Plant and Soil **255:** 487–494, 2003.

Respiration activity of ectomycorrhizas from *Cenococcum geophilum* and *Lactarius* sp. in relation to soil water potential in five beech forests

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Received 11 February 2002. Accepted in revised form 18 March 2003

Key words: Cenococcum geophilum, ectomycorrhiza, *Fagus silvatica*, *Lactarius* sp., respiration activity, soil water potential

Abstract

Forest trees are involved in root symbioses with hundreds of species of ectomycorrhizal fungi which constitute functional guilds able to improve the water and mineral nutrition of host trees. In temperate ecosystems, water shortage is a main factor limiting tree vitality. To assess how soil water conditions affected the physiological state of beech (*Fagus silvatica* L.) ectomycorrhizal roots, we monitored glucose respiration of two ectomycorrhizal types (*Lactarius* sp. and *Cenococcum geophilum*) during two complete growing seasons. Five stands of contrasting soil conditions were chosen in north-eastern France. The top soil horizons were equipped with micropsychrometers for measuring water potential and temperature. Glucose respiration on individual ectomycorrhizas was measured *in vitro* by trapping [^{14}C]-CO_2 from radiolabelled glucose. For soil water potential < -0.2 MPa, the potential respiration activity of *C. geophilum* ectomycorrhizas was significantly less altered than that of *Lactarius* sp. ectomycorrhizas, indicating that *C. geophilum* is more likely than *Lactarius* sp. to maintain the physiological integrity of beech roots facing drought stress.

Introduction

At the soil–root interface of temperate and boreal forest ecosystems, ectomycorrhizal (ECM) fungi are known to play a fundamental role by enhancing the water and mineral nutrition of the host plant and promoting its growth. ECM fungi are particularly well adapted to mobilise sparse heterogeneous resources such as mineral cations, phosphorus and nitrogen from the soil (Smith and Read, 1997). They also play an important role in the water status of trees (Garbaye and Guehl, 1997; Morte et al., 2001).

These functions are ensured by a high diversity of fungi: a single host tree may interact with hundreds of ECM fungal species (Dahlberg, 2001; Taylor et al., 2000), each species being represented by different genotypes (Debaud et al., 1995). This high biodiversity of ECM fungi corresponds to a broad range of complementary functional abilities which is thought to be important to ecosystem functioning (Baxter and Dighton, 2001; Cairney, 1999; Leake, 2001). As stated by Loreau et al. (2001), at least a minimum number of species is essential for ecosystem functioning under constant conditions and a larger number of species is probably essential for maintaining the stability of ecosystems processes in a changing environment.

The increasing availability of powerful molecular markers has led to the extensive study of the genetic structure of ECM communities (Dahlberg, 2001; Horton and Bruns, 2001). This flood of genetic information is outpacing our ability to understand the biological meaning of the genetic variability (Read, 2000). Moreover, inter- (and intra-) specific differences in the functional roles played by ECM fungi in forest ecosystems remain poorly studied. Little is

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known about *in situ* physiological activities of ECM fungi as a result of technical difficulties (Buscot et al., 2000) and confounding factors in experiments (Leake, 2001). ECM communities undergo quick shifts of their functional guilds in response to rapid environmental variations (Lilleskov et al., 2001; Peter et al., 2001; Shi et al., 2002). This is a major difficulty in assessing the functional role of the ECM community in forest ecosystems on the long term.

Water availability is the main environmental factor limiting tree growth in temperate forest ecosystems. The mechanisms by which the fungus modifies host-plant water relations remain poorly known, and studies addressing this question have mainly considered endomycorrhizal fungi (for a review see Augé, 2001). Nevertheless, the water status is seemingly modified by ectomycorrhizal fungi either by a direct effect on water uptake (Agerer, 1991; Garbaye and Guehl, 1997; Muhsin and Zwiazek, 2001; Unestam and Sun, 1995) and nutrient uptake (Morte et al., 2000) or by an indirect effect through stomatal (Mason et al., 1999; Morte et al., 2000, 2001; Nardini et al., 2000) or osmotic (Wartinger et al., 1994) regulations. The effects of global climate change in ecosystem functioning are of increasing interest and several evidences indicate that a deeper understanding of root dynamics (Norby and Jackson, 2000) and shifts in mycorrhizal communities (Treseder and Allen, 2000) are critical to describing the integrated response of ecosystems. In northeastern France, the crisis in the 1990s in beech vitality in lowland areas was apparently mainly due to water shortage (Badeau et al., 1997), and questions about the role of the ECM communities under such water-stress conditions should be addressed. The ECM communities have been well described in these lowland beech forests: Voiry (1981) reported the frequent occurrence of 43 morphological types, with a prominence of *Cenococcum geophilum* Fr., *Lactarius* sp. and *Hebeloma* sp. Blaise and Garbaye (1983) also described *C. geophilum* to be the most frequent (43% of relative abundance) ECM fungus in these forests.

The abundance of the ubiquitous and cosmopolitan ascomycete *C. geophilum* might provide a decisive advantage to beech root systems since several studies report that some *C. geophilum* isolates are more resistant to drought than many other ECM fungi (Coleman et al., 1989; Mexal and Reid, 1973; Neves-Machado, 1995; Theodorou, 1978). Still, while the pure culture response of ectomycorrhizal fungi to imposed water stress has been well studied (Coleman et al., 1989; Mexal and Reid, 1973; Neves Machado, 1995), fewer studies have dealt with the *in situ* behaviour of different ECM morphotypes facing limiting water conditions. Nevertheless, Shi et al. (2002) have shown that different mycorrhizal types responded to drought differently in terms of their patterns of occurrence/abundance and their physiological responses. Pigott (1982b), by observing the fine structure of *C. geophilum* ectomycorrhizas using electron micrography, showed that they remained alive throughout long periods of drought and pointed out that this resistance was apparently related to the ability of the fungal partner to withstand dessication (Pigott, 1982a). We therefore hypothesized that the better drought resistance of *C. geophilum* would preserve the functional status of *C. geophilum* ectomycorrhizal tips. Thus, in post-drought periods, *C. geophilum* ectomycorrhizas, unlike other morphotypes, would be readily able to absorb available water again. Colonisation by *C. geophilum* would constitute a major advantage for roots in post-drought periods.

Simple tests to assess the vitality (i.e., physiological status) of ectomycorrhizas originating from the field are lacking (Niederer et al., 1989). In the present study, we have used a micro-radiorespirometry approach (Al Abras et al., 1988) sensitive enough to assess the physiological activity of single ectomycorrhizal tips. This allowed us to compare the [^{14}C] glucose respiration of single ectomycorrhizas of the black *C. geophilum* morphotype and another dominant morphotype (*Lactarius*-type) sampled in five different beech stands in northeastern France. The sites have been selected for their contrasting climatic and edaphic conditions. We evaluated the effect of soil factors, such as soil water potential and soil temperature, on the physiological status of beech ectomycorrhizal roots.

Materials and methods

Experimental stands and sampling patterns

Investigations were performed on mycorrhizal roots of 80–100 year-old mature beech (*Fagus silvatica* L.) managed as even-aged, periodically thinned stands in northeastern France. Five experimental stands (about 1000 m^2 each) were chosen along a 250 km transect of contrasted soil conditions (Table 1). These sites have previously been used to assess the genetic structure of *C. geophilum* populations (Jany et al., 2002). Each stand contained five 15 m^2 randomly localized plots

Table 1. Location and characteristics of the studied beech stands

	La Crête	Amance	Charmois	Hennezel	Tendon
Coordinates	5° 21′ 25″ E	6° 20′ 25″ E	6° 16′ 12″ E	6° 11′ 45′ ′ E	6° 41′ 25″ E
	48° 12′ 15″ N	48° 44′ 25″ N	48° 6′ 40″ N	48° 2′ 30′ ′ N	48° 43′ 15″ N
Elevation	370 m	280 m	400 m	420 m	570 m
Parent material	limestone	loess	sandstone	sandstone	granite
Texture	clayey	loam	sandy loam	sandy loam	sandy
Humus type	eutrophic mull	mesotrophic mull	mesotrophic mull	mesotrophic mull	mor moder
pH	6.5	4.6	4.8	4.4	3.8

Table 2. Soil temperature and water potential for the 1999 and 2000 growing seasons

Stands	Date	Soil temperature (°C)*	Water soil potential (MPa)*
La Crête	Jul 1 –1999	14	–0.20
	Oct 14 – 1999	11.5	–0.20
	Jun 14 – 2000	16	–0.19
	Jul 19 – 2000	13.5	–0.13
	Aug 23 – 2000	14	–0.63
	Sep 27 – 2000	13	–0.19
Amance	Jul 8 – 1999	14.5	–0.13
	Aug 3 –1999	15.5	–0.62
	Oct 21 – 1999	8.5	–0.39
	May 31 – 2000	11	–0.12
	Jun 20 – 2000	16.5	–0.57
	Aug 9 – 2000	15	–0.32
	Sep 13 – 2000	16	–0.72
Charmois	May 20 –1999	9.5	–0.20
	Jul 15 – 1999	16	–0.11
	Aug 8 – 1999	13.5	–0.14
	Oct 7 – 1999	14	–0.14
	Jun 7 – 2000	13	–0.20
	Jul 12 – 2000	12.5	–0.20
	Aug 17 – 2000	17	–0.24
	Sep 20 – 2000	13	–0.14
Hennezel	Jun 17 – 1999	14.5	–0.18
	Jul 21 – 1999	16	–0.14
	Aug 26 – 1999	16.5	–0.56
	Jun 24 – 2000	10.5	–0.20
	Jul 5 – 2000	14	–0.40
	Aug 2 – 2000	16	–0.13
	Sep 6 – 2000	16	–0.66
Tendon	Jun 10 – 1999	11	–0.26
	Jul 29 – 1999	16.5	–0.54
	Sep 2 – 1999	15.5	–0.55
	Oct 27 – 1999	11	–0.16
	May 17 – 2000	13	–0.19
	June 28 – 2000	12.5	–0.49
	July 26 – 2000	14	–0.18
	Aug 30 – 2000	14.5	–0.15

* Mean values averaged from five measures among the plot.

(3 × 5 m) equipped with a micropsychrometer probe PST-55-15-SF (Wescor, Ut, USA) set horizontally 5–10 cm deep in the A_1 horizon. Roots were sampled during two complete growing seasons according to the time-pattern shown in Table 2. One stand was visited at each sampling date, and soil cores were taken from the inorganic soil layer in the A_1 horizon (about 10 cm deep) in each of the five instrumented plots. At the same time, soil water potential and temperature were measured in each plot by connecting the micropsychrometric probe to a H33-T Dew Point microvoltmeter (Wescor). Soil samples were placed in separate plastic bags, transported on ice and processed immediately after arrival at the laboratory, i.e. 3 h at most after harvest.

Ectomycorrhiza preparation

Samples were soaked in water for 30 min and the roots were washed in running water on a 0.5 mm sieve. Live root tips were examined under a stereo-microscope and *C. geophilum* and *Lactarius*-type ectomycorrhizas were identified, using macroscopic and microscopic features (Agerer, 1995; Ingleby, 1990). Two *C. geophilum* ectomycorrhizas and two *Lactarius*-type ectomycorrhizas were harvested for each sampled core and immediately processed.

Microradiorespirometry assay

Each single ectomycorrhiza was introduced in a rubber-stopped 10 mL Erlenmeyer flask equipped with a side-arm and a hanging polypropylene center well (Kimble-Kontes, NJ, USA), and incubated overnight (15 h) at 25 °C, on an orbital shaker (150 rpm), in 1 mL of modified Pachlewski sterile medium (Pachlewski and Pachlewska, 1974) containing 1.6 m*M*

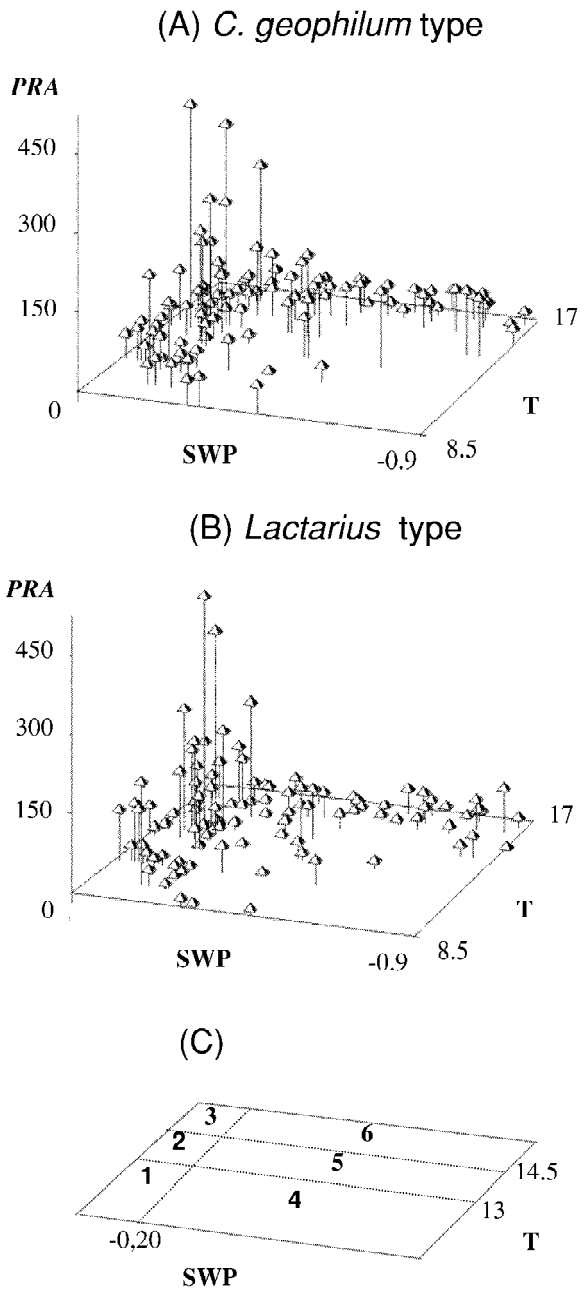

Figure 1. Distribution of respiration activities (*PRA*) in CPM per pg (ergosterol) of single *C. geophilum* (A) or *Lactarius*-type (B) ectomycorrhizal tips, in relation to the soil water potential (SWP) in MPa and the temperature (T) in °C. Each ectomycorrhiza was sampled from *in situ* conditions. Respiration activities were assessed using a micro-radiorespirometry method and soil water potential and soil temperature were measured for each sample using field micropsychrometers. Six distinct soil conditions (1 – 6) for which the difference between *PRA* of *C. geophilum* and *Lactarius*-type ectomycorrhizas was empirically found to be the most significant are represented in (C).

[1-^{14}C]glucose (0.5 μCi). Antibiotics were not added to the incubation medium since tests comparing radiorespirometry assay using rifampicin and/or chloramphenicol did not show difference with assays without any antibiotics added to the incubation medium. The respired [1-^{14}C]CO_2 was trapped in the center well of the flask that contained 100 μL of Kombutron, a CO_2-trapping scintillation fluid (Kontron Analytic, Zurich, Switzerland). After incubation, 200 μL of trichloroacetic acid were added through the rubber stop of the the side-arm using a syringe, in order to release residual CO_2 possibly dissolved in the incubation medium. The 100 μL of the CO_2-trapping scintillation fluid were added to 5 mL of Ecolite scintillation liquid (ICN Pharmaceuticals, Ca, USA) and counted directly in a 1900-CA TriCarb liquid scintillation analyser (Packard Instrument Company, Il, USA). Ectomycorrhizas were then assayed for ergosterol content.

Determining ergosterol content by HPLC

Extraction of ergosterol was done as described by Martin et al. (1990). Ergosterol, a specific component of fungal membrane (Weete, 1974), reflects the live fungal biomass (Martin et al., 1990; Nylund and Wallander, 1992). Considering that the variable component in the compared beech ectomycorrhiza was the fungal partner, we expressed the potential respiration activity (*PRA*) values per picogram of ergosterol content in the whole ectomycorrhiza. For equivalent weight, *C. geophilum* (collection isolate 147–54 from Sweden, CentraalBureau voor Schimmelcultures, The Netherlands) was found to contain 50% more ergosterol than *L. subdulcis* (isolate BRI 2 from eastern France, our collection). *PRA* of *C. geophilum* ectomycorrhizas is therefore underrated when comparing *C. geophilum* and *Lactarius* sp.

Data analysis

Potential respiration activities of single ectomycorrhizas (*PRA*) were expressed in CPM (count per minute) per pg of ergosterol. The nonparametric and paired Wilcoxon signed-rank test (Siegal and Castellan, 1988) which makes no assumptions about the distribution of the data, was used to test the hypothesis that there was a difference between *PRA* of *C. geophilum* and *Lactarius* type ectomycorrhizas. A pair of data consisted of the *PRA* values for a *Lactarius*-type mycorrhiza and a *C. geophilum* mycorrhiza from the

same sample. Differences could be tested when the number of paired data (n) was ≥ 6.

Results

Soil parameters

Investigations carried out on five beech stands during two complete growing seasons (1999 and 2000) addressed a wide range of soil conditions (sandy granite to clayey limestone). Temperature and water potential of the A_1 soil horizon were variable throughout the growing seasons (Table 2). Soil temperatures ranged between 9 °C and 17 °C, whereas soil water potential ranged from −0.05 to −0.9 MPa. Most soil water potential values were in the higher potentials (≥ -0.2 MPa), and values lower than −0.8 MPa were measured only four times throughout our study. The lowest soil water potentials were recorded when the soil temperatures were the highest but high soil water potentials (≥ -0.2 MPa) were also observed in the range of high temperatures (Figure 1).

Ectomycorrhizal types

The *Lactarius*-type, with smooth, branched and yellowish ectomycorrhizas was dominant in the five beech stands. According to Agerer (1995) and Prévost and Pargney (1995), this morphotype corresponds to ectomycorrhizas formed by *Lactarius subdulcis* with beech; however, we did not check this identification by molecular techniques. The *C. geophilum* morphotype (Agerer, 1995) with thick, straight, unbranched and dark hyphae emanating from a black, dense ectomycorrhizal mantle, represented 10 to 40% of all root tips depending on the sampling date.

Potential respiration activity (PRA) *of ectomycorrhizas*

PRA of *C.geophilum* and *Lactarius*-type ectomycorrhizas was highly variable throughout the two growing seasons ranging from zero up to 500 CPM pg^{-1} ergosterol. The distribution of *PRA* values was very asymmetric, with 80% of data lower than 100 CPM pg^{-1} ergosterol. *PRA* was not related with the sampling date, but was dependent on soil water potential and temperature (Figure 1).

By combining different ranges of soil temperatures and water potentials, we defined six distinct conditions for which the difference between *PRA* of the two ectomycorrhizal types was found to be significant or not using Wilcoxon signed-rank test (Figure 1; Table 3). No difference were found between *PRA* of *C. geophilum* ectomycorrhizas and *Lactarius*-type ectomycorrhizas for conditions 1, 2 and 3, whereas *PRA* of *C. geophilum* ectomycorrhizas were higher than *PRA* of *Lactarius*-type ectomycorrhizas for conditions 4, 5 and 6 (according to the soil conditions as defined in Table 3). These differences would be even more pronounced if *PRA* was expressed on a fungal biomass basis instead of ergosterol content, because we know that *C. geophilum* contains 50% more ergosterol than *L. subdulcis*.

The difference in *PRA* between *C. geophilum* and *Lactarius*-type ectomycorrhizas varied among the five stands (Table 4). *PRA* was found to be higher for *C. geophilum* than for *L. subdulcis* only for ectomycorrhizas originating from La Crête and Amance, but there was no significant difference between *C. geophilum* and *Lactarius*-type for ectomycorrhizas originating from Charmois, Hennezel and Tendon.

Two nuclear rDNA ITS ribotypes (A_c and B_c) were distinguished among these five beech stands, and ribotype B_c was dominant in La Crête and Amance whereas ribotype A_c was detected in Charmois and very dominant in Hennezel and Tendon (Jany et al., 2002).

Discussion

In the current study, we have assessed glucose respiration of single ectomycorrhizal tips to compare the physiological activity of *C. geophilum* and *Lactarius* ectomycorrhizas in five beech stands with contrasting soil conditions and throughout two growing seasons.

Glucose oxidation can provide information about metabolic activity of ectomycorrhizas (Al Abras et al., 1988; Garrett et al., 1982; Hacskaylo, 1965; Marshall and Perry, 1987). In the present study, the high variability of *PRA* values showed high differences of metabolic activity among ectomycorrhizal tips. The lack of normal distribution of *PRA* values when plotted against soil water potential and soil temperature suggests that many factors (e.g., host tree photosynthetic activity, nutrient availability, etc) other than those monitored here (soil temperature and soil water potential) can alter the metabolic activity of ectomycorrhizas. However, our data indicate that alteration of *PRA* by drought plays an important role in this limita-

Table 3. Difference of PRA between *C. geophilum* (*Cg*) and *Lactarius* type (*Lt*) ectomycorrhizas among different soil water potential and soil temperature ranges

Soil conditions # [a]	Soil water potential	Soil temperature	*PRA* of *Cg* vs PRA of *Lt*
1	0 to –0.2 MPa	8 – 13 °C	n.s. ($P > 0.05$) [b]
2	0 to –0.2 MPa	13 – 14.5 °C	n.s. ($P > 0.05$)[b]
3	0 to –0.2 MPa	14.5 – 17 °C	n.s. ($P > 0.05$)[b]
4	–0.2 to –0.9 MPa	8 – 13 °C	$Cg > Lt$ ($P < 0.01$)[b]
5	–0.2 to –0.9 MPa	13 – 14.5 °C	$Cg > Lt$ ($P < 0.05$)[b]
6	–0.2 to –0.9 MPa	14.5 – 17 °C	$Cg > Lt$ ($P < 0.01$)[b]
Overall range	0 to –0.9 MPa	8 – 17 °C	$Cg > Lt$ ($P < 0.01$)[b]

[a] See also Figure 1(C).
[b] Wilcoxon signed rank test.

Table 4. Difference of PRA between *C. geophilum* (*Cg*) and *Lactarius* type (*Lt*) ectomycorrhizas for different soil water potential and temperature ranges among the five sampling stand and for the overall sampling

Soil water potential	Soil temperature	La Crête (B_c)[a]	Amance (B_c)[a]	Charmois (A_c)[a]	Hennezel (A_c)[a]	Tendon (A_c)[a]	Overall sampling
0 to –0.2 MPa	8 – 13 °C	[e]	[e]	$Lt > Cg$[d]	[e]	$Cg > Lt$ [c]	n.s. [b]
0 to –0.2 MPa	13 – 14.5 °C	$Cg > Lt$ [b]	[e]	n.s. [b]	[e]	$Lt > Cg$[c]	n.s. [b]
0 to –0.2 MPa	14.5 – 17 °C	[e]	[e]	n.s. [b]	n.s. [b]	[e]	n.s. [b]
–0.2 to –0.9 MPa	8 – 13 °C	[e]	[e]	[e]	[e]	$Cg > Lt$ [d]	$Cg > Lt$ [c]
–0.2 to –0.9 MPa	13 – 14.5 °C	$Cg > Lt$ [c]	[e]	[e]	[e]	[e]	$Cg > Lt$[d]
–0.2 to –0.9 MPa	14.5 – 17 °C	[e]	$Cg > Lt$ [c]	[e]	n.s. [b]	$Cg > Lt$ [c]	$Cg > Lt$ [c]
0 to –0.9 MPa	8 – 17 °C	$Cg > Lt$ [c]	$Cg > Lt$ [c]	n.s. [b]	n.s. [b]	n.s. [b]	$Cg > Lt$ [c]
overall range							

[a] Dominant ribotype of *Cenococcum geophilum* in the site according to Jany et al., 2002.
[b] Non significant with $P > 0.05$, Wilcoxon signed rank test.
[c] $P < 0.01$, Wilcoxon signed rank test.
[d] $P < 0.05$, Wilcoxon signed rank test.
[e] Not calculated because number of paired data (PRA of *Cg* ectomycorrhiza and PRA of *Lt* ectomycorrhiza) < 6.

tion as shown by the reduced *PRA* values for soil water potential < -0.2 MPa.

Furthermore, glucose respiration allows comparison of different ectomycorrhizal fungal species because glucose is known to be universally delivered in the apoplast by hydrolysis of sucrose originating from the plant partner (Nehls et al., 2001). In the present study, *PRA* of *C. geophilum* ectomycorrhizas was significantly higher than *PRA* of *Lactarius* sp. ectomycorrhizas at low soil water potentials (< -0.2 MPa) suggesting that *C. geophilum* ectomycorrhizas have a higher metabolic activity at low soil water potential than those of *Lactarius* sp..

The meaning of such differences relies on the correspondence of a high metabolic potential with optimum functioning (especially water and nutrient uptake). Indeed, a high metabolic rate implies a higher cost to the host, but provides means to face drought by maintaining responses such as accumulation of compatible solutes (Brown, 1990) or energy spilling through some metabolic pathways as mannitol cycle (Jenings and Burke, 1990) which is particularly important in *C. geophilum* (Martin et al., 1985). The latter suggestion is in accordance with the hypothesis of the better resilience to drought of *C. geophilum* compared to other ectomycorrhizal fungi (Mexal and Reid, 1973; Neves Machado, 1995; Theodorou, 1978). Pigott (1982 a,b) argues that *C. geophilum* would provide an adaptative advantage to the colonized roots because it preserves an intact absorbing system for longer periods of time, allowing the uptake of water and ions to resume immediatly in favourable post-drought periods, whereas dead ectomycorrhizas involving other fungi have to be replaced by new ones.

It is remarkable that, in the two sites where *C. geophilum* expresses best its difference with *Lactarius* sp. (La Crête and Amance), our previous genetical survey revealed that the ribotype B_c is dominant. In contrast, in Charmois, Hennezel and Tendon (where *C. geophilum* does not express it) the ribotype A_c is dominant. Although other factors can obviously be involved (interaction with soil type, diversity of *Lactarius* sp., etc.), this suggests an intraspecific functional diversity of *C. geophilum*. This would be in accordance with the results of Coleman et al. (1989) and Neves Machado (1995) with pure mycelial cultures.

Acknowledgements

This study was part of JL Jany's PhD. project funded by a scholarship from the Office National des Forêts (O.N.F.) and the Région Lorraine. We thank J.L. Churin and P. Vion for their assistance in the field surveys, F. Colin for assistance in preparing Figure 1 and M. Buée for stimulating discussions.

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Section editor: M. Jones