CAROLINE ROCHON

DISTRIBUTION ET PRODUCTIVITÉ DE DEUX CHAMPIGNONS ECTOMYCORHIZIENS (*CANTHARELLUS CIBARIUS* VAR. *ROSEOCANUS* ET *HYPOMYCES LACTIFLUORUM/RUSSULA BREVIPES*) EN PEUPLEMENTS DE PIN GRIS DE L'EST DU CANADA

Thèse présentée
à la Faculté des études supérieures et postdoctorales de l'Université Laval
dans le cadre du programme de doctorat en sciences forestières
pour l'obtention du grade de Philosophiae Doctor (Ph.D.)

DÉPARTEMENT DES SCIENCES DU BOIS ET DE LA FORÊT
FACULTÉ DE FORESTERIE, DE GÉOGRAPHIE ET DE GÉOMATIQUE
UNIVERSITÉ LAVAL
QUÉBEC

2011

Résumé

Les champignons ectomycorhiziens forment des symbioses racinaires avec des arbres des forêts boréales. Malgré leur importance dans cet écosystème, leurs exigences en matière d'habitat demeurent mal connues. Deux champignons comestibles, une chanterelle (*Cantharellus cibarius* var. *roseocanus* Redhead, Norvell & Danell) et la dermatose des russules (*Hypomyces lactifluorum* (Schwein.) Tul. & C.Tul. / *Russula brevipes* Peck.), ont été étudiés dans cette thèse. Trois expériences ont été menées en peuplements aménagés et non aménagés de pin gris (*Pinus banksiana* Lamb.). Cette étude visait à caractériser les paramètres environnementaux qui influencent la production de carpophores chez ces champignons, à déterminer l'impact d'une peturbation forestière sur ces derniers et à préciser l'importance de la phénologie du pin gris pour la fructification de *C. cibarius* var. *roseocanus*. Les résultats relient la distribution (présence/absence des carpophores) et la productivité (biomasse fraîche et densité des carpophores) de ces champignons à des paramètres du sol, du peuplement, de la végétation et du climat. L'aménagement de sentiers n'a pas augmenté la production de carpophores de dermatose des russules, mais l'a maintenu durant les périodes de faibles précipitations. La productivité de ce champignon présente des corrélations positives avec l'abondance de plantes intolérantes à l'ombre et l'ammonium extractible et négatives avec le pH du sol. La productivité de *C. cibarius* var. *roseocanus* s'équivaut entre le peuplement aménagé et non aménagé malgré l'absence de carpophores dans les sentiers du peuplement aménagé. L'association végétale *Solidago puberula – Comptonia peregrina – Pinus banksiana* et la présence de mousses représente un habitat propice à la fructification de cette chanterelle, alors que la présence de plantes éricacées la défavoriserait. Les précipitations et la température de l'air ont aussi un impact sur la quantité de carpophores. Le pic de fructification de *C. cibarius* var. *roseocanus* suit la transition du bois juvénile vers le bois mature. Durant la saison de croissance, la respiration du carpophore est synchronisée avec la respiration totale du sol et ces deux respirations sont corrélées avec les variations de températures du sol. Les résultats permettront de mieux prédire la distribution et la productivité de ces espèces en peuplements de pin gris. Ces connaissances contribueront au développement et à l'exploitation durable de cette ressource.

Abstract

Ectomycorrhizal fungi form root symbioses with boreal tree species. Despite their importance in that ecosystem, their requirements in term of habitat remain unknown. Two edible mushrooms, a chanterelle (*Cantharellus cibarius* var. *roseocanus* Redhead, Norvell & Danell) and the lobster mushroom (*Hypomyces lactifluorum* (Schwein.) Tul. & C.Tul. / *Russula brevipes* Peck.) were studied in this thesis. Three experiments were conducted in managed and unmanaged jack pine (*Pinus banksiana* Lamb.) stands. This research aimed to characterize the ecological parameters related to the production of sporocarps of these fungi, to determine the impact of a specific forest disturbance on the latter and to specify the importance of jack pine phenology on the pattern of *C. cibarius* var. *roseocanus* carpophore production. Results allowed us to link the mushroom distribution (sporocarp presence/absence) and the productivity (fresh sporocarp biomass and sporocarp density) to specific soil, stand, plant and meteorological parameters. Trail management did not increase lobster mushroom carpophore production but maintained it during periods of reduced precipitation. Productivity of this fungus was positively related to the abundance of shade-intolerant plant species and to extractable ammonium concentration, and negatively related to soil pH. *C. cibarius* var. *roseocanus* sporocarp productivity was similar for the managed and the unmanaged stands despite the absence of carpophore on trails of the managed stand. The *Solidago puberula – Comptonia peregrina– Pinus banksiana* association and mosses presence indicated high-quality environments for chanterelle fructification, whereas ericaceous species presence restricted it. Rainfall and air temperature also had an impact on the carpophore productivity. The *C. cibarius* var. *roseocanus* fructification peak followed the earlywood–latewood transition within days. Over the growing season the carpophore respiration was in synchrony with the total soil respiration and these respirations were correlated to the soil temperature patterns. Results will enable the prediction of the distribution and the productivity of these species in jack pine stands. This knowledge will contribute to the sustainable development and use of this natural resource.

Avant-Propos

Mes études doctorales ont été dirigées par Damase P. Khasa et co-dirigées par J. André Fortin et David Paré. Elles ont été réalisées grâce au soutien financier du Conseil national de recherches en sciences naturelles et en génie du Canada (projet stratégique # 306898-04 accordé au Professeur Yves Piché et collaborateurs de l'Université Laval).

La présente thèse est constituée des cinq chapitres. Le chapitre 1 et le chapitre 5 présentent, respectivement, l'introduction et la conclusion de l'ensemble de la thèse. Cette thèse comporte trois manuscrits (chapitres 2, 3, 4) qui ont été ou seront soumis pour publication dans des revues scientifiques. Les articles ont été rédigés en anglais, mais sont accompagnés, dans ce mémoire, d'un résumé en français. Pour chacun de ces manuscrits, j'ai agi à titre de première auteure. Cela signifie que j'ai procédé aux analyses statistiques des données expérimentales, à la présentation des données et à la rédaction du texte. Les co-auteurs de ces articles ont également apporté leur contribution que ce soit en supervisant mes travaux sur le terrain, en effectuant certains travaux de laboratoire, en me conseillant lors de l'analyse des données ou en révisant mes manuscrits. J'adresse mes sincères remerciements à ces co-auteurs qui sont Damase P. Khasa (chapitres 2, 3 et 4), J. André Fortin (chapitres 2, 3 et 4), David Paré (chapitre 2, 3 et surtout 4) et Nellia Pélardy (chapitre 3).

Chapitre 2

Rochon, C., Paré, D., Khasa, D.P., and Fortin, J.A. 2009. Ecology and management of the lobster mushroom in an eastern Canadian jack pine stand. Canadian Journal of Forest Research **39**(11): 2080-2091.

Cet article a été soumis et publié dans la revue *Canadian Journal of Forest Research*.

Chapitre 3

Rochon, C., Paré, D., Pélardy, N., Khasa, D.P., and Fortin, J.A. 2011. Ecology and productivity of *Cantharellus cibarius* var. *roseocanus* in two eastern Canadian jack pine stands. Botany.

Cet article a été soumis et accepté pour publication le 25 juillet 2011 dans la revue *Botany*.

Chapitre 4

Rochon C., Paré D., Khasa D.P., and Fortin, J.A. 2011. Linking mushroom fructification with tree phenology and belowground carbon fluxes using *Cantharellus cibarius* var. *roseocanus* as a model in an eastern Canadian jack pine stand. Tree physiology.

Ce chapitre a été soumis le 1 juin 2011 pour publication dans la revue *Tree physiology*.

Remerciements

Maintenant que tout est terminé, les bonnes et les moins bonnes journées, les instants de découragements et de frustrations et les moments de satisfaction et de réussite, j'aimerais en quelques mots remercier toutes les personnes qui m'ont appuyé durant ces 6 années de doctorat. Je voudrais d'abord remercier la personne qui m'a résolument appuyé et encouragé et qui a cru en moi durant tout mon doctorat, J. André Fortin. M. Fortin, sans vous je n'aurais jamais pu mener à bien ce projet. Merci pour votre générosité et votre patience et merci d'avoir partagé votre passion pour les champignons avec moi.

Je tiens également à remercier mon directeur de recherche, Damase P. Khasa, d'avoir accepté de me faire confiance et de m'avoir donné l'opportunité de faire cette thèse. M. Khasa votre capacité de travail, votre sourire et vos encouragements m'ont bien souvent motivé à continuer mes travaux de recherche.

Je me dois également de remercier mon deuxième co-directeur, David Paré, qui a toujours pris le temps de répondre à mes nombreuses questions et interrogations. Je lui suis reconnaissante d'avoir partagé ses connaissances et son expérience. David, merci pour ton acharnement et ta rigueur qui ont permis l'amélioration de la qualité de mes manuscrits.

La totalité des expériences de cette thèse ont été réalisées sur un site privé, le domaine de la rivière Mistassini, et sur les sites avoisinants. Je tiens donc à remercier les propriétaires de ce domaine, Céline Marceau et Alain Blais, pour nous avoir appuyé, conseillé, logé et souvent nourri au cours des trois étés passés chez eux. Votre accueil chaleureux et vos conversations singulières m'ont permis d'apprécier chaque moment passé au Lac! Merci aussi à Marie-Lou Gagnon et Jessica Gagnon pour leur aide et présence.

Bien sûr, je remercie aussi toutes celles et tous ceux, et ils sont nombreux, qui m'ont aidé dans la mise en place et la gestion des parcelles expérimentales, dans la collecte des données, dans l'analyse des échantillons de sol et des carottes d'arbre et dans le développement des marqueurs moléculaires. Je pense notamment à Andrew Coughlan, André Gagné, Nellia Pélardy, Karine Bertrand et Marie-Ève Beaulieu à l'Université Laval et à Alain Courcelles, Sébastien Dagnault et Ricardo Morin au Service Canadien des forêts,

(SCF-RNCan). Finalement, j'adresse un merci tout spécial à Christine Roussel-Roy, alors étudiante au premier cycle au Département de biologie de la Faculté des sciences et génie et qui a travaillé pendant trois ans sur tous les aspects de ce projet. Christine merci pour ton dévouement et ton efficacité!

J'en profite aussi pour remercier le docteur Sylvie Richard, Stéphane Gariépy et mon superviseur actuel, Normand Laflamme au Service Canadien des forêts (SCF-RNCan), pour m'avoir laissé la possibilité de travailler tout en continuant mon doctorat. Sans votre ouverture d'esprit, je n'y serais pas arrivé.

Je voudrais aussi remercier Shannon Berch (B.C. Ministry of Forests and Range, Research Branch) d'avoir participé de loin à l'évolution de mon doctorat et de m'avoir accueilli pour mon stage doctoral (Thank you for everything Shannon). Je la remercie également d'avoir accepté de faire partie de mon comité d'évaluation, de même que Line Lapointe (Université Laval) et Normand Villeneuve (Ministère des Ressources naturelles et faune)

Finalement, un merci tout particulier à mes proches. À mes parents et à mon frère, qui ont toujours cru en moi et en mes projets hors du commun, à mes amis en qui je peux avoir une confiance absolue et qui continue à égayer ma vie jour après jour, mais surtout à Julien et à notre fils Raphaël, mes amours sans qui la vie ne serait pas aussi bien, merci, merci, merci d'être là pour moi. Je vous aime tellement!

À tous les passionnés de champignons…

Table des matières

Liste des tableaux

Liste des figures

Chapitre 1. Introduction générale

Depuis le XX^{e} siècle, l'importance de l'industrie des champignons comestibles a considérablement augmenté à l'échelle planétaire (Pilz et al. 2002). De nos jours, des milliers de personnes œuvrent dans cette industrie et les échanges totalisent des milliards de dollars annuellement (Pilz et al. 2002). Deux champignons ectomycorhiziens (ECM) comestibles, une espèce de chanterelle (*Cantharellus cibarius* var. *roseocanus* Redhead, Norvell & Danell) et un complexe formé d'un champignon qui parasite un autre champignon (*Hypomyces lactifluorum* (Schwein.) Tul. & C.Tul. et *Russula brevipes* Peck.), sont le sujet de cette thèse. Ces deux champignons forestiers ont un fort potentiel économique au Québec, mais leur écologie et leur rôle dans la forêt boréale restent encore méconnus. De plus, malgré les avancées effectuées les liens entre la distribution des champignons ECM et les différents paramètres environnementaux demeurent difficiles à établir puisque qu'ils n'interagissent pas seuls avec ces champignons. En vue d'un développement et d'une exploitation durable de cette ressource au sein d'un aménagement forestier raisonné, nous avons entrepris une étude sur trois ans de ces deux champignons dans leur milieu naturel (dans un peuplement aménagé et un non aménagé de pin gris (*Pinus banksiana* Lamb.)). Cette étude visait à caractériser les paramètres environnementaux et à déterminer l'impact d'une perturbation forestière sur la production de carpophores de deux espèces de champignons ECM ainsi qu'à préciser l'importance de la phénologie du pin gris pour la fructification de *C. cibarius* var. *roseocanus*. Une meilleure compréhension de l'écologie de ces deux espèces encouragera une gestion durable de cette ressource. Ainsi, nous avons mis en relation la distribution (présence/absence des carpophores) et la productivité (biomasse fraîche des carpophores et densité des carpophores) des deux champignons ECM avec un grand nombre de paramètres environnementaux : des conditions météorologiques, des variables du sol et des caractéristiques d'un peuplement de pin gris et de sa végétation associée. La phénologie de l'arbre hôte en lien avec la phénologie de fructification de *C. cibarius* var. *roseocanus* a aussi été étudiée dans le but de mesurer l'impact de la phénologie de l'arbre sur la respiration de la rhizosphère, sur la respiration du carpophore et sur les variations de fructification du champignon. Ainsi, les flux de carbone de la plante et du sol au cours de la

saison de croissance sont des variables importantes dans ce travail de recherche. Cette introduction résume les connaissances essentielles à la compréhension des chapitres de recherche de cette thèse (chapitres 2, 3 et 4), soit des connaissances sur le cycle du carbone, sur le pin gris et la forêt boréale, sur les liens entre les perturbations anthropiques et naturelles et la productivité des champignons ECM et sur les deux champignons à l'étude.

Le cycle du carbone et les processus des écosystèmes terrestres

Parce que des augmentations importantes ont été observées dans les niveaux de CO_2 atmosphérique depuis les années 1950, probablement un indicateur de changements climatiques, le cycle du carbone dans les écosystèmes terrestres est devenu un thème majeur de recherche en écologie (Schimel 1995). Les écosystèmes terrestres absorbent le CO_2 atmosphérique par la photosynthèse, l'immobilisent dans la biomasse aérienne et dans les sols sous forme de matière organique en décomposition et le libèrent dans l'atmosphère par le processus de respiration. À l'échelle mondiale, la photosynthèse et la respiration représentent d'énormes flux de carbone qui se contrebalancent approximativement (Schimel 1995). L'échange net du carbone dans un écosystème (net ecosystem exchange) réfère à l'équilibre entre le carbone fixé par les plantes (photosynthèse) et le carbone libéré par les plantes et les microbes (respiration) (Schuur and Trumbore 2006). En fait, le carbone pénètre dans l'écosystème forestier par la photosynthèse et en ressort soit rapidement par la respiration des racines et de la rhizosphère (en moins d'une année), soit plus lentement par la décomposition de la litière des feuilles, des racines et de la matière organique du sol (après plusieurs années) (Kozlowski 1992; Trumbore 1993). Le carbone photosynthétique qui n'est pas respiré par la plante est utilisé pour le stockage, la croissance des tissus ou encore par les organismes symbiotiques et les herbivores (Trumbore 2006). La respiration de l'écosystème est divisée en deux types: la respiration de la canopée et la respiration souterraine, aussi appelée la respiration du sol.

La respiration du sol est une composante importante des échanges de CO_2 entre les écosystèmes forestiers et l'atmosphère (Borken et al. 2006). Cependant, dû à sa variabilité spatiale, saisonnière et interannuelle élevée, elle est difficilement quantifiable à l'échelle de l'écosystème (Andersen et al. 2005; Gaumont-Guay et al. 2006). Néanmoins, Janssens et al.

(2001) ont réussi à estimer que la portion de la respiration qui provient du sol équivaudrait à 69% de la respiration de l'écosystème total (i.e. des plantes et du sol) et Davidson et al. (2006) considèrent qu'elle se situerait entre 30 et 80% de la respiration totale. La respiration du sol résulte de deux grandes sources : hétérotrophe et autotrophe. La respiration hétérotrophe entraîne un dégagement de CO_2 par les microorganismes du sol qui décomposent la litière (feuilles et résidus de racines) et la matière organique, alors que la respiration autotrophe correspond aux processus métaboliques de la plante et des mycorhizes associées à ses racines qui libèrent du CO_2 dans la rhizosphère (Cisneros-Dozal et al. 2006).

La partition de la respiration totale du sol entre ces deux composantes (flux autotrophe et hétérotrophe) est complexe puisque les flux de CO_2 mesurés intègrent plusieurs processus distincts et varient selon la méthodologie utilisée et les caractéristiques étudiées. Hanson et al. (2000) ont passé en revue les approches qui évaluaient la proportion attribuable à la respiration autotrophe et concluent qu'elle constitue de 32% à 60% du CO_2 libéré par le sol. Cependant, cette proportion attribuée à chaque flux n'est pas constante au cours d'une saison de croissance (Trumbore 2006). Afin de calculer la contribution relative de ces deux flux à la respiration totale du sol, différentes approches sont utilisées :

1) *La collecte de données continues des flux de CO_2 et le développement de modèles* (par example Davidson et al. 2006; Tang et al. 2005). Ces techniques extrapolent les mesures du flux de CO_2 pour les différentes composantes (i.e., la respiration totale du sol, la respiration hétérotrophe et la respiration autotrophe), soit par incubations du sol, par excision des racines et plus récemment par l'utilisation de chambres de respiration et de la technique de covariance des turbulences (eddy covariance) (Bergeron et al. 2009).

2) *L'application de mesures isotopiques, dont le radiocarbone* (Borken et al. 2006; Cisneros-Dozal et al. 2006; Schuur and Trumbore 2006). Cette approche se base sur les différences qui existent entre la signature isotopique de la respiration autotrophe et hétérotrophe. Le rapport C_{14}/C_{12} du carbone fixé récemment est proche de celui du CO_2 de l'atmosphère actuelle (respiration autotrophe) alors que celui du carbone

trouvé dans la litière et dans la matière organique du sol est plus élevé ce qui reflète un temps de résidence plus long (respiration hétérotrophe).

3) *Les manipulations expérimentales qui suppriment la respiration autotrophe* (Gadgil and Gadgil 1971; Högberg et al. 2001; Scott-Denton et al. 2006). Ces manipulations ont pour objectif de couper l'apport des racines à une partie du sol, soit en effectuant une tranchée autour de ce sol, soit en enlevant le phloème de l'arbre (tree girdling), ce qui détruit son habileté à transférer le carbone des feuilles vers les racines.

Des équations empiriques qui relient les flux saisonniers et annuels de CO_2 aux variations de température et d'humidité ont été développées (Trumbore 2006). Cependant, la succession végétale, les perturbations, la phénologie de la croissance des feuilles et des racines, la sénescence (Davidson et al. 2006) et l'apport de substrats (Schuur and Trumbore 2006) seraient aussi des facteurs importants. De plus, en ce qui concerne les flux de CO_2 du sol, la température et l'humidité du sol ont tendance à masquer l'importance des mécanismes photosynthétiques qui apportent les hydrates de carbone des feuilles aux racines (Kuzyakov and Gavrichkova 2010). Ainsi, la respiration hétérotrophe serait davantage sous contrôle environnemental (température et humidité du sol principalement) ce qui la rendrait plus variable, alors que la respiration autotrophe serait plus constante dans le temps parce qu'influencée par les mécanismes journaliers et saisonniers de la photosynthèse (Tang et al. 2005). Dans les écosystèmes forestiers, le carbone absorbé au début de la saison de croissance par les nouvelles pousses provient des réserves d'hydrates de carbone des arbres. Par la suite, ces réserves sont moins utilisées et en fin de saison ce ne sont que les produits plus récents de la photosynthèse qui sont utilisés pour le développement des racines (Kozlowski 1992).

Comme les racines avec lesquelles ils forment une symbiose, les champignons mycorhiziens utilisent les glucides issus de la photosynthèse comme source d'énergie (Fahey et al. 2005), notamment lors du développement des carpophores (i.e. la partie visible du champignon qui se trouve habituellement à la surface du sol). La fructification de

ces carpophores dépend de la photosynthèse de la plante hôte, et non du carbone accumulé dans le mycélium (i.e. l'appareil végétatif souterrain du champignon, formé de filaments généralement blanchâtres, les hyphes) (Lamhamedi et al. 1994). En forêt boréale, plusieurs espèces ligneuses vivent en associations symbiotiques avec des champignons ectomycorhiziens et éricoïdes (Allen 1991). Lors de la symbiose ectomycorhizienne, les champignons forment des organes symbiotiques, appelés ectomycorhizes, avec les radicelles des espèces ligneuses. Cette symbiose permet le partage des ressources entre les deux organismes, la plante hôte fournissant l'énergie sous forme d'hydrates de carbone en échange de nutriments apportés par le champignon (Smith and Read 2008). Plusieurs études effectuées en milieu naturel suggèrent que les champignons ECM contribuent à certaines fonctions primordiales de l'écosystème dont le cycle du carbone, la mobilisation des nutriments provenant de la matière organique et des sols minéraux et la liaison entre les arbres à partir d'un réseau mycorhizien commun (Courty et al. 2010).

En forêt boréale, les produits de la photosynthèse sont davantage exportés dans les organes souterrains de l'arbre, et indirectement aux champignons ECM, lors de la deuxième moitié de la saison de croissance. Le développement des racines et des carpophores atteint alors son apogée (Fortin and Lamhamedi 2009). Godbout et Fortin (1990) ont d'ailleurs démontré qu'une diminution de la photopériode intensifie la production de carpophores de *Laccaria bicolor* (Maire) Orton. Cette baisse de photopériode correspondrait à un changement de l'allocation des produits de la photosynthèse des bourgeons vers les racines puis vers les tissus fongiques où les hydrates de carbone sont rapidement convertis en tréhalose, mannitol ou glycogène (Finlay and Söderström 1992; Rousseau and Reid 1989). De plus, les études effectuées en laboratoire suggèrent que dépendamment du moment dans la saison de croissance, 1% à 21% de la production primaire nette d'une plante serait assimilée par les champignons ECM (Hobbie 2006). En fait, même si la proportion de la respiration qui correspond à la respiration des champignons est difficile à séparer de la respiration des racines (Rousseau and Reid 1989), Heinemeyer et al. (2007) estiment que durant l'automne le flux de CO_2 du sol serait divisé comme suit : 60% proviendrait des organismes hétérotrophes, 25% viendrait des hyphes de champignons ECM et 15% résulterait des racines des plantes (de la respiration autotrophe).

Ainsi, le mycélium des champignons contribuerait de manière significative aux flux élevés mesurés occasionnellement dans les sols et des taux de respiration élevés pourraient être reliés au développement de carpophores (Borken et al. 2006).

La forêt boréale du Canada

La forêt boréale, ou taïga, forme un vaste anneau de forêts septentrionales de conifères (pins, sapins et épinettes) qui couvre sans discontinuité les zones subarctiques de l'Amérique de Nord et de l'Eurasie (Moore et al. 1998). Cette forêt est répartie sur 12,2 millions de km^2, dont 36% se trouve en Amérique du Nord, principalement au Canada (Black et al. 2005). Près de 60% du carbone de la biosphère terrestre est emmagasiné dans les forêts tropicales, tempérées et boréales. La forêt boréale représente, à elle seule, le deuxième biome en importance, après les forêts ombrophiles tropicales, en termes de productivité primaire nette, de réserve totale de carbone et de potentiel de séquestration du carbone (Grace 2005). La forêt boréale a donc une influence majeure sur le cycle global du carbone.

Cette forêt est dominée majoritairement par des gymnospermes de la famille des Pinaceae dont les genres *Pinus* et *Picea* forment de vastes peuplements forestiers, souvent monospécifiques (Read et al. 2004). Capables de s'acclimater à une vaste gamme de températures et d'humidités, les arbres qui y poussent sont adaptés à survivre sur des sols pauvres en nutriments (Moore et al. 1998). Ces adaptations sont favorisées par la composition de la litière des forêts de conifères. En effet, la litière d'aiguilles favorise des conditions acides qui ralentissent l'évapotranspiration et la décomposition de la matière organique déjà freinées par les faibles températures hivernales (Henry 2002). Les résidus organiques des plantes s'accumulent donc, soit en une couche d'humus sur le dessus du sol ou en tourbe qui peut s'étendre jusqu'à des profondeurs considérables (Whittaker 1970). Cela favorise la croissance d'espèces à croissance lente, dont les conifères, qui sont capables d'accumuler certains nutriments, dont le calcium (Ca), l'azote (N), le phosphore (P) et le potassium (K) (Henry 2002).

Le pin gris (*Pinus banksiana* Lamb.) a l'aire de répartition la plus vaste du Canada et est considéré comme le pin le plus représentatif de ce milieu (Farrar 1995). Cette essence de lumière se développe habituellement après feu sur de grandes superficies. Cette espèce s'est adaptée à cette fréquente perturbation naturelle en conservant ses semences dans des cônes sérotineux dont l'ouverture est déclenchée par la chaleur générée durant un feu. Le pin gris occupe habituellement des stations peu fertiles et sèches comme les eskers, les terrains sableux grossiers, les sols superficiels et les affleurements rocheux (Cayford and McRae 1983). Il peut être retrouvé en peuplement pur ou mélangé avec d'autres essences de lumière comme le bouleau à papier (*Betula papyrifera* Marsh.) et le peuplier faux tremble (*Populus tremuloides* Michx.) et sa strate arbustive est composée en grande partie d'espèces de la famille des Éricacées (*e.g. Vaccinium* spp. ou *Ledum groenlandicum*) (Farrar 1995). Enfin, la vaste majorité des arbres des forêts tempérés et boréales sont colonisées par les champignons ECM (Read 1991). C'est le cas du pin gris dont les racines seraient colonisées à 90% par ces champignons (Visser 1995). Il a d'ailleurs été montré que le nombre de morphotypes ectomycorhiziens augmente progressivement avec l'âge des peuplements de pin gris jusqu'à se stabiliser autour de 122 ans. Une chronoséquence de champignons ECM en fonction de l'âge du peuplement a aussi été établie. Les espèces trouvées dans ces peuplements incluent des espèces pionnières telles que *Coltricia perennis*, des espèces « multi-âges » dont *Suillus brevipes* et des espèces identifiées principalement dans des peuplements matures comme *Lactarius* spp. et *Russula* spp. (Visser 1995).

La capacité des conifères à vivre en milieu difficile tient autant aux qualités de leurs parties aériennes qu'à celles de leurs organes souterrains. Ils disposent de systèmes racinaires multitrophiques hautement dépendant de leur symbiose avec certains champignons telluriques. Les associations les plus communes avec les espèces ligneuses sont réalisées par des champignons supérieurs de l'embranchement des ascomycètes et des basidiomycètes (Molina et al. 1992). Il est actuellement estimé que 5 000 espèces végétales et 20 000 à 25 000 espèces fongiques sont impliquées dans la symbiose ectomycorhizienne (Rinaldi et al. 2008). Elle joue donc un rôle déterminant dans la dynamique écologique des écosystèmes. Les champignons ECM occupent une place capitale dans le maintien de la

fertilité des sols car ils continuent le processus de décomposition après que les bactéries et les actinomycètes aient cessé d'être actifs (Brady 1990). Ils jouent aussi un rôle dans l'altération des minéraux en pénétrant les matériaux solides grâce à leur réseau d'hyphes (Hoffland et al. 2004). Ils affectent aussi les processus de formation d'humus et la stabilisation des agrégats particulièrement dans les sols acides (Walse et al. 1998). La grande diversité fongique et le nombre de fructifications observées en forêt boréale du printemps jusqu'à la fin de l'automne démontrent d'ailleurs que les champignons ECM sont adaptés à leur environnement et qu'ils occupent différentes fonctions écologiques occupées dans ce biome.

Les champignons ectomycorhiziens

Un champignon supérieur est un organisme composé d'un réseau filamenteux d'hyphes microscopiques, appelé mycélium, qui se développe dans les horizons organiques et minéraux du sol. Ces organismes forment soit des associations symbiotiques ou font du saprophytisme sur la matière organique du sol ou du parasitisme sur les racines des plantes supérieures. En forêts tempérées et boréales de même qu'en forêts tropicales et subtropicales, plusieurs espèces de champignons vivent en symbiose avec les arbres en formant des associations ectomycorhiziennes. Lors de ces associations, les champignons ectomycorhiziens (ECM) recouvrent les racines de l'hôte d'un manchon fongique et leurs hyphes pénètrent entre les cellules racinaires du cortex pour former le réseau de Hartig (Figure 1.1). Ce réseau sert d'interface alimentaire où l'hôte apporte à l'endophyte les sucres et autres substances de croissance produits lors de la photosynthèse alors que le champignon favorise, par son système d'hyphes dans le sol, l'absorption d'eau et le prélèvement des nutriments pour l'hôte (Le Tacon 1982; Smith and Read 2008). Cette source de carbone permet à plusieurs champignons ECM de produire des fructifications. La fructification, ou carpophore, est la structure reproductive de ces champignons. Il existe environ 150 000 espèces de champignons supérieurs, mais seules quelques dizaines possèdent des carpophores ayant un intérêt culinaire (Pilz and Molina 2000).

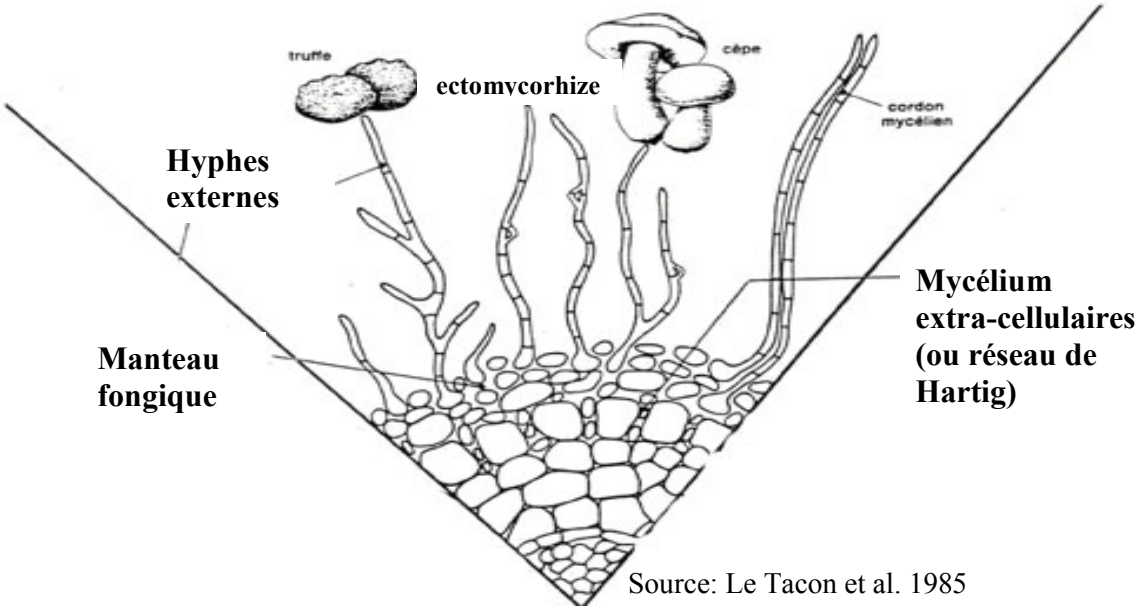


Figure 1.1. Les structures des champignons ectomycorhiziens.

De façon générale, par leur capacité à décomposer les résidus organiques, les champignons sont les plus versatiles et possiblement les plus persistants de tous les micro-organismes du sol. Ils dégradent la cellulose, l'amidon, les résines, la lignine, les protéines, les sucres et plusieurs autres composés simples. Ils sont considérés comme fonctionnant plus efficacement que les bactéries, particulièrement dans les sols acides, car ils transforment une proportion plus élevée de résidus à l'intérieur de leurs propres tissus (jusqu'à 50% comparativement à 20% pour les bactéries) (Brady 1990). De plus, les champignons ECM auraient des propriétés biochimiques et physiologiques qui les rendraient hautement efficaces à récupérer les sources organiques de d'azote (N) et de phosphore (P) dans les horizons de surface du sol, horizons qui seraient favorables au développement des ectomycorhizes en raison de la présence des racines fines de l'hôte, d'humus et d'une concentration élevée en CO_2 (Meyer 1973). Grâce à ces propriétés spécifiques, les champignons ECM limiteraient donc la disponibilité de certains nutriments qui seraient autrement destinées à la communauté des décomposeurs (Read et al. 2004).

Toutefois, ils ne peuvent pas oxyder l'ammonium (NH_4^+) en nitrate (NO_3^-) pas plus qu'ils ne peuvent fixer l'azote (N_2). La spécificité des champignons ECM réside dans le fait

qu'ils doivent obligatoirement utiliser des sucres simples provenant des racines comme source d'énergie. Ils ont cependant conservé une importante capacité saprophytique (i.e. qu'ils sont capables de se nourrir de matière organique morte) (Read et al. 2004). En plus de leur rôle primordial dans la décomposition des résidus végétaux de la forêt boréale, plusieurs champignons permettent à leurs hôtes de survivre grâce à leur capacité à récupérer les nutriments même dans les milieux pauvres (Read and Perez-Moreno 2003). Les plantes dominantes dépendent donc largement de la capacité de leurs partenaires fongiques à récupérer et à absorber les nutriments, de sorte que ces symbioses contrôlent en partie les cycles nutritifs, la productivité, la composition en espèces et le fonctionnement des écosystèmes. En contrepartie, puisque les champignons ECM ne peuvent pas faire de photosynthèse et qu'ils doivent obtenir leurs nutriments d'autres organismes vivants ou morts, les forêts sont essentielles à leur survie et à leur productivité.

La productivité des champignons ECM : impacts anthropiques et naturels

Historiquement, les fructifications des champignons comestibles constituaient une source locale de nourriture et de médicaments alternatifs (Boa 2004), mais depuis le XX^{e} siècle, l'importance de l'industrie du champignon comestible a considérablement augmentée à l'échelle planétaire (Pilz et al. 2002). En Amérique du Nord, les champignons étaient traditionnellement cueillis depuis d'innombrables années, mais la demande s'est considérablement accrue vers la fin des années 1980 (Pilz and Molina 2000). À cette époque la commercialisation des champignons forestiers comestibles a pris de l'ampleur, ce qui a transformé les manières de cueillir, de vendre et de manipuler ces champignons (Amaranthus and Pilz 1996; Arnolds 1995). De nos jours, des milliers de personnes œuvrent dans l'industrie du champignon ; cueilleurs, acheteurs et consommateurs forment un marché alternatif où les échanges totalisent des milliards de dollars annuellement (Pilz et al. 2002). À titre d'exemple, le commerce mondial de la chanterelle rapporterait à lui seul entre 1.25 et 1.67 milliards de dollars chaque année (Hall and Yun 2000; Watling 1997). Depuis ces travaux, cette industrie a connu un essor important. Au Québec, l'abondance de champignons suscite de plus en plus l'intérêt du marché international et plusieurs champignons à haute valeur commerciale sont dorénavant cueillis et vendus. Ce potentiel

commercial a favorisé le développement de projets visant à mieux identifier les conditions environnementales propices à leur fructification (par example Gévry and Villeneuve 2009; Pinna et al. 2010).

À l'échelle planétaire la production naturelle de plusieurs espèces de champignons comestibles a décliné durant le dernier siècle. À titre d'exemples, mentionnons la truffe (*Tuber melanosporum* Vitt.) en France et le Matsutake (*Tricholoma matsutake* (Vittad.) Sacc.) en Asie. La pollution de l'air, les courtes rotations entre les coupes forestières, les coupes à blanc, la destruction de la litière forestière et une cueillette excessive de champignons sont quelques-unes des raisons évoquées pour ce déclin (Arnolds 1991). Divers travaux ont été effectués sur la côte Ouest canadienne et américaine (par example Bergemann and Largent 2000; Kranabetter et al. 2005; Pilz and Molina 2002; Wiensczyk et al. 2002; Wurtz et al. 2005) afin d'éviter qu'un déclin des populations ne survienne dans ces régions suite à l'exploitation intensive de la ressource et afin de mieux comprendre le rôle des champignons, principalement des espèces comestibles, dans les différents écosystèmes terrestres. Ces études ont permis de mieux comprendre la biologie et l'écologie d'espèces à fort potentiel commercial dont le *Tricholoma magnivelare* (Peck) Redhead, la *Cantharellus formosus* Corner et les *Morchella* spp. Une étude sur 30 ans a même démontré qu'une cueillette intensive des champignons ne diminue pas la production future ni la diversité en champignons (Egli et al. 2006). Cependant, malgré les avancées effectuées peu de connaissances ont été acquises concernant l'impact des conditions du site (le sol, le climat), du peuplement forestier (l'âge, la densité, la hauteur) et de l'aménagement forestier (dont les éclaircies) sur la productivité des champignons ECM à long terme (Alexander et al. 2002). En fait, les liens entre la distribution des champignons ECM et les paramètres environnementaux demeurent difficiles à établir puisque les principales variables du sol (pH, matière organique et taux de saturation du complexe argilo-humique) n'interagissent pas seules avec ces champignons (Hansen 1988; O'Dell et al. 1999). En effet, d'importantes variables telles que la capacité d'échange cationique, les caractéristiques de l'humus (Nantel and Neumann 1992), la présence d'aiguilles au sol (Bergemann and Largent 2000), les mousses (Carleton and Read 1991) et l'ouverture de la canopée (Pilz et al. 2006) ont été étudiées et identifiées comme des variables qui favorisent

la fructification des champignons ECM. Finalement, la physiologie, l'écologie, la nutrition, la distribution et la productivité des champignons ECM restent peu connus et il est souvent difficile de savoir si la structure d'une population est reliée à l'hétérogénéité retrouvée sur un micro-site ou aux caractéristiques biologiques et génétiques de l'espèce (Fiore-Donno and Martin 2001).

D'autres études ont plutôt tenté de mesurer l'impact de l'aménagement forestier sur la production épigée et la diversité des champignons comestibles afin de favoriser une utilisation durable de cette ressource (Durall et al. 2006; Kranabetter and Kroeger 2001). Il a d'abord été montré que le prélèvement d'arbres dans un peuplement forestier affecte grandement les autres ressources présentes, dont la productivité fongique et la croissance future des arbres (Alexander et al. 2002; Molina et al. 1993). Durall et al. (1999) ont établi que les coupes partielles, à l'exception des petites trouées, provoquent une diminution immédiate de la richesse ectomycorhizienne, diminution qui peut perdurer plusieurs années. De plus, l'établissement de nouvelles espèces d'arbres suite à une coupe à blanc changerait la composition de la communauté des champignons ECM plutôt que de réduire le pourcentage de racines colonisées (Jones et al. 2003). Pilz et al. (2006) ont souligné que le nombre et le poids des fructifications de *Cantharellus* spp. diminuent de façon significative l'année qui suit une coupe forestière. Enfin, d'autres études ont montré que lorsque des arbres matures sont laissés sur place lors de coupes forestières, une proportion importance des racines ectomycorhizées reste active (Goodman and Trofymow 1998; Harvey et al. 1980). En outre, certaines espèces spécifiques de champignons, dont le champignon pionnier *Thelephora* spp., semblent préférer les peuplements ouverts qui ont été coupés au cours des dernières années (Durall et al. 1999; Smith and Read 2008). Malgré ces études, nos connaissances sur l'écologie des champignons ECM forestiers restent minimes alors que l'importance accordée à la ressource mycologique ne cesse de croître. Dans ce contexte, un plus grand nombre d'études sur les données écologiques des espèces fongiques en relation avec leur environnement s'avèrent donc nécessaire pour guider le développement et l'aménagement durable de cette ressource (Berch et al. 2007).

Finalement, mentionnons que l'absence de carpophores sur un site ne signifie pas nécessairement que le champignon n'y est pas présent. C'est pourquoi il importe de regarder à la fois la formation des carpophores et la présence de racines ectomycorhizées. De plus, il n'existe, à ce jour, qu'une faible correspondance entre les espèces dominantes au niveau des carpophores et la diversité des espèces ectomycorhiziennes présentes sur le système racinaire (Horton and Bruns 2001). Un résumé de l'état des connaissances est présenté ici pour les deux espèces des champignons ECM comestibles faisant l'objet de cette thèse.

Cantharellus cibarius var. *roseocanus* : état des connaissances

Cantharellus cibarius var. *roseocanus* Redhead, Norvell & Danell est un basidiomycète de la famille des Cantharallaceae (Corner 1966). Cette famille se divise en deux genres *Craterellus* et *Cantharellus* (Dahlman et al. 2000). Les chanterelles (*Cantharellus* spp.) sont des champignons ECM qui s'associent de façon symbiotique avec les racines d'une grande variété d'hôtes en Amérique du Nord, dont les bouleaux (*Betula spp*.), les chênex (*Quercus spp.*), les hêtres (*Fagus spp*.), les épinettes (*Picea spp*.), les pins (*Pinus spp*.), les pruches (*Tsuga spp.*), les sapins (*Abies spp.*) et les Pseudotsuga (*Pseudotsuga spp.*) (Pilz et al. 2003). Les différentes espèces de chanterelles ont été trouvées en Europe, dans les trois Amériques, dans le cercle arctique de l'hémisphère nord, en Afrique, dans la chaîne de l'Himalaya et en Thaïlande. Les chanterelles sont d'ailleurs parmi les champignons ECM les plus consommés sur la planète (Danell 1999). À notre connaissance, *Cantharellus cibarius* var. *roseocanus* n'avait jamais été identifiée au Québec avant cette étude et les seules autres mentions connues de la *C. cibarius var roseocanus* proviennent de la côte Pacifique du Canada et des États-Unis (Pacific Forest Centre Herbarium (DAVFP) 2011; Dunham et al. 2003).

La plupart des connaissances actuelles sur le genre *Cantharellus*, incluant *C. cibarius* var. *roseocanus*, proviennent de l'espèce *C. cibarius* Fr. (Danell 1999). Basé sur ces connaissances, il a été établi que les chanterelles produisent annuellement des spores de façon continue sur une période variant de un à deux mois. Les fructifications croissent lentement (2 à 5 cm/mois) et survivent en moyenne 44 jours au sol (Largent and Sime

1995). *Cantharellus cibarius* fructifie surtout dans des peuplements de 20 à 60 ans, mais cela peut varier selon le climat et la rapidité de croissance des arbres hôtes (Tanino et al. 2005). De plus, bien que les thalles de *C. cibarius* soient pérennes et que les premières fructifications apparaissent autour de la mi-juillet et disparaissent à la fin octobre, la production peut varier énormément d'années en années et de sites en sites (Danell 1994)., Les carpophores de *C. cibarius* vivent habituellement dans des sols dérivés de limon, de tilt fluvioglaciaire, de roches sédimentaires ou de granite et préfèrent des sols bien drainés avec un pH avoisinant 4.5 et un contenu en matière organique ne dépassant pas 10% dans les deux premiers cm du sol (Jansen and van Dobben 1987; Rangel-Castro 2001). Cependant, peu d'informations existent quant à la façon dont ces espèces colonisent les sols puisque le mycélium est diffus et que les hyphes ne se regroupent pas pour former des structures visibles (Pilz et al. 2003). Finalement, l'habitat typique de *C. cibarius* consisterait en un sol forestier pauvre en nutriments, bien drainé et recouvert d'une végétation de sous-bois où les mousses et les lichens seraient les espèces végétales dominantes (Jansen and van Dobben 1987).

La production de carpophores de la plupart des champignons ECM est hautement dépendante de la température et des conditions d'humidité du sol (Tyler 1985). Les variations saisonnières et interannuelles de productivité seraient grandement influencées par les conditions météorologiques du milieu. Par exemple, des températures printanières chaudes et un mois de juin pluvieux permettraient une fructification hâtive de *C. cibarius* (Danell 1994) et des températures estivales chaudes seraient corrélées avec une abondance élevée de carpophores de *C. formosus* (Norvell et al. 1996). De plus, une étude de 14 ans en Irlande a montré qu'un haut taux d'humidité durant la période de fructification, en particulier durant la seconde moitié de la saison de croissance des arbres, favoriserait la poursuite de la croissance des basidiomycètes sans qu'ils ne se dessèchent (Eveling et al. 1990). Finalement, il semblerait que lors des années sèches, les peuplements riches en débris de bois présenteraient de meilleures chances de développer des fructifications de chanterelles, puisque l'humidité du sol est retenue par le bois en décomposition (Pilz et al. 2003).

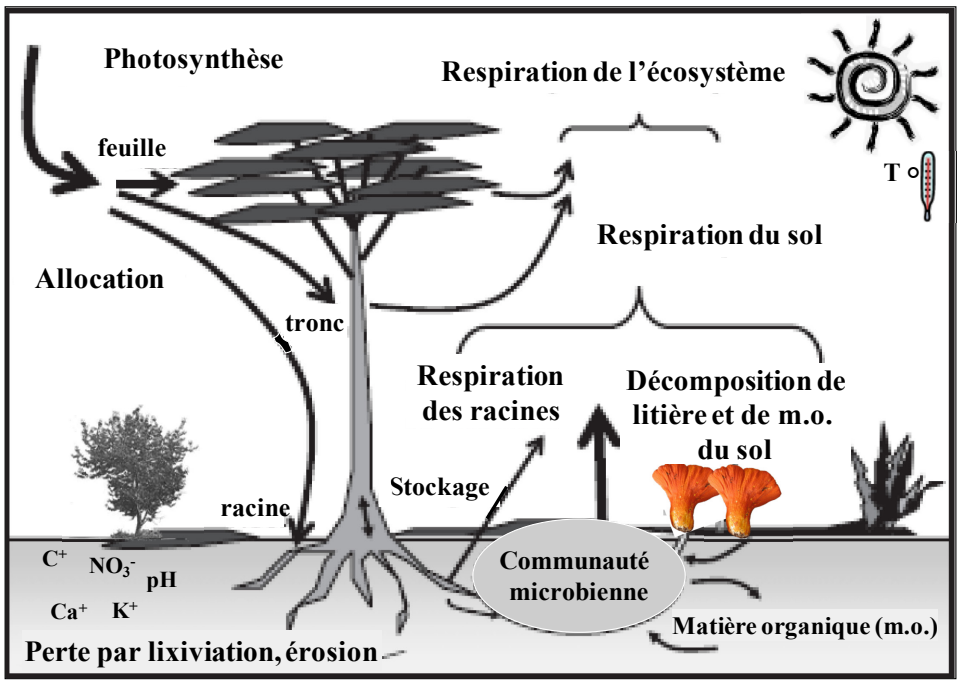
Dermatose des russules : un champignon qui parasite un champignon

La dermatose des russules est un organisme complexe qui résulte de l'infection des basidiomycètes *Russula* sp. ou de *Lactarius* sp. par l'ascomycète *Hypomyces lactifluorum* (Schwein.) Tul. & C.Tul. *Hypomyces lactifluorum* est un champignon de couleur orangée à rouge qui appartient à la famille des Hypocreaceae. Ce genre regroupe des mycoparasites dont treize espèces s'attaquent aux champignons à lames (Rogerson and Samuels 1994). Ces mycoparasites ont tendance à s'attaquer de manière spécifique à une famille ou un genre d'hôte. L'espèce *H. lactifluorum* croît uniquement sur la surface des lactaires et des russules, ce qui hypertrophie le chapeau et déforme le pied et les lames de l'individu parasité (Bessette et al. 1997). En fait, *H. lactifluorum* transforme la surface du chapeau de son hôte en un hyménium d'ascomycète. Ce complexe basidiomycète-ascomycète restera solide durant la libération des ascopores puis il y aura dépérissement graduel de la fructification (Rogerson and Samuels 1994).

Jusqu'à ce jour, dans les grandes populations de *H. lactifluorum*, l'hôte avait pu être identifié comme étant *Russula brevipes* Peck. ou *Lactarius piperatus* (L.) Pers. (Rogerson and Samuels 1994). Plus récemment, *Russula delica* Fr. et *Russula chloroides* (Kromb.) Bres. ont aussi été identifiés par biologie moléculaire (marqueurs spécifiques) comme d'autres hôtes de *H. lactifluorum* (J.M. Moncalvo, conservateur en chef, Royal Ontario Museum, département d'histoire naturelle, Toronto, Canada, comm. pers., 2006). Inconnu en Europe, des mentions du complexe *Russula/Hypomyces* ont été rapportées au Mexique, au Canada, au Guatemala et aux États-Unis (Rogerson and Samuels 1994). Ce parasite est connu pour revenir année après année dans la même population de lactaires ou de russules. Le mécanisme d'infection de ce parasite est encore mal connu. Le parasite pourrait provenir du sol, possiblement infecté, et intégrerait le mycélium du champignon hôte à un très jeune âge par un mécanisme encore méconnu (Hanlin 1963). Ainsi la phase sexuée (téléomorphe) du parasite a été observée dans le sol et sur le champignon parasité, alors que malgré les nombreux essais effectués pour tenter d'observer la germination de l'ascospore, aucun stade asexué (la phase anamorphe) n'est encore connu (Kuo 2011; Rogerson and Samuels 1994).

Les russules *(Russula* spp.), les principales espèces hôtes du parasite *H. lactifluorum*, sont des champignons ECM qui s'associent symbiotiquement avec de nombreuses espèces ligneuses. L'hôte le plus commun, *R. brevipes*, est retrouvés dans les peuplements matures de conifères ou d'espèces mixtes (Durall et al. 2006; Villeneuve et al. 1989). La dermatose des russules se trouve donc principalement dans ces peuplements matures, mais ses carpophores sont typiquement ensevelis sous les débris de conifères (Bessette et al. 1997). De plus, l'apparition de carpophores de cette espèce serait favorisée par le piétinement et la présence de remblais le long des chemins forestiers (Villeneuve 2000).

La Figure 1.2 résume les différents thèmes abordés dans cette introduction : le cycle du carbone dans l'écosystème, la respiration du sol, la phénologie du pin gris, la présence des deux espèces spécifiques de champignons ECM en forêt boréale et l'impact des perturbations anthropiques et des paramètres environnementaux sur la productivité des champignons ECM. Cette introduction est une mise en contexte des chapitres 2,3 et 4 de cette thèse qui tentent d'approfondir les connaissances sur les champignons *H. lactifluorum/R. brevipes* et *Cantharellus cibarius* var. *roseocanus* dans leur environnement naturel.



Adapté de Trumbore 2006

Figure 1.2. Les différents paramètres environnementaux de la forêt boréale et leur relation avec les champignons ECM.

Objectifs et hypothèses de la thèse

L'objectif général de cette thèse est d'étudier l'impact de paramètres environnementaux et d'une perturbation forestière sur deux espèces de champignons ECM, *C. cibarius* var. *roseocanus* et le complexe *R.brevipes/H.lactifluorum,* et de préciser l'importance de la phénologie du pin gris (*P. banksiana*) pour la fructification de *C. cibarius* var. *roseocanus*. Une meilleure compréhension de l'écologie de ces deux espèces enouragera une gestion durable de cette ressource. En effet, mieux définir l'habitat de ces deux espèces, les paramètres environnementaux qui influencent leur présence et leur productivté ainsi que leurs liens avec la phénologie de l'arbre hôte sont des éléments nécessaires à l'identification de zones mycologiques à fort potentiel commercial et à leur intégration dans les aménagements forestiers comme zones d'exploitation, de cueillete ou de conservation de la ressource mycologique.

Le défi principal de cette thèse consistait à travailler avec un dispositif expérimental situé en forêt naturelle afin d'obtenir des données précises et valables sur ces deux

champignons ECM. Cela s'est avéré d'une grande complexité compte tenu des innombrables interactions écologiques, difficilement contrôlables, qui influençaient ces champignons. Afin de répondre à cet objectif général et aux objectifs spécifiques de chaque chapitre, trois études distinctes ont été effectuées sur un même site expérimental situé en peuplement de pin gris de l'est du Québec.

Au chapitre 2, nous avons étudié 54 parcelles de carpophores de dermatoses des russules situées dans deux peuplements de pin gris durant trois saisons de croissance. Les objectifs spécifiques de ce chapitre étaient de (1) déterminer les paramètres écologiques (sol, peuplement, végétation et climat) qui sont reliés à la distribution (présence/absence) et à la productivité (i.e. la biomasse fraîche et la densité) des carpophores de la dermatose des russules, et (2) comparer la productivité de ce champignon dans trois environnements forestiers (sentiers, bandes de forêt entre les sentiers et forêt non aménagée).

Dans le chapitre 3, nous avons étudié 45 parcelles de carpophores de *C. cibarius* var. *roseocanus*, durant les mêmes trois saisons de croissance (2005, 2006 et 2007) et à l'intérieur des mêmes peuplements de pin gris qu'au chapitre 2. L'objectif spécifique du chapitre 3 était de déterminer quels paramètres écologiques de la forêt de pin gris de l'est du Canada influencent la distribution et la productivité de *C. cibarius* var. *roseocanus*. Les hypothèses testées dans ce chapitre étaient que (1) le mycélium de *C. cibarius* var. *roseocanus* se situe dans les horizons organiques du sol en raison de la disponibilité des nutriments, (2) la productivité des carpophores de ce champignon est influencée par des paramètres spécifiques du sol, du peuplement et de la végétation, mais les fluctuations trouvées entre les trois années doivent être attribuées aux variations météorologiques. (3) Les carpophores sont présents dans une éconiche spécifique car ils sont trouvés en association avec certaines micro-conditions du site.

Après avoir identifié les principaux paramètres environnementaux influençant la productivité et la distribution des carpophores de *C. cibarius* var. *roseocanus* dans un peuplement de pin gris au chapitre 3, le chapitre 4 se concentre plus précisément sur les relations qui existent entre la phénologie d'un champignon ECM et de son hôte, en utilisant une chanterelle (*C. cibarius* var. *roseocanus*) et un pin (*P. bansksiana*) comme modèles.

Seules 12 des 45 parcelles préalablement sélectionnées ont été utilisées pour cette expérience. L'objectif de ce dernier chapitre visait à déterminer comment les variations de production de carpophores d'un champignon ECM, la respiration autotrophe du sol et la respiration des carpophores de ce même champignon sont liées à la phénologie de l'arbre hôte. Pour atteindre cet objectif, nous avons considéré la transition du bois juvénile vers le bois mature et les taux de respiration du sol, répartis en respiration totale du sol, respiration autotrophe et respiration des carpophores. Les hypothèses testées dans ce chapitre étaient (1) la transition entre le bois juvénile et mature de l'arbre hôte, qui correspond au moment où un grand nombre de composés photosynthétiques sont transférés aux racines, entraîne un pic de fructification de champignons, (2) Un apport supplémentaire de C dans le sol suite à la transition du bois juvénile vers le bois mature est détecté par une augmentation de la respiration autotrophe du sol et stimule la production de carpophores de *C. cibarius* var. *roseocanus.* (3) la respiration mesurée sur des carpophores de *C. cibarius* var. *roseocanus* suit les variations journalières et saisonnières de la respiration souterraine.

Références

Alexander, S.J., Pilz, D., Weber, N.S., Brown, E., and Rockwell, V.A. 2002. Mushrooms, trees, and money: value estimates of commercial mushrooms and timber in the Pacific Northwest. Environmental Management **30**(1): 129-141.

Allen, M.F. 1991. The Ecology of Mycorrhizae. Cambridge University Press, Cambridge, UK.

Amaranthus, M., and Pilz, D. 1996. Productivity and sustainable harvest of wild mushrooms. *In :* Managing forest ecosystems to conserve fungus diversity and sustain wild mushroom harvests. *Edited by* D. Pilz, and R. Molina. Gen. Tech. Rep. PNW-GTR-371, US Department of Agriculture, Forest Service, Pacific Northwest Research Station, Portland, OR., USA. pp. 42-61.

Andersen, C.P., Nikolov, I., Nikolova, P., Matyssek, R., and Häberle, K.-H. 2005. Estimating "autotrophic" belowground respiration in spruce and beech forests: decreases following girdling. European Journal of Forest Research **124**(3): 155-163.

Arnolds, E. 1991. Decline of ectomycorrhizal fungi in Europe. Agriculture, Ecosystems & Environment **35**(2-3): 209-244.

Arnolds, E. 1995. Conservation and management of natural populations of edible fungi. Canadian Journal of Botany **73**(S1): S987-S998.

Berch, S.M., Ka, K.-H., Park, H., and Winder, R. 2007. Development and potential of the cultivated and wild-harvested mushroom industries in the Republic of Korea and British Columbia. B.C. Journal of Ecosystems and Management **8**(3): 53-75.

Bergemann, S.E., and Largent, D.L. 2000. The site specific variables that correlate with the distribution of the Pacific Golden Chanterelle, *Cantharellus formosus*. Forest Ecology and Management **130**(1-3): 99-107.

Bergeron, O., Margolis, H.A., and Coursolle, C. 2009. Forest floor carbon exchange of a boreal black spruce forest in eastern North America. Biogeosciences **6**(9): 1849-1864.

Bessette, A.E., Bessette, A.R., and Fischer, D.W. 1997. *Hypomyces* and allies. *In :* Mushrooms of northeastern North America. Syracuse University Press, Syracuse, N.Y., USA. pp. 529-532.

Black, T.A., Gaumont-Guay, D., Jassal, R.S., Amiro, B.D., Jarvis, P.G., Gower, S.T., Kelliher, F.M., Dunn, A., and Wofsy, S.C. 2005. Measurement of CO_2 exchange between Boreal forest and the atmosphere. *In :* The carbon balance of forest biomes. *Edited by* H. Griffiths, and P.-G. Jarvis. Taylor & Francis Group, New York, N.Y., USA. pp. 156-195.

Boa, E. 2004. Wild edible fungi: a global overview of their use and importance for people. Food and Agriculture Organization of the United Nations, Non-Wood Forest Products Series No. 17. Rome, Italy.

Borken, W., Savage, K., Davidson, E.A., and Trumbore, S.E. 2006. Effects of experimental drought on soil respiration and radiocarbon efflux from a temperate forest soil. Global Change Biology **12**(2): 177-193.

Brady, N.C. 1990. Organisms of the Soil. 10th edition. *In :* The nature and properties of soils. Macmillan Publishing Company, New York. pp. 253-277.

Carleton, T.J., and Read, D.J. 1991. Ectomycorrhizas and nutrient transfer in conifer-feather moss ecosystems. Canadian Journal of Botany **69**(4): 778-785.

Cayford, J.H., and McRae, D.J. 1983. The ecological role of fire in jack pine forests. *In :* The role of fire in northern circumpolar ecosystems. *Edited by* R.-W. Wein, and D.-A. MacLean. John Wiley & sons, New York, N.Y., USA. pp. 183-199.

Cisneros-Dozal, L.M., Trumbore, S.E., and Hanson, P.J. 2006. Partitioning sources of soil-respired CO_2 and their seasonal variation using a unique radiocarbon tracer. Global Change Biology **12**(2): 194-204.

Corner, E.J.H. 1966. A monograph of Cantharelloid fungi. Oxford University Press, Oxford, UK.

Courty, P-E., Buée, M., Diedhiou, A.G., Frey-Klett, P., Le Tacon, F., Rineau, F., Turpault, M.P., Uroz, S., and Garbaye, J. 2010. The role of ectomycorrhizal communities in forest ecosystem processes: new perspectives and emerging concepts. Soil Biology and Biochemistry **42**(5): 679-698.

Dahlman, M., Danell, E., and Spatafora, J.W. 2000. Molecular systematics of *Craterellus*: cladistic analysis of nuclear LSU rDNA sequence data. Mycol. Res. **104**(4): 388-394.

Danell, E. 1994. Formation and growth of the ectomycorrhiza of *Cantharellus cibarius*. Mycorrhiza **5**(2): 89-97.

Danell, E. 1999. *Cantharellus*. *In :* Ectomycorrhizal fungi: key genera in profile. *Edited by* J.W.G. Cairney, and S.M. Chambers. Springer-Verlag, New York, N.Y., USA. pp. 253-267.

Davidson, E.A., Richardson, A.D., Savage, K.E., and Hollinger, D.Y. 2006. A distinct seasonal pattern of the ratio of soil respiration to total ecosystem respiration in a spruce-dominated forest. Global Change Biology **12**(2): 230-239.

Dunham, S.M., O'Dell, T.E., and Molina, R. 2003. Analysis of nrDNA sequences and microsatellite allele frequencies reveals a cryptic chanterelle species *Cantharellus cascadensis* sp. nov. from the American Pacific Northwest. Mycological Research **107**(10): 1163-1177.

Durall, D.M., Gamiet, S., Simard, S.W., Kudrna, L., and Sakakibara, S.M. 2006. Effects of clearcut logging and tree species composition on the diversity and community composition of epigeous fruit bodies formed by ectomycorrhizal fungi. Canadian Journal of Botany **84**(6): 966-980.

Durall, D.M., Jones, M.D., Wright, E.F., Kroeger, P., and Coates, K.D. 1999. Species richness of ectomycorrhizal fungi in cutblocks of different sizes in the Interior Cedar-Hemlock forests of northwestern British Columbia: sporocarps and ectomycorrhizae. Canadian Journal of Forest Research **29**(9): 1322-1332.

Egli, S., Peter, M., Buser, C., Stahel, W., and Ayer, F. 2006. Mushroom picking does not impair future harvests - results of a long-term study in Switzerland. Biological Conservation **129**(2): 271-276.

Eveling, D.W., Wilson, R.N., Gillespie, E.S., and Bataillé, A. 1990. Environmental effects on sporocarp counts over fourteen years in a forest area. Mycological Research **94**(7): 998-1002.

Fahey, T.J., Tierney, G.L., Fitzhugh, R.D., Wilson, G.F., and Siccama, T.G. 2005. Soil respiration and soil carbon balance in a northern hardwood forest ecosystem. Canadian Journal of Forest Research **35**(2): 244-253.

Farrar, J.L. 1995. Les arbres du Canada. Fides et le Service canadien des forêts, Ottawa, Canada.

Finlay, R., and Söderström, B. 1992. Mycorrhiza and carbon flow to the soil. *In :* Mycorrhizal functioning: an integrative plant fungal process. *Edited by* M. F. Allen. Chapman & Hall, New York, N.Y., USA. pp. 134-160.

Fiore-Donno, A.-M., and Martin, F. 2001. Populations of ectomycorrhizal *Laccaria amethystina* and *Xerocomus* spp. show contrasting colonization patterns in a mixed forest. New Phytologist **152**(3): 533-542.

Fortin, J.A., and Lamhamedi, M.S. 2009. Ecophysiology and sporocarp development of ectomycorrhizal basidiomycetes associated with boreal forest gymnosperms. *In :* Advances in mycorrhizal science and technology. *Edited by* D. P. Khasa, Y. Piché, and A.P. Coughlan. National Reseach Council of Canada, Ottawa, Canada. pp. 161-173.

Gadgil, R.L., and Gadgil, P.D. 1971. Mycorrhiza and litter decomposition. Nature **233**(5315): 133.

Gaumont-Guay, D., Black, T.A., Griffis, T.J., Barr, A.G., Morgenstern, K., Jassal, R.S., and Nesic, Z. 2006. Influence of temperature and drought on seasonal and interannual variations of soil, bole and ecosytem respiration in a boreal aspen stand. Agricultural and Forest Meteorology **140**(1-4): 203-219.

Gévry, M.F., and Villeneuve, N. 2009. Ecology and management of edible mycorrhizal mushrooms in eastern Canada. *In :* Advances in mycorrhizal science and technology. *Edited by* D. P. Khasa, Y. Piché, and A.P. Coughlan. National Research Council of Canada, Ottawa, Canada. pp. 175-191.

Godbout, C., and Fortin, J.A. 1990. Cultural control of basidiome formation in *Laccaria bicolor* with container-grown white pine seedlings. Mycological Research **94**(8): 1051-1058.

Goodman, D.M., and Trofymow, J.A. 1998. Distribution of ectomycorrhizas in micro-habitats in mature and old-growth stands of Douglas-fir on southeastern Vancouver Island. Soil Biology and Biochemistry **30**(14): 2127-2138.

Grace, J. 2005. Role of forest biomes in the global carbon balance. *In :* The carbon balance of forest biomes. *Edited by* H. Griffiths, and P.-G. Jarvis. Taylor & Francis Group, New York, N.Y., USA. pp. 19-45.

Hall, I.R., and Yun, W. 2000. Edible mushrooms as secondary crops in forests. Quarterly Journal of Forestry **94**(4): 299-304.

Hanlin, R.T. 1963. Morphology of *Hypomyces lactifluorum*. Botanical Gazette **124**(6): 395-404.

Hansen, P.A. 1988. Prediction of macrofungal occurrence in Swedish beech forests from soil and litter variable models. Plant Ecology **78**(1-2): 31-44.

Hanson, P.J., Edwards, N.T., Garten, C.T., and Andrews, J.A. 2000. Separating root and soil microbial contributions to soil respiration: a review of methods and observations. Biogeochemistry **48**(1): 115-146.

Harvey, A.E., Larsen, M.J., and Jurgensen, M.F. 1980. Partial cut harvesting and ectomycorrhizae: early effects in Douglas-fir-larch forests of western Montana. Canadian Journal of Forest Research **10**(3): 436-440.

Heinemeyer, A., Hartley, I.P., Evans, S.P., Carreira de la Fuentes, J.A., and Ineson, P. 2007. Forest soil CO_2 flux: uncovering the contribution and environmental responses of ectomycorrhizas. Global Change Biology **13**(8): 1786-1797.

Henry, J.D. 2002. Canada's boreal forest. Smithonian Natural History Series, Smithonian Institution Press, Washington, D.C., USA.

Hobbie, E.A. 2006. Carbon allocation to ectomycorrhizal fungi correlates with belowground allocation in culture studies. Ecology **87**(3): 563-569.

Hoffland, E., Kuyper, T.W., Wallander, H., Plassard, C., Gorbushina, A.A., Haselwandter, K., Holmström, S., Landeweert, R., Lundström, U.S., and Rosling, A. 2004. The role of fungi in weathering. Frontiers in Ecology and the Environment **2**(5): 258-264.

Högberg, P., Nordgren, A., Buchmann, N., Taylor, A.F.S., Ekblad, A., Högberg, M.N., Nyberg, G., Ottosson-Löfvenius, M., and Read, D.J. 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature **411**(6839): 789-792.

Horton, T.R., and Bruns, T.D. 2001. The molecular revolution in ectomycorrhizal ecology: peeking into a black-box. Molecular Ecology **10**(8): 1855-1871.

Jansen, E., and van Dobben, H.F. 1987. Is decline of *Cantharellus cibarius* in the Netherlands due to air pollution? Ambio **16**(4): 211-213.

Janssens, I.A., Lankreijer, H., Matteucci, G., Kowalski, A.S., Buchmann, N., Epron, D., Pilegaard, K., Kutsch, W., Longdoz, B., Grünwald, T., et al. 2001. Productivity overshadows temperature in determining soil and ecosystem respiration across European forests. Global Change Biology **7**(3): 269-278.

Jones, M.D., Durall, D.M., and Cairney, J.W.G. 2003. Ectomycorrhizal fungal communities in young forest stands regenerating after clearcut logging. New Phytologist **157**(3): 399-422.

Kozlowski, T.T. 1992. Carbohydrate sources and sinks in woody plants. The Botanical Review **58**(2): 107-222.

Kranabetter, J.M., Friesen, J., Gamiet, S., and Kroeger, P. 2005. Ectomycorrhizal mushroom distribution by stand age in western hemlock – lodgepole pine forests of northwestern British Columbia. Canadian Journal of Forest Research **35**(7): 1527-1539.

Kranabetter, J.M., and Kroeger, P. 2001. Ectomycorrhizal mushroom response to partial cutting in a western hemlock - western redcedar forest. Canadian Journal of Forest Research **31**(6): 978-987.

Kuo, M. 2003. *Hypomyces lactifluorum*: the lobster mushroom. Retrieved from the MushroomExpert.Com Web site: www.mushroomexpert.com/hypomyces_lactifluorum.html [accessed April 15 2011].

Kuzyakov, Y., and Gavrichkova, O. 2010. Time lag between photosynthesis and carbon dioxide efflux from soil: a review of mechanisms and controls. Global Change Biology **16**(12): 3386-3406.

Lamhamedi, M.S., Godbout, C., and Fortin, J.A. 1994. Dependence of *Laccaria bicolor* basidiome development on current photosynthesis of *Pinus strobus* seedlings. Canadian Journal of Forest Research **24**(9): 1797-1804.

Largent, D.L., and Sime, A.D. 1995. A preliminary report on the phenology, sporulation and lifespan in *Cantharellus cibarius* and *Boletus edulis* basidiomes in Patrick's Point State Park. *In :* Symposium proceedings, 43rd annual meeting of the California Forest Pest Council. *Edited by* D.H. Adams, J.E. Rios and A.J. Stere. Sacramento, CA., USA. Le Tacon, F. 1982. Perspectives de la maîtrise de la mycorhization en sylviculture. *In :* Les mycorhizes, partie intégrante de la plante: biologie et perspectives d'utilisation. *Edited by* S. Gianinazzi, V. Gianinazzi-Pearson, and A. Trouvelot. Proceedings of a seminar, 5–6 May 1982 at Dijon, France, les Colloques de l'INRA, no. 13. pp. 273–285.

Le Tacon, F., Jung, G., Mugnier, J., Michelot, P., and Mauperin, C. 1985. Efficiency in a forest nursery of an ectomycorrhizal fungus inoculum produced in a fermentor and entrapped in polymeric gels. Canadian Journal of Botany **63**(9): 1664-1668.

Meyer, F.H. 1973. Distribution of ectomycorrhizae in native and man-made forests. *In :* Ectomycorrhizae, their ecology and physiology. *Edited by* G.C. Marks, and T.T. Kozlowski. Academic Press, New York, N.Y., USA. pp. 79–106.

Molina, R., Massicotte, H., and Trappe, J.M. 1992. Specificity phenomena in mycorrhizal symbiosis: community-ecological consequences and practical implications. *In :* Mycorrhizal functioning: an integrative plant fungal process. *Edited by* M. F. Allen. Chapman & Hall, New York, N.Y., USA. pp. 357-423.

Molina, R., O'Dell, T.E., Luoma, D., Amaranthus, M., Castellano, M., and Russell, K.M. 1993. Biology, ecology, and social aspects of wild edible mushrooms in the forests of the Pacific Northwest: a preface to managing commercial harvest. Gen. Tech. Rep. PNW-GTR-309, US Department of Agriculture, Forest Service, Pacific Northwest Research Station, Portland, OR., USA.

Moore, R., Clark, W.D., and Vodopich, D.S. 1998. Biomes. *In :* Botany. WCB/McGraw-Hill, New York, N.Y., USA. pp. 783-804.

Nantel, P., and Neumann, P. 1992. Ecology of ectomycorrhizal-basidiomycete communities on a local vegetation gradient. Ecology **73**(1): 99-117.

Norvell, L., Roger, J., Lindgren, J., and Kopecky, F. 1996. Study 8: Oregon *Cantharellus* study project, 1986-1996. *In :* Managing forest ecosystems to conserve fungus diversity and sustain wild mushroom harvests. *Edited by* D. Pilz, and R. Molina. Gen. Tech. Rep. PNW-GTR-371, US Department of Agriculture, Forest Service, Pacific Northwest Research Station, Portland, OR., USA. pp. 69-72.

O'Dell, T.E., Ammirati, J.F., and Schreiner, E.G. 1999. Species richness and abundance of ectomycorrhizal basidiomycete sporocarps on a moisture gradient in the *Tsuga heterophylla* zone. Canadian Journal of Botany **77**(12): 1699-1711.

Pacific Forest Centre Herbarium (DAVFP). 2011. Forest Pathology Herbarium. Available: www.pfc.cfs.nrcan.gc.ca/biodiversity/herbarium/index_e.html [accessed April 02 2011].

Pilz, D., and Molina, R. 2000. Wild edible mushroom research and monitoring in the Pacific Northwest United States. *In :* Les champignons forestiers: récolte, commercialisation et conservation de la ressource. *Edited by* J.A. Fortin, and Y. Piché. CRBF, Université Laval, Québec, Canada, 22 et 23 février 1999. pp. 7-11.

Pilz, D., and Molina, R. 2002. Commercial harvests of edible mushrooms from the forests of the Pacific Northwest United States: issues, management, and monitoring for sustainability. Forest Ecology and Management **155**(1-3): 3-16.

Pilz, D., Molina, R., Danell, E., Waring, R., Rose, C., Alexander, S., Luoma, D., Cromack, K.J., and Lefevre, C. 2002. SilviShrooms: predicting edible mushroom productivity using forest carbon allocation modelling and immunoassays of ectomycorrhizae. *In :* Proceedings of the Second International Conference on Edible Mycorrhizal Mushrooms: edible mycorrhizal mushrooms and their cultivation. 3-6 July 2001, Christ Church, New Zeland. *Edited by* I.R. Hall, Y. Wang, A. Zambonelli, and E. Danell, [CD-ROM]. New Zealand Institute for Crop & Food Research Limited, Christ Church, New Zealand.

Pilz, D., Molina, R., and Mayo, J. 2006. Effects of thinning young forests on chanterelle mushroom production. Journal of Forestry **104**(1): 9-14.

Pilz, D., Norvell, L., Danell, E., and Molina, R. 2003. Ecology and management of commercially harvested chanterelle mushrooms. Gen. Tech. Rep. PNW-GTR-576, US Department of Agriculture, Forest Service, Pacific Northwest Research Station, Portland, OR., USA.

Pinna, S., Gévry, M.F., Côté, M., and Sirois, L. 2010. Factors influencing fructification phenology of edible mushrooms in a boreal mixed forest of Eastern Canada. Forest Ecology and Management **260**(3): 294-301.

Rangel-Castro, J.I. 2001. Eco-physiology of the ectomycorrhizal mushroom *Cantharellus cibarius*: carbon and nitrogen metabolism, and the interactions with bacteria and mycophagous inverterbrates. M.Sc. thesis. Department of Forest Mycology and Pathology, Sweden University of Agricultural Sciences, Uppsala, Sweden.

Read, D.J. 1991. Mycorrhizas in ecosystems. Cellular and Molecular Life Sciences **47**(4): 376-391.

Read, D.J., Leake, J.R., and Perez-Moreno, J. 2004. Mycorrhizal fungi as drivers of ecosystem processes in heathland and boreal forest biomes. Canadian Journal of Botany **82**(8): 1243-1263.

Read, D.J., and Perez-Moreno, J. 2003. Mycorrhizas and nutrient cycling in ecosystems - a journey towards relevance? New Phytologist **157**(3): 475-492.

Rinaldi, A.C., Comandini, O., and Kuyper, T.W. 2008. Ectomycorrhizal fungal diversity: separating the wheat from the chaff. Fungal Diversity **33**(1): 1-45.

Rogerson, C.T., and Samuels, G.J. 1994. Agaricicolous species of *Hypomyces*. Mycologia **86**(6): 839-866.

Rousseau, J.V.D., and Reid, C.P.P. 1989. Measurement of carbon cost in ectomycorrhizae. *In :* Applications of continuous and steady-state methods to root biology. *Edited by* J.G. Torrey, and L.J. Winship. Kluwer Academic Publishers, Dordrecht, The Netherlands. pp. 183-196.

Schimel, D.S. 1995. Terrestrial ecosystems and the carbon cycle. Global Change Biology **1**(1): 77-91.

Schuur, E.A.G., and Trumbore, S.E. 2006. Partitioning sources of soil respiration in boreal black spruce forest using radiocarbon. Global Change Biology **12**(2): 165-176.

Scott-Denton, L.E., Rosenstiel, T.N., and Monson, R. K. 2006. Differential controls by climate and substrate over the heterotrophic and rhizospheric components of soil respiration. Global Change Biology **12**(2): 205-216.

Smith, S.E., and Read, D.J. 2008. Mycorrhizal symbiosis, 3rd ed. Academic Press, London, UK.

Tang, J., Baldocchi, D.D., and Xu, L. 2005. Tree photosynthesis modulates soil respiration on a diurnal time scale. Global Change Biology **11**(8): 1298-1304.

Tanino, K.K., Ivanochko, G., Jessup, C., Nelson, J., and Hrycan, W. 2005. Stage I: sustainable harvest of wild mushrooms in Nothern Saskatchewan: final report. Saskatchewan Agriculture Development Fund, University of Saskatchewan, Saskatoon, SK., Canada.

Trumbore, S.E. 1993. Comparison of carbon dynamics in tropical and temperate soils using radiocarbon measurements. Global Biogeochemical Cycles **7**(2): 275-290.

Trumbore, S.E. 2006. Carbon respired by terrestrial ecosystems - recent progress and challenges. Global Change Biology **12**(2): 141-153.

Tyler, G. 1985. Macrofungal flora of Swedish beech forest related to soil organic matter and acidity characteristics. Forest Ecology and Management **10**(1-2): 13-29.

Villeneuve, N. 2000. Diversité et productivité des champignons forestiers: les apports de la recherche et de l'inventaire. *In :* Les champignons forestiers: récolte, commercialisation et conservation de la ressource. *Edited by* J. A. Fortin, and Y. Piché. CRBF, Université Laval, Québec, Canada, 22 et 23 février 1999. pp. 91-100.

Villeneuve, N., Grandtner, M.M., and Fortin, J.A. 1989. Frequency and diversity of ectomycorrhizal and saprophytic macrofungi in the Laurentide Mountains of Quebec. Canadian Journal of Botany **67**(9): 2616-2629.

Visser, S. 1995. Ectomycorrhizal fungal succession in jack pine stands following wildfire. New Phytologist **129**(3): 389-401.

Walse, C., Berg, B., and Sverdrup, H. 1998. Review and synthesis of experimental data on organic matter decomposition with respect to the effect of temperature, moisture, and acidity. Environmental Reviews **6**(1): 25-40.

Watling, R. 1997. The business of fructification. Nature **385**(6614): 299-300.

Whittaker, R.H. 1970. Communities and ecosystems. Macmillan Company, New York, N.Y. USA.

Wiensczyk, A.M., Gamiet, S., Durall, D.M., Jones, M.D., and Simard, S.W. 2002. Ectomycorrhizae and forestry in British Columbia: a summary of current research and conservation strategies. B.C. Journal of Ecosystems and Management **2**(1): 1-20.

Wurtz, T.L., Wiita, A.L., Weber, N.S., and Pilz, D. 2005. Harvesting morels after wildfire in Alaska. Research Note PNW-RN-546, US Department of Agriculture, Forest Service, Pacific Northwest Research Station, Portland, OR., USA.

Chapitre 2. Ecology and management of the lobster mushroom in an eastern Canadian jack pine stand

Cet article a été publié en langue anglaise dans la revue *Canadian Journal of Forest Research*. L'usage du point décimal est donc de rigueur.

Rochon, C., Paré, D., Khasa, D.P., and Fortin, J.A. 2009. Ecology and management of the lobster mushroom in an eastern Canadian jack pine stand. Canadian Journal of Forest Research **39**(11): 2080-2091.

Résumé

La dermatose des russules, un organisme résultant de l'infection de *Russula* spp. par *Hypomyces lactifluorum* (Schwein.) Tul. & C.Tul., est commune dans les forêts boréales canadiennes et possède un bon potentiel commercial. Établie dans un peuplement de *Pinus banksiana* Lamb. aménagé pour la production de champignons, cette étude visait à : (1) documenter la productivité de carpophores (densité, biomasse par unité de surface) durant trois saisons, (2) comparer la productivité dans trois environnements forestiers (sentiers, bandes de forêt entre les sentiers et forêt non aménagée), (3) établir les paramètres écologiques reliés à la productivité, et (4) caractériser les microhabitats où les carpophores sont présents à l'aide de descripteurs du sol et de la végétation. La densité du champignon avait tendance à être plus élevée dans les sentiers que sous couvert, mais la biomasse fraîche était plus élevée dans les bandes de forêt, sauf en 2006, année où les précipitations ont été faibles au milieu de l'été. L'aménagement de sentiers n'a pas augmenté la production du champignon mais a permis de la maintenir durant les périodes de faibles précipitations. La productivité était positivement reliée à l'abondance des espèces de plantes intolérantes à l'ombre et à l'ammonium extractible et négativement reliée au pH du sol. Dans le cadre de cette étude, les microhabitats dans lesquels des carpophores étaient présents avaient un pH acide, une disponibilité élevée en phosphore, une faible présence de *Kalmia angustifolia* L. et de petites trouées dans le couvert végétal occupées par des espèces intolérantes à l'ombre. Cette étude constitue un premier pas vers la compréhension de l'écologie de la dermatose des russules et des impacts des pratiques forestières sur ce champignon.

Abstract

The lobster mushroom, an organism resulting from the infection of *Russula* spp. by *Hypomyces lactifluorum* (Schwein.) Tul. & C.Tul., is common to Canadian boreal forests and has good commercial potential. Within a *Pinus banksiana* Lamb. stand managed for mushroom production, this study aimed to: (1) document carpophore productivity (density, biomass per area) during three seasons, (2) compare productivity among three forest conditions (trails, forest strips between trails and unmanaged forest), (3) establish ecological parameters related to productivity, and (4) define microhabitats where carpophores are present by using soil and vegetation descriptors. Mushroom density tended to be higher on the trails than under the canopy, but fresh biomass was higher in forest strips except in 2006 when midsummer precipitation was low. Trail management did not increase mushroom production but maintained it during periods of reduced precipitation. Productivity was positively related to the abundance of shade-intolerant plant species and to extractable ammonium, and negatively related to soil pH. Within the present study conditions, microhabitats suitable for the presence of carpophores had low pH, high available phosphorus, low *Kalmia angustifolia* L. presence, and small canopy gaps with shade-intolerant species. This study was the first step toward understanding the ecology and impacts of forest practices on the lobster mushroom.

Introduction

The world market for wild edible forest ectomycorrhizal (ECM) fungi was estimated at $2.5 billion in 2002 and is rapidly growing (Yun and Hall 2004). Among the species of interest is the lobster mushroom, a common boreal forest mushroom with high potential commercial value. This mushroom results from parasitism by *Hypomyces lactifluorum* (Schw. ex Fr.) Tul., an ascomycete (Nectriaceae; Hypocreales), on *Russula* spp. or *Lactarius* spp. *Hypomyces lactifluorum* is an orange to reddish orange fungus that grows on the host basidiocarp, deforming its cap, stalk, and gills (Bessette et al. 1997). Host species have been attributed to *Lactarius piperatus* (Scop. ex Fr.) S. F. Gray and most commonly to *Russula brevipes* Pk. (Rogerson and Samuels 1994). However, recently, using molecular markers, two other host species were identified, *Russula delica* Fr. and *Russula chloroides* (Krombh.) Bres. (Rizvi et al. 2007). The distribution of the *Russula-Hypomyces* complex is limited to North America; it has been recorded in every provinces of Canada, as well as in several parts of the United States, Mexico, and Guatemala (Rogerson and Samuels 1994).

Russula spp. hosting the parasite are ECM fungi symbiotically associated with the roots of a large number of tree species, where both partners take advantage of the association. For example, *R. brevipes* tree hosts are members of the genera Betula, Picea, Pinus, Populus, Quercus, and possibly others (Gabel et al. 2004). Since ECM fungi depend on neighbouring host trees for the development and production of their sporocarps, some studies have demonstrated that harvesting the trees has negative impacts on ECM sporocarp formation (Kranabetter and Kroeger 2001) and that ECM richness decreases as the distance from intact forest increases (Durall et al. 1999). On the other hand, other studies have shown that in partial cutting where mature trees are deliberately left on site, a high number of active ECM root tips are maintained (e.g. Harvey et al. 1980). Furthermore, some specific mushroom types seem to prefer open stands that have been logged in the past (Smith and Read 2008).

Fungi respond to main soil attributes, such as pH, percent organic matter, and base saturation, and also to other soil variables present in lower concentration, such as nitrogen (N) mineralization (Hansen 1988). Important variables such as exchangeable acidity, duff

depth, humus and needle cover (Bergemann and Largent 2000), moss abundance and composition (Amaranthus and Russell 1996), and canopy cover (Pilz et al. 2006), have been established as important variables related to mushroom fructification. While progress has been made through these studies, it is not yet well understood how site conditions (soil fertility, climate), stand conditions (age, density, growth rate), and management activities affect mushroom productivity over time (Pilz et al. 2002). The knowledge gap on the ecology of wild edible mushrooms could obstruct efforts to manage this resource (Amaranthus and Pilz 1996).

As for the lobster mushroom, it is usually harvested in natural forests without any attempts to improve its productivity. The present study took advantage of a commercial mushroom harvesting operation where 42 ha of a jack pine stand are managed to maximize valuable sporocarp productivity, facilitate harvesting, and assure sustainability of the resource. This offered a unique opportunity to conduct this study.

This research aims to characterize, within a jack pine stand, the ecological conditions related to lobster mushroom presence and productivity, including fresh sporocarp biomass and sporocarp density. It also intends to determine the impact of a specific forest thinning operation on mushroom fructification in order to propose silvicultural treatments likely to improve production, harvesting and sustainability of this resource. Specific objectives of this study were to (1) document the productivity (fresh mushroom biomass and density per area) during three consecutive seasons, (2) compare mushroom productivity among three forest conditions (trails and forest strips in a managed stand and an unmanaged stand), (3) determine the ecological parameters (soil, stand, vegetation and meteorological) related to productivity, and finally (4) find soil and vegetation micro-site conditions related to the presence and absence of carpophores.

The host mushroom, *R. brevipes*, is found predominantly in mature stands (Villeneuve et al. 1989), and it is usually found partially buried in conifer debris even when infected by *H. lactifluorum* (Bessette et al. 1997). Based on our observations on species site preferences, we hypothesized that in eastern Canada, lobster mushroom sporocarps occupy

a specific econiche that could consist of woody debris, open canopy, sandy soil and specific plant associations, including *Pinus banksiana* Lamb. and *Picea mariana* (Mill.) Britton, Sterns & Poggenb., but excluding invasive and soil nutrient competitive plants, such as *Kalmia angustifolia* L. Because these conditions are mainly found on forest trails, we suggest that lobster mushroom fructification can be favoured by trail development (logged forest strips of 3.5 m x 200 m).

Materials and methods

Site descriptions

This study was conducted in two slightly different jack pine stands of the boreal forest near Girardville, Québec, Canada (49°07'N; 72°35'W) (Figure 2.1). The first stand was located in the Domaine de la Rivière Mistassini private forest. For the purpose of this paper, this site was named the "managed stand". The second stand was established on public land, 3 km north of the first stand and will be referred to as the "unmanaged stand". With an elevation ranging from 150 to 250 m above sea level, the mean annual temperature and precipitation recorded are 0.80 °C and 1015 mm respectively. These climatic data were computed by the algorithms in the BioSIM model (Régnière and St-Amant 2007).

Stands were located within the continuous boreal forest sub-zone where, according to the BioSIM model, the average number of degree days (>5 °C) is 1516. Vegetation in this sub-zone was mainly composed of mature softwood species and light-seeking hardwoods. Both stands studied were dominated by jack pine (*Pinus banksiana*), but while the managed forest naturally regenerated following a wildfire in 1945, the unmanaged forest was logged 40 years ago. The soil of both stands was formed on a coarse sand fluvial deposit and was classified as an Orthic Humo-Ferric Podzol with a mor humus layer varying in depth from 0.0 to 11.0 cm. Jack pine occurs extensively in the Canadian boreal forest, often on droughty, nutrient-poor, sandy soils (Farrar 1995). Stand characteristics are presented in Table 2.1.

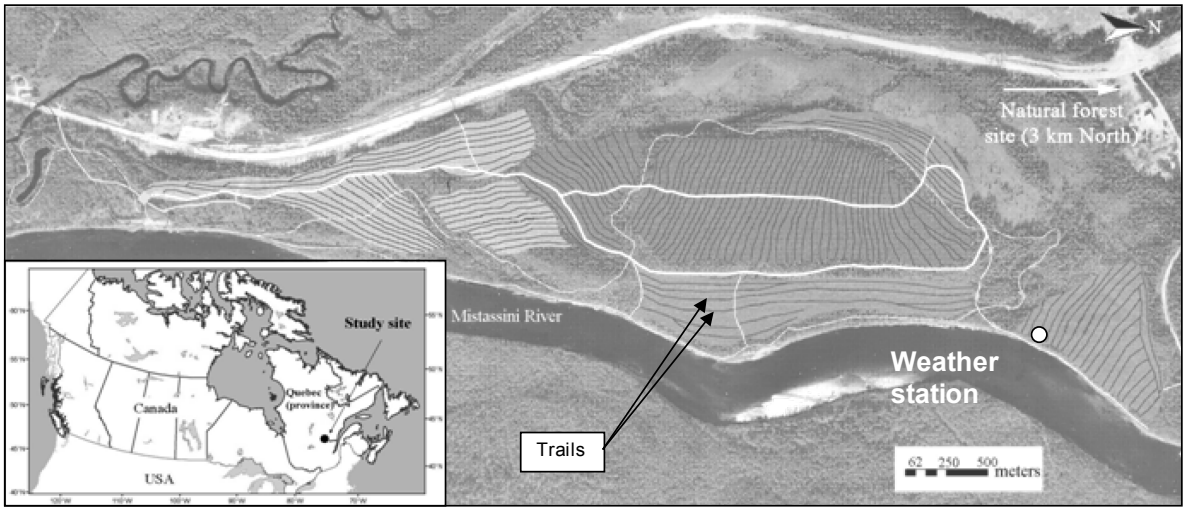


Figure 2.1. Aerial photograph showing the managed stand studied in the Lac Saint-Jean region, Québec, Canada. The unmanaged stand is situated 3 km north of the managed stand. Arrows point to harvesting trails. The zone between the arrows (between the trails) is a forest strip. ○, Weather station location.

Site management and silvicultural treatment

Observation plots were established in 2005 in the two above mentioned stands. In the managed stand during the fall of 2004 and the spring of 2005, selective logging was conducted in strips that were 3.5 m wide and 15 m apart. This generated approximately 175 trails (approximately 200 m long x 3.5 m wide) totalling 35 km of trails, on an overall area of 12.3 ha. Boles were harvested and branches were chipped and spread on trails. The other stand was left unmanaged for the last 40 years. These two stands allowed us to compare lobster mushroom fructification on a natural site with that on a site that was managed to facilitate mushroom harvesting.

Table 2.1. Specific locations and stand characteristics of the two sites studied in the eastern Canadian boreal forest from 2005 to 2007.

Site No.	Site name	Size of the site	Latitude	Longitude	Vegetation type	No. of trees /ha	Stand age (years)	Stand average height (m)	Stand average DBH (cm)
1	Managed stand	2.0 km^2	49°07'N	72°35'W	*P. banksiana*	489	62	16.2	19.0
2	Unmanaged stand	2.4 km^2	49°08'N	72°36'W	*P. banksiana*	628	40	15.7	18.8

In 2005, approximately 150 locations containing fruit bodies of lobster mushrooms were found within the study area, 24 were found in the unmanaged stand, 49 on the trails of the managed stand, 41 on forest strips in the managed stand and 36 at the edge between trails and forest. The edge was a 60 cm zone at the border of the trails and the forest strips, which served as a buffer between the two locations. To ensure a clear separation between trails and forest strips, plots found along the edge were not kept as observation plots. Within these locations, 54 lobster mushroom colonies at least 20 m apart were randomly chosen to establish observation plots of 5 m^2 (2.25 m x 2.25 m). Eleven plots were located in the unmanaged forest, 24 on the managed forest trails, and 19 on forest strips of the managed forest. In addition, 62 control plots, without fruit bodies, were equally located within the two stands, since one of the objectives was to define factors linked with mushroom presence-absence. These were randomly selected to be representative of the study site. To avoid the presence of the lobster mushroom in the control plots, 13 control plots were eliminated over the 2006 and 2007 harvesting seasons because of fruit bodies presence within 10 m of those plots. In total, 54 lobster mushroom plots and 49 control plots (a total of 103 observation plots) were established over the entire study site. These plots were surveyed over three full growing seasons (2005, 2006, and 2007), starting in mid-June until the end of September each year, except in 2005 when observations started at the end of July. The unmanaged site was partially logged during the spring of 2007, which resulted in the removal of three lobster mushroom plots the third year of the project.

Fruit body characteristics

Total numbers of lobster mushroom fruit bodies found inside plots and their degree of maturity were recorded every week over the three growing seasons. For 10 fruit bodies, observations were conducted to locate the mushroom point of insertion at the base of its stipe in the soil profile. Every two weeks, all fruit bodies were harvested to measure the average size of fruit bodies (cm per m^2), fresh and dry mushroom biomass (average sporocarp mass (g)/m^2), mushroom density (number of fruit bodies/m^2), and moisture content of fruit bodies (%). However, during the 2005 growing season, only half of the fruit bodies were harvested, the other half were used to determine the mushroom life-span, calculated from the first day of fruit body appearance until its complete disappearance. The results obtained for fresh and dry biomass and for fruit body average size followed similar patterns. Therefore, only the results of fresh biomass are presented, since it is the value of interest for commercial harvesters (Pilz et al. 2006). Moreover, because the moisture content data did not vary significantly (mean of 89%), results will not be presented. Hence, for the purpose of this study, productivity can be described by two parameters, namely fresh mushroom biomass and mushroom density.

Inventory of the managed stand mushroom production

To evaluate the 2007 overall production of the managed stand and to validate the productivity results obtained between forest conditions (trails and forest strips) in plots, an inventory covering a large area was conducted. Seventeen transects (7 m wide by 200 m long) were chosen randomly then established across the managed stand. Fruit bodies were harvested weekly, counted, and weighed, distinguishing the ones found directly on the managed forest trails from those located between the trails. The transects chosen represented approximately 7% of the managed territory, and data obtained were extrapolated to the entire managed stand.

Ecological parameters

Soil sampling

In 2005, mineral and organic soil cores (0-10 cm of the soil layer) measuring 10 cm x 10 cm were taken in each plot, and organic matter thickness was recorded to the nearest centimetre. To reduce variability imposed by the weather, soil samples were taken within 2 days during the second week of August. Because of the high number of plots used in this study, only one core per plot was taken in one harvesting season. Assuming that potential seasonal fluctuations would not hide differences between tree species, soil type, or stand age, one core per plot was considered sufficient to give a general idea of soil characteristics (Bauhus et al. 1998).

Samples were frozen at -20 °C until analysis. Organic layer samples were sieved at 4 mm and mineral soil at 2 mm to remove coarse woody debris and roots. Moisture content was then determined on subsamples by drying to constant mass at 65 °C. Other subsamples were dried at room temperature and pH was determined in distilled water and in KCl solutions (Carter 1993). Mineral N (NO_3^- and NH_4^+) concentrations were determined from a 2 mol/L KCl extraction, using flow injection analysis for quantification (Tecator FIA Star 5020) (Keeney and Nelson 1982). Bray-2 extractable P (phosphorus) was analyzed spectrophotometrically (Bray and Kurtz 1945). Exchangeable cations (sodium, manganese, iron, aluminum, potassium, magnesium and calcium) were extracted with 0.1 mol/L $BaCl_2$ and their concentrations were determined by atomic absorption (Hendershot and Duquette 1986). The sum of these cations was considered as the cation exchange capacity (CEC). To assess carbon:nitrogen ratio (C:N), total C and N concentrations were determined on a CNS analyzer (Leco Corporation) (Kowalenko 2001). Soil texture (sand, silt, and clay percentage) was determined with the hydrometer method.

Vegetation characterization

On each 2.25 m x 2.25 m plot, the percentages (%) of ground cover by lichens, mosses, and individual species of herbs, shrubs, and trees, as well as the cover of epigeous woody

debris were determined and all plant species were recorded (Bonham 1989). Those percentages of vegetation cover are referred to as vegetation variables.

Stand characteristics, such as average tree diameter (cm), basal area (m^2/ha) and tree density (trees/ha) were assessed in a radius of 5 m around each plot with a diameter at breast height (DBH) tape. Only trees with a DBH greater than 7 cm were considered and recorded to the nearest centimetre (Bonham 1989). Basal area for all tree species was determined for each plot, using the equation proposed by Nissen and Midmore (2002). The height of trees with diameters greater than 7 cm was estimated with a Suunto clinometer to the nearest 0.1 m. Tree age was estimated at 60 years, the age of the last wildfire, for the managed stand and at 40 years for the unmanaged stand based on 10 randomly selected trees (Bonham 1989). Canopy cover percentages (expressed by the opening of the canopy and the percentage of transmissible light) were obtained for each plot from photographs of the canopy taken with a fish-eye lens mounted on a 35 mm camera located at the centre of the plot at 1 m height. The scientific image processing software Gap Light Analyser Version 2.0 was used to process and analyze the digital hemispheric canopy images (Frazer et al. 1999). The software extracts canopy structure data (gap fraction, canopy openness, effective leaf area index (LAI)) and gap light transmission indices.

To identify the ecological conditions that are related to mushroom productivity (fresh mushroom biomass and mushroom density) and lobster mushroom microhabitat (comparison between plots with and without lobster mushroom fruit bodies), soil, stand, and vegetation parameters were used.

Meteorological parameters

A portable field meteorological station was installed in the managed stand (Figure 2.1). General meteorological parameters (air temperature (°C) at ground level, mineral soil temperature (°C) at 5 cm depth, total rainfall (mm), and soil relative humidity (%) at 12 cm depth) were measured from this meteorological station. The portable meteorological station took measurements every 5 min, except for rainfall. Data were taken on the managed forest trails and between the trails from mid-June to the end of September, over the 3-year project.

However, some data were missing from May to August 2005 and for July and August 2006. Data were gathered using records from five meteorological stations situated 21-62 km away from our study site. Microhabitat variables for precise information on each plot were alse gathered. Watchdog temperature data loggers, placed in the middle of the plots, captured soil temperatures at 5 cm depth every hour and were kept in place over the entire length of the project (except from September 24, 2006 to June 15, 2007). Moreover, as data loggers could not be installed in every plot, soil temperature was also monitored weekly by manually inserting a probe to 5 cm depth. Soil relative humidity was also taken weekly inside each plot at 12 cm depth with a Time Domain Reflectometry (TDR) logger 300 (Spectrum Technologies).

Statistical analysis

To achieve homoscedasticity and normality of the residuals, log or square root transformation of data was applied when necessary. A rank transformation was applied for the 2005 mushroom density data, since none of the regular transformations yielded normality of data.

The experimental design was a two-stage nested design because the observation plots were nested in the locations (unmanaged stand, trails, and forest strips). The control plots were not included in these analyses. The statistical model can be defined as follows:

[1] $y_{ijk} = \mu + \tau_i + \beta_{j(i)} + \varepsilon_{(ij)k}$

where y_{ijk} is the response for observation plots j in i locations during interval k; μ is the overall mean; τ_i is the location effect; $\beta_{j(i)}$ is the observation plot effect in the location; and $\varepsilon_{(ij)k}$ is the residual error.

To compare fresh mushroom biomass and mushroom density in different locations (unmanaged stand, trails and forest strips in the managed stand) over the 3-year study, an analysis of variance with repeated measurement of a two-stage nested design followed by LSD comparison tests were used on the observation plots (n = 54). Additionally, to evaluate the 2007 fresh mushroom biomass and mushroom density obtained during the managed stand mushroom inventory, analysis of variance followed by T-tests was used to

compare those parameters on the managed forest trails and in the zone between the trails. Analyses of variance were performed using a mixed-model analysis (SAS Proc Mixed SAS, version 9.1 (SAS Institute Inc. 2004)).

The relationships between lobster mushroom fresh biomass and density, with soil, stand, vegetation, and meteorological parameters, were explored using stepwise multiple regressions and redundancy analyses. The significance level chosen for all tests was α = 0.05 and n = 54. Stepwise multiple regression analyses were performed using SAS, version 9.1, and Pearson correlation's coefficients were used to determine significant correlations. Redundancy analyses (RDA) were performed using Canoco version 4.5 (ter Braak and Smilauer 1998). The Monte Carlo permutation test was used to select the best combination of explanatory variables. However, because results obtained for the two types of analyses were similar, we chose to present only the results obtained with RDA.

Analyses were performed both for combined and individual years. Two dependent variables were used for these analyses, fresh mushroom biomass and mushroom density. They were analyzed separately at first and then simultaneously. Soil parameters in the mineral soil and in the organic soil as well as meteorological, stand and vegetation parameters were considered as independent variables. The soil parameters were C:N ratio, extractable P, CEC, available N (NO_3^- and NH_4^+), pH, percentage of light reaching the forest floor, and percentage of sand, clay and silt. The stand parameters were basal area, tree density, tree height and organic matter thickness; the vegetation parameters were percent area coverage of tree canopy, understory vegetation and lichens and mosses found in the plots. The meteorological parameters were the cumulative sum of degree days, weekly mean air temperature, weekly mean soil temperature, weekly total rainfall, and weekly mean relative humidity.

Stepwise discriminant analyses (n = 103) allowed us to compare plots containing lobster mushroom fruit bodies with control plots (plots without any fruit bodies) independent of the plot locations. The objective of the analysis was to determine which

combination of habitat variables (soil, stand, and vegetation parameters) best predicted the fructification of this mushroom. All tests were performed using SAS, version 9.1.

Results

Fruit body characteristics and production

Early in July, before the appearance of parasitized fruit bodies, basidiomata of non-infected *R. brevipes* were observed at the periphery of the observation plots. *R. brevipes* was identified by J.M. Moncalvo (Senior Curator, Royal Ontario Museum, Departement of Natural History, Toronto, Canada, personal communication, 2006) as the *H. lactifluorum* host in the study site. Occasionally *R. brevipes* only partially covered by *H. lactifluorum* were observed. Observations showed that fruit bodies typically protruded from the soil, and the base of the stipes was covered with sand from the eluvial horizon, suggesting that their ectomycorrhizae develop in the B and/or C horizons of the soil.

From August 10 to September 29, 2005, observation plots produced a total of 228 lobster mushroom basidiomata (Figure 2.2). The fresh mushroom biomass was not recorded for the first year of the experiment. In 2006, observation plots yielded 135 fruit bodies between July 11 and September 23 (Figure 2.2), for a total fresh biomass of 10.77 kg. In 2007, between July 10 and September 18, 244 fruit bodies were observed in the plots (Figure 2.2), and fresh mushroom biomass was 19.82 kg. Because of colony development, 17 plots in 2006 and 26 plots in 2007 were expanded or slightly moved to include the full production of the thallus.

The first fruit bodies were observed on July 10-11 in 2006 and 2007 in the observation plots located on the trails as opposed to July 25 in the observation plots located in the forest strips and unmanaged stand. The average life span of this mushroom was 13 days, with some mushrooms surviving less than 5 days and others more than 30 days. In 2005 and 2007, the emergence of new fruit bodies was reduced to a very low level by the beginning of September as compared with 2006 when this phase was reached much earlier, i.e. by the

middle of August. Total mushroom production was also much reduced as compared with that of 2007 (Figure 2.2).

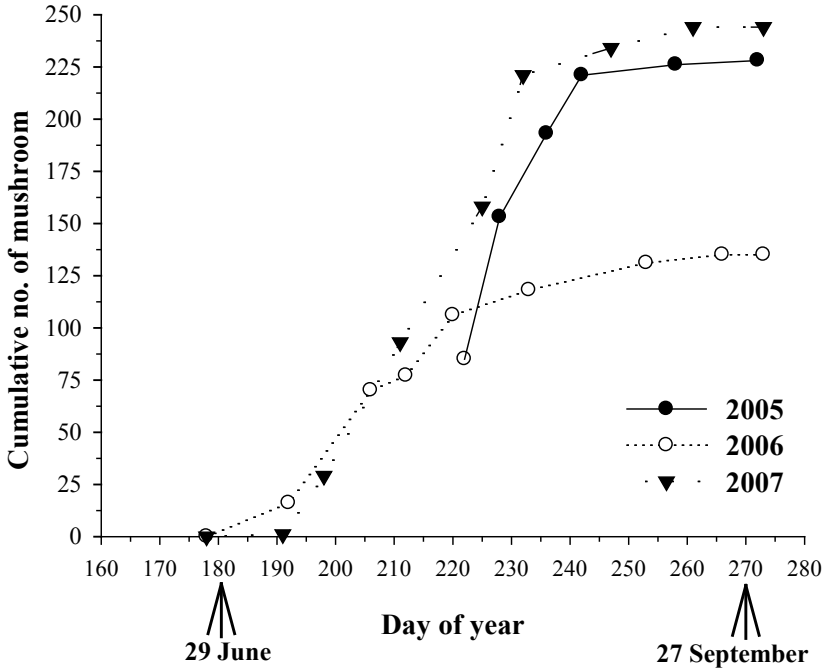


Figure 2.2. Cumulative number of lobster mushrooms harvested weekly in the observation plots from 10 July (day of year 191) to 30 September (day of year 272) for the years 2005 to 2007. Due to technical problems, the first harvesting date was 9 August (day of year 222) for the year 2005.

Silvicultural treatment impact on the lobster mushroom

In 2005 and 2007, no significant differences were found in mushroom densities between locations (trails, forest strips between the trails, unmanaged stand) (Figure 2.3a). However, in 2006, mushroom density was significantly lower under the canopy (between the trails and unmanaged stand), whereas this difference was not observed on the trails. In 2005 and 2006, the number of fruit bodies tended to be higher on the trails than in the forest strips and in the unmanaged stand (Figure 2.3a), but this difference was not significant ($P = 0.19$), so the number of fruit bodies was considered to be similar in every location.

Mushroom biomass was significantly higher in forest strips between the trails than on the trails, except in 2006, a year of reduced midsummer rainfall (July 29 - August 28) (Figure 2.3b). For 2005 and 2007, biomasses were lower on the trails than in the forest strips and in the unmanaged stand. No significant effect of the year of sampling was

detected in the forest strips for fresh mushroom biomass (Figure 2.3b). In the unmanaged stand, biomass tended to be higher in 2005.

In 2005 and 2007, mushroom density and fresh mushroom biomass on the trails had an opposite relative difference: while mushroom density on the trails was high, fresh mushroom biomass was low.

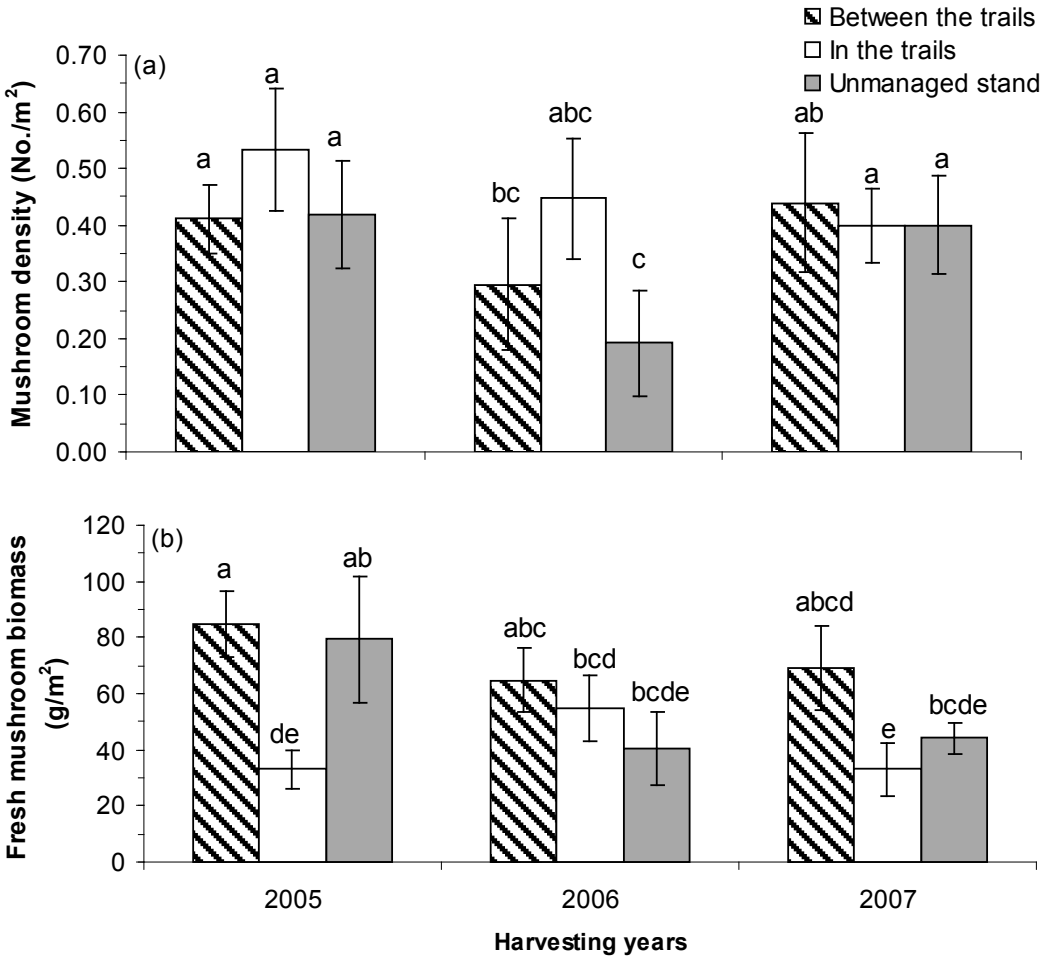


Figure 2.3. (a) Lobster mushroom density and (b) fresh mushroom biomass found in the observation plots from the beginning of the fruiting season in 2005 until the end of the fruiting season in 2007. Different letters represent significant differences under a least significant difference test at α=0.05. The error bars represent the standard error.

The 2007 mushroom inventory revealed a fresh mushroom production of 21.6 kg/ha independent of the locations (on the trails or in the forest strips between the trails). Besides, as a complement to the results obtained in the observation plots (Figure 2.3a), the mushroom inventory showed that mushroom density over the entire study area was

significantly higher on the trails than in the forest strips between the trails (Figure 2.4a). However, contrary to the observation plots, the mushroom inventory indicated that fresh mushroom biomass production is not significantly different between the two environments (P = 0.14) (Figure 2.4b). Because the inventory covered a greater area and is potentially less biased, we suggest that the overall productivity of these two environments is relatively similar.

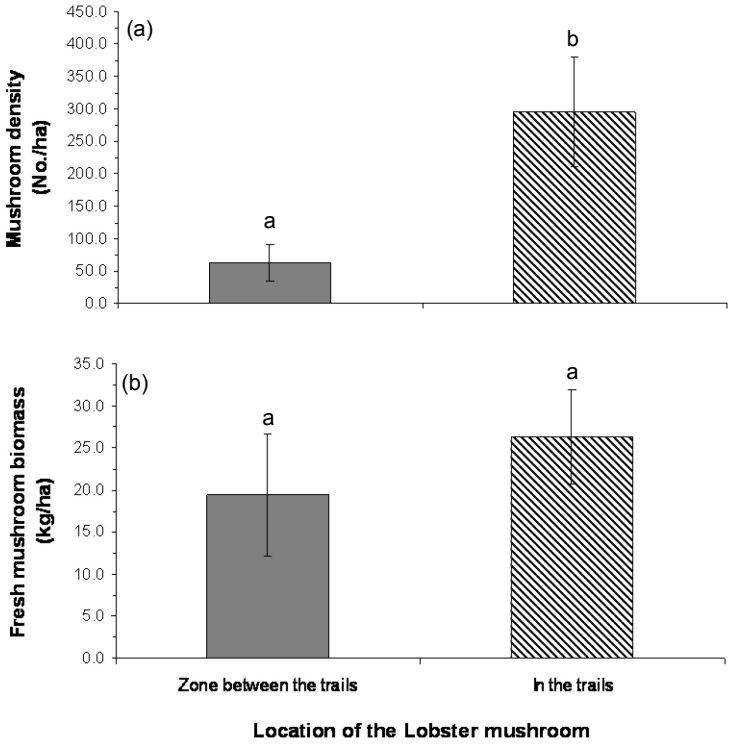


Figure 2.4. The 2007 lobster mushroom inventory over the entire privately managed study site compared (a) the mushroom density and (b) the fresh mushroom biomass found on the trails and in the forest strips between the trails. Different letters represent significant differences under a T-test at α=0.05. The error bars represent the standard error.

Influence of ecological parameters

RDA and stepwise multiple regressions confirmed that the fresh mushroom biomass and mushroom density were significantly affected by stand characteristics, soil variables and vegetation variables (percentage of plant cover) (Figure 2.5).

The first and second axes of the mushroom density and biomass had a total variance of 51.0% (32.8% for the first axis and 18.2% for the second axis) (Figure 2.5). Both axes were

a weighted average of all the variables, with a high incidence of percentage cover of *Betula papyrifera* and *Pinus banksiana* on axis 1, and a high incidence of CEC $_{(organic\ layer)}$, pH $_{(organic\ layer)}$ and percentage cover of *Pinus banksiana* on axis 2. According to the forward selection (a variable selection procedure), mushroom density had significant positive correlations with percentage cover of *Prunus pensylvanica* (0.36), *B. papyrifera* (0.31), *Alnus rugosa* (0.30), *Populus tremuloides* (0.22), and weak negative correlations with percentage cover of *Carex* sp. (-0.06) and NH_4^+ $_{(mineral\ layer)}$ (-0.04) (Figure 2.5). As for fresh mushroom biomass, the forward selection method revealed significant correlations with NH_4^+ $_{(organic\ layer)}$ (0.41) and pH $_{(mineral\ layer)}$ (-0.28) (Figure 2.5).

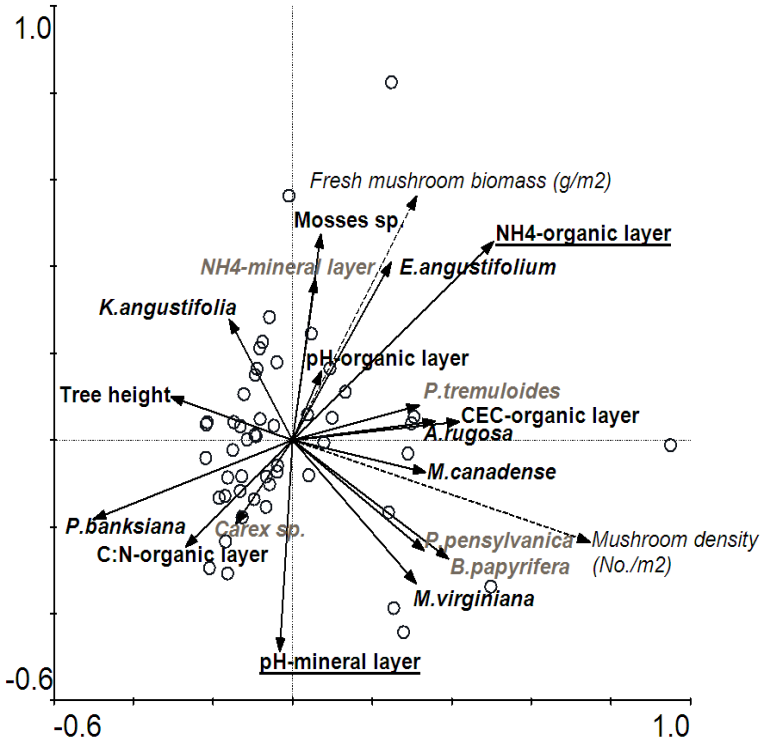


Figure 2.5. Correlation biplot showing the relationship between ecological parameters and fresh mushroom biomass and density in the 3 year average lobster mushroom experimental plots. It represents the forward selection and the best fit correlations. The underlined parameters are the best combination of explanatory variables for fresh biomass and the parameters in grey are the best explanatory variables for mushroom density. The circles represent the lobster mushroom plots. The complete names for the vegetation parameters are: Alnus rugosa, Betula papyrifera, Carex sp., Epiolobium angustifolium, Kalmia angustifolia, Maianthemum canadense, Medeola virginiana, mosses species, Pinus banksiana, Populus tremuloides, and Prunus pensylvanica.

Moreover, based on the information given in Figure 2.5, parameters were grouped together. The first group contained parameters showing positive correlations with fresh mushroom biomass: percentage cover of moss species and *Epilobium angustifolium*, NH_4^+ concentration in both soil layers, and pH $_{(organic\ layer)}$. A second set of parameters, percentage cover of *Pinus banksiana* and *Carex* sp., C:N ratio $_{(organic\ layer)}$ and pH $_{(mineral\ layer)}$ showed negative correlations with fresh mushroom biomass. A third set of parameters, percentage cover of *Populus tremuloides* and *A. rugosa* and CEC $_{(organic\ layer)}$ were positively correlated with fresh mushroom biomass and mushroom density. Another group was formed by percentage cover of *Maianthemum canadense*, *B. papyrifera*, *Prunus pensylvanica* and *Medeola virginiana* as they were positively correlated with the mushroom density. Finally, tree height and percentage cover of *Kalmia angustifolia* and *Pinus banksiana* formed a group negatively correlated with mushroom density.

Influence of meteorological parameters

Meteorological data were also analyzed, but no significant correlations or obvious relations could be drawn. Despite this, it was possible to determine that the 2006 total rainfall was higher than that of the other 2 years except from July 29 to August 28 when mushroom fructification was at its peak (Figure 2.6). This period of reduced rainfall could explain why the 2006 total mushroom production was lower than that of the other 2 years and levelled off by the beginning of August (Figure 2.2). It also reflects the influence of rainfall events days before lobster mushroom fructification.

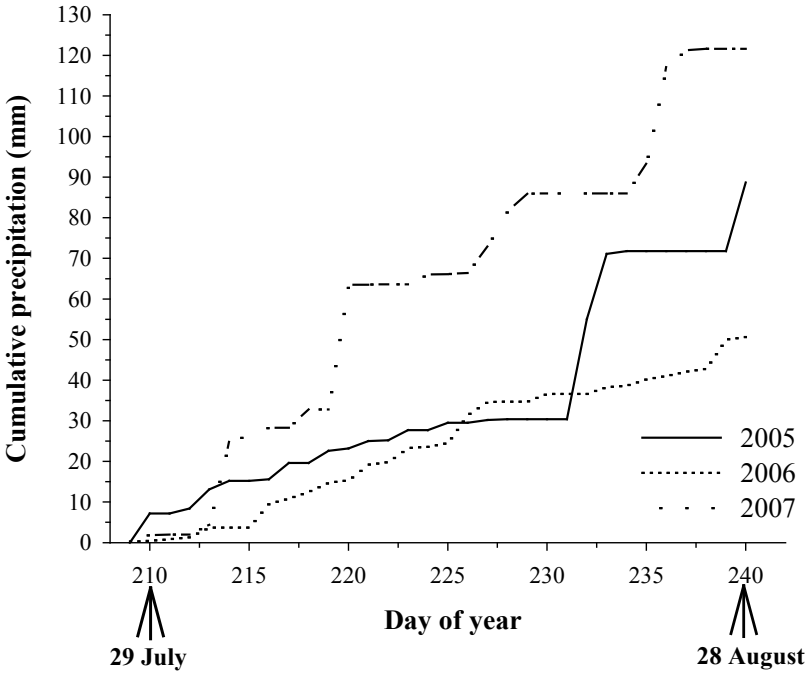


Figure 2.6. Cumulative precipitation taken in 2005, 2006, and 2007 at five weather stations near the study site during the lobster mushroom fructification peak period (29 July to 28 August).

Lobster mushroom microhabitat

To detect parameters that influence the mushroom habitat (comparison between plots with and without lobster mushroom fruit bodies), stepwise discriminant analyses were performed on 46 soil, stand and vegetation parameters (Table 2.2). Soil and stand parameters that were highly correlated with the lobster mushroom habitat were, in order of stepwise selection, pH $_{\text{(organic layer)}}$ (0.37), stand opening (-0.44) and extractable P $_{\text{(organic layer)}}$ (0.48). Vegetation parameters that were significantly correlated with the lobster mushroom habitat were, in order of importance, percentage cover of *B. papyrifera* (0.25), *K. angustifolia* (-0.32), lichen species (0.40) and *Comptonia peregrina* (0.45). These selected parameters appear to reflect the use of a specific habitat by the lobster mushroom.

Data showed that using the discriminant function with the pedological and stand variables as predictors, 64.8% of lobster mushroom plots and 74.4% of the control plots were correctly classified (Table 2.3). The discriminant function using vegetation variables correctly classified 80.8% of the mushroom plots and 53.5% of the control plots.

Table 2.2. Variables selected by Stepwise discriminant analyses as potential habitat descriptors for the lobster mushroom among 18 pedological and stand parameters (first analysis) and 28 vegetation parameters (second analysis).

Analyses	Step	Selected parameter	F value to enter	Wilks' λ^a	Pr > λ^a	a[b]	r[c]
Pedological and stand parameters	1	$pH_{(organic\ layer)}$	15.10	0.863	0.002	0.636	0.370
	2	Stand opening	6.86	0.804	0.01	-0.507	-0.443
	3	Extractable $P_{(organic\ layer)}$	3.93	0.772	0.05	0.434	0.4780
Vegetation parameters (% cover)	1	*Betula papyrifera*	5.97	0.940	0.016	0.479	0.246
	2	*Kalmia angustifolia*	4.12	0.899	0.045	-0.716	-0.317
	3	Lichen species	6.69	0.838	0.011	0.647	0.403
	4	*Comptonia peregrina*	4.67	0.797	0.033	-0.499	0.451

[a]Wilks' λ values and associated p-values refer to multivariate analysis of a variance model (Manova) as measured by λ.
[b]a is the standardized canonical discriminant function coefficient.
[c]r is the correlation of the variable with the discriminant function (structure coefficient).

Table 2.3. Proportion of plots correctly and incorrectly classified by the discriminant analyses based on pedological, stand, and vegetation parameters.

Analyses	Total number of plots	Plots classified as lobster mushroom plots	Plots classified as control plots
Pedological and stand parameters	54 lobster mushroom plots	35/54 (64.8%)	19/54 (35.2%)
	43 control plots	11/43 (25.6%)	32/43 (74.4%)
Vegetation parameters (% cover)	52 lobster mushroom plots	42/52 (80.8%)	10/52 (19.2%)
	43 control plots	20/43 (46.5%)	23/43 (53.5%)

Discussion

Fruit body characteristics production

Colony development

Our results illustrated that lobster mushroom fruit bodies situated on the trails appeared earlier than those between the trails or in the unmanaged stand. The canopy opening that brings more light and heat to the soil could favour early mushroom production (Arnolds 1995) and might facilitate nutrient uptake by the mushrooms (Cheng et al. 2005). This explained early production as well as a higher mushroom density on trails.

As seen here and in other studies, the parasitic ascomycete *H. lactifluorum* appeared in succeeding years in the same population of *R. brevipes* (Bessette et al. 1997). Moreover, the 2006 and 2007 colony expansions suggest that the lobster mushroom had the ability to fruit in slightly different places every year, which could help them take advantage of new nutrient sources in the soil.

Our field observation confirmed that the lobster mushroom fruit body emerges quickly from the soil in 1 day and survived for an average of 13 days. This development pattern is comparable to that of species, such as *Boletus* sp. (Martinez Peña 2003) which is a fast-growing species with a short life-span as opposed to slow growing species, such as *Cantharellus* sp. that persist for an average of 44 days or more (Pilz et al. 2003).

Rainfall influence

The number of fruit bodies found in a site is often strongly governed by weather (Jansen and Van Dobben 1987). In 2005 and 2007, 2 years showing regular events of rainfall from April to November, cumulative mushroom production followed a sigmoid pattern. In 2006, seasonal cumulative production levelled off by the beginning of August. Cumulated 2006 precipitation was the highest of the 3 years except for this period during which only superficial rain events occurred (from July 29 to August 28). Low 2006 mushroom density in forest strips between the trails and in the unmanaged stand and a higher 2006 density on the trails suggested that midsummer precipitation is critical to mushroom production. It also indicated that a closed canopy may greatly reduce mushroom production by intercepting water from superficial rain events and that deeply occurring mycorrhizae of the fungus did not receive an adequate amount of water. The presence of woody debris on the trails might have favoured mushroom production during the 2006 drought because woody debris have the capacity to preserve soil humidity (Harvey et al. 1978) and allow fungi to survive in soils during periods of limited moisture availability (Tedersoo et al. 2003).

Silvicultural treatment impact on the lobster mushroom

To our knowledge, this study site designed for mushroom management is unique. Trails created for lobster mushroom harvest allowed us to compare its productivity in terms of fresh biomass and density in different environments. The two survey methodologies that were used (3-year plot inventory and one-season linear survey) showed slightly different results, but both revealed that trail openings affected fresh mushroom biomass and density. Moreover, even if the overall productivity did not differ significantly from one location to the other, there was a tendency for a greater number of fruit bodies to develop on the trails while the fresh biomass seemed to be higher in forest strips. The production of numerous small fruit bodies on the trails and of a reduced number with heavier mass in forest strips was identified as a pattern for this species and may indicate how the lobster mushroom adapts to disturbed areas. Nevertheless, those conclusions need to be used with caution because locations (trails, forest strips between the trails, and unmanaged stand) were not replicated. Results are only valid for those two specific stands.

The overall fresh lobster mushroom production in the managed area was 21.6 $kg \cdot ha^{-1} \cdot year^{-1}$. When compared with other wild edible mushrooms, this estimate is at the higher end of production rates. *Cantharellus formosus* had an estimated production from 1.0 to 25.0 $kg \cdot ha^{-1} \cdot year^{-1}$ in the Olympic Peninsula, Washington State, USA (Pilz et al. 1998). For *Tricholoma magnivelare,* estimates ranged from 4.3 (Pilz et al. 1999) to 11.8 $kg \cdot ha^{-1} \cdot year^{-1}$ (Alexander et al. 2002) in the Pacific Northwest region of the USA and in Blue Mountains of Oregon, *Morchella* sp. varied between 1.0 and 3.5 $kg \cdot ha^{-1} \cdot year^{-1}$ depending on the disturbance and stand age (Alexander et al. 2002).

Influence of ecological parameters

Each species of ECM fungus has unique ecological requirements, tolerances and physiological capabilities (Read 1991). Moreover, depending on the chosen variables, whether it is mushroom abundance, weight (Pilz et al. 2006) or presence-absence (Bergemann and Largent 2000), the environmental parameters influencing mushroom fructification could differ. As observed here and in other studies, ECM fungi are most

likely to be influenced by a combination of vegetation and direct and indirect soil influences (Nantel and Neumann 1992).

Fresh mushroom biomass was strongly associated with the presence of NH_4^+ and negatively associated with C:N ratio in both soil layers and with acidic soil conditions (low pH). The accessibility to N by ECM fungi is the main growth-limiting factor in the boreal forest (Read 1991). However, most ECM fungi in those forests are adapted to acidic soil conditions (Molina and Palmer 1982) where they are highly efficient at scavenging for organic sources of N in surface soil horizons (Read et al. 2004). NH_4^+ is generally recognized as the most readily usable source of N for most ECM fungi (Smith and Read 2008).

Early-stage shade-intolerant species, such as *B. papyrifera*, *Populus tremuloides*, *Prunus pensylvanica*, and *A. rugosa* were the most important parameters associated with mushroom density. This is inconsistent with reported observations of Russulaceae growing in undisturbed old-growth forests (Bergemann and Miller 2002) and being absent or not abundant in early regeneration stands (Bradbury et al. 1998). A possible explanation for this discrepancy is that the relationships identified in this study are only applicable to managed forest stands with small canopy openings, such as that of trails. Trail development could also explain the negative correlations found between *Pinus banksiana*, tree height, and mushroom density, as this development reduced the number of mature trees, mainly *Pinus banksiana*, growing on a site. Moreover, mushroom fructification on trails indicated profound differences between parasitized and nonparasitized *R. brevipes*, with the lobster mushroom in this study showing a capacity to grow in light-disturbed areas, such as trails surrounded by forest strips, that was not observed on nonparasitized *R. brevipes* in the study by Bergemann and Miller (2002). Several facotrs might explain those differences such as changes in the mushroom needs, ecology or reproduction but such factors need to be tested.

Lobster mushroom microhabitat

When comparing plots containing lobster mushroom fruit bodies with control plots (plots without any fruit bodies) independent of the plot locations, pedological and stand parameters identified as important predictors of lobster mushroom habitat were pH $_{(organic\ layer)}$, extractable P $_{(organic\ layer)}$ and stand opening. Vegetation parameters, such as percentage cover of *B. papyrifera*, *K. angustifolia*, lichen species, and *C. peregrina* were also good predictors. We suggest that the lobster mushroom is adapted to acidic soil conditions where according to Read et al. (2004) it could access major nutrients such as P and N from a wide range of organic sources. This mushroom is found in small, light-disturbed areas, such as trails and gaps in stand openings that are colonized by early stage species such as *B. papyrifera*. *Kalmia angustifolia* is not often present in the lobster mushroom habitat, and this could be explain by its ericoid mycorrhizae that compete for nutrients and suppress the formation of other fungi on roots (Yamasaki et al. 1998). Jansen and Van Dobben (1987) found that in typical nutrient-poor and sandy forest soils, mushroom decline is often accompanied by a decrease in lichen cover indicating drastic changes in the environment. Our study confirms this, as the presence of lichen was a good indicator of the lobster mushroom habitat. *Comptonia peregrina* is a N-fixing plant that does well on disturbed sites and in openings in coniferous forests (Hendrickson 1986). The presence of *C. peregrina* could provide a source of available N for the mushroom while *K. angustifolia* may reduce N mineralization (LeBel et al. 2008) and increase the amount of soil N sequestered as tannin–protein complexes (Joanisse et al. 2009).

Conclusion

This research aimed to provide a better understanding of the ecology and management of a complex organism, the lobster mushroom. An overall yearly fresh mushroom production average of 21.6 kg/ha was observed. Furthermore, an interaction between midsummer precipitation and stand management was noted. Mushroom production on harvested trails was not dependent on rainfall, whereas production under forest canopy appears to be strongly influenced by rainfall. Stand management had an opposite influence on mushroom density (number of carpophores/m^2) and on fresh mushroom biomass, as abundant small fruit bodies were growing on the trails and fewer but heavier ones were

found in the forest strips between the trails. Overall, trail construction did not increase lobster mushroom production but maintained it. During wet periods, one sampling method indicated greater productivity in the forest strips, while this difference was less apparent during the dry midsummer period of 2006. Also, the 2007 inventory method that covered more ground indicated that mushroom density was higher on the trails, but no certainty on total productivity differences between the treatments could be found. It may therefore be concluded that managing trails for mushroom production does not stimulate mushroom production. Nevertheless, the advantage of a trail system for systematically harvesting mushrooms and the ease with which they can be found on the trails are factors that should be considered.

Within the fairly homogeneous conditions of a jack pine forest on a coarse sandy soil, the habitat for lobster mushroom fructification was characterized by acidic soil conditions, high extractable P, and the presence of small forest gaps with early-stage species, such as *B. papyrifera*, the N-fixing plant *C. peregrina*, and without *K. angustifolia*. Within the area containing fructifications, mushroom density was positively correlated to percentage cover of *A. rugosa, B. papyrifera*, *Populus tremuloides* and *Prunus pensylvanica*, whereas fresh mushroom biomass was positively correlated with NH_4^+ and negatively correlated with pH. Whether these factors are really causal agents of greater fructification remains however to be tested experimentally before recommendations for fertilization or plant species management can be made.

Acknowledgements

This research was made possible by a Natural Sciences and Engineering Research Council of Canada strategic grant. We are grateful for the technical assistance provided by Karine Bertrand, Andrew Coughlan, André Gagné, Julie Godbout, Nellia Pélardy, Christine Roussel-Roy at Université Laval, and Alain Courcelles and Sébastien Dagnault at the Laurentian Forestry Centre of Natural Resources Canada. We would also like to thank our collaborators in the field study, Céline Marceau and Alain Blais, for their generous help and patience during this 3-year study and for letting us use their land. We appreciate the useful

comments and recommendations provided by Catherine Ste-Marie of Natural Resources Canada.

References

Alexander, S.J., Pilz, D., Weber, N.S., Brown, E., and Rockwell, V.A. 2002. Mushrooms, trees, and money: value estimates of commercial mushrooms and timber in the Pacific Northwest. Environmental Management **30**(1): 129-141.

Amaranthus, M., and Pilz, D. 1996. Productivity and sustainable harvest of wild mushrooms. *In :* Managing forest ecosystems to conserve fungus diversity and sustain wild mushroom harvests. *Edited by* D. Pilz, and R. Molina. Gen. Tech. Rep. PNW-GTR-371, US Department of Agriculture, Forest Service, Pacific Northwest Research Station, Portland, OR., USA. pp. 42-61.

Amaranthus, M., and Russell, K. 1996. Effect of environmental factors and timber and mushroom harvest on *Cantharellus* production on the Olympic Peninsula, Washington State. *In :* Managing forest ecosystems to conserve fungus diversity and sustain wild mushroom harvests. *Edited by* D. Pilz, and R. Molina. Gen. Tech. Rep. PNW-GTR-371, US Department of Agriculture, Forest Service, Pacific Northwest Research Station, Portland, OR., USA. pp. 73-74.

Arnolds, E. 1995. Conservation and management of natural populations of edible fungi. Canadian Journal of Botany **73**(S1): S987-S998.

Bauhus, J., Paré, D., and Côté, L. 1998. Effects of tree species, stand age and soil type on soil microbial biomass and its activity in a southern boreal forest. Soil Biology and Biochemistry **30**(8-9): 1077-1089.

Bergemann, S.E., and Largent, D.L. 2000. The site specific variables that correlate with the distribution of the Pacific Golden Chanterelle, *Cantharellus formosus*. Forest Ecology and Management **130**(1-3): 99-107.

Bergemann, S.E., and Miller, S.L. 2002. Size, distribution, and persistence of genets in local populations of the late-stage ectomycorrhizal basidiomycete, *Russula brevipes*. New Phytologist **156**(2): 313-320.

Bessette, A.E., Bessette, A.R., and Fischer, D.W. 1997. *Hypomyces* and allies. *In :* Mushrooms of northeastern North America. Syracuse University Press. Syracuse, N.Y., USA. pp. 529-532.

Bonham, C.D. 1989. Measurements for terrestrial vegetation. Wiley-Interscience, New York. N.Y., USA.

Bradbury, S.M., Danielson, R.M., and Visser, S. 1998. Ectomycorrhizas of regenerating stands of lodgepole pine (*Pinus contorta*). Canadian Journal of Botany **76**(2): 218–227.

Bray, R.H., and Kurtz, L.T. 1945. Determination of total, organic and available forms of phosphorus in soils. Soil Science **59**(1): 39-45.

Carter, M.R. 1993. Soil sampling and methods of analysis. CRC Press, Boca Raton, FL., USA.

Cheng, S., Widden, P., and Messier, C. 2005. Light and tree size influence belowground development in yellow birch and sugar maple. Plant Soil **270**(1): 321-330.

Durall, D.M., Jones, M.D., Wright, E.F., Kroeger, P., and Coates, K.D. 1999. Species richness of ectomycorrhizal fungi in cutblocks of different sizes in the Interior Cedar-Hemlock forests of northwestern British Columbia: sporocarps and ectomycorrhizae. Canadian Journal of Forest Research **29**(9): 1322-1332.

Farrar, J.L. 1995. Trees in Canada. Canadian Forest Service. Fitzhenry & Whiteside Ltd., Ottawa, Ont., Canada.

Frazer, G.W., Canham, C.D., and Lertzman, K.P. 1999. Gap Light Analyser (GLA), Version 2.0: imaging software to extract canopy structure and gap light transmission indices from true-colour fisheye photographs, users manual and program documentation. Simon Fraser University, Burnaby, B.C., and the Institute of Ecosystem Studies, Millbrook, N.Y.

Gabel, A., Ebbert, E., and Lovett, K. 2004. Macrofungi Collected from the Black Hills of South Dakota and Bear Lodge Mountains of Wyoming. American Midland Naturalist **152**: 43-62.

Hansen, P.A. 1988. Prediction of macrofungal occurrence in Swedish beech forests from soil and litter variable models. Plant Ecolology **78**(1-2): 31-44.

Harvey, A.E., Jurgensen, M.F., and Larsen, M.J. 1978. Seasonal distribution of ectomycorrhizae in a mature Douglas-fir/larch forest soil in western Montana. Forest Science **24**(2): 203-208.

Harvey, A.E., Larsen, M.J., and Jurgensen, M.F. 1980. Partial cut harvesting and ectomycorrhizae: early effects in Douglas-fir-larch forests of western Montana. Canadian Journal Forest Research **10**(3): 436-440.

Hendershot, W.H., and Duquette, M. 1986. A simple barium chloride method for determining cation exchange capacity and exchangeable cations. Soil Science Society of America **50**(3): 605-608.

Hendrickson, O.Q. 1986. Invasion of clear-cuttings by the actinorhizal plant *Comptonia peregrina*. Canadian Journal Forest Research **16**(4): 872-874.

Jansen, E., and van Dobben, H.F. 1987. Is decline of *Cantharellus cibarius* in the Netherlands due to air pollution? Ambio **16**(4): 211-213.

Joanisse, G.D., Bradley, R.L., Preston, C.M., and Bending, G.D. 2009. Sequestration of soil nitrogen as tannin-protein complexes may improve the competitive ability of sheep laurel (*Kalmia angustifolia*) relative to black spruce (*Picea mariana*). New Phytologist **181**(1): 187-198.

Keeney, D.R., and Nelson, D.W. 1982. Nitrogen-inorganic forms. *In :* Methods of soil analysis Part 2. Chemical and microbiological properties. *Edited by* R.H. Miller, and D.R. Keeney. American Society of Agronomy, Madison, Wis., USA. pp. 643-698.

Kowalenko, C.G. 2001. Assessment of Leco CNS-2000 analyzer for simultaneously measuring total carbon, nitrogen, and sulphur in soil. Communications in Soil Science and Plant Analysis **32**(13-14): 2065-2078.

Kranabetter, J.M., and Kroeger, P. 2001. Ectomycorrhizal mushroom response to partial cutting in a western hemlock - western redcedar forest. Canadian Journal Forest Research **31**(6): 978-987.

LeBel, P., Thiffault, N., and Bradley, R.L. 2008. *Kalmia* removal increases nutrient supply and growth of black spruce seedlings: An effect fertilizer cannot emulate. Forest Ecology and Management **256**(10): 1780-1784.

Martinez Peña, F. 2003. Producción y aprovechamiento de *Boletus edulis Bull.:Fr.* en un bosque de *Pinus sylvestris L*. Bases para la ordenación y valoración económica del recurso micológico forestal. Junta de Castilla y León, Soria. Spain.

Molina, R., and Palmer, J.G. 1982. Isolation, maintenance, and pure culture manipulation of ectomycorrhizal fungi *In :* Methods and principles of mycorrhizal research. *Edited by* N.C. Schenck. The American Phytopathological Society, APS Press, St. Paul, MN., USA. pp. 115-129.

Nantel, P., and Neumann, P. 1992. Ecology of ectomycorrhizal-basidiomycete communities on a local vegetation gradient. Ecology **73**(1): 99-117.

Nissen, T.M., and Midmore, D.J. 2002. Stand basal area as an index of tree competitiveness in timber intercropping. Agroforestry Systems **54**(1): 51-60.

Pilz, D., Molina, R., and Liegel, L. 1998. Biological productivity of chanterelle mushrooms in and near the Olympic Peninsula Biosphere Reserve. Ambio Special Report **9**: 8-13.

Pilz, D., Smith, J., Amaranthus, M.P., Alexander, S., Molina, R., and Luoma, D. 1999. Managing the commercial harvest of the American matsutake and timber in the southern Oregon Cascade Range. Journal of Forestry **97**(3): 4-11.

Pilz, D., Molina, R., Danell, E., Waring, R., Rose, C., Alexander, S., Luoma, D., Cromack, K.J., and Lefevre, C. 2002. SilviShrooms: predicting edible mushroom productivity using forest carbon allocation modelling and immunoassays of ectomycorrhizae. *In :* Proceedings of the Second International Conference on Edible Mycorrhizal Mushrooms: Edible mycorrhizal mushrooms and their cultivation. 3-6 July 2001, Christ Church, New Zeland. *Edited by* I.R. Hall, W. Yun, E. Danell and A. Zambonelli. [CD-ROM]. New Zealand Institute for Crop and Food Research Limited, Christ Church, N.Z.

Pilz, D., Norvell, L., Danell, E., and Molina, R. 2003. Ecology and management of commercially harvested chanterelle mushrooms. Gen. Tech. Rep. PNW-GTR-576, US Department of Agriculture, Forest Service, Pacific Northwest Research Station, Portland, OR., USA.

Pilz, D., Molina, R., and Mayo, J. 2006. Effects of thinning young forests on chanterelle mushroom production. Journal of Forestry **104**(1): 9-14.

Read, D.J. 1991. Mycorrhizas in ecosystems. Cellular and Molecular Life Sciences **47**(4): 376-391.

Read, D.J., Leake, J.R., and Perez-Moreno, J. 2004. Mycorrhizal fungi as drivers of ecosystem processes in heathland and boreal forest biomes. Canadian Journal of Botany **82**(8): 1243-1263.

Régnière, J., and St-Amant, R. 2007. Stochastic simulation of daily air temperature and precipitation from monthly normals in North America north of Mexico. International Journal of Biometeorology **51**(5): 415-430.

Rizvi, L., Didudk, M., and Moncalvo, J.M. 2007. Identifying the host of lobster mushrooms. *In :* Colloque Mycorhizes 2007, Institut de recherche en biologie végétale Montréal, Que., Canada. Poster of the Department of Ecology and Evolution, University of Toronto, Toronto, Ont. Canada.

Rogerson, C.T., and Samuels, G.J. 1994. Agaricicolous species of *Hypomyces*. Mycologia **86**(6): 839-866.

SAS Institute Inc. 2004. SAS/STAT® 9.1 user's guide, Vols 1-7. SAS Institute Inc., Cary, N.C.

Smith, S.E., and Read, D.J. 2008. Mycorrhizal symbiosis. 3rd ed. Academic Press, London. UK.

Tedersoo, L., Koljalg, U., Hallenberg, N., and Larsson, K.-H. 2003. Fine scale distribution of ectomycorrhizal fungi and roots across substrate layers including coarse woody debris in a mixed forest. New Phytologist **159**(1): 153-165.

ter Braak, C.J.F., and Smilauer, P. 1998. CANOCO® reference manual and user's guide to Canoco® for Windows: software for canonical community ordination (version 4). Microcomputer Power, Ithaca, N.Y., USA.

Villeneuve, N., Grandtner, M.M., and Fortin, J.A. 1989. Frequency and diversity of ectomycorrhizal and saprophytic macrofungi in the Laurentide Mountains of Quebec. Canadian Journal of Botany **67**(9): 2616-2629.

Yamasaki, S.H., Fyles, J.W., Egger, K.N., and Titus, B.D. 1998. The effect of *Kalmia angustifolia* on the growth, nutrition, and ectomycorrhizal symbiont community of black spruce. Forest Ecology and Management **105**(1-3): 197-207.

Yun, W., and Hall, I.R. 2004. Edible ectomycorrhizal mushrooms: challenges and achievements. Canadian Journal of Botany **82**(8): 1063-1073.

Chapitre 3. Ecology and productivity of *Cantharellus cibarius* var. *roseocanus* in two eastern Canadian jack pine stands

Cet article a été soumis en langue anglaise pour publication dans la revue *Botany*. L'usage du point décimal est donc de rigueur.

Rochon, C., Paré, D., Pélardy, N., Khasa, D.P., and Fortin, J.A. Ecology and productivity of *Cantharellus cibarius* var. *roseocanus* in two eastern Canadian jack pine stands. Botany. (Accepté le 25 juillet 2011).

Résumé

Malgré l'importance économique des chanterelles, leurs exigences en matière d'habitat demeurent peu connues. La productivité des carpophores de *Cantharellus cibarius* var. *roseocanus* Redhead, Norvell & Danell a été mesurée au cours de trois saisons de croissance dans deux peuplements de *Pinus banksiana* Lamb. en forêt boréale. L'objectif était de déterminer comment la variabilité du peuplement, des associations végétales, du sol et des conditions météorologiques était reliée à la productivité des carpophores. L'ADN de cette espèce a été détecté dans les horizons organiques et minéraux du sol. La productivité des carpophores ne semblait pas différente entre les peuplements, mais l'absence de colonies sur les sentiers d'un des sites pourrait refléter des conditions micro-environnementales non convenables à la croissance de cette chanterelle. Dans les conditions actuelles du site, les microhabitats favorables présentaient une forte densité du peuplement, un rapport C : N du sol élevé ainsi que la présence fréquente de mousses. L'association *Solidago puberula* Nutt. – *Comptonia peregrina* (L.) Coulter – *Pinus banksiana*, la présence de lichens et des teneurs en argile et limon les plus élevées possible sur ce sol sableux modérément acide indiquaient des milieux productifs pour cette chanterelle, alors que la présence de plantes éricacées était négativement corrélée. Des corrélations positives ont été trouvées entre le total des précipitations une semaine avant la fructification, la température de l'air deux semaines avant la fructification et la productivité des carpophores. Les résultats mettent en évidence les conditions favorisant les fructifications de *C. cibarius* var. *roseocanus* à l'intérieur de ces peuplements.

Abstract

Despite the economical importance of chanterelles, much remains to be known about their habitat requirements. *Cantharellus cibarius* var. *roseocanus* Redhead, Norvell & Danell sporocarp productivity was measured during three growing seasons in two *Pinus banksiana* Lamb. stands of the boreal forest. The objective was to determine how the variability in stand, plant association, edaphic and meteorological conditions was related to carpophore productivity. DNA of this species was detected in organic and mineral soil horizons. Sporocarp productivity was similar for both stands, but the absence of colonies on trails in one of the sites is likely to reflect microenvironmental conditions that are unsuitable for chanterelle growth. Under the prevailing site conditions, preferred micro-habitats were characterized by high stand density, high C:N ratio and a frequent moss presence. The *Solidago puberula* Nutt. – *Comptonia peregrina* (L.) Coulter – *Pinus banksiana* association, lichen presence and as much clay and silt contents as can possibly be found on this moderately acidic sandy soil indicated high-quality environments for this chanterelle, whereas ericaceous species presence was negatively correlated. Positive correlations between total rainfall one week prior to fructification, air temperature two weeks prior to fructification and carpophore productivity were established. Results highlight the specific conditions favourable to *C. cibarius* var. *roseocanus* fructifications within these stands.

Introduction

Wild edible mushrooms have been harvested for as long as humans have foraged in woodlands. However, in the later part of the 20th century, mushroom commercialization became global, and annual international trade is now worth billions of dollars. Commercially harvested sporocarps of *Cantharellus* spp. have been estimated to be worth US$1.67 billion/year on the world market (Watling 1997). Since chanterelles grow in symbiotic associations with fine roots of trees forming ectomycorrhiza (ECM) (Smith and Read 2008), forests are essential to their survival and productivity (Pilz et al. 2003). Forest management practices and excessive mushroom harvesting could have negative impacts on the sustainability of both partners (Amaranthus and Pilz 1996), therefore more ecological information on specific econiche and ecological parameters influencing fungal species in their natural environement are needed.

Chanterelles belong to the Cantharellaceae family that is divided into two genera *Craterellus* and *Cantharellus,* among which are found the *Cantharellus* species (Corner 1966; Dahlman et al. 2000). *Cantharellus cibarius* Fr. was first lumped under a single Latin binomen, but recent researches have made it clear that *C. cibarius* is not as ubiquitous as once believed. Arora and Dunham (2008) and Dunham et al. (2003b) have shown that this common morphology masks a species complex. *Cantharellus* spp. are found on every continent having the appropriate host tree, namely Asia, Africa, America, Oceania and Europe, and are among the most widely consumed ectomycorrhizal mushrooms (Danell 1999). In eastern North America, they form ectomycorrhizal associations with a wide range of host tree genera, including oak (*Quercus spp.*), beech (*Fagus spp*.), birch (*Betula spp*.) and various conifers (Pilz et al. 2003). Recently, a new *Cantharellus* of the *cibarius* group was identified and described by Redhead et al. (1997) as *C. cibarius* var. *roseocanus* Redhead, Norvell & Danell. In North America, this colourful yellowish pink hoary coating species was first identified in Western Canada and USA in association with conifers (mainly *Picea* sp. but also *Abies* sp., *Tsuga* sp. and *Pinus* sp.) (Redhead et al. 1997).

Only a few studies on the habitat requirements of chanterelle species have been conducted and most data concerning the ecology of the genus *Cantharellus*, including *C. cibarius* var. *roseocanus,* are derived from *C. cibarius* (Danell 1999). It is recognized that *Cantharellus* spp. tend to fruit most abundantly in stands aged 41-60 years, but also occur in very young stands (less than 20 years of age) and older forests (60 to 200 years old) (Tanino et al. 2005), and fruit on a variety of soils derived from limestone, glacial till, sedimentary rock and weathered granite (Pilz et al. 2003). Moreover, *Cantharellus* spp. respond to the main soil variables (pH, drainage and organic matter) (Jansen and van Dobben 1987) and to other variables such as humus characteristics (Nantel and Neumann 1992) and nitrogen deposition (Wallenda and Kottke 1998). Moreover, other important variables such as duff depth and needle cover (Bergemann and Largent 2000), vascular associates (mosses) as well as soil temperature, air temperature, moisture variables (Amaranthus and Russell 1996) and canopy cover (Pilz et al. 2006) have also been examined and established as important variables for the fructification of chanterelles. However, it is not yet well understood how site conditions (e.g., soil aspect), stand conditions (e.g., age, stand density, tree height), weather and management activities (e.g., thinning) affect mushroom productivity of specific species in their own habitat (Alexander et al. 2002).

Since Redhead et al. (1997), no specific study on *C. cibarius* var. *roseocanus* has been completed. Because *C. cibarius* var. *roseocanus* occurs in coniferous forests as does *C. cibarius,* we assumed that basic ecological information on the variety *C. cibarius* var. *roseocanus* should be similar to that of *C. cibarius*. In the Netherlands *Cantharellus cibarius* occurs mostly on well-drained sandy soils (Jansen and van Dobben 1987) while in Sweden it was associated with a low N content and a pH ranging from 4.0 to 5.5 (Rangel-Castro 2001). Moreover, in boreal forests, chanterelle fruiting bodies usually begin to appear in the middle of July until late October (Danell 1994) and production can vary greatly from year to year and from site to site (Egli et al. 2006).

The main objective of this research was to determine which ecological conditions prevailing in an eastern North American jack pine forest were related to *C. cibarius* var.

roseocanus presence and productivity (i.e., fresh sporocarp biomass and sporocarp density). Specifically, our hypotheses were: (1) *C. cibarius* var. *roseocanus* mycelium is present in the organic layers of the soil horizons where nutrients are most available; (2) *C. cibarius* var. *roseocanus* sporocarp productivity is influenced by specific soil, stand and vegetation parameters and fluctuations found between the 3 years of the study can be attributed to meteorological variations; (3) Sporocarps are present in a particular econiche because they are related to specific soil, stand and vegetation micro-site conditions.

Materials and methods

Site description and management

The study was conducted in two jack pine stands of the boreal forest near Girardville, Québec, Canada (49°07'N; 72°35'W) (for a map of the study site, see Rochon et al. (2009)). The first stand was located in the Domaine de la Rivière Mistassini private forest and will be referred to as the "private stand". In this 2 km^2 area, selective logging was conducted in strips that were approximately 200 m long x 3.5 m wide during the fall of 2004 and the spring of 2005. This generated approximately 175 trails (15 m apart) totalling 35 km of trails, on an overall area of 0.12 km^2. Boles were harvested and branches were chipped and spread on the trails. The second stand was established on public land, 3 km north of the first stand, and will be referred to as the "public stand". The size of this second stand was estimated at 2.4 km^2. They were located in the continuous boreal forest subzone. Both stands studied were dominated by jack pine (*Pinus banksiana*) associated with black spruce (*Picea mariana* (Mill.) Britton, Sterns & Poggenb.). Both stands are of fire origin and have approximately 20 years of difference, but as their heights were similar (16.2 for the private stand and 15.7 for the public stand), we considered both stands to be very similar and excluded successional effect. For specific stand characteristics, see Rochon et al. (2009).

Distribution of the observation plots

Observation plots were established in 2005 in both stands. Between 2005 and 2007, 72 locations containing fruiting bodies of *C. cibarius* var. *roseocanus* were found in the study

area; 42 were located in the public stand and 30 were in the private stand. From these locations, 45 plots were randomly chosen to establish observation plots. *Cantharellus cibarius* var. *roseocanus* plots were at least 20 m apart and their size varied from 1 to 144 m^2 depending on how disperse fruiting bodies were within the plot. Plot area generally corresponded to an ellipse with major and minor axes estimated from the two longest distances between fruiting bodies. Plots were established over the entire study site: 21 were located in the public stand and 24 in the private stand. In the private stand, plots were located in the forest strips between the trails.

In addition, 50 control plots (chanterelle-free plots) of 5 m^2 (2.25 x 2.25 m) were distributed equally in the stands since one of the objectives was to define factors linked with mushroom presence/absence. These were randomly selected on the map of the study stands. In 2006 and 2007, seven control plots were eliminated because they were either lost or chanterelle fruiting bodies were found within 7 m of those plots. To avoid the presence of mushroom mycelium in control plots, a 7 m threshold was chosen based on Dunham et al. (2003a) who suggested that genets of *C. formosus* Corner had mean widths of 3.2 ± 3.6 m. In total, 88 observation plots (45 *C. cibarius* var. *roseocanus* plots and 43 control plots), located at least 20 m apart, were established over the entire study site. These plots were visited weekly from mid-June until the end of September during three years (2005, 2006 and 2007), except for 2005 when observations started on August 11 due to late establishment of the experimental design.

Mushroom molecular identification and detection in soil profiles

In 2007, to confirm that *C. cibarius* var. *roseocanus* was absent from control plots, soil cores were taken twice (before mushroom fructification (July 6, 2007) and during mushroom fructification (July 30, 2007) from 11 control plots in areas with high *C. cibarius* var. *roseocanus* density. To detect the presence of *C. cibarius* var. *roseocanus* mycelium in soil horizons, soil cores were taken from five randomly chosen chanterelle plots. Five soil cores were taken before mushroom fructification and seven soil cores, among which two had a carpophore at the soil surface, were taken during mushroom fructification. In those five chanterelle plots, 10 additional soil cores were collected to

observe *C. cibarius* var. *roseocanus* ectomycorrhiza morphology and distribution in the soil profile. Each soil core was randomly sampled within a plot.

Soil cores were obtained by placing a plastic sleeve into a metal cylinder (4.5 cm diameter x 29 cm height) that was driven into the soil and then removed from it. Within 24 hours after sampling, soil cores were frozen at -20^{o}C until DNA extractions. In the laboratory, each core was aseptically extracted from its sleeve and separated into different horizons according to the Canadian System of Soil Classification for Orthic Humo-Ferric Podzol soil (Soil Classification Working Group 2002). There were two organic horizons (F and H) and two mineral horizons (Ae (eluviation horizon) and B (accumulation horizon)). Each horizon was mixed, sieved at 4 mm to remove coarse woody debris and roots, and immediately subsampled for DNA extraction. DNA was extracted using the Power soil DNA kit (MO Bio Laboratories, Inc., Carlsbad, CA, USA) following the manufacturer's instructions, except for the final elution which was carried out with 50 µL of elution buffer instead of 100 µL.

Sequences of *C. cibarius*, *C. formosus*, *C. subaldibus* Smith & Morse and *C. cibarius* var. *roseocanus* available from GenBank and first sequenced using universal primers ITS1F and ITS4 (Gardes and Bruns 1993) were aligned and compared with designed ITS primers specific to *C. cibarius* var *roseocanus*. Primers RS1A (5'-ATAGAGCCGTTCCAGTTGGGC-3') and RS1R (5'-AGCAATTACCCCAAGCAAGC-3') were designed using Primer3 v.0.4.0 (http://frodo.wi.mit.edu/primer3) and synthesized by Invitrogen (Invitrogen, Carlsbad, CA, USA). GenBank (NCBI) was queried using the BLASTn algorithm (http://www.ncbi.nlm.nih.gov/BLAST/) to detect sequences highly similar to those primers. Moreover, the designed primers were tested on other chanterelle species to verify their specificity.

The designed primers were then used to detect *C. cibarius* var. *roseocanus* presence in soil samples. Amplification reactions were performed in a 20 µL mixture volume containing 25 ng of DNA from soil, 1X of PCR buffer, 1.5 mM of $MgCl_2$, 0.1 mg of BSA, 200 µM of each dNTP, 0.4 µM of primer pair, and 0.025 units of *Taq* DNA polymerase.

They were performed using the designed primers under the following conditions: one cycle of 3 min at 94°C, 34 cycles of 1 min at 94°C followed by 1 min at 66°C and 1 min at 72°C, followed by a final extension at 72°C for 10 min. PCR products were analyzed on 1% agarose gels. A threshold concentration, under which the PCR test could not detect the mushroom, of 1.55 x 10^{-5}g/g was established using serial dilutions of lyophilised powder of *C. cibarius* var *roseocanus* individuals in sterilized sand.

Cantharellus cibarius var. *roseocanus* characteristics and productivity

The total number of fruiting bodies found in the plots and their degree of maturity were recorded weekly over three chanterelle growing seasons, which typically starts in mid-July and finishes at the end of September. During the 2005 growing season, only half of the fruiting bodies were harvested and the other half were left on-site and marked with methylene blue on the cap to avoid double counting as suggested by Straatsma et al. (2001). Since Egli et al. (2006) demonstrated that systematic harvesting had no impact on subsequent sporocarp production, all fruit bodies were harvested in 2006 and 2007. Average maximum and minimum cap diameters (cm), fresh mushroom biomass (mushroom mass (g)/total plot area (m^2)) and mushroom density (number of fruiting bodies/total plot area (m^2)) were measured. Since correlations can be obtained between cap diameter and weight (Pilz et al. 1998), we chose to present the biomass results only. Moreover, dry mass was not assessed to allow the owners of the private property to sell the freshly harvested chanterelles. However, for the purpose of this study, biological productivity can be described as the total number (i.e., mushroom density) or weight (i.e., mushroom biomass) of fruit per unit area during the course of a fruiting season (Pilz and Molina 2002).

The overall productivity or landscape productivity (i.e., the estimated quantity of fruiting bodies located in the entire study site) of the stands was also extrapolated from *C. cibarius* var. *roseocanus* harvested in the plots. It was estimated based on an inventory made in 2005 that the plots represented 75% of the fruiting bodies found in the private stand and 50% of those found in the public stand. Those estimates allowed us to extrapolate the overall productivity from the biological productivity.

In 2005, to determine *C. cibarius* var. *roseocanus* lifespan, sporocarps located in the non-harvested plots were followed through their entire growth period, from August 11 until September 30. In total the lifespan of 235 sporocarps located in 15 different plots was determined and used to establish the lifespan average for *C. cibarius* var. *roseocanus* sporocarps in the study site.

Influence of ecological parameters

To identify which ecological parameters are related to *C. cibarius* var. *roseocanus* productivity and carpophore's presence in the microhabitat, organic and mineral soil, stand, vegetation and meteorological parameters were measured. Details about data gathering are presented in Rochon et al. (2009) but briefly, in 2005, we assessed pH in distilled water and in KCl solutions; mineral N (NO_3^- and NH_4^+) concentrations from a 2 mol/L KCl extraction; Bray-2 extractable P (phosphorus); cation exchange capacity (CEC) determined by the sum of exchangeable cations (sodium, manganese, iron, aluminum, potassium, magnesium and calcium) extracted with 0.1 mol/L $BaCl_2$; carbon:nitrogen ratio (C:N) determined on a CNS analyzer; and soil texture for the mineral fraction only (fine sand, coarse sand, silt and clay percentage) using the hydrometer method.

The following vegetation variables were recorded in each plot: the percentage (%) of ground covered by lichens of two undistinguishable species(*Cladonia stellaris* (Opiz) Pouzar & Vězda and *Cladonia rangiferina* (L.) Weber ex F.H. Wigg.)), moss (*Pleurozium schreberi* (Brid) Mitt.), and individual species of herbs, shrubs and trees (Bonham 1989). The percentage of exposed soil with epigeous woody debris was also measured.

Stand characteristics such as tree diameter (cm) and height (cm), basal area (m^2/ha) and tree density (trees/ha) were assessed for all tree species with a diameter at breast height (DBH) greater than 7 cm, in a 5 m radius around each plot centre. Canopy cover percentages (expressed by the opening of the canopy and the percentage of transmissible light) were obtained for each plot following the protocol reported elsewhere (Rochon et al. 2009).

Meteorological variables measured every 5 min by a field portable meteorological station installed in the private stand were: air temperature (°C) at ground level, mineral soil temperature (°C) at 5 cm depth, and soil relative humidity (%) at 12 cm depth. Total rainfall (mm) was recorded during rain events. Moreover, since Norvell et al. (1996) suggested that micrometeorological data would prove more informative in tracking sporocarp production of basidiomycetes than generalized weather data, watchdog temperature data loggers that captured soil temperatures at 5 cm depth every hour were installed, and soil relative humidity (%) at 12 cm depth was monitored weekly inside each plot.

Statistical analysis

To achieve homoscedasticity and normality of the residuals, log transformation on data was applied when necessary. This was a completely randomized experimental design used to compare fresh mushroom biomass (g/total plot area (m^2)) and mushroom density (number of fruiting bodies/total plot area (m^2)) in the two locations. Hierarchical experiments with repeated measurements followed by LSD (least significant difference) comparison tests were used in the observation plots (n = 44). Analyses of variance were performed using a mixed-model analysis (SAS Proc Mixed, version 9.1 (SAS Institute Inc. 2004)).

The relationships between *C. cibarius* var. *roseocanus* productivity and soil, stand, vegetation and meteorological parameters were explored using stepwise multiple regressions. The significance level chosen for all tests was $\alpha = 0.05$ and n = 44 because only *C. cibarius* var. *roseocanus* plots were used. Multiple regression analyses were performed for combined years. Two dependent variables were used for these analyses: fresh mushroom biomass and mushroom density. The two stands were analyzed as a whole. Multiple regression analyses were performed and Pearson's correlation coefficients were used to determine significant correlations.

Soil parameters in the mineral and organic soil as well as meteorological, stand and vegetation parameters were considered as independent variables. The soil parameters were C:N ratio, extractable P, CEC, available N (NO_3^- and NH_4^+), pH, percentage of light reaching the forest floor, and percentage of fine sand, coarse sand, clay and silt. The stand parameters were basal area, tree density, tree height and organic matter thickness, and the vegetation parameters were the percentage cover of trees, understory vegetation, exposed soil with epigeous wood debris, lichens and mosses found in the plots. Species that occurred only once were removed from the analyses (Nantel and Neumann 1992). The meteorological parameters were the cumulative sum of degree-days, weekly mean, maximum and minimum air temperature, weekly total rainfall calculated from the meteorological station data, and weekly mean, maximum and minimum soil temperature and soil relative humidity found in the observation plots. To determine which meteorological parameter influenced *C. cibarius* var. *roseocanus* productivity and when, analyses were done at 0 shift (i.e., analyses were run when mushroom fructification and meteorological data coincided with the calendar date) and at 1 to 8 weeks shift (e.g., analyses were run when meteorological data were shifted by 1 week prior to the calendar date initiation of mushroom fructification) during both years.

Stepwise discriminant analyses (n = 100) were performed to find predictors that best discriminated plots containing *C. cibarius* var. *roseocanus* fruiting bodies from control plots, independently of the plot location (private/public stands). These analyses aimed at determining which combination of parameters (soil, stand and vegetation) best predicted *C. cibarius* var. *roseocanus* habitat. Independent parameters were the same as in the multiple regression analyses except that the percentages of sand, clay and silt were removed from the analyses due to some missing data. All univariate and multivariate tests were performed using SAS, version 9.1.

Results

Mushroom molecular identification and detection in soil profiles

The *Cantharellus* identified in the study site was *C. cibarius* var. *roseocanus* (J.M. Moncalvo, Senior Curator, Royal Ontario Museum, Department of Natural History, Toronto, Canada, personal communication, 2006). In this study, the designed primers were highly specific to *C. cibarius* var. *roseocanus* when compared to other chanterelle species. Moreover, sequences of amplicons having 99% similarity with sequences in GenBank were identified as belonging to *C. cibarius* var *roseocanus* (Dunham et al. 2003b). At the threshold concentration of 1.55×10^{-5} g/g, gel analyses of PCR products using the designed primers did not reveal the presence of any *C. cibarius* var. *roseocanus* in soil layers of the 11 control plots located in areas of high *C. cibarius* var. *roseocanus* density. Based on these analyses, *C. cibarius* var. *roseocanus* was considered absent or present in undetectable quantities in the control plots of the study site.

Cantharellus cibarius var. *roseocanus* DNA was detected before and during mushroom fructification in the soil of 4 out of the 5 chanterelle plots tested. In 75% of the plots sampled July 6 (before mushroom fructification), *C. cibarius* var. *roseocanus* mycelium was detected in the Ae (eluviation) and B (accumulation) horizons, and in 25% of the plots it was detected in the F horizon (Figure 3.1). During mushroom fructification, July 30, DNA was detected in the Ae horizon in half of the plots, in the B horizon in 60% of the plots, and in the F and H horizons in 20% of the plots (Figure 3.1). Moreover, in the two samples with a fruit body present at the soil surface, *C. cibarius* var. *roseocanus* DNA was present in the F and H horizons. The ten additional soil cores used to detect mycorrhiza in the soil horizons showed, upon visual observation, that *C. cibarius* var. *roseocanus* ectomycorrhiza were present exclusively in the organic horizons.

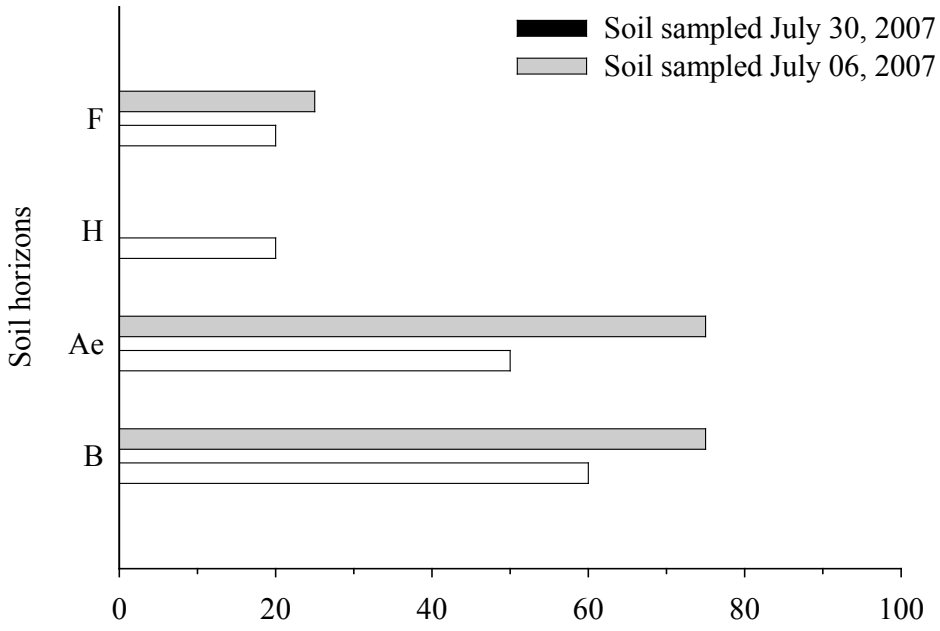


Figure 3.1. Proportion of *C. cibarius* var. *roseocanus* plots where DNA was detected in each soil horizon (F, H, Ae and B). Black bars are for soils sampled before mushroom fructification (July 6, 2007) and grey bars are for soils sampled during mushroom fructification (July 30, 2007).

C. cibarius var. *roseocanus* characteristics and productivity

In 2006 and 2007, the first fruiting bodies were observed between July 10 and 12 in the plots (Figure 3.2). Data are not available for 2005 as fruiting bodies were not recorded before August 11. The average lifespan of *C. cibarius* var. *roseocanus* was 35 days, with 11% of the mushrooms surviving less than 20 days and 35% living over 40 days.

The yearly overall sporocarp productivity of the study site was estimated from the production obtained in the plots and varied, depending on the year and site, from 0.23 kg/hectare to 3.85 kg/hectare. From August 11 to September 29, 2005, biological productivity was 12.88 g/m^2 in the private stand and 17.12 g/m^2 in the public stand (Table 3.1). In 2006, observation plots yielded 42.04 g/m^2 in the private stand and 47.05 g/m^2 in the public stand between July 10 and September 23 (Table 3.1). Peak mushroom fructification occurred during the week of August 12 in 2005 and during the week of July 25 in 2006 (Figure 3.2). In 2005 and 2006, the emergence of new fruiting bodies started to slow down at the end of August (August 29 in 2006 and August 31 in 2005) and was

reduced to a very low level by the end of September (Figure 3.2). From July 10 to September 17, 2007, fresh mushroom biomass in the plots varied from 24.94 g/m^2 in the private stand to 41.56 g/m^2 in the public stand (Table 3.1). The 2007 fructification of *C. cibarius* var. *roseocanus* was delayed compared with 2005 and 2006 (Figure 3.2). The first fruiting bodies were observed in mid-July, and then the emergence of fruiting bodies almost stopped for two weeks at the beginning of August. The fructification peak happened during the week of August 13 and new fruiting bodies were still emerging on September 17 (last data recorded) (Figure 3.2).

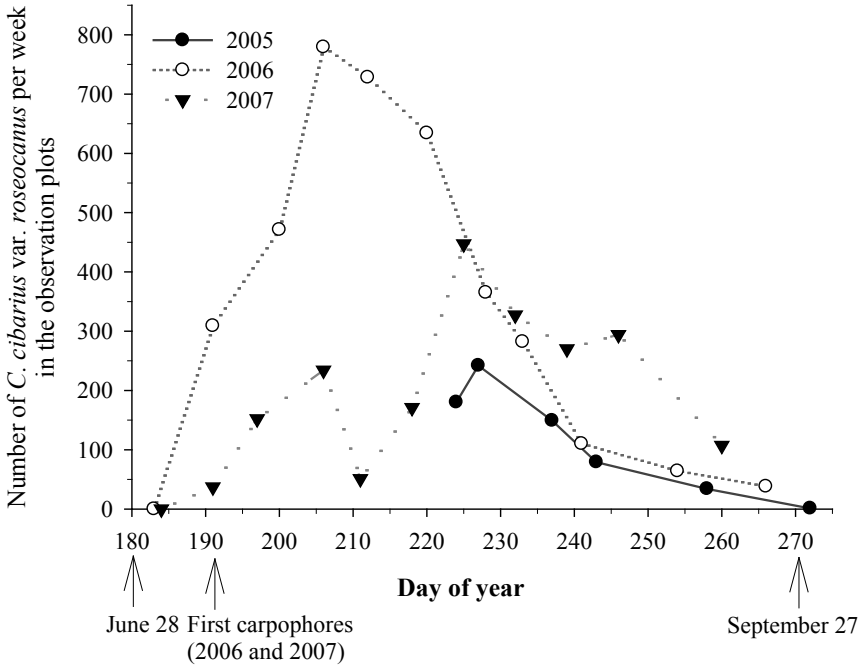


Figure 3.2. Number of *C. cibarius* var. *roseocanus* harvested weekly in the observation plots from July 10 (calendar day 191) to September 30 (calendar day 272) for the years 2005 to 2007. Due to the late establishment of the experimental design, the first harvesting date was August 11 (calendar day 224) in 2005.

Table 3.1. Biological productivity (expressed as *C. cibarius* var. *roseocanus* fresh mushroom biomass and sporocarp density in the plots) for the years 2005, 2006 and 2007.

	Private stand plots[a]			Public stand plots		
	2005	2006	2007	2005	2006	2007
Fresh mushroom biomass (g/total plot area (m^2))	12.88	42.04	24.94	17.12	47.05	41.56
Sporocarp density (no./total plot area (m^2))	1.45	4.69	2.73	1.22	4.13	3.79

[a] Private stand plots are located in the forest strips between trails.

Comparison between the two stands

No significant differences in mushroom density (number of fruit bodies/total plot area (m^2)) and fresh mushroom biomass (g/total plot area (m^2)) were found between the two stands within the same year during the 3-year study (Figure 3.3). Thus, even with the trail opening in the private stand, the two stands were considered similar and were analysed together in the following section (influence of ecological parameters). Moreover, mushroom density and fresh biomass were significantly higher in 2006 than in 2005 in both locations. Additionally, no colonies of *C. cibarius* var. *roseocanus* growing exclusively on the trails were found during the 3-year study.

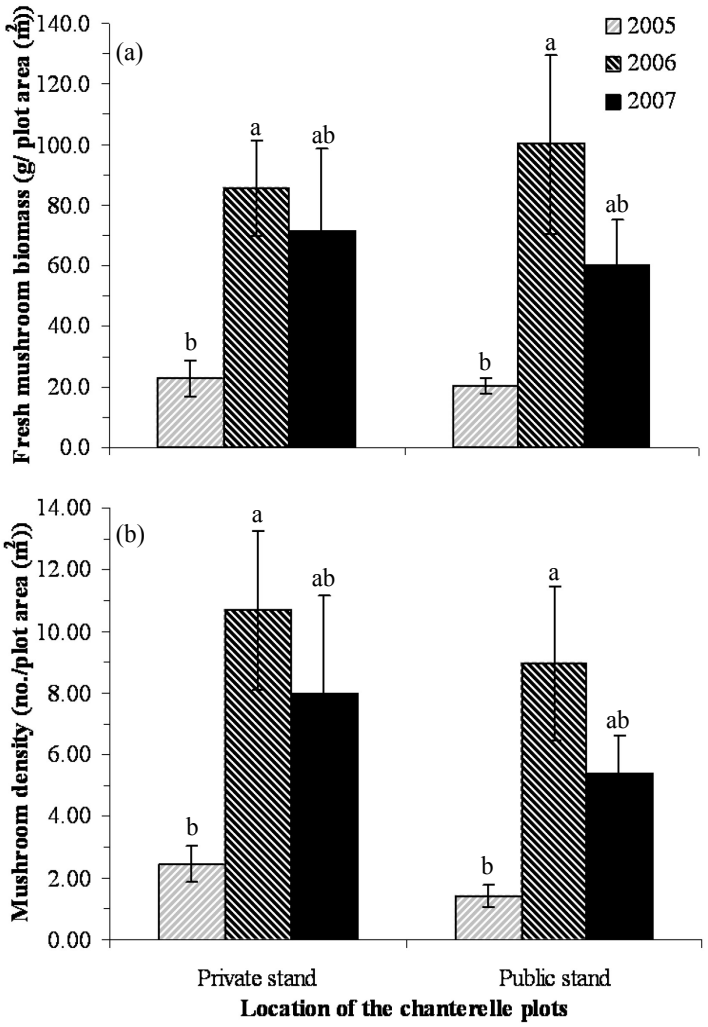


Figure 3.3. (a) Fresh *C. cibarius* var. *roseocanus* biomass and (b) *C. cibarius* var. *roseocanus* density found in the observation plots from the beginning of the fruiting season in 2005 until the end of the fruiting season in 2007. Different letters represent significant differences under a least significant difference (LSD) test at α=0.05. The error bars represent the standard error.

Influence of ecological parameters

Soil and stand characteristics

To detect which combination of parameters best predicted *C. cibarius* var. *roseocanus* habitat (comparison between plots with and without *C. cibarius* var. *roseocanus* fruiting bodies) independently of plot location, discriminant analyses were performed on 18 soil and stand parameters. At each step a one-way ANOVA was performed, and the parameter with the highest *F*-to-enter value was selected. Soil and stand parameters that were highly

correlated with *C. cibarius* var. *roseocanus* habitat were, in order of importance, stand density (0.46), C:N ratio $_{(organic\ layer)}$ (0.45), NH_4^+ $_{(mineral\ layer)}$ (-0.40), pH $_{(mineral\ layer)}$ (0.34), pH $_{(organic\ layer)}$ (0.16), and NO_3^- $_{(mineral\ layer)}$ (-0.12) (Table 3.2). Table 3.3 shows that when using the discriminant function with the soil and stand parameters as predictors, 68.9% of *C. cibarius* var. *roseocanus* plots and 72.1% of the control plots were correctly classified.

Stepwise multiple regressions included three additional soil parameters: percentage of coarse sand (1.0-0.25 mm), fine sand (0.11-0.05 mm), and clay and silt. Results for mushroom biomass and density were similar, thus only the results for mushroom density are presented. According to the stepwise selection method, significant positive correlations were found with percentage of clay and silt (0.75), NO_3^- $_{(mineral\ layer)}$ (0.29) and pH $_{(organic\ layer)}$ (0.34), and negative correlations were detected with the percentage of coarse sand (-0.50) and stand density (-0.30) (Model 1, Table 3.4). While the stepwise analysis detected high correlations with those parameters, the statistical regression for the mushroom density only included percentage of clay and silt (which average in the soil was estimated at 13.7%), NO_3^- $_{(mineral\ layer)}$ and C:N ratio (Model 1, Table 3.4). It should be noted that NO_3^- was found in extremely low concentrations in both soil layers, varying from 0.0 mg/g to 0.0085 mg/g.

Table 3.2. Parameters selected by stepwise discriminant analyses as potential habitat predictors for *C. cibarius var. roseocanus* (control vs chanterelle plots) among 18 stand and soil parameters (first analysis) and 23 vegetation parameters (second analysis).

Analyses	Step	Selected parameter	F value	Pr > F	Wilks' λ^a	Pr > λ^a	a^b	r^c
Stand and soil parameters	1	Stand density	5.51	0.02	0.94	0.021	0.41	0.46
	2	pH $_{(mineral\ layer)}$	3.22	0.08	0.91	0.015	0.65	0.34
	3	NH_4^+ $_{(mineral\ layer)}$	4.30	0.04	0.86	0.006	-0.64	-0.40
	4	pH $_{(organic\ layer)}$	2.60	0.11	0.84	0.004	0.51	0.16
	5	NO_3^- $_{(mineral\ layer)}$	3.94	0.05	0.80	0.002	-0.54	-0.12
	6	C:N ratio $_{(organic\ layer)}$	3.13	0.08	0.77	0.001	0.43	0.45
Vegetation parameters (% cover)	1	Exposed soil with woody debris	24.92	<0.0001	0.78	<0.0001	1.62	-0.64
	2	*Solidago puberula*	8.00	0.006	0.71	<0.0001	-0.53	0.22
	3	*Pteridium aquilinum*	6.27	0.01	0.66	<0.0001	0.34	-0.23
	4	*Ledum groenlandicum*	3.82	0.05	0.63	<0.0001	0.37	-0.17
	5	*Picea mariana*	3.88	0.05	0.60	<0.0001	0.36	-0.27
	6	Moss (*P. schreberi)*	2.80	0.10	0.58	<0.0001	0.79	0.49

[a] Wilks' λ values and associated p-values refer to multivariate analysis of a variance model (Manova) as measured by λ.
[b] a is the standardized canonical discriminant function coefficient.
[c] r is the correlation of the parameter with the discriminant function (structure coefficient).

Table 3.3. Proportion of *C. cibarius* var. *roseocanus* and control plots correctly and incorrectly classified by the discriminant analyses based on stand, soil, and vegetation parameters.

Analyses	Total number of plots	Plots classified as *C. cibarius* var. *roseocanus* plots	Plots classified as control plots
Stand and soil parameters	45 *C. cibarius* var. *roseocanus* plots	31/45 (68.9%)	14/45 (31.1%)
	43 control plots	12/43 (27.9%)	31/43 (72.1%)
Vegetation parameters (% cover)	45 *C. cibarius* var. *roseocanus* plots	42/45 (93.3%)	3/45 (6.7%)
	43 control plots	14/43 (32.6%)	29/43 (67.4%)

Table 3.4. Best models selected with stepwise multiple regressions analyses for *C. cibarius var. roseocanus* density (no. of fruit bodies / total plot area (m^2)) among observation plots containing fruiting bodies for the years 2005, 2006 and 2007 combined.

Model	Ecological parameters	Value of the parameter			Value for the model	
		Correlation coefficients	SE[a]	P-value	R-square	P-value
Model 1: Soil parameters						
	Percentage of clay and silt	0.75	0.13	0.0005	0.70	0.0001
	NO_3^- (mineral layer	0.29	718.0	0.02		
	C:N ratio (mineral layer)	0.04	0.08	0.13		
	Stand density[b]	-0.30	.	.	.	N.I.[c]
	Percentage of coarse sand (0.25-1.0 mm)[b]	-0.50	.	.	.	N.I.
	pH (organic layer)[b]	0.34	.	.	.	N.I.
Model 2: Vegetation parameters (% cover)						
	Solidago puberula	0.24	0.09	0.05	0.16	0.03
	Cornus canadensis	0.23	0.005	0.09		
	Lichens (*C. stellaris* and *C. rangiferina*)[b]	0.21	.	.	.	N.I.
	Linnaea borealis[b]	0.22	.	.	.	N.I.
	Vaccinium angustifolium[b]	-0.21	.	.	.	N.I.
	Comptonia peregrina[b]	0.18	.	.	.	N.I.
	Kalmia angustifolia[b]	-0.17	.	.	.	N.I.
Model 3: Meteorological parameters[d]						
	Air temperature (°C) 2 weeks prior to fructification	0.74	0.03	<0.0001	0.74	<0.0001
	Total rainfall (mm) 1 week prior to fructification	0.50	0.004	0.003		

[a] SE is the standard error.
[b] Parameter chosen by the stepwise selection method but not included in the regression model.
[c] N.I. = Not included in the regression model
[d] Meteorological parameters are for 2006 and 2007 only and data were analysed for each week.

Vegetation parameters

The parameters selected as the most discriminant between the plots with and without mushrooms were, in order of importance, percentage of exposed soil with woody debris, percentage cover by *Solidago puberula* Nutt., *Pteridium aquilinum* (Linné) Kuhn, *Ledum groenlandicum* (Oeder) Kron & Judd, *Picea mariana* and moss (*Pleurozium schreberi*) (Table 3.2). Moreover, Table 3.3 shows that when using the discriminant function with the vegetation parameters as predictors, 93.3% of *C. cibarius* var. *roseocanus* plots and 67.4% of the control plots were correctly classified.

Stepwise multiple regressions showed similar results for mushroom biomass and density, thus only the results for mushroom density are presented. According to the stepwise selection method, mushroom density was significantly correlated with *Solidago puberula* (0.24), *Cornus canadensis* L. (0.23), the percentage cover of lichens (*C. stellaris* and *C. rangiferina*) (0.21), *Linnaea borealis* L. (0.22), *Vaccinium angustifolium* Aiton (-0.22), *Comptonia peregrina* (L.) Coulter (0.18) and *Kalmia angustifolia* L. (-0.17). *Solidago puberula* and *Cornus canadensis* were the only parameters included in the regression model (Model 2, Table 3.4).

Meteorological parameters

To determine which meteorological parameters influenced *C. cibarius* var. *roseocanus* productivity (using mushroom density), nine parameters were analysed at 0 shift (i.e., analyses were run when productivity and meteorological data coincided with the calendar date) and at 1 to 8 weeks shift. Only the years 2006 and 2007 were considered; 2005 was not included in the analyses due to the late mushroom harvest and missing meteorological data. The best meteorological parameters that explain mushroom density in both stands over a 2-year study were total rainfall (mm) one week prior to fructification and air temperature (°C) two weeks prior to fructification (Model 3, Table 3.4).

The dynamics of rainfall events, air temperature and emergence of mushroom fructification for 2006 and 2007 are shown in Figure 3.4. The year 2006 had a mean annual rainfall of 752.0 mm. During that year, many small rain events were recorded from July 2 to July 10 (date of the first fruiting body emergence), and 10.5 mm of cumulated rain were recorded one week prior to the highest mushroom density peak (July 25) (Figure 3.4a). Moreover, higher temperatures than the daily average were recorded two weeks prior to this peak (Figure 3.4c). The absence of rainfall from August 8 to August 13, 2006 (day 220 to day 225), was followed by a decrease in mushroom density on August 16 (day 228) (Figure 3.4a). In 2007, mean annual rainfall was 543.5 mm, and three mushroom density peaks were observed on July 22, August 8 and September 3 (days 205, 225 and 246). Each of those peaks was preceded by rainfall events 7 to 10 days prior to the peak (11.4 mm, 17.8

mm and 27.9 mm of cumulated rain, respectively) (Figure 3.4b). Furthermore, two weeks prior to the highest mushroom density peak in 2007, a temperature increase was recorded (Figure 3.4d).

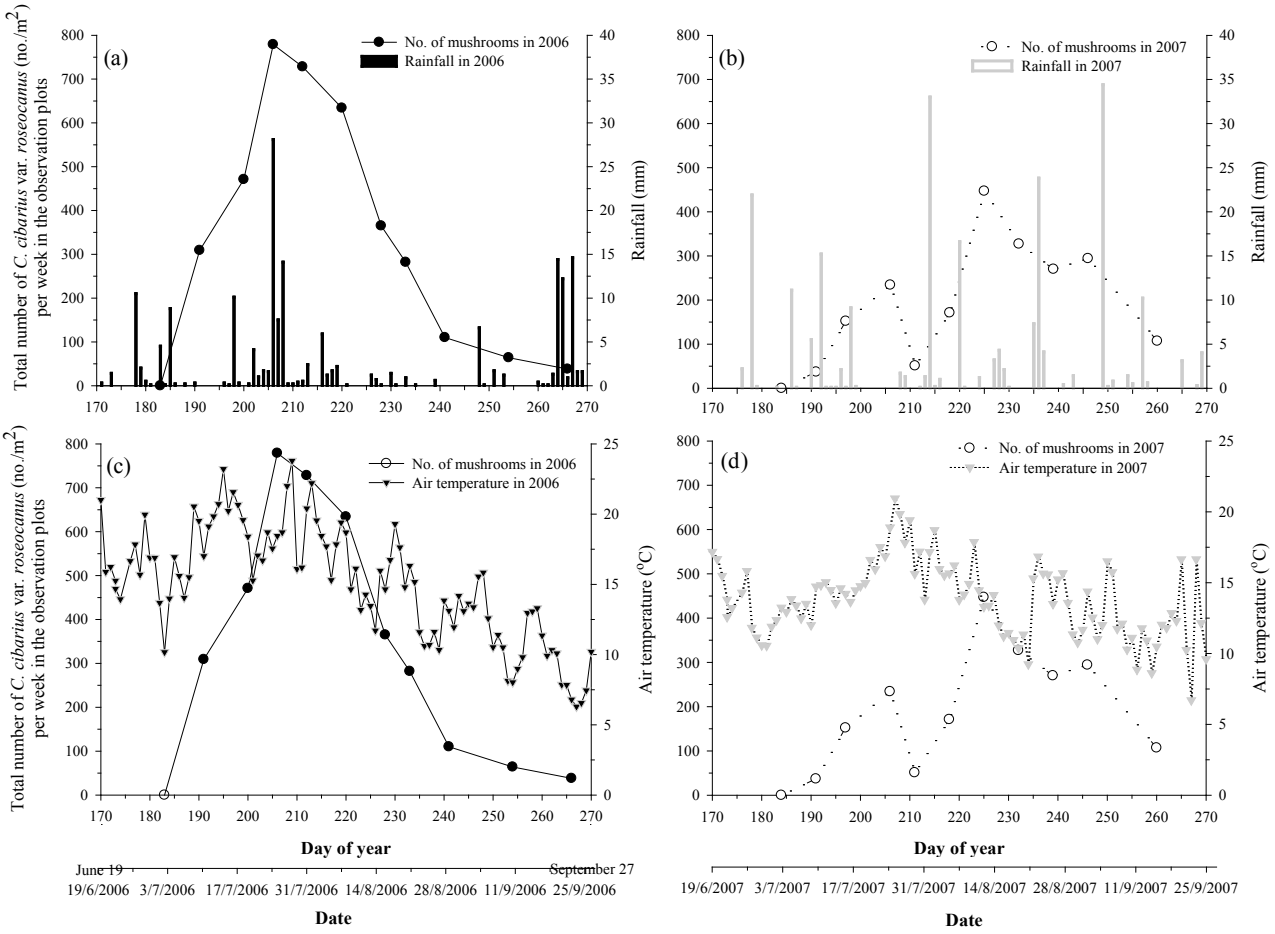


Figure 3.4. Number of *C. cibarius* var. *roseocanus* harvested weekly in the observation plots associated with (a) daily rainfall in 2006, (b) daily rainfall in 2007, (c) average daily air temperature in 2006, and (d) average daily air temperature in 2007 at the study site weather station from June 19 to September 27.

Discussion

Mushroom molecular identification and detection in soil profiles

This study confirms the presence of *C. cibarius* var. *roseocanus* in Quebec's boreal forest by molecular identification. It also reveals that the mycelium of this chanterelle species is abundant in the top mineral soil, but it can also develop in the organic horizon,

especially at the time of fructification. The presence of ectomycorrhiza in the organic horizons mostly near fruit bodies corroborates the fact that mycelium was detected in the organic horizons at the time of fructification. Our results support those of Rosling et al. (2003) who found that the organic layer and mineral horizon (especially the one located immediately beneath the organic layer) are intensively exploited by ECM. However, further observations are required to confirm the exact patterns of *C. cibarius* var. *roseocanus* in soil horizons.

Cantharellus cibarius var. *roseocanus* characteristics and productivity

During this 3-year study, fruit bodies of *C. cibarius* var. *roseocanus* seemed to first appear in mid-July. A peak was then detected between the end of July and mid-August, and the decline in production happened at the end of August. In North America, *Cantharellus* spp. are normally considered as species fruiting late in the summer (e.g. Pilz et al. 2006), but in the eastern Canadian forest they seem to start fruiting earlier (Spahr 2009). The average lifespan found in our observations plots (35 days) shows that *C. cibarius* var. *roseocanus* lifespan is similar to other *Cantharellus sp.* as it is consistent with Pilz et al. (2003) who stated that chanterelle are long-lived mushrooms that persist for approximately 44 days. However, Tanino et al. (2005) mentioned in their correspondence with several international experts that chanterelles may live up to 84 days (with an average lifespan of 49 days). These findings corroborate our own personal observation.

The average overall sporocarp productivity (or landscape productivity) in the study site varied, depending on the year and stand, from 0.23 kg/ha to 3.85 kg/ha. Pilz et al.'s (1998) results were similar to ours, ranging from 0.076 kg/ha to 21.95 kg/ha, with an average of 2.52 kg/ha. However, in a 6-year study undertaken in the Central Cascade Range (Oregon, USA), average productivity of *C. formosus* and C. *subalbidus* across stands was 9.79 kg/ha/year (Pilz et al. 2003). The warmer climate, allowing an extended productivity, possibly favoured a higher average productivity than ours.

It seems that biological productivity varied greatly between years in the study site, with the year 2006 being a period favourable to *C. cibarius* var. *roseocanus* productivity and the

year 2005 being less productive. Those variations were likely due to meteorological conditions, but might also have been caused by the late harvest in the 2005 plots. This suggests that intensive sampling over a minimum of 3 to 8 years, as proposed by Gardes and Bruns (1996), could give us a better insight into species productivity and ecology over time.

Comparison between the two stands

No significant difference in terms of productivity was found between locations (public and private stands). This revealed that *C. cibarius* var. *roseocanus* colonies were not disturbed by small changes in their environment, as long as host trees remained present in the stand. However, the lack of *C. cibarius* var. *roseocanus* colonies growing exclusively on trails in one of the stands seems to reflect a negative impact of trail opening on this mushroom, supporting the idea that mycorhizal fungi are best adapted to prevailing conditions in natural forest sites (Meyer 1973). A low number of sporocarps in canopy gaps were also detected by Grebenc et al. (2009). The trail opening, which involved tree removal and mulching of branches, might have created microenvironmental conditions that were not suitable for *C. cibarius* var. *roseocanus* growth. This could not be quantified as no data had been recorded prior to removing trees. Nevertheless, those conclusions need to be used with caution because locations were not replicated. Results are only valid for the two specific stands studied. Therefore, a much larger study with additional replicates designed to evaluate the impact of trails on *C. cibarius* var. *roseocanus* is needed.

Influence of ecological parameters

Soil and stand characteristics

Many factors associated with forests are collectively responsible for influencing ECM development, including plant species composition, forest structure (Ishida et al. 2007), stand age and soil nutrients (Avis et al. 2003), but ECM fungi are most likely to be affected by a combination of vegetation and of direct and indirect soil parameters (Nantel and Neumann 1992). Depending on the chosen variables (mushroom density, biomass or

mushroom presence/absence), the ecological parameters influencing mushroom fructification may differ (Rochon et al. 2009). However, in this study, these three variables were similar for soil and stand parameters.

Cantharellus cibarius var. *roseocanus* was present in high density mature stands but the productivity was higher in the open canopy where *S. puberula* and *P. schreberi* were most abundant. Because chanterelles obtain their carbohydrate nutrition from living trees through a symbiotic association (Pilz et al. 2003), they need tree partners to grow and produce basidiomata, which explains why they were found mainly where stand density was high. No other stand characteristics were identified as good indicators of *C. cibarius* var. *roseocanus* presence or density in plots. This result is supported by Twieg et al. (2009), who found that tree cover variables were weakly correlated with ECM communities.

The pH in both soil layers was a good predictor of *C. cibarius* var. *roseocanus* presence in the habitat and was significantly correlated with mushroom productivity: mushroom density increased with the pH (up to a pH of 5.8). *Cantharellus cibarius* var. *roseocanus* seems to grow in a moderately acidic environment (on soils with a pH ranging from 4.0 to 5.8) as does *C. cibarius* in the Netherlands (Jansen and van Dobben 1987).

The C:N ratio was a good predictor of chanterelle presence in a stand, and mushroom productivity increased with a higher C:N ratio. Our results suggest that *C. cibarius* var. *roseocanus* was mostly associated with mature stands where, as mentioned by Olsson et al. (1996), the C:N ratio is higher and the presence of mineralizable N is lower than in disturbed areas. Moreover, our results are in agreement with those of Jansen and van Dobben (1987) who showed that *C. cibarius* has a preference for soils with lower mineral N content.

NH_4^+ and NO_3^- were negatively associated with *C. cibarius* var. *roseocanus* presence in the stands but as NO_3^- increased in the *C. cibarius* var. *roseocanus* plots, mushroom productivity also increased. As shown in Avis et al. (2003), sporocarps production generally declines with increasing fertilization (e.g. *Amanita* sp., *Boletus* sp., and

Cortinarius sp.). However, the relation between NO_3^- and mushroom productivity is neither consistent with Kernaghan et al. (2003), who found no effect of N on ECM communities in boreal mixed forests, nor with Rangel-Castro (2001), who demonstrated that *C. cibarius* vegetative mycelia had a preference for NH_4^+ over other sources of N. Because soil C:N can be considered as a good predictor of soil available N (negatively) and showed an opposite trend to mineral N concentrations and because the amounts of mineral N (NH_4^+ and NO_3^-) measured were extremely low (0.0 mg/g to 0.0085 mg/g), the dissimilarities between our and other results suggest that a direct effect of N availability should be considered with caution.

In the study site, a positive correlation was found between *C. cibarius* var. *roseocanus* productivity and the percentage of clay and silt in the soil, and a negative correlation was found with the percentage of coarse sand. These results reveal a difference between *C. cibarius* of the Netherlands, which is found on sandy and well-drained forest soils (Jansen and van Dobben 1987), and *C. cibarius* var. *roseocanus*, which grows in fine sandy environments that contain clay and silt (which amount was averaged at 13.7% in the soil of the study site).

Vegetation parameters

Several plant species had a high importance for the chanterelle presence in the habitat and were associated with mushroom productivity. Vegetation parameters influencing mushroom productivity differed from the habitat (presence/absence) predictors, except for *S. puberula*. *Solidago puberula*, *P. banksiana* (the main host species of *C. cibarius* var. *roseocanus* in the study site) and *C. peregrina*, a N-fixing plant, form a common association found in the Lac St-Jean sandy plains (Marie-Victorin 1995). Therefore, this association and the soil conditions related to it (well-drained acidic soils with fine sand) are likely to represent a high-quality environment for *C. cibarius* var. *roseocanus*.

Some vegetation parameters represented inadequate habitats for *C. cibarius* var. *roseocanus*, as for example: exposed soils with woody debris, which are mainly found in trails; *P. aquilinum,* which is found in dry areas; *L. groenlandicum*, which is found in

swampy habitats; and *P. mariana*, which was situated in an inadequate habitat for this mushroom in this study. Despite the fact that *Kalmia angustifolia* and *Vaccinium angustifolium* were abundant in the jack pine stands, these ericaceous species were not frequently observed in association with *C. cibarius* var. *roseocanus* possibly because they are known to be competitors for nutrients and possible suppressors of ECM root formation (Yamasaki et al. 1998). Moreover, it has been shown that *K. angustifolia* litter increases the amount of soil N sequestered as tannin-protein complexes, which decreases soil available N (LeBel et al. 2008) and may give a competitive advantage to *K. angustifolia* by favouring ericoid mycorrhizas associated with the former (Joanisse et al. 2009).

Pleurozium schreberi and *C. canadensis,* which are often found in moist mossy habitats, were good indicators of *C. cibarius* var. *roseocanus* habitat. Lavoie et al. (2007) found that *P. schreberi* brings a better N and P status to the soil, which may have favoured the presence of the mushroom in mossy areas. Nevertheless, a positive correlation between lichen (*C. stellaris* and *C. rangiferina*) and *C. cibarius* var. *roseocanus* was detected in this study. Our results support those of Jansen and Van Dobben (1987) who found that in typical nutrient-poor and sandy forest soils, *C. cibarius* decline is often accompanied by a decrease in lichen cover due to changes in the environment.

Meteorological parameters

Sporocarp productivity of most fungi is highly dependent on temperature and soil moisture conditions (Tyler 1985). In other studies, it has been suggested that warm spring weather and a rainy June promote early fruiting (Danell 1994), that warm summer temperatures are correlated with high chanterelle numbers (Norvell et al. 1996), and that high soil humidity during the fruiting season allows chanterelle mushrooms to continue growing without drying out (Eveling et al. 1990). However, to our knowledge, this is the first study to demonstrate statistically on such a short-term period (2 years) the influence of meteorological parameters on a specific chanterelle species. Our data suggest positive correlations between total rainfall a week prior to fructification, air temperature two weeks prior to fructification, and *C. cibarius* var. *roseocanus* density. Indeed, in 2006 and 2007, it was observed that mushroom fructification peaks were preceded by rainfall events seven to

ten days prior to a peak and by temperature increases two weeks prior to a peak. Martínez de Aragón et al. (2007) also found a relationship between mushroom production and rainfall, but obtained better correlations when they added mean evapotranspiration for the months of September and October and mean soil temperature in August. Our results were obtained from a period of observation covering only two seasons and locations were not replicated. A longer-term study with more replicates could further validate the influence of rainfall and temperature on *C. cibarius* var. *roseocanus* fructification.

Conclusion

This study identified for the first time in Eastern Canada, *C. cibarius* var. *roseocanus* and indicated that it is dominant in the top mineral soil layer but that it also develops in the organic horizon, especially when fructifying. The overall yearly fresh mushroom production varied depending on the year and stand from 0.23 kg/ha to 3.85 kg/ha, which is similar to what has been found in the western USA for other chanterelle species. It was nevertheless, similar in both stands studied, so it seems that trail openings did not directly affect mushroom productivity on an area basis. However, the lack of *C. cibarius* var. *roseocanus* colonies growing exclusively on trails likely reflects microenvironmental conditions that may not be suitable for this mushroom's growth.

Under the homogeneous conditions of a jack pine stand located on sandy soil, the potential habitat for *C. cibarius* var. *roseocanus* was characterized by specific soil, vegetation and meteorological conditions, amongst which the *S. puberula – C. peregrina – P. banksiana* association represented a high-quality environment for the mushroom. However, whether these factors truly are causal agents of greater fructification or whether they reflect true drivers of sporocarp productivity remains to be tested more thoroughly and over a longer-term period before recommendations can be made for stand management.

Acknowledgements

This research was made possible by the Natural Sciences and Engineering Research Council of Canada strategic project # 306898-04 granted to Professor Yves Piché and collaborators at Université Laval. We are grateful for the molecular identification financial

support obtained through Yves Piché's laboratory as well as the technical assistance provided by Karine Bertrand, Andrew Coughlan, André Gagné, Julien Prunier and Christine Roussel-Roy at Université Laval, and Alain Courcelles and Sébastien Dagnault at the Laurentian Forestry Centre of Natural Resources Canada. We also thank our collaborators in the field study, Céline Marceau and Alain Blais, for their generous help and patience during this 3-year study and for letting us use their land.

References

Alexander, S.J., Pilz, D., Weber, N.S., Brown, E., and Rockwell, V.A. 2002. Mushrooms, trees, and money: value estimates of commercial mushrooms and timber in the Pacific Northwest. Environmental Management **30**(1): 129-141.

Amaranthus, M., and Pilz, D. 1996. Productivity and sustainable harvest of wild mushrooms. *In :* Managing forest ecosystems to conserve fungus diversity and sustain wild mushroom harvests. *Edited by* D. Pilz, and R. Molina. Gen. Tech. Rep. PNW-GTR-371, US Department of Agriculture, Forest Service, Pacific Northwest Research Station, Portland, OR., USA. pp. 42-61.

Amaranthus, M., and Russell, K. 1996. Effect of environmental factors and timber and mushroom harvest on *Cantharellus* production on the Olympic Peninsula, Washington State. *In :* Managing forest ecosystems to conserve fungus diversity and sustain wild mushroom harvests. *Edited by* D. Pilz, and R. Molina. Gen. Tech. Rep. PNW-GTR-371, US Department of Agriculture, Forest Service, Pacific Northwest Research Station, Portland, OR., USA. pp. 73-74.

Arora, D., and Dunham, S.M. 2008. A new, commercially valuable chanterelle species, *Cantharellus californicus* sp. nov., associated with live oak in California, USA. Economic Botany **62**(3): 376-391.

Avis, P.G., McLaughlin, D.J., Dentinger, B.C., and Reich, P.B. 2003. Long-term increase in nitrogen supply alters above- and below-ground ectomycorrhizal communities and increases the dominance of *Russula* spp. in a temperate oak savanna. New Phytologist **160**(1): 239-253.

Bergemann, S.E., and Largent, D.L. 2000. The site specific variables that correlate with the distribution of the Pacific Golden Chanterelle, *Cantharellus formosus*. Forest Ecology and Management **130**(1-3): 99-107.

Bonham, C.D. 1989. Measurements for terrestrial vegetation. Wiley-Interscience, New York, NY., USA.

Corner, E.J.H. 1966. A monograph of Cantharelloid fungi. Oxford University Press, Oxford, UK.

Dahlman, M., Danell, E., and Spatafora, J.W. 2000. Molecular systematics of *Craterellus*: cladistic analysis of nuclear LSU rDNA sequence data. Mycol. Res. **104**(4): 388-394.

Danell, E. 1994. Formation and growth of the ectomycorrhiza of *Cantharellus cibarius*. Mycorrhiza **5**(2): 89-97.

Danell, E. 1999. *Cantharellus*. *In :* Ectomycorrhizal fungi: key genera in profile. *Edited by* J.W.G. Cairney and S.M. Chambers. Springer-Verlag, New York, N.Y., USA. pp. 253-267.

Dunham, S.M., Kretzer, A., and Pfrender, M.E. 2003a. Characterization of Pacific golden chanterelle (*Cantharellus formosus*) genet size using co-dominant microsatellite markers. Molecular Ecology **12**(6): 1607-1618.

Dunham, S.M., O'Dell, T.E., and Molina, R. 2003b. Analysis of nrDNA sequences and microsatellite allele frequencies reveals a cryptic chanterelle species *Cantharellus cascadensis* sp. nov. from the American Pacific Northwest. Mycological Research **107**(10): 1163-1177.

Egli, S., Peter, M., Buser, C., Stahel, W., and Ayer, F. 2006. Mushroom picking does not impair future harvests - results of a long-term study in Switzerland. Biological Conservation **129**(2): 271-276.

Eveling, D.W., Wilson, R.N., Gillespie, E.S., and Bataillé, A. 1990. Environmental effects on sporocarp counts over fourteen years in a forest area. Mycological Research **94**(7): 998-1002.

Gardes, M., and Bruns, T.D. 1993. ITS primers with enhanced specificity for basidiomycetes -application to the identification of mycorrhizae and rusts. Molecular Ecology **2**(2): 113-118.

Gardes, M., and Bruns, T.D. 1996. Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: above- and below-ground views. Canadian Journal of Botany **74**(10): 1572-1583.

Grebenc, T., Christensen, M., Vilhar, U., Čater, M., Martín, M.P., Simončič, P., and Kraigher, H. 2009. Response of ectomycorrhizal community structure to gap opening in natural and managed temperate beech-dominated forests. Canadian Journal of Forest Research **39**(7): 1375-1386.

Ishida, T.A., Nara, K., and Hogetsu, T. 2007. Host effects on ectomycorrhizal fungal communities: insight from eight host species in mixed conifer-broadleaf forests. New Phytologist **174**(2): 430-440.

Jansen, E., and van Dobben, H.F. 1987. Is decline of *Cantharellus cibarius* in the Netherlands due to air pollution? Ambio **16**(4): 211-213.

Joanisse, G.D., Bradley, R.L., Preston, C.M., and Bending, G.D. 2009. Sequestration of soil nitrogen as tannin-protein complexes may improve the competitive ability of sheep laurel (*Kalmia angustifolia*) relative to black spruce (*Picea mariana*). New Phytologist **181**(1): 187-198.

Kernaghan, G., Widden, P., Bergeron, Y., Légaré, S., and Paré, D. 2003. Biotic and abiotic factors affecting ectomycorrhizal diversity in a boreal mixed-woods. Oikos **102**(3): 497-504.

Lavoie, M., Paré, D., and Bergeron, Y. 2007. Relationships between microsite type and the growth and nutrition of young black spruce on post-disturbed lowland black spruce sites in eastern Canada. Canadian Journal of Forest Research **37**(1): 62-73.

LeBel, P., Thiffault, N., and Bradley, R.L. 2008. *Kalmia* removal increases nutrient supply and growth of black spruce seedlings: An effect fertilizer cannot emulate. Forest Ecology and Management **256**(10): 1780-1784.

Marie-Victorin, F. 1995. Flore laurentienne, 3^{e} éd. Les Presses de l'Université de Montréal, Montréal, Qué., Canada.

Martínez de Aragón, J., Bonet, J.A., Fischer, C.R., and Colinas, C. 2007. Productivity of ectomycorrhizal and selected edible saprotrophic fungi in pine forests of the pre-Pyrenees mountains, Spain: predictive equations for forest management of mycological resources. Forest Ecology and Management **252**(1-3): 239-256.

Meyer, F.H. 1973. Distribution of ectomycorrhizae in native and man-made forests. *In :* Ectomycorrhizae, their ecology and physiology. *Edited by* G.C. Marks, and T.T. Kozlowski. Academic Press, New York, N.Y., USA. pp. 79–106.

Nantel, P., and Neumann, P. 1992. Ecology of ectomycorrhizal-basidiomycete communities on a local vegetation gradient. Ecology **73**(1): 99-117.

Norvell, L., Roger, J., Lindgren, J., and Kopecky, F. 1996. Study 8: Oregon *Cantharellus* study project, 1986-1996. *In :* Managing forest ecosystems to conserve fungus diversity and sustain wild mushroom harvests. *Edited by* D. Pilz, and R. Molina. Gen. Tech. Rep. PNW-GTR-371, US Department of Agriculture, Forest Service, Pacific Northwest Research Station, Portland, OR., USA. pp. 69-72.

Olsson, B.A., Staaf, H., Lundkvist, H., Bengtsson, J., and Kaj, R. 1996. Carbon and nitrogen in coniferous forest soils after clear-felling and harvests of different intensity. Forest Ecology and Management **82**(1-3): 19-32.

Pilz, D., Molina, R., and Liegel, L. 1998. Biological productivity of chanterelle mushrooms in and near the Olympic Peninsula Biosphere Reserve. Ambio Special Report **9**: 8-13.

Pilz, D., and Molina, R. 2002. Commercial harvests of edible mushrooms from the forests of the Pacific Northwest United States: issues, management, and monitoring for sustainability. Forest Ecology and Management **155**(1-3): 3-16.

Pilz, D., Norvell, L., Danell, E., and Molina, R. 2003. Ecology and management of commercially harvested chanterelle mushrooms. Gen. Tech. Rep. PNW-GTR-576, US Department of Agriculture, Forest Service, Pacific Northwest Research Station, Portland, OR., USA.

Pilz, D., Molina, R., and Mayo, J. 2006. Effects of thinning young forests on chanterelle mushroom production. Journal of Forestry **104**(1): 9-14.

Rangel-Castro, J.I. 2001. Eco-physiology of the ectomycorrhizal mushroom *Cantharellus cibarius*: carbon and nitrogen metabolism, and the interactions with bacteria and mycophagous inverterbrates. M.Sc. thesis. Department of Forest Mycology and Pathology, Sweden University of Agricultural Sciences, Uppsala, Sweden.

Redhead, S.A., Norvell, L.L., and Danell, E. 1997. *Cantharellus formosus* and the Pacific golden chanterelle harvest in western North America. Mycotaxon **65**(1) : 285-322.

Rochon, C., Paré, D., Khasa, D.P., and Fortin, J.A. 2009. Ecology and management of the lobster mushroom in an eastern Canadian jack pine stand. Canadian Journal of Forest Research **39**(11): 2080-2091.

Rosling, A., Landeweert, R., Lindahl, B.D., Larsson, K.-H., Kuyper, T.W., Taylor, A.F.S., and Finlay, R.D. 2003. Vertical distribution of ectomycorrhizal fungal taxa in a podzol soil profile. New Phytologist **159**(3): 775-783.

SAS Institute Inc. 2004. SAS/STAT® 9.1 user's guide, Volumes 1-7. SAS Institute Inc., Cary, NC.

Smith, S.E., and Read, D.J. 2008. Mycorrhizal symbiosis, 3rd ed. Academic Press, London, UK.

Soil Classification Working Group. 2002. The Canadian System of Soil Classification, 3rd ed. NRC Research Press, Ottawa, Ont., Canada

Spahr, D.L. 2009. Edible and Medicinal Mushrooms of New England and Eastern Canada. North Atlantic Books, Berkeley, CA., USA.

Straatsma, G., Ayer, F., and Egli, S. 2001. Species richness, abundance, and phenology of fungal fruit bodies over 21 years in a Swiss forest plot. Mycological Research **105**(5): 515-523.

Tanino, K.K., Ivanochko, G., Jessup, C., Nelson, J., and Hrycan, W. 2005. Stage I: sustainable harvest of wild mushrooms in Northern Saskatchewan: final report. Saskatchewan Agriculture Development Fund, University of Saskatchewan, Saskatoon, SK.

Twieg, B.D., Durall, D.M., Simard, S.W., and Jones, M.D. 2009. Influence of soil nutrients on ectomycorrhizal communities in a chronosequence of mixed temperate forests. Mycorrhiza **19**(5): 305-316.

Tyler, G. 1985. Macrofungal flora of Swedish beech forest related to soil organic matter and acidity characteristics. Forest Ecology and Management **10**(1-2): 13-29.

Wallenda, T., and Kottke, I. 1998. Nitrogen deposition and ectomycorrhizas. New Phytologist **139**(1): 169-187.

Watling, R. 1997. The business of fructification. Nature **385**(6614): 299-300.

Yamasaki, S.H., Fyles, J.W., Egger, K.N., and Titus, B.D. 1998. The effect of *Kalmia angustifolia* on the growth, nutrition, and ectomycorrhizal symbiont community of black spruce. Forest Ecology and Management **105**(1-3): 197-207.

Chapitre 4. Linking mushroom fructification with tree phenology and belowground carbon fluxes using *Cantharellus cibarius* var. *roseocanus* as a model in an eastern Canadian jack pine stand

Ce chapitre a été soumis pour publication dans la revue *Tree physiology*. L'usage du point décimal est donc de rigueur.

Rochon C., Paré D., Khasa D.P., and Fortin, J.A. 2011. Linking mushroom fructification with tree phenology and belowground carbon fluxes using *Cantharellus cibarius* var. *roseocanus* as a model in an eastern Canadian jack pine stand. Tree physiology. (soumis le 1 juin 2011).

Résumé

Les champignons ectomycorhiziens (ECM) forment des symbioses racinaires avec les espèces d'arbres boréales et acquièrent des portions substantielles de leurs réserves d'énergie par les arbres. Durant la deuxième moitié de la saison de croissance, les champignons ECM consacrent d'importantes ressources à la production de carpophores. L'objectif principal de cette étude est de déterminer les relations entre la production de carpophores d'un champignon ECM (*Cantharellus cibarius* var. *roseocanus* Redhead, Norvell & Danell) et la phénologie de l'arbre hôte (*Pinus banksiana* Lamb.). Le moment de transition entre le bois juvénile et mature de l'arbre, ce qui correspond au moment où davantage de composés photosynthétiques sont accessibles pour les racines, a été suivi durant deux saisons de croissance ainsi que la production de carpophores et les taux de respiration du sol, répartis en respiration totale du sol (RTS), respiration autotrophe (RA) et respiration des carpophores (RC). L'apparition des premiers carpophores arrivait avant la transition du bois juvénile vers le bois mature, mais leur pic d'abondance arrivait dans les jours suivant cette transition Durant toute la saison de croissance, la RC était significativement plus élevée que la RTS par unité de surface et était synchronisée avec elle. La RTS et la RC étaient fortement corrélées avec les variations de températures. La RC journalière de 2006 atteignait un sommet vers 1700 h, ce qui correspondait à un décalage de 3-4 heures avec la RTS. Ce pic de respiration n'a pas été détecté en 2007. Le taux de CO_2 corrigé à une température constante de 10°C n'était pas significativement différent entre la période avant et après la transition du bois juvénile vers le bois mature pour le RTS, la RA et la RC ce qui suggère que le taux de respiration de la rhizosphère n'est pas différent entre ces deux stades phénologiques. La synchronicité entre le pic d'abondance des carpophores de *C. cibarius* var. *roseocanus* et la transition entre le bois juvénile et mature suggère que la fructification est reliée à la phénologie de l'arbre et à la disponibilité des hydrates de carbone lors de leur allocation dans la rhizosphère. Cependant, la forte dépendance de la respiration du sol (RTS et RC) aux variations de température du sol et l'absence apparente d'influence des stades phénologiques de l'arbre hôte laissent présumer que la quantité d'hydrates de carbone utilisée par les champignons ECM, particulièrement pour la production de carpophores, est fonction de la demande du champignon durant son stade reproductif.

Abstract

Ectomycorrhizal (ECM) fungi form root symbioses with boreal tree species and acquire substantial portions of their energy supply from trees. During the second half of the growing season, ECM fungi devote important resources to the production of carpophores. The main objective of this study is to determine the relationships between carpophore production of an ECM mushroom (*Cantharellus cibarius* var. *roseocanus* Redhead, Norvell & Danell) and the phenology of the host tree (*Pinus banksiana* Lamb.). The timing of earlywood-latewood transition in trees, which corresponds to the moment when more photosynthetic assimilates are accessible to roots, was assessed during two growing seasons together with carpophore production and soil respiration rates which were partitioned into total soil respiration (TSR), autotrophic soil respiration (AR) and carpophore respiration (CR). The first carpophores appeared earlier than the earlywood-latewood transition but the greatest abundance of carpophores happened within days of this transition.. Over the growing season, CR was significantly higher than TSR on a per area basis and was in synchrony with it. TSR and CR were strongly related to the temperature patterns. The 2006 daily CR peaked at 1700 h, corresponding to a 3-4 hour time lag with the TSR peak. Such pattern was not detected in 2007. The CO_2 efflux, corrected to a constant temperature of 10°C, did not significantly differ between the time periods before and after the earlywood-latewood transition for TSR, AR and CR, suggesting that the respiration rate of the rhizosphere is not different in these two tree phenological states. The synchronicity between the peak in the abundance of *C. cibarius* var. *roseocanus* fructifications and the early-wood latewood transition suggests that fructification is related to the host tree phenology and to the availability of carbohydrates for belowground allocation. However, the strong dependence of soil respiration component fluxes (TSR and CR) to soil temperature and the lack of apparent influence of the host tree physiological state on these fluxes also suggest that the amount of carbohydrates used by ECM fungi, especially for carpophore production, is driven by the fungal demand during its reproductive stage.

Introduction

Soil respiration is one of the major carbon fluxes from the terrestrial biosphere into the atmosphere and typically contributes 30–80% of annual total ecosystem respiration (i.e. aboveground and belowground respiration) in forests (Borken et al. 2006; Davidson et al. 2006). Difficulties in quantifying carbon (C) allocation from trees to ectomycorrhizal (ECM) fungi as well as determining the contribution of ECM fungi to forest soil CO_2 efflux (Heinemeyer et al. 2007) have prevented a precise assessment of their importance to forest ecosystems carbon exchanges (Hobbie 2006), but due to seasonal variations these estimates are expected to fluctuate during the growing season with a higher rate mid-August (Langlois and Fortin 1984). Moreover, it seems that during the autumn, the soil CO_2 flux components could be divided into 60% soil heterotrophic, 25% ECM mycelium and 15% root fluxes (Heinemeyer et al. 2007). Borken et al. (2006) suggested that belowground mycelium of fungi may contribute to the large fluxes occasionally measured even without fruiting bodies. They also noticed high soil respiration rates related to the development of fruiting bodies in their study site.

Soil respiration consists of functionally two different components: autotrophic respiration (CO_2 derived from root plus associated respiration from mycorrhizae and microorganisms) and heterotrophic respiration (CO_2 released during decomposition of soil organic matter) (Tang et al. 2005). Autotrophic respiration is linked to the supply of photosynthetic products from plants (Cisneros-Dozal et al. 2006). Hanson et al. (2000) reviewed the various methods applied to evaluate autotrophic respiration and concluded that it varies from 32% to 60% of the annual total CO_2 released from soils. One widely used approach in forest ecosystems to determine in situ autotrophic respiration is by inserting root exclusions to sever roots and then measure CO_2 efflux in and outside the exclusion. This method provides autotrophic respiration estimates that are similar to the ones obtain from other methods (Vogel and Valentine 2005).

Stem girdling experiments have shown that autotrophic soil respiration is driven by recently assimilated carbon (Högberg et al. 2001). Kuzyakov and Gavrichkova (2010)

indicated in their review that the lag between photosynthesis C capture and release by soil autotrophic respiration was about 4-5 days for large trees. They considered that available photosynthate was the main driver of this flux and that soil temperature is an indirect factor that conditions respiration rates. Moreover, in the boreal forest, at the beginning of the growing season aerial parts offer a stronger C sink than roots but once shoot growth is completed carbohydrates are then more available for export to other parts of the tree, including roots and mycorrhizal fungi. It is during the second half of the growing season (mid-August) that ECM fungi develop most of their carpophores, suggesting that the host plant, by controlling the availability of carbohydrates, exerts an influence on the fruiting of ECM fungi (Langlois and Fortin 1984, Godbout and Fortin 1990).

The annual stem growth trends observed in boreal and temperate areas of the northern hemisphere are very similar: a positive exponential growing phase followed by a phase in which growth rate decreases, resulting in a sigmoid-shaped pattern (Deslauriers et al. 2003b). The annual transition from earlywood to latewood formation is a conspicuous developmental switch in temperate region trees. Latewood is induced during the later part of the growing season, when cell division activity in the cambial meristem declines. It involves an increase in wall thickening of the cambial derivatives (Uggla et al. 2001). The initiation of latewood formation is associated with the cessation of apical and needle growth at a time when current-year needles have become net exporters of photosynthetic assimilate to the roots (Larson 1960). The completion of tree growth coincides with the findings of Li et al. (2002) that mobile carbon pool in the roots in September is nearly twice that in May and is likely to lead to a peak in the fruiting body production.

Cantharellus cibarius var. *roseocanus* Redhead, Norvell & Danell belongs to the genus *Cantharellus* of the Canthaerallaceae family (Corner 1966). *C. cibarius* var. *roseocanus* is an ECM fungus that grows in symbiotic associations with fine roots of trees as shown for other ECM species (Smith and Read 2008). Forests are essential to their survival and fruiting as the ECM mycelial system is strongly dependent on current assimilates (Högberg et al. 2001). In the boreal forest, the dominant plants depend upon the abilities of their fungal partners to recover nutrients, thus the symbioses control nutrient cycles,

productivity, species composition, and functioning of these ecosystems (Read et al. 2004). To our knowledge, no study on the physiology of a specific ECM species *in situ* and its relation to host tree phenology has been done. Thereby, questions on the role of ECM fungi in forest soils have been raised (Borken et al. 2006) such as: are there possible episodic pulses of soil carbon dioxide (CO_2) efflux during periods of prolific mushroom production; does the belowground mycelium contribute to the large fluxes occasionally measured in soil; and to what extent can fungi store carbon to use for rapid growth of fruiting bodies,. The first step to resolve these issues is to identify how plants allocate C among respiration, storage, growth and transfer to other organisms, such as fungi, using specific models (Trumbore 2006).

The objective of this research was to determine how the pattern of carpophore production of an ECM fungus (*Cantharellus cibarius* var. *roseocanus*) as well as soil rhizospheric and carpophore respiration, are related to the phenology of the host tree (*Pinus banksiana* Lamb.). To reach this objective we studied timing of earlywood-latewood transition in trees and the CO_2 belowground fluxes. Our hypotheses were: (1) the earlywood-latewood transition of the host tree, which corresponds to the moment when trees export their photosynthetic assimilates to roots, leads to a high *C. cibarius* var. *roseocanus* fructification peak; (2) This greater C allocation to roots following the earlywood-latewood transition of the host tree is detected by a higher autotrophic soil respiration; (3) *C. cibarius* var. *roseocanus* CO_2 fluxes measured from the carpophores follow the soil CO_2 fluxes on a daily and weekly basis.

Materials and methods

Study area

The study was conducted in two jack pine stands of the boreal forest near Girardville, Québec, Canada (49°07'N; 72°35'W) (for a map of the study site, see Rochon et al. (2009)). The first stand was located in the Domaine de la Rivière Mistassini private forest. The size of this stand was estimated at 2 km^2. The second stand was established on a public land 3 km north of the first stand and its size was estimated at 2.4 km^2. Both stands studied

were located in the continuous boreal forest subzone and were dominated by jack pine (*Pinus banksiana*) associated with black spruce (*Picea mariana* (Mill.) Britton, Sterns & Poggenb.). Forest age structure was dominated by cohorts that originated following wildfire in 1945 for the first site and heavy cutting around 1959 for the second site. With an elevation ranging from 150 to 250 m above sea level, the mean annual temperature and precipitation are 0.80°C and 1015 mm, respectively. Even though they are 3 km apart, these two stands were considered to be similar for the purposes of this experiment, therefore data were analyzed together. Additional stand characteristics details can be found in Rochon et al. (2009).

Plot selection

Forty-five *Cantharellus cibarius* var. *roseocanus* plots varying from 1 to 144 m^2, depending on how dispersed fruiting bodies were within a colony, were randomly established in 2005. The protocol used to establish these plots was reported elsewhere Rochon et al. (2011). Within these plots, 12 were randomly selected in 2006 to extract wood core samples of 12 mature *Pinus banksiana* trees (mean height, 15.0 m; mean diameter at breast height (DBH), 20.1 cm) and to measure soil respiration (including heterotrophic and autotrophic respiration) and *C. cibarius* var. *roseocanus* respiration. Due to some missing data, only 10 of these trees were selected for the analyses. However, all *C. cibarius* var. *roseocanus* plots were used to determine sporocarp production (described as weekly number of fresh mushrooms found in plots).

Wood core sampling

A surgical bone-sampling needle DBMNI-1501 (aiguille d'aspiration, Inter V Médical, Montréal, Que.) called Trephor was used for the extraction of small cores of wood and bark (Rossi et al. 2006a) in the 10 *Pinus banksiana* trees previously mentioned. The cores were 2 mm in diameter and 15-20 mm long, containing four to six rings. Microcore extraction was used because its small diameter inflicted very small wounds that allowed repetitive sampling during the year without causing any tree reactions (Forster et al. 2000). Wood cores were extracted every week for two growing seasons (2006 and 2007) from mid-June to mid-September. Samples were collected in a spiral fashion up the stems at approximating

breast height (1.3 m). Wood cores were always taken at least 5 cm apart from each other to avoid hitting resin ducts on adjacent cores. Microcores presenting ring development malformation were not analyzed. Samples usually contained the previous four or five tree rings and the developing annual layer with the cambial zone and adjacent phloem. Dead outer bark was removed before sampling. The collected microcores were placed in Eppendorf microtubes with an ethanol solution (50% in water) and stored at 5°C to avoid tissue deterioration. All samples were then processed within a maximum of 3 weeks. Each sample was oriented by marking the transverse side with a pencil under a stereo-microscope at 10–20 magnification. The microcores were dehydrated with successive immersions in ethanol and D-limonene, embedded in paraffin and transverse sections of 6–10 μm thickness were cut with a rotary microtome (Rossi et al. 2006a).

Ring development analysis was then conducted. Sections were stained for 10 min with cresyl violet acetate (0.16% in water) and kept in water to assess the ring development. Sections were then observed under normal and polarized light at a magnification of 400–500 to differentiate the developing xylem cells. For each tree, the total number of cells was counted along three radial files and the total number averaged and used to assess overall timing of xylem growth. The total number of cells included cells in the phases of radial enlargement, cell wall thickening, and mature cells (Deslauriers et al. 2003a). The number of cells in earlywood and latewood phase and the moment when the cells shifted from earlywood to latewood were recorded.

The Gompertz equation was fitted to establish the cell increase profile for *Pinus banksiana* as proposed in Rossi et al. (2006b) and Rossi et al. (2003) and can be defined as:

$$[1] \quad y = a\exp\left(-e^{(\beta-\kappa t)}\right)$$

where y is the weekly cumulative sum of cells, t the time computed in Julian days, a the upper asymptote (maximum growth expressed as cell number), β the x-axis placement parameter and κ the rate of change of the shape (Cheng and Gordon 2000). The calculations were done with fitted data using NLIN (nonlinear regression, Marquardt iterative option) of the SAS statistical package. This method regresses the residuals onto the model partial derivatives with respect to the Gompertz parameters until the estimates converge (SAS

Institute Inc. 2004). Examination of the R-square and the asymptotic t-statistic for the parameters showed that the Gompertz function was appropriate for describing growth and time relationships (Huang et al. 1992).

Soil respiration

In each of the 12 selected plots, six white polyvinylchloride (PVC) collars, three 4-cm-long collars and three 30-cm-long collars all with a 10 cm internal diameter, were placed into the soil to the level of the forest floor to measure total soil respiration (TSR) and heterotrophic respiration (HR) respectively. PVC collars were used to minimize soil disturbance during measurements while maintaining a tight seal on the soil (Fahey et al. 2005). Thirty-cm-long collars were used to sever a large proportion of roots (Vogel and Valentine 2005). All collars were left in place during the entire duration of the experiment (i.e. 2 years). Collars were separated from each other by approximately 80 cm between each small collar and between each long collar and by approximately 105 cm between a small collar and its correspondent long collar. Moreover, in each plot the first collars (one long and one small) were always situated 110 cm from the host jack pine tree. Soil respiration measurements began approximately two weeks after installation of the collars.

Soil respiration was measured between 0900 and 1400 h to minimize effects of potential diurnal influence on observed data (Scott-Denton et al. 2006) on a weekly basis from June 29 to September 12, 2006 and from July 3 to September 18, 2007. Soil respiration rates were measured with a portable open photosynthetic system (LI-6400, Li-Cor, Lincoln, Nebraska, USA) that had a vented, closed, soil chamber attachment (Li-Cor, Model-6400-09). The chamber system was used to monitor the increasing rate in CO_2 concentration in the sealed collar. The CO_2 concentration was first measured in the ambient air adjacent to the collar. Then, the chamber was placed over the collar and allowed to equilibrate for about 1 min. A pump circulated air at a rate of 0.5 L min^{-1} during all the flux readings. Over the interval of three flux readings on a single collar, the concentration of CO_2 in the headspace of the chamber spanned a range of ± 0.2 μmol CO_2 m^{-2} s^{-1} from the ambient atmospheric concentration near the soil surface. The average of the two last measures was used to determine the respiration rate. Autotrophic respiration (AR) was

estimated by subtracting the heterotrophic respiration (HR) from the total soil respiration (TSR) collar.

Soil temperature at 5 cm depth was manually measured next to each collar by inserting a temperature probe. Soil relative humidity at 12 cm depth was also taken next to each collar with a Time Domain Reflectometry (TDR) logger 300 (Spectrum Technologies). The CO_2 efflux, corrected to a constant temperature of 10°C to account for seasonal changes that are not related to changes in temperature, was estimated for each plot every week using the equation of Lavigne et al. (2003):

$$[2] \quad SR_{10} = \frac{SR}{e^{b(Ts-10)}}$$

where SR_{10} is the average respiration rate at 10°C for each plot and year, SR is the observed soil respiration, coefficient b describes the rate of change of SR with a unit change of Ts, and T_s is the soil temperature at 5 cm depth. Coefficient b was assigned a value of 0.0693 as proposed by Lavigne et al. (2003) according to the work of Rayment and Jarvis (2000).

We computed the daily soil respiration (SR_{daily}) between measurement days by linearly interpolating values of SR_{10}, and summed these estimates to compute annual SR (SR_{annual}).We used values of SR_{10} computed with equation 2 to calculate SR_{daily} using the equation proposed by Lavigne et al. (2003):

$$[3] \quad SR_{daily} = \sum_{hour} SR_{10} e^{0.0693(Ts-10)} 3600 \frac{12}{1\,000\,000}$$

where T_s, the soil temperature at 5 cm depth, is recorded hourly in each plot by a Watchdog temperature data logger, multiplying by 3600 to convert time from seconds to hours and by 12/1000000 to convert C from micromoles to grams; and the summation is performed for each hour of the day.

C. cibarius var. *roseocanus* respiration

Two experiments were used to estimate the *C. cibarius* var. *roseocanus* CO_2 fluxes. In the first experiment, one additional collar including a fruiting body of *C. cibarius* var.

roseocanus was installed in July 2006 in each of the 12 plots already chosen to measure soil respiration. CO_2 fluxes of these *C. cibarius* var. *roseocanus* collars, which included soil respiration, were measured from the first appearance of a fruiting body until its disappearance. The protocol used to measure the carpophore respiration (CR) was the same as the one described in the soil respiration section except that 4-cm-long collars were not permanently installed around fruiting bodies but rather temporarily installed every time a flux was measured. The SR_{10} correction was also applied to avoid seasonal trends due to soil temperature and SR_{daily} was calculated.

In the second experiment, six *C. cibarius* var. *roseocanus* colonies were randomly chosen amongst all colonies to measure the CO_2 fluxes of three fruiting bodies and two soil samples over a 15-hour period. The period between 2200 and 0700 h was not included as Liu et al. (2006) had shown a distinct day/night pattern for soil respiration with a switch around 0800 and 2000 h. A site was visited only once to measure fruiting body and soil respiration over a 15-hour period, therefore six sites were visited over a 2-year period on the following dates: 17/08/2006; 28/08/2006; 11/09/2006; 26/07/2007; 21/08/2007; and 04/09/2007. Temperature data were inaccurate on the 28/08/2006 therefore this sampling date was removed as the SR_{10} correction could not be calculated. The protocol used to measure fruiting body respiration over a 15-hour period was the same as the one described in the soil respiration section except that five 4-cm-long collars were installed for a period of 15 hours only. The SR_{10} correction was also applied to avoid seasonal trends due to soil temperature.

C. cibarius var. *roseocanus* production

The total number of fruiting bodies found in all 45 plots was recorded every week over the growing seasons 2006 and 2007. This allowed us to estimate the mushroom production of the study site (number of fresh fruiting bodies/week) and to determine the beginning of *C. cibarius* var. *roseocanus* fructification as well as the peak of production.

Statistical analysis

The statistical differences between the average weekly fluxes of the CR and the TSR as well as between the average daily fluxes of the CR and the TSR were separated by least significant difference (LSD) tests on repeated measurements. The statistical differences between the CO_2 average fluxes corrected to a constant temperature of 10°C (SR_{10}) before the earlywood-latewood transition and after the earlywood-latewood transition of TSR, AR and CR were also separated by least significant difference (LSD) tests on the 2006 and 2007 repeated measurements. Analyses of variance were performed using a GLM model analysis (SAS Proc GLM, version 9.1 (SAS Institute Inc. 2004)).

The relationships between TSR or *C. cibarius* var. *roseocanus* weekly respiration and meteorological parameters were explored using stepwise multiple regressions. The significance level chosen for all tests was $\alpha = 0.05$ and $n = 12$. Multiple regression analyses were performed for combined years (2006 and 2007). Two dependent variables were used for these analyses: TSR and weekly CR. The two stands were analyzed as a whole. Multiple regression analyses were performed and Pearson's correlation coefficients were used to determine significant correlations.

The meteorological parameters were the cumulative sum of degree-days, weekly mean, maximum and minimum air temperature, weekly total rainfall calculated from the meteorological station data, and weekly mean, maximum and minimum soil temperature and soil relative humidity found in the observation plots. To determine which meteorological parameter influenced both types of respiration and when, analyses were done at 0 shift (i.e., analyses were run when respiration measurements and meteorological data coincided with the calendar date) and at 1 to 8 weeks shift (e.g., analyses were run when meteorological data were shifted by 1 week prior to the calendar date of respiration measurements).

Results

Patterns of cambial cell development and relationships with the fructification of *C. cibarius* var. *roseocanus*

For a given site and year, the ring width increase of each tree began and ended at the same time and the mean pattern was found to fit the Gompertz function as shown by the R^2 (Figure 4.1, Table 4.1). For both years, the total number of cells regularly increased until a mean maximum number, defined as the upper asymptote, was reached. The cell production rate was approximately the same for 2006 and 2007 (0.32 cell/day and 0.36 cell/day respectively) (Table 4.1). However, the 2006 and 2007 curves were slightly different as the total number of cells formed each year was different, with asymptotes of 44.52 cells in 2006 and 63.53 cells in 2007 (Figure 4.1, Table 4.1). Depending on the year, the upper asymptote was reached at different dates in August and seemed to arrive earlier when the earlywood-latewood transition was earlier (Table 4.2). Cell production rates declined after the end of July, corresponding to latewood cell production, which started July 26 in 2006 and August 7 in 2007 (Table 4.2).

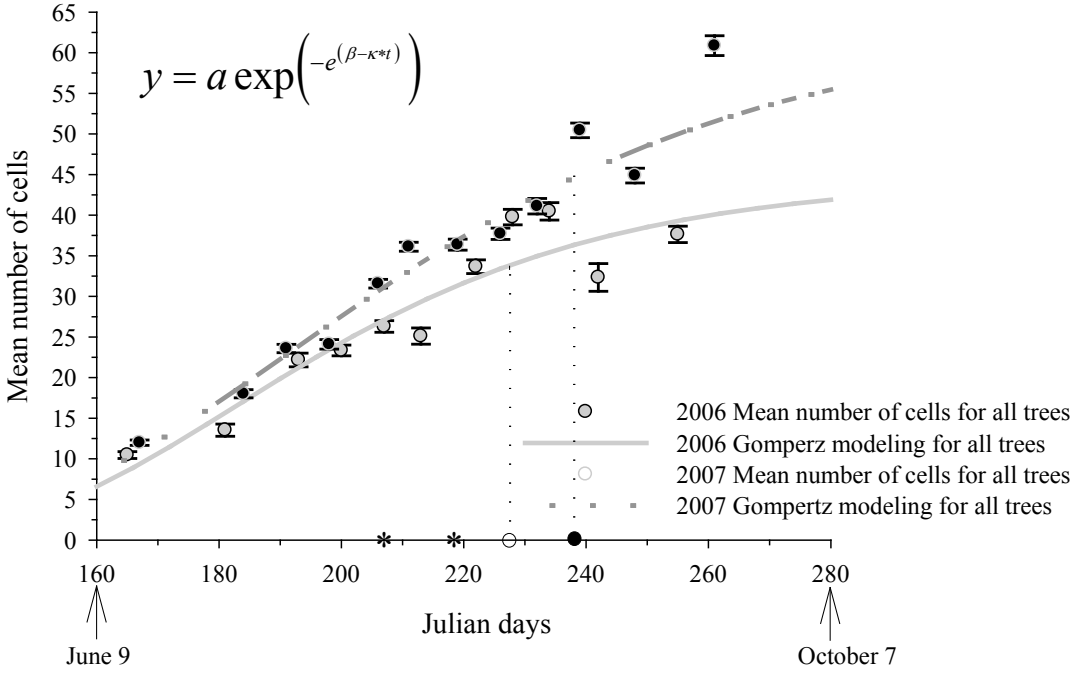


Figure 4.1. Cell number increase from the beginning of the growing season: 2006 and 2007 mean total cell number of ten trees counted each week and their general logistic pattern. * = date of the earlywood-latewood transition (day 207 in 2006 and day 219 in 2007), ○ = date when the cells reached the upper asymptote in 2006 (day 228), ● = date when the cells reached the upper asymptote in 2007 (day 239). The error bars represent the standard error.

Table 4.1. Parameters of the Gompertz equation fitted for every year.

Gompertz parameters	2006	2007
R^2 (%)	0.88	0.95
α	44.52	63.53
β	5.24	4.36
κ	0.03	0.02
r (cell/day)	0.32	0.36

Note: α= upper asymptote of the maximum number of cells at t_i; β = x-axis placement parameter; κ = rate of change parameter; r = rate of cell formation by day.

Table 4.2. Date of principal events of the growing season.

Observation	2006	2007
Beginning of radial enlargement (xylem formation)	June 14[b]	June 16[b]
Earlywood-latewood transition (date when latewood is first produced)	July 26	August 7
Cell number reaching the upper asymptote	August 16	August 27
Beginning of *C. cibarius* var. *roseocanus* fructification[a]	July 10-12	July 10-12
Maximum of *C. cibarius* var. *roseocanus* fructification[a]	July 26	August 13

Note: The values show the date on which 50% of the sample trees had the specified characteristics.
[a] From Rochon et al. (2011).
[b] Date of first measurement, xylem formation could have started earlier.

In 2006, the first fruiting bodies were observed between July 10 and 12 in the plots (Table 4.2). Peak of mushroom fructification occurred during the week of July 26 in 2006 and the emergence of new fruiting bodies started to slow down at the end of August (Figure 4.2b). The 2007 fructification of *C. cibarius* var. *roseocanus* was delayed compared with 2006. The first fruiting bodies were observed in mid-July as in 2006, then the emergence of fruiting bodies almost stopped for two weeks at the beginning of August (Figure 4.2b). The fructification peak happened during the week of August 13 and new fruiting bodies were still emerging on September 17, 2007 (last data recorded).

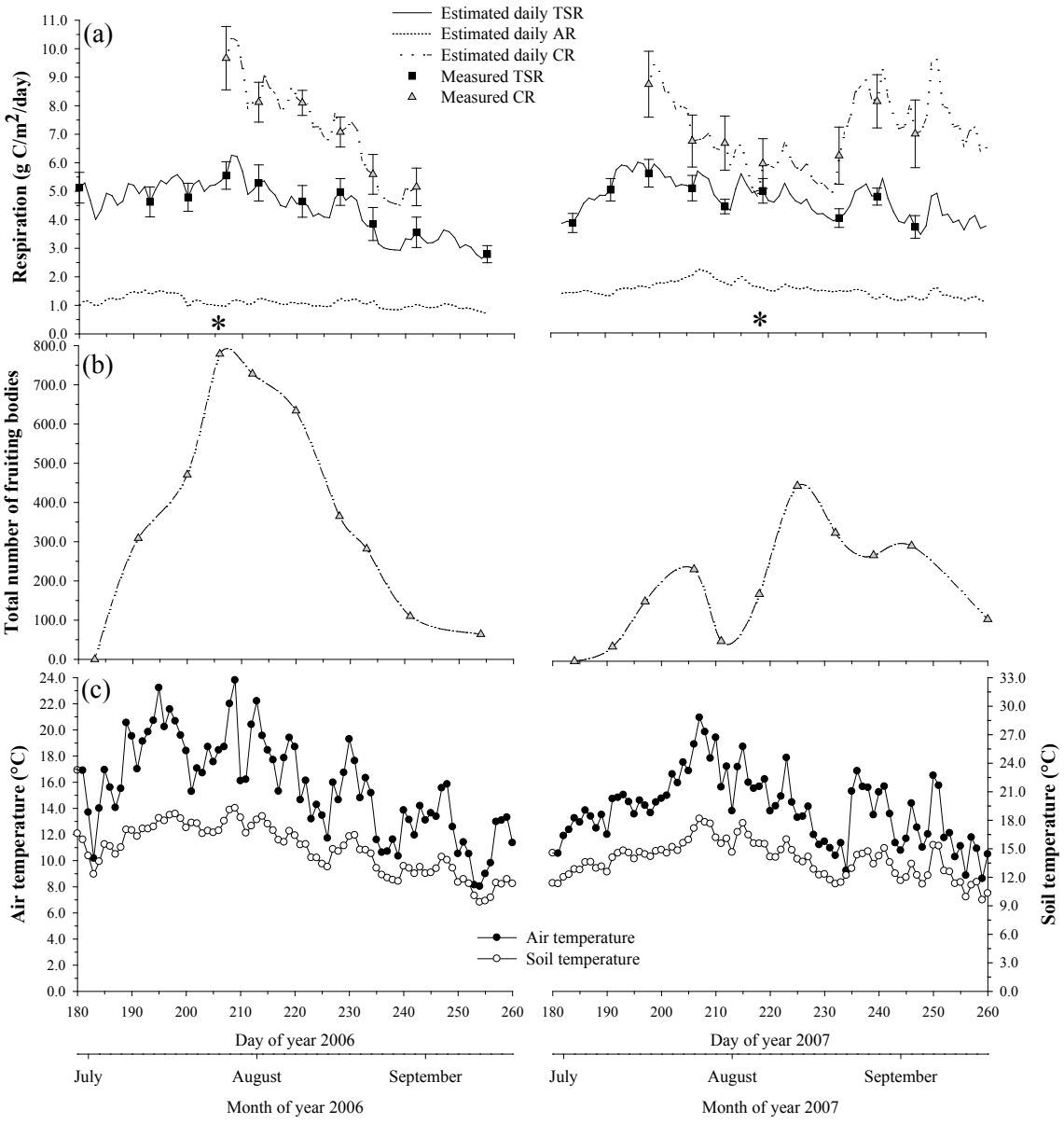


Figure 4.2. (a) Modeled and measured daily total soil respiration (TSR), autotrophic respiration (AR) and *C. cibarius* var. *roseocanus* respiration (CR) (which included TSR) in 2006 and 2007 using the SR_{daily} equation proposed by Lavigne et al. (2003), (b) Total number of *C. cibarius* var. *roseocanus* harvested weekly in the observation plots, (c) Daily air and soil temperature. The error bars represent the standard error. * = Dates of the earlywood-latewood transition.

The earlywood-latewood transition was later in 2007 than in 2006 as was the *C. cibarius* var. *roseocanus* maximum production of fruiting bodies (Table 4.2). Moreover, the mushroom fructification peak happened at the same date as the earlywood-latewood

transition in 2006 and 6 days later in 2007. The beginning of the fructification did not change regardless of the year and the date of earlywood-latewood transition.

Soil respiration

For the total soil respiration (TSR), no recurrent peaks were detected during the two years, but the scale of variation in TSR was similar for both years. Moreover, the TSR followed specific meteorological parameters as detected by stepwise multiple regressions. Therefore, the best meteorological parameters to explain TSR were soil temperature (°C), and the weekly maximum air temperature (°C) at the time of measurements as well as total rainfall (mm) one week prior to measurements (Model 1, Table 4.3; Figure 4.2c). No link with soil humidity was detected.

The 2006 summer maximum TSR (5.5 g C m^{-2} day^{-1}) was measured July 26 and a second peak was detected August 16 (Figure 4.2a). The 2007 summer maximum TSR of 5.6 g C m^{-2} day^{-1} was measured July 17 and a second peak happened August 28 (Figure 4.2a). The annual TSR (TSR_{annual}) varied from 311 to 553 g C m^{-2} $year^{-1}$ depending on the year and the stand with a mean TSR_{annual} at 426 g C m^{-2} $year^{-1}$ (Table 4.4). For the TSR_{annual}, no significant differences were detected between years although the second stand had significantly higher CO_2 fluxes under a LSD test ($\alpha = 0.01$) than the first stand (Table 4.4). The mean CO_2 efflux at 10°C (SR_{10}) of TSR was not significantly different before and after the earlywood-latewood transition (Table 4.5).

Table 4.3. Best models selected with stepwise multiple regressions analyses for total soil respiration (TSR) and *C. cibarius* var. *roseocanus* respiration (CR) (g C m^{-2} day^{-1}) calculated from the measured data obtained from the 12 observation plots for the years 2006 and 2007 combined.

Model	Meteorological parameters	Value of the parameter			Value of the model	
		Correlation coefficients	SE[a]	P-value	R-square	P-value
Model 1: Total soil respiration						
	Soil temperature (°C)	0.74	0.10	<0.001	0.74	<0.001
	Maximum weekly air temperature (°C)	0.54	0.06	0.04		
	Total rainfall (mm) 1 week prior to respiration measurements	0.37	0.01	0.01		
Model 2: *C. cibarius* var. *roseocanus* respiration						
	Soil temperature (°C)	0.54	0.15	0.02	0.29	0.04

[a] SE is the standard error.

Table 4.4. Annual total soil respiration (TSR_{annual}) and annual autotrophic respiration (AR_{annual}) calculated from measured and interpolated data obtained from the 12 observation plots.

Stand	Year	TSR_{annual} (g C/m^2/year)	AR_{annual} (g C/m^2/year)
1	2006	310.84 (a)	73.94 (b)
	2007	329.93 (a)	95.54 (ab)
2	2006	553.41 (b)	150.11 (ab)
	2007	510.14 (b)	196.53 (a)

The different letters in parentheses represent significant differences under a least significant difference (LSD) test at α=0.01. Comparisons were performed only between TSR_{annual} results and between AR_{annual} results.

Table 4.5. Mean CO_2 efflux at 10°C (SR_{10}) of total soil respiration (TSR), autotrophic respiration (AR) and carpophore respiration (CR) calculated from measured data obtained from the 12 observation plots.

Year	Earlywood/ latewood transition	Mean TSR (g C/m^2/day)	SE	Mean AR (g C/m^2/day)	SE	Mean CR (g C/m^2/day)	SE
2006	Before	4.85 (a)	±0.52	1.11 (a)	±0.37	.	.
	After	4.19 (a)	±0.51	1.08 (a)	±0.30	6.81	.
2007	Before	4.80 (a)	±0.38	1.61 (a)	±0.29	7.12 (a)	±1.97
	After	4.14 (a)	±0.34	1.25 (a)	±0.25	6.64 (a)	±2.90

The different letters in parentheses represent significant differences under a least significant difference (LSD) test at α=0.01. Comparisons were performed between TSR results and between AR results for 2006 and 2007, and between CR results for the year 2007 only.

The autotrophic respiration (AR) was more stable than the two other types of respiration (TSR and heterotrophic respiration (HR)) throughout the experiment varying from 0.7 to 2.0 g C m^{-2} day^{-1} over the years 2006 and 2007 (Figure 4.2a). The difference between TSR and HR revealed that between 24% and 33% of the summer respiration can be attributed to the rhizospheric component of the soil. The annual AR (AR_{annual}) varied from 74 to 197 g C m^{-2} $year^{-1}$ depending on the year and the stand with a mean respiration of 129 g C m^{-2} $year^{-1}$ but no significant differences were detected under a LSD test (α = 0.01) (Table 4.4). Moreover, the AR could not be correlated to any specific meteorological parameters under stepwise multiple regressions and the mean CO_2 efflux at 10°C (SR_{10}) of AR was not statistically different before and after the earlywood-latewood transition (Table 4.5).

The emergence of the first fruiting bodies (between July 10 and 12) did not correspond to any respiration peak (TSR or AR) for 2006 and 2007. However, in 2006 the mushroom fructification peak (week of July 26) corresponded to the first TSR peak (5.5 g C m^{-2} day^{-1}) (Figures 4.2a, 4.2b). The mushroom fructification peak also corresponded to the date on which 50% of the sampled trees had started their earlywood-latewood transition (Table 4.2). In 2007, a minor mushroom fructification peak (week of July 26) was associated with the first autotrophic peak (2.0 g C m^{-2} day^{-1}) (Figures 4.2a, 4.2b). Moreover, the 2007 major fructification peak, on August 13, could not be linked to any measured respiration peak as no data were recorded that week. However, estimated data showed a respiration peak on August 11, due to an air and soil temperature increase (Figures 4.2a, 4.2c). This strengthened the idea of an existing link between the mushroom fructification and the TSR, but no direct link with the AR was detected. The 2007 earlywood-latewood transition started 6 days before the fructification peak of August 13 (Table 4.2).

C. cibarius var. *roseocanus* respiration

Weekly mushroom respiration

In 2006 and 2007, comparisons between collars containing one fruiting body of *C. cibarius* var. *roseocanus* and collars without any fruiting body were undertaken from the

end of June until mid-September. The average life of the fruiting bodies growing in the collars was estimated at 33.4 days in 2006 and 28.3 days in 2007. Using a LSD test on measured data, collars containing fruiting bodies and soil respiration had significantly higher ($\alpha = 0.01$) CO_2 fluxes (mean = 4.8 µmol CO_2 m^{-2} s^{-1} in 2006 and 5.4 µmol CO_2 m^{-2} s^{-1} in 2007) when compared with collars without fruiting bodies (mean = 3.1 µmol CO_2 m^{-2} s^{-1} in 2006 and 3.5 µmol CO_2 m^{-2} s^{-1} in 2007 for TSR) (Figure 4.3). Moreover, weekly CR followed one specific meteorological parameter, soil temperature (°C) at the time of measurements, as detected by stepwise multiple regressions (Model 2, Table 4.3).

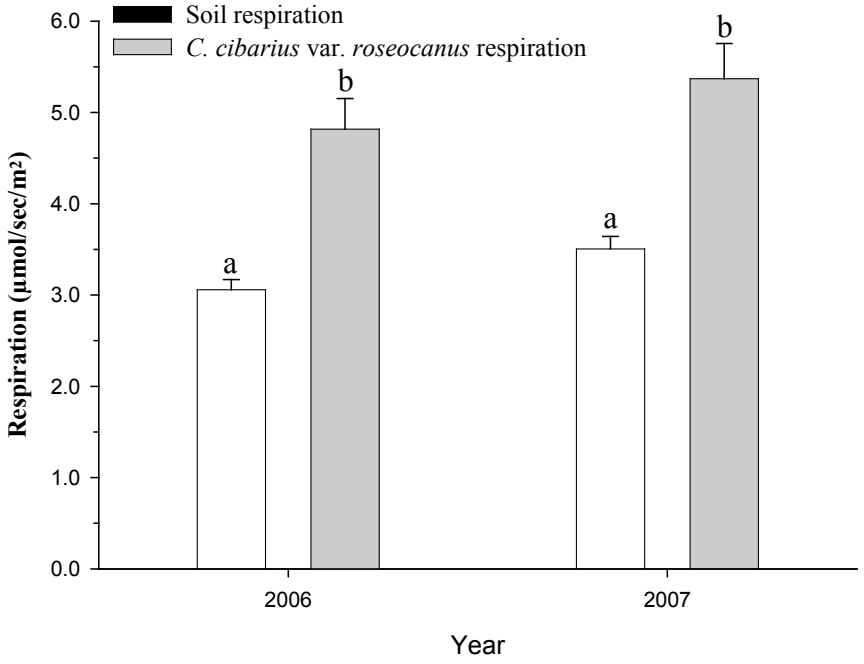


Figure 4.3. Averaged total soil respiration (TSR) and *C. cibarius* var. *roseocanus* respiration (which include soil respiration) (CR) measured in the 12 observation plots from June 29 to September 23, 2006 and from July 3 to September 18, 2007. Different letters represent significant differences under a least significant difference (LSD) test at α=0.01. The error bars represent the standard error.

In 2006, the CR peak (9.7 g C m^{-2} day^{-1}), which happened the week of July 26, corresponded to the first TSR peak (Figure 4.2a). This CR peak also corresponded to the earlywood-latewood transition (Table 4.2). In 2007, the two highest measured mushroom respiration fluxes (July 17 and August 28) corresponded to two measured TSR peaks (Figure 4.2a). A third peak detected from the estimated data was recorded September 9 for the CR, the TSR and the AR and was linked to a temperature increase (Figures 4.2a, 4.2b).

The earlywood-latewood transition, which had started on August 7, was not linked to any CR peak (Table 4.2; Figure 4.2a) and the mean CO_2 efflux at 10°C (SR_{10}) of CR was not statistically different before and after the earlywood-latewood transition (Table 4.5). Moreover, in 2006 and 2007, no direct link between AR and CR was detected.

Daily mushroom respiration

Exploratory measurements aiming at comparing TSR and CR on a daily basis (15-hour period) were undertaken in 2006 and 2007. The period between 2200 and 0700 h was monitored on August 17, 2006 but observations showed that fluxes were stable (variation of less than 1 µmol m^{-2} s^{-1}), thus no further monitoring was done for that period. Moreover, *C. cibarius* var. *roseocanus* had significantly higher ($\alpha = 0.01$) CO_2 fluxes (mean = 4.4 µmol CO_2 m^{-2} s^{-1} in 2006 and 4.2 µmol CO_2 m^{-2} s^{-1} in 2007) when compared with total soil CO_2 fluxes (mean = 2.3 µmol CO_2 m^{-2} s^{-1} in 2006 and 2.2 µmol CO_2 m^{-2} s^{-1} in 2007) under a LSD test on measured data. 2006 and 2007 *C. cibarius* var. *roseocanus* CO_2 fluxes were similar, varying from 3.30 to 5.50 µmol CO_2 m^{-2} s^{-1} (Figure 4.4). In 2006, measured CR had a regular pattern with a respiration peak at the end of the day (around 1700 h) (Figure 4.4a). In 2007, a CR peak was detected around 1100-1200 h but data fluctuation between measurements resulted in high standard errors, therefore no clear conclusion could be made for the CR that year (Figure 4.4b). TSR pattern varied from 1.56 µmol CO_2 m^{-2} s^{-1} to 2.81 µmol CO_2 m^{-2} s^{-1} in 2006 and from 1.96 µmol CO_2 m^{-2} s^{-1} to 2.45 µmol CO_2 m^{-2} s^{-1} in 2007 with the highest values found between 1300 and 1400 h for both years (Figure 4.4).

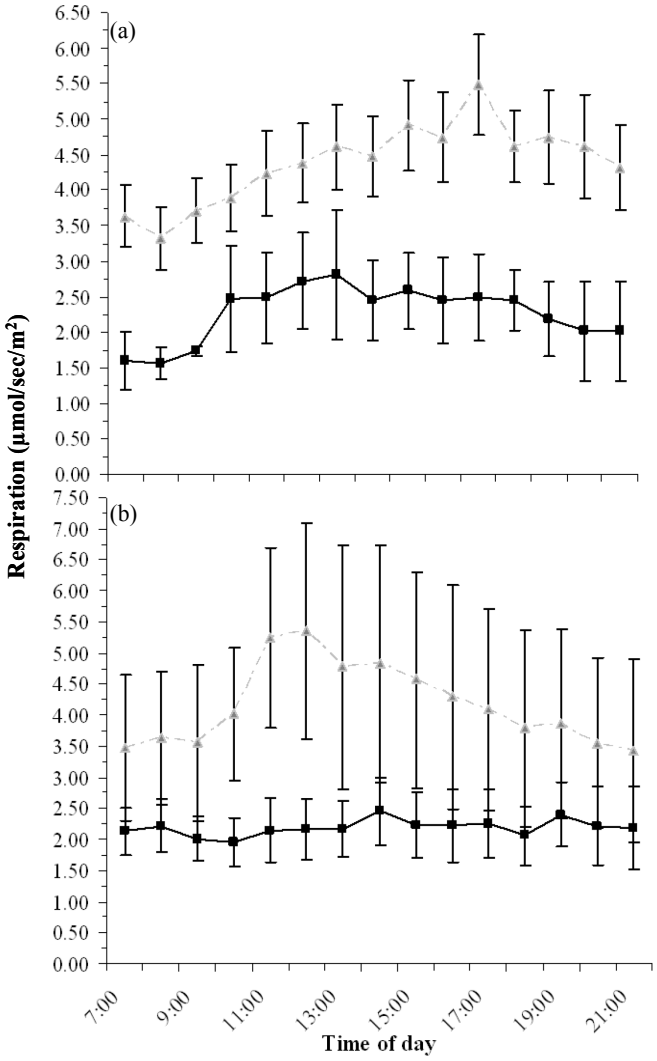


Figure 4.4. Daily total soil respiration (TSR) (—■—) and *C. cibarius* var. *roseocanus* respiration (CR) (—▲—) from 0700 to 2100 h in (a) 2006 and (b) 2007. The error bars represent the standard error.

Discussion

Patterns of cambial cell development and relationships with the fructification of *C. cibarius* var. *roseocanus*

The sigmoidal pattern of cell development in the cambium observed in temperate and boreal forests of the northern hemisphere is typical of all biological limiting growth processes and is fixed in ontogeny (Klingenberg 1998). To our knowledge, this is the first study to demonstrate that *Pinus banskiana* cell formation in growth ring fitted the sigmoidal pattern using the Gompertz function. From the annual growth pattern found for

the host tree, *P. banskiana*, important phenological traits were determined among which the time of maximum growth corresponding to the moment of transition from growth acceleration to growth deceleration (the upper asymptote) (Rossi et al. 2006b). Depending on the year, the mean maximum cell number reaching the upper asymptote occurred on August 16, 2006 and August 27, 2007 and seems to be reached earlier when the earlywood-latewood transition was earlier (July 16, 2006 and August 7, 2007). After this point, cell number fluctuation was induced by sampling in different positions on the trees as demonstrated by Deslauriers et al. (2003a). Moreover, variations in the transition time did not seem to be related to the start of the radial enlargement and, as shown by Deslauriers et al. (2003a), even when the growing season started sooner the earlywood-latewood shift was not sooner. Indeed, this annual transition from earlywood to latewood formation, where latewood is induced during the later part of the growing season, is a conspicuous developmental switch in temperate region trees (Uggla et al. 2001). Moreover, at the same period, Langlois and Fortin (1984) observed an increased C flow towards the roots after cessation of height growth.

Cantherallus cibarius var. *roseocanus* fructification peak happened on the same date as the earlywood-latewood transition in 2006 and 6 days later in 2007, but this transition did not seem to influence the beginning of the fructification as it remained the same in 2006 and 2007 (i.e. between July 10 and 12). The peak of the forest mushroom season coincided with the completion of growth of associated trees. With its long lifespan and early fruiting (mid-July), *C. cibarius* var. *roseocanus* is an exception to most ECM fungi, but this species also benefitted from this C contribution as its production peak corresponded with the earlywood-latewood transition. Indeed, at that moment, trees change their carbon balance strategy and start to direct more of their photosynthates to roots (Larson 1960). This enhanced C allocation to the roots may benefit the ECM fungi, including *C. cibarius* var. *roseocanus*, and play an important role in the timing of basidiome production as the production of a higher number of fruiting bodies occurred after mid-August (Fortin and Lamhamedi 2009). In agreement, Högberg et al. (2001), who observed that the ectomycorrhizal mycelial system is strongly dependent on current assimilates, and Kuikka et al. (2003), who found that the biomass of ECM fruiting bodies positively correlates with

starch concentration in fine roots, supported this idea of a mushroom fructification peak following the earlywood-latewood transition, which correspond to a slow down of wood production.

Soil respiration

It is not surprising that the TSR generally followed the annual soil and air temperature patterns. Our results are similar to those of many studies showing that the maximum soil CO_2 rates always corresponded to the highest annual soil temperature (reviewed in Davidson et al. (2006)) but contrary to those studies, ours did not show any correspondence to soil moisture content. Overall, our instantaneous soil respiration measurements were in the lower range of the values measured by Bergeron et al. (2009) in a black spruce forest located at a similar latitude but our TSR_{annual} values, which ranged from 311 to 553 g C m^{-2} $year^{-1}$, were close to the estimates of Striegl and Wickland (2001) for a mature jack pine stand and that of Bergeron et al. (2009) and of Gaumont-Guay et al. (2008) for black spruce sites, which were estimated at 710 and 611 g C m^{-2} $year^{-1}$, respectively.

No patterns or peaks of AR were observed. Moreover, the changes in tree phenology during the earlywood-latewood transition did not cause an AR increase as expected. It is important to note that the method used is prone to large uncertainties induced by the subtraction of heterotrophic fluxes from TSR fluxes. Also, the use of small-area root exclusion samplers can be subject to underestimation of autotrophic contributions (Jassal and Black 2006). Despite these constraints, we estimated that 24 to 33% of the summer respiration was attributed to the rhizospheric component of the soil. Most published studies on C allocation found similar results, among which Fahey et al. (2005) estimated root respiration to account for 40% of soil respiration using a trenching method and Scott-Denton et al. (2006) calculated that tree root respiration constituted between 31% and 44% of mid-summer respiration.

The emergence of the first fruiting bodies (July 10-12) did not correspond to any TSR peak for 2006 and 2007, but the 2006 mushroom fructification peak (week of July 26) corresponded to the first TSR peak. The 2006 mushroom fructification peak also

corresponded to the earlywood-latewood transition. In 2007, estimated data suggested a TSR peak during the *C. cibarius* var. *roseocanus* fructification peak, and the earlywood-latewood transition started 6 days before this fructification peak (August 11). These results support those of Högberg et al. (2001), who suggested that root respiration was more dependent on C import in the late growing season. According to Druebert et al. (2009), carbon stored in root systems was used only if the supply of recent assimilates was restricted, suggesting that trees were able to actively govern the carbon supply of the associated fungi. Godbout and Fortin (1990) also proposed that fruiting body production is driven by the phenological and physiological states of the host tree. Our results show that the peak in the emergence of mushroom fructification is close to the earlywood-latewood transition. Once fructification occurs, respiration rate of the carpophores (CR) is greatly dependent on micrometeorological conditions. Soil rhizospheric respiration (AR), like TSR and CR, does not appear to be influenced by the host tree physiological stage (before or after the earlywood-latewood transition). These results support the concept of an ECM demand of C supply from host trees (Courty et al. 2010) with an initiation of ECM fungi fructification controlled by host phenology.

C. cibarius var. *roseocanus* respiration

Weekly mushroom respiration

This study demonstrated that collars containing fruiting bodies had significantly higher CO_2 fluxes when compared with collars without fruiting bodies. Borken et al. (2006) also found a high TSR rate related to the development of fruiting bodies from a *Russula* species. They suggested that belowground mycelium of these fungi may contribute to the large fluxes occasionally measured in some experiments. Moreover, a high C allocation to ECM fungi is expected in low-nutrient coniferous stands (Hobbie 2006) where mycorrhizae might receive 20 to 40% of total host-plant photosynthates, with most transferred to soil mycelium (Smith and Read 2008). This is supported by the work of Heinemeyer et al. (2007) who divided soil CO_2 flux into soil heterotrophic, ECM hyphal, and root fluxes and concluded that ECM mycelium can contribute substantially more to soil CO_2 flux than do roots.

We found a high CR peak the first week of the mushroom fructification, which was synchronized with a TSR peak. These results led us to suspect that *C. cibarius* var. *roseocanus* fruiting bodies had a high respiration rate induced by punctual high TSR flux. Heinemeyer et al. (2007) demonstrated that under certain conditions ECM respiration appeared to be highly dependent on assimilate supply. Moreover, they observed that the ECM contribution to soil respiration tended to decrease after the main fruiting body production. This was also recorded for the year 2006 in our study.

Our 2006 and 2007 results suggested that CR peaks corresponded to TSR peaks. Högberg et al. (2002) showed that the dominant flux of C to and through roots sustains a fast soil C cycle, i.e. the respiratory activities of roots and their mycorrhizal fungi. This raised the possibility of a link between CR and TSR. However, the occurrence of *C. cibarius* var. *roseocanus* fruiting bodies in early July (before the major C supply from host trees) as well as some unaccountable CR peaks during the summers of 2006 and 2007 led us to suspect that either there is a very slow transfer of C from the host tree to the mushroom (Fortin and Lamhamedi 2009) or, under certain conditions, this fungus was able to seek other sources of C as suggested by Read et al. (2004) for other ECM fungi. Borken et al. (2006) demonstrated that some belowground mycela were utilizing C that is more enriched in $\Delta^{14}C$ than recent photosynthetic products, thus supporting the idea that some ECM fungi might be capable of saprophytic activities. This raises the possibility that ECM fungi could store carbon to use for rapid growth of fruiting bodies but further research is needed to evaluate the function of ECM fungi in the decomposition of soil organic matter in forests (Borken et al. 2006).

Daily mushroom respiration

Tang et al. (2005) demonstrated that TSR under the tree was strongly correlated with tree photosynthesis, but with a time lag of 7-12 hours that may be equivalent to the time needed for the translocation of photosynthates (mainly carbohydrates) from leaves to roots. However, even if Heinemeyer et al. (2007) demonstrated that the diurnal cycle of TSR followed the diurnal cycle of temperature, we observed that the TSR under the tree seemed to be out of phase with soil temperature. This indicated that TSR was driven by

photosynthesis first and secondly by soil temperature and moisture (Tang et al. 2005). In 2006, we found that CR had a regular pattern with a peak at the end of the day (around 1700 h), but such a pattern could not be detected in 2007. However, TSR was found to be at its highest around 1300-1400 h for both years. We hypothesized that this 3-4 hour time lag found only in 2006 is the time needed for the photosynthates to be translocated from the roots to the fungi. However, because the sensitivity of respiration responding to photosynthesis is small, the above time lag and the correlation between respiration (TSR and CR) and photosynthesis could be suppressed by more sensitive drivers such as temperature and moisture as shown by Tang et al. (2005). Although we demonstrated the existence of a relationship between TSR, CR and photosynthesis, further research on fungal respiration (including the mycelium and the fruiting body respiration) is necessary to better understand the weekly and daily fungal respiration patterns.

Conclusion

To our knowledge, this is the first study to demonstrate that *P. banksiana* cell formation in growth ring fit the sigmoidal pattern observed for temperate and boreal tree species of the northern hemisphere. Moreover, this study shows that *C. cibarius* var. *roseocanus* fructification is greater following the earlywood-latewood transition within days but that the beginning of the mushroom fructification did not change regardless of the year and the date of earlywood-latewood transition. This raises the possibility that some ECM fungi, including *C. cibarius* var. *roseocanus*, may be able to access the C of the host tree even when the supply for belowground organisms is low. It is also possible that they can store carbon to use for rapid growth of fruiting bodies or that they access a carbon source through saprophytic activities, but further research is needed to confirm C source used by these fungi.

We estimated that between 24 and 33% of the TSR was attributed to the rhizospheric component of the soil. CR and TSR generally followed the annual soil and air temperature patterns in 2006 and 2007. Results suggested that CR and AR are not dependent on the host tree physiological state because their corrected CO_2 efflux to a constant temperature of

10°C did not differ between the time period before and after the earlywood-latewood transition.

Collars containing fruiting bodies had higher CO_2 fluxes than collars without fruiting bodies on a weekly basis. In 2006 and 2007, the main CR peaks corresponded to TSR peaks. Moreover, the 2006 daily fruiting bodies respiration had a regular pattern with a respiration peak at the end of the day (around 1700 h), which corresponded to a 3-4 hour time lag with the TSR, but such a pattern could not be detected in 2007 probably due to more sensitive drivers such as temperature and moisture. We hypothesized that this 3-4 hour time lag is the time needed for the photosynthetic products to be translocated from the roots to the fungi.

Even if the fructification pattern of *C. cibarius* var. *roseocanus* is somewhat related to tree phenology, results indicated that AR and CR are not dependent on tree phenological state and this supports the mycocentric view that when a plant is in surplus, the C is at least partly allocated according to the C-demand of the fungus rather than according only to the demand of the C autotrophic host (Robinson and Fitter 1999).

Acknowledgements

This research was made possible by the Natural Sciences and Engineering Research Council of Canada strategic project # 306898-04 granted to Professor Yves Piché and collaborators at Université Laval in 2005. We are grateful for the technical assistance provided by Marie-Ève Beaulieu, Karine Bertrand, Andrew Coughlan, André Gagné, Nellia Pélardy and Christine Roussel-Roy at Université Laval, and Sébastien Dagnault at the Laurentian Forestry Centre of Natural Resources Canada. We also thank our collaborators in the field study, Alain Blais, Jessica Gagnon and Céline Marceau, for their help and patience during this 3-year study and for letting us take measurements on their land.

References

Bergeron, O., Margolis, H.A., and Coursolle, C. 2009. Forest floor carbon exchange of a boreal black spruce forest in eastern North America. Biogeosciences **6**(9): 1849-1864.

Borken, W., Savage, K.E., Davidson, E.A., and Trumbore, S.E. 2006. Effects of experimental drought on soil respiration and radiocarbon efflux from a temperate forest soil. Global Change Biology **12**(2): 177-193.

Cheng, C., and Gordon, I.L. 2000. The Richards function and quantitative analysis of germination and dormancy in meadowfoam (*Limnanthes alba*). Seed Science Research **10**(3): 265-277.

Cisneros-Dozal, L.M., Trumbore, S.E., and Hanson, P.J. 2006. Partitioning sources of soil-respired CO_2 and their seasonal variation using a unique radiocarbon tracer. Global Change Biology **12**(2): 194-204.

Corner, E.J.H. 1966. A monograph of Cantharelloid fungi. Oxford University Press, Oxford, UK.

Courty, P.-E., Buée, M., Diedhiou, A.G., Frey-Klett, P., Le Tacon, F., Rineau, F., Turpault, M.P., Uroz, S., and Garbaye, J. 2010. The role of ectomycorrhizal communities in forest ecosystem processes: new perspectives and emerging concepts. Soil Biology and Biochemistry **42**(5): 679-698.

Davidson, E.A., Richardson, A.D., Savage, K.E., and Hollinger, D.Y. 2006. A distinct seasonal pattern of the ratio of soil respiration to total ecosystem respiration in a spruce-dominated forest. Global Change Biology **12**(2): 230-239.

Deslauriers, A., Morin, H., and Begin, Y. 2003a. Cellular phenology of annual ring formation of *Abies balsamea* in Quebec boreal forest (Canada). Canadian Journal of Forest Research **33**(2): 190-200.

Deslauriers, A., Morin, H., Urbinati, C., and Carrer, M. 2003b. Daily weather response of balsam fir (*Abies balsamea* (L.) Mill.) stem radius increment from dendrometer analysis in the boreal forests of Québec (Canada). Trees - Structure and Function **17**(6): 477-484.

Druebert, C., Lang, C., Valtanen, K., and Polle, A. 2009. Beech carbon productivity as driver of ectomycorrhizal abundance and diversity. Plant, Cell & Environment **32**(8): 992-1003.

Fahey, T.J., Tierney, G.L., Fitzhugh, R.D., Wilson, G.F., and Siccama, T.G. 2005. Soil respiration and soil carbon balance in a northern hardwood forest ecosystem. Canadian Journal of Forest Research **35**(2): 244-253.

Forster, T., Schweingruber, F.H., and Denneler, B. 2000. Increment puncher: a tool for extracting small cores of wood and bark from living trees. IAWA Journal **21**(2): 169-180.

Fortin, J.A., and Lamhamedi, M.S. 2009. Ecophysiology and sporocarp development of ectomycorrhizal basidiomycetes associated with boreal forest gymnosperms. *In :* Advances in mycorrhizal science and technology. *Edited by* D. P. Khasa, Y. Piché, and A.P. Coughlan. National Reseach Council of Canada, Ottawa, Canada. pp. 161-173.

Gaumont-Guay, D., Black, T.A., Barr, A.G., Jassal, R.S., and Nesic, Z. 2008. Biophysical controls on rhizospheric and heterotrophic components of soil respiration in a boreal black spruce stand. Tree Physiology **28**(2): 161-171.

Godbout, C., and Fortin, J.A. 1990. Cultural control of basidiome formation in *Laccaria bicolor* with container-grown white pine seedlings. Mycol. Res. 94: 1051-1058.

Hanson, P.J., Edwards, N.T., Garten, C.T., and Andrews, J.A. 2000. Separating root and soil microbial contributions to soil respiration: a review of methods and observations. Biogeochemistry **48**(1): 115-146.

Heinemeyer, A., Hartley, I.P., Evans, S.P., Carreira de la Fuentes, J.A., and Ineson, P. 2007. Forest soil CO_2 flux: uncovering the contribution and environmental responses of ectomycorrhizas. Global Change Biology **13**(8): 1786-1797.

Hobbie, E.A. 2006. Carbon allocation to ectomycorrhizal fungi correlates with belowground allocation in culture studies. Ecology **87**(3): 563-569.

Högberg, P., Nordgren, A., and Ågren, G.I. 2002. Carbon allocation between tree root growth and root respiration in boreal pine forest. Oecologia **132**(4): 579-581.

Högberg, P., Nordgren, A., Buchmann, N., Taylor, A.F.S., Ekblad, A., Högberg, M.N., Nyberg, G., Ottosson-Löfvenius, M., and Read, D.J. 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature **411**(6839): 789-792.

Huang, S., Titus, S.J., and Wiens, D.P. 1992. Comparison of nonlinear height-diameter functions for major Alberta tree species. Canadian Journal of Forest Research **22**(9): 1297-1304.

Jassal, R.S., and Black, T.A. 2006. Estimating heterotrophic and autotrophic soil respiration using small-area trenched plot technique: theory and practice. Agricultural and Forest Meteorology **140**(1-4): 193-202.

Klingenberg, C.P. 1998. Heterochrony and allometry: the analysis of evolutionary change in ontogeny. Biological Reviews of the Cambridge Philosophical Society **73**(1): 79-123.

Kuikka, K., Härmä, E., Markkola, A., Rautio, P., Roitto, M., Saikkonen, K., Ahonen-Jonnarth, U., Finlay, R., and Tuomi, J. 2003. Severe defoliation of Scots pine reduces reproductive investment by ectomycorrhizal symbionts. Ecology **84**(8): 2051-2061.

Kuzyakov, Y., and Gavrichkova, O. 2010. Time lag between photosynthesis and carbon dioxide efflux from soil: a review of mechanisms and controls. Global Change Biology **16**(12): 3386-3406.

Langlois, C.G., and Fortin, J.A. 1984. Seasonal variations in the uptake of [^{32}P]phosphate ions by excised ectomycorrhizae and lateral roots of *Abies balsamea*. Can. J. For. Res. 14: 412-415.

Larson, P.R. 1960. A physiological consideration of the springwood summerwood transition in red pine. Forest Science **6**(2): 110-122.

Lavigne, M.B., Boutin, R., Foster, R.J., Goodine, G., Bernier, P.Y., and Robitaille, G. 2003. Soil respiration responses to temperature are controlled more by roots than by decomposition in balsam fir ecosystems. Canadian Journal of Forest Research **33**(9): 1744-1753.

Li, M., Hoch, G., and Körner, C. 2002. Source/sink removal affects mobile carbohydrates in *Pinus cembra* at the Swiss treeline. Trees - Structure and Function **16**(4): 331-337.

Liu, Q., Edwards, N.T., Post, W.M., Gu, L., Ledford, J., and Lenhart, S. 2006. Temperature-independent diel variation in soil respiration observed from a temperate deciduous forest. Global Change Biology **12**(11): 2136-2145.

Rayment, M.B., and Jarvis, P.G. 2000. Temporal and spatial variation of soil CO_2 efflux in a Canadian boreal forest. Soil Biology and Biochemistry **32**(1): 35-45.

Read, D.J., Leake, J.R., and Perez-Moreno, J. 2004. Mycorrhizal fungi as drivers of ecosystem processes in heathland and boreal forest biomes. Canadian Journal of Botany **82**(8): 1243-1263.

Robinson, D., and Fitter, A. 1999. The magnitude and control of carbon transfer between plants linked by a common mycorrhizal network. Journal of Experimental Botany **50**(330): 9.

Rochon, C., Paré, D., Khasa, D.P., and Fortin, J.A. 2009. Ecology and management of the lobster mushroom in an eastern Canadian jack pine stand. Canadian Journal of Forest Research **39**(11): 2080-2091.

Rochon, C., Paré, D., Pélardy, N., Khasa, D.P., and Fortin, J.A. 2011. Ecology and productivity of the *Cantharellus cibarius* var. *roseocanus* in an eastern Canadian jack pine stand. Botany. (Submitted).

Rossi, S., Anfodillo, T., and Menardi, R. 2006a. Trephor: a new tool for sampling microcores from tree stems. IAWA Journal **27**(1): 89-97.

Rossi, S., Deslauriers, A., Anfodillo, T., Morin, H., Saracino, H., Motta, R., and Borghetti, M. 2006b. Conifers in cold environments synchronize maximum growth rate of tree-ring formation with day length. New Phytologist **170**(2): 301-310.

Rossi, S., Deslauriers, A., and Morin, H. 2003. Application of the Gompertz equation for the study of xylem cell development. Dendrochronologia **21**(1): 33-39.

SAS Institute Inc. 2004. SAS/STAT® 9.1 user's guide, Volumes 1-7. SAS Institute Inc., Cary, NC.

Scott-Denton, L.E., Rosenstiel, T.N., and Monson, P.K. 2006. Differential controls by climate and substrate over the heterotrophic and rhizospheric components of soil respiration. Global Change Biology **12**(2): 205-216.

Smith, S.E., and Read, D.J. 2008. Mycorrhizal symbiosis, 3rd ed. Academic Press, London, UK.

Striegl, R.G., and Wickland, K.P. 2001. Soil respiration and photosynthetic uptake of carbon dioxide by ground-cover plants in four ages of jack pine forest. Canadian Journal of Forest Research **31**(9): 1540-1550.

Tang, J., Baldocchi, D.D., and Xu, L. 2005. Tree photosynthesis modulates soil respiration on a diurnal time scale. Global Change Biology **11**(8): 1298-1304.

Trumbore, S.E. 2006. Carbon respired by terrestrial ecosystems - recent progress and challenges. Global Change Biology **12**(2): 141-153.

Uggla, C., Magel, E., Moritz, T., and Sundberg, B. 2001. Function and dynamics of auxin and carbohydrates during earlywood/latewood transition in Scots pine. Plant Physiology **125**(4): 2029.

Vogel, J.G., and Valentine, D.W. 2005. Small root exclusion collars provide reasonable estimates of root respiration when measured during the growing season of installation. Canadian Journal of Forest Research **35**(9): 2112-2117.

Chapitre 5. Conclusion générale

Deux champignons ectomycorhiziens (ECM) de la forêt boréale (*Cantharellus cibarius* var. *roseocanus* Redhead, Norvell & Danell et *Hypomyces lactifluorum* (Schwein.) Tul. & C.Tul. / *Russula brevipes* Peck.) ont été étudiés dans cette thèse. Ces deux espèces ont été trouvées dans les sols sableux des peuplements matures de pin gris (Pinus banksiana Lamb.) de l'est du Canada. Comme presque tous les champignons ECM (Eveling et al. 1990), elles étaient influencées par les variables météorologiques, particulièrement par la température de l'air et la pluviométrie. Par contre, ces deux espèces étaient trouvées dans des milieux différents à l'intérieur d'un même peuplement. En effet, *C. cibarius* var. *roseocanus* préfèrait les sols bien drainés avec un pH avoisinant 4.5 et vivait généralement sous-couvert forestier à proximité des arbres hôtes, des mousses et des lichens . *H. lactifluorum*/*R. brevipes*, mieux connus sous le nom de dermatose des russules, est une espèce plus généraliste qui était adaptée à plusieurs types de sols, de peuplements et de végétation.

Des études se sont intéressées aux différents aspects écologiques susceptibles d'influencer les champignons ECM. Les travaux de Wiensczyk et al. (2002) résument les différentes stratégies de conservation proposées pour préserver ces espèces qui sont menacées par la surexploitation, les coupes forestières et la pollution. Durall et al. (2006) et Kranabetter and Kroeger (2001) ont tenté de mesurer l'impact de l'aménagement forestier sur la production épigée et la diversité des champignons comestibles. D'autres études ont permis de mieux comprendre la biologie et l'écologie d'espèces à fort potentiel commercial dont *Cantharellus formosus* Corner et *Morchella* spp. Finalement, certaines ont étudié le rôle des communautés ectomycorhiziennes dans les écosystèmes forestiers et l'allocation du carbone (C) aux champignons ECM (Courty et al. 2010; Hobbie 2006). Cependant, malgré les avancés effectuées, la physiologie, l'écologie, la distribution et la productivité de la plupart des champignons ECM restent peu connus et les liens entre ces champignons (leur présence et leur productivité) et les paramètres environnementaux ou la phénologie de l'arbre hôte demeurent encore difficiles à établir.

Cette thèse s'insère dans ce contexte et porte une attention particulière aux caractéristiques d'un peuplement, à sa végétation de sous-étage et à son sol en relation avec deux espèces de champignons ECM (*C. cibarius* var. *roseocanus* et *H. lactifluorum*/*R. brevipes*,). Elle tient aussi compte d'une perturbation anthropique (l'ouverture de sentiers) et des variations climatiques trouvées en peuplement de pin gris (température du sol et de l'air, humidité du sol et pluviométrie). Puisque que dans cette étude des interactions écologiques, de nombreuses variables et leurs interactions se chevauchent, plusieurs variables ont été utilisées simultanément (multivariables). Enfin, cette thèse s'intéresse, à une échelle fine, à l'importance de la phénologie de l'arbre pour un champignon ECM (*C. cibarius* var. *roseocanus).*

Sommaire des résultats

Le chapitre 2 de cette thèse a montré que l'aménagement de sentiers n'a pas augmenté la production de carpophores de dermatose des russules, qui était en moyenne de 21.6 kg/ha, mais a permis de la maintenir durant les périodes de faibles précipitations. La productivité, définie comme la biomasse fraîche par unité de surface et la densité de carpophores par unité de surface, était supérieure en présence d'espèces de plantes intolérantes à l'ombre (*Populus tremuloides* Michx., *Prunus pensylvanica L.f., Betula papyrifera* Marsh), d'ammonium extractible (NH_4^+), et diminuait avec une augmentation du pH. De plus, les carpophores de cette espèce sont présents dans certains microhabitats alors qu'ils sont absents dans d'autres. Un pH faible, une disponibilité élevée en phosphore (P), une faible présence de *Kalmia angustifolia* L. et de petites trouées dans le couvert forestier occupées par des espèces intolérantes à l'ombre et par la plante fixatrice d'azote (N) *Comptonia peregrina* (L.) Coulter favoriseraient la présence de carpophores. Finalement, cette étude démontre que certains aménagements durables, et potentiellement rentables, pourraient être développés afin de favoriser la présence et une augmentation de la productivité d'espèces de champignons ECM généralistes.

Le chapitre 3 de cette thèse a confirmé la présence de *C. cibarius* var. *roseocanus* au Québec grâce à l'utilisation de marqueurs moléculaires (détection d'ADN spécifique) et a montré qu'elle se développe dans les horizons organiques et minéraux du sol. Menée dans

deux peuplements de pin gris (dont un incluait 35 km de sentiers), cette étude a aussi permis de déterminer que la productivité de *C. cibarius* var. *roseocanus* au cours de trois années de croissance variait de 0.23 kg/ha à 3.85 kg/ha. Malgré l'absence de différence significative de productivité entre les peuplements, l'absence de colonies sur les sentiers semblait indiquer que certaines conditions micro-environnementales étaient inadéquates à la fructification de cette chanterelle. De plus, les principales caractéristiques du peuplement et du sol, la végétation associée et les conditions météorologiques reliées à la productivité et à la présence/absence de cette espèce sur un site ont été identifiées. Ainsi, une forte densité du peuplement, un rapport C : N du sol élevé et la présence de mousses formaient un micro-habitat favorable à la présence de *C. cibarius* var. *roseocanus* dans les peuplements étudiés. L'association végétale *Solidago puberula* Nutt. – *Comptonia peregrina – Pinus banksiana* et la présence de lichens et de fortes teneurs en argile et limon sur un sol sableux modérément acide semblaient caractéristiques des milieux productifs à la fructification de cette chanterelle, alors que la présence de plantes éricacées (*Kalmia angustifolia* et *Vaccinium angustifolium*) pourrait limiter le développement des carpophores. Finalement, la quantité totale des précipitations et la température de l'air précédent d'une semaine à deux semaines la fructification auraient un impact sur la quantité de carpophores à venir.

Le chapitre 4 de cette thèse a mis en évidence les relations entre la phénologie de l'arbre hôte (*P. banksiana*) et celle d'un champignon ECM (*C. cibarius* var. *roseocanus*). Même si les premiers carpophores apparaissaient avant la transition du bois juvénile vers le bois mature (i.e. le moment où davantage de composés photosynthétiques sont transférés aux racines), le pic de fructification du champignon suivait cette transition moins d'une semaine plus tard. De plus, durant toute la saison de croissance la respiration du carpophore était synchronisée avec la respiration totale du sol, tout en étant significativement plus élevée. Les variations de ces deux respirations étaient fortement corrélées avec les variations de températures du sol et de l'air. 24 à 33% de la respiration totale du sol correspondait à la composante rhizosphérique du sol, mais nous n'avons pas réussi à relier les pics de respiration autotrophe du sol aux pics de fructification et de respiration du champignon. En 2006, la respiration journalière des carpophores de *C. cibarius* var.

roseocanus atteignait un sommet en soirée, ce qui correspondait à un décalage de 3 à 4 heures avec la respiration totale du sol qui est elle-même décalée de 7 à 12 heures avec la photosynthèse de l'arbre (Tang et al. 2005). Cependant, ce pic de respiration n'a pas été détecté en 2007. Cette étude a aussi permis de démontrer que la formation annuelle des cernes de croissance de *P. banskiana* correspond au schéma sigmoïdal observé chez les autres espèces de conifères des forêts tempérés et boréales de l'hémisphère nord.

Pertinence de l'étude

Le dispositif expérimental utilisé pour l'étude sur la dermatose des russules (chapitre 2) était unique. En effet, des sentiers avaient été aménagés par les propriétaires du domaine où l'étude s'effectuait afin de favoriser la croissance de ce champignon. Cet aménagement original nous a permis de comparer la productivité de la dermatose des russules dans trois environnements différents (i.e. une forêt non aménagée, des sentiers et des bandes de forêt entre les sentiers) et de vérifier l'impact de cet aménagement forestier sur la fructification de ce champignon. Ainsi, nous avons montré que les carpophores étaient plus gros, mais moins nombreux dans les bandes de forêt entre les sentiers et plus petits, mais plus nombreux dans les sentiers, mais que la productivité du peuplement ne variait pas malgré ces différences. Le chapitre 2 de cette thèse a aussi mis en évidence pour la première fois les paramètres environnementaux qui influencent la productivité et la présence/absence de ce champignon en milieu naturel. Villeneuve (2000) suggérait que les carpophores de la dermatose des russules sont fréquemment trouvés ensevelis sous les débris de conifères et Bessette et al. (1997) affirmaient que les chemins forestiers favoriseraient leur présence ce que nos résultats ont confirmé en peuplements de pin gris. Finalement, nos observations ont indiqué que cette espèce généraliste possédait la capacité de fructifier à des endroits légèrement différents chaque année, ce qui lui faciliterait l'accès à des nouvelles sources de nutriments dans le sol.

Le chapitre 3 de cette thèse a permis de confirmer la présence de *C. cibarius* var. *roseocanus* au Québec. Cette chanterelle avait été identifiée par Redhead et al. (1997) dans l'ouest de l'Amérique du Nord, mais n'avait pas été répertoriée dans l'est. De plus, nos résultats qui confirment la présence de *C. cibarius* var. *roseocanus* dans les horizons

organiques et minéraux d'un podzol humo-ferrique complètent l'étude fait par Read et al. (2004) sur la distribution verticale des mycorhizes échantillonnées dans les différents horizons d'un sol podzolique. Comme au chapitre 2, cette étude nous a permis de déterminer les paramètres environnementaux qui influencent la productivité et la présence/absence de ce champignon en milieu naturel. Nos résultats vont dans le même sens que les travaux effectués par Bergemann and Largent (2000) sur *Cantharellus formosus* Corner qui stipulaient que la présence/absence de cette espèce à des endroits spécifiques était dû à l'épaisseur et au pourcentage de couverture apporté par l'humus et à l'acidité du sol. Par contre, puisque nous avons utilisé un plus grand nombre de paramètres environnementaux que ces auteurs, nous avons pu montrer que l'habitat de *C. cibarius* var. *roseocanus* en peuplement de pin gris était caractérisé par une association végétale particulière ainsi que par d'autres paramètres spécifiques. Cette étude a aussi prouvé statistiquement l'influence des paramètres météorologiques sur la fructification des chanterelles alors que celle-ci avait été suggérée par plusieurs auteurs dont Danell en 1994.

Les études des chapitres 2 et 3 de cette thèse se distinguent des études préalables sur l'écologie des champignons ECM par différents aspects. Premièrement, ces études utilisaient simultanément trois variables associées à la fructification des champignons : la biomasse fraîche, la densité et la présence/absence des carpophores à l'échelle d'un peuplement (échelle fine), alors que les autres études n'utilisaient en général qu'un ou deux de ces critères (comme par exemple Bergemann and Largent 2000; Pilz et al. 2006). L'utilisation de l'une ou l'autre de ces variables peut modifier les résultats obtenus comme nous l'avons constaté en analysant les résultats des deux études. Par exemple, au chapitre 3 de cette thèse, nous avons obtenu des résultats contradictoires pour les nitrates (NO_3^-) qui étaient négativement corrélés avec la présence de *C. cibarius* var. *roseocanus* dans le peuplement alors qu'ils favorisaient sa productivité. Deuxièmement, aux chapitres 2 et 3 de cette thèse, un plus grand nombre de paramètres environnementaux que dans les études préalables ont été utilisés chez les deux espèces de champignons, soit 18 paramètres de peuplement et de sol, 23 paramètres de végétation associée et 9 paramètres liés aux conditions météorologiques. Troisièmement, afin d'approfondir les connaissances sur l'écologie et de proposer des recommandations spécifiques à chaque espèce, nous n'avons

étudié qu'une espèce de champignon ECM à la fois plutôt que plusieurs espèces simultanément. En effet, chaque espèce de champignon ECM a des besoins écologiques, des niveaux de tolérances aux stress et des capacités physiologiques uniques (Read 1991).

Le chapitre 4 de cette thèse est plus exploratoire que les études des chapitres 2 et 3. À notre connaissance, plusieurs études avaient examiné la phénologie de l'arbre et le développement du bois initial et final (Deslauriers et al. 2003; Rossi et al. 2006) et d'autres s'étaient concentrées sur la respiration du sol et l'apport des champignons ECM aux flux de CO_2 des sols forestier (Heinemeyer et al. 2007; Högberg et al. 2001). Cependant, aucune étude n'avait tenté de lier la phénologie d'un arbre à celle d'un champignon ECM. De plus, à part Borken et al. (2006) qui mentionnaient une augmentation de la respiration du sol en présence de carpophores de *Russula* sp. sur leur site d'étude, la majorité des recherches sur les flux de C souterrains ne tiennent pas compte de la présence de carpohpores ou du mycélium lors des mesures de respiration du sol. De notre côté, nous avons utilisés simultanément la technique qui mesure la respiration du sol et celle qui détermine la date de transition entre le bois juvénile et le bois mature. Cela a permis d'établir pour la première fois des liens entre la respiration totale de sol, la transition du bois juvénile vers le bois mature de l'arbre et la fructification des carpophores de *C. cibarius* var. *roseocanus*. Cette étude est aussi la première à mesurer le dégagement de CO_2 des carpophores de *C. cibarius* var. *roseocanus* à une échelle journalière et hebdomadaire.

Limites de l'étude

Comme dans toute recherche, nous sommes conscients que cette étude présente certaines limites. Aux chapitres 2 et 3, un peu plus de 50 parcelles de champignons de dermatoses des russules et 45 parcelles de *C. cibarius var. roseocanus* avaient été installées sur les deux peuplements. Par contre, il n'y avait pas de répétition formelle de ces peuplements ce qui empêchait de comparer la productivité de *C. cibarius var. roseocanus* entre le peuplement aménagé et le peuplement non aménagé et de déterminer si les résultats obtenus étaient dus à l'aménagement de sentiers ou simplement à des différences entre les peuplements. Nos résultats devront donc être confirmés dans d'autres peuplements équivalents. De plus, au chapitre 3 en raison de l'absence de données prises avant

l'aménagement de sentiers, nous n'avons pas pu expliquer l'absence de carpophores de chanterelles dans les sentiers. Lors de la sélection de futurs sites expérimentaux, il serait hautement souhaitable d'avoir des répétitions des sites aménagés et non aménagés pour mieux évaluer l'impact des aménagements forestiers sur la productivité des champignons ECM.

Dans les chapitres 2, 3 et 4 de cette thèse nous avons principalement utilisé le carpophore comme indicateur de la productivité et de la présence/absence du champignon sur le site. Hormis, le chapitre 3 où la présence d'ADN a été cherchée dans les différents horizons du sol des parcelles de *C. cibarius* var. *roseocanus* et des parcelles témoins, nous n'avons pas considéré les structures souterraines des champignons. Pourtant l'absence de carpophores sur un site ne signifie pas nécessairement que le champignon n'y est pas présent (Horton and Bruns 2001). C'est pourquoi il importe de regarder à la fois la formation des carpophores et la présence de racines ectomycorhizées. Lors de travaux futurs sur la dermatose des russules, sur les chanterelles ou sur tout autre champignon ECM, l'utilisation des structures souterraines en plus des carpophores permettrait de confirmer les résultats.

Bien que l'utilisation de plusieurs paramètres environnementaux ait permis d'identifier ceux qui sont les plus importants pour la présence/absence et la productivité de la dermatose des russules et de *C. cibarius* var. *roseocanus*, cela a aussi amené des contraintes aux chapitres 2 et 3 de cette thèse. Le problème majeur était que plusieurs de ces paramètres influençaient simultanément ces champignons tout en interagissant les uns par rapport aux autres. Ainsi, parce qu'il nous était impossible d'isoler ces paramètres environnementaux dans le temps et dans l'espace, nous ne pouvions pas prédire quel paramètre influençait la productivité du champignon à un moment donné de la saison de croissance; la réponse étant probablement un mélange de tous ces paramètres à la fois. Le trop peu de répétition dans l'espace et dans le temps, nous a empêché d'effectuer des analyses spatiales telles que celles utilisées par Fortin et Dale (2005). En fait, nous avions trop peu d'échantillons de sol, d'humidité et d'ADN du sol pour entreprendre ces analyses. Ainsi, même s'il est coûteux et difficile à mettre en place, l'échantillonnage d'un plus grand

nombre de données à l'intérieur d'une même saison de croissance devrait être envisagé pour les expériences futures.

Cette étude a été effectuée sur une période de trois années. Cela correspond au temps minimum recommandé pour une étude sur les champignons ECM puisqu'il est habituellement suggéré de mener ces études sur des périodes plus longues, de 3 à 8 ans selon Gardes and Bruns (1996) et jusqu'à 30 ans selon Egli et al. (2006). Aux chapitres 3 et 4, prolonger la longueur des études aurait permis de valider l'influence de la pluviométrie et de la température sur la fructification et sur la respiration de *C. cibarius* var. *roseocanus* et d'évaluer l'impact de la création de sentiers sur la productivité des deux champignons à long terme.

Au chapitre 4 de cette thèse, plusieurs raisons pouvaient expliquer l'absence de pic de respiration autotrophe du sol durant les deux saisons de croissance : la respiration autotrophe du sol n'était pas mesurée directement (elle était obtenue en soustrayant la respiration hétérotrophe de la respiration totale du sol), l'utilisation de petits collets expérimentaux contribuait à sous-estimer cette respiration (Jassal and Black 2006) ou encore l'absence de croissance des racines. De plus, en 2007 aucun lien entre la respiration des carpophores et la date de transition entre le bois juvénile et le bois mature n'a été décelé alors que ce lien était très clair en 2006. Cela est peut-être dû au fait que les mesures n'ont pas pu être prises durant une semaine, mais cela montre probablement davantage que plusieurs autres paramètres, principalement météorologiques, influençaient la relation entre la phénologie de l'arbre et le champignon ECM. Notre étude a servi à repérer les liens existants entre la phénologie de l'arbre hôte et celle de son champignon ECM, mais des travaux utilisant d'autres techniques (comme les marqueurs isotopes radioactifs ou les chambres de respiration) permettraient de suivre de façon très précise l'allocation du C de l'arbre hôte aux champignons ECM et de décrire les modalités de respiration de ces champignons.

Perspectives de recherche

Suite aux travaux de recherche présentés dans les chapitres 2, 3 et 4 de cette thèse, plusieurs études complémentaires pourraient être effectuées, que ce soit pour détailler les structures du complexe *H. lactifluorum*/*R. brevipes*, pour tester les facteurs environnementaux qui influencent la présence et la productivité de *H. lactifluorum*/*R. brevipes* et de *C. cibarius* var. *roseocanus*, pour analyser l'impact d'aménagements forestiers sur les champignons ECM ou pour comprendre l'allocation du C d'un arbre hôte jusqu'au champignon ECM. Le Tableau 5.1 résume les questions de recherche auxquelles pourraient répondre de futurs travaux.

Table 5.1. Questions pour les recherches futures.

L'impact d'un aménagement forestier
- Quel est l'impact à long terme de l'aménagement de sentiers sur *H. lactifluorum*/*R. brevipes*?
- Quel est l'impact à court et à long terme de la création de sentiers sur *C. cibarius* var. *roseocanus*?

Les paramètres environnementaux
- Quelle est la contribution relative de chaque paramètre préalablement sélectionné sur la productivité de ces deux champignons ECM? Sur la distribution de ces deux champignons ECM?
- L'importance de ces paramètres pourrait-elle être déterminée expérimentalement par des essais en laboratoire, en serre ou en milieu naturel?
- Pourquoi les espèces éricacées inhibent-elles la présence des champignons ECM dans les parterres forestiers? Et par quel moyen?
- Quels sont les nutriments qui pourraient favoriser une augmentation de la productivité des champignons ECM (N, P et micronutriments)?

La productivité et la présence des champignons ECM à l'intérieur d'un peuplement
- Est-ce que la présence et la productivité de ces deux champignons ECM changent dans le temps et dans l'espace à l'intérieur d'autres peuplements ou d'autres sites?

Les structures souterraines des champignons
- Est-ce que la distribution des deux champignons ECM sur le site d'étude serait confirmée par l'étude d'autres structures (mycélium, mycorhizes, racines d'arbres)?
- Des paramètres environnementaux supplémentaires seraient-ils identifiés par l'étude d'autres structures?
- La phénologie de l'arbre a-t-elle un impact sur les structures souterraines des champignons ECM?
- Quels sont les horizons précisément occupés par *H. lactifluorum*/*R. brevipes*?

La phénologie de l'arbre et les champignons ECM
- Les arbres sont-ils capables de céder du C au champignon symbiotique à certains moments clés?
- L'utilisation du marquage d'isotopes radioactif (C_{14}) ou autres méthodes moléculaires pourrait-elle permettre de préciser les flux de C entre l'arbre et le champignon ECM? Par exemple, combien de temps y a-t-il entre la photosynthèse et l'arrivé du C dans les structures souterraines, dont le champignon? Quelle est la grandeur du réseau souterrain qui est influencé par un arbre?
- Quelle proportion du C du carpophore provient de la photosynthèse et quelle proportion provient de la dégradation de la matière organique du sol? Quelle est la source préférentielle de C d'origine hétérotrophe dans les carpophores (litière récente, bois mort, matière organique stable du sol)?

Changements climatiques
- Comment *H. lactifluorum*/*R. brevipes* et *C. cibarius* var. *roseocanus* réagiront-elles à une augmentation de la température terrestre? À une augmentation ou à une diminution des précipitations?
- Un changement des espèces arborescentes aurait-il un impact négatif ou positif sur la distribution des champignons ECM en forêt boréale?

Références

Bergemann, S.E., and Largent, D.L. 2000. The site specific variables that correlate with the distribution of the Pacific Golden Chanterelle, *Cantharellus formosus*. Forest Ecology and Management **130**(1-3): 99-107.

Bessette, A.E., Bessette, A.R., and Fischer, D.W. 1997. *Hypomyces* and allies. *In :* Mushrooms of northeastern North America. Syracuse University Press, Syracuse, N.Y., USA. pp. 529-532.

Borken, W., Savage, K.E., Davidson, E.A., and Trumbore, S.E. 2006. Effects of experimental drought on soil respiration and radiocarbon efflux from a temperate forest soil. Global Change Biology **12**(2): 177-193.

Courty, P.-E., Buée, M., Diedhiou, A.G., Frey-Klett, P., Le Tacon, F., Rineau, F., Turpault, M.P., Uroz, S., and Garbaye, J. 2010. The role of ectomycorrhizal communities in forest ecosystem processes: new perspectives and emerging concepts. Soil Biology and Biochemistry **42**(5): 679-698.

Danell, E. 1994. Formation and growth of the ectomycorrhiza of *Cantharellus cibarius*. Mycorrhiza **5**(2): 89-97.

Deslauriers, A., Morin, H., and Begin, Y. 2003. Cellular phenology of annual ring formation of *Abies balsamea* in Quebec boreal forest (Canada). Canadian Journal of Forest Research **33**(2): 190-200.

Durall, D.M., Gamiet, S., Simard, S.W., Kudrna, L., and Sakakibara, S.M. 2006. Effects of clearcut logging and tree species composition on the diversity and community composition of epigeous fruit bodies formed by ectomycorrhizal fungi. Canadian Journal of Botany **84**(6): 966-980.

Egli, S., Peter, M., Buser, C., Stahel, W., and Ayer, F. 2006. Mushroom picking does not impair future harvests - results of a long-term study in Switzerland. Biological Conservation **129**(2): 271-276.

Eveling, D.W., Wilson, R.N., Gillespie, E.S., and Bataillé, A. 1990. Environmental effects on sporocarp counts over fourteen years in a forest area. Mycological Research **94**(7): 998-1002.

Fortin, M.J., and Dale, M. 2005. Spatial Analysis: a guide for ecologists. Cambridge University Press, Cambridge, U.K.

Gardes, M., and Bruns, T.D. 1996. Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: above- and below-ground views. Canadian Journal of Botany **74**(10): 1572-1583.

Heinemeyer, A., Hartley, I.P., Evans, S.P., Carreira de la Fuentes, J.A., and Ineson, P. 2007. Forest soil CO_2 flux: uncovering the contribution and environmental responses of ectomycorrhizas. Global Change Biology **13**(8): 1786-1797.

Hobbie, E.A. 2006. Carbon allocation to ectomycorrhizal fungi correlates with belowground allocation in culture studies. Ecology **87**(3): 563-569.

Högberg, P., Nordgren, A., Buchmann, N., Taylor, A.F.S., Ekblad, A., Högberg, M.N., Nyberg, G., Ottosson-Löfvenius, M., and Read, D.J. 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature **411**(6839): 789-792.

Horton, T.R., and Bruns, T.D. 2001. The molecular revolution in ectomycorrhizal ecology: peeking into a black-box. Molecular Ecology **10**(8): 1855-1871.

Jassal, R.S., and Black, T.A. 2006. Estimating heterotrophic and autotrophic soil respiration using small-area trenched plot technique: theory and practice. Agricultural and Forest Meteorology **140**(1-4): 193-202.

Kranabetter, J.M., and Kroeger, P. 2001. Ectomycorrhizal mushroom response to partial cutting in a western hemlock - western redcedar forest. Canadian Journal of Forest Research **31**(6): 978-987.

Pilz, D., Molina, R., and Mayo, J. 2006. Effects of thinning young forests on chanterelle mushroom production. Journal of Forestry **104**(1): 9-14.

Read, D.J. 1991. Mycorrhizas in ecosystems. Cellular and Molecular Life Sciences **47**(4): 376-391.

Read, D.J., Leake, J.R., and Perez-Moreno, J. 2004. Mycorrhizal fungi as drivers of ecosystem processes in heathland and boreal forest biomes. Canadian Journal of Botany **82**(8): 1243-1263.

Redhead, S.A., Norvell, L.L., and Danell, E. 1997. *Cantharellus formosus* and the Pacific golden chanterelle harvest in western North America. Mycotaxon **65**(1): 285-322.

Rossi, S., Deslauriers, A., and Anfodillo, T. 2006. Assessment of cambial activity and xylogenesis by microsampling tree species: an example at the Alpine timberline. IAWA Journal **27**(4): 383-394.

Tang, J., Baldocchi, D.D., and Xu, L. 2005. Tree photosynthesis modulates soil respiration on a diurnal time scale. Global Change Biology **11**(8): 1298-1304.

Villeneuve, N. 2000. Diversité et productivité des champignons forestiers: les apports de la recherche et de l'inventaire. *In :* Les champignons forestiers: récolte, commercialisation et conservation de la ressource. *Edited by* J. A. Fortin, and Y. Piché. CRBF, Université Laval, Québec, Canada, 22 et 23 février 1999. pp. 91-100.

Wiensczyk, A.M., Gamiet, S., Durall, D.M., Jones, M.D., and Simard, S.W. 2002. Ectomycorrhizae and forestry in British Columbia: A summary of current research and conservation strategies. B.C. Journal of Ecosystems and Management **2**(1): 1-20.