Mycorrhiza (2007) 17:547–550
DOI 10.1007/s00572-007-0113-x

SHORT NOTE

Differential ability of ectomycorrhizas to survive drying

Magali di Pietro · Jean-Louis Churin · Jean Garbaye

Received: 27 November 2006 / Accepted: 23 January 2007 / Published online: 17 February 2007

Abstract To test the hypothesis that, depending on the fungal symbiont, ectomycorrhizas are differentially affected by severe drought stress, we developed a simple method to quantify the loss of vitality of excised ectomycorrhizal tips subjected to drying under controlled conditions. The method uses 96-well microtitration plates with one single ectomycorrhizal tip per well, and is based on measuring the loss of volume and the loss of electrolytes before and after the imposed stress. This approach very significantly discriminated the two ectomycorrhizal morphotypes formed with beech (*Fagus silvatica*) by *Lactarius subdulcis* and *Cenococcum geophilum*, which confirmed the ability of the latter fungal species to protect roots against desiccation already suggested by previous works. The new method should contribute to the present effort in deciphering the functional diversity of complex ectomycorrhizal communities.

Keywords Ectomycorrhiza · Drought stress · Loss of electrolytes

Introduction

Most of the fine roots of forest trees are packed in the uppermost horizons of the soil, which are rich in organic matter and where nutrient cycling is most intense. In temperate and boreal forests, these absorbing fine roots are symbiotically associated with a wide diversity of ectomycorrhizal fungi, which contribute to mobilizing nutrients and transferring them to the trees. However, the upper horizon is also the part of the soil that suffers most from drought in summer, when water is limiting and the demand by the trees is high. This results in the periodical death of ectomycorrhizas (Courty et al. 2006), which have to form again when drought ends before being able to resume nutrient uptake. Therefore, because the regeneration of fine roots is carbon-costly, it is an adaptive advantage for the trees to have ectomycorrhizas that can survive and remain active longer during drought periods.

However, this character is somewhat distinct from other mechanisms involved in water supply and conservation by ectomycorrhizas and which cannot be tested on excised root tips, such as uptake and conduction by rhizomorphs (Lamhamedi et al. 1992), hydraulic conductivity of the soil–root interface and osmotic adjustment (Guehl et al. 1992), or modification in sugar metabolism (Shi et al. 2002).

In this work, we hypothesized that ectomycorrhizal types differ in their ability to survive in a drying soil. To test this hypothesis, we developed a method to quantify this character, and we used it to compare two ectomycorrhizal types common and often dominant in beech forests in central Europe.

Materials and methods

Roots were collected from the A_1 horizon (mull-type humus, about 5 cm thick) in a mature beech stand (*Fagus silvatica* L.) on a luvic cambisol (mull type humus, pH 4.6 in the A_1 horizon) in July and August 2006. They were gently washed in tap water and observed with a dissecting microscope. The ectomycorrhizas formed by *Lactarius subdulcis* (Pers.: Fr.) S. F. Gray and *Cenococcum geophilum* Fr. were morphologically identified according to Agerer's *Colour Atlas of*

M. di Pietro · J.-L. Churin · J. Garbaye (✉)
INRA, UMR 1136 Interactions Arbres-Microorganismes,
54280 Champenoux, France
e-mail: garbaye@nancy.inra.fr

Ectomycorrhizae (Agerer 1987). One vital ectomycorrhizal tip (1–2 mm long) was excised and placed individually, with 100 µl of distilled water, in the wells of a clear, 96-well microtitration plate with flat bottoms. The ectomycorrhizal tips were considered as vital when turgid, not shrunken or discolored and, in the case of *C. geophilum*, when the black, thick, emanating hyphae typical of this species were still abundant (they turn brittle in senescent ectomycorrhizas and break easily at washing). Fourteen ectomycorrhizal tips in 14 wells were used for each assay on each ectomycorrhizal type, keeping two empty wells per type (thus, it would have been possible to assay six ectomycorrhizal types simultaneously instead of two). The plates were kept covered at 4°C overnight to be further processed the next day because the following steps of the procedure take a whole day.

The next day, the covered plates were scanned and the projected surface area of each tip was measured using the WinRhizo scanner and image analysis software (Regent Instruments, Quebec, Canada) as described by Buée et al. (2005). The tips were then transferred into another plate containing 100 µl of fresh distilled water per well, using soft tweezers (Roth Gmbh+Co, Karlsruhe, Germany). This new plate was shaken (60 rpm) at 25°C for 20 min in the Inkubator 1000 (Heidolph, Schwabach, Germany). The contents of the 14 wells per morphotype were then pooled (1.4 ml) for measuring the conductivity of the solution using the ECTestr low+ conductivity meter (Fisher Bioblok Scientific, Illkirch, France). When the conductivity of the distilled water used to fill the wells was not zero, the value was used to correct the conductivity readings; this was rarely necessary according to the sensitivity of the instrument. The corrected conductivity values (C) were expressed in microsiemens. The ectomycorrhizal tips were removed from the wells with the soft tweezers and transferred into a third plate with empty (dry) wells, after blotting on dry filter paper. This plate was put in a flat, 600-ml Tupperware© box (600 ml, 23×14×3 cm) with an air-tight lid, together with three 3.5-cm Petri dishes containing an oversaturated solution of $Ca(NO_3)_2$ or $CaCl_2$, providing atmosphere relative humidity of 31 or 51%, respectively. The box was kept at 25°C without shaking for 2 or 3 h. At the end of this period of time, the plate was scanned with the WinRhizo to determine the projected surface area of each apex after desiccation. The plate must be kept covered during measurement to prevent further drying in the atmosphere of the laboratory. Each well was then filled with 100 µl of distilled water, the plate was shaken (60 rpm) at 25°C for 20 min, and the conductivity of the solution was measured as previously described.

This procedure provided two types of results. First, the relative loss of projected surface area (S) during the desiccation process ([S before drying − S after drying] / S before drying); this is an indicator of the loss of water by the ectomycorrhizal tips, resulting in cell collapse and shrinking. Second, the specific loss of electrolytes ([C after drying − C before drying] / S before drying); this is an indicator of cell damage associated with the loss of turgor (McKay 1992; Jaglo-Ottosen et al. 1998).

Five series of experiments were performed with ectomycorrhizas collected from July 18 to August 29, involving two stress intensities (31 and 51% relative humidity) and two stress durations (2 and 3 h). One to five replicates (of 14 tips each) were tested for each treatment, depending on the number of ectomycorrhizas found in each root sample. ANOVA was performed (using Statview 5.0, SAS Institute, Cary, NC, USA) on the data from the three experiments with at least two replicates for both ectomycorrhizal types, and Fisher's least significant difference was used to compare the two ectomycorrhizal types.

Results

Table 1 shows that the drying treatment resulted in relative loss of projected surface area (ΔS / $S_{initial}$) ranging from 0.416 to 0.665 for *L. subdulcis* and from 0.065 to 0.490 for *C. geophilum*, while the conductance of the immersion water, reported to the prestress surface area (specific loss of electrolytes: ΔC / $S_{initial}$) ranged from 1,424 to 2,700 for *L. subdulcis* and from 0 to 831 for *C. geophilum*. In the five series of experiments, these two indices were always lower for the latter ectomycorrhizal type, indicating that the vitality of *C. geophilum* ectomycorrhizas was less affected by the treatments than that of *L. subdulcis* ectomycorrhizas.

However, Fig. 1 shows that the two indices did not similarly discriminate the two ectomycorrhizal morphotypes: while ΔC / $S_{initial}$ very consistently discriminated the two sets of plots on the graph independently of the sampling date and of the experimental conditions, ΔS / $S_{initial}$ displayed a higher variability and poorer discriminating power.

From the methodological point of view, we can therefore conclude that the desiccation treatment of 31% relative humidity for 3 h efficiently reveals differences in the ability of different types of ectomycorrhizas to remain vital when subjected to drought stress, and that the specific loss of electrolytes is a more reliable indicator of ectomycorrhizal integrity than the relative loss of surface area.

Discussion

Lactarius subdulcis is a beech-specific fungal symbiont that forms branched, monopodial ectomycorrhizal clusters with a smooth mantle. This morphotype belongs to the "contact exploration type" according to Agerer (2001). *Cenococcum geophilum* is not host-specific and forms tiny, jet-black,

Table 1 Relative loss of projected surface area (ΔS / $S_{initial}$) and specific loss of electrolytes (ΔC / $S_{initial}$) of *L. subdulcis* and *C. geophilum* beech ectomycorrhizas subjected to controlled drought stress (results of five different experiments)

Sampling date	Replicate number	Stress duration (h)	Stress intensity (% relative humidity)	ΔS / $S_{initial}$		ΔC / $S_{initial}$ (μS cm^{-2})	
				Ls	Cg	Ls	Cg
Jul. 18	*Ls*3, *Cg*2	2	51	0.665 (2.7)[a]	0.442 (13.8)	2,143 (13.8)[a]	160 (6.5)
Jul. 18	*Ls*3, *Cg*1	2	51	0.479 (8.6) nd	0.415	2,700 (19.7) nd	831
Aug. 2	*Ls*3, *Cg*1	2	31	0.505 (4.7) nd	0.490	2,087 (13.3) nd	0
Aug. 17	*Ls*3, *Cg*3	3	31	0.549 (2.2)[b]	0.065 (8.7)	1,982 (11.1)[a]	326 (>100)
Aug. 29	*Ls*5, *Cg*5	3	51	0.416 (34.1) NS	0.251 (6.5)	1,424 (18.2) NS	503 (>100)

Mean values of individual test run with 14 ectomycorrhizal tips (see text) and replicated one to five times, depending on the experiment. Values in brackets (when more than two replicates) are variation indices calculated as standard deviation / mean × 100 and expressed in percent. The variation index is higher than 100% in two cases because *C. geophilum* sometimes had nil ΔC values. ANOVA was performed on the data from the three experiments with at least two replicates for *C. geophilum*, and Fisher's least significant difference was used to compare the two ectomycorrhizal types

LsL. subdulcis, *CgC. geophilum*, *NS* difference not significant (p>0.05), *nd* ANOVA not done

[a] Difference significant at the probability level of 0.01

[b] Difference significant at the 0.001 probability level

hairy and unbranched ectomycorrhizas with a wide range of tree species in all ectomycorrhizal forests worldwide. This morphotype belongs to Agerer's "medium distance exploration type." Together, *L. subdulcis* and *C. geophilum* ectomycorrhizas usually represent a high proportion (sometimes up to 50%, Le Tacon, personal communication) of all morphotypes in the ectomycorrhizal community in a beech forest. Both of these ectomycorrhizal types have hydrophilic mantle and hyphal surfaces (Unestam 1991; Unestam and Sun 1995), but *C. geophilum* displays additional characters that may contribute to drought tolerance, such as the accumulation of melanin and thick, microfibrillar and gelatinous cell walls (Pigott 1982a). In that, *C. geophilum* (an Ascomycete) resembles many lichen-forming Ascomycetes, in which the fungal hyphae swell and become gelatinous in water, thus storing water and keeping the lichen tissue moist longer after rain.

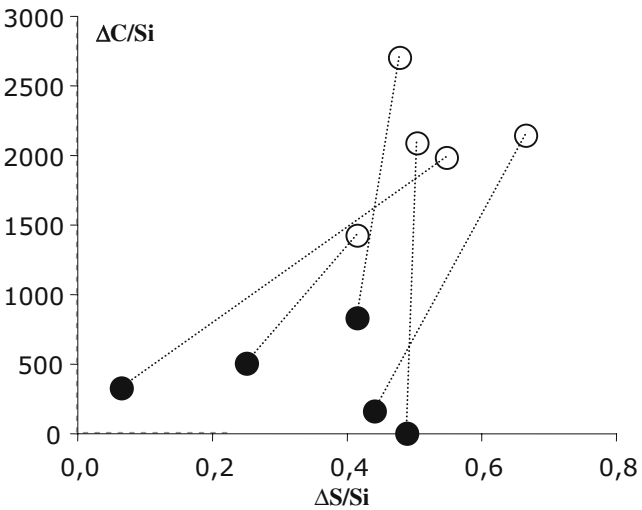

Fig. 1 Specific loss of electrolytes (*ΔC/Si*, μS cm^{-2}) plotted against relative loss of projected surface area (*ΔS/Si*) of *L. subdulcis* (*open circles*) and *C. geophilum* (*solid circles*) beech ectomycorrhizas subjected to controlled desiccation. The two plots from one experiment are linked by a *dotted line* (see Table 1 for experimental conditions). ΔC = conductivity after drying − conductivity before drying. *Si* initial projected surface area (before drying). ΔS = projected surface area before drying − projected surface area after drying

Here, we compared these two morphotypes for their ability to survive controlled drying in a desiccating atmosphere that mimics the conditions in the voids of a drying soil. We assessed their vitality before and after the drying treatment using two criteria: the loss of electrolytes and the loss of volume (shrinking). We optimized the experimental conditions such as to maximize the response. We found that *C. geophilum* ectomycorrhizas always stood the stress better than *L. subdulcis* ectomycorrhizas.

These results validate our hypothesis that ectomycorrhizal morphotypes can differ in their loss of integrity and vitality when subjected to a severe drought stress. This is of great ecological significance at a time when deciphering the functional complexity of ectomycorrhizal communities in connection with the global climate change is a key challenge. More specifically, our results directly confirm the early observations by Pigott (1982b), Neves-Machado (1995), and Jany et al. (2003), which suggested that *C. geophilum* was a symbiont particularly efficient in protecting the absorbing short roots of forest trees against drought damage. Screening a wider range of ectomycorrhizal types with our test could also eventually contribute to finding other drought-tolerant types, for instance when selecting fungal strains for the controlled mycorrhization of plantation forests in dry areas (Neves-Machado 1995).

Finally, from the methodological point of view, the method proposed here is cheap, simple, rapid, and can be run in routine together with the multienzymatic activity assays developed by Pritsch et al. (2004) and Courty et al. (2005) to address the functional diversity of ectomycor-

rhizal communities. It can be operational as it has been described here, but there is no doubt that future works under various conditions and with a diversity of ectomycorrhizal types may contribute to improve it.

References

Agerer R (1987) Colour atlas of ectomycorrhizae. Einhorn-Verlag Eduard Dietenberger, Munich

Agerer R (2001) Exploration types of ectomycorrhizae—a proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. Mycorrhiza 11:107–114

Buée M, Vairelles D, Garbaye J (2005) Year-round monitoring of diversity and potential metabolic activity of the ectomycorrhizal community in a beech (*Fagus silvatica*) forest subjected to two thinning regimes. Mycorrhiza 15:235–245 DOI 10.1007/s00572-004-0313-6.x

Courty PE, Pritsch K, Schloter M, Hartmann A, Garbaye J (2005) Activity profiling of ectomycorrhiza communities in two forests using multiple enzymatic tests. New Phytol 167:309–319

Courty PE, Pouységur R, Buée M, Garbaye J (2006) Laccase and phosphatase activities of the dominant ectomycorrhizal types in a lowland oak forest. Soil Biol Biochem 38:1219–1222 DOI 10.1016/j.soilbio.2005.10.005

Guehl JM, Garbaye J, Wartinger AM (1992) The effect of ectomycorrhizal status on plant–water relations and sensitivity of leaf gas exchange to soil drought in Douglas fir (*Pseudotsuga menziesii*) seedlings. In: Read DJ, Lewis DH, Fitter AH, Alexander IJ (eds) Mycorrhizas in ecosystems. CAB International, Oxfordshire, pp 323–332

Jaglo-Ottosen KR, Gilmour SJ, Zarka DG, Schabenberger O, Thomashow MF (1998) *Arabidopsis CBF1* overexpression induces *COR* genes and enhances freezing tolerance. Science 280:104–106

Jany JL, Martin F, Garbaye J (2003) Respiration activity of ectomycorrhizas from *Cenococcum geophilum* and *Lactarius* sp. in relation to soil water potential in five beech forests. Plant Soil 255:487–494

Lamhamedi MS, Bernier PY, Fortin JA (1992) Hydraulic conductance and soil water potential at the soil–root interface of *Pinus pinaster* seedlings inoculated with different dicaryons of *Pisolithus* sp. Tree Physiol 10:231–244

McKay (1992) Electrolyte leakage from fine roots of conifer seedlings: a rapid index of plant vitality following cold storage. Can J For Res 22(9):1371–1377

Neves-Machado MH (1995) La mycorhization contrôlée d'*Eucalyptus globulus* au Portugal et l'effet de la sécheresse sur la symbiose ectomycorhizienne de cette essence. Ph.D. thesis, University of Nancy 1

Pigott CD (1982a) Fine structure of mycorrhiza formed by *Cenococcum geophilum* Fr. on *Tilia cordata* mill. New Phytol 92:501–512

Pigott CD (1982b) Survival of mycorrhiza formed by *Cenococcum geophilum* Fr in dry soils. New Phytol 92:513–517

Pritsch K, Raidl S, Marksteiner E, Blaschke H, Agerer R, Schloter M, Hartmann A (2004) A rapid and highly sensitive method for measuring enzyme activities in single mycorrhizal tips using 4-methylumbelliferone labelled fluorogenic substrates in a microplate system. J Microbiol Methods 58:233–241

Shi L, Guttenberger M, Kottke I, Hampp R (2002) The effect of drought on mycorrhizas of beech (*Fagus sylvatica* L.): changes in community structure, and the content of carbohydrates and nitrogen storage bodies of the fungus. Mycorrhiza 12:303–311

Unestam T (1991) Water repellency, mat formation and leaf-stimulated growth of some ectomycorrhizal fungi. Mycorrhiza 1:13–20

Unestam T, Sun YP (1995) Extramatrical structures of hydrophobic and hydrophilic ectomycorrhizal fungi. Mycorrhiza 5:301–311