM) counterparts, but that this enhanced photoassimilation did not result in increased plant growth. Thus, the authors concluded that the C gain observed in M plants is probably channelled to the C sink developed by the mycobiont. Estimated C costs of the AM symbiosis are well documented in the literature (e.g. Pang & Paul 1980; Kucey & Paul 1982; Snellgrove *et al*. 1982; Koch & Johnson 1984; Douds *et al*. 1988; Wang *et al*. 1989) and many authors consider that 4-20% of the total C fixed by an AM plant is used by the mycobiont (e.g. Bago *et al*. 2000a; Douds *et al*. 2000; Graham 2000).

Considering the improved mineral acquisition efficiency of M plants compared to NM plants, the growth of M plants in general is improved. Phosphorus is a key element in the photosynthesis (Salisbury & Ross 1985; Marschner 1990) and therefore reduced P contents in the shoot (e.g. in NM plants) may directly affect plant growth. Furthermore, high foliar phosphate concentrations enable the translocation of C compounds towards other plant organs (Herold 1980). Thus, changes in the nutritional status of M plants may result in a changed C budget. Because of the different nutritional status of M and NM plants it is therefore difficult to assess the cost of the M symbiosis by comparing M and NM plants (Pang & Paul 1980). One possible solution to this problem is to supply P to NM plants as a means of obtaining plants of a similar size and P content (Kucey & Paul 1982; Snellgrove *et al*. 1982; Wright *et al*. 1998a,b). However, as arbuscular mycorrhization not only affects P uptake but also the uptake of a wide range of nutrients such as N, S, Cu, Zn or Ni (Marschner 1990; Smith & Read 1997), results obtained using this experimental approach must also be interpreted with caution. Moreover, M plants have been shown to be more resistant towards soil-borne pathogens (Dehne 1982; St-Arnaud *et al*. 1995), indicating metabolic (not necessarily nutrient induced) changes. In order to overcome the differences between M and NM plants, a number of studies have used split-root systems (Koch & Johnson 1984; Douds *et al*. 1988; Wang *et al*. 1989). Using this technique, the plant’s root

system is equally divided between two compartments, one of which is subsequently inoculated with a M fungus, hence allowing a comparison of the C sink strength of M and NM roots of the same plant.

Most studies concerning C partitioning in M plants have not emphasized the possible importance of the effect of the fungal species and the host plant involved in the AM symbiosis. However, Pearson *et al*. (1993, 1994) showed that *S. calospora* and *Glomus* sp. [WUM 10(1)] exhibit different colonization patterns in relation to root carbohydrate concentration. More recently, van der Heijden *et al*. (1998), in a study of the impact of fungal diversity on plant diversity, showed that different plant species benefit from different AM fungal species. In an attempt to investigate some of the possible mechanisms underlying these findings, we studied the ^{14}C partitioning in M and NM split-root systems of two economically important plants, barley and sugar maple, to estimate the C sink strength capacity of each of three AM fungi.

2.5 Materials and methods

2.5.1 Experimental design

The split-root system developed by Wyss *et al*. (1991) and modified by Vierheilig *et al*. (2000a) was used. Two compartments, compartments B and C, contained the two halves of the root system of the study plant. An inoculum compartment, compartment A, which comprised bean plants (*Phaseolus vulgaris* L. cv. Sun Gold) inoculated with one of the AM fungi under study (see below) or not (control) was attached to compartment B which contained the half of the root system to be inoculated. A Nylon screen (60 µm mesh) separated compartments A and B and compartments B and C were separated by a PVC plate.

2.5.2 Biological material and growing conditions

The AM fungi *Gigaspora rosea* Nicolson & Schenck (DAOM 194757, ECORC, Agriculture and Agri-Food Canada, Ottawa, Canada), *Glomus intraradices* Smith &

Schenck (DAOM 197198) and *Glomus mosseae* (Nicolson & Gerdemann) Gerd. & Trappe (BEG 12; La Banque Européenne des Glomales, International Institute of Biotechnology, Kent, UK) were used.

Barley seeds (*Hordeum vulgare* L. cv. Salome) were germinated in vermiculite. After 3 d, seedlings were transferred to the split-root system (two primary roots per compartment). Both compartments B and C contained a steam-sterilized mixture of sand:soil:turface (vol:vol:vol, 1:1:1). Plants were grown for 3 wk, in the presence of the inoculum compartment A, in a growth chamber (photoperiod: 16 h; light: 300 μmol m^{-2} s^{-1} PAR (photosynthetically active radiation); temperature: 23/19°C day/night; RH: 50 %, no mineral fertilization) prior to labelling with ^{14}C. To facilitate the obtainment of optimal M levels, no mineral fertilization was added.

Sugar maple seeds (*Acer saccharum* Marsh.) were collected in October 2000 in a sugar maple forest near Québec City. After 3 months of cold stratification (4°C), seeds were germinated in perlite. Seedlings were transferred to pots (100 ml) containing a steam-sterilized mixture of sand:sugar maple forest soil:turface (vol:vol:vol, 1:2:1) and grown under glass (photoperiod: 14 h; light: $\geq$ 300 μmol m^{-2} s^{-1} PAR; temperature: 25/17°C day/night; RH: not controlled; no mineral fertilization). Six wk after germination, the primary root was cut 2-3 cm below the root-shoot interface to promote lateral root production. Three wk later, the seedlings were transferred to the split-root system. Plants were grown for 3 wk, in the presence of the inoculum compartment A, under the greenhouse conditions outlined above, prior to labelling with ^{14}C.

In order to increase the number of replicates, the experiment was repeated over time. Therefore, for both plant species, three repetitions of the experiment were performed with five replicates per treatment and per repetition giving a total of 15 plants for each of the four treatments (three fungal species and one control).

2.5.3 $^{14}CO_2$ labelling

Compartment A was removed before labelling. Each plant shoot was place inside a 945-ml (18×20 cm) transparent freezer bag (Ziploc®) together with a 29.5-ml cup containing a basic solution of 37 kBq (1 μCi) $NaH^{14}CO_3$ (Amersham Pharmacia Biotech). The plastic bag was then closed and a sealing compound placed around the shoot stem. Gaseous $^{14}CO_2$ was produced by injecting 1 ml lactic acid (85%) into the cup. Plants were exposed for 2 h in a growth chamber (see above for conditions). After the pulse period, the bags were removed under a venting fume-hood and the plants returned to a growth chamber.

After a 24-h chase period the two halves of the split-root systems were harvested separately and fresh weights recorded. For each repetition, sub-samples of roots from two of the five replicates (plants) were collected from both compartments, weighed, and assessed for M colonization. The rest of the root systems were oven dried (24 h at 65°C), weighed, and used to determine the level of radioactivity. Barley roots were digested with the tissue solubilizer NCS, and sugar maple roots were ground in liquid nitrogen and digested according to the technique described by Clifford *et al*. (1973). Radioactivity was assessed by liquid scintillation spectrometry. Counts were standardized with a quench curve and expressed in dpm. The presence of radioactivity in the substrate was determined from 1 g of the sand:soil:turface mix after digestion in NCS.

Results were expressed as a percentage of total ^{14}C and as a percentage of total root dry weight in the M and NM compartments. Corrections were made for the root samples taken to determine M colonization levels.

2.5.4 Effect of AM fungal species on growth of barley

The effect of *Gi. rosea*, *G. intraradices* and *G. mosseae* on the growth of barley was tested. Their effect on sugar maple was not tested in this study because this species showed a large, within treatment, growth variation in the previous split-root experiment.

Four-d-old barley seedlings were transferred to compartment B (non-divided) of the system described above. Four compartments, each containing 13 seedlings, were used. The seedlings of each compartment were inoculated or not by attaching inoculum compartments containing either M bean plants colonized by *Gi. rosea*, *G. intraradices* or *G. mosseae*, or NM plants, and grown in a growth chamber (see above for conditions). After 7 d, 10 plants of each of the four treatments were transferred to individual pots (700 ml) containing the steam-sterilized barley substrate (see above). The presence of M colonization was verified on the three remaining plants.

The pots were transferred to the greenhouse (see above for conditions). Plants were grown in a randomized complete block design. Plants were watered weekly with a 10% Hoagland solution (200 ml per pot) and with deionized water as needed. In order to study the effect of the fungal treatments with time, harvests were performed after 4 and 8 wk. M colonization levels (% root length colonized by M fungus) were determined for all plants on a fresh root sample. Shoots were dried (24 h at 65°C) and weighed. Shoot dry weight was expressed as a percentage of the control.

2.5.5 Measurement of mycorrhizal colonization levels

Barley root samples were stained using the ink and vinegar technique (Vierheilig *et al*. 1998) and M colonization levels were measured according to Newman (1966). Sugar maple root samples were stained with trypan blue (Koske & Gemma 1989) and M colonization levels were assessed according to McGonigle *et al*. (1990). The M colonization levels were expressed as the percentage root length colonized by AM fungi regardless of the fungal structures.

2.5.6 Statistical analyses

Split-root experiment data from barley and sugar maple were analysed separately. The data for the percentage of radioactivity allocated to each compartment were arcsin-transformed. Data from compartments B and C in the control plants were compared using a paired *t*-test to test the validity of our experimental design. The calculated *P* values were 0.77 for barley

and 0.72 for sugar maple showing no differences due to the experimental design. Therefore, further statistical analyses only considered the data from inoculated sides, which were analysed by two-way ANOVA, with treatment and repetition as main factors.

Shoot dry weight was analysed separately for the two harvests by one-way ANOVA, with fungal species as the treatment factor. A posteriori comparisons were made using LSD tests.

2.6 Results

2.6.1 Mycorrhizal colonization levels

Three wk after inoculation, the inoculated sides of the split-root systems of barley (Fig. 2.1a-c) and sugar maple (Fig. 2.2a) were colonized regardless of the M fungus used. No colonization was observed in roots from NM control plants or in roots from the non-inoculated compartment C of the split-root system.

2.6.2 ^{14}C partitioning

No radioactivity was detected in the growth substrate (<60 dpm g^{-1} dry soil), therefore, all the data analyses refer to the radioactivity measured in the roots.

2.6.2.1 Barley host plants

The root systems of two barley plants were damaged during labelling. Consequently, the statistical analyses were performed on a total of 58 plants. The analysis of variance

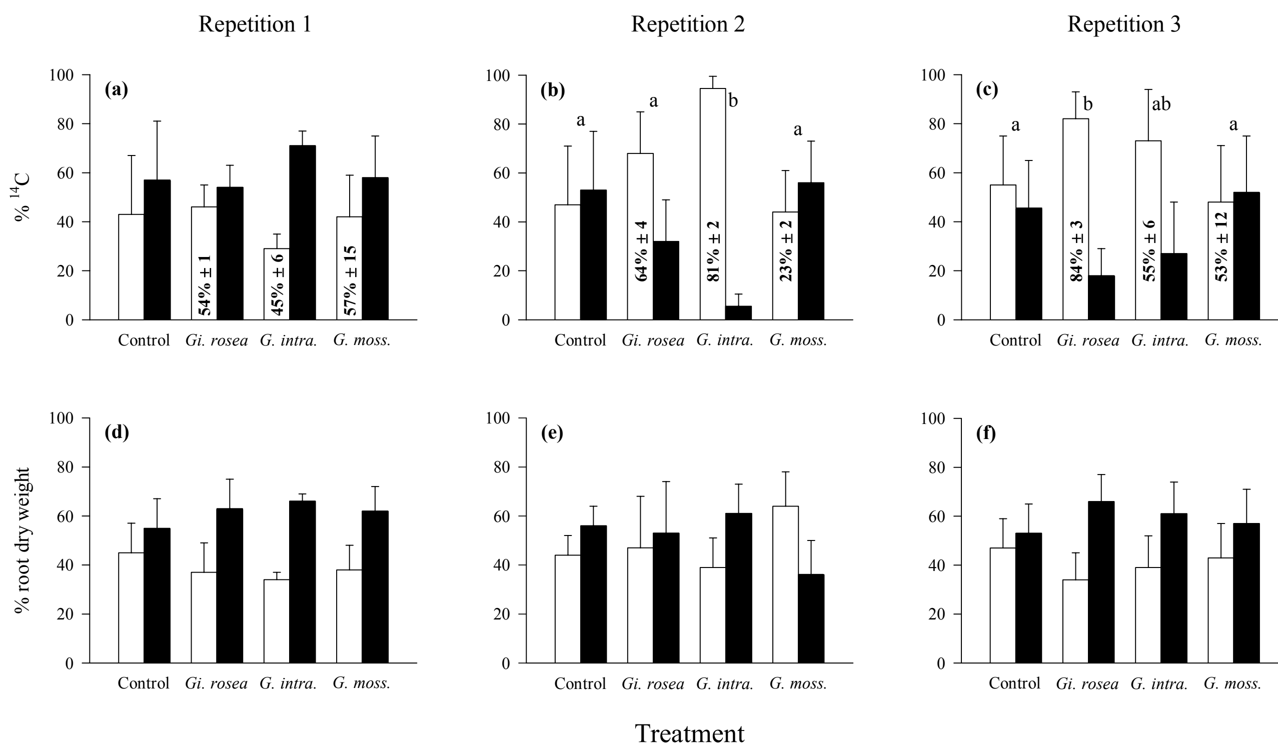

Figure 2.1. (a-c) Mean total ^{14}C (± 1 SD) partitioned and (d-f) mean percentage dry weight (± 1 SD) of M (open bars) and NM (solid bars) barley roots, for each repetition, in control (compartments B and C), *Gigaspora rosea*, *Glomus intraradices* and *Glomus mosseae* treatments. Mycorrhizal colonization levels (± 1 SD) are shown within M bars (a-c). When the ANOVA within the repetition was significant (b,c) an LSD test was performed (treatments with the same letter are not statistically different). *Gi. rosea*=*Gigaspora rosea*; *G. intra.*=*Glomus intraradices*; *G. moss.*=*Glomus mosseae*.

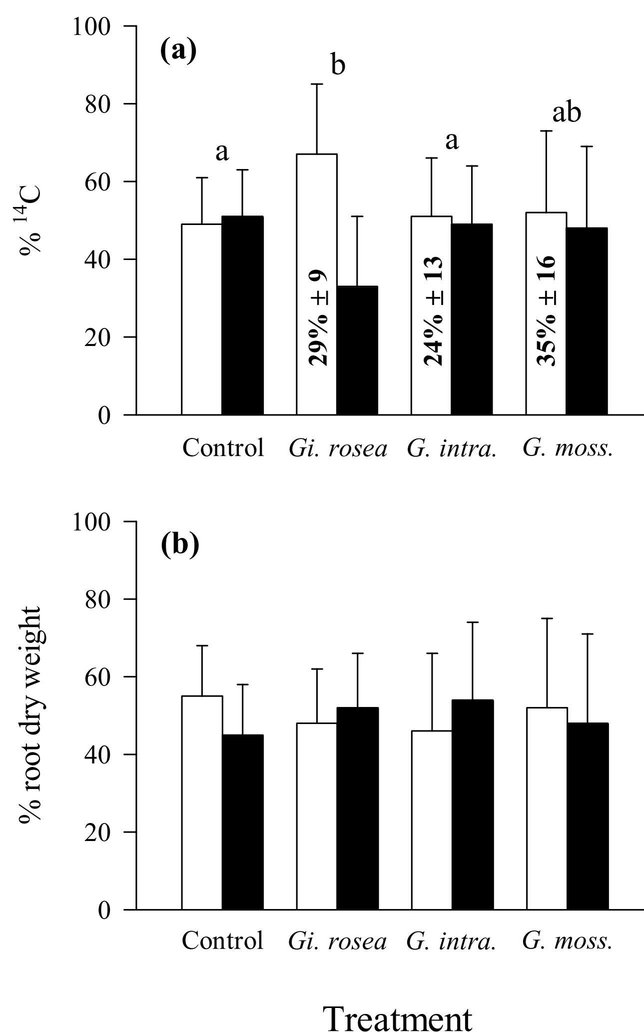

Figure 2.2. (a) Mean total ^{14}C (± 1 SD) partitioned and (b) mean percentage dry weight (± 1 SD) of M (open bars) and NM (solid bars) sugar maple roots in control (compartments B and C), *Gigaspora rosea*, *Glomus intraradices* and *Glomus mosseae* treatments. Mycorrhizal colonization levels (± 1 SD) are shown within M bars (a). As treatment showed significant differences (a) an LSD test was performed (treatments with the same letter are not statistically different). Abbreviations are as in Fig. 2.1.

revealed strong treatment and repetition effects on ^{14}C partitioning and a strong treatment×repetition interaction (Table 2.1). Therefore, data obtained using the three different fungi were analysed separately for each repetition. Significant one-way ANOVA tests were followed by a LSD test. The percentage of ^{14}C allocated to M roots was significantly higher than control roots (P value < 0.05) in plants inoculated with *G. intraradices* in repetition 2 and with *Gi. rosea* in repetition 3 (Table 2.1, Fig. 2.1b,c). When inoculated with *G. mosseae*, the percentage of ^{14}C allocated to M roots never differed significantly from control roots (Fig. 2.1a-c).

Table 2.1. Degrees of freedom (DF), mean sum of squares (MS), *F* values, and *P* values of (a) a two-way ANOVA and (b) one-way ANOVAS for each repetition performed on percentage ^{14}C allocated to M barley roots (data arcsin-transformed).

	Source of variation		DF	MS	*F* value	*P* value
a	Treatment (Treat.)		3	0.3959	8.08	0.0002
	Repetition (Repet.)		2	0.6994	14.27	0.0000
	Treat. × Repet.		6	0.2797	5.71	0.0002
	Error		46	0.0490	-	-
b	Repet. 1	Treat.	3	0.0349	1.04	0.4029
		Error	15	0.0335	-	-
	Repet. 2	Treat.	3	0.6567	13.74	0.0001
		Error	15	0.0478	-	-
	Repet. 3	Treat.	3	0.2337	3.61	0.0365
		Error	16	0.0647	-	-

Partitioning of ^{14}C in plants colonized by *Gi. rosea* and *G. intraradices* appeared to be correlated with M colonization levels: the higher the M colonization level, the stronger the sink for carbohydrates (Fig. 2.1a-c). By contrast, ^{14}C partitioning in plants colonized by *G. mosseae* (Fig. 2.1a-c) was not affected by M colonization levels and was always equally

divided between M and NM roots. Data from the six plants (two per repetition) used to determine M colonization levels were used to perform linear correlation plots representing arcsin[% ^{14}C in M roots/100] as a function of M colonization levels. The r^2 values were 0.73 (P=0.03) for *Gi. rosea* (Fig. 2.3a) and 0.75 (P=0.03) for *G. intraradices* (Fig. 2.3b) confirming the presence of a significant correlation between these two parameters. By contrast, the r^2 value was 0.37 (P=0.20) for *G. mosseae* (data not shown).

Increased ^{14}C partitioning in M roots did not correspond to increased root dry weight when compared to NM roots (Fig. 2.1d-f). In fact, M roots often represented a lower percentage of the total root dry weight that their NM counterparts in all four treatments, but treatment effect on percentage root dry weight was not statistically significant (F=2.65, P=0.06).

2.6.2.2 Sugar maple host plants

During the 3 wk following the inoculation, leaves of 23 of the sugar maple seedlings exhibited extensive necrotic zones or showed no capacity to fix $^{14}CO_2$ (absence of ^{14}C in the leaves after labelling). Consequently, the statistical analyses were performed on a total of 37 plants. The analysis of variance revealed a treatment effect on ^{14}C partitioning but no effect of repetition and no treatment×repetition interaction (Table 2.2). Therefore, data from all repetitions for each fungal species were pooled for comparison with the control data. *Gigaspora rosea* increased ^{14}C partitioning towards M roots (Table 2.2, Fig. 2.2a) while root dry weight was not affected by mycorrhization (Fig. 2.2b). Furthermore, there was no correlation between % ^{14}C in M roots and M colonization levels (r^2=0.01, P=0.88). *Glomus intraradices* and *G. mosseae* neither modified ^{14}C partitioning nor root dry weight between M and NM roots (Fig. 2.2a,b).

2.6.3 Growth of mycorrhizal barley

Significant differences in shoot dry weight were observed (P=0.03) 4 wk after transfer to individual pots. Shoot dry weight in *Gi. rosea* and *G. intraradices* treatments were significantly lower (63% ± 14 and 66% ± 12, respectively) than the control, while shoot dry weight of plants inoculated with *G. mosseae* did not differ from the other treatments

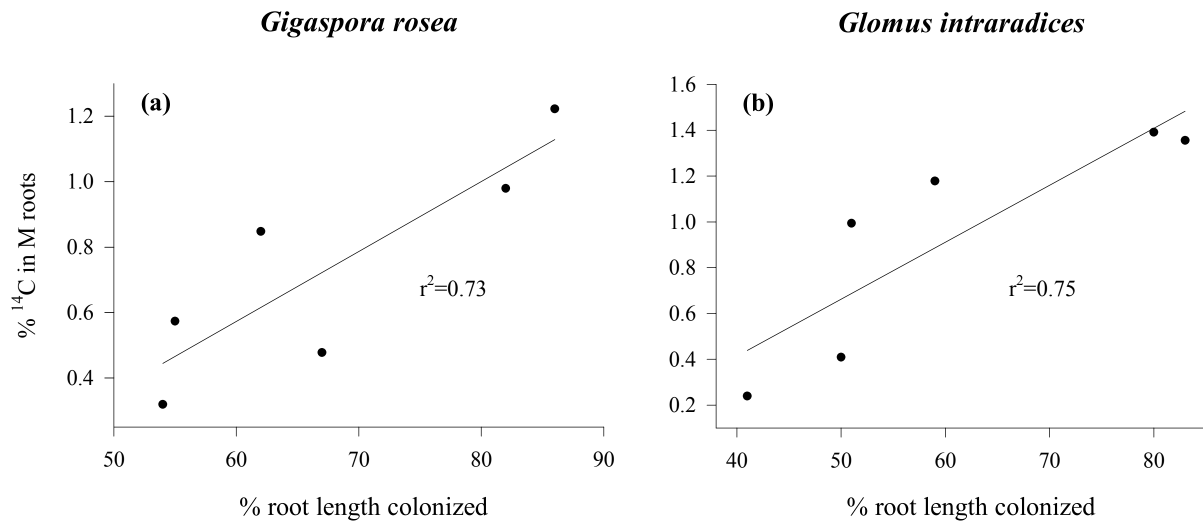

Figure 2.3. Relationship between percentage ^{14}C allocated to M roots (arcsin-transformed) and the percentage of barley root length colonized by (a) *Gigaspora rosea* and (b) *Glomus intraradices*. Dots represent the two out of five plants per repetition used to assess M colonization levels. r^2 values of the correlation are presented.

Table 2.2. Degrees of freedom (DF), mean sum of squares (MS), *F* values, and *P* values of a two-way ANOVA performed on percentage ^{14}C allocated to M sugar maple roots (data arcsin-transformed).

Source of variation	DF	MS	*F* value	*P* value
Treatment (Treat.)	3	0.1935	3.55	0.0288
Repetition (Repet.)	2	0.0061	0.11	0.8942
Treat. × Repet.	6	0.0459	0.84	0.5501
Error	25	0.0545	-	-

(78%°±°12 of control). At the second harvest (8 wk after transfer to pots) significant differences in shoot dry weight were also observed (*P*=0.02). Shoot dry weight in the *G. intraradices* treatment was significantly lower (67% ± 21) than the control, while *Gi. rosea* and *G. mosseae* treatments did not differ from the other treatments (85% ± 8 and 82% ± 7 of control, respectively). All the inoculated plants were extensively colonized by AM fungi (data not shown).

2.7 Discussion

In the present study we have shown that fungal and plant species are major factors influencing the C partitioning between M and NM roots grown in a split-root system. In previous experiments with similar split-root systems the effect of one AM fungal strain on the C partitioning between M and NM roots of one (Douds *et al*. 1988; Wang *et al*. 1989) or two plant species (Koch & Johnson 1984) was studied. In our experiments, we used two morphologically and phenologically different host plant species: barley, a rapidly growing herbaceous annual and sugar maple, a slow growing woody perennial. The ability of three different AM fungal species, from two genera, to alter the C sink strength of the root system was tested on each of the two plant species. Thus, in our experiment C partitioning in split-root systems was studied in six AM fungus-host plant combinations.

The C partitioning in M and NM roots of the two plant species showed different patterns depending on the AM fungal partner. The three AM fungi which were used not only showed different C sink strength capacities but the sink strength of one, *G. intraradices*, also seemed to be host plant dependent. The results for *G. intraradices* differ from those obtained by Koch & Johnson (1984), who showed that *G. intraradices* had no effect on ^{14}C-labelled photosynthate partitioning between M and NM roots in two citrus cultivars. In the present study, *Gi. rosea* and *G. intraradices* showed a strong C sink capacity in barley. In both fungi the sink strength was positively correlated with colonization levels. Low root colonization resulted in no increase in C transfer to M roots. This is in accordance with the results of Thomson *et al*. (1990) that showed a positive relationship between the soluble carbohydrate concentration in the roots of *Trifolium subterraneum* and the percentage of root colonization by *S. calospora* and *G. fasciculatum.* In our study, the variability of M levels between repetitions in barley was probably due to variations in the inoculation capacity of the M bean plants used (Pearson *et al*. 1994). Compared to colonization levels in barley, root colonization by *Gi. rosea* in sugar maple seedlings was low. Nevertheless, the C transfer to M roots indicated that *Gi. rosea* was a strong C sink. However, in contrast to the experiment with barley, *G. intraradices*, with a similar level of root colonization to *Gi. rosea*, was not a strong sink for C in sugar maple and *G. mosseae* showed a low C sink capacity in barley and sugar maple. M levels in barley colonized by *G. mosseae* were low in comparison to *Gi. rosea* and *G. intraradices*, and this could explain the weak sink strength of this fungus. These M levels are, however, comparable with those reported in the study of Vierheilig *et al*. (2000a) in which the same fungal strains and the same host plant (barley cv. Salome) were used. The present results thus support the idea that a given AM fungus may have totally different effects depending on the plant species (van der Heijden *et al*. 1998) with which it is associated.

Differences in C partitioning in M and NM roots cannot be attributed to differences in root dry weight. As expected and as previously observed in fresh barley roots (Vierheilig *et al*. 2000a), the M root dry weight was lower than the NM root dry weight of barley plants colonized by *Gi. rosea* and *G. intraradices*. By contrast, in repetition 2, barley plants inoculated with *G. mosseae* showed higher M root dry weight than NM root dry weight. In

sugar maple, mycorrhization had no impact on root dry weight, regardless of the AM fungus. This could perhaps be explained by the fact that sugar maple is a slow growing tree and that the time between the mycorrhization and harvest of sugar maples might have been too short to detect differences in dry weight between M and NM compartments.

What is the implication of AM fungi of different sink strength on host plants? A permanent strong C sink is probably unfavourable to plants in which growth is C limited (e.g. under low light conditions), but it is likely that the strength of the C sink decreases once the fungus is well established in the host roots and once it has extensively colonized the mycorrhizosphere. Conversely, a fungus with low C sink strength is expected to be profitable to its host only if it efficiently improves the uptake of mineral nutrients. The study of the effects of the three AM fungi tested on the growth and mineral nutrition of barley are complementary to the C partitioning experiments. It showed that the plants harvested at 4 wk had a decreased shoot dry weight when inoculated with the two species showing a strong C sink capacity, *Gi. rosea* and *G. intraradices*, while *G. mosseae* did not cause any growth depression. After 8 wk *G. intraradices* was the only AM fungus still suppressing plant growth. Analyses of mineral foliar concentrations did not give significant differences (data not shown). It appears that the mycorrhization of barley (cv. Salome) by the strong C sink *Gi. rosea* is costly during initial establishment and that it can deprive plants of notable amounts of C. This is probably linked with the fact that species within the genus *Gigaspora* rapidly form a dense and extensive mycelial network (Dodd *et al*. 2000; Hart & Reader 2002). Moreover, it has been noted that *S. calospora*, another member of the Gigasporaceae, has particularly high C requirement in comparison to *Glomus* species (Thomson *et al*. 1990; Pearson *et al*. 1993). *Glomus intraradices*, the other fungus showing a strong C sink capacity in barley, probably used plant derived C for the formation of large quantities of intraradical vesicles inside colonized roots (Peng *et al*. 1993). Similar growth depression in M plants has previously been reported for *G. intraradices* (Peng *et al*. 1993; Marschner & Crowley 1996; Pozo *et al*. 2002) and for many other AM fungal species (e.g. Schenck & Smith 1982; Boyetchko & Tewari 1995; Graham & Abbott 2000; Taylor & Harrier 2000). *Glomus mosseae*, which apparently was a low C sink, also produced plants of slightly smaller size than the control. The present results suggest that AM C cost is

influenced by the development state of the fungal partner and perhaps also by the developmental state of the plant.

In conclusion, this is, to the best of our knowledge, the first report of C partitioning in M and NM split-root systems involving different host plant species colonized by different AM fungi. This work emphasizes the importance of considering both plant and fungal species when studying the C sink strength capacity of AM fungi or the C cost induced by M colonization. This study also highlights the fact that an individual AM fungus might be either a strong or weak C sink depending on the plant host. Furthermore, in several AM fungal species M colonization levels appear to be an important factor determining fungal C sink strength. Finally, an expensive AM fungus may not necessarily be a disadvantage to a plant host if there are long-term fitness gains.

2.8 Conclusion

L'utilisation du système split-root facilite l'étude de la force de puits de C des champignons MA car il permet de mesurer la répartition de C chez des racines M et NM au sein d'une même plante. Grâce à l'emploi de cet outil, il a été possible de comparer pour la première fois dans une même étude la force de puits de trois champignons MA chez deux espèces végétales et d'obtenir une combinaison de six couples plante/champignon. À la lumière de ce chapitre, il apparaît que la force de puits de C des champignons MA varie non seulement en fonction de l'espèce fongique mycorhizatrice mais également en fonction de l'espèce végétale hôte. Les trois espèces fongiques étudiées ici présentaient trois patrons de répartition de C différents. Il en résulte qu'un champignon MA (par ex. *G. intraradices*) peut être qualifié à la fois un puits de C de forte ou de faible capacité selon l'espèce végétale à laquelle il est associé. De plus, chez certaines espèces fongiques MA les taux de mycorhization apparaissent être un facteur déterminant de la force de puits de C. Enfin, les mesures de croissance et l'analyse des nutriments effectuées sur les plantes en serres ont montré qu'un champignon MA coûteux n'est pas nécessairement un désavantage pour une plante s'il peut engendrer d'importants bénéfices à long terme.

CHAPITRE III

Variable carbon-sink strength of different *Glomus mosseae* strains colonizing barley roots

3.1 Avant-propos

Le texte de ce chapitre est la version révisée d'un manuscrit accepté sous forme de brève communication à la revue Canadian Journal of Botany (Revue Canadienne de Botanique) (Lerat, Lapointe, Piché & Vierheilig). Horst Vierheilig a participé à la phase expérimentale de ce travail et à la rédaction de cet article. Une partie de son contenu a fait l'objet d'une présentation orale au Colloque Canadien sur les Mycorhizes qui s'est tenu en avril 2002 à Québec.

3.2 Résumé

La répartition racinaire de carbone (C) a été étudiée chez de l'orge (*Hordeum vulgare* L.) colonisé par une parmi trois souches du champignon mycorhizien à arbuscules (MA) *Glomus mosseae* (Nicolson et Gerdemann) Gerd. et Trappe. Les racines de chaque plante ont été équitablement réparties entre les deux compartiments d'un système en racines dédoublées et un côté a été inoculé avec l'une des trois souches du champignon MA. Vingt-trois jours après inoculation, les parties aériennes d'orge ont été marquées avec du $^{14}CO_2$. Vingt-quatre heures plus tard, les plantes ont été récoltées et le ^{14}C a été analysé séparément dans les racines mycorhizées (M) et non mycorhizées (NM). La répartition du ^{14}C entre les côtés M et NM variait entre les souches fongiques: BEG 54 était un puits de C fort, BEG 55 était un puits de C modéré alors que BEG 12 montrait une force de puits similaire à celle des plantes témoins. Les différences de force de puits de C observées se reflétaient en

différences de biomasse végétale sèche. La biomasse végétale sèche totale des plantes inoculées par BEG 12, BEG 54 et BEG 55 représentait respectivement 81,3%, 65,3% et 73,4% de la biomasse des plantes témoins. Cet article rend compte pour la première fois d'une variation de souche dans la répartition du C chez des plantes M dans un système en racines dédoublées.

3.3 Summary

Root carbon (C) partitioning was investigated in barley (*Hordeum vulgare* L.) colonized by one of three strains of the arbuscular mycorrhizal fungus (AMF) *Glomus mosseae* (Nicolson & Gerdemann) Gerd. & Trappe. The roots of each plant were evenly divided between two compartments of a split-root system and one side was inoculated with one of the three AMF strains. Twenty-three d after inoculation barley shoots were labeled with $^{14}CO_2$. Twenty-four h later plants were harvested and the mycorrhizal (M) and non-mycorrhizal (NM) roots were analyzed separately for ^{14}C. Partitioning of C between M and NM sides differed between the fungal strains: BEG 54 was a strong C sink, BEG 55 was a moderate strong C sink and BEG 12 showed similar C-sink strength as the non-inoculated control plants. The observed differences in C-sink strength mirrored differences in plant dry biomass. Total plant dry biomass of plants inoculated with BEG 12, BEG54 and BEG 55 represented 81.3%, 65.3% and 73.4% of the biomass of the control plants, respectively. This paper is the first report of an AMF strain-specific variation of C partitioning in M plants in a split-root system.

3.4 Introduction

Arbuscular mycorrhizal fungi (AMF) belong to a ubiquitous group of Zygomycetes and form a symbiotic association with the roots of most land plants. AMF improve plant nutrition mainly by transferring phosphorus (P) from the soil to the plant. The host plants provide the fungi with carbohydrates (Smith & Read 1997).

The variability of the effect of different AMF genera, species or even different strains of the same species on plant nutritional parameters, such as growth and P-acquisition, is well

documented (Graham *et al*. 1982; Giovannetti & Gianinazzi-Pearson 1994; Smith *et al*. 2000; Burleigh *et al*. 2002), however, few data are available concerning the variability of the carbohydrate demand of different AMF. Carbon (C)-sink strength studies by Pang & Paul (1980), Kucey & Paul (1982), Snellgrove *et al*. (1982), Koch & Johnson (1984), Douds *et al*. (1988) and Wang *et al*. (1989) suggest that the cost of supporting AMF symbionts represents 4 to 20% of the total C fixed by the plant. In these studies, experimental conditions or the AMF used varied and therefore no comparison of C-sink strength between AMF has been possible. However, Pearson & Jakobsen (1993) compared the consumption of $^{14}CO_2$ in plants colonized by one of three species of AMF and found differences between treatments in the proportion of ^{14}C allocation below ground.

The C-sink strength of three AMF, *Gigaspora rosea*, *Glomus intraradices* and *Glomus mosseae*, was compared in a recent study using a split-root system (Lerat *et al*. 2003). The split-root system allows the study of C partitioning between mycorrhizal (M) and non-mycorrhizal (NM) roots of the same plant and thus avoids the comparison of M and NM plants with a potentially different mineral status. Using this system with barley and sugar maple, Lerat *et al*. (2003) observed clear differences in the sink strength of the tested fungi. *Gigaspora rosea* exhibited a high sink strength capacity in both host plants and *G. intraradices* had a strong sink capacity in barley but not in sugar maple. These results therefore showed that the sink strength of an AMF can vary between different host plants. The third AMF, *G. mosseae*, showed a low sink strength in both plant species tested. The C-sink strength of AMF can thus vary greatly between fungal species.

The goal of the present work was to investigate, using three strains of *G. mosseae* from different geographic regions, whether the low sink strength of *G. mosseae* colonized roots is a common feature of this species or whether it is strain specific.

3.5 Materials and methods

3.5.1 Biological material and growing conditions

Barley (*Hordeum vulgare* L. cv. Salome) seeds were surface-sterilized in 0.75% sodium hypochlorite (5 min), rinsed with tap water and germinated in vermiculite.

After 4 d the seedlings were transferred to a split-root system (two roots per compartment) containing a steam-sterilized (40 min, 120°C) mixture of silicate sand, Turface and agricultural soil (v:v:v/1:1:1). After transfer of the barley plants, roots on one side of the split-root system were inoculated with one of the three tested *G. mosseae* strains using the inoculum compartment method of Vierheilig *et al*. (2000a). Briefly, the inoculum compartments contained bean plants (*Phaseolus vulgaris* L. cv. Sun Gold) colonized by one of three *Glomus mosseae* (Nicolson & Gerdemann) Gerd. & Trappe strains identified by their BEG (La Banque Européenne des Glomales; International Institute of Biotechnology; Kent; GB) number: i) BEG 12; isolated in England, ii) BEG 54, isolated in Indonesia and iii) BEG 55, isolated in the Philippines. The inoculum compartments and the split-root compartments are separated by a nylon screen that allows fungal hyphae, but not roots, to pass. Control plants were in contact with an inoculum compartment containing NM bean plants. The plants were grown in a growth chamber (day/night cycle: 16 h (23°C)/8h (19°C), light: 300 µmol m^{-2} s^{-1} PAR, relative humidity: 50%) with no additional mineral fertilization.

3.5.2 $^{14}CO_2$ labelling

Twenty three d after inoculation, the inoculum compartments were removed and the barley shoots were labeled with 37 kBq (1 µCi) $^{14}CO_2$ for 2 h as described in Lerat *et al*. (2003). After a 24-h chase period the two halves of each split-root system were harvested separately and the fresh biomass recorded. Mycorrhizal roots of each plant were sub-sampled, weighed, and used to assess mycorrhizal colonization levels. The rest of the root systems was oven dried, weighed, and used to determine radioactivity levels. Shoots were oven dried and weighed. Dried roots were digested with tissue solubilizer (NCS) at 60°C overnight. Radioactivity was assessed by liquid scintillation spectrometry. Counts were

standardized with a quench curve and expressed in dpm. The presence of radioactivity in the substrate of inoculated and non-inoculated compartments was also assessed after digestion of approximately 1 g of substrate in NCS. The experiment was repeated twice with five replicates per repetition and per treatment giving a total of 10 plants per treatment.

Carbon partitioning results were expressed as percentage of total ^{14}C and as percentage of total root dry biomass in the inoculated and non-inoculated compartments. Corrections were made for root sub-samples used for the determination of M colonization levels. Because dry biomass differed between repetitions, shoot, root and total dry biomass were expressed as a percentage of the mean of the control plants within repetition.

3.5.3 Measurement of root colonization

Mycorrhizal root samples from each inoculated plant and randomly selected NM root samples were cleared by boiling in 10% KOH and stained by boiling in a 3% ink (Shaeffer; black)/vinegar (5% acetic acid) solution (Vierheilig *et al*. 1998; Vierheilig *et al*. 2000b). Stained roots were observed with a light microscope and the percentage of root colonization was determined using the method of Newman (1966).

3.5.4 Statistical analyses

The percentages of radioactivity allocated to each of the M and NM compartments were arcsin transformed. ^{14}C partitioning to the inoculated sides (M compartment) was compared using two-way ANOVA, with *G. mosseae* strain as the treatment factor and repetition in time (block) as the other factor. Shoot, root and total dry biomass were analyzed by two-way ANOVA, with fungal strain as the treatment factor. *A posteriori* comparisons were made using LSD tests.

3.6 Results

Three wk after inoculation, the inoculated sides of the split-root systems of barley were heavily colonized regardless of the *G. mosseae* strain used (Fig. 3.1a). No colonization was observed in non-inoculated root samples.

No radioactivity was detected in the growth substrate (<60 dpm g^{-1} dry soil), therefore, all the data analyses refer to the radioactivity measured in the roots.

^{14}C partitioning was affected by treatment (P=0.01) but not by repetition (P=0.66). Greater amounts of ^{14}C were found in M roots of the BEG 54 treatment compared to the control and BEG 12 treatments, while the partitioning of ^{14}C towards M roots of the BEG 55 treatment was not significantly different from partitioning observed in the other treatments (Fig. 3.1a). A paired *t*-test analysis (results not shown) revealed no significant differences between root dry biomass of M and NM sides (data arcsin-transformed) in any treatment (Fig. 3.1b).

In all fungal treatments total and shoot dry biomass were lower than the control (Table 3.1). The BEG 54 treatment generated significantly smaller plants than the BEG 12 treatment while the BEG 55 plants did not significantly differ in size from the two other fungal treatments. Root dry biomass was unaffected by the BEG 12 treatment, however, the BEG 54 and BEG 55 treatments produced less root dry biomass than the control plants.

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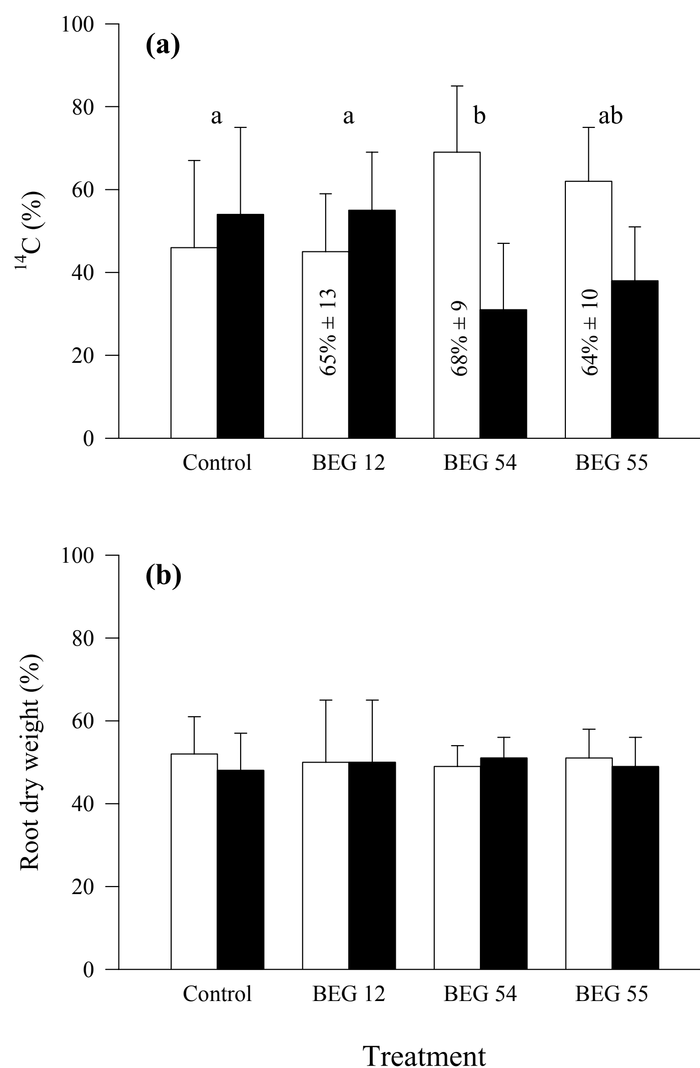

Figure 3.1. (a) Mean total ^{14}C (± 1 SD) partitioning and (b) mean percentage dry biomass (± 1 SD) between M (open bars) and NM (solid bars) barley roots in control and *Glomus mosseae* BEG 12, BEG 54 and BEG 55 treatments. Means are calculated from the sum of the two repetitions. Treatments with the same letter are not statistically different. Mycorrhizal colonization levels (± 1 SD) are shown within M bars (a).

Table 3.1. Dry biomass (± 1 SD) of control and *Glomus mosseae* BEG 12, BEG 54 and BEG 55 treatments expressed as percentage of control. Means are calculated from the sum of the two repetitions. Treatments within a row with the same letter are not statistically different.

Dry biomass	Control	BEG 12	BEG 54	BEG 55
Shoot**	100.0 [a]	74.6 ± 11.3 [b]	59.6 ± 12.0 [b]	72.7 ± 14.4 [b]
Roots*	100.0 [a]	95.7 ± 21.1 [a]	80.8 ± 31.3 [b]	77.5 ± 23.7 [b]
Total**	100.0 [a]	81.3 ± 9.1 [b]	65.3 ± 15.4 [c]	73.4 ± 14.8 [bc]

note: *, $P<0.05$; **, $P<0.001$

3.7 Discussion

While the existence of species-specific differences in the C-sink strength capacity of AMF has been shown (Pearson & Jakobsen 1993; Lerat *et al*. 2003), the present study using three *G. mosseae* strains confirms that differences in C-sink strength may also occur at the intra-specific level. The *G. mosseae* strain BEG 12 showed a low C-sink strength capacity, which confirms the results obtained by Lerat *et al*. (2003). The other two *G. mosseae* strains, BEG 54 and BEG 55, showed C-sink strength capacities similar to those described for two other AMF species, *G. intraradices* and *Gi. rosea* (Lerat *et al*. 2003). The differences observed in the C-sink strength capacity between the *G. mosseae* strains was correlated to differences in plant biomass. While barley plants colonized by BEG 12, the strain with the lowest sink strength capacity, had a similar biomass to the NM controls, plants colonized by strain BEG 54, the strain with the highest sink strength capacity, showed a clear suppression of shoot growth. Previous studies have also demonstrated that plants colonized by certain AMF may show no growth stimulation and may even show a reduction in growth when compared to non-inoculated controls (e.g. Marschner & Crowley 1996; Pozo *et al*. 2002; Lerat *et al*. 2003). This may be linked to the aggressiveness of the fungus with regards to its ability to colonize the root system and to the level of its

requirement for host plant-derived C (Graham & Abbott 2000; Pozo *et al*. 2002). As AMF are obligate biotrophs, depending on host plant C for establishment of the symbiosis and subsequent growth and nutrient uptake (Smith & Read 1997; Vierheilig *et al*. 2002), the measurements of C-sink strength capacity of a given mycobiont has the potential to provide an insight into the metabolic activity (including respiration) and the extraradical hyphal development and spore production of the fungus. Different C-sink strength may explain intra-specific AMF growth differences (Graham *et al*. 1982; Giovannetti & Gianinazzi-Pearson 1994).

The different C-sink strength capacities observed are likely to reflect strain specific genetic variations. Although Lanfranco *et al*. (1995) and Lloyd-Macgilp *et al*. (1996) showed intra-specific genetic variability for a number of AMF, the analysis of ribosomal DNA of various strains of *G. mosseae* has revealed this species to be genetically similar world-wide (Lloyd-Macgilp *et al*. 1996). This suggests that this technique is insufficient to reveal species-specific differences in metabolic capacity. However, recently developed DNA-micro-array techniques will allow the mechanisms underlying the C-sink strength capacities of different AMF to be studied in greater depth.

In conclusion, the present study has shown that the weak C-sink strength of *G. mosseae* BEG 12 reported by Lerat *et al*. (2003) is strain- and not species-specific.

3.8 Conclusion

Les résultats présentés ici montrent que la faible force de puits de C de la souche *G. mosseae* BEG 12 est spécifique à cette souche et non à l'espèce. Ce chapitre confirme la faible capacité de force de puits exercée par BEG 12 sur l'orge. En revanche, les deux autres souches testées, BEG 54 et BEG 55, affichent des forces de puits de C plus élevées que BEG 12 et différentes entre elles. Alors que l'analyse génétique de la sous-unité ribosomale 18S a conduit à la conclusion que cette espèce était identique à travers le monde, nous avons été en mesure de détecter une variabilité intra-spécifique de la demande

en C chez *G. mosseae* révélant l’existence d’un métabolisme différant parmi les souches de ce champignon MA.

CHAPITRE IV

Arbuscular mycorrhizal colonization and spore populations associated with *Acer saccharum* exhibiting either good or poor crown regrowth following ice storm damage

4.1 Avant-propos

Le texte de ce chapitre a été soumis à la revue Canadian Journal of Forest Research (Revue Canadienne de Recherche Forestière) (Lerat, Coughlan, Piché & Lapointe) et une partie de son contenu a été présentée au congrès Mycorhizes 2000 tenu à Rivière-du-Loup. Andrew P. Coughlan a participé à la partie analytique ainsi qu'à la rédaction de ce travail.

4.2 Résumé

Le statut mycorhizien à arbuscules (MA) d'érables à sucre (*Acer saccharum*) matures touchés par une vague de verglas en janvier 1998 dans deux parcelles du sud du Québec a été étudié à l'automne 1999. Deux catégories d'arbres ont été définies selon le mode de reprise de croissance de leur houppier après le verglas: les arbres à bonne reprise de croissance et les arbres à mauvaise reprise de croissance. Le statut MA de 12 arbres de chaque catégorie a été étudié à partir d'échantillons de racines fines et la population de spores MA du sol quantifiée. Le pourcentage de colonisation racinaire par des hyphes internes, des pelotons mycéliens (coïls), des vésicules ainsi que le pourcentage de colonisation totale était plus élevé dans les racines des arbres à mauvaise reprise de croissance. Le pourcentage de colonisation racinaire par des arbuscules ne variait pas selon le niveau de reprise des arbres. L'examen des spores a permis d'élargir les aires de distribution connue de *Glomus arborense* McGee et *G. warcupii* McGee de l'Australie à l'Amérique du Nord. Cependant, aucune différence notable en densité de spores et

composition d'espèces de champignons MA entre les arbres à bonne et mauvaise reprise de croissance n'a été détectée. Bien que la raison exacte de l'état de santé des arbres à mauvaise reprise de croissance reste floue, il est émis l'hypothèse selon laquelle les champignons MA pourraient contribuer à la capacité de reprise de croissance observé.

4.3 Abstract

The arbuscular mycorrhizal (AM) status of mature sugar maple trees (*Acer saccharum*) damaged by an ice storm in January 1998 was investigated in autumn 1999 in two plots in southern Québec. Two categories of trees were defined according to the regrowth patterns of their crown following the ice storm event: good and poor regrowth trees (RT). The AM status of 12 trees within each category was studied from fine root samples and the AM spore population of the soil quantified. Percentage root colonization by internal hyphae, coils, vesicles and total colonization was higher in the roots of poor RT. Percentage root colonization by arbuscules did not differ between good and poor RT. The spore survey allowed us to extend the known ranges of *Glomus arborense* McGee and *G. warcupii* McGee from Australia to North America. However, we detected no significant differences in AM fungi spore density and species composition between good and poor RT. Although the exact reason of the health status of poor RT is unclear, we hypothesize that AM fungi might contribute to the observed regrowth pattern.

4.4 Introduction

The arbuscular mycorrhizal (AM) symbiosis is an association between most terrestrial plants and a class of fungi (Glomeromycota) which occurs in the roots of host plants (Schüssler *et al*. 2001). AM fungi are normally considered to improve plant mineral nutrition (in particular phosphorus (P)), water uptake, and resistance to root pathogens (Smith & Read 1997). In return, the plant supplies the AM fungi with carbohydrates derived from photoassimilation. The fungus being heterotrophic depends on a host-derived C supply. The cost of supporting AM fungi, in terms of photoassimilates, has been estimated in many studies (e.g. Pang & Paul 1980; Kucey & Paul 1982; Snellgrove *et al*. 1982; Koch & Johnson 1984; Douds *et al*. 1988; Wang *et al*. 1989) and several consider

that 4-20% of the total C fixed by host plants may be used by the AM fungi (Bago *et al*. 2000a; Douds *et al*. 2000; Graham 2000).

Sugar maple is symbiotically associated with arbuscular mycorrhizal (AM) fungi (Yawney & Schultz 1990; Cooke *et al*. 1993). To the best of our knowledge, the status of mycorrhizal association in sugar maple has never been studied in a natural disturbance context. Between the 4th and 10th of January 1998, the worst ever ice storm of Canadian history (Statistics Canada 1998) struck the Central and Eastern parts of the country. The province of Québec was the most seriously affected, with freezing rain exceeding 100 mm at certain locations (Irland 1998). The total area of Québec's forests affected by the ice storm reached 1.8 million ha of which 32% (567 737 ha) were heavily damaged (Boulet *et al*. 2000). Hooper *et al*. (2001) estimated that in a sugar maple forest of a heavily damaged region (Mont Saint-Hilaire, southwestern Québec) the ice storm brought down 33.6 m^3 of woody debris (fallen branches) per hectare.

Following the storm, the Quebec Ministry of Natural Resources surveyed the regrowth of mature deciduous trees in permanent plots installed prior to the event. The residual canopy volume of these trees in the plots was assessed during the summers of 1998 and 1999. In the present study, sugar maple trees which showed an increase in crown volume between 1998 and 1999 were categorized as good regrowth trees (RT) and sugar maple trees showing no variation or a decrease of crown volume were categorized as poor RT. Trees of both categories were found randomly distributed across the sites with good and poor RT occurring as close neighbors (personal observation), which excluded any possible micro-environmental differences as an explanation for the observed regrowth responses. Several reasons may lie behind the observed poor regrowth pattern: poor vigor of certain individuals before the ice storm, the intrusion of rust from wounds or subtle genetic differences. However, from another stand point, regrowth (as growth) is a process that requires important carbon (C) resources (Poorter & Villar 1997) and, therefore, limitations in C supply could also explain the occurrence of poor RT. Because different AM fungi may have different C requirements (Lerat *et al*. 2003) and different colonization levels might also translate into different C requirements (Thomson *et al*. 1990; Lerat *et al*. 2003), we

wanted to test whether AM fungi could partly influence the regrowth capacity of sugar maple trees. In the present paper, the AM status of good and poor RT in two plots of southern Québec struck by the 1998 ice storm was investigated. This includes a survey of AM colonization in fine roots and fungal spore production nearby maple roots.

4.5 Materials and methods

4.5.1 Plots and tree selection

The sampled plots are located in the Montérégie region (southern Québec), where the 1998 ice storm damages were at their worst and the ice thickness exceeded 80 mm (Irland 1998). In November 1999, two permanent survey plots set up by the Québec Ministry of Natural Resources in which mature trees had been severely damaged by the ice storm were selected. The two plots (400 m^2) were ca. 1.5 km apart. The proportion of residual crown of the trees in the two plots was visually estimated using interval classes of 10%. The 100% value corresponded to a tree without damage while the 0% corresponded to a tree which had lost its entire crown.

Plot I, located in Saint-Damase (45°30’15’’N, 73°02’50’’W), is managed as a sugarbush and tapped annually. Sugar maple (*Acer saccharum* Marsh.) is the dominant hardwood species in the stand. A few yellow birch trees (*Betula alleghaniensis* Britton) also occur in the plot. The mean diameter at breast height of sugar maple trees studied in Plot I was 320 mm ± 35 (± 1 SD). The soil (pH 5.3) was coarse loamy sand with a thin mull type humic horizon.

Plot II, located in Rougemont (45°29’45’’N, 73°03’40’’W), is a former sugarbush that is no longer tapped. Sugar maple is the dominant hardwood species in the stand. Yellow birch and American beech (*Fagus grandifolia* Ehrh.) occur as companion species (15-20% of mature trees). The mean diameter at breast height of sugar maple trees studied in Plot II was 347 mm ± 112. The soil (pH 5.4) was a medium sandy loam with a thin (3 cm) mull type humic horizon.

Selected trees had been numbered and clearly identified when the plots were installed. Six good RT and six poor RT were chosen in each plot according to their regrowth pattern. Mean crown volume (± 1 SD) was 20% ± 20 in 1998 and 38% ± 23 in 1999 for good RT, and 33% ± 17 in 1998 and 29% ± 16 in 1999 for poor RT.

4.5.2 Sampling

To assess AM fungi colonization, two fine root samples were collected from each maple. To insure that the fine roots were connected to the selected tree, two primary coarse roots radiating from the trunk were partly dug out until the root divided into fine feeder roots. Root samples were bagged with soil to avoid dehydration.

To assess the AM fungal spore population four 1 L, 15-cm deep soil cores (Klironomos *et al*. 1993; Klironomos 1995; Moutoglis & Widden 1996) were taken around each good and poor RT. In order to increase the probability that the collected spores were produced by AM fungi in association with the sampled sugar maple trees, two of the cores were taken at the same place as the fine roots samples. The other two soil cores were collected at 4 m from the sugar maple trunk (Coughlan *et al*. 2000). The latter cores were collected in a zone where there was no other sugar maple trunk within a 4-m distance or where the neighboring tree was an ectomycorrhizal American beech (Plot II). All root and soil samples were stored at 4°C prior to processing (3 to 6 months).

4.5.3 Quantification of mycorrhizal colonization

Fine roots were stained using the method of Koske & Gemma (1989) but modified to include clearing in 10% KOH at 90°C for 60 min (Coughlan *et al*. 2000). Fungal colonization was assessed microscopically according to the grid line intersect method of McGonigle *et al*. (1990). The presence or absence of colonization was noted together with the type of AM feature observed: internal hypha, coil, arbuscule and/or vesicle. Total colonization levels were calculated as the total number of intersects where AM structures were observed/total intersects observed (at least 100 per root sample) (Zahka *et al*. 1995). The mean AM colonization levels of each tree were calculated from the two root samples.

4.5.4 Spore analysis

An homogenized subsample of ca. 100 g of each soil sample (n=96) was used to determine the spore populations. Spores were extracted following the classical wet sieving and decanting method (Gerdemann & Nicolson 1963). A 850-µm mesh sieve was used to discard coarse debris and a nest of five sieves of different mesh size (500-250-106-75-45 µm) were used to retain sporocarps and spores. Final spore extraction was done using sucrose gradient procedure described in Coughlan *et al*. (2000).

Prior to determination of spore abundance, a collection of morphologically different spores was obtained from a large number of the 96 soil samples. Identification of AM taxa was performed with the collaboration of Dr. Yolande Dalpé (ECORC, Ottawa, Canada). Spores were identified to species using both original descriptions and by comparison with the DAOM mycological herbarium and type specimens (Ottawa). Voucher specimens were mounted in polyvinyl alcohol - lactic acid - glycerol (PVLG) (Koske & Tessier 1983) and deposited in the DAOM herbarium.

Spore identification and quantification was done using a dissecting microscope and a compound microscope. All spores were counted and noted. Another soil subsample (about 50 g) was weighed, dried overnight at 60°C, and weighed again to enable spore population to be expressed as the number of spores kg^{-1} dry soil. Mean spore abundance was calculated from the four soil samples collected around each tree.

4.5.5 Statistical analysis

The AM colonization data were analyzed by comparing the means of 12 good RT and 11 poor RT (roots of one tree were not suitable for AM fungi observation). Fungal structures (internal hyphae, coils, arbuscules and vesicles) were first analyzed by two-way MANOVA, with plot and health status of sugar maple trees as main factors, prior to be analyzed individually (with total colonization) by two-way ANOVA. Linear regressions were performed on mycorrhizal colonization levels and diameter at breast height to test for a tree

size effect. Spore abundance data for each species were log-transformed prior to analysis by two-way ANOVA, with plot and health status of sugar maple trees as main factors. The frequency of spores of AM species around the good and poor RT was analyzed by logistic regression.

4.6 Results

4.6.1 Mycorrhizal colonization

The MANOVA revealed that mycorrhizal colonization was affected by tree health (P=0.02) but not by the plot (P=0.76) or the interaction tree health×plot (P=0.39). The percentage of root length occupied by internal hyphae, coils and vesicles and the total root length colonized by AM fungi was significantly higher in poor RT (Fig. 4.1). The percentage of root length occupied by arbuscules was not significantly different between good and poor RT. Vesicles were the most frequently observed AM structures in both tree health categories (Fig. 4.1). Coils were observed much less frequently.

Independently of health status and plot, there was no linear correlation between diameter at breast height and the percentage root length colonized by internal hyphae (r^2=0.04, P=0.51), coils (r^2=0.04, P=0.51), arbuscules (r^2=0.01, P=0.73), vesicles (r^2=0.09, P=0.33) and total colonization (r^2<0.01, P=0.91).

4.6.2 Spore survey

Eleven AM taxa occurred relatively frequently in the soil samples (Fig. 4.2): eight were identify to the species level: *Acaulospora lacunosa* Morton, *Sclerocystis rubiformis* Gerd. & Trappe, *Glomus aggregatum* Schenck & Smith emend. Koske, *Glomus arborense* McGee, *Glomus constrictum* Trappe, *Glomus fasciculatum* (Thaxter sensu Gerd.) Gerd. & Trappe, *Glomus macrocarpum* Tulasne & Tulasne, and *Glomus warcupii* McGee. Three other unidentified *Glomus* species were also observed and noted as *Glomus* sp1, *Glomus* sp2 and *Glomus* sp3. Rarely observed spores of the *Glomus* genus were grouped under

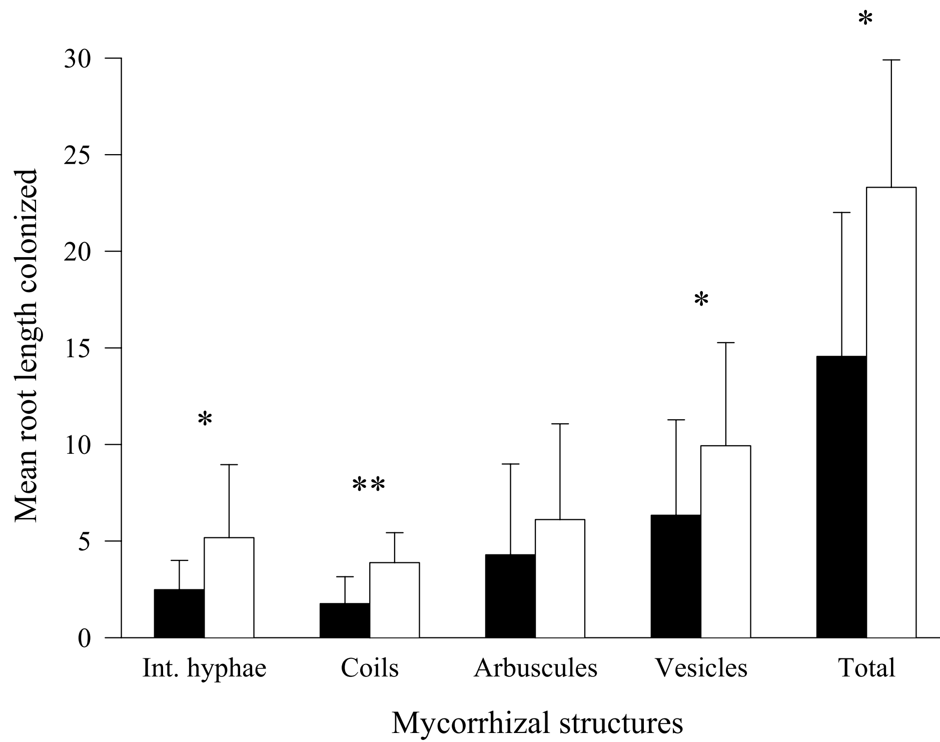

Figure 4.1. Mean percentage (± 1 SD) AM root colonization by internal hyphae, coils, arbuscules and vesicles and percentage total AM root colonization as a function of tree health (good regrowth, solid bars; poor regrowth, open bars; n=23). *, $P<0.05$; **, $P<0.01$.

Glomus spp. The spores of *A. lacunosa* were the most frequent, the most abundant, and the most easily recognizable of the genus *Acaulospora*. Spores from other species within this genus (including *Acaulospora tuberculata* Janos & Trappe) were classified in *Acaulopora* spp.

Spore abundance (log[spores kg^{-1} dry soil]) did not differ significantly ($P \geq 0.08$) with tree health for any of the observed taxa; the spores of two species *A. lacunosa* ($P=0.02$) and *Glomus* sp3 ($P=0.01$) were more abundant in Plot I. However, certain trends were observed. For example, *G. arborense* was never found around poor RT ($P=0.08$), and there seemed to be more spores kg^{-1} dry soil of *A. lacunosa* ($P=0.15$) around good RT and less spores kg^{-1} dry soil of *Glomus* sp3 ($P=0.17$) around poor RT (Fig. 4.2). Logistic regression of the frequency of occurrence of a given AM fungal species in proximity to a tree of a given health category (Fig. 4.3) was not significant ($P>0.07$).

4.7 Discussion

The anatomy of mycorrhizal structures (arbuscules, coils, vesicles and internal hyphae) observed in the roots of sugar maple trees affected by the 1998 ice storm were similar to those described by Cooke *et al*. (1992) and Klironomos (1995) under natural conditions. This suggests that crown damage had no impact on AM fungal morphology structures. However, in our samples the total percentages of AM root colonization were lower (average: 19% ± 8) than those reported for autumn-collected sugar maple roots in other field studies (between 40% and 70%) (Brundrett & Kendrick 1988; Cooke *et al*. 1992; Klironomos *et al*. 1993; Klironomos 1995). In recent studies, Ouimet *et al*. (1995) and Coughlan *et al*. (2000) showed that the intensity of AM colonization in sugar maple appears to be positively correlated to soil pH. However, in similar pH conditions (between pH 5 and pH 6), total AM root colonization measured by Coughlan *et al*. (2000) on 6-month-old sugar maple seedlings was higher (around 40%) than those of the present study indicating that the low AM root colonization we observed were not related to acidic soil pH. Although we have no data on the AM root colonization levels prior to the ice storm,

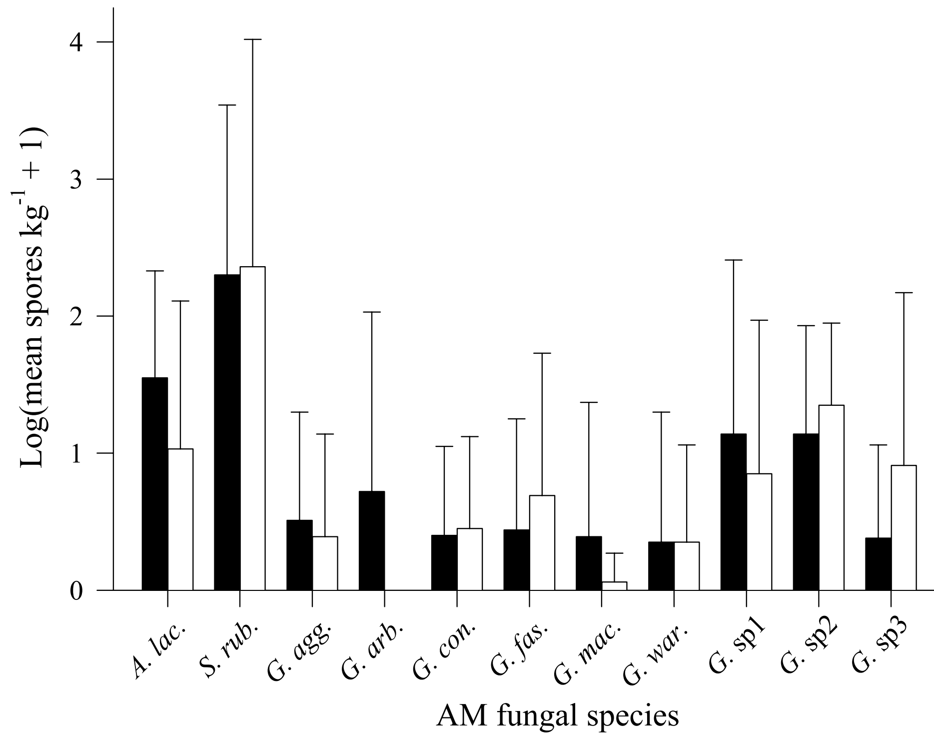

Figure 4.2. Log-transformed mean spore number per kg of dry soil (± 1 SD) for the most frequently observed AM taxa as a function of tree health (good regrowth, solid bars; poor regrowth, open bars). The 11 fungi are *Acaulospora lacunosa* (*A. lac.*), *Sclerocystis rubiformis* (*S. rub.*), *Glomus aggregatum* (*G. agg.*), *G. arborense* (*G. arb.*), *G. constrictum* (*G. con.*), *G. fasciculatum* (*G. fas.*), *G. macrocarpum* (*G. mac.*), *G. warcupii* (*G. war.*), *Glomus* sp1, *Glomus* sp2, and *Glomus* sp3.

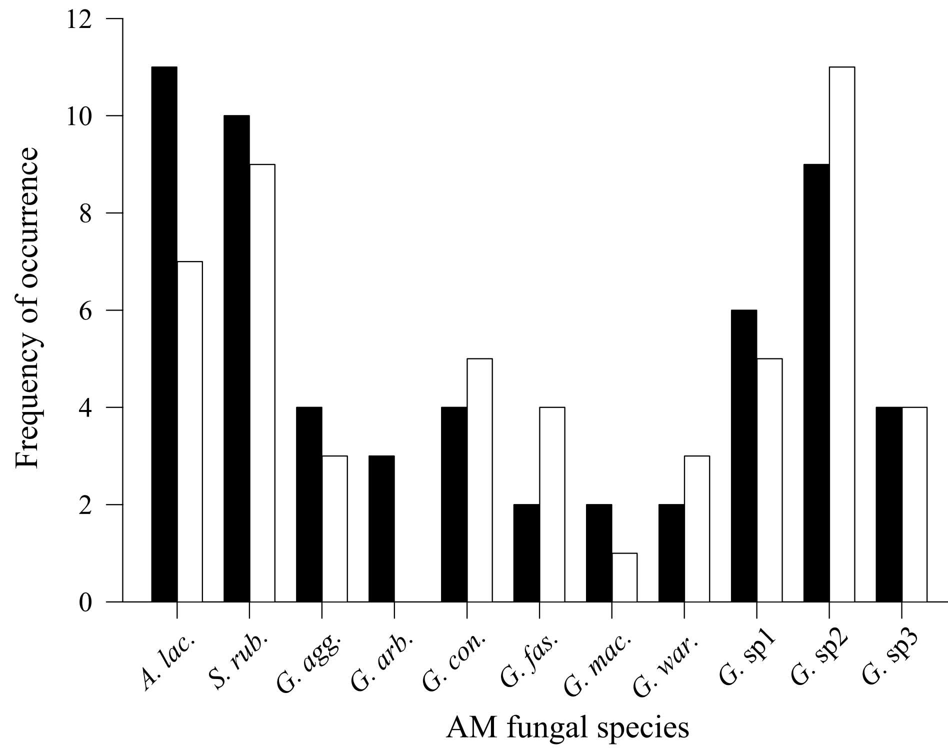

Figure 4.3. Frequency of occurrence of the most frequently observed AM taxa as a function of tree health (good regrowth, solid bars; poor regrowth, open bars). Abbreviations are as in Fig. 4.2.

this tends to suggest that partial crown loss in sugar maple may induce a decrease in AM colonization as observed by Spitko *et al*. (1978) in sugar maple decline. Furthermore, many studies have shown that clipping and defoliation have negative effects on the development of AM fungi (e.g. Daft & El-Giahmi 1978; Same *et al*. 1983; Allsopp 1998) and that development of AM fungi in the roots can be correlated to C supply (Thomson *et al*. 1990). Thus, the low AM root colonization of the sugar maple trees struck by the ice storm was probably due to a diminution of the quantity of C available to the AM fungi caused by the destruction of an important volume of their crown.

Differences in mycorrhizal colonization patterns were detected between good and poor RT independently of plot factor and diameter at breast height (tree size). This is inconsistent with Ouimet *et al*. (1995) who stated that the health of (declining) sugar maples was not related to root colonization. However, Cooke *et al*. (1993) suggested a possible relationship between sugar maple health and condition of the mycorrhizal association. In the present study, poor RT root samples showed higher percentage occurrence of vesicles, coils and internal hyphae than good RT. However, there was no significant variation in the percentage of arbuscules between good and poor RT. This contrasts with the studies of Spitko *et al*. (1978), Cooke *et al*. (1992) and Duckmanton & Widden (1994) where fewer arbuscules were observed in stressed sugar maples. Present results indicate that mycorrhizal symbiosis in both good and poor RT was functional as arbuscules are widely considered to be the site of transfer of mineral nutrients from AM fungi toward plants (Smith & Read 1997). However, the exact localization of the site(s) of C transfer from the plant to the fungus is less clear. In the *Paris*-type mycorrhiza formed by sugar maple, coils are thought to contribute to C transfer between symbionts (Klironomos 1995; Smith & Read 1997). Thus, the increased percentages of coils in the roots of poor RT could reflect a more elevated C transfer from poor RT to AM fungi. Increased percentage root length colonized by AM coils has been also reported in ozone-stressed sugar maples (Duckmanton & Widden 1994).

In the present study, the level of vesicle differed between the two categories of trees and were higher in poor RT. An increase in the number of vesicles has also been reported in

sugar maples in response to diverse sources of stress including crown decline (Spitko *et al*. 1978; Cooke *et al*. 1992), base cation amendment (Cooke *et al*. 1993), ozone exposure (Duckmanton & Widden 1994) and harsh soil conditions (Klironomos 1995). Increased vesicle production in poor RT could partly be explained by one or both of two hypotheses. The first one is that good and poor RT differ in the composition of the AM species colonizing the majority of the root system. The cost of the AM symbiosis has been shown to fluctuate with the AM fungal species involved in the symbiosis (Lerat *et al*. 2003) and can reach 20% of total plant photosynthates (Wang *et al*. 1989). Also, it is generally accepted that vesicles are C storage organs and are probably costly to produce in terms of C. Could poor RT have been associated with 'expansive' fungi which drained significantly more carbohydrates for the production of larger quantities of vesicles than good RT? If so, this would have deprived poor RT from relatively important C resources. The second hypothesis is that poor RT exhibited a poorer health status for reasons not related to AM fungi. Several authors (Biermann & Linderman 1983; Bonfante-Fasolo 1984) suggested that vesicles function as propagules and have a strong inoculum potential. In such a case, an AM fungus harbored by a poor RT, may have responded to those more severe stressful conditions and invested in reproduction by producing more vesicles. In either case, this higher investment into vesicles reduced the amount of C available for crown regrowth and could contribute to maintain poor RT in their lower health status.

Despite the low AM root colonization, eleven AM fungal taxa were identified in the spore survey. The sampling period (early November) was appropriate to a spore survey since AM spore populations in sugar maple forests peak in the autumn (Klironomos *et al*. 1993; Moutoglis & Widden 1996). After a review of the literature (Klironomos *et al*. 1993; Moutoglis *et al*. 1995; Zahka *et al*. 1995; Moutoglis & Widden 1996; Coughlan *et al*. 2000) the AM species diversity of the studied soils appears to be high and is only lower than in Coughlan *et al*. (2000). Also, four AM fungal species were formally recorded for the first time in a sugar maple forest soil: *A. lacunosa*, *A. tuberculata*, *G. arborense* and *G. warcupii*. The list of AM species living in sugar maple stands is probably far from exhaustive since this tree occupies a wide diversity of sites (Godman *et al*. 1990) and only a few surveys have been published yet. Furthermore, the presence of certain AM fungal

species is not necessarily revealed by spore counts because some species only sporulate under particular conditions (Coughlan *et al*. 2000), some do not necessarily sporulate in the autumn (Koske *et al*. 1997) and some others seem not to produce spores at all (McGee 1989). The species *G. arborense* and *G. warcupii* are newly recorded in North America. These two species were originally described from Australia, both associated to forest plants (McGee 1986). However, sporocarps of *G. warcupii* were not observed. *A. lacunosa* was the most frequently observed AM fungal species in the 96 soil samples. This species was originally described from high aluminum and low pH soil (Morton 1986). According to previous maple forest surveys, *Acaulospora* species seem to be well adapted to acidic sugar maple soils (Klironomos *et al*. 1993; Moutoglis & Widden 1996). The three relatively frequent taxa *Glomus* sp1, *Glomus* sp2 and *Glomus* sp3 remained unidentified and may belong to new taxa.

The spore population study did not allow the detection of differences in the occurrence of any of the AM fungal species between good and poor RT. Mature sugar maples are the dominant AM plants of sugar maple forests but AM understory species also produce important spore populations (Merryweather & Fitter 1998) and isolated AM species may be preferably associated with the herbaceous species living in this habitat. In an attempt to partly overcome this problem two of the soil samples were collected from the rhizosphere of the sampled fine roots. Moutoglis *et al*. (1995) directly observed spores attached to sugar maple roots. However, this method was not relevant for our study, in which several AM fungal species (e.g. the *Acaulospora* genus) produce mature spores which detach from their suspensor hyphae. The presence or absence of spores did not reveal differences in the presumed composition of the AM population of good and poor RT. This could be explained by the low number of trees sampled (n=24). Furthermore, for the reasons given above, the observation of spores only confirms the presence of a species that has sporulated. Molecular techniques may help to identify non-sporulating species. Simon *et al*. (1992) were the first to develop such an approach to identify AM fungi within roots of host plants. However, the use of PCR-based methods on field-collected material has been restricted to a limited number of studies (e.g. Clapp *et al*. 1995; Helgason *et al*. 1999). Also, the primer

sequences used in the latter studies may not be appropriate for the detection of all AM fungal species (Redecker 2000).

In conclusion, this paper highlights certain differences in mycorrhizal condition in sugar maple trees struck by the 1998 ice storm and showing different regrowth patterns. Although the exact cause of a higher mycorrhizal colonization in poor RT remains unknown, the results suggest that AM fungi might contribute to the poor regrowth status of these trees. This study supports the importance of considering AM fungal morphology and not only the total colonization status when studying AM relationship with tree health. The spore survey data did not allow us to confirm our hypotheses that good and poor RT have different AM fungal populations. However, it led to the discovery of two AM fungal species previously recorded only in Australia and the discovery of three as yet unnamed *Glomus* spp.

4.8 Conclusion

Ce chapitre met en évidence des différences dans la condition mycorhizienne d'érables à sucre touchés par le verglas de 1998 et montrant différents patrons de reprise de croissance. Bien que la cause exacte de taux de colonisation mycorhizienne plus élevés chez les arbres à mauvaise reprise de croissance demeure irrésolue, les résultats suggèrent que les champignons MA aient pu contribuer au mauvais état de santé observé chez ces arbres. Cette étude soutient l'importance de tenir compte de la morphologie des structures MA lorsque l'on étudie la santé d'arbres en relation avec les champignons MA et non pas uniquement le degré de colonisation racinaire totale. L'analyse des spores n'a pas permis de confirmer notre hypothèse de départ selon laquelle les arbres à bonne et à mauvaise reprise de croissance avaient différentes populations de champignons MA. Néanmoins, ceci a mené à la découverte de deux espèces fongiques MA auparavant signalées uniquement en Australie ainsi que trois espèces de *Glomus* n'ayant pas été décrites à ce jour.

Conclusion générale

L'utilisation d'une espèce ligneuse pérenne telle que l'érable à sucre, couplée à l'emploi d'espèces herbacées (l'érythrone d'Amérique et l'orge), a permis d'étudier divers aspects des relations source/puits de C dans la symbiose MA en se positionnant à différents niveaux d'intégration. L'échelle la plus fine se place au niveau des systèmes racinaires de plantules d'érable à sucre et de plants d'orge, alors que l'échelle la plus élevée se situe au niveau de l'écologie d'érables à sucre matures. En ajoutant, à un degré intermédiaire, l'écophysiologie de juvéniles d'érables à sucre sur le terrain, il a été possible d'embrasser une vue d'ensemble des relations source/puits de C de la symbiose mycorhizienne de l'érable à sucre.

Dans le premier chapitre, les relations source/puits de C ont été étudiées dans un contexte particulier et novateur. Pour la première fois, une étude a pu mettre en évidence l'existence de transferts de C entre plantes connectées par des champignons MA en conditions naturelles. Contrairement à toutes les études réalisées auparavant dans ce domaine, la relation source/puits gouvernant la force et le sens des transferts de C n'avait pas d'origine artificielle comme l'ombrage ou la suppression du feuillage de la plante destinée à être receveuse. Dans notre étude, la force génératrice de transfert a été l'utilisation de deux espèces végétales aux phénologies distinctes, l'érable à sucre sous sa forme juvénile et l'érythrone d'Amérique. C'est la formation d'organes chez ces espèces à un moment précis de l'année qui crée ici le besoin en C.

Il est plausible qu'une érablière, ou tout autre écosystème, soit le théâtre d'autres relations source/puits calquées sur ce modèle. En théorie, des juvéniles d'érable à sucre vivant dans l'ombre des sous-bois pourraient également recevoir du C de la part de leurs géniteurs, c'est-à-dire les érables à sucre adultes occupant l'étage supérieur de la canopée, et ce, tout au long de la saison de croissance. De telles extrapolations expliqueraient en partie comment les érables à sucre juvéniles supportent pendant de nombreuses années les conditions de lumière limitantes régnant au niveau de leur étage ou pourquoi certaines espèces semblent cohabiter de façon préférentielle. Cependant, les quantités de C

transférées entre plantes demeuraient faibles ce qui invite en conséquence à adopter une position prudente quant à de telles spéculations.

Si l’on se place au niveau de l’écosystème, le champignon MA agit comme une sorte de canalisation reliant deux plantes par laquelle transite du C. Il se voit alors réduit au simple rôle d’intermédiaire ou de médiateur. Par contre, d’un point de vue mycocentrique, nous sommes en réalité en présence d’un système composé de deux relations source/puits: une première relation plante donneuse/champignon et une seconde relation champignon/plante receveuse. Ces relations se situent certes à deux échelles différentes, les volumes de C transférés dans la première relation étant nettement supérieurs à ceux de la seconde. Il est cependant intéressant de signaler que dans un tel dispositif le champignon joue un double rôle: celui de puits, quand il reçoit du C en provenance de la plante donneuse, et celui de source, lorsqu’il en fournit à la plante receveuse.

À la suite des expériences réalisées au printemps, il est apparu que la magnitude du transfert de C à partir des érythrones était corrélée au niveau de stockage en amidon de leur organe de réserve, le corme. En d’autres termes, il semble que l’érythrone ne délivre du C aux juvéniles d’érable à sucre par le biais de champignons MA qu’une fois le remplissage du corme complété. Cela signifie que l’érythrone utilise en priorité le C qu’il a fixé pour combler ses propres puits avant d’en exporter l’excédent aux champignons MA et indirectement aux juvéniles d’érable à sucre. D’un point de vue physiologique, il semble logique que le remplissage des puits de C se fasse par ordre de force décroissant. Les racines d’érythrone étant développées et mycorhizées à l’automne (Lapointe & Molard 1997), leur demande en C au printemps est relativement faible. Le puits que représente le remplissage des réserves d’amidon de l’érythrone est par conséquent nettement plus fort que celui constitué par les racines mycorhizées et le C est ainsi majoritairement dirigé vers le corme plutôt que vers les racines. Il s'avérerait donc ici que le champignon MA, contrairement aux champignons pathogènes ou parasites, n’exerce aucun contrôle direct sur le volume de C qui lui est alloué mais dépend strictement de ce que la plante hôte est en mesure de lui procurer. Ainsi, la quantité de C perçue par le champignon MA n’obéirait qu’à la simple loi de l’offre et de la demande.

Cependant, il existerait des champignons parvenant à mobiliser des quantités de C considérables au détriment de leur plante hôte. Des exemples où des plantes mycorhizées affichent des taux de croissance inférieurs à ceux de plantes non mycorhizées sont fréquents dans la littérature. Ainsi, les deux chapitres suivants ont été consacrés à vérifier si la loi de l'offre et de la demande exposée précédemment était universelle dans la symbiose MA ou si cette loi pouvait être modifiée en fonction des partenaires mycorhiziens. Ici encore, le choix de la méthode à employer est apparu essentiel car la volonté de combiner plusieurs espèces (ou souches) fongiques et plusieurs espèces végétales rendait l'expérimentation passablement lourde. De plus, la comparaison de plantes mycorhizées et non mycorhizées, dont le statut nutritionnel peut différer, devait être écartée. Le système à racines dédoublées («split-root») s'est montré être l'outil adéquat à de telles études car il autorise la comparaison de racines mycorhizées et de racines non mycorhizées au sein d'une même plante. Aussi, grâce au faible volume qu'il occupe, le système «split-root» permet de travailler avec de nombreuses plantes dans un espace restreint. L'extension de l'utilisation de cet outil pourrait faciliter l'étude de nombreux aspects de la physiologie des mycorhizes.

Dans un premier temps, les travaux réalisés avec le système en «split-root» ont montré que la force de puits de C des champignons MA n'est pas la même d'une espèce à l'autre. *Gigaspora rosea* est apparue comme ayant une forte capacité de puits de C aux premières étapes de la colonisation avec les deux espèces végétales testées (l'érable à sucre et l'orge) alors qu'à l'opposé, des racines mycorhizées par *Glomus mosseae* ne puisaient pas plus de C que des racines non mycorhizées. Le troisième champignon MA testé, *Glomus intraradices*, s'est avéré particulièrement intéressant car sa force de puits variait avec l'espèce végétale hôte. Une telle variabilité indique que toute généralisation portant sur le coût en C des champignons MA est difficile. Il est concevable que certains champignons ont évolué afin d'exploiter de façon optimale les ressources carbonées des plantes indigènes à leur environnement. D'un autre côté, on pourrait tenir le raisonnement inverse et supposer que ce sont les plantes qui, en cherchant à optimiser leur bilan énergétique, se sont adaptées à contenir au mieux les champignons MA avec lesquelles elles vivaient en symbiose. On

est certainement plus proche de la réalité en suggérant que plantes et champignons ont évolué ensemble au sein d'écosystèmes, développant une phénologie complémentaire pour atteindre finalement une juste combinaison des contrôles de l'utilisation du C.

La capacité d'attraction du C semble avoir une répercussion sur la plante, à tout le moins au cours des étapes précoces de la mycorhization. Par exemple, chez les plants d'orge mycorhizés avec la souche fongique BEG 54 de *Glomus mosseae* (3ème chapitre), la masse sèche des parties aériennes était plus faible que chez les plants des autres traitements. Il semble donc que ce champignon est parvenu à dériver d'importantes quantités de C au détriment de sa plante hôte. Ceci confirme que dans une telle association la plante exerce peu de contrôle sur la quantité de C qu'elle fournit au champignon. Ces résultats sont contradictoires des interprétations faites dans le premier chapitre où les érythrones semblaient contrôler l'alimentation en C de leurs champignons MA. Or, dans le cas en question, on travaillait avec des plantes dans leur milieu d'origine, elles étaient en association avec des champignons MA indigènes. Il serait par conséquent intéressant de tester cette souche sur des espèces natives de l'endroit exact où elle a été isolée afin de vérifier si ces plantes sont capable de maîtriser la force de puits apparente d'un tel champignon.

Les travaux réalisés sur des érables à sucre adultes en situation de perturbation naturelle ont mis en jeu des plantes hôtes et leurs champignons MA indigènes. Chez les érables à sucre affichant une mauvaise reprise de croissance, les taux de mycorhization étaient plus élevés que chez les arbres à bonne reprise de croissance. Au cours des expériences réalisées à l'aide du système «split-root» la force de puits de C était corrélée aux taux de colonisation racinaire chez les deux champignons MA s'étant révélés être des puits forts avec l'orge, *Gi. rosea* et *G. intraradices*. Des taux de mycorhization plus élevés chez les érables à sucre à mauvaise reprise de croissance pourraient refléter une mobilisation plus importante des réserves carbonées par les partenaires fongiques et ce, chez des individus dont le houppier semble déjà en difficulté de régénération. Notre hypothèse suggérant que ces arbres soient en symbiose avec des champignons différents de ceux des arbres à bonne reprise de croissance va à l'encontre des propositions formulées précédemment voulant que des

champignons MA et des plantes indigènes à un milieu donné aient trouvé un équilibre symbiotique quasiment parfait. Or, l'analyse taxinomique des populations de spores de champignons MA récoltées autour de ces arbres n'a pas permis de détecter des différences de composition d'espèces entre nos deux catégories d'arbres. Il est donc préférable de conclure que ces arbres sont colonisés par les mêmes champignons et que seuls les niveaux de colonisation racinaires diffèrent entre les deux catégories d'érables à sucre. Les champignons MA sont sensibles aux hormones végétales (Vierheilig & Piché 2002) et sont donc capables de détecter un certain niveau de stress. Les partenaires fongiques des érables à faible reprise de croissance auraient donc perçu le mauvais état de santé de ces arbres et auraient tiré au maximum profit des ressources carbonées de leurs hôtes avant que ceux-ci ne s'affaiblissent encore d'avantage. Cependant, dans l'un ou l'autre de ces cas, il apparaît que dans certaines conditions naturelles, contrairement à ce qui a été proposé au vu des résultats du transfert de C, les champignons MA sont capables de déjouer le contrôle exercé par la plante sur les quantités de C qu'elle leur délivre.

Tous ces travaux ont montré que les relations source/puits de C dans la symbiose mycorhizienne sont des processus physiologiques complexes et variables dans le temps. L'un des paramètres importants gérant ce type de relation est le facteur espèce, voire souche, aussi bien au niveau végétal qu'au niveau fongique. Il est également apparu que le développement de certains champignons MA est étroitement corrélé à la quantité de C qui leur est allouée. L'érable à sucre est une espèce ligneuse qui au cours de sa vie rencontre différents niveaux de lumière (du sol jusqu'au plus haut de la canopée) se reflétant par différents niveaux de nutrition carbonée. Il serait donc intéressant d'étudier s'il existe chez l'érable à sucre une succession dans la composition en espèces des champignons MA au cours de sa croissance, depuis le stade plantule jusqu'au stade arbre dominant, en passant par un stade intermédiaire. Les techniques de la biologie moléculaire sont aujourd'hui suffisamment avancées pour se présenter comme étant l'outil idéal qui permettra de répondre à cette question. L'autre élément essentiel qui ressort de ces recherches est l'existence en conditions naturelles de transferts de C entre plantes connectées par des champignons MA. Ce point mériterait d'être étudié plus en profondeur afin de sonder l'étendue de ce type de transferts chez les plantes partageant un même habitat et, plus

particulièrement, il serait intéressant de vérifier si des arbres adultes vivant en pleine lumière peuvent alimenter en C les plantes des sous-étages et ainsi participer au maintien et au renouvellement des écosystèmes.

Bibliographie

Allsopp N. (1998). Effect of defoliation on the arbuscular mycorrhizas of three perennial pasture and rangeland grasses. *Plant and Soil* **202**: 117-124.

Arnebrant K., Ek H., Finlay R.D. & Söderström B. (1993). Nitrogen translocation between *Alnus glutinosa* (L.) Gaertn. Seedlings inoculated with *Frankia* sp. and *Pinus contorta* Doug. Ex Loud seedlings connected by a common ectomycorrhizal mycelium. *New Phytologist* **124:** 213-242.

Bago B., Pfeffer P.E. & Shachar-Hill Y. (2000a). Carbon metabolism and transport in arbuscular mycorrhizas. *Plant Physiology* **124:** 949-957.

Bago B., Shachar-Hill Y. & Pfeffer P.E. (2000b). Dissecting carbon pathways in arbuscular mycorrhizas with NMR spectroscopy. *Dans*: Current advances in mycorrhizae research. Podila G.K., Douds D.D., eds. APS Press, St Paul, Minnesota, USA. pp. 111-126.

Bécard G., Doner L.W., Rolin D.B., Douds D.D. & Pfeffer P.E. (1991). Identification and quantification of trehalose in vesicular-arbuscular mycorrhizal fungi by *in vivo* ^{13}C NMR and HPLC analyses. *New Phytologist* **118:** 547-552.

Bethlenfalvay G.J., Reyes-Solis M.G., Camel S.B. & Ferrera-Cerrato R. (1991). Nutrient transfer between the root zones of soybean and maize plants connected by a common mycorrhizal mycelium. *Physiologia Plantarum* **82:** 423-432.

Bidartondo M.I., Redecker D., Hijri I., Wiemken A., Bruns T.D., Dominguez L., Sersìc A., Leake J.R. & Read D.J. (2002). Epiparasitic plants specialized on arbuscular mycorrhizal fungi. *Nature* **419:** 389-392.

Biermann B. & Linderman R.G. (1983). Use of vesicular-arbuscular mycorrhizal roots, intraradical vesicles and extraradical vesicles as inoculum. *New Phytologist* **95:** 97-105.

Bonfante-Fasolo P. (1984). Anatomy and morphology of VA Mycorrhizae. *Dans*: VA mycorrhiza. CRC Press, Boca Raton, Florida. pp. 5-33.

Boulet B., Trottier F. & Roy G. (2000). L'aménagement des peuplements forestiers touchés par le verglas. Gouvernement du Québec, Ministère des Ressources naturelles. Code de diffusion: 2000-30.69.

Boyetchko S.M. & Tewari J.P. (1995). Susceptibility of barley cultivars to vesicular-arbuscular mycorrhizal fungi. *Canadian Journal of Plant Science* **75:** 269-275.

Brownlee C., Duddridge J.A., Malibari A. & Read D.J. (1983). The structure and function of mycelial systems of ectomycorrhizal roots with special reference to their role in forming inter-plant connections and providing pathways for assimilate and water transport. *Plant and Soil* **71:** 433-443.

Brundrett M.C. & Kendrick B. (1988). The mycorrhizal status, root anatomy, and phenology of plants in a sugar maple forest. *Canadian Journal of Botany* **66:** 1153-1173.

Brundrett M.C. & Kendrick B. (1990a). The roots and mycorrhizas of herbaceous woodland plants. I. Quantitative aspects of morphology. *New Phytologist* **114:** 457-468.

Brundrett M.C. & Kendrick B. (1990b). The roots and mycorrhizas of herbaceous woodland plants. II. Structural aspects of morphology. *New Phytologist* **114:** 469-479.

Brundrett M.C., Piché Y. & Peterson R.L. (1984). A new method for observing the morphology of vesicular-arbuscular mycorrhizae. *Canadian Journal of Botany* **63:** 2128-2134.

Bücking H. & Heyser W. (2001). Microautoradiographic localization of phosphate and carbohydrates in mycorrhizal roots of *Populus tremula* × *Populus alba* and the implications for transfer processes in ectomycorrhizal associations. *Tree Physiology* **21:** 101-107.

Burleigh S.H., Cavagnaro T. & Jakobsen I. (2002). Functional diversity of arbuscular mycorrhizas extends to the expression of plant genes involved in P nutrition. *Journal of Experimental Botany* **53:** 1593-1601.

Canham C.D. (1988). Growth and canopy architecture of shade-tolerant trees: response to canopy gaps. *Ecology* **69:** 786-795.

Clapp J.P., Young J.P.W., Merryweather J. & Fitter A.H. (1995). Diversity of fungal symbionts in arbuscular mycorrhizas from a natural community. *New Phytologist* **130:** 259-265.

Clifford P.E., Marshall C. & Sagar G.R. (1973). An examination of the value of ^{14}C-urea as a source of $^{14}CO_2$ for studies of assimilate distribution. *Annals of Botany* **37:** 37-44.

Cooke M.A., Widden P. & O'Halloran I. (1992). Morphology, incidence and fertilization effects on the vesicular-arbuscular mycorrhizae of *Acer saccharum* in a Quebec hardwood forest. *Mycologia* **84:** 422-430.

Cooke M.A., Widden P. & O'Halloran I. (1993). Development of vesicular-arbuscular mycorrhizae in sugar maple (*Acer saccharum*) and effects of base-cation amendment on vesicle and arbuscule formation. *Canadian Journal of Botany* **71:** 1421-1426.

Coughlan A.P., Dalpé Y., Lapointe L. & Piché Y. (2000). Soil pH-induced changes in root colonization, diversity, and reproduction of symbiotic arbuscular mycorrhizal fungi from healthy and declining maple forests. *Canadian Journal of Forest Research* **30:** 1543-1554.

Cox G. & Tinker P.B. (1976). Translocation and transfer of nutriments in vesicular-arbuscular mycorrhizas. I. The arbuscule and phosphorus transfer: a quantitative ultrastructural study. *New Phytologist* **77:** 371-378.

Daft M.J. & El-Giahmi A.A. (1978). Effect of arbuscular mycorrhiza on plant growth. VIII. Effects of defoliation and light on selected hosts. *New Phytologist* **80:** 365-372.

Dehne H.W. (1982). Interactions between vesicular-arbuscular mycorrhizal fungi and plant pathogens. *Phytopathology* **72:** 1115-1119.

Dexheimer J., Marx C., Gianinazzi-Pearson V. & Gianinazzi S. (1985). Ultracytological studies on plasmalemma formations produced by host and fungus in vesicular-arbuscular mycorrhizae. *Cytologia* **50:** 461-471.

Dodd J.C., Boddington C.L., Rodriguez A., Gonzalez-Chavez C. & Mansur I. (2000). Mycelium of arbuscular mycorrhizal fungi (AMF) from different genera: form, function and detection. *Plant and Soil* **226:** 131-151.

Douds D.D., Johnson C.R. & Koch K.E. (1988). Carbon cost of the fungal symbiont relative to net leaf P accumulation in a split-root VA mycorrhizal symbiosis. *Plant Physiology* **86:** 491-496.

Douds D.D., Pfeffer P.E. & Shachar-Hill Y. (2000). Carbon partitioning, cost, and metabolism of arbuscular mycorrhizas. *Dans*: Arbuscular mycorrhizas: physiology and function. Kapulnik Y., Douds D.D., eds. Kluwer Academic Publishers, Dordrecht, The Netherlands. pp. 107-129.

Duckmanton L. & Widden P. (1994). Effect of ozone on the development of vesicular-arbuscular mycorrhizae in sugar maple saplings. *Mycologia* **86:** 181-186.

Duddridge J.A., Malibari A. & Read D.J. (1980). Structure and function of mycorrhizal rhizomorphs with special reference to their role in water transport. *Nature* **287:** 834-836.

Ellsworth D.S. & Reich P.B. (1992). Leaf mass per area, nitrogen content and photosynthetic carbon gain in *Acer saccharum* seedlings in contrasting forest light environments. *Functional Ecology* **6:** 423-435.

Finlay R.D. & Read D.J. (1986). The structure and function of the vegetative mycelium of ectomycorrhizal plants. I. Translocation of ^{14}C-labelled carbon between plants interconnected by a common mycelium. *New Phytologist* **103:** 143-156.

Francis R. & Read D.J. (1984). Direct transfer of carbon between plants connected by vesicular-arbuscular mycorrhizal mycelium. *Nature* **307:** 53-56.

Frey B. & Shüepp H. (1992). Transfer of symbiotically fixed nitrogen from berseem (*Trifolium alexandrium* L.) to maize via vesicular-arbuscular mycorrhizal hyphae. *New Phytologist* **122:** 447-454.

Gallaud I. (1905). Études sur les mycorhizes endotrophes. *Revue Générale de Botanique* **17:** 5-48, 66-83, 123-135, 223-239, 313-325, 425-433, 479-500.

Gerdemann J.W. & Nicolson T.H. (1963). Spores of mycorrhizal endogone species extracted from soil by wet sieving and decanting. *Transactions of the British Mycological Society* **46:** 235-244.

Gianinazzi S., Gianinazzi-Pearson V. & Dexheimer J. (1979). Enzymatic studies on the metabolism of vesicular-arbuscular mycorrhiza. III. Ultrastructure localisation of acid and alkaline phosphate in onion roots infected with *Glomus mosseae* (Nicol. & Gerd.). *New Phytologist* **82:** 127-132.

Giovannetti M. & Gianinazzi-Pearson V. (1994). Biodiversity in arbuscular mycorrhizal fungi. *Mycological Research* **98:** 705-715.

Godman R.M., Yawney H.W. & Tubbs C.H. (1990). *Acer saccharum* Marsh. Sugar maple. *Dans*: Silvics of North America. U.S. Department of Agriculture. Agriculture handbook, Washington DC, USA. pp. 78-91.

Graham J.H. (2000). Assessing costs of arbuscular mycorrhizal symbiosis in agroecosystems. *Dans*: Current advances in mycorrhizae research. Podila G.K., Douds D.D., eds. APS Press, St. Paul, USA. pp. 127-140.

Graham J.H. & Abbott L.K. (2000). Wheat responses to aggressive and non-aggressive arbuscular mycorrhizal fungi. *Plant and Soil* **220:** 207-218.

Graham J.H., Linderman R.G. & Menge J.A. (1982). Development of external hyphae by different isolates of mycorrhizal *Glomus* spp. in relation to root colonization and growth of troyer citrange. *New Phytologist* **91:** 183-189.

Graves J.D., Watkins N.K., Fitter A.H., Robinson D. & Scrimgeour C. (1997). Intraspecific transfer of carbon between plants linked by a common mycorrhizal network. *Plant and Soil* **192:** 153-159.

Harley J.L. & Smith S.E. (1983). Mycorrhizal symbiosis. Academic Press, London.

Hart M.M. & Reader R.J. (2002). Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. *New Phytologist* **153:** 335-344.

Hayman D.S. (1983). The physiology of vesicular-arbuscular endomycorrhizal symbiosis. *Canadian Journal of Botany* **61:** 944-963.

Helgason T., Fitter A.H. & Young J.P.W. (1999). Molecular diversity of arbuscular mycorrhizal fungi colonising *Hyacinthoides non-scripta* (bluebell) in a seminatural woodland. *Molecular Ecology* **8:** 659-666.

Herold A. (1980). Regulation of photosynthesis by sink activity - the missing link. *New Phytologist* **86:** 131-144.

Ho I. & Trappe J.M. (1973). Translocation of ^{14}C from *Festuca* plants to their endomycorrhizal fungi. *Nature* **244:** 30-31.

Hooper M.C., Arii K. & Lechowicz M.J. (2001). Impact of a major ice storm on an old-growth hardwood forest. *Canadian Journal of Botany* **79:** 70-75.

Irland L.C. (1998). Ice storm 1998 and the forests of the Northeast: a preliminary assessment. *Journal of Forestry* **96:** 32-40.

Jabaji-Hare S., Deschêne A. & Kendrick B. (1984). Lipid content and composition of vesicles of a vesicular-arbuscular mycorrhizal fungus. *Mycologia* **76:** 1024-1030.

Klironomos J.N. (1995). Arbuscular mycorrhizae of *Acer saccharum* in different soil types. *Canadian Journal of Botany* **73:** 1824-1830.

Klironomos J.N., Moutoglis P., Kendrick B. & Widden P. (1993). A comparison of spatial heterogeneity of vesicular-arbuscular mycorrhizal fungi in two maple-forest soils. *Canadian Journal of Botany* **71:** 1472-1480.

Koch K.E. & Johnson C.R. (1984). Photosynthate partitioning in split-root citrus seedlings with mycorrhizal and non-mycorrhizal root systems. *Plant Physiology* **75:** 26-30.

Koske R.E. & Gemma J.N. (1989). A modified procedure for staining roots to detect VA mycorrhizas. *Mycological Research* **92:** 486-488.

Koske R.E. & Tessier B. (1983). A convenient permanent slide mounting medium. *Mycological Society of America Newsletter* **34:** 59.

Koske R.E., Gemma J.N. & Jackson N. (1997). Mycorrhizal fungi associated with three species of turfgrass. *Canadian Journal of Botany* **75:** 320-332.

Kucey R.M.N. & Paul E.A. (1982). Carbon flow, photosynthesis, and N_2 fixation in mycorrhizal and nodulated faba beans (*Vicia faba* L.). *Soil Biology and Biochemistry* **14:** 407-412.

Küppers M. (1984). Carbon relations and competition between woody species in a Central European hedgerow. I. Photosynthetic characteristics. *Oecologia* **64:** 332-343.

Lanfranco L., Wyss P., Marachi C. & Bonfante P. (1995). Generation of RAPD-PCR primers for the identification of isolates of *Glomus mosseae*, an arbuscular mycorrhizal fungus. *Molecular Ecology* **80:** 622-629.

Lapointe L. & Molard J. (1997). Costs and benefits of mycorrhizal infection in a spring ephemeral, *Erythronium americanum*. *New Phytologist* **135:** 491-500.

Lei T.T. & Lechowicz M.J. (1990). Shade adaptation and shade tolerance in saplings of three *Acer* species from eastern North America. *Oecologia* **84:** 224-228.

Lerat S., Gauci R., Catford J.G., Vierheilig H., Piché Y. & Lapointe L. (2002). ^{14}C transfer between the spring ephemeral *Erythronium americanum* and sugar maple saplings via arbuscular mycorrhizal fungi in natural stands. *Oecologia* **132:** 181-187.

Lerat S., Lapointe L., Gutjahr S., Piché Y. & Vierheilig H. (2003). Carbon partitioning in a split-root system of arbuscular mycorrhizal plants is fungal and plant species dependent. *New Phytologist* **157:** 589-595.

Lloyd-Macgilp S.A., Chambers S.M., Dodd J.C., Fitter A.H., Walker C. & Young J.P.W. (1996). Diversity of the ribosomal internal transcribed spacers within and among isolates of *Glomus mosseae* and related mycorrhizal fungi. *New Phytologist* **133:** 103-111.

Marschner H. (1990). Mineral nutrition of higher plants. Academic Press, London, UK.

Marschner P. & Crowley D.E. (1996). Root colonization of mycorrhizal and non-mycorrhizal pepper (*Capsicum annuum*) by *Pseudomonas fluorescens* 2-79RL. *New Phytologist* **134:** 115-122.

McGee P.A. (1986). Further sporocarpic species of *Glomus* (Endogonaceae) from South Australia. *Transactions of the British Mycological Society* **87:** 123-129.

McGee P.A. (1989). Variation in propagule numbers of vesicular-arbuscular mycorrhizae in a semi-arid soil. *Mycological Research* **92:** 28-33.

McGonigle T.P., Miller M.H., Evans D.G., Fairchild G.L. & Swan J.A. (1990). A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytologist* **115:** 495-501.

Merryweather J. & Fitter A.H. (1998). The arbuscular mycorrhizal fungi of *Hyacinthoides non-scripta*. II. Seasonal and spatial patterns of fungal populations. *New Phytologist* **138:** 131-142.

Morton J.B. (1986). Three new species of *Acaulospora* (Endogonaceae) from high aluminum, low pH soils in West Virginia. *Mycologia* **78:** 641-648.

Morton J.B., Bentivenga S.P. & Bever J.D. (1995). Discovery, measurement and interpretation of diversity in arbuscular endomycorrhizal fungi (Glomales, Zygomycetes). *Canadian Journal of Botany* **73:** S23-S32.

Moutoglis P. & Widden P. (1996). Vesicular-arbuscular mycorrhizal spore populations in sugar maple (*Acer saccharum* marsh. L.) forests. *Mycorrhiza* **6:** 91-97.

Moutoglis P., Klironomos J.N., Widden P. & Kendrick B. (1995). Direct observation of spores of vesicular-arbuscular mycorrhizal fungi growing on sugar maple roots in the field, using sodium hexametaphophate as a soil dispersant. *Mycologia* **87:** 419-423.

Newman E.I. (1966). A method of estimating the total length of root in a sample. *Journal of Applied Ecology* **3:** 139-145.

Newman E.I. (1988). Mycorhizzal links between plants: their functioning and ecological significance. *Advances in Ecological Research* **18:** 243-270.

Newman E.I. & Eason W.R. (1993). Rates of phosphorus transfer within and between ryegrass (*Lolium perenne*) plants. *Functional Ecology* **7:** 242-248.

Ouimet R., Camiré C. & Furlan V. (1995). Endomycorrhizal status of sugar maple in relation to tree decline and foliar, fine-roots, and soil chemistry in Beauce region, Québec. *Canadian Journal of Botany* **73:** 1168-1175.

Pang P.C. & Paul E.A. (1980). Effects of vesicular-arbuscular mycorrhiza on ^{14}C and ^{15}N distribution in nodulated fababeans. *Canadian Journal of Soil Science* **60:** 241-250.

Pearson J.N., Abbott L.K. & Jasper D.A. (1993). Mediation of competition between two colonizing VA mycorrhizal fungi by the host plant. *New Phytologist* **123:** 93-98.

Pearson J.N., Abbott L.K. & Jasper D.A. (1994). Phosphorus, soluble carbohydrates and the competition between two arbuscular mycorrhizal fungi colonizing subterranean clover. *New Phytologist* **127:** 101-106.

Peng S., Eissenstat D.M., Graham J.H., Williams K. & Hodge N.C. (1993). Growth depression in mycorrhizal *Citrus* at high-phosphorus supply. *Plant Physiology* **101:** 1063-1071.

Pinior A., Wyss U., Piché Y. & Vierheilig H. (1999). Plants colonized by AM fungi regulate further root colonization by AM fungi through altered root exudation. *Canadian Journal of Botany* **77:** 891-897.

Poorter H. & Villar R. (1997). The fate of acquired carbon in plants: Chemical composition and construction costs. *Dans*: Plant resource allocation. Bazzaz F.A., Grace J. eds. Academic Press, San Diego, USA. pp. 39-72.

Pozo M.J., Cordier C., Dumas-Gaudot E., Gianinazzi S., Barea J.M. & Azcon-Aguilar C. (2002). Localized versus systemic effect of arbuscular mycorrhizal fungi on defence responses to *Phytophtora* infection in tomato plants. *Journal of Experimental Botany* **53:** 525-534.

Read D.J., Koucheki H.K. & Hodgson J. (1976). Vesicular-arbuscular mycorrhiza in natural vegetation systems I. The occurrence of infection. *New Phytologist* **77:** 641-653.

Redecker D. (2000). Specific PCR primers to identify arbuscular mycorrhizal fungi within colonized roots. *Mycorrhiza* **10:** 73-80.

Robinson D. & Fitter A.H. (1999). The magnitude and control of carbon transfer between plants linked by a common mycorrhizal network. *Journal of Experimental Botany* **50:** 9-13.

Rousseau J.V.D., Sylvia D.M. & Fox A.J. (1994). Contribution of ectomycorrhiza to the potential nutrient-absorbing surface of pine. *New Phytologist* **128:** 639-644.

Salisbury F.B. & Ross C.W. (1985). Plant physiology. Wadsworth Publishing Company, Belmont, California, USA.

Same B.I., Robson A.D. & Abbott L.K. (1983). Phosphorus, soluble carbohydrates and endomycorrhizal infection. *Soil Biology and Biochemistry* **15:** 593-598.

Sancholle M., Dalpé Y. & Grandmougin-Ferjani A. (2001). Lipids of mycorrhizae. *Dans*: The Mycota IX. Hock B. ed., Springer. Berlin, Germany. pp. 63-93.

Schenck N.C. & Smith G.S. (1982). Responses of six species of vesicular-arbuscular mycorrhizal fungi and their effects on soybean at four soil temperatures. *New Phytologist* **92:** 193-201.

Schüssler A., Schwartzzott D. & Walker C. (2001). A new phylum, the *Glomeromycota*: phylogeny and evolution. *Mycological Research* **105:** 1413-1421.

Shachar-Hill Y., Pfeffer P.E., Douds D.D., Osman S.F., Doner L.W. & Ratcliffe R.G. (1995). Partitioning of intermediary carbon metabolism in vesicular-arbuscular mycorrhizal leek. *Plant Physiology* **108:** 7-15.

Simard S.W., Jones M.D., Durall D.M., Perry D.A., Myrold D.D. & Molina R. (1997a). Reciprocal transfer of carbon isotopes between ectomycorrhizal *Betula papyrifera* and *Pseudotsuga menziesii*. *New Phytologist* **137:** 529-542.

Simard S.W., Perry D.A., Jones M.D., Myrold D.D., Durrall D.M. & Molina R. (1997b). Net transfer of carbon between ectomycorrhizal tree species in the field. *Nature* **388:** 579-582.

Simon L., Lalonde M. & Bruns T.D. (1992). Specific amplification of 18S fungal ribosomal genes from vesicular-arbuscular endomycorrhizal fungi colonizing roots. *Applied and Environmental Microbiology* **58:** 291-295.

Simon L., Bousquet J., Lévesque R.C. & Lalonde M. (1993). Origin and diversification of endomycorrhizal fungi and coincidence with vascular land plants. *Nature* **363:** 67-69.

Smith F.A., Jakobsen I. & Smith S.E. (2000). Spatial differences in acquisition of soil phosphate between two arbuscular mycorrhizal fungi in symbiosis with *Medicago truncatula*. *New Phytologist* **147:** 357-366.

Smith S.E. & Read D.J. (1997). Mycorrhizal symbiosis, second edition. Academic Press, London, UK.

Snellgrove R.C., Splittstoesser W.E., Stribley D.P. & Tinker P.B. (1982). The distribution of carbon and the demand of the fungal symbiont in leek plants with vesicular-arbuscular mycorrhizas. *New Phytologist* **92:** 75-87.

Solaiman M.D.Z. & Saito M. (1997). Use of sugars by intraradical hyphae of arbuscular mycorrhizal fungi revealed by radiorespirometry. *New Phytologist* **136:** 533-538.

Spitko R.A., Tattar T.A. & Rohde R.A. (1978). Incidence and condition of vesicular-arbuscular mycorrhizae infections in the roots of sugar maple in relation to maple decline. *Canadian Journal of Forest Research* **8:** 375-379.

St-Arnaud M., Hamel C., Caron M. & Fortin J.A. (1995). Endomycorhizes VA et sensibilité des plantes aux maladies: synthèse de la littérature et mécanismes d'interaction potentiels. *Dans*: La symbiose mycorhizienne. Fortin J.A., Charest C., Piché Y., eds. Éditions ORBIS, Frelighsburg, Québec, Canada. pp. 51-87.

Statistics Canada (1998). The St. Lawrence River valley 1998 ice storm: maps and facts. Occasional, catalogue n° 16F0021XIB.

Taylor J. & Harrier L. (2000). A comparison of nine species of arbuscular mycorrhizal fungi on the development and nutrition of micropropagated *Rubus idaeus* L. cv. Glen Prosen (Red Raspberry). *Plant and Soil* **225:** 53-61.

Taylor R.J. & Pearcy R.W. (1976). Seasonal patterns of the CO_2 exchange characteristics of understory plants from a deciduous forest. *Canadian Journal of Botany* **54:** 1094-1103.

Taylor T.M. & Osborn J.M. (1995). The importance of fungi in shaping the paleoecosystem. *Review of Palaeobotany and Palynology*. **90:** 249-262.

Thomson B.D., Robson A.D. & Abbott L.K. (1990). Mycorrhizas formed by *Gigaspora calospora* and *Glomus fasciculatum* on subterranean clover in relation to soluble carbohydrate concentrations in roots. *New Phytologist* **114:** 217-225.

Trouvelot A., Kough J.L. & Gianinazzi-Pearson V. (1986). Mesure du taux de mycorhization VA d'un système radiculaire. Recherche de méthodes d'estimation ayant une signification fonctionnelle. *Dans*: Physiological and genetical aspects of mycorrhizae. INRA Publications, Paris. pp. 217-221.

van der Heijden M.G.A., Klironomos J.N., Ursic M., Moutoglis P., Streitwolf-Engel R., Boller T., Wiemken A. & Sanders I.R. (1998). Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* **396:** 69-72.

Vierheilig H. & Piché Y. (2002). Signalling in arbuscular mycorrhiza: facts and hypotheses. *Dans*: Flavonoids in cell function. Buslig B., Manthey J., eds. Kluwer Academic/Plenum Publishers, New York, USA. pp. 23-39.

Vierheilig H., Coughlan A.P., Wyss U. & Piché Y. (1998). Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. *Applied and Environmental Microbiology* **64:** 5004-5007.

Vierheilig H., Garcia-Garrido J.M., Wyss U. & Piché Y. (2000a). Systemic suppression of mycorrhizal colonization of barley roots already colonized by AM fungi. *Soil Biology and Biochemistry* **32:** 589-595.

Vierheilig H., Walters M., Wyss U., Samson J., Strack D. & Piché Y. (2000b). Cyclohexenone derivate- and phosphate-levels in split-root systems and their role in the systematic suppression of mycorrhization in precolonized barley plants. *Journal of Plant Physiology* **157:** 593-599.

Vierheilig H., Bago B., Lerat S. & Piché Y. (2002). Shoot-produced, light-dependent factors are partially involved in the expression of the arbuscular mycorrhizal (AM) status of AM host and non-host plants. *Journal of Plant Nutrition and Soil Science* **165:** 21-25.

Walker C. & Trappe J.M. (1993). Names and epithets in the Glomales and Endogonales. *Mycological Research* **97:** 339-344.

Wang G.M., Coleman D.C., Freckman D.W., Dyer M.I., McNaughton S.J., Acra M.A. & Goeschl J.D. (1989). Carbon partitioning patterns of mycorrhizal versus non-mycorrhizal plants: real-time dynamic measurements using $^{11}CO_2$. *New Phytologist* **112:** 489-493.

Wittigham J. & Read D.J. (1982). Vesicular-arbuscular mycorrhiza in natural vegetation system. III. Nutrient transfer between plants with mycorrhizal connections. *New Phytologist* **90:** 277-284.

Wright D.P., Read D.J. & Scholes J.D. (1998a). Mycorrhizal sink strength influences whole plant carbon balance of *Trifolium repens* L. *Plant, Cell and Environment* **21:** 881-891.

Wright D.P., Scholes J.D. & Read D.J. (1998b). Effects of VA mycorrhizal colonization on photosynthesis and biomass production of *Trifolium repens* L. *Plant, Cell and Environment* **21:** 209-216.

Wu B., Nara K. & Hogetsu T. (2001). Can ^{14}C-labeled photosynthetic products move between *Pinus densiflora* seedlings linked by ectomycorrhizal mycelia? *New Phytologist* **149:** 137-146.

Wyss P., Boller T. & Wiemken A. (1991). Phytoalexin response is elicited by a pathogen (*Rhizoctonia solani*) but not by a mycorrhizal fungus (*Glomus mosseae*) in bean roots. *Experientia* **47:** 395-399.

Yawney W.J. & Schultz R.C. (1990). Anatomy of a vesicular-arbuscular endomycorrhizal symbiosis between sugar maple (*Acer saccharum* Marsh) and *Glomus etunicatum* Becker & Gerdemann. *New Phytologist* **114:** 47-57.

Zahka G.A., Bagget K.L. & Wong B.L. (1995). Inoculum potential and other VAM fungi parameters in four sugar maple forests with different levels of stand dieback. *Forest Ecology and Management* **75:** 123-134.