INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

Bell & Howell Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

UMI®

The effects of age and size of canopy gaps on the mycorrhizae of yellow birch (*Betula alleghaniensis* Britton) and sugar maple (*Acer sacchrum* Marsh.) from deciduous forests of the Québec city region

Tonia De Bellis

A Thesis

in

the Department

of

Biology

Presented in Partial Fulfilment of the Requirements

for the Degree of Master of Science at

Concordia University

Montréal, Québec, Canada

July 2000

ABSTRACT

The effects of age and size of canopy gaps on the mycorrhizae of yellow birch (*Betula alleghaniensis* Britton) and sugar maple (*Acer saccharum Marsh.*) from deciduous forests of the Québec city region

Tonia De Bellis

Effects of different sized and different aged canopy gaps on the ectomycorrhizae of yellow birch and vesicular-arbuscular mycorrhizae of sugar maples collected from two deciduous forests in central Québec were examined. The colonization of maple roots by arbuscules, vesicles, and coils were quantified. Colonization by ectomycorrhizal fungi was quantified and characterized using morphotyping and molecular techniques. In the 1-yr old 1000 m^2 cuts, colonization increased in the maple roots as the season progressed. In October, there were more arbuscules in the seedlings from the gaps *vs* those from the uncut areas. No differences in ECM colonization between uncut and 1-year old gaps were noted in the yellow birch seedlings and high levels of colonization occurred throughout the season. In the 5- and 10-yr old gaps, colonization in both species increased as the season progressed, and higher colonization levels were present in the large (~100 m^2) *vs* small naturally occurring gaps (0-2 m^2). Based on morphotypes, species richness on yellow birch was unaffected by gap size and age, but diversity was slightly greater in the older gaps. Sixteen different ECM morphotypes were found, 10 of which were characterized using molecular methods, yielding 17 different restriction patterns. These results emphasize the importance of using molecular methods to back up morphological assays of ectomycorrhizal species richness.

ACKNOWLEDGEMENTS

I'd like to recognize and extend my deepest appreciation and respect to Dr. Widden for his constant instruction and encouragement, for allowing me to play in his lab for all these years and calling it "work".

I'd also like to thank my committee members Dr. Albert and Dr. Varin, for actually reading my thesis, and providing helpful insight. Thanks also to Christian Messier and FCAR for their financial support of this project.

Many thanks also go out to Nadine Costanzo and Marc Béland, for teaching me the A-B-C's of mycorrhizae, and for teaching me the needed lab techniques right from the start. My acknowledgements, would be incomplete without recognizing the unexpendable assistance in the lab and/or field from Joel Coburn, Luz Tavera-Mendosa, Goldie Marmor, Cathy Calogeropoulos, Sébastien Gougeon, Jean-Pierre Ricard, Patricia Gomez, Nadia, Mom and Dad.

The molecular part of this project would not have been accomplished without the help of Frédérick Marsolais, Jason Boyd, Satinder Gidda, and Pascale Gaudet.

Also, I would like to offer my thanks to Dr. Dighton and Amy Tuininga for their help with the ectomycorrhizae, and Dr. Kapoor for his help in making the colour plates.

Mike P. and friends for providing me with wonderful music.....,

& Jack for everything else.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

In natural ecosystems, plant roots can be associated with a wide range of fungi. Fungi can form parasitic, neutral or mutualistic relationships with plants. One of the most common and widespread plant-fungus associations are the mycorrhizae. Mycorrhizal fungi form symbiotic associations with plants. Mycorrhizae can form on virtually all plants from a wide variety of environments ranging from temperate to tropical forests, agricultural farmlands, heathlands, savannas, grasslands, and alpine and arctic tundras (Smith and Read 1997; Read 1991). Mycorrhizae colonize the roots of up to 90% of all vascular plants (Ozinga et al. 1997), making this relationship the rule among plants rather than the exception.

These symbioses are characterized by the bi-directional movement of nutrients, where carbon flows to the fungus and inorganic nutrients move to the plant (Smith and Read 1997). The fungi increase a plant's access to soil nutrients by extending their thin fungal hyphae beyond the root absorption zone, exploring a much greater soil area. This increase in nutrient uptake has led to increased growth rates and survivorship in mycorrhizal plants over non-mycorrhizal plants (Allen and Allen 1990; Davies and Call 1990; Haselwandter and Bowen 1996). Mycorrhizae are divided into 2 main categories based on their morphology. There are the endomycorrhizae which penetrate into the plant root cells, and the ectomycorrhizae (ECM) which penetrate in between root cells and form a sheath of fungal material that surrounds the plant's fine root tips. There are several groups of endomycorrhizae such as the ericoid and orchid mycorrhizae, but the most ancient and abundant type is the vesicular-arbuscular mycorrhiza (VAM). VAM

fungi form within the roots of up to two-thirds of all plant species (Newsham et al. 1995). There are about 150 different species of fungi forming the VAM association, all of which belong to the fungal phylum Zygomycota from the order Glomales (Brundrett 1991). The term "vesicular-arbuscular" comes from the two main structures the fungus produces within the cortical plant root cells. The VAM fungi form vesicles, which are round balloon-like structures that act as storage organs or as resting spores (Bonefante-Fasolo 1986). They also produce arbuscules, which are formed by many dichotomously branching hyphae, that resemble little tree-like structures within the cortical root cells (Bonfante-Fasolo 1986). It is at the arbuscule interface that nutrient transfer between plant and fungus occurs (Smith and Smith 1996). Another common fungal structure that forms within the cortical root cells is the coil, which is simply a looped arrangement of fungal hyphae. The main benefit imparted to the plant by the VAM fungi is an increase in the acquisition of several nutrients such as Ca, S, NO_3^-, Cu, Zn and especially P (Allen 1996; Marschner and Dell 1994). VAM fungi can also produce phosphatases which can solubilize phosphorus from sources that would otherwise be unavailable to plants (Read 1991). Other benefits include increased drought tolerance, increased pathogen resistance, and increased protection against toxic elements present in the soil (Newsham et al. 1995). VAM fungi are not very host specific and can therefore colonize the roots of a wide range of plants including herbs, shrubs and trees (Smith and Smith 1996). Some of the trees that are colonized by VAM fungi are the elms, ashes, walnuts, cottonwoods and, maples (O'Dell et al. 1993).

Ectomycorrhizae are formed by a wide range of fungi including thousands of species from the Basidiomycota, as well as many species from the Ascomycota and

Zygomycota (Brundrett 1991). The ectomycorrhizal root is characterized by three main structures. There is a sheath or mantle of fungal hyphae, which encloses the root, that is involved in nutrient storage. The Hartig net, is made up of fungal hyphae that penetrate between the first few layers of cortical root cells. In the Hartig net, the hyphal walls are in very close contact with those of the plant and it is at this interface that nutrient transfer occurs (Smith and Read 1997). The third main structural component of the ECM is the extensive extramatrical mycelium, which grows from the mantle and penetrates into the soil. As part of their extramatrical mycelium, ECM fungi form mushrooms which are a product of sexual reproduction. The mushrooms produce millions of spores which can be dispersed long distances by the wind. The ECM fungi can also supply a plant with several nutrients such as P, N, K, and Ca (Marshner and Dell 1994). Some ECM fungi can produce cellulases and phosphatases, and many can produce potent acid carboxypeptidases, giving them the ability to mobilize nitrogen from protein sources (Read 1991). ECM fungi are also involved in water uptake, resistance to toxic elements in the soil and pathogen resistance (Smith and Read 1997). ECM fungi mainly colonize the roots of tree species, such as the pines, spruces, hemlocks, oaks, beeches, firs, and birches (O'Dell et al 1993). There are over 5000 species of ECM fungi (Molina et al. 1992), which show varying degrees of host specificity. The majority can colonize nearly any host plant while others can be host specific (O'Dell et al. 1993), and it is not uncommon to find several different fungi colonizing the roots of a single tree.

Mycorrhizal fungi colonize the fine roots of most plants found in North American deciduous forests, and the importance of these associations is well recognized.

Therefore, when considering which management techniques should be used in a forest, their impact on the mycorrhizal community must be evaluated. Silvicultural practices such as clear-cutting, where large areas of forest are cut, are known to affect a forest's diverse flora and fauna. It is for this reason that the forestry community of eastern North America has developed a new cutting system based on the natural regeneration processes that occur in a forest. This practice is called selective cutting, a process in which many small areas are cleared throughout a forest. Foresters selectively cut areas with older damaged trees or where commercially unimportant species are present, creating different sized gaps to encourage the growth of shade-intolerant species. This practice has been adopted in Québec's mixed yellow birch sugar maple forests to encourage the growth of the commercially important yellow birch.

The optimum light level for the growth and root development of birch seedlings is 45-50% of full sunlight (Erdeman 1990). The importance of the gap openings created by the selective cutting technique is to create a light environment sufficient to support and encourage the growth of yellow birch. Sugar maples are very shade tolerant and can survive periods of suppression (Goodman et al. 1990). Sugar maple seedlings can grow fairly rapidly and can form solid root systems even in low light levels (Runke 1984). It has been reported that abundant yellow birch and sugar maple seedlings germinate in the gaps created by selective cutting (Majcen and Richard 1992). However it has also been noted that a large portion of the yellow birch saplings established in the cuts die before the next cutting cycle (i.e. in 15-20 years), while the sugar maple saplings have a much higher survival rate (Messier, pers. comm.). The long-term survival of both of these species may depend on their ability to compete with one another under the

differing light regimes created by the different sized gaps. A possible factor in the ability of these species to compete with one-another may be through the effectiveness of their mycorrhizal systems. The effects of the canopy gaps created by selective cutting on the mycorrhizal community in Québec's yellow birch sugar maple forests has not been examined.

VAM fungi are obligate biotrophs, therefore they are dependent on the host plant for their source of carbon. Disturbances such as selective cutting, where host plants are removed from the system, are bound to disrupt the underground mycelial-plant links. Disturbances to the fungal mycelial networks can reduce inoculum potential and thus reduce colonization rates (Read and Birch 1988). These impacts may be felt most at the seedling establishment phase (Read and Birch 1988). Seedlings may be depending on the pre-existing underground network as a source of inoculum such that they can quickly become colonized and benefit by the increased nutrient uptake at a critical point in their life. In a laboratory study, it was shown that seedlings had higher rates of colonization when grown next to adult plants that were colonized than if grown alone in soil with VAM inoculum (Eissenstat and Newman 1990). In the field, propagules of VAM fungi include spores, and root fragments containing hyphae and vesicles (Brundrett 1991). However, the principal source of inoculum is the extraradical network of soil hyphae (Brundrett 1991; Kabir et al. 1997); thus, knowing how long this underground network remains viable after a disturbance is of key importance. In California, the colonization rates of the roots of 1- to 5- month old bishop pine seedlings growing in a site that was disturbed by a wildfire were examined (Horton et al. 1998). The wildfire burned 4942 hectares, yet, one month post-fire, VAM structures were present in the

roots of seedlings. The number of VAM seedlings colonized was higher in areas where there was an abundance of resprouting VAM hosts. Horton et al. (1998) state that the fungi colonizing the seedlings were fungi that survived the fire and were not fungi that dispersed into the site after the fire.

Information on host removal and its effects on ECM fungi is more extensive, since these fungi colonize the roots of many commercially important tree species. The effects of clear-cutting on ECM colonization are quite variable. Parke et al. (1984), found lower mycorrhizal colonization rates on seedlings collected from clear-cuts than those collected from the uncut forest. In a 6 hectare burned clear-cut, not only was ECM colonization reduced within the clear-cut but, ECM activity was also reduced for at least 7.6 m into the adjacent stand (Harvey et al. 1980a). Another study looked at the ECM colonization of Northern red oak in relation to differing levels of overstory removal (Zhou et al. 1997). Within the first growing season, ECM colonization was actually greater in the trees collected in the 50% canopy removal treatment than in the clear-cut and uncut plots. However, by the second growing season there was no difference in ECM colonization in the trees collected from the 75%, 25%, and 0% (uncut) canopy removal treatments, but all three of these treatments had significantly higher levels of colonization than the trees collected from the clear-cut (Zhou et al. 1997). Other studies show no real difference in ECM colonization rates in clear-cut *vs* uncut forest. Pilz and Perry (1984), observed no difference in colonization rates between seedlings collected from one year old burned and unburned clear-cuts, and those growing in uncut forest. Visser et al. (1998), reported up to 90-100% mycorrhizal colonization on the roots of aspen growing in a clear-cut and uncut mixedwood site in Alberta, Canada.

In smaller gaps, created in a 95-year-old lodgepole pine stand in eastern Wyoming, Parsons et al (1994) monitored the changes in densities of ECM communities. Different sized gaps were created by removing, 1-, 5-, 15-, and 30-tree clusters. In this study, the colonization levels decreased as the gap size increased. However, the ground cover in this forest was sparse, very few understory plants were present, and the roots of the logepole pine formed the dominant belowground biomass component. Thus, when a gap was created, very few plant roots remained to help maintain the underground mycelium. In a forest in northwestern British Colombia, the ECM community in various sized forest openings was examined on naturally regenerating western hemlock seedlings (Kranabetter and Wylie 1998). Gaps of 50 - 75 m in diameter with few or no residual trees were analyzed, and sampling occurred along transects that cut across each gap. Transects that fell within 20 m of residual trees were discarded. A gradual decrease in fungal types was noted from the edge of the cut to the interior of the gap. Fungal species richness decreased from 13.1 types at the edge of the gap, to 7.8 types present in the center (Kranabetter and Wylie 1998). From the above mentioned studies it is clear that the size of the cut is not the only factor affecting the ECM community. Other factors such as surrounding understory vegetation, regeneration rates and mechanisms, length of time the cleared area remains barren, and severity of the forest soil disturbance may all play important roles.

Various physical and chemical factors can also affect the distribution of mycorrhizal fungi. When a gap is created, the light regime of the seedlings growing in

the gap will certainly be much greater than those growing under a forest canopy. Mycorrhizal fungi obtain their carbon from the host plant, thus, a plant's photosynthetic ability and the rate at which it translocates photosynthate to roots can strongly affect the development of mycorrhizae. Reduced light levels have been associated with reduced mycorrhizal colonization in many VAM and ECM plants (Bethlenfalvay and Pacovsky 1983; Björkman 1970; Daft and El-Giahmi 1977; Furlan and Fortin 1977). A laboratory study has shown that reductions in light levels did not affect total VAM colonization levels but levels of arbusclues found in the cells were significantly reduced (Franken and Gnädinger 1994). Hayman (1974) found larger and more arbuscules in the roots of onion plants exposed to higher light levels.

Canopy openings can also affect soil temperature and moisture, which, in turn can increase soil nutrient flow and pH (Harvey et al. 1980b). Each mycorrhizal fungus has different physiological requirements, environmental tolerances, and microhabitat preferences, thus altered environments often result in the replacement of one mycorrhizal fungal species by another (Perry et al. 1989). Different mycorrhizal fungi have different temperature optima (Cline et al. 1987; Forbes et al 1996; Grey 1991; Raju et al. 1990), hence different soil temperatures can cause shifts in the fungal species colonizing roots (Parke et al. 1983; Raju et al. 1990). Liming of soils is a practice used by foresters to alleviate soil acidity. The application of lime will increase soil pH and will release nutrients into the soil (Hausenbuiller 1985). Many studies have shown that mycorrhizal colonization usually decreases with the addition of fertilizers (Arnebrant and Söderstörm 1992; Marx et al. 1977; Newton and Pigott 1991; Sanders et al. 1975, Termorshuizen 1993). Abbott and Robson (1991), report that a change in soil pH from

4.5 to 7.5 by the addition of lime, did not change the total VAM colonization levels of plant roots but a change in fungal species colonizing the roots was observed. The effects of liming on ECM communities varies according to the duration of treatment and the amount of lime applied. Long term, higher dose studies reveal changes in types of ECM found in limed treated forests (Anderson and Söderstörm 1995; Erland and Söderstörm 1991), while a study by Karén and Nylund (1996) showed that moderate levels of lime did not alter the ECM community of the forest.

To fully assess the impact of selective cutting on VAM fungi it is important not only to look at root colonization levels but to quantify which fungal structures are present in the roots. In examining VAM fungi in plants in the field, it has recently been shown that there is a correlation between the amount of vesicles, coils, and arbuscules found within the plant root cells with the environment in which the plants are growing. Studies with sugar maples have shown that when a tree is under stress (i.e. low soil pH, exposed to UV-B, or high ozone), the proportion of vesicles found in the root cells increase while the numbers of arbuscules decrease (Costanzo 1999; Dukmanton and Widden 1994; Klironomos 1995; Klironomos and Allen 1995). It is at the arbuscule interface that nutrient transfer occurs, thus, a reduction in arbuscule numbers would indicate that the uptake of nutrients is reduced. The fungus may even become parasitic to a plant by storing carbon for its own use by producing many vesicles. A sign of a healthy mycorrhizal symbiosis is one which has a high arbuscule to vesicle ratio.

Another way of obtaining an increased understanding of mycorrhizae in the field, is by documenting the seasonal patterns of colonization throughout the year.

Information on the relationship between mycorrhizal phenology and host phenology is important to the understanding of the role of mycorrhizal fungi in the field. Mullen and Schmidt (1993), examined the development of the VAM fungi in *Ranunculus adoneus* over a 2-year period. They observed a peak in arbuscule numbers in the month of August. *R.adoneus* is an alpine plant, and it stores most of its nutrients late in the season, such that they can be used for growth the following season (Mullen and Schimdt 1993). The results from that study suggest that the VAM fungi play an important role in the life cycle of *R. adoneus*. However studies of this sort do not always show obvious trends. The VAM colonization of six different plants from a meadow in Yorkshire, England was followed for a 2-year period (Sanders and Fitter 1992a). In this study no correlation between mycorrhizal and plant phenology could be made. A reasons for this finding may be that different types of fungi colonize the roots at different times of the year, and each fungus may affect the plant in different ways. Thus the inability to find any trends may be due to shifts in the types of fungi colonizing the roots throughout the year (Sanders and Fitter 1992a). Identification of the VAM fungi present in roots can be achieved with molecular tools (Clapp et al. 1995; Clapp et al 1999; Helgason et al. 1999), but it has proven to be a time consuming and intricate task. Thus the identification of the VAM fungi in the sugar maples was not attempted in this study.

To assess the quality of an ECM association, an overall quantification is needed along with an examination of the fungal diversity present on the plant roots (Meier 1991). Sometimes changes may be subtle and the quantification of ECM fungi on the root tips alone will not pick up any differences. There may be differences in the types of fungi colonizing the roots while the total colonization rate will not be affected. Different

fungi have different physiological traits, and each fungus can benefit a plant in different ways, so changes in fungal types must be recorded. For example, Dighton and Skeffinton (1987), looked at the effects of acid rain on the ECM fungi colonizing Scots pine seedlings. Reductions of two different fungal types were noted. These two types of fungi produce an abundant amount of extramatrical hyphae, therefore reductions in their numbers would lead to loss of absorption area available for nutrient uptake (Dighton and Skeffinton 1987). A similar pattern was observed by Saikkonen et al (1999), where they looked at the effects of defoliation on the ECM of Scots pines. Over 98% of the fine roots analysed were colonized in all treatments but they did see a change in the types of ECM fungi colonizing the roots in the different treatment. ECM morphotypes with thick mantles and rhizomorphs were replaced with smooth ECM types with no or few rhizomorphs in the defoliated trees. The defoliation must reduce the amount of photosynthate available to the below-ground sinks, thus the fungi respond to this change by producing less carbon-demanding morphotypes. The opposite occurred in a study which looked at the effects of increased CO_2 levels on *Betula papyrifera* Marsh. saplings (Godbold and Berntson 1997). Significant changes in the composition of ECM morphotypes, towards morphotypes with more emanating hyphae and rhizomorphs, were observed in the elevated CO_2 plots. Changes in ECM fungal diversity in response to changing environments have also been reported in many other studies (Meier 1991; Qui et al. 1993).

In the past, descriptions of ECM fungal communities were based on the amounts of mushrooms present on the forest floor. The results of many studies have shown that mushroom fruiting aboveground is a poor indicator of what types of fungi are actually

colonizing the roots of plants belowground (Dahlberg et al. 1997; Danielson 1984; Gardes and Bruns 1996a; Gehring et al. 1998; Jansen and De Nie 1988; Visser 1995). Inconsistencies between fruiting bodies and ECM colonizing root tips may be because fruiting bodies are very dependent on environmental factors such as soil moisture for their development, and many ECM fungi do not form conspicuous fruiting bodies but are hypogeous. Morphotyping is a common method used to differentiate different types of ECM fungi on root tips and is based on morphological characteristics of the ECM such as texture, branching pattern and mantle type. However a more reliable method of identification of the ECM fungal types is to use molecular techniques based on the polymerase chain reaction (Gardes and Bruns 1996b). Many fungi can change in physical appearance throughout their developmental stages (Mehmann et al 1995), thus studies based on morphotyping alone can be misleading. A classification of fungi based on morphology may not be as thorough a method of identification as the molecular based method. DNA is relatively stable and does not change at different stages of a fungus' development. The DNA region most often used in the analysis of differentiating ECM fungal types is the internal transcribed spacer (ITS) region of the ribosomal DNA. The intraspecific variation in the ITS region has been reported to be very low, while the degree of interspecific variation is high, making this region well suited for the identification of ECM species (Gardes et al. 1993; Kårén et al. 1997). Other advantages of using the ITS region are that it can be rapidly amplified with universal primers and it is present in the genome in multiple copies; therefore, amplification from a small DNA sample is possible (Gardes et al. 1993). Comparisons of restriction fragment length polymorphism (RFLP) of the PCR amplified ITS region of the rDNA with known RFLP patterns is a fast and reliable way to confirm the identification of the suspect ECM types

(Mehmann et al. 1995).

It is not known how the mycorrhizae of sugar maple and yellow birch are affected by selective cutting. Information on the ECM fungal community colonizing birch roots in Québec forests is also scarce. The objectives of this study were to: 1) Assess the mycorrhizal status of sugar maple seedlings growing in different sized and different aged gaps, and in uncut forested areas throughout the growing season. 2) Assess the ECM status of yellow birch seedlings growing in different sized and different aged gaps, and in uncut areas throughout the growing season. 3) Examine the mycorrhizal response to the differing environmental parameters created by the canopy openings (i.e. increased light levels and changes in soil temperatures) and to the application of lime. 4) Characterize the ECM fungi colonizing the roots of the birch seedling by morphological and molecular methods. 5) Create a database of RFLP patterns of the ITS region of the ECM fungi found on the birch roots, which can later be used for other studies of the ECM communities in deciduous forests around the same geographical region.

MATERIALS AND METHODS

Site description

Seedlings were collected at two birch-beech-sugar maple forests in central Québec. One site is situated in the Réserve faunique de Portneuf in Rivière à Pierre, Québec (47°04'N and 72°15' W). Sugar maple (*Acer saccharum* Marsh.) and yellow birch (*Betula alleghaniensis* Britton), make up 75% of the vegetation cover. Other common trees present include American beech (*Fagus grandifolia* Ehrh.), red pine (*Picea rubens* Sarg.), red maple (*Acer rubrum* L.), and balsam fir (*Abies balsamea* (L.) Mill.). Understory vegetation includes mountain maple (*Acer spicatum* Lam.), striped maple (*Acer pennsylvanicum* L.), and Canada yew (*Taxus canadensis* Marsh.). The site is characterized by an undifferentiated till of approximately 1m in depth. The soil is an orthic humo-ferric podzol type with an average pH of 3.9-4.2 in the upper 10 cm. The altitude ranges between 320 to 400 m (Ricard 1999). Fifty 1000 m^2 gaps were created in November and December 1996, of which 9 were used in this study. Within each gap a 7 X 7 m limed quadrat was set up in July 1997 and 1998. Each quadrat, including a 0.5 m buffer strip surrounding it (total area of 64 m^2), received a mixture of 3.2 kg of crushed dolomite powder, containing $CaCO_3$ 92% (Ca:36%) and $MgCO_3$ 0.76% (Mg:0.35%), plus 0.16 kg of KCl.

The second site is located at the Station Forestière in Duchesnay, Québec (46°55' N and 71°40' W). The altitude ranges between 200-300 m, mean annual precipitation is 1220 mm and average minimum and maximum temperatures range between -12°C in January to 28°C in July. The canopy is dominated by sugar maple, yellow birch and American beech. Dominant understory vegetation include Canada

yew, striped maple and a few mountain maples. The soil is a brunisol with a pH of 4.94 (Ricard 1999). At this site, sampling took place in two locations; at one location gaps were cut in fall 1988, and at the second location gaps were created in fall 1994. From the 2 locations, seedlings were collected from gaps of 2 sizes (small, naturally occurring gaps = 0-2 m^2, large = ~100 m^2).

Collection

Entire yellow birch and sugar maple seedlings at Rivière à Pierre were collected by digging them up with a trowel. One year old yellow birch seedlings and 1-2 yr old sugar maple seedlings were randomly selected. Some soil was left around the roots to prevent them from drying out. Each seedling was placed in a Ziploc© bag and stored in the fridge (4°C) until it was processed. In Duchesnay, the whole sapling was removed from the soil with a shovel. Soil was left around the roots of each tree to prevent dessication. Saplings were left in a bag and stored at 4 °C. In the lab the soil was washed from the roots and portions of each root system was randomly collected for mycorrhizal analysis.

Seedlings at Rivière à Pierre were collected at the beginning of June, August and October. Seedlings were randomly collected from nine 1000 m^2 gaps. In June and August, 3 sugar maple and 3 yellow birch seedlings were collected from within each gap. As well, 3 sugar maple and 3 yellow birch seedlings were collected in uncut areas surrounding each gap. A total of 144 seedlings were collected for each month. In October, 5 sugar maple and yellow birch seedlings were collected from each gap. Also, 5 seedlings of each species were collected from the 7 X 7 m limed plots, and 5

seedlings of each species were collected from uncut areas surrounding each gap. A total of 210 seedlings were collected. For each seedling collected in the gaps, its location from the edge of the cut was recorded.

Seedlings in Duchesnay were collected in November 1997, and the end of April, June, July, August, September, and October 1998. Seedlings of ~1 m in height were randomly selected. In November 1997 and October 1998, 5 sugar maple, and 5 yellow birch seedlings were collected from gaps of two sizes (i.e. small = 0-2 m^2, and large = ~100 m^2) from the 2 locations. At one location, gaps were created in 1988 and at the other gaps were created in 1994, resulting in gaps of 5 and 10 years old. A total of 20 sugar maples and 20 yellow birch were collected for these two months. For the other sampling dates, 3 saplings of each species were collected from the different sized gaps at the 2 locations, for a total of 12 seedlings of each species. A total of 100 trees of each species were sampled at this site.

Light measurements

In Rivière à Pierre, the Photosynthetic Photon Flux Density (PPDF), was measured in August 1998, in each of the nine 1000 m^2 gaps, and in the surrounding control areas. The light measurements were taken at a height of 20 cm above the forest floor. In Duchesnay, the light measurements were taken in September 1999. When the seedlings were sampled the previous year, a stake with each seedling's height was left at the exact spot from which they were taken. The light measurements the following year, were taken at each seedling's height. The light measurements were collected under overcast sky conditions according to the method described by Messier et al. 1998.

The light measurements in the forest were obtained with a hand-held LI-190 point quantum sensor (LI-COR. inc., Lincoln, NE). Continuous light measurements of the above canopy PPDF from an adjacent opening were recorded using a LI-1000 datalogger.

Soil temperature and pH

For each seedling collected at Rivière à Pierre, soil temperature was obtained by placing a thermometer next to the selected seedling at a soil depth of ~ 2 cm. A sample of soil surrounding each seedling was placed in the Ziploc© bags when seedlings were collected. The soil was stored at 4°C until processed. Distilled water was added to the soil to form a soil paste and the soil pH was the recorded using a Piccolo ATC pH meter (Hanna intruments).

Staining and quantification of VAM structures in the sugar maple roots

For the seedlings collected in Duchesnay, some of the finer branches of the root system were preserved in formalin-alcohol-acetic acid (FAA) at room temperature for a minimum of 24 h. Complete root systems of the younger seedlings collected at Rivière à Pierre were preserved in FAA solution. The preserved roots were placed in OmniSette® Tissue Cassettes (Fisher Scientific, Pittsburg, PA) and were cleared by autoclaving for 35 min at 103.4 kPa in 10% KOH. The autoclave step was repeated 3 times, each time the 10% KOH solution was changed. After these steps the roots were gently rinsed with tap water and then bleached with 30% hydrogen peroxide for 1 h. The roots were rinsed again with tap water, acidified in 15% HCl for 15 min and stained in 0.15% Chorazol Black E at 90°C for 12-15 min. The roots were left overnight in a

destaining solution of 50% glycerol. The roots were mounted on slides in gycerine jelly and squashed with a cover slip. The VAM fungi were examined using a Nikon Optiphot differential interference contrast (DIC) microscope at a magnification of either 200X or 400X. The colonization rate for each root sample was obtained by the magnified grid intersect method (McGonigle et al. 1990). One hundred intersects were evaluated, and at each intersect the presence or absence of arbuscules, vesicles, and coils were noted. The colonization by each fungal structure was determined by counting the number of intersects in which it was present. Total colonization levels were obtained by counting all intersects that had at least one fungal structure present.

Observation and quantification of the ECM on the birch roots

A portion of each birch root system (~ 1-2 g) was sampled for all seedlings collected from the Duchesnay site. Roots free of soil were placed in plastic vials in distilled water and were stored a maximum of 48 h before being quantified and morphotyped. Roots were cut into ~3-5 cm pieces and laid out in an INTEGRID™ Petri Dish (Becton Dickson Labware, Lincoln Park, NJ). The quantification of the ECM colonization rates was performed using a Wild Heerburrg dissecting microscope with magnifications of 128-800 X. Percent colonization was obtained by a grid line intersect method where 100 root tips for each sample were analyzed for the presence or absence of ECM. Each intact root tip was categorized on the basis of some macroscopic and microscopic features. Each fungal type was characterized on several macroscopic features such as color, branching pattern, texture, external hyphae, tip shape (Goodman et al. 1998). Microscopic features included mantle type, the presence and types of cystidia the presence of clamps were noted (Goodman et al. 1998).

Whole root systems of the seedlings from Rivière à Pierre were evaluated for ECM colonization. When root systems were too large (i.e over 500 root tips), the root branches were cut into ~2 cm pieces and were laid on a numbered INTEGRID Petri Dish (Beckton Dicson Labware, Lincoln Park, NJ). Fifteen randomly selected squares were chosen for quantification, all root tips present in the 15 squares were quantified for ECM. Classification of each fungal morphotype was as described above for the Duchesnay samples.

Collection of fungal material for DNA extraction

Two to five root tips of each morphotype were placed in 1.5 ml eppendorf tubes and lyophilized. A number of replicate samples for each morphotype were collected. Lyophilized ECM tips were stored in the freezer at -20°C until DNA was extracted.

DNA extraction

Three hundred µl of a 2X CTAB lysis buffer (Gardes and Bruns 1993) was added to a 1.5 ml eppendorf tube containing the lyophilized ECM tips. The buffer-suspended samples were frozen in liquid nitrogen and crushed with a micropestle three times. After the final crushing, 100 µl of a 200 µg/ml solution of proteinase K in TE buffer with 0.5% SDS (Sambrook et al 1989) were added to each tube. Samples were then incubated at 64°C for 1 h, then one volume of Tris saturated phenol-choloroform-isoamyl alcohol (24:24:1, v/v/v) was added to each tube, the tubes were mixed and the phases were separated by centrifugation (14962 x g) for 1min. The aqueous phase was placed into a clean eppendorf tube and 1 volume of chloroform-isoamyl alcohol (24:1, v/v) was added, and the tubes were mixed and centrifuged. The aqueous phase was

placed into a clean tube and 100 µl of a 50 µg/ml solution of RNAse in TE buffer (Sambrook et al 1989) was added and samples were incubated at 37°C for 1 h. One volume of Tris saturated phenol-chloroform-isoamyl alcohol (24:24:1, v/v/v) was added to each tube, mixed and centrifuged. The aqueous phase was removed and 1 volume of chloroform-isoamyl alcohol (24:1, v/v) was added, mixed and centrifuged. The aqueous phase was placed into a clean tube and 0.8 volume of cold isopropanol was added. To this solution, 0.1 volume of sodium acetate (3M, pH 8.0) was added and samples were placed in the freezer at –20°C for 30 min. The samples were then centrifuged at max speed (13000 rpm, 14962 x g) for 15 min. The supernatant was discarded and the pellet was washed with 800 µl of cold 70% ethanol. Samples were again centrifuged at max speed for 15 min. The supernatant was removed and samples were dried at room temperature. DNA was re-suspended in 50 µl of sterile HPLC (Fisher Scientific, Pittsburg, PA) water and incubated at 37°C for 1 h. Samples were stored at 4°C until used.

PCR amplification and RFLP analysis

A number of DNA dilutions (undiluted, 1:10 and 1:100) were tested to determine the efficiency of amplification. The reactions were carried out in a final volume of 50 ul containing: 200 µM of each dNTP, 50 µM KCl, 10 mM Tris-HCl (pH 9), 3 mM $MgCl_2$, 30 pmol of each primer (ITS4 : 5'- TCC TCC GCT TAT TGA TAT GC –3' and ITS1-F: 5' CTT GGT CAT TTA GAG GAA GTA A – 3')(Gardes and Bruns 1996b)), and 0.75 units of Taq DNA polymerase. Each tube was briefly mixed in vortex mixer, centrifuged (14962 x g) for 1 min and a drop of mineral oil was added to prevent evaporation. The tubes were placed either in a MiniCycler PTC-150-16 or PTC-100 Programmable

Thermal Cycler (MJ Research Inc., Watertown, Massachusetts). The thermal parameters used started with an initial denaturation step of 94°C for 85s which was followed by 35 amplification cycles of denaturation, annealing, and extension. The thermal parameters of the first 13 cycles were 95°C for 35s, 55.5°C for 55s, and 72°C for 45s. Cycles 14-26 and 27-35 used the same denaturation and annealing parameters but the extension steps were increased to 120s for cycles 14-26, and to 180s for cycles 27-35. After the final cycle, the samples were incubated at 72°C for 10 min (Gardes and Bruns 1993). Controls with no DNA were run with every series of amplifications to test for the presence of contamination in reagents and reaction buffers. Also a positive control was run with every amplification to test for the proper functioning of all the reagents and buffers in the PCR mix.

Restriction digests of the amplified DNA were performed on unpurified PCR products. Ten µl of PCR product was combined with 9.5 µl water, 1.5 µl buffer (supplied by the manufacturer), 0.2 µl BSA and 0.5 µl restriction endonuclease (10 units/ul), either Mbo I, Hinf I, and Taq I (Promega Corporation, Madison, WI). Each tube was incubated for 1.5 h at each enzyme's optimal temperature. Digested DNA was loaded onto a 2% Agarose gel (ICN Biomedicals, Aurora, Ohio). To stain the gels, 0.25 µl of 1% ethidium bromide was added to 25 ml of TBE buffer (Sambrook et al 1989) containing 0.5 g dissolved agarose before the gels were poured. Gels were photographed under UV light. A DNA molecular weight marker VIII (Boehringer Mannheim) was used to determine fragment lengths.

Statistical analysis

All statistical analyses were performed using SPSS software (Norusis 1997). For the data collected from the sugar maples at both sites, a t-test and a one way analysis of variance were used to detect differences in colonization levels between treatments within a single sampling month. When the data did not fit the normal distribution an arcsine transformation was performed. A Tukey HSD test ($p<0.05$) was used to detect any treatment differences. A two-way ANOVA was used to detect any overall differences in colonization levels with date and treatment.

The data obtained for the total ECM colonization levels on the birch roots at Rivière à Pierre was not normally distributed, thus, non-parametric tests were used to analyse these results. Mann Whitney U and the Kruskal-Wallis test were done to investigate any differences in mycorrhizal colonization between treatments within each sampling month. Differences in ECM colonization of the birch at Duchesnay within a sampling month were analysed using a t-test or a one-way ANOVA. A two-way ANOVA was used to detect any differences between ECM colonization rates and sampling date and treatment for the whole data set.

ECM Diversity & Richness

Diversity indices for the ECM morphotypes for each group of birch trees in Duchesnay and Rivière à Pierre were calculated. Simpson's diversity was calculated by using the following formula: Simpson's index = $1/\sum_{i=1}^{s} P_i^2$ (Begon et al. 1996). When calculating the diversity and richness indices, each morphotype was considered as a single taxon.

RESULTS

Rivière à Pierre

Soil pH, soil temperature and light regime

The results of the t-test on soil pH show no significant differences between treatments for the months of June and August. Soil temperatures were significantly higher in the gaps *vs* the uncut areas for the month of June, but no differnces in soil temperature were noted from the soils collected from the different treatments in August. In October, soil temperatures in the uncut forest areas were also significantly lower than those in the gaps. In the gaps, soil pH in the limed plots was significantly higher than that of the unlimed areas and uncut forest (Appendix 1). Although significant differences in soil temperature and pH were found in soil data, the differences between the treatments that were significant were quite small (ie. ~ 2°C and 0.01 pH units), such slight variations between treatments are not likely to affect fungal colonization. However, the % PPDF levels measured from the nine 1000 m^2 gaps ranged from 26.4-89.1%, while the range of light levels that the seedlings collected in the control plots were exposed to ranged from 0.8-18.4%.

VAM colonization

The fine roots of 237 out of 243 maples sampled were mycorrhizal. A steady increase in VAM colonization was noted throughout the season. No relationship between VAM colonization and the distance each maple seedling was sampled from the gap edge was detected (r^2=0.016, $p>0.05$). The 2-way ANOVA results on the whole data set show that there are some highly significant differences in total VAM colonization, arbuscular colonization and coil colonization levels between treatment groups and between the

different sampling periods (Table 1). However the treatment x date term in all 3 tests was not significant, thus there was no interaction between date and treatment. Overall, higher colonization rates were present in the seedlings collected in the gaps *vs* those collected in the uncut areas. Analysis of the data collected in a single month revealed that significantly higher levels of total VAM colonization and arbuscular colonization were observed in the seedlings collected in the gaps over the uncut areas for the months of June and October (Fig.1). Coil colonization levels did not differ between groups throughout the period of study (Fig. 2a). For the month of August, there were no differences in colonization levels between the gap and uncut areas for all fungal structures quantified. The Tukey HSD post hoc tests revealed that total colonization, coil colonization, and arbuscular colonization levels for the month of October were significantly higher in the seedlings collected in the gaps and limed plots over the seedlings collected in the uncut areas. Total VAM colonization levels, arbuscular and coil colonization levels of the limed seedlings did not differ from those in the seedlings collected in the gaps. No statistical tests were performed on the vesicle colonization data since it was not normally distributed even after the arc sine transformation. Vesicle levels did increase slightly as the season progressed, however their total numbers remained low throughout the sampling season (Fig. 2b).

ECM Colonization

There were no significant differences in total ECM colonization levels between the gap, uncut and limed plots within each sampling date during the course of this study (Fig. 3). ECM colonization levels of the yellow birch roots were very high throughout the season. Although not significant, total ECM colonization levels were always slightly higher in the

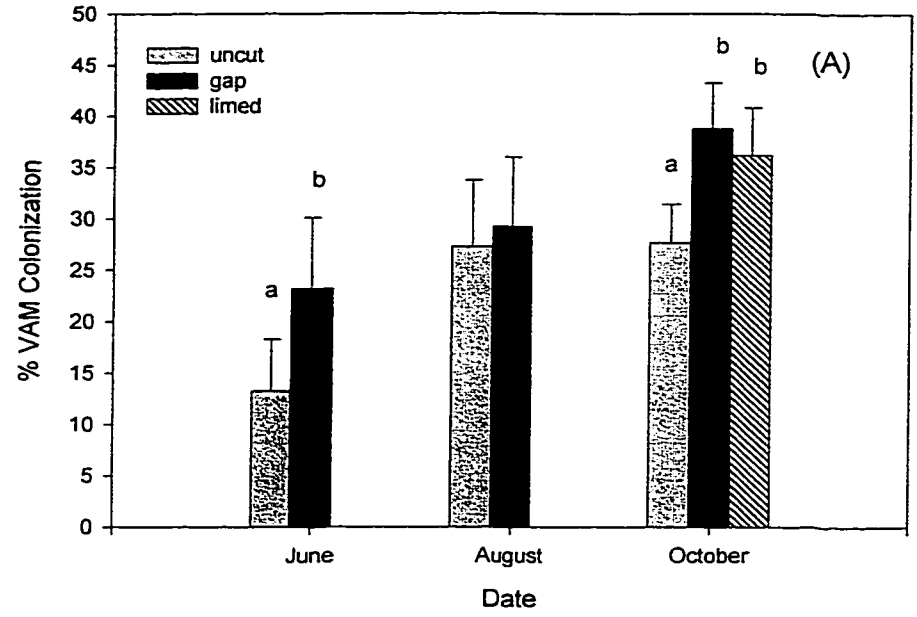
Table 1. P-values from the 2-way analysis of variance of VAM colonization of the sugar maples collected at Rivière à Pierre.

Dependant variable	date	gap size	date x gap size
Arbuscules	.000	.004	.271
Coils	.000	.007	.449
Total colonization	.000	.002	.194

Figure 1a. Total VAM colonization of sugar maple roots collected from Rivière à Pierre.

Figure 1b. Arbuscular colonization of sugar maple roots collected from Rivière à Pierre.

Bars are means + 95% confidence intervals (n=27: June and August, n=45: October)


50
45
40
35
30
25
20
15
10
5
0
% VAM Colonization
uncut
gap
limed
(A)
a
b
a
b
b
June
August
October
Date

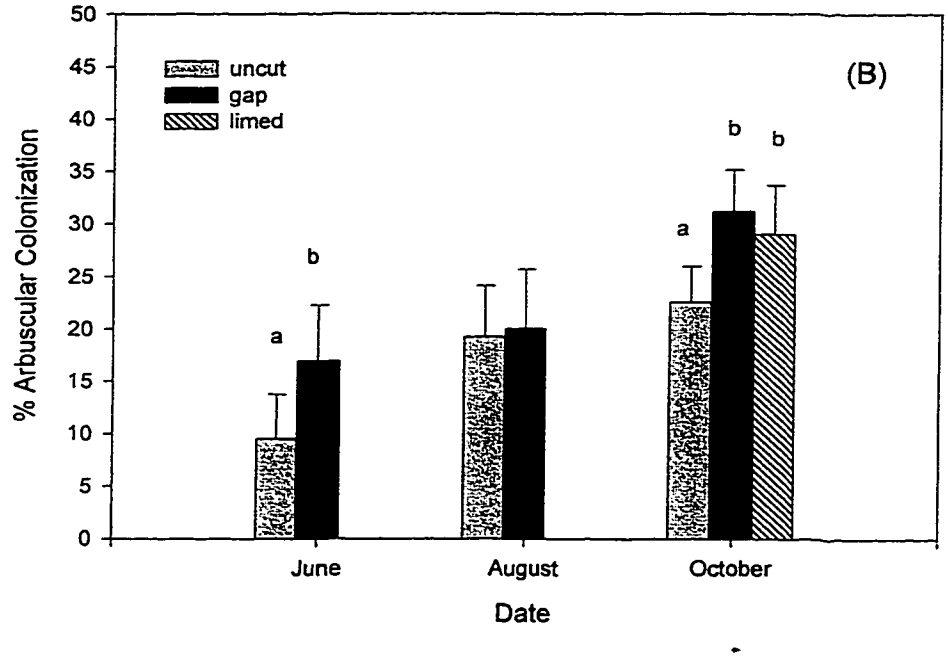
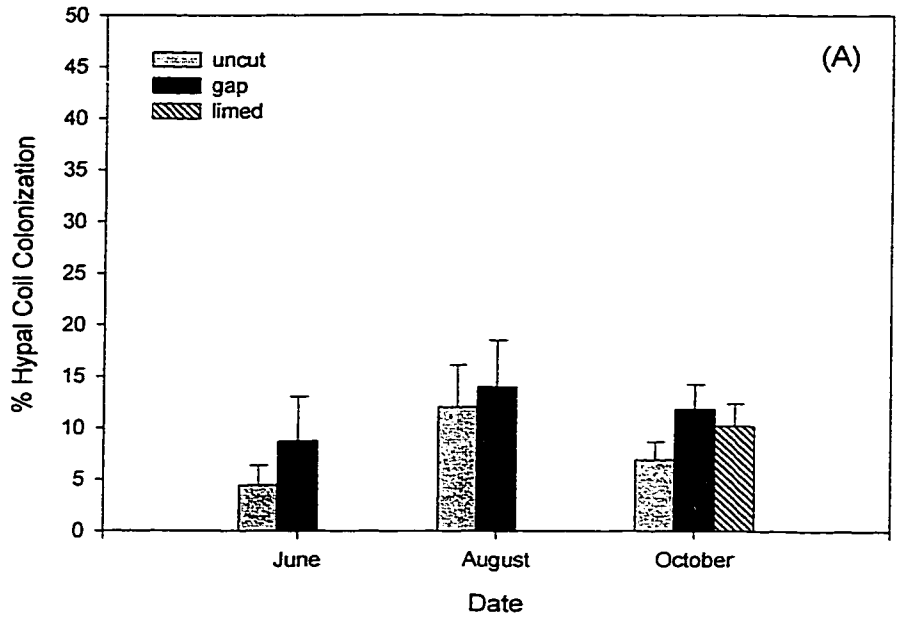

50
45
40
35
30
25
20
15
10
5
0
% Arbuscular Colonization
uncut
gap
limed
(B)
a
b
a
b
b
June
August
October
Date

Figure 2a. Hyphal coil colonization of sugar maples collected in Rivière à Pierre.

Figure 2b. Vesicular colonization of sugar maples collected in Rivière à Pierre.

Bars represent means + 95% confidence intervals (n=27: June and August, n=45: October)


(A)
uncut
gap
limed
% Hypal Coil Colonization
50
45
40
35
30
25
20
15
10
5
0
June
August
October
Date

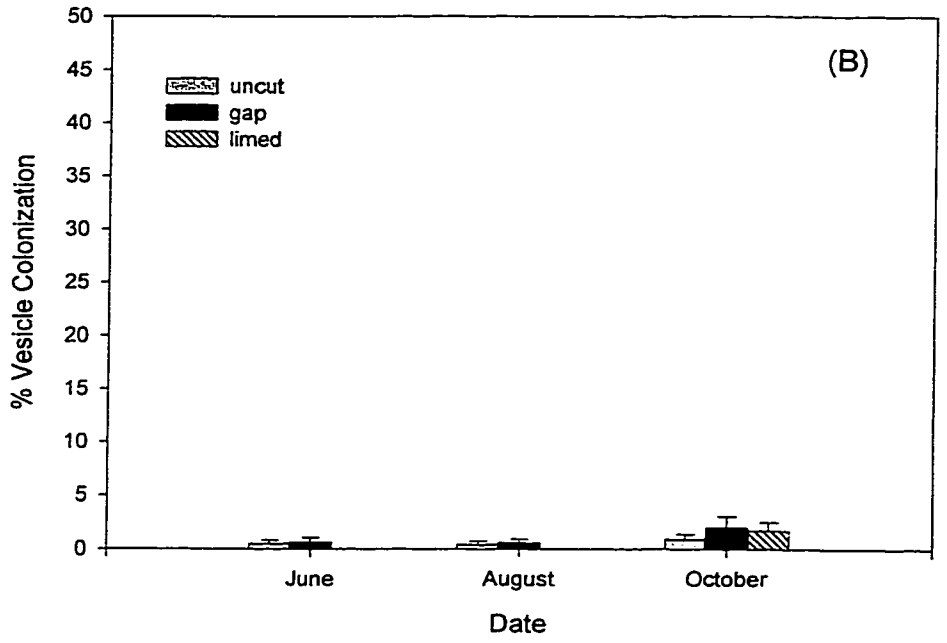
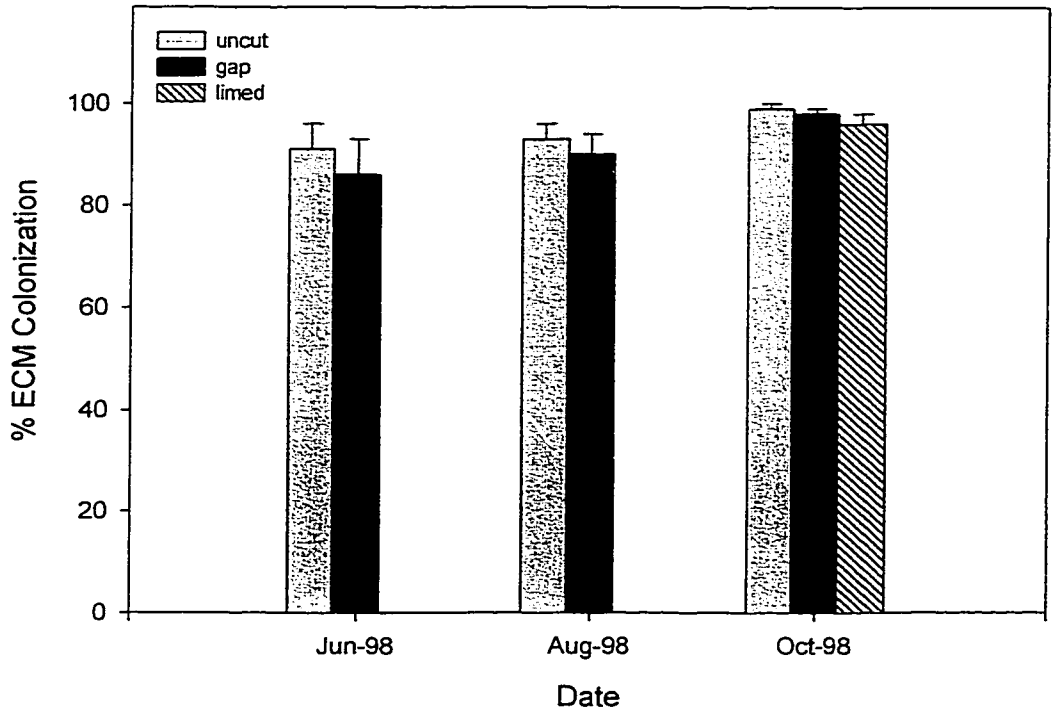

(B)
uncut
gap
limed
% Vesicle Colonization
50
45
40
35
30
25
20
15
10
5
0
June
August
October
Date

Figure 3. ECM colonization of the yellow birch roots from Rivière à Pierre.

Bars represent means + 95% confidence intervals (n=27: June and August, n=45: October)


uncut
gap
limed
100
80
60
40
20
0
% ECM Colonization
Jun-98
Aug-98
Oct-98
Date

control seedlings than in seedlings collected from the gaps. No shifts in morphotype distributions were noted from the different treatments sampled. Morphotype M1 was dominant at all sampling dates and in all treatments. All other morphotypes were present in very low frequencies compared to morphotype M1 (Fig. 4a). Diversity and richness of ECM morphotypes did not differ between treatments (Table 2).

Duchesnay.

Light measurements

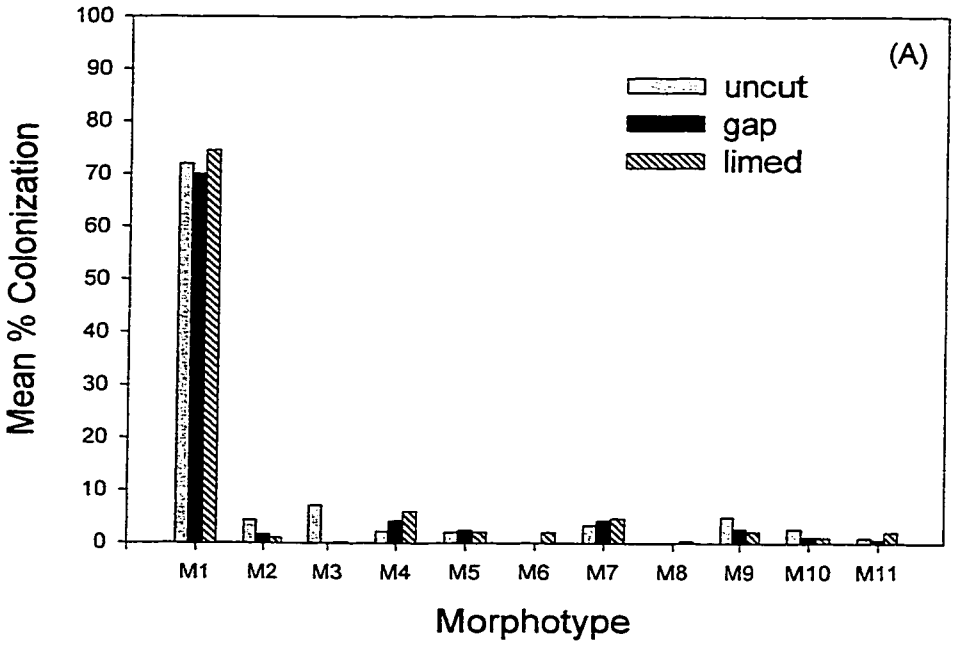
The light levels in the large gaps were significantly higher than that in the small gaps (p=.002, Table 3).

VAM colonization

There were no differences in VAM colonization between the maple seedlings collected from the 10- and 5-yr old gaps, therefore the data for these 2 groups were pooled. Mean total VAM colonization, arbuscular colonization, and coil colonization varied significantly with date and gap size (Table 4). Total VAM colonization and arbuscular colonization peaked at the end of the season (Fig. 5). In the seedlings collected in 1998, low colonization levels were seen in the months of April, July and August, there was a slight peak in the month of June, with significantly higher levels occurring in the months of September and October. The general trend in VAM colonization with higher colonization levels in the seedlings collected in the large gaps over those collected in the small gaps is present across the season. Analysis of colonization differences of each VAM structure quantified within each sampling month reveal no differences between small and large gaps, except for the month of November where coil colonization in the

Figure 4a. Mean morphotype abundance for all treatments from Rivière à Pierre.

Figure 4b. Mean morphotype abundance for the large and small gaps from Duchesnay.


(A)
uncut
gap
limed
Mean % Colonization
100
90
80
70
60
50
40
30
20
10
0
M1
M2
M3
M4
M5
M6
M7
M8
M9
M10
M11
Morphotype

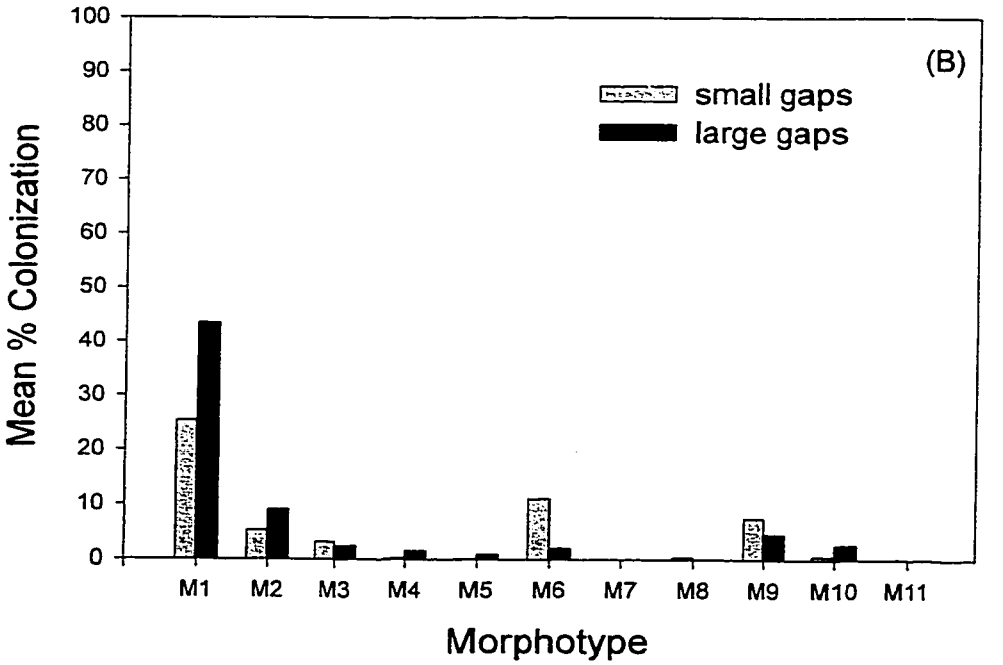

(B)
small gaps
large gaps
Mean % Colonization
100
90
80
70
60
50
40
30
20
10
0
M1
M2
M3
M4
M5
M6
M7
M8
M9
M10
M11
Morphotype

Table 2. Morphotype diversity and richness of the ECM fungi on the yellow birch seedlings collected from Rivière à Pierre and Duchesnay.

Site:	**Rivière à Pierre**		**Duchesnay**	
Treatment	control	gap	small gap	large gap
Richness	15	15	14	14
Simpson's Diversity	1.6	1.7	2.7	2.7

Table 3. Light measurements in the different sized gaps from Duchesnay

	Sugar maples		**Yellow birch**	
gap size	small	large	small	large
mean light (%PPDF)	2.30[a]	7.30[b]	.96[a]	5.84[b]

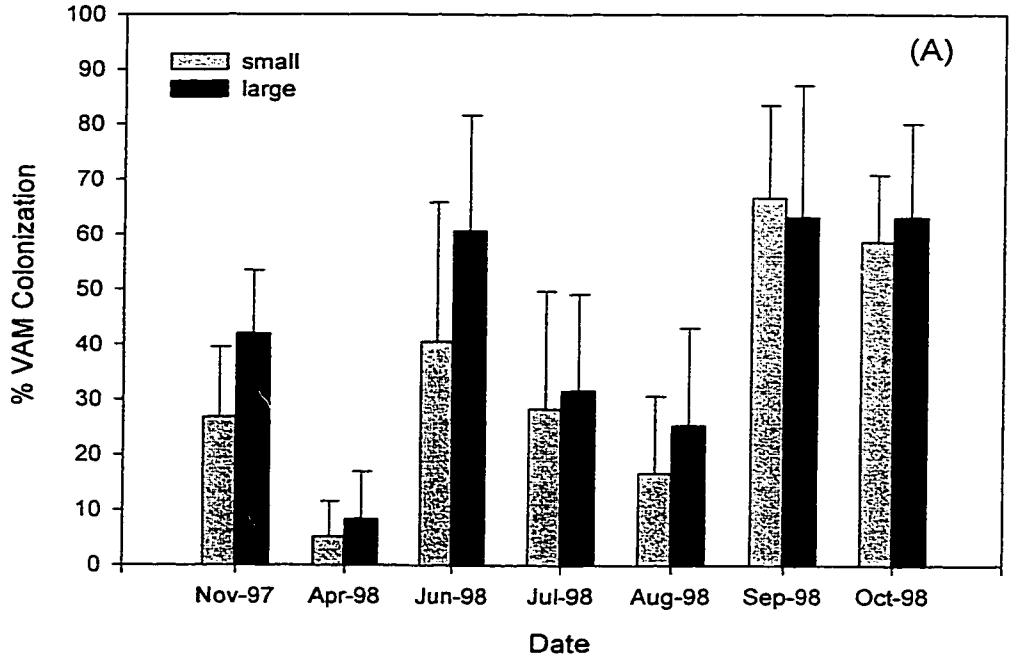
Table 4. P-values from the 2-way analysis of variance of the data on VAM colonization for sugar maples collected at Duchesnay.

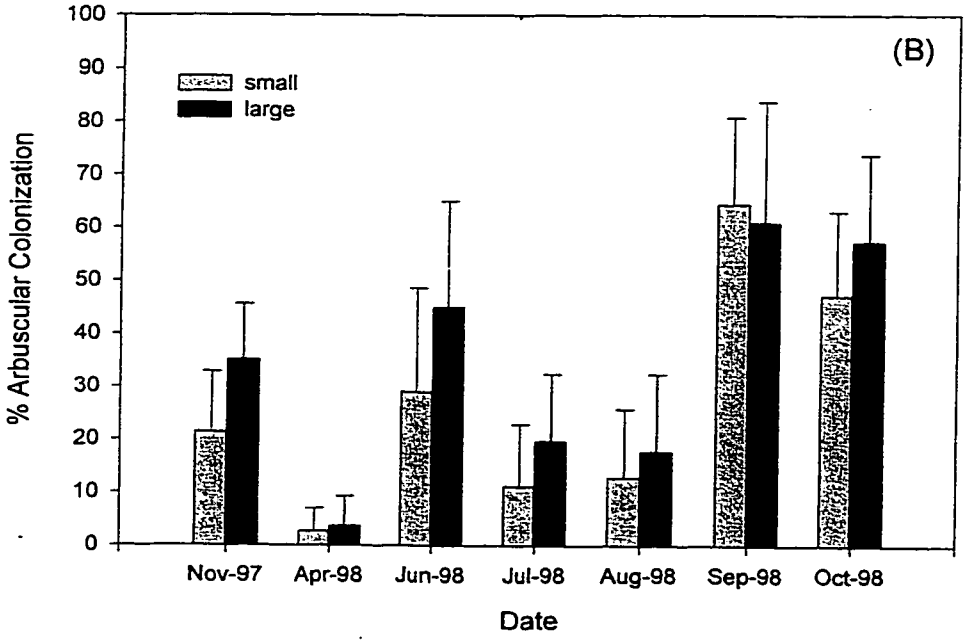
Dependant variable	**date**	**gap size**	**date x gap size**
Arbuscules	.000	.037	.751
Coils	.000	.320	.409
Total colonization	.000	.028	.727

Figure 5a. Total VAM colonization of sugar maple roots collected from Duchesnay.

Figure 5b. Arbuscular colonization of sugar maple roots collected from Duchesnay.

Bars represent means + 95% confidence intervals (n=10: November and October, n=6: for all other months)


(A)
small
large
% VAM Colonization
100
90
80
70
60
50
40
30
20
10
0
Nov-97
Apr-98
Jun-98
Jul-98
Aug-98
Sep-98
Oct-98
Date


(B)
small
large
% Arbuscular Colonization
100
90
80
70
60
50
40
30
20
10
0
Nov-97
Apr-98
Jun-98
Jul-98
Aug-98
Sep-98
Oct-98
Date

large gaps were significantly higher than those in the small gaps (Fig. 6a). Vesicles were present in low numbers throughout the season in both large and small gaps (Fig. 6b).

ECM colonization

Data collected from the birch seedlings in the 10- and 5-yr old gaps were not significantly different, thus the results were pooled. Two-way analysis of variance of the complete data set indicated that there were significant differences in mean ECM colonization rates between sampling dates (p=.000) and between gap sizes (p=.000), and the date x gap size interaction was not significant (p=.963). Overall, colonization was significantly higher in the large gaps. Except for the month of April (p=.036), there were no significant differences in colonization between the large and small gaps within a single month. The lack of any differences between treatments within a single month may be due to the high variation and low sample size for each group. Fig. 7 shows that ECM colonization increased as the season progressed. The morphotype diversity and richness did not differ between gap sizes (Table 2).

Morphological identification of mycorrhizas

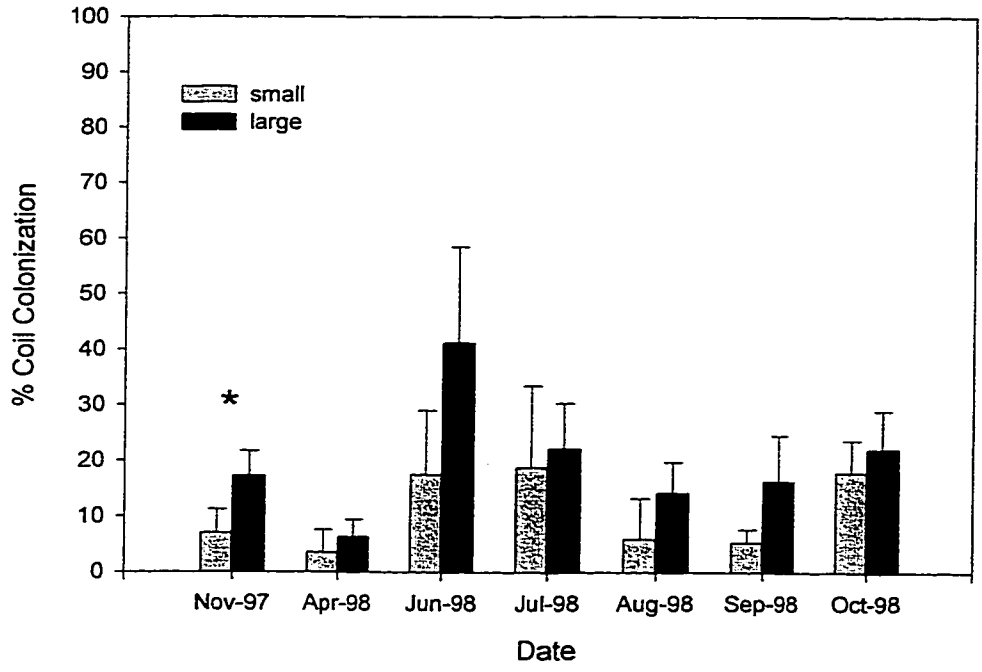
Based on macroscopic features, a total of 16 different mycorrhizal morphotypes were found on the birch rootlets collected from both sites. In Duchesnay, 14 different morphotypes were found and 15 different morphotypes were found at Rivière à Pierre. Thirteen morphotypes were common to both sites. Three of the 14 morphotypes found on the Duchesnay birch seedlings, were present on only 11 out of the 100 (11 %)

Figure 6a. Hyphal coil colonization of sugar maple roots collected in Duchesnay.

Figure 6b. Vesicular colonization of the sugar maple roots collected in Duchesnay.

Bars represent means + 95% confidence intervals (n=10: November and October, n=6: for all other months)

* indicates significant differences between groups, within a single month


100
90
80
70
60
50
40
30
20
10
0
% Coil Colonization
small
large
*
Nov-97
Apr-98
Jun-98
Jul-98
Aug-98
Sep-98
Oct-98
Date

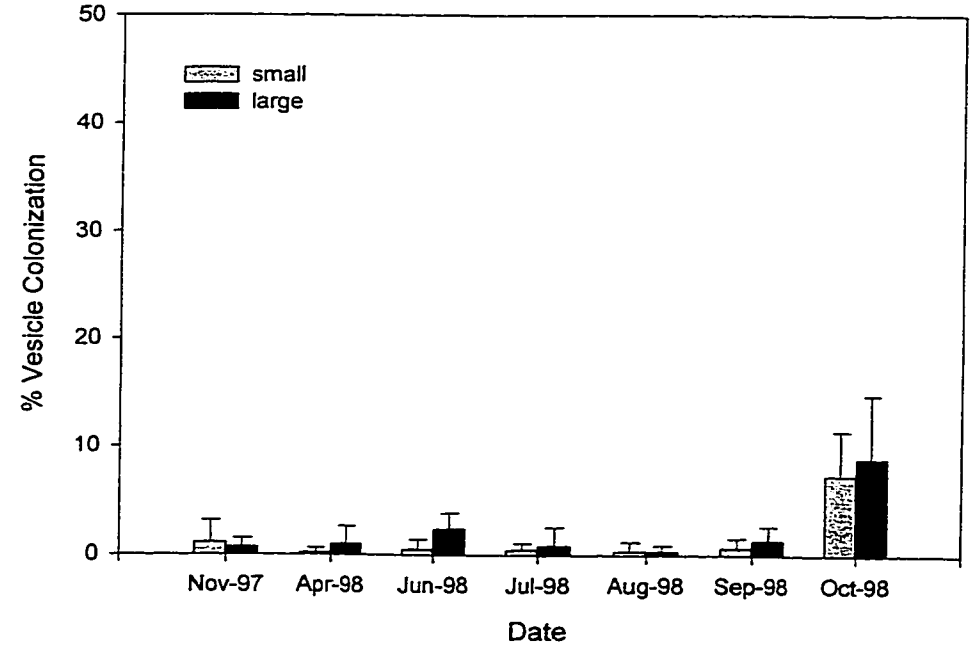
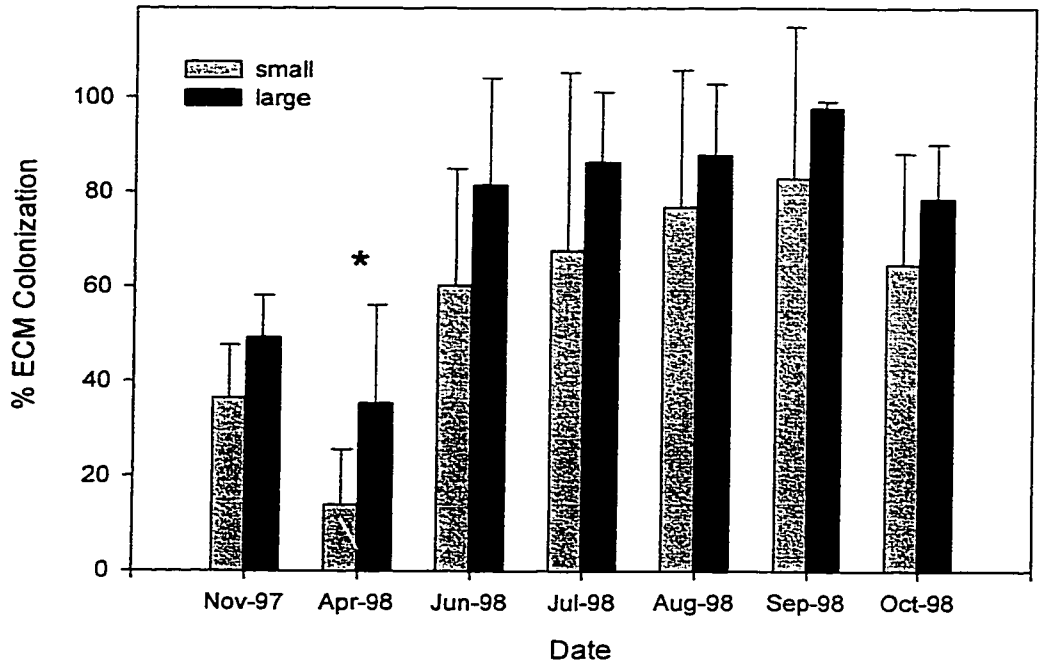

50
40
30
20
10
0
% Vesicle Colonization
small
large
Nov-97
Apr-98
Jun-98
Jul-98
Aug-98
Sep-98
Oct-98
Date

Figure 7. ECM colonization of the yellow birch from Duchesnay.

Bars represent means + 95% confidence intervals (n=10: November and October, n=6: for all other months)

* indicates significant differences between groups, within a single month.


small
large
100
80
60
40
20
0
% ECM Colonization
*
Nov-97
Apr-98
Jun-98
Jul-98
Aug-98
Sep-98
Oct-98
Date

seedlings collected. Four out of the 15 morphotypes found on the seedlings collected at Rivière à Pierre were present on 19 out of the 243 (7.8%) seedlings. The 11 remaining morphotypes present at both sites were more common and are shown in Figs. 8 - 30. The main distinguishing characters used to differentiate the various morphotypes are listed in Table 5. By far the most frequent type was Morphotype 1 (MI). It was present on the rootlets of 92 out of 100 seedlings from Duchesnay and 238 out of 243 seedlings at Rivière à Pierre. Mean colonization of the 11 more common morphotypes for each site are shown in Fig. 4.

DNA Amplification and RFLP Analysis

Several DNA samples from each morphotype were extracted and a total of 88 different samples were amplified (Table 6). Amplified fragments ranged from 680 to 950 bp. DNA from ten different morphotypes was successfully amplified and a total of 17 different RFLP-types were found. 'RFLP-type' refers to the groups of DNA samples that had similar RFLP patterns when digested with all three enzymes. All three restriction enzymes used were needed to distinguish the different RFLP-types. The most common morphotype M1 was comprised of 6 different RFLP-types (Fig. 31), and morphotype M2 consisted of three different RFLP-types (Fig. 32). All other morphotypes consisted of only one RFLP-type (Fig. 33).

Figure 8 - 9. Morphotype M1 (mag X 360)

Figure 10. Younger ECM tip of Morphotype M1 (mag X 420)

Figure 11. Morphotype M2 with attached rhizomorphs (mag X 420)

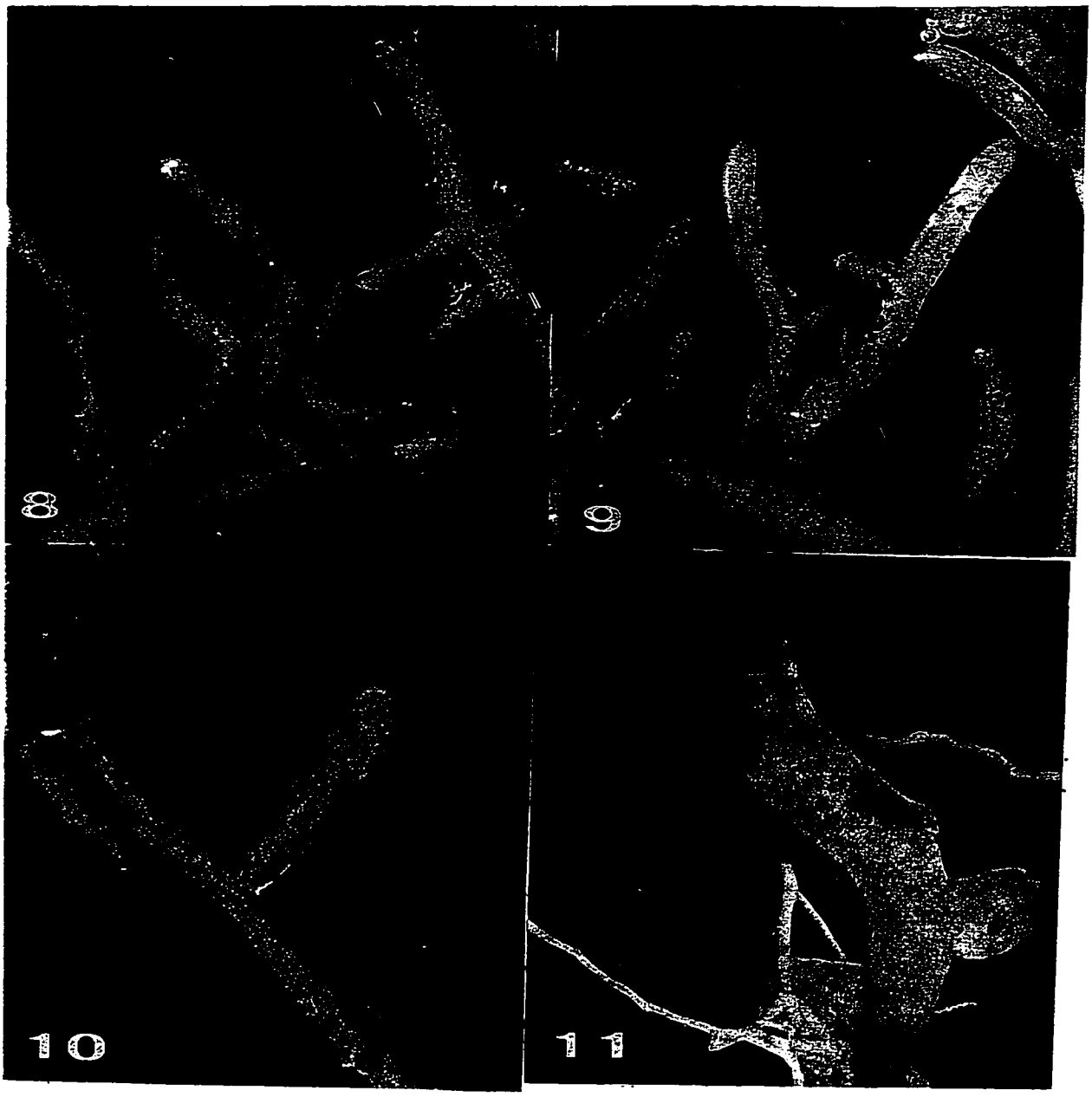

8
9
10
11

Figure 12. Ectomycorrhizal system of morphotype M2 (mag X 320).

Figure 13-14. Morphotype M3 (mag X 320).

Figure 15. Lustrous, cottony texture of morphotype M3 (mag X 800).

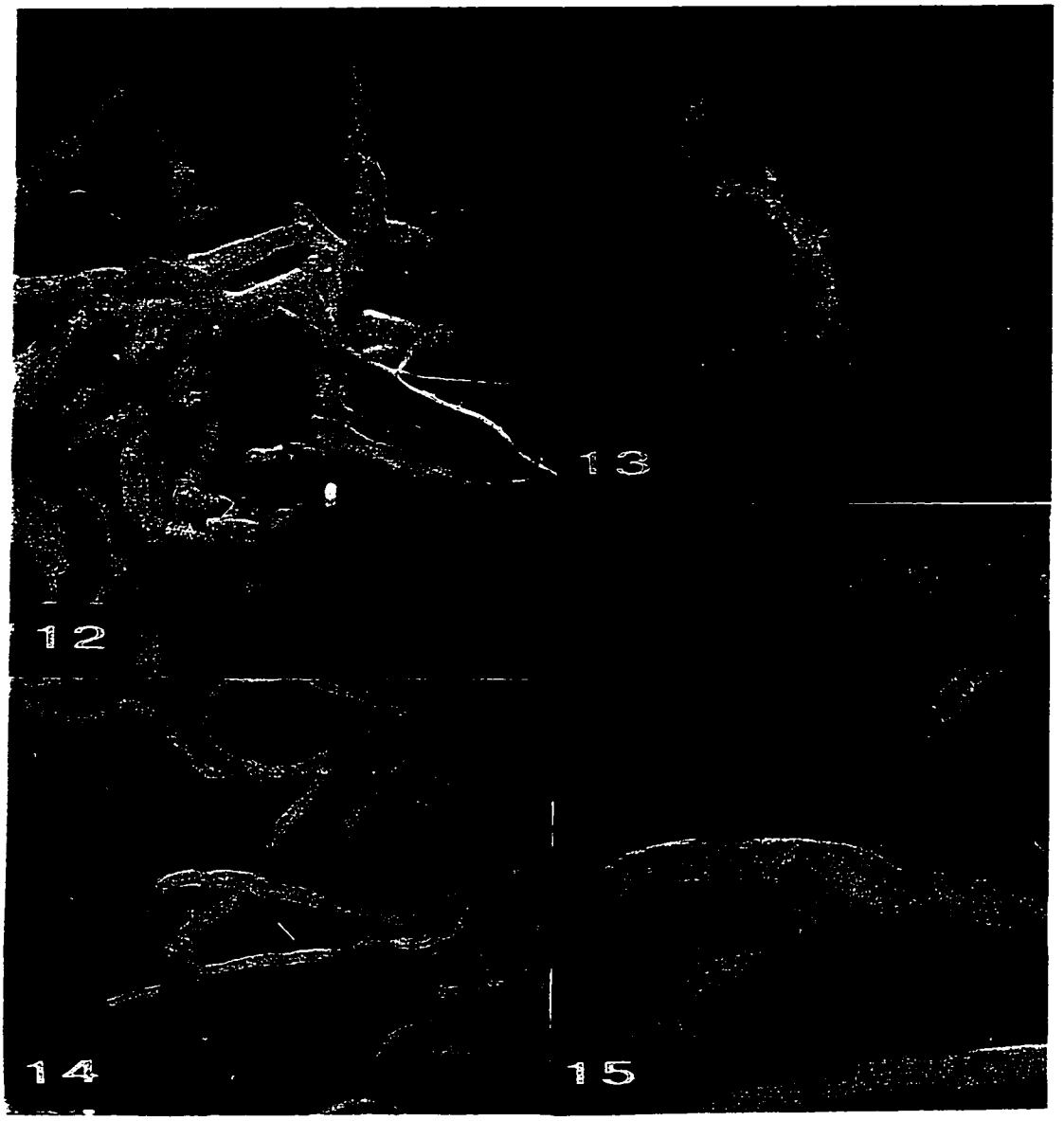

12
13
14
15

Figure 16. ECM system of morphotype M4 (mag X 320).

Figure 17. Lustrous, felty ECM tips of morphotype M4 (mag X 800).

Figure 18 - 19. Branched and unbranched tips of morphotype M5 (mag X 800).

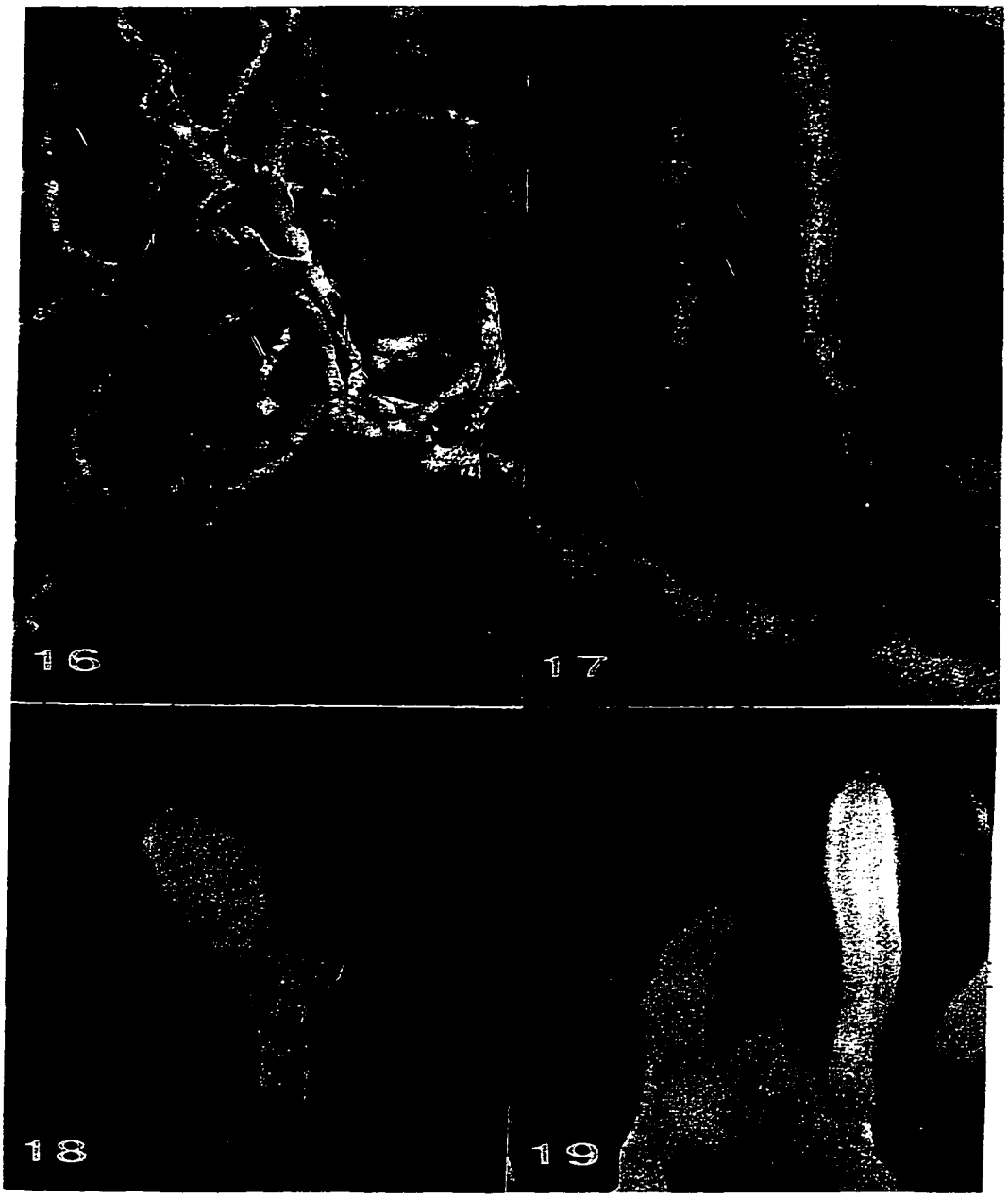

16
17
18
19

Figure 20. System of three tips of morphotype M6 (mag X 800).

Figure 21. Lustrous tip of morphotype M6 (mag X 800).

Figure 22. Ectomycorrhizal system of morphotype M7 (mag x 320).

Figure 23. Single tip of morphotype M7 (mag X 800).

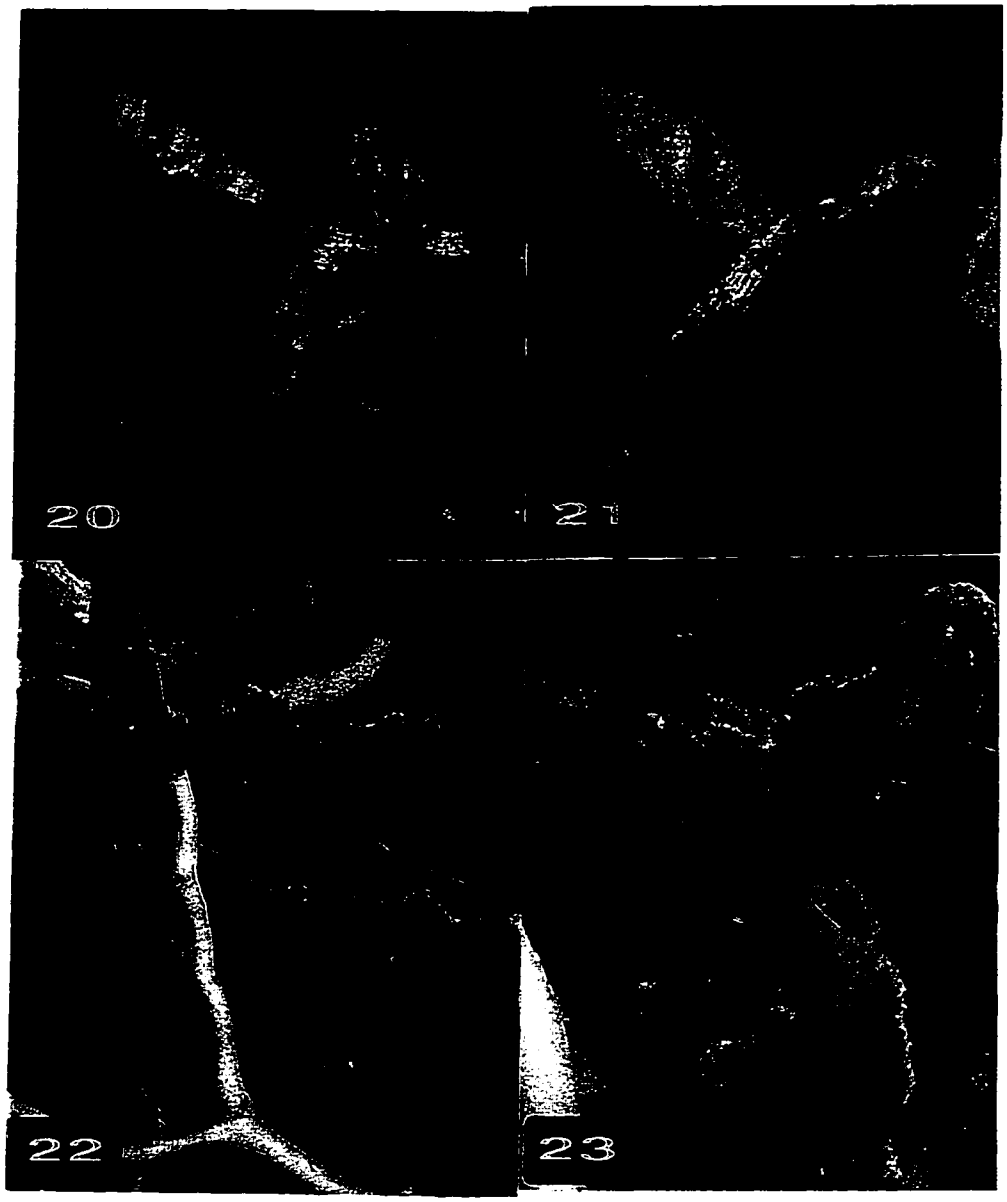

20
21
22
23

Figure 24 - 25. Morphotype M8 (mag X 320).

Figure 26. Morphotype M9, *Cenococcum sp.* (mag X 320).

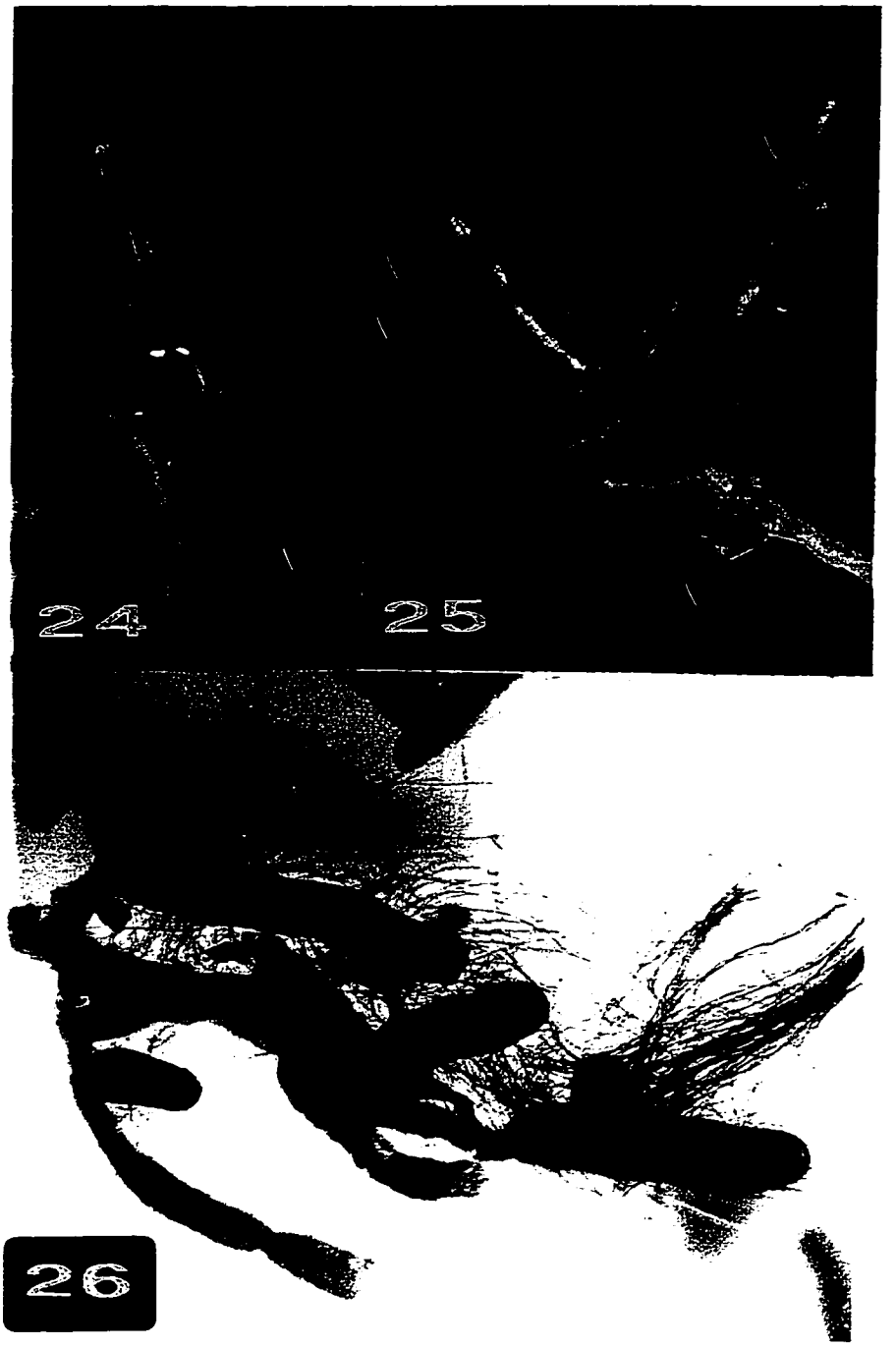

24
25
26

Figure 27. ECM system of morphotype M10 (mag X 320).

Figure 28. Younger ECM tips of morphotype M10 (mag X 800).

Figure 29. ECM system of morphotype M11 (mag X 320).

Figure 30. Lustrous ECM tip of morphotype M11 (mag X 800).

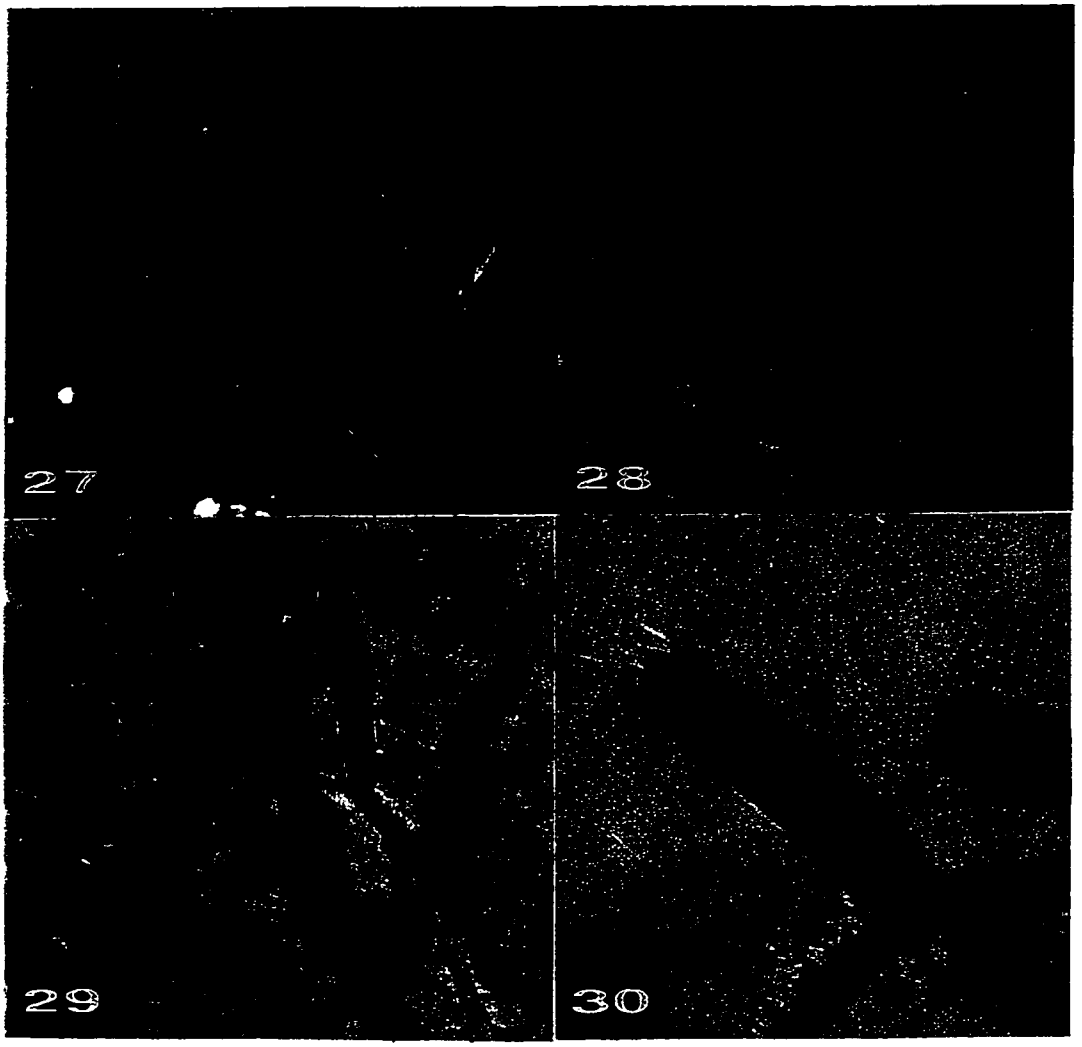

27
28
29
30

Table 5 . Brief description of the distinguishing morphological characters of some of the different mycorrhizal morphotypes of yellow birch.

#	Color, Lustre & Mantle Texture	Mantle Type☼	Branching Pattern	R*	CC**	differentiating features & comments
M1	orange-beige, smooth	outer: ns inner: i-is	monopodial pinnate	-	-	May have few short thin hyphae
M2	beige with white lustrous outer layer, felty	ns	monopodial pyramidal	+	-	
M3	white, cottony	ns	monopodial pinnate	-	-	
M4	orange lustrous, felty	ns	irregular	-	+	
M5	orange- beige, cottony	ns	monopodial pinnate	-	+	
M6	orange-beige, slightly lustrous, peach-fuzz texture	ns	not branched	-	-	has bottle-shaped, straight neck cystidia

Table 5. Continued

#	Color, Lustre & Mantle Texture	Mantle Type☼	Branching Pattern	R*	CC**	differentiating features & comments
M7	orange-brown	ns	monopodial pinnate	-	-	layer of hyphae around mantle, which held onto soil particles, giving it the "dirty" appearance
M8	bronze, shiny, smooth	ns	monopodial pyramidal	-	-	
M9	black, lustrous, with long stringy hyphal strands	rs	monopodial pinnate	-	-	*Cenococcum* sp.
M10	brown, a little shiny smooth	rs	monopodial pinnate	-	-	
M11	dark brown, lustrous smooth	outer: ns inner: i-is	monopodial pinnate	-	-	

Note: descriptive terminology used (except colour) taken from Goodman et al 1996.
*R= Rhizomorhs
**CC=Clamp connections
☼ : ns= net synenchyma
i-is= interlocking irregular synenchyma
rs= regular synenchyma

Table 6. ITS length and fragment lengths of each RFLP-type

Morpho-type	# DNA samples extracted	RFLP-Type(s)	# of samples/ RFLP type	uncut band size	Fragment lengths: Hinf I	Taq I	Mbo I
M1	47	1	2	680	2 X 320	310/210	400/200
		2	8	750	410/330	340/170	440/140
		3	11	750	490/210	420/290	380/330
		4	15	700	380/200	320/310	400/215
		5	2	800	400/380	320/310	220
		6	9	700	370/220	300/220	380/220
M2	14	1	10	700	260/220/120	180/140	350/215/120
		2	2	700	330/320	280	260/150
		3	2	700	450/260	320	260/150
M3	3	1	3	680	2 X 320	310/270	400/190
M4	3	1	3	800	450/290	320	260/145
M5	4	1	4	750	300/210/150	300/235	290/240
M6	4	1	4	720	380/360	360/330	450/210

Table 6. continued

Morpho-type	# DNA samples extracted	RFLP-Type(s)	# of samples/ RFLP type	uncut band size	Fragment lengths: Hinf I	Taq I	Mbo I
M8	2	1	2	720	400/300	215/165	280/215
M9	3	1	3	950	600/170	290/230/190/160	480/310
M10	3	1	3	750	380/210/150	360/320	260/230
M11	5	1	5	720	200/190/150	360/240	260/230/120

Figure 31. Gel figures showing the 6 different RFLP-types obtained when the amplified ITS region of the rDNA of morphotype M1 was digested with:

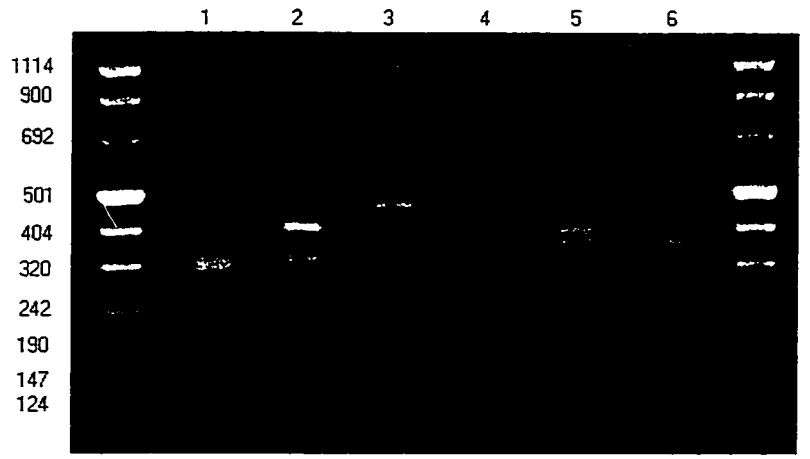
31 a. Hinf 1.

31 b. Mbo 1.

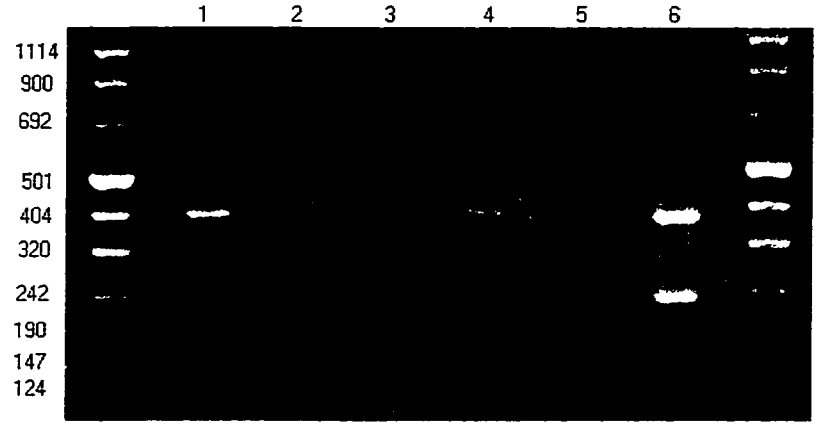
31 c. Taq 1.

In the first and last lane of each gel lies the molecular weight marker (bands are labelled in bp).

a


1 2 3 4 5 6
1114
900
692
501
404
320
242
190
147
124

b


1 2 3 4 5 6
1114
900
692
501
404
320
242
190
147
124

c

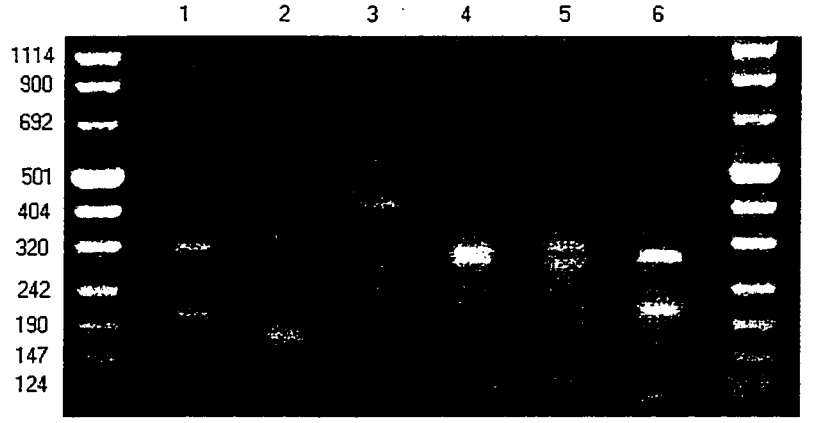

1 2 3 4 5 6
1114
900
692
501
404
320
242
190
147
124

Figure 32. Gel figures showing the 3 different RFLP-types obtained when the amplified ITS region of the rDNA of morphotype M2 was digested with:

32 a. Hinf 1.

32 b. Mbo 1.

32 c. Taq 1.

In the first and last lane of each gel lies the molecular weight marker (bands are labelled in bp).

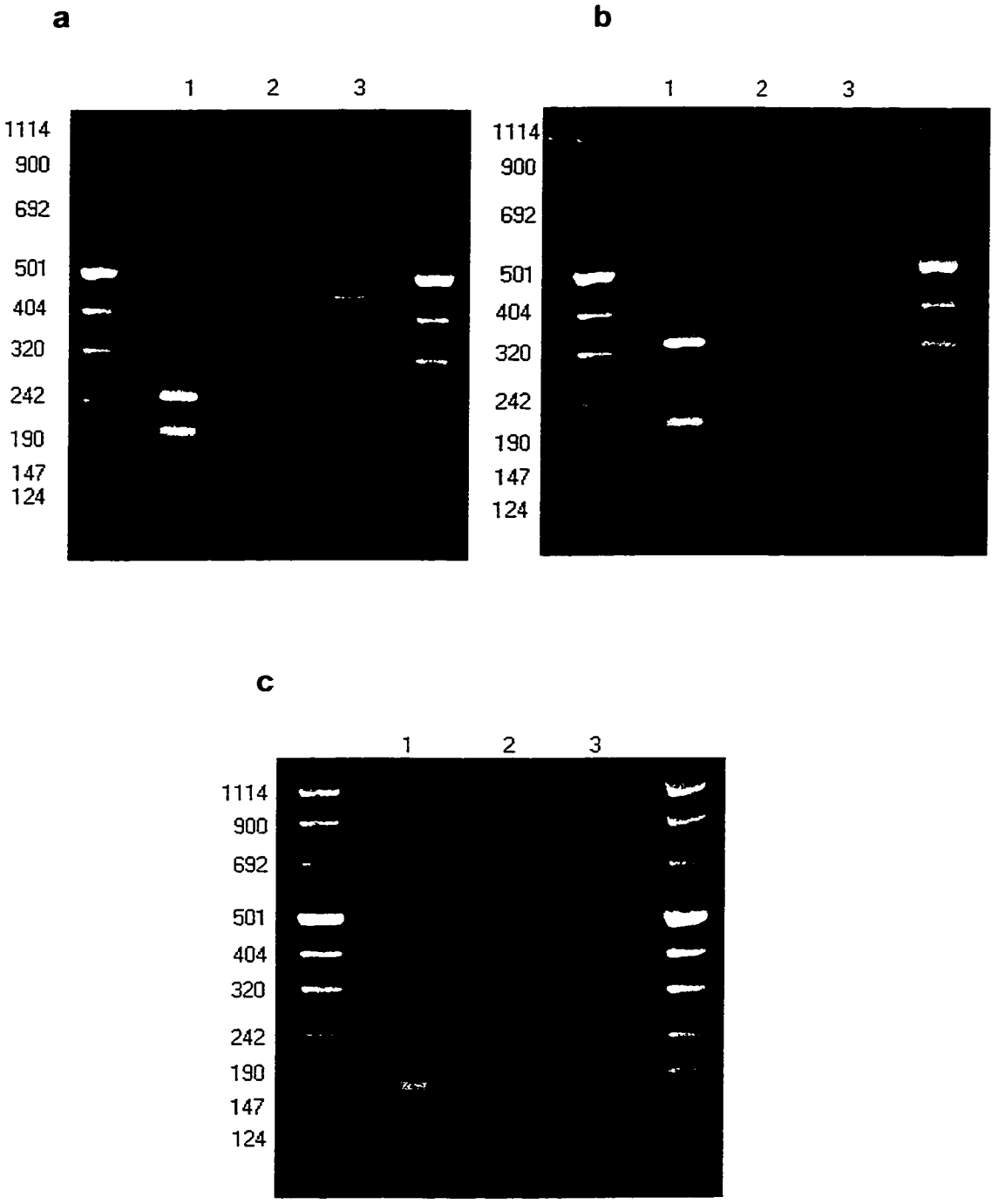

a
1
2
3
1114
900
692
501
404
320
242
190
147
124
b
1
2
3
1114
900
692
501
404
320
242
190
147
124
c
1
2
3
1114
900
692
501
404
320
242
190
147
124

Figure 33. RFLP analysis of the amplified ITS region of rDNA from morphotypes M3-M6, M8-M11 digested with:

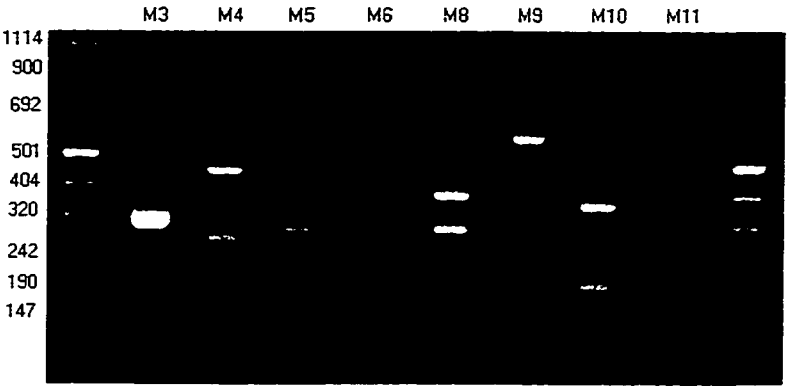
33 a. Hinf 1.

33 b. Mbo 1.

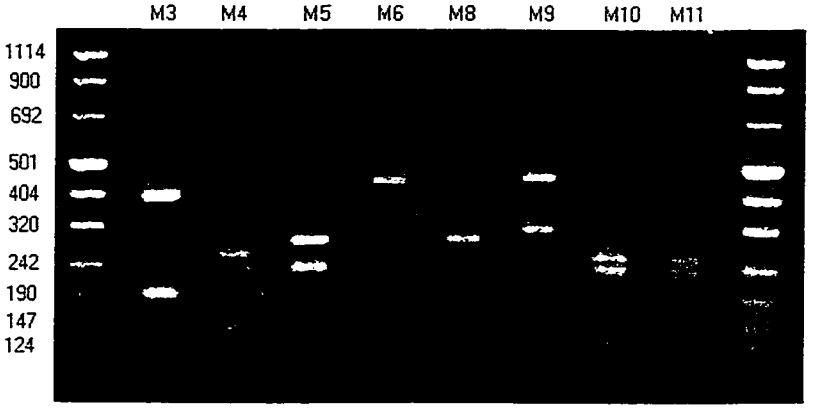
33 c. Taq 1.

In the first and last lane of each gel lies the molecular weight marker (bands are labelled in bp).

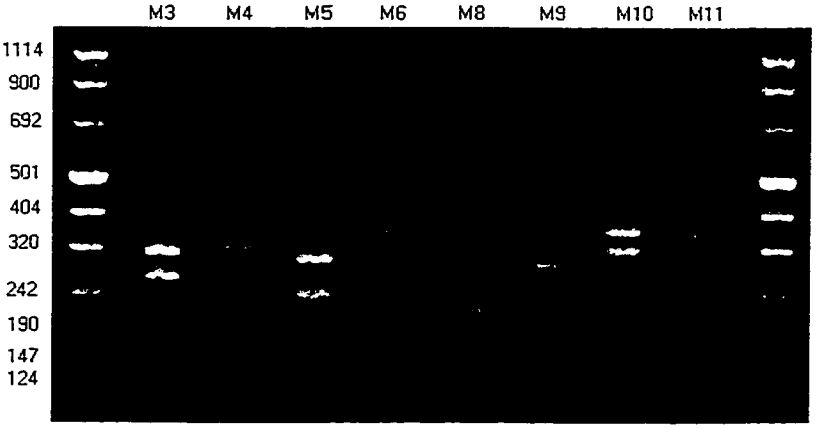
a


M3
M4
M5
M6
M8
M9
M10
M11
1114
900
692
501
404
320
242
190
147

b


M3
M4
M5
M6
M8
M9
M10
M11
1114
900
692
501
404
320
242
190
147
124

c


M3
M4
M5
M6
M8
M9
M10
M11
1114
900
692
501
404
320
242
190
147
124

Discussion

Selective cutting did not seem to negatively affect the mycorrhizal colonization in the seedlings collected in Duchesnay, nor in Rivière à Pierre. The high levels of colonization at both sites provide evidence that inoculum potential was not lost by the creation of the gaps. The ECM colonization in the 1-year-old seedlings collected in the 1000 m^2 gaps at Rivière à Pierre did not differ from that in the 1-year seedlings collected in the uncut areas. However, in the older saplings collected in Duchesnay, ECM colonization was higher in the saplings collected in the larger gaps than in those collected from the smaller sized gaps. Changes in colonization levels between seedlings collected in the gap and uncut areas were detected in the 1-2 year old maples, but no changes in colonization levels were detected in the 1-year old birch, this may suggest that the VAM fungi may be more sensitive to the changes created by selective cutting than the ECM fungi.

Percent ECM colonization of the birch seedlings at Rivière à Pierre was 86 to 99% in all treatments over the whole study period. The forest soil was not seriously disrupted following gap creation; thus the already established mycelial network most likely colonized the newly germinating seedlings. Various other factors may have also contributed to the maintenance of indigenous fungi. Yellow birch seedlings germinated within the gaps the following June, only 6 months after the cuts. This prompt regeneration of yellow birch as well as other ectomycorrhizal species such as beech, may be an important factor in sustaining the indigenous fungi. Maintaining native ECM inoculum is very important, for studies have shown that nursery seedlings colonized with easily culturable ECM fungi planted into cleared areas, do not perform as well as

seedlings colonized with native ECM fungi (Zhou et al. 1997). Also, stumps of mature beech and yellow birch trees that were cut the previous fall were left within the gaps and it has been stated that excised roots can remain viable for at least 9 months under field conditions (Ferrier and Alexander 1985). Their persistence may be dependent on the amount of carbohydrate reserves in the root tissue at the time of the cut. Root carbohydrate reserves show seasonal trends, with peaks in spring and fall (Ferrier and Alexander 1985) and in this study the gaps were created in the fall. Yellow birch sprouted from the stumps left in the gaps, hence there were enough carbohydrates to support the growth of lateral branches; these reserves may also contribute to the persistence of the ECM fungi. Similar findings were observed by Visser et al. (1998), where ECM colonization of aspen roots (which can form root sprouts) was 90-100% in both clear-cut and control areas and the maintenance of the ECM inoculum was attributed to the lack of severe forest soil disturbance, and the persistence of the mycorrhizal roots. Also, the mycorrhizal types colonizing the roots on the seedlings in the gaps were similar to those present on the roots of the seedlings collected from the uncut areas. Again our results resemble those of Visser et al (1998), who also observed no differences in the types of fungi on the aspen from the cut and uncut forest. However, our results do differ from those reported by Durall et al (1999), and Kranabetter and Wylie (1998), who report decreases in ECM fungal richness on the roots of logepole pine and western hemlock in the cut areas over those in the undisturbed forest. The size of the gaps in the Kranabetter and Wylie (1998) study ranged from ~2000-4400 m^2, but Durall et al (1999), found reductions in fungal richness in gaps of 900 - 5000 m^2. Neither lodgepole pine nor western hemlock can form root sprouts or lateral branches from stumps; thus, after a cut the roots are depleted of their

carbon stores and gradually die. However, the birch and aspen roots are quickly supported by the newly formed above ground parts, therefore remaining alive along with the ECM fungi. ECM morphotype diversity was slightly higher in the older seedlings collected in Duchesnay over the 1-yr old seedlings from Rivière à Pierre. The orange-smooth morphotype did not dominate the system in Duchesnay as much as it did in Rivière à Pierre, which resulted in the higher diversity. Results from other studies also show that older seedlings tend to host a more diverse flora of mycorrhizal species over younger seedlings (Dahlberg and Stenström 1991; Danielson and Pruden 1989).

In Duchesnay, higher ECM colonization levels were found in the birch seedlings collected in the larger gaps. Mycorrhizal development depends in part on photosynthate production (Hacskaylo 1973), the light regime of the seedlings collected from the larger gaps was significantly higher than those collected from the smaller gaps. This difference in the aboveground environment may explain the higher levels of ECM colonization in the larger gaps. The lime application did not affect the mycorrhizal colonization rates. Very little lime was added, and soil pH did not change enough to affect the ECM fungi.

The main phase of ECM activity is often thought to be at the end of the growing season rather than at the beginning (Smith and Read 1997). Jones et al. (1991), showed that carbon allocation to the belowground tissues in beech is highest at the end of a growing season. Photosynthate is probably not limiting later in the season when all leaves have emerged, also it is at this time that the main flush of root growth occurs in many tree species (Jones et al. 1991; Smith and Read 1997). The highest ECM colonization in Duchesnay was noted at the end of the season in the months of August

and September. By our last sampling date, the ECM fungi on the roots were beginning to senesce, thus root colonization levels decreased. In Rivière à Pierre however, ECM colonization rates were high throughout the whole season. The seedlings collected at this site were younger than those collected at Duchesnay. They had smaller root systems and thus were probably more likely to be fully colonized. It is very beneficial for a seedling to be highly colonized as it has been proven that seedlings with high ECM colonization early in the season can quickly capture nutrients, and are more likely to survive (Perry 1987). Mycorrhizal seedlings also have an added advantage as they are colonized by the already established underground fungal network. Many forest trees are connected by the underground fungal network. It has been suggested that subordinate plants such as seedlings can increase their nutrient uptake by the ECM fungi, but don't have a high carbon drain because the older, larger trees that are also connected by the same mycelium supply most of the carbon to the fungus (Newman 1988; Simard et al. 1997).

The birch seedlings at both sites were highly colonized and the short roots were dominated by a single morphotype. Dominance of one or a few fungal morphoytpes within a specific area is a common feature in ECM community structure. Examination of the ECM community in a 40-year-old Sitka spruce forest in Europe, revealed that one dominant morphotype colonized the roots of over 70% of the roots analyzed (Taylor and Alexander 1989). In another study of the ECM community of mature Pinyon pines in northern Arizona, 15-19 different morphotypes were found at the various sites, but at each site only one or a few types were abundant (Gehring et al. 1998). Goodman and Trofymow (1998) looked at the ECM fungi from an old growth and mature stand of Douglas fir in southern Vancouver. In this study 69 different types were found but only 9

types colonized over 70% of all root tips. Twelve different morphotypes were found on the roots of Douglas fir in disturbed and undisturbed forests in west central Oregon, and of these 12 types, only 2 types colonized more than 2/3 of all root tips (Pilz and Perry 1984). A study on aspen regeneration in a clear-cut in Alberta, also showed that a total of 22 different types were found but the ECM community was dominated by 4 different types (Visser et al 1998). In my study a total of 16 different morphotypes were observed with a single type dominating. Reasons for this dominance may be that in all studies mentioned, no severely damaging disturbance has occurred in these forest soils to physically disrupt the underground mycelium, thus a few morphoytpes may have become well adapted in an environment that has remained relatively stable for long periods of time. Another reason for this high degree of dominance, may be a result of the ability of the fungi to expand through the soil and spread over large areas. Because of this, colonies of the same species can overlap and their mycelia anastomise, thus a single species can cover a large area. These already established fungi also have the added advantage when competing for sites on new roots because they are already receiving carbon from their host plant (Brundrett 1991; Dahlberg and Stedid 1995). In such forests invasion of new fungal types may be difficult.

The majority of the ectomycorrhizal root tips analyzed in this study were colonized by a smooth orange-beige morphotype (M1) that neither formed rhizomorphs nor supported an extensive array of external hyphae. A fungus with an extensive external mycelium may be costly for a seedling to support, thus, a smooth type may be the cost efficient alternative. Roots of the seedlings were present in the top few centimeters of the forest soil, close to litter accumulation and nutrients. Vast amounts of external hyphae may not be required to efficiently capture soil nutrients, rather the

seedlings may benefit by supporting a smooth type that has the ability to exude enzymes which can break down, release and absorb nutrients from the surrounding organic materials. ECM fungi not only aid in the uptake of nutrients but can also store them in the mantle for later use. Studies have shown that smooth ECM types on pine and beech roots can produce large quantities of surface bound phosphatases which can hydrolyse organic and inorganic phosphates and release orthophosphate which is absorbed and stored in the fungal sheath (Read 1984). Absorption rates of the mycorrhizal plants were 5 times greater than in the non-mycorrhizal plants (Read 1984). The seedlings in this study may benefit from their association with the smooth orange-beige type by increasing their nutrient uptake and storage abilities.

Based in morphological assessments the ECM community seemed to be dominated by a single species. This result must be interpreted with caution since that single morphotype consisted of 6 different RFLP-types. In a study such as this one where thousands of root tips were quantified in a single day, a very detailed examination of every single root tip was not possible. Basing the identification of different fungal types on a few key morphological characters may not be an efficient method of identifying a fungus to species level. From recently published material, it seems very possible that fungi that are morphologically similar may in fact be different species. In a study of the ECM community of spruce stands treated with different levels of lime, 60-80% of the roots were colonized by a smooth brown morphotype that consisted of several different RFLP-types (Jonsson et al. 1999c). In a study of the ECM species composition in seedlings and mature Scots pines in boreal forests in northern Sweden, 20 different fungal morphotypes were found, while 43 different ECM taxa were

distinguished using molecular methods (Jonsson et al. 1999a). In another study of ECM fungal diversity in a spruce stand in Switzerland, 18 different fungal types were differentiated using morphological features while characterization with molecular tools resulted in 23 different RFLP-types (Mehmann et al. 1998). From these studies it seems very likely that several fungal species can look very much alike. Past studies on ECM fungal richness based on morphotyping may underestimate species numbers. For example in a study of the ECM fungal community of Scots pines in northern Sweden, 135 different ECM taxa were distinguished using ITS-RFLP analysis, quite a large number of different types compared to most studies based on morphotyping (Jonsson et al. 1999b). Accordingly, in this study, changes in ECM species composition following gap creation may have been overlooked. Relatively few tips were successfully amplified for RFLP analysis, thus, fungal richness within these study sites may have been underestimated. Species richness in the above-mentioned studies have all been based on RFLP analysis rather than actual sequence data. Differences in RFLP patterns may not necessarily define a new species, but results from other published studies (Gardes et al. 1991; Kårén et al. 1997), do indicate that intraspecific variation of the ITS region of ECM fungi is very low. Therefore, this method does seem to be a fairly quick and practical method for the identification of ECM fungi.

This study is a first in a continuing series of projects that will be conducted at these two study sites. Currently, an extensive mushroom survey of the area is being conducted and ECM fungi colonizing the mature yellow birch trees are being collected. The ECM tips and mushrooms are will be processed for DNA analysis. Once this work is complete, there will be some reference material to compare these RFLP-types to, which may allow further identification. According to a crude identification based on

comparisons with previously published photographs of known ECM types, the dominant morphotype seems to be *Russula* sp. (Goodman et al 1998).

The VAM colonization rates in the sugar maples collected at both sites also were not reduced by the cuts. VAM fungal preservation in the gaps may also be a result of the relatively minor level of soil disturbance coupled with quick regeneration and the presence of many VAM hosts. Sugar maples, striped maples, and red maples were all present in the gaps the following spring, along with many herbaceous VAM plants. Arbuscule levels were significantly higher in the maples collected in the large gaps in Duchesnay and in the 1000 m^2 gaps in Rivière à Pierre, over the maples collected in the small gaps or control areas. High arbuscule numbers coupled with low vesicle numbers is a sign of a healthy mycorrhizal association. Many other studies have reported a decrease in arbuscule levels with an increase in vesicle numbers in sugar maples that are in a state of decline (Cooke et al. 1992; Costanzo 1999; Duckmanton and Widden 1994). Cooke et al. (1992), and Kliromomos (1995), looked at the VAM colonization in sugar maples growing in acidic soils and observed levels of vesicular colonization between 15 to over 30%. In the whole of this study, % vesicular colonization never rose above 10%, while arbuscule colonization rates peaked at 60% in Duchesnay and 23% in Rivière à Pierre.

Information on VAM fungal colonization in maples from natural environments is scarce. Brundrett and Kendrick (1988) looked at total VAM colonization rates of sugar maples from a forest in southwestern Ontario, and colonization rates peaked in September in both seedling and mature sugar maples. Klironomos (1995), examined the roots of mature maples from forests in southern Ontario, and total colonization along

with arbuscular, vesicular, and coil colonization levels were observed. Total colonization levels varied from 30-70%, while arbuscular levels peaked at ~17%. Arbuscular levels in our study were higher than those reported by Klironomos (1985), but similar to those reported by Costanzo (1999). Costanzo (1999), examined the effects of various stressors on the mycorrhizae of 2-yr-old sugar maple seedlings growing in pots with forest soil. In the control (non-stressed, healthy) seedlings, average arbuscular levels for the month of September were ~60% and vesicular levels were about 5%.

At the end of the sampling season (September and October), colonization levels in Duchesnay were much higher than those observed at Rivière à Pierre. VAM colonization levels can be affected by environmental conditions (Cooke et al. 1992; Duke et al. 1994, Klironomos 1995), thus differences in colonization rates may be partially attributed to the environmental differences between the two sites. Also the seedlings collected at Rivière à Pierre were much younger than those collected at Duchesnay, thus differences in tree age may also contribute to differences in colonization.

In both sites, VAM colonization increased as light levels increased. It is well documented that the development of VAM is influenced by the amount of light received by the host plant (Bethlenfalvay and Pacovsky 1983; Furlan and Fortin 1977; Gerdeman 1968; Hayman 1974; Nemec 1987; Pearson et al. 1991; Son and Smith 1988). Colonization rates are lower in low light conditions and increase in high light conditions. These effects are generally attributed to a plant's photosynthetic abilities and carbon stores. Not only did we see increases in total VAM colonization rates in the high light

plots, but the number of arbuscules also increased. This phenomenon has also been observed in other studies (Franken and Gnädinger 1994; Pearson et al. 1991; Hayman 1974). Since arbuscules are the site of nutrient transfer, seedlings collected in the gaps are probably benefitting more from the VAM fungi by an increase in nutrient uptake. As with the ECM fungi, VAM colonization rates for the seedlings growing in the limed plots did not differ from those in the untreated areas.

VAM colonization in sugar maples growing in natural ecosystems can fluctuate widely over a single growing season, and colonization can also vary from one season to the next (Brundrett and Kendrick 1988; Cooke et al. 1992). Exact causes for these shifts are still uncertain, but according to the results of recent studies (Brundrett and Kendrick 1988; Klironomos 1995; Mullen and Shmidt 1993; Sanders and Fitter 1992a), environmental conditions and plant growth patterns appear to play important roles. In Duchesnay, a slight increase in arbuscular colonization is noted in June. At this time maple seedlings start to leaf out, and nutrient demand may increase. VAM colonization decreases in July and August, and peaks in September and October. At the end of the season soil nutrient availability increases hence increased colonization rates may benefit the plant at this point in the season. Supporting high levels of fungal colonization only at times of increased nutrient demand, or when soil nutrient levels increase may be an advantageous strategy for the plant. Having high colonization levels all year long may become carbon draining (Sanders and Fitter 1992b). In Rivière à Pierre not much can be said on the seasonal fluctuation of the VAM fungi, as sampling occurred only 3 times. However, a steady increase in colonization levels was observed as the season progressed. These findings are similar to those of Brundrett and Kendrick (1988), who

also report a steady increase in root colonization levels in sugar maple seedlings as the season progressed.

The higher arbuscular colonization levels in the sugar maples growing in large gaps over those collected in small gaps or uncut areas, suggests that they may have a more beneficial mycorrhizal system (Klironomos and Allen 1995). The selective cutting technique does encourage the growth of yellow birch seedlings in the initial stages after gap creation but, as years go by, the maples may always be at a competitive advantage. A possible reason for this may be due to their mycorrhizal association that can adapt to the differing light levels, which will allow them to efficiently capture nutrients from the soil. This will help the maples grow to maturity, and they will eventually begin to shade out the surrounding vegetation including the yellow birch. Sugar maples are shade tolerant, thus, they can outcompete the yellow birch as the canopy becomes denser. A way to ensure longer life spans for the yellow birch may be to selectively cut away some of the sugar maples when light levels become too low for the birch. Other possible methods to encourage the yellow birch may be in creating larger sized gaps which will delay canopy closure, providing a favorable light environment for the birch for a longer period of time. Another method may be to remove the maple stumps at the time of the cut. This will eliminate the formation of maple sprouts, again slowing down canopy closure, and will eliminate their shading of the surrounding yellow birch seedlings.

CONCLUSIONS

Selective cutting has no negative effect on mycorrhizal community structure of yellow birch and sugar maple. Reasons for this may include quick regeneration of the mycorrhizal hosts coupled with minor levels of soil disruption and relatively small gap size.

Although the VAM fungi seem to be more flexible and quickly adapted to environmental changes, much more data is needed to draw any firm conclusions. Differences in colonization between gap and control areas were observed in the maples but not in the birch seedlings collected at Rivière à Pierre. Types of ECM fungi colonizing the roots of birch seedlings from the gaps did not differ from those in the uncut forest areas. Overall, both VAM and ECM fungal colonization rates increased with increasing light levels.

Another important point emphasized by these results is that in order to achieve a reliable assessment of ECM community structure, either a very detailed morphotyping technique should be used or the DNA from many samples of ECM tips should be characterized.

REFERENCES

Abbott, L.K. and Robson, A.D. 1991. Factors influencing the occurrence of vesicular-arbuscular mycorrhizas. Agriculture, Ecosystems and Environment 35:121-150.

Allen, E.B. and Allen, M.F. 1990. The mediation of competition by mycorrhizae in successional and patchy environments. *In*: Perspectives on Plant Competition. J.B. Grace and D. Tilman (eds.), Academic Press Inc., Harcourt Brace Jovanovich - Publishers. San Diego, California. pp.365-389.

Allen, M.F. 1996. The ecology of arbuscular mycorrhizas: a look back into the 20th century and a peek into the 21st. Mycological Research 100(7): 769-782.

Anderson, S. and Söderström, B. 1995. Effect of lime ($CaCO_3$) on ectomycorrhizal colonization of *Picea abies* (L) Karst. seedlings planted in a spruce forest. Scandinavian Journal of Forest Research 10: 149-154.

Arnebrant, K. and Söderström, B. 1992. Effects of different fertilizer treatments on ectomycorrhizal colonization potential in two Scots pine forests in Sweden. Forest Ecology and Management 53: 77-89.

Begon, M., Harper, J.L. and Townsend, C.R. 1996. Ecology- 3rd Edition. Blackwell Science Ltd., Oxford.

Bethlenfalvay, G.J. and Pacovsky, R.S. 1983. Light effects in mycorrhizal soybeans. Plant Physiology 73: 969-972.

Björkman, E. 1970. Forest Tree Mycorrhizas - The conditions for its formation and the significance for tree growth and afforestation. Plant and Soil 32: 589-610.

Bonfante-Fasolo, P. 1986. Anatomy and morphology of VA mycorrhizae. *In:* VA mycorrhizae. C. Powell and D.J. Bagyaraj (eds). CRC Press. Inc., Boca Rontan, Florida, pp. 5-33.

Brundrett, M. 1991. Mycorrhizas in natural ecosystems. Advances in Ecological Research 21: 171-313.

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

Cline, M.L., France, R.C. and Reid, C.P.P. 1987. Intraspecific and interspecific growth variation of ectomycorrhizal fungi at different temperatures. Canadian Journal of Botany 65: 869-875.

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

Clapp, J.P., Fitter, A.H. and Young, J.P.W. 1999. Ribosomal small subunit sequence variation within spores of an arbuscular mycorrhizal fungus, *Scutellospora* sp. Molecular Ecology 8: 915-921.

Cooke, M.A., Widden, P. and O'Halloran, I. 1992. Morphology, incidence and fertilization effects on the vesicular-arbuscular mycorrhizae of *Acer saccharum* in a Québec hardwood forest. Mycologia 84(3): 422-430.

Costanzo, N. 1999. The effects of stress on biomass, soluble sugar concentrations and VA mycorrhizal colonization in sugar maple seedlings (*Acer saccharum* Marsh.). MSc Thesis, Concordia University, Montreal, Canada.

Daft, M.J. and El-Giahmi, A.A. 1977. Effect of arbuscular mycorrhiza on plant growth. New Phytologist 80: 365-372.

Dahlberg, A. and Stenström, E. 1991. Dynamic changes in nursery and indigenous mycorrhiza of *Pinus sylvestris* seedlings planted out in forest and clearcuts. Plant and Soil 136: 73-86.

Dahlberg, A. and Stendid, J. 1995. Spatiotemporal patterns in ectomycorrhizal populations. Canadian Journal of Botany 73(Suppl. 1):S1222-S1230.

Dahlberg, A., Jonsson, L. and Nylund, J-E. 1997. Species diversity and distribution of biomass above and below ground among ectomycorrhizal fungi in an old-growth Norway spruce forest in south Sweden. Canadian Journal of Botany 75: 1323-1335.

Danielson, R.M. 1984. Ectomycorrhizal associations in jack pine stands in northeastern Alberta. Canadian Journal of Botany 62: 932-939.

Danielson, R.M. and Pruden, M. 1989. The ectomycorrhizal status of urban spruce. Mycologia 81 (3): 335-341.

Davies, F.T. and Call, C.A. 1990. Mycorrhizae, survival and growth of selected woody plant species in lignite overburden in Texas. Agriculture, Ecosystems and Environment 31: 243-252.

Dighton, J. and Skeffinton, R.A. 1987. Effects of artificial acid precipitation on the mycorrhizas of scots pine seedlings. New Phytologist 107: 191-202.

Duckmanton, L. and Widden, P. 1994. Effect of ozone on the development of vesicular-arbuscular mycorrhizae in sugar maple seedlings. Mycologia 86: 181-186.

Duke, S.E., Jackson, R.B. and Caldwell, M.M. 1994. Local reduction of mycorrhizal arbuscule frequency in enriched soil microsites. Canadian Journal of Botany 72:998-1001.

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: 1322-1332.

Eissenstat, D.M. and Newman, E.I. 1990. Seedling establishment near large plants: effects of vesicular-arbuscular mycorrhizas on the intensity of plant competition. Functional Ecology 4: 95-99.

Erdmann, G. G. 1990. *Betula alleghaniensis* Britton yellow birch. Silvics of North America, Department of Agriculture, Washington, DC. Volume 2 : 133-147.

Erland, S. and Söderström, B. 1991. Effects of lime and ash treatments on ectomycorrhizal infection of *Pinus sylvestris* L. seedlings planted in a pine forest. Scandinavian Journal of Forest Research 6: 519-525.

Ferrier, R.C. and Alexander, I.J. 1985. Persistence under field conditions of excised fine roots and mycorrhizas of spruce. In: Ecological Interactions in Soil. A.H. Fitter, D. Atkinson, D.J. Read, and Usher, M.B. (eds.), Blackwell, London, pp.175-179.

Forbes, P.J., Ellison, C.H. and Hooker, J.E. 1996. The impact of arbuscular mycorrhizal fungi and temperature on root system development. Agronomie 16: 617-620.

Franken, P. and Gnädinger, F. 1994. Analysis of parsley arbuscular endomycorrhizas: infection development and mRNA levels of defense-related genes. Molecular Plant-Microbe Interactions 7(5): 612-620.

Furlan, V. and Fortin, J.A. 1977. Effect of light intensity on the formation of vesicular-arbuscular endomycorrhizas on *Allium cepa* by *Gigaspora calospora*. New Phytologist 79: 335-340.

Gardes, M., White, T.J., Fortin, J.A., Bruns, T.D. and Taylor, J.W. 1991. Identification of indigenous and introduced symbiotic fungi in ectomycorrhizae by amplification of nuclear and mitochondrial ribosomal DNA. Canadian Journal of Botany 69: 180-190.

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: 113-118.

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

Gardes, M. and Bruns, T.D. 1996b. ITS-RFLP matching for identification of fungi. *In*: Species Diagnostic Protocols: PCR and Other Nucleic Acid Methos. J.P. Clapp (ed.). Humana Press, Totpwa, New Jersey, pp.177-192.

Gehring, C.A., Theimer, T.C., Whitham, T.G. and Keim, P. 1998. Ectomycorrhizal fungal community structure of pinyon pines growing in two environmental extremes. Ecology 79(5): 1562-1572.

Gerdemann, J.W. 1968. Vesicular-arbuscular mycorrhiza and plant growth. Annual Review of Phytopathology 79: 397-418.

Godbold, D.L. and Berntson, G.M. 1997. Elevated atmospheric CO_2 concentration changes ectomycorrhizal morphotype assemblages in *Betula papyrifera*. Tree Physiology 17: 347-350.

Godmann, R., M., Yawney, H., W. and Tubbs, C., H. 1990. *Acer saccharum* Marsh. sugar maple. Silvics of North America, Department of Agriculture, Washington, DC. Volume 2 : 78-91.

Goodman, D.M., Durall, D.M., Trofymow, J.A. and Berch, S.M. 1998. A Manual of Concise Descriptions of North American Ectomycorrhizae. Mycologue Publications, B.C.

Goodman, D.M. and Trofymow, J.A. 1998. Comparisons of communities of ectomycorrhizal fungi in old-growth and mature stands of Douglas-fir at two sites on southern Vancouver Island. Canadian Journal of Forest Research 28: 574-581.

Grey, W.E. 1991. Influence of temperature on colonization of spring barleys by vesicular arbuscular mycorrhizal fungi. Plant and Soil 137: 181-190.

Hacskaylo, E. 1973. Carbohydrate physiology. *In:* Ectomycorrhizae - Thier Ecology and Physiology. G.C. Marks and T.T. Kozlowski (eds.), Academic Press, New York, pp.207-230.

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

Harvey, A.E., Jurgensen, M.F. and Larsen, M.J. 1980a. Clearcut harvesting and ectomycorrhizae: survival of activity on residual roots and influence on a bordering forest stand in western Montana. Canadian Journal of Forest Research 10: 300-303.

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

Hausenbuiller, R.L. 1985. Soil Science - Principles & Practices. 3rd Edition. Wm. C. Brown Publishers. Dubuque, Iowa. pp.610.

Haselwanter, K. and Bowen, G.D. 1996. Mycorrhizal relations in tree for agroforestry and land rehabilitation. Forest Ecology and Management 81: 1-17.

Hayman, D.S. 1974. Plant growth responses to vesicular-arbuscular mycorrhiza. New Phytologist 73: 71-80.

Horton, T.R., Cázares, E. and Bruns, T.D. 1998. Ectomycorrhizal, vesicular-arbuscular and dark septate fungal colonization of bishop pine (*Pinus muricata*) seedlings in the first 5 months of growth after wildfire. Mycorrhiza 8: 11-18.

Jansen, A.E. and De Nie, H.W. 1988. Relations between mycorrhizas and fruitbodies of mycorrhizal fungi in Douglas fir plantations in The Netherlands. Acta Bot. Neerl. 37(2):243-249.

Jones, M.D., Durall, D.M. and Tinker, P.B. 1991. Fluxes of carbon and phosphorus in willow ectomycorrhizas and their changes with time. New Phytologist 119: 99-106.

Jonsson, L., Dahlberg, A., Nilson, M-C., Kårén, O. ans Zackrisson, O. 1999a. Continuity of ectomycorrhizal fungi in self-regenerating boreal *Pinus sylvestris* forests studied by comparing mycobiont diversity on seedlings and mature trees. New Phytologist 142: 151-162.

Jonsson, L., Dahlberg, A., Nilsson, M-C., Zackrisson, O. and Kårén, O. 1999b. Ectomycorrhizal fungal communities in late-successional Swedish boreal forests, and their composition following wildfire. Molecular Ecology 8: 205-215.

Jonsson, T., Kokalj, S., Finlay, R. and Erland, S. 1999c. Ectomycorrhizal community structure in a limed spruce forest. Mycological Research 103(4): 501-508.

Kabir, Z., O'Halloran, I.P. and Hamel, C. 1997. Overwinter survival of arbuscular mycorrhizal hyphae is favored by attachment to roots but diminished by disturbance. Mycorrhiza 7:197-200.

Kårén, O. and Nylun, J-E. 1996. Effects of N-free fertilization on ectomycorrhiza community structure in Norway spruce stands in Southern Sweden. Plant and Soil 181: 295-305.

Kårén, O., Högberg, N., Dahlberg, A., Jonsson, L. and Nylund, J-E. 1997. Inter- and intraspecific variation in the ITS region of rDNA of ectomycorrhizal fungi in Fennoscandia as detected by endonuclease analysis. New Phytologist 136: 313-325.

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

Klironomos, J.N. and Allen, M.F. 1995. UV-B-mediated changes on below ground communities associated with the roots of *Acer saccharum*. Functional Ecology 9: 923-930.

Kranabetter, J.M. and Wylie, T. 1998. Ectomycorrhizal community structure across forest openings on naturally regenerated western hemlock seedlings. Canadian Journal of Botany 76: 189-196.

Majcen, Z. and Richard, Y. 1992. Résultats après 5 ans d'un essai de coupe de jardinage dans une érablière. Canadian Journal of Forest Research 22: 1623-1629.

Marshner, H. and Dell, B. 1994. Nutrient uptake in mycorrhizal symbiosis. Plant and Soil 159: 89-102.

Marx, D.H., Hatch, A.B. and Mendicino, J.F. 1977. High fertility decreases sucrose content and susceptibility of loblolly pine to ectomycorrhizal infection by *Pisolithus tinctorius*. Canadian Journal of Botany 55: 1569-1574.

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

Mehmann, B., Egli, S., Braus, G.H. and Brunner, I. 1995. Coincidence between molecularly or morphologically classified ectomycorrhizal morphotypes and fruitbodies in a spruce forest. *In*: Biotechnology of Ectomycorrhizae: Molecular Approaches. V.Stocchi, P. Bonfante and M. Nuti (eds). Plenum Press New York, pp. 41-52.

Meier, S. 1991. Quality versus Quantity: optimizing evaluation of ectomycorrhizae for plants under stress. Environmental Pollution 73: 205-216.

Messier, C., Parent, S. and Bergeron, Y. 1998. Effects of overstory and understory vegetation on the understory light environment in mixed boreal forests. Journal of Vegetation Science 9:511-520.

Molina, R., Massicotte, h. and Trappe, J.M. 1992. Specificity phenomena in mycorrhizal symbiosis: community-ecological consequences and practical implications. In: Mycorrhizal Functioning - an Integrated Plant-Fungal process. M.F. Allen (ed.), Chapman and Hall, New York, pp. 357-423.

Mullen, R.B. and Schmidt, S.K. 1993. Mycorrhizal infection, phosphorus uptake, and phenology in *Ranunculus adoneus*: implications for the functioning of mycorrhizae in alpine systems. Oecologia 94:229-234.

Nemec, S. 1987. VA mycorrhizae in horticultural systems. *In:* Ecophysiology of VA mycorrhizal Plants. G.R. Safir (ed.), CRC Press, Florida.

Newman, E.I. 1988. Mycorrhizal Links Between Plants: Their Functioning and Ecological Significance. Advances in Ecological Research 18: 243-270.

Newsham, K.K., Fitter, A.H. and Watkinson, A.R. 1995. Multi-functionality and biodiversity in arbuscular mycorrhizas. Trends in Ecology and Evolution 10: 407-411.

Newton, A.C. and Pigott, C.D. 1991. Mineral nutrition and mycorrhizal infection of seedling oak and birch - II. The effects of fertilizers on growth, nutrient uptake and ectomycorrhizal infection. New Phytologist 117: 45-52.

Norusis, M.J. 1997. SPSS® 7.5 - Guide to Data Analysis. Prentice Hall Inc., New Jersey.

O'Dell, T.E., Castellano, M.A. and Trappe, J.M. 1993. Biology and Application of Ectomycorrhizal Fungi. *In*: Soil Mircobial Ecology- Applications in Agriculture and Environmental Management. pp.379-416.

Ozinga, W.A., Van Andel, J. and McDonnell-Alexander, M.P. 1997. Nutritional soil heterogeneity and mycorrhiza as determinants of plant species diversity. Acta Bot. Neerl. 46(3): 237-254.

Parke, J. L., Linderman, R.G. and Trappe, J.M. 1983. Effect of root zone temperature on ectomycorrhiza and vesicular-arbuscular mycorrhiza formation in disturbed and undisturbed forest soils of southwest Oregon. Canadian Journal of Forest Research 13: 657-665.

Parke, J.L., Linderman, R.G. and Trappe, J.M. 1984. Inoculum potential of ectomycorrhizal fungi in forest soil from southwest Oregon and northern California. Forest Science 30:300-304.

Parsons, W.F.J., Miller, S.L. and Knight, D.H. 1994. Root-gap dynamics in a lodgepole pine forest: ectomycorrhizal and nonmycorrhizal fine root activity after experimental gap formations. Canadian Journal of Fprest Research 24: 1531-1538.

Pearson, J.N., Smith, S.E. and Smith, F.A. 1991. Effect of photon irradiance on the development and activity of VA mycorrhizal infection in *Allium porrum*. Mycological Research 95(6): 741-746.

Perry, D.A., Molina, R. and Amaranthus, M.P. 1987. Mycorrhizae, mycorrhizospheres, and reforestation: current knowledge and research needs. Canadian Journal of Forest Research 17: 929-940.

Perry, D.A., Amaranthus, M.P., Borchers, J.G., Borchers, S.L. and Brainerd, R.E. 1989. Bootstrapping in Ecosystems. BioScience 39:230-237.

Pilz, D.P. and Perry, D.A. 1984. Impact of clearcutting and slash burning on ectomycorrhizal associations of Douglas-fir seedlings. Canadian Journal of Forest Research 14: 94-100.

Qui, Z., Chappelka, A.H., Somers, G.L., Lockaby, B.G. and Meldahl, R.S. 1993. Effects of ozone and simulated acidic precipitation on ectomycorrhizal formation on loblolly pine seedlings. Environmental and Experimental Botany 33(3): 423-431.

Raju, P.S., Clark, R.B., Ellis, J.R. and Maranville, J.W. 1990. Effects of species of VA-mycorrhizal fungi on growth and mineral uptake of sorghum at different temperatures. Plant and Soil 121: 165-170.

Read, D.J. and Birch, C.P.D. 1988. The effects and implications of disturbance of mycorrhizal mycelial systems. Proceedings of the Royal Society of Edinburgh 94B: 13-24.

Read, D.J. 1984. The structure and function of the vegetative mycelium of mycorrhizal roots. In: The Ecology and physiology of the fungal mycelium - symposium of the British Mycological Society. D.H. Jennings and A.D.H. Rayner (eds.), Cambridge University Press, Cambridge, pp. 215-240.

Read, D.J. 1991. Mycorrhizas in Ecosystems. Experientia 47: 376-391

Ricard, J-P. 1999. Rapport de terrain 1997-8: Projet CRSNG Stratégique. Unpublished report, GREF-Interuniversitaire, Montreal, Quebec. .

Runke, R.R. 1984. Development of woody vegetation in treefall gaps in beech-sugar maple forest. Holarctic Ecology 7: 157-164.

Saikkonen, K., Ahonen-Jonnarth, U., Markkola, A-M., Helander, M., Tuomi, J., Roitto, M. and Ranta, H. 1999. Defoliation and mycorrhizal symbiosis: a functional balance between carbon sources and below-ground sinks. Ecology Letters 2: 19-26.

Sambrook, J., Fritsh, E.F. and Maniatis, T. 1989. Molecular Cloning - A laboratory Manual. 2nd Edition. Cold Spring Harbor Laboratory Press, New York.

Sanders, F.E. 1975. The Effects of Foliar- Applied Phoshate on the Mycorrhizal Infections of Onion roots. In: Endomycorrhizas- Proceedings of a Symposium held at the University of Leeds. Sanders, F.E., Mosse, B. and Tinker, P.B. (eds.). Academic Press, London. pp 261- 276.

Sanders, I.R. and Fitter, A.H. 1992a. The ecology and functioning of vesicular-arbuscular mycorrhizas in co-existing grassland species. New Phytologist 120: 517-524.

Sanders, I.R. and Fitter, A.H. 1992b. The ecology and functioning of vesicular-arbuscular mycorrhizas in co-existing grassland species - II. Nutrient uptake and growth of vesicular-arbuscular mycorrhizal plants in a semi-natural grassland. New Phytologist 120: 525-533.

Simard, S.W., Perry, D.A., Jones, M.D., Myrold, D.D., Durall, D.M. and Molina, R. 1997. Net transfer of carbon between ectomycorrhizal tree species in the field. Nature 388: 579-582.

Smith, F.A. and Smith, S.E. 1996. Mutualism and parasitism: diversity in function and structure in the "arbuscular" (VA) mycorrhizal symbiosis. Advances in Botanical Reasearch 22: 1-43.

Smith, S.E. and Read, D.J. 1997. Mycorrhizal Symbiosis - 2nd Edition. Academic Press, London. pp.605.

Son, C.L. and Smith, S.E. 1988. Mycorrhizal growth responses: interactions between photon irradiance and phosphorus nutrition. New Phytologist 108: 305-314.

Taylor, A.F.S. and Alexander, I.J. 1989. Demography and population dynamics of ectomycorrhizas of sitka spruce fertilized with N. Agriculture, Ecosystems and Environment 28:493-496.

Termorshuizen, A.J. 1993. The influence of nitrogen fertilizers on ectomycorrhizas and their fungal carpophores in young stands of *Pinus sylvestris*. Forest ecology an Management 57: 179-189.

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

Visser, S., Maynard, D. and Danielson, R.M. 1998. Response of ecto-and arbuscular mycorrhizal fungi to clear-cutting and the application of chipped aspen wood in a mixedwood site in Alberta, Canada. Applied Soil Ecology 7: 257-269.

Zhou, M., Sharik, T.L., Jurgensen, M.F. and Richter, D.L. 1997. Ectomycorrhizal colonization of *Quercus rubra* seedlings in response to vegetation removals in oak and pine stands. Forest Ecology and Management 93: 91-99.

Appendix 1. Mean temperature and pH of the soil samples collected from Rivière à Pierre.

Month	Treatment	Temp (°C)	pH
June	uncut	6.9[a]	3.9[a]
	gap	8.6[b]	4.0[a]
August	uncut	17.3[a]	4.1[a]
	gap	18.2[a]	4.2[a]
October	uncut	8.5[a]	4.1[a]
	gap	9.5[b]	4.0[a]
	limed	9.3[b]	4.2[b]