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Review

Cross talks at the morphogenetic, physiological and gene regulation levels between the mycobiont *Piloderma croceum* and oak microcuttings (*Quercus robur*) during formation of ectomycorrhizas

Sylvie Herrmann [a], François Buscot [b,*]

[a] *Department of Community Ecology (BZF), UFZ Centre for Environmental Research, Leipzig-Halle, Theodor-Lieser-Strasse 4, D-06120 Halle/S., Germany*

[b] *Department of Soil Ecology (BOOEK), UFZ Centre for Environmental Research, Leipzig-Halle, Theodor-Lieser-Strasse 4, D-06120 Halle/S., Germany*

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Abstract

Ectomycorrhiza, a symbiosis between soil fungi and the rootlets of major forest trees, is characterized by well defined anatomical traits but also encompasses a wide range of ecological and physiological situations. Functional studies of this symbiosis therefore address different kinds of systems. Here we review works done on an experimental model with micropropagated oak cuttings infected in a Petri dish system with the basidiomycote *Piloderma croceum*. The model is characterized by a high demand for carbohydrates by the fungus and the only differentiating of mycorrhizas with plants having a sufficient carrying capacity in terms of photoassimilate production. Already during the pre-mycorrhizal stage symbiotic interactions between the partners are observed at the morphogenetic and physiological levels and are influenced by the typical endogenous rhythmic development of the plant with alternating growth flushes in the shoot and in the roots. The system was used for first molecular and transcriptomic studies based on a subtractive suppressive hybridization, a macro-array experiment and the research for specific genes.

Keywords: *Piloderma croceum*; *Quercus robur*; Oak microcuttings; Ectomycorrhiza; Gene expression

Contents

* Corresponding author. Tel.: +49 0 345 558 5221; fax: +49 0 345 558 5449.
E-mail address: francois.buscot@ufz.de (F. Buscot).

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1. Introduction

Ectomycorrhizas (EM) are associations between soil fungi and fine roots of trees characterized by a differentiated symbiotic structure made of an external hyphal mantel around the root tip and hyphae that penetrate between cortical cells of the root to built an apposition structure called the Hartig net (Smith and Read, 1997; Fig. 1E). The tree partners of EM are members of a limited number of families (e.g. Fagaceae, Betulaceae, Pinaceae, Salicicaceae, Myrtaceae, Dipterocarpaceae) (Smith and Read, 1997) to which however the major forest species of the boreal and temperate regions as well as some important trees of the tropics are belonging. The fungal partners of EM are members of diverse families of the asco- and basidiomycotes that also comprise saprotrophic and sometimes even parasitic species. The particular diversity structure of both the fungal and the tree partners reflects that the potential to form EM was acquired and lost many times during evolution (Hibbett et al., 2000). This trait is a major difference between EM and arbuscular mycorrhizas (AM), the other prominent category of mycorrhizal associations, which are much more ancient (Redecker et al., 2000) and probably have an almost monophyletic origin. In this context, it makes sense that studies on formation and function of EM are performed on a diversity of associations that all have their sense due to the wide range of biological and ecological traits encountered in ectomycorrhizal symbioses.

In the early 50th, pioneer works on EM functions used excised roots from the field (Harley and McCready, 1950). They were rapidly completed by experiments done in more or less controlled systems with soils, simple substrates such as sand or peat or liquid culture media, which allow synthesizing EM between given plant and fungal partners (for the techniques, see the review by Peterson and Chakravarty, 1991). One of these systems is the Petri dish one, in which axenic small plants are inoculated with fungal pure cultures on a defined agar medium (Fig. 1C). Although artificial, this system brings the major advantages of enabling to control the physical and chemical culture parameters, being strictly gnotobiotic and allowing the precise characterization of physiological and molecular events related to each step of the symbiosis establishment and function (Martin et al., 1995, 2001).

Some Petri dish models use young plantlets obtained after germination of surface sterilized seeds (Kottke et al., 1987; Wong and Fortin, 1988; Hilbert et al., 1991; Burgess et al., 1996; Malajczuk et al., 1990). This procedure presents a double disadvantage. The plant material is genetically inhomogeneous and the particular physiology of seedlings is not representative of the one displayed by trees during most of their long lifespan. This latter point is especially critical as the duration of EM synthesis experiments in Petri dishes is inherently limited to approximately 2.5–3 months, a time interval in which tree seedlings are reduced to cotyledons plus almost one or two additional shoot units and a simple root system consisting of a poorly ramified main root (see for example the system with eucalypts used by Burgess et al., 1996; or Duplessis et al., 2005). Another approach consists in using micropropagated plants, which are genetically homogenous, depleted of cotyledons and present a higher level of shoot and root complexity as well as a physiology that is more representative of the one of adult trees (see for example the systems with poplar used by Grunze et al., 2004 and with oaks (Herrmann et al., 1998; Fig. 1C)).

The present article summarizes and discusses own works done in a Petri dish system using micropropagated and rooted oaks. First the particularities of the plant model and also of *Piloderma croceum,* the fungal partner used in most experiments, are presented. The following sections of the article consider successively the interactions between the partners in EM synthesis experiments at the morphogenetic and physiological levels, before summarizing the actual results obtained at the molecular level. The discussion evaluates the place and the perspectives of our model for research on EM formation and functioning.

2. Developmental traits of *Quercus robur* and particularities of its micropropagation for EM synthesis experiments

2.1. Rhythmic growth of oaks

Apart the mentioned advantages in terms of genetic homogeneity, absence of cotyledons and development level, micropropagated oaks present a rhythmic growth. Here we review the morphogenetic basement of this trait

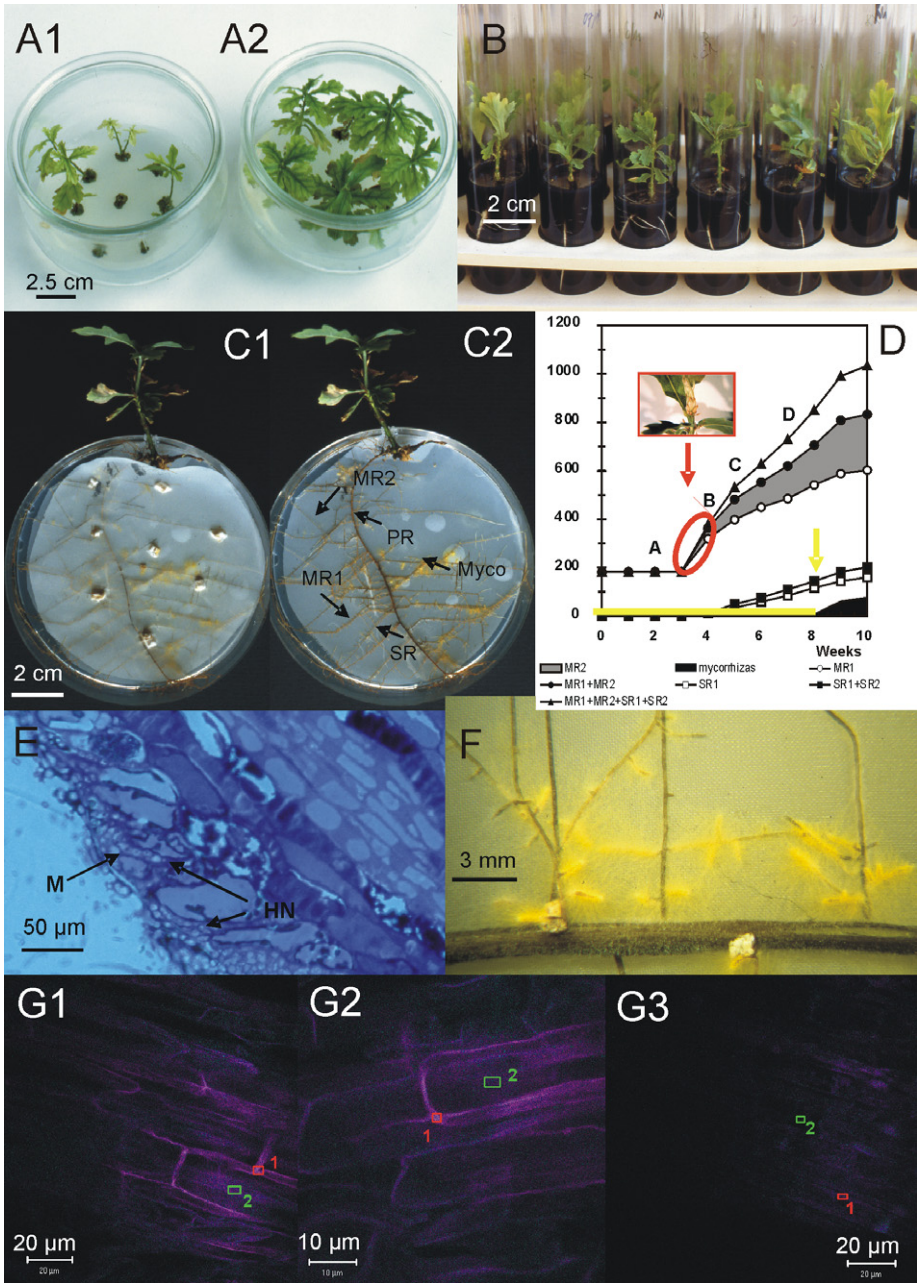

Fig. 1. Micropropagated shoots of *Quercus robur* L. obtained after (A1) predominant subcultures of axillaries and (A2) alternated subcultures of axillaries and apicals. (B) Rooting of microcuttings on activated charcoal medium (see Herrmann et al., 1998). Petri dish system for mycorrhizal synthesis between *Quercus robur* and *Piloderma croceum* with the fungal inoculates (C1) and after removal of the upper nylon sheet that carries these inoculates (C2); PR, principal roots; MR1, MR2, 1st and 2nd-order mother roots; SR, short roots; Myco, yellow pigmented mycorrhizas. (D) Diagram representing the alternation between root and shoot growth flushes in oak microcuttings and the dynamic of EM formation with *P. croceum*; elongation of MR1 and MR2 (mm), formation of SR (number), A = bud rest period, B = bud swelling, C = bud out bursting, D = epinasty, harvest time of roots for molecular analyses is indicated with a red arrow, mycorrhization beginning is indicated with a yellow arrow. (E) Longitudinal section of mycorrhizas with typical Mantel (M) and Hartig net (HN) structures. (F) Formation of mycorrhizas at root tips of MR and SR. Auto-fluorescence detected by confocal laser scanning microscopy (for preparation see Herrmann et al., 2004) in inoculated roots having contact (G1) or not (G2) with hyphae compared to non-inoculated roots (G3).

and its interest for producing an unlimited number of plantlets with synchronous development.

The genus *Quercus* belongs to the family of the Fagaceae. A relation between tree architecture and growth dynamic has been described by Hallé et al. (1978). In European and American Fagaceae there is a good connection between the architectural model and the genus. Oaks grow according to the Rauh model, chestnut trees according to the Massart model and beeches according to the Troll model (Soumoy et al., 1996). The architecture model of Rauh is characterized by a rhythmic growth (Hallé et al., 1978). Klebs (1917) demonstrated for the first time that oaks present a regular succession of growth units when cultivated under controlled and uniform conditions (25 °C

and long days). He considered this behavior to be identical to the rhythmic growth previously described for tropical trees. Numerous works trying to elucidate the determinism of endogenous rhythmic growth in trees used the oak as model tree (Champagnat et al., 1986a,b; Favre and Juncker, 1989; Barnola et al., 1990). In seedlings younger than 3 months, the root system is characterized by a strong hierarchy resulting in a herring-bone pattern (Lavarenne, 1968). At this stage one orthogeotropic tap root displaying a linear growth dominates (Lavarenne, 1968; Belgrand et al., 1987; Harmer, 1990; Alaoui-Sossé et al., 1994) over semi-plagiotropic lateral roots in which slight growth fluctuations are noted (Belgrand et al., 1987). This rhythmic tendency comes to a full expression only in 2-year-old seedlings that typically display alternations of growth flushes in roots and shoot (Reich et al., 1980). On oak cuttings, Riedacker and Belgrand (1983) showed a reduced hierarchy in the regenerated root systems with a prolonged growth of the laterals, but did not analyze the rhythmic development aspect. In the oak microcuttings used in our works, the development of the root system is highly comparable to the one of older seedlings or cuttings (Herrmann et al., 1998). Hierarchy between the principal (or main) root and the lateral roots is reduced and within the lateral roots an endogenous rhythmic growth alternates with shoot episodic development (Herrmann et al., 1998).

2.2. Consequences of the endogenous rhythmic growth for establishing a long term in vitro propagation

Establishment of an *in vitro* shoot culture system involves four successive stages: the *isolation* of the initial explants with decontamination and growth of performed buds, the *stabilization* resulting in uniform and continuous propagation, the *optimization* for an intense production and finally the *rooting and acclimatization* of the produced plants. Plants displaying a growth dynamics determined by strong episodic shoot growth flushes like oaks are the most recalcitrant species because shoot cultures maintain their rhythmic habit and cannot be readily stabilized to display a uniform and continuous growth under *in vitro* conditions (McCown, 2000). *In vitro* propagation of *Quercus robur* from stem explants was established in the eightieths (Chalupa, 1984; Vieitez et al., 1985; Favre and Juncker, 1987; Meier-Dinkel, 1987). In oaks, the cytokinin BAP and the macronutrient composition of the media play an important role in the expression of the episodic growth and the stabilization of the culture (Favre and Juncker, 1989). These authors also found an alternation of subcultures on media with high and low nitrogen content to be crucial to insure the long-term culture of initial explants (Juncker and Favre, 1994). During our 15 years experience in oak micropropagation on low nitrogen content medium we could observe that alternating subcultures of apical and axillaries (Fig. 1A1 and A2) was required for an optimal expression of the rhythmic growth and to avoid degeneration and loss of the clone. No alternation in medium was necessary using this procedure. Since exactly two decades (1986–2006) the *Quercus robur* clone DF159 from Favre and Juncker (1987) is successfully propagated and the endogenous rhythmic growth characteristic for adult oak trees still expressed. These oak microcuttings offer the opportunity to study the impact of endogenous rhythmic growth of trees in the field of mycorrhizal research for the first time.

3. Particularities of *Piloderma croceum* as model fungus for studying EM synthesis experimentally

3.1. Systematic, biology and ecology

More than 6000 fungal species form EM in nature, but only a reduced number of them that are easy to maintain in pure culture and to handle for EM synthesis have been used, and their number is even smaller when strictly considering Petri dish models (see Section 1). There is no exhaustive list of EM fungi compatible with oaks, but literature data indicate that oaks form EM with a wide range of fungi that was even found higher than the one on birch in a field study (Newton, 1991). Further diversity analyses reported about 140 EM taxa on single plots for different oak species (Richard et al., 2004; Walker et al., 2005).

We did most of our experiments with the basidiomycote *Piloderma croceum*. The genus *Piloderma* belongs to the Corticiaceae the taxonomy of which has recently been revised by Larsen et al. (1997). In particular both taxa *P. croceum* Erikss. & Hjortst and *Piloderma bicolor* (Peck) sensu Jül. were unified in the unique species *Piloderma fallax* (Libert) Stalp. In our work we use the old nomenclature as our isolate (729 of the TUMY type collection of the Spezielle Botanik-Mykologie in Tübingen, Germany) is registered under the name *P. croceum*.

P. fallax is one of the only two species within the genus *Piloderma* which have been shown to form EM, the other one being *Piloderma byssinum* (Melin, 1936; Björkman, 1942; Erdtman, 1948). This resupinate ectomycorrhizal fungus forms thin (<1 mm) inconspicuous sporocarps that are easily overlooked (Smith and Read, 1997) and therefore neglected in broad range ecological studies. For the same reason, molecular studies which originally focused on comparing EM community on roots with above ground sporocarps were missing until recently. Nevertheless *P. fallax* has been investigated for much more than seventy years from the point of view of its distribution and autecological traits (Erland and Taylor, 1999). Its unusual bright yellow pigment called corticrocin (Erdtman, 1948; Schreiner et al., 1998) makes it particularly easy to recognize (Fig. 1C and F). Biomass quantification of *P. fallax* is based on ergosterol content (Ekblad et al., 1998) or on the real-time TaqMan PCR technology (Raidl et al., 2005). Its distribution is worldwide (Melin, 1936; Björkman, 1942; Eriksson et al., 1981; Smith and Read, 1997; Larsen et al., 1997) and it is described as a broad-host range mycobiont that can associate to a variety of conifers and hardwood trees

(Eriksson et al., 1981; Larsen, 1983; Brand, 1991; Smith et al., 2000). Both mycorrhizal *Piloderma* species are commonly associated with old forest stands (Dighton and Mason, 1985; Bradbury et al., 1998; Goodman and Trofymow, 1998). *P. fallax* occurs more often in soils with increased amount of coarse woody debris (Smith et al., 2000) and is considered as late-stage fungus (Dighton and Mason, 1985). While the genus *Piloderma* displays a wide distribution between mineral and organic horizons, *P. fallax* is restricted to the organic one (Christy et al., 1982; Goodman and Trofymow, 1998; Rosling et al., 2003) and provides its host plants with nutrients of organic origin. *P. fallax* is characterized by a low growing mycelium highly sensitive to temperature (Erland and Finlay, 1992). Mycelium extension is increased when the fungus is connected with host plants (Erland et al., 1990), and Dahlberg and Stenström (1991) demonstrated connections with mature host plants to be important for efficient colonization of young seedlings.

3.2. Piloderma as model system for experiments

Parallel to field investigations, *Piloderma* was studied under experimental or axenic culture conditions and used to synthesize EM with various hosts plants in numerous morphological and physiological studies (Ho and Zak, 1979; Nylund and Unestam, 1982; Ramstedt et al., 1986; Kottke et al., 1987; Hutchison and Piché, 1995; Herrmann et al., 1998, 2004; Chen et al., 2003; Mahmood et al., 2003) and in some molecular works (Krüger et al., 2004; Frettinger et al., 2004, 2006a,b). The higher demand of carbohydrate for late-stage EM colonizer like *P. fallax* was shown in laboratory studies (Gibson and Deacon, 1990; Hutchison and Piché, 1995). Herrmann et al. (1998) confirmed that *P. fallax* is a strong sink for assimilates. The fate of carbohydrates in *Piloderma* EM was examined by Ramstedt et al. (1986) who showed glucose and fructose from the host to be stored as mannitol by the fungus. The presence of acid phosphatase on the surface of *P. fallax* hyphae led Ho and Zak (1979) to hypothesize a capability of mobilizing organic bound P. The ability to mobilize P and Ca from apatite and ash was demonstrated more recently for different *Piloderma* spp. (Wallander et al., 1997; Arocena and Glowa, 2000; Mahmood et al., 2003; Rosling et al., 2004; Hagerberg et al., 2005), and the role of laccase-like genes in nutrient mobilization has been discussed by Chen et al. (2003).

4. Morphogenetic and physiological interactions between *Q. robur* and *P. croceum*

4.1. Morphological effects in the pre-mycorrhizal stage with *P. croceum*

The first thoroughly characterization of the interaction between *Q. robur* and *P. croceum* at the morphogenetic level was made in an experimental series also considering the early stage EM fungus *Paxillus involutus* in parallel (Herrmann et al., 1998). *P. involutus* is growing relatively rapidly compared to *P. croceum* and began to form EM after 6 weeks against 8 for *P. croceum*. In this experiment series, we used rather small plants that were only able to display one growth flush during the 10 week experiment and did not grow synchronously. However, the respective influence of both mycobionts on plant development in reference to a non inoculated control treatment could be compared by analyzing all individual plants at a similar stage of their development, i.e. at the end of the first growth flush in the Petri dish system, which is marked by a slow down of the roots growth. At this stage, the treatment with *P. croceum* had a significant positive effect on the growth and production of all lateral root types (Fig. 1C2) and on the total leaf area in contrast to both the control plants and those inoculated with *P. involutus* (Table 1). The stimulation of the root and leaf growth was not paralleled by increases in dry weight, so that the specific root length and the specific leaf area were increased. *P. croceum* also appeared to suppress plant internal developmental correlations during the first growth flush. This concerned in particular correlations between the root and the shoot development (Herrmann et al., 1998; Table 1). Noteworthy was that all these morphogenetic effects of *P. croceum* were already observable after 8 weeks of co-culture, that is at a time at which the EM formation was only beginning with this late stage fungus. Thus these effects are to be considered as pre-mycorrhizal.

4.2. Manipulating the onset of EM formation with IAA and activated charcoal

This first work was completed in a second study consisting in manipulating the onset of the EM coupled with physiological analyses (Herrmann et al., 2004). In this case, we used synchronously growing microcuttings that achieved two complete growth flushes within 10 weeks. The later trait was obtained by optimizing the transfer of the microcuttings between the tubes used for their rooting to the Petri dish system (Fig. 1B and C) by early protecting the plant shoot part in a moistened plastic bags until its transfer into the growth chamber (Herrmann et al., 2004). This led to an enhanced surviving of the leaves induced before inoculation and finally to get more vigorous plants with a higher shoot development compared to our first work. Additionally the survival rate was enhanced, which allowed to select plants growing synchronously in the repeats of each treatment (see Herrmann et al., 2004). Based on the known implication of auxins on the development of EM (Slankis, 1973; Gay et al., 1994) and positive effect of activated charcoal on mycorrhiza synthesis (Kottke et al., 1987; Roth-Bejerano et al., 1990), we tested these substances on our system. They both reduced the onset of EM formation from 9 to 5 weeks (Table 1) and led to a higher EM formation. However, it appeared that

Table 1
Synopsis of the morphological and physiological effects of the EM fungus *Piloderma croceum* on oak microcuttings (*Quercus robur*) in a Petri dish system

	P. croceum (Herrmann et al., 1998)	*P. croceum* (Herrmann et al., 2004)			*P. croceum* + r.h. (Herrmann et al., 2004)		
		Alone	+AC	+IAA	EM early	EM late	No EM
Root development							
Root elongation							
Principal roots	→	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Lateral roots	↗	↗	→	→	n.d.	n.d.	n.d.
1st order mother roots	↗	↗	→	→	n.d.	n.d.	n.d.
2nd order mother roots	↗	↗	→	↗	n.d.	n.d.	n.d.
1st and 2nd order short roots	↗	↗	↗	→	n.d.	n.d.	n.d.
Whole root system	↗	↗	→	→	n.d.	n.d.	n.d.
Root dry weight	→	→	→	→	n.d.	n.d.	n.d.
Specific root length	↗	↗	→	→	n.d.	n.d.	n.d.
Shoot development					n.d.	n.d.	n.d.
Total leaf area	↗	↗	→	→	↗	→	→
Leaf dry weight	→	↗	→	→	n.d.	n.d.	n.d.
Specific leaf area	↗	→	→	→	n.d.	n.d.	n.d.
Total plant development							
Plant dry weight	→	↗	→	→	n.d.	n.d.	n.d.
Root/shoot	→	→	→	↗	n.d.	n.d.	n.d.
Development correlations							
MR1 growth onset/delay until MR1 V_{max}	Suppressed	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
MR1 growth onset/delay until shoot stage B	Suppressed	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
MR1 growth onset/delay until shoot stage D	Suppressed	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Delay until MR1 V_{max}/delay until shoot stage B	Maintained	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Delay until MR1 V_{max}/delay until shoot stage C	Suppressed	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
EM formation after (weeks)	8	9	5	5	6	9	No
Photosynthesis criteria	n.d.	n.d.	n.d.	n.d.			
Optimal quantum yield	n.d.	n.d.	n.d.	n.d.	↗	↗	↗
Effective quantum yield	n.d.	n.d.	n.d.	n.d.	↗	↗	↗
Chlorophyll $a + b$	n.d.	n.d.	n.d.	n.d.	↗	↗	↗

All analyzes were performed after 9–10 weeks of co-cultivation. For the exact values and experimental details refer to Herrmann et al. (1998, 2004). AC, activated charcoal at 2% w/v; IAA, indol-acetic-acid at 5 μM; r.h., manipulation of the relative humidity around the plant shoot part at 45% and 95% to obtain inoculated plants forming EM after the 1st growth flush (6 weeks = EM early), the 2nd growth flush (9 weeks = EM late) or not forming EM (no EM).

this accelerated EM formation was related to a disappearance of most morphogenetic effects of *P. croceum* on the oak in case of long pre-mycorrhizal stage (Table 1).

4.3. Physiological effect of *P. croceum* in the pre-mycorrhizal stage

In this second work we also manipulated the humidity around the shoot part of the microcuttings during the EM synthesis experiments and found a reduced r.h. of 45% to trigger EM formation up to the first growth flush, while a humidity close to saturation (r.h. = 95%) led to no EM formation in a majority of repeats or to mycorrhization only after the 2nd growth flush in most of the other repeats. This assay was used for physiological analyses in which we compared non-inoculated control plants, with inoculated plant forming EM after the 1st or the 2nd growth flush or not forming EM at all. The analyzed criteria were on the one hand directly related to photosynthesis activity and consisted in the optimal and effective quantum yield and in the chlorophyll amount (see Seaton and Walker, 1990). On the other hand, the impairment of the photosynthetic apparatus under oxidative stress during the curse of the co-cultures was followed by characterizing the non-photochemical quenching (see Li et al., 2000). For all criteria we found the inoculation with *P. croceum* and not the EM formation itself to trigger photosynthesis of the oak microcuttings and to protect its photosystem during the whole time span of the assay (Table 1). An important observation was that the plants able to form EM already after the 1st growth flush were all characterized by a significantly higher leaf surface at this stage (Table 1). Because the parameters characterizing the photosystem were similar in all inoculated plants, this indicates that these plants displayed a higher photoassimilation.

The latter result shows differentiation of EM by the late-stage fungus *P. croceum* to depend on the photoassimilation level of the plant. On the other hand, the morphogenetic (triggered growth, see Table 1) and physiological (stimulation and protection of the photosynthetic apparatus, see Table 1) impacts observed in the long pre-mycorrhizal stage when less developed plants were inoculated

are clear symbiotic effects that occur prior to the differentiation of the EM mycorrhizal tissues. On the basis of both results, we propose the interpretation that with *P. croceum,* EM tissues (i.e. Hartig net and hyphal mantle) only differentiate when plants have reached a certain carrying capacity in terms of sufficient photoassimilate production for supporting a fully differentiated EM interface, and that for too small plants, acquisition of this carrying capacity is triggered by symbiotic effects in the pre-mycorrhizal stage. This thesis is supported by the fact that when EM formation was advanced artificially by giving auxin or activated charcoal to the medium, the plant growth stimulating effects by the fungus were suppressed as if the limited available resources were entirely used for the EM differentiation and functioning (Table 1). Consistent with this interpretation line are also the facts that *P. croceum* is known to have a high demand for carbohydrates (Hutchison and Piché, 1995; Gibson and Deacon, 1990), that glucose dominates in root exudates (Jones and Darrah, 1996; Lugtenberg et al., 1999; Toal et al., 2000) and that in forests mature trees delivering carbohydrates support EM colonization on young seedlings (Dahlberg and Stenström, 1991; Erland and Taylor, 1999).

4.4. Impact of the endogenous rhythmic plant growth on EM formation

If this carrying capacity thesis is correct, it can be hypothesized that the rhythmic plant growth, which is paralleled by shifts in the balance of internal plant resource allocation between shoot and root, should rule the dynamic of EM formation especially on plants with limited size as the ones used in our system. In fact we noticed that in our first two experience series (Herrmann et al., 1998, 2004) the onset of the EM formation occured during a root flush coinciding with the end of a shoot flush, i.e. during a resource sink in the root part. This relation was investigated more systematically in a third work in which we observed the mycorrhization patterns of oak microcuttings inoculated with different mycobionts in relation to the plant growth rhythm (Buscot and Herrmann, 2004). We observed that according to the fungal partner the growth rhythm may matter or not, and that when it matters, this leads to quite different mycorrhization patterns according to the phase of the growth rhythm at which the EM form. All these patterns could be interpreted in terms of competition between the fungus and the roots in relation to the available resources at different stages of the rhythmic plant growth. For example with *Laccaria amethystea* roots formed monopodial EM with limited growth during shoot flushes *vs.* branched EM and lateral root elongation during root flushes (Buscot and Herrmann, 2004). During root flushes *Pisolithus tinctorius* formed abundant herring-bone mycorrhiza displaying elongation along the lateral roots and densely pinate mycorrhizal systems at their extremity at the end of the root flush. During shoot flushes EM formation was reduced and the two described patterns were not realized.

At this point of our work, the morphogenesis of oak microcuttings, their growth rhythm and its impact on the mycorrhization dynamics were well characterized, especially with the EM fungus *P. croceum*. The fact that this model mycobiont displayed typical mycorrhizal effects (plant growth increase, modified development and photosynthesis response) during its long pre-symbiotic stage is not so surprising. In EM, there is no systematic relationship between the mycorrhization level or the intensity of the EM infection and the effects on functions such as plant growth (Marx, 1979) or protection against root pathogens (Buscot et al., 1992). In wet soils, mycorrhizal effects are observed without any differentiation of mycorrhizal tissues (Smith and Read, 1997). For studying the gene regulation in EM symbioses, the pre-mycorrhizal stage of the association between *P. croceum* and oak microcuttings offers a physiological homogenous material in comparison to fully developed EM with their different functional zones (Kottke and Oberwinkler, 1986).

5. Gene regulation in microcuttings pre-mycorrhizal and mycorrhizal with *P. croceum*

5.1. Demonstration of a specific gene regulation in pre-mycorrhizal roots

To date, four molecular studies have been conducted on our model system. The first one aimed at showing that in addition to its morphogenetic and physiological pre-mycorrhizal effects, *P. croceum* also modifies the expression of symbiosis related genes in roots prior to any EM tissue formation. To assess this hypothesis we made a subtractive suppressive hybridization (SSH) between pre-mycorrhizal microcuttings on the one hand and non inoculated plants or fungal pure cultures on the other one (Krüger et al., 2004) and gathered about 250 clones. The RNA was extracted from whole root systems gathered after week 4, that is during a root elongation flush and at stage B of the shoot development (Fig. D). After performing an independent screening of a corresponding differential expression by reverse Northern, we sequenced 55 confirmed up regulated clones. We analyzed in details 29 that had a length higher than 150 nucleotides, from which one half could be identified after sequence comparison to available data banks (Table 2). These clones displayed analogies to known proteins with putative functions that can be expected in functional symbioses. For example, among six clones corresponding to signal perception and transduction one displayed strong homologies to receptor like proteins transmitting signals from the cytoplasm to the nucleus in *Arabidopsis thaliana* (Table 2) and involved in early defense in *Saccharomyces pombe* (see discussion in Krüger et al., 2004). One of four clones corresponding to stress and defense was a metallothionein, which has also been found during the formation of EM in *Betula pendula* (Johansson et al., 2004). Three identified clones corresponded to

Table 2
List of gene clones with differential expression: (1) in a subtractive suppressive experiment using all roots of oak microcuttings (*Quercus robur* L.) regenerated in the pre-mycorrhizal stage after inoculation with the basidiomycota *Piloderma croceum* (see Krüger et al., 2004), (2) in a macroarray experiment with the same kind of material but with differentiating between lateral (LR) and principal (PR) roots (see Frettinger et al., 2006a)

GeneBank Accession No.	Database matches	New total root system (1)	New PR (2)	New LR (2)
	Signal perception and transmission-transduction pathway			
AJ616018	Ca^{2+}-transporting ATPase-like protein (*Arabidopsis thaliana*)	+		
AJ577265	Pheromone receptor-like protein (*Arabidopsis thaliana*)	+		
AJ616018	Ca^{2+}-transporting ATPase-like protein (*Arabidopsis thaliana*)	+		
AJ580022	Peptidylprolyl cis-trans isomerase (*Triticum aestivum*)	+		
AJ580027	Putative protein kinase (*Arabidopsis thaliana*)	++		
AJ580031	Putative protein kinase (*Arabidopsis thaliana*)	++		
CT010119	GPI-protein transamidase complex subunit			−
CT010146	Putative kinase		+	+
CT010137	Putative serine/threonine protein kinase			−
CR627577	ATP synthase epsilon subunit		−	
CR627507	S-phase kinase-associated protein 1A		−	
CR627979	Protein translation factor SUI1 homolog			−
CR627971	Putative glycosyl hydrolase family protein		−	+
	Stress defense			
AJ580022	Peptidyl-prolyl *cis-trans* isomerase (*Triticum aestivum*)	+		
AJ577263	Metallothionein-like protein (*Quercus suber*)	+		
AJ577266	Formate dehydrogenase (*Hordeum vulgare*)	+		
AJ580024	Serine carboxypeptidase III (*Hordeum vulgare*)	+		
CR628301	Thaumatin-like protein PR5		+	+
CT010142	Proline-rich protein PRP1		++	++
CR627833	Aquaporin			+
CR627776	Late embryogenesis abundant protein Lea5		+	++
CR627822	Pollen specific protein SF21		+	+
CT010009	Dormancy-associated protein		+	+
CR627834	Metallothionein-like protein type 2		+	+
CR627847	Metallothionein-like protein type 3		−	+
CT010129	Iron transport protein 2			−
CT010139	Thioredoxin H-type		+	+
CR627830	Nonsymbiotic hemoglobin		++	−
CR627712	Class I heat shock protein		−	
CT010145	Heat shock protein 17.4		−	
CT009912	NADH dehydrogenase		−	+
CR627826	Phosphoenolpyruvate carboxykinase			−
CR627568	Defender against cell death 1		−	−
CR628014	Early light-induced protein			−
	Recognition and growth response			
AJ577264	Expansin-like protein (*Arabidopsis thaliana*)	+		
AJ580024	Serine carboxypeptidase III (*Hordeum vulgare*)	+		
AJ580030	Rev interacting protein mis3-like (*Arabidopsis thaliana*)	+		
AJ580034	Hypothetical protein (*Arabidopsis thaliana*), similarity to inhibitor of apoptosis protein gb|U45881 from *Drosophila melanogaster*	+		
	Protein synthesis and transcription			
AJ580029	Poly(A)-specific ribonuclease (PAN1) (*Saccharomyces cerevisiae*)	++		
AJ580035	Ribosomal protein L17 (*Castanea sativa*)	+		
CT010115	40S ribosomal protein S23			−
CR627513	40S ribosomal protein S28		−	
CR627691	40S ribosomal protein S21		−	−
CR628064	40S ribosomal protein S11-1		−	
CR627961	50S ribosomal protein L14			− −
CR627517	60S ribosomal protein L13			−
CR627508	60S ribosomal protein L18a		−	−
CT010155	60S ribosomal protein L17		−	
CR628254	60S ribosomal protein L23		−	
CR628009	60S ribosomal protein L24		−	−
CR627566	Histone H3.3			−
CR627506	Histone H4			−.−
CT010120	Histone H3.2		−	−

(*continued on next page*)

Table 2 (*continued*)

GeneBank Accession No.	Database matches	New total root system (1)	New PR (2)	New LR (2)
CR628250	*Castanea mollissima* 10 tRNA-Lys (trnK) gene		−	
CR627985	Cysteine proteinase RD19a precursor			−
CT010130	APS reductase		−	
	Carbon metabolism			
AJ580028	Phosphoglyceromutase (*Ricinus communis*)	++		
CR627824	Small subunit of ribulose-1,5-bisphosphate			−
CR627808	Ribulose bisphosphate carboxylase		−	+
CR627918	Galactinol synthase		-	+
CR627903	Glyceraldehyde 3-phosphate dehydrogenase		−	
CR627667	Cp10-like protein			−
	Lipid metabolism			
CT010117	Acyl-CoA-binding protein		−	
CR627835	PVR3-like protein		−	
	No function			
AJ873930	No match		−	−
AJ873932	No match		−	− −
AJ580023	No match	++		
AJ580025	No match	+		
AJ580026	No match	+		
AJ580032	No match	+		
AJ580033	No match	++		
AJ580036	No match	+		
AJ580037	No match	++		
AJ580038	No match	+		
AJ580039	No match	+		
AJ580040	No match	+		
AJ580041	No match	++	++	++
AJ580042	No match	+		
AJ580043	No match	++		
AJ580045	No match	++		
AJ580046	No match	++	−	−
CT010122	Hypothetical protein CBG16863			− −
CT010138	Hypothetical protein		+	
CR627824	No match 1			−
CR627775	No match 2		++	+
CT010123	No match 3		−	
CT010116	No match		−	
CT010121	No match		−	
CR627669	No match			− −
CT010131	No match		−	
CR627615	No match		−	
CT010132	No match			−
CT010133	No match			−
CT010143	No match			−
CT009838	No match		−	+
CT010149	No match		−	−
CR628002	No match			−
CR628146	No match		+	−
CT010025	No match		-	+
CT010150	No match			− −
CT010158	No match		++	+
CT010061	No match		−	
CT010156	No match			+
CT009915	No match		+	− −
CT010147	No match		−	−

+ or −, up or down regulation lower than 2-fold; ++ or −−, up or down regulation between 2- and 4-fold.

prevention cell death (for example a clone with strong homologies to an apoptosis inhibitor) which makes sense in functional symbiotic tissues (Krüger et al., 2004; Table 2). The fourth clone related to recognition and growth response corresponds to an expansin gene (Table 2) already described in the early phase of other symbioses (Cosgrove et al., 2002) and that could indicate the onset cell wall reorganizations prior to differentiation of the Hartig net.

Consistent with this hypothesis was the observation of an enhanced auto-fluorescence in cell walls of pre-mycorrhizal roots (Fig. 1G). One clone displayed an homology with a rev interactive and another one with the ribosomal protein L17 of chestnut (Table 2), which indicates an enhanced translation activity (Boris-Lawrie et al., 2001). In total, the picture drawn by the found expression pattern, that is signal perception followed by a mix of stress and recognition reactions and by functional responses, fits well with an early symbiotic functionality previously detected at the morphologic (Herrmann et al., 1998) and physiological levels (Herrmann et al., 2004).

5.2. Implication act of fungal auxins and of plant chitinases

The next two molecular works consisted in targeted investigations to verify precise hypotheses. The first one was an experiment to assess the implication of the auxin indole-3-acetic acid (IAA) in the pre-mycorrhizal effect of *P. croceum*. It relied on the fact that giving IAA to the system accelerates EM formation by *P. croceum* (Herrmann et al., 2004, see also Section 4) but also on the well established production of IAA in EM fungi (Ulrich, 1960; Gay et al., 1992) and the known involvement of IAA in EM formation (Slankis, 1973; Rudawska and Kieliszewska-Rokicka, 1997). Microcuttings were either inoculated with *P. croceum*, treated by 5 μM IAA or let as control. Cultivation was performed until a phase of maximal root extension after 4 weeks (Fig. 1D) and followed by RNA extraction. Finally a reverse Northern analysis was performed with the 55 cDNA (for experimental details see Frettinger et al., 2004) of the SSH clone bank obtained by Krüger et al. (2004). In this experiment the gene expression was no more assessed in the whole root system but specifically on lateral roots that are able to form EM, i.e. mother and short roots (see Fig. 1C2). This might explain that only 67% of the SSH genes were found expressed in at least one treatment. About one third of these regulated genes responded to both treatments either in the same or in an opposite way, which is remarkable considering that only one concentration of IAA was tested. Less than 10% of the clones only answered to the IAA treatment. Two of these clones encode phosphoglycerate mutase already known to answer to IAA and in parasitic interactions (Mazarei et al., 2003). In total, the experiment supported the idea that *P. croceum* might act on gene regulation in roots at least partially via auxins. However within a given gene functional group, we often found no coherence in the expression patterns. For example in the treatment with *P. croceum* inoculation we confirmed the up regulation found in the SSH experiment (Krüger et al., 2004) for both genes encoding calmodulin and calcium ATPase, two proteins related to calcium concentration regulation in cells (DeRuijter et al., 1998; Snedden and Fromm, 2001) and that have been shown to play a role in plant-microbe symbioses and to answer to auxin induction (Yang and Poovaiah, 2000). However the answer of these clones to the auxin treatment was a lowered transcript level, which pin points the low mimetic character of applicating once a unique dosis of IAA compared to the biotic interactions occurring over weeks after the fungal inoculation.

In a second targeted work, we looked for a possible chitinase gene over expression in pre-mycorrhizal root cells (Frettinger et al., 2006b). A chitinase activity makes sense in relation to the formation of a mycorrhizal interface that requires a reorganization of the fungal cell wall. While it was detected at the enzymatic level in both AM (Bestel-Corre et al., 2002) and EM (Hodge et al., 1996), a specific transcription of class III chitinases had only been shown in AM (Salzer et al., 2000) before our study. We hypothesized that gene expression could occur already in pre-mycorrhizal roots in our system. Because the gene expression profiles in the total root system and in lateral roots had been shown to differ in our previous two analyses (Krüger et al., 2004; Frettinger et al., 2004) we compared principal and lateral roots. In a first part of the work, we amplified, cloned and sequenced two class III chitinase genes in the oak (*QrchitIII-1* and *QrchitIII-2*; Frettinger et al., 2006b). Only the transcript level of *QrchitIII-1* was found significantly increased in lateral roots of oak microcuttings in both a semi quantitative RT-PCR and a quantitative real time RT-PCR. No expression was found in principal roots.

5.3. Gene regulations profiles in lateral vs. and principal pre-mycorrhizal roots

The last molecular work done on the model system aimed at extending the regulation study to a broader range of genes and to systematize the comparison between different root categories (Frettinger et al., 2006a). Lateral *vs.* main roots of plant pre-mycorrhizal with *P. croceum* and of control plants were gathered for RNA extraction at the end of the 1st root growth flush, i.e. approximately after four weeks of cultivation (see corresponding development stage on Fig. 1D). In addition, a further subset of inoculated plants was cultivated until the onset of EM formation, which occurred during weeks 5 and 6, and the mycorrhizal part of their lateral roots was used for RNA extraction. The expression analysis relied on a macroarray containing the 55 clones of the SSH but also 1866 EST of a bank gathered from leaves of *Quecus petrea* buds (Derory et al., 2006). In addition for more than half of the clones found significantly differentially expressed, semi quantitative RT-PCR was done for confirmation, and for some genes also a quantitative real time PCR assessment was performed (Frettinger et al., 2006a). The macro array comparison between pre-mycorrhizal lateral and principal roots revealed 75 clones to be differentially expressed in at least one of the root type (Table 2). The genes can be ordered into five functional categories plus one group of clones with unknown function (Table 2). As in our SSH study, those genes displaying homology to known proteins were consistent with a symbiotic interaction. For example

in the category of stress defense, we found a member of PR5 proteins (Table 2) that are involved in reaction against pathogens (Asiegbu et al., 2005) and induce permeability changes in fungal cell walls (Roberts and Selitrennikoff, 1990). We also found an aquaporin over expression, which is consistent with a similar finding in mycorrhizal poplars and correspond to the higher demand for water in mycorrhizal plants (Marjanovic et al., 2005). Respectively, 55 and 51 clones were found differentially expressed in lateral and in principal roots. Only 28% of the transcripts had a similar expression in both types of roots, and notable differences were found (compare the last two columns of Table 2). These differences are resumed in Table 3. If considering the totality of the 75 revealed transcripts, about 60% of them displayed an opposite or no regulation in the other root category, but the dissimilarity was higher (75%) for clones encoding proteins involved in signal perception and transduction and lower (38% and 47% for principal and lateral roots, respectively) for the ones involved in stress and defense reactions (Table 3). The dissimilarity values for transcripts involved in lipid and sugar metabolism should not be interpreted due to the low number of concerned clones. This regulation dissimilarity between both types of roots suggests that besides a systemic response to fungal infection, the root system responds specifically in its different part. For example it makes sense that the transcript level of the clone corresponding to aquaporin was up regulated in the lateral roots that are devoted to water absorption and can develop EM but not in the principal roots (Table 2). An important result of the study was that the transcript found regulated in pre-mycorrhizal roots displayed an almost similar expression in differentiated EM (Frettinger et al., 2006a), reinforcing the morphological and physiological indications that at least partial symbiotic functions occurs already in the pre-mycorrhizal stage. In this line, we found approximately 70% of the transcripts to be down regulated in pre-mycorrhizal roots (Table 3). It is known that in mycorrhizas many functions are transferred to the fungal partner during the symbiosis (Ouziad et al., 2005). Down regulations were especially pronounced for those genes that encode signal perception and transduction (75% for both lateral and principal roots), sugar (67% and 100%, respectively), lipid and protein (100%) metabolism (see Table 3). In contrast, the highest part of genes involved in stress response was up regulated (respectively, 67% and 62%).

6. General discussion and perspectives for further molecular investigations

6.1. Place of the Q. robur/P. croceum model in research on EM

Advanced works on molecular regulation in EM should be performed on model organisms that are easy to handle in symbiosis experiments and for which broad genetic information is available and both mutation and transformation systems established. From this point of view, the basidiomycota *Hebeloma cylindrosporum* is undoubtedly an adequate model as it is transformable and as a large number of EST is available (see review by Marmeisse et al., 2004). Similarly, *Laccaria bicolor,* the first EM fungus that has been fully sequenced just recently (http://genome.jgi-psf.org/Lacbi1/Lactbi1.home.html) is the most promising model fungus actually. It is of interest that we could synthesize EM between *Laccaria* and oak microcuttings (Buscot and Herrmann, 2004). On the plant side *Populus* is the candidate of choice as it has also been fully sequenced and is transformable (Martin et al., 2004). However, if the term ectomycorrhiza corresponds to a defined symbiotic anatomical structure (see Section 1 and Fig. 1E), it encompasses a wide range of associations with diverse morphological, ecological and physiological traits (Smith and Read, 1997). This justifies works on further model systems even if they do not fulfill all requirements mentioned above at the moment. Indeed, neither *Piloderma* nor *Quercus* have been transformed actually.

Table 3
Comparative expression profiles in six functional categories of gene clones from *Quercus petraea* (Derory et al., 2006) in a macro-array experiment comparing lateral and principal roots of *Quercus robur* microcuttings during pre-mycorrhizal interaction with the ectomycorrhizal fungus *Piloderma croceum* (after Frettinger et al., 2006a)

Gene groups (number of clones and % of the total)	Number of clones expressed in pre-mycorrhizal roots			
	Lateral roots (LR)		Principal roots (PR)	
	Clone number (+%/−%)	% Differently regulated in PR	Clone number (+%/−%)	% Differently regulated in LR
Total (75 = 100%)	**53** (+34/−66%)	58	**51** (+29/−71)	61
Stress defense (17 = 23%)	**15** (+67/−33)	47	**13** (+62/−38)	38
Signal pers. & trans. (7 = 9%)	**4** (+25/−75)	75	**4** (+25/−75)	75
Sugar metabolism (5 = 7%)	**3** (+33/−67)	100	**3** (−100)	100
Lipid metabolism (2 = 3%)	**0**	–	**2** (−100)	100
Prot. synth. transc. (16 = 21%)	**10** (−100)	60	**10** (−100)	60
Unknown (28 = 37%)	**21** (+29/−71)	62	**19** (+32/−68)	58

(+%/−%), percent of up and down regulated clones; Signal pers. & trans., signal perception, and transmission-transduction pathway; Prot. synth. Transc., protein synthesis and transcription.

Our model system is actually the sole enabling to consider the impact of endogenous plant rhythmic development, and we have demonstrated clearly with a diversity of mycobionts that this trait matters for EM formation and functions (Herrmann et al., 2004; Buscot and Herrmann, 2004). At the fungus level, we use the mycobiont *P. croceum* (or *P. fallax*) that is characterized by a high carbohydrate demand (Gibson and Deacon, 1990; Hutchison and Piché, 1995). There is good experimental evidence of a balance in C-resources between shoot and roots in trees and of its ruling the fate of EM formation and functioning (Rygiewicz and Andersen, 1994; Markkola et al., 2004). The experiments resumed in Section 1 indicate differentiation of EM by *P. croceum* to occur on plants having an adequate carrying capacity in terms leave development and photoassimilate production (Herrmann et al., 2004). Noteworthy are the characteristic symbiotic morphogenetic and physiological effects during the pre-mycorrhizal phase (see Table 1). In addition, we also have evidence of nutrient transfers between the partners in this early phase and that the balance between their respective resources rules the cross talk in the pre-mycorrhizal stage and the establishment and functions of differentiated EM (Herrmann et al., in preparation). This cross talk has also been detected in a system at a higher experimental scale in which *P. fallax* was colonizing different substrates (Rosling et al., 2004). All these particularities of the model system *Q. robur*/*P. croceum* justified the undertaking of molecular investigations.

6.2. Position of our molecular and transcriptomic approach, perspectives

Current molecular studies on EM follow two main lines. The first one consists in a targeted approach that aims to reveal the genes involved in a precise pathway already characterized at the physiological level and to study their regulation. Good examples here are the works that have been achieved on the regulation of the C-transfer between trees and EM fungi (Grunze et al., 2004; Nehls et al., 2001) as well as those to characterize the uptake and transport of nitrogen by the fungus and its translocation to the plant (Javelle et al., 2003; Benjdia et al., 2006). The second way is a less targeted approach that bases on the production of more or less large gene banks or EST collections such as the ones with 3964 ESTs for the basidiomycota *Paxillus involutus* and 2532 ESTs for birch (*Betula pendula*) developed by Johansson et al. (2004). Such banks can serve to develop microarrays for following transcriptomic responses to the EM state in fungi or plant (Peter et al., 2003; Johansson et al., 2004; Wright et al., 2005; LeQuéré et al., 2005), or the response of EM to specific environmental factors such as heavy metals (Jacob et al., 2004). These banks may also be the starting point for regulation studies of one gene in response to EM inoculation or a given biological compound such as auxins in order to enlighten its function (Balasubramanian et al., 2002; Reddy et al., 2003; Tagu et al., 2003).

The *Q. robur*/*P. croceum* system did not serve for studying specific physiological functions but allows following and manipulating the morphologic and physiological crosstalks between the partners before and during the EM symbiosis taking the partner development and its impact on the available resources and their sharing into account. In addition, the model proceeds comparatively slowly through the different steps of symbiosis establishment for which clear morphological markers exist. One especially interesting trait not described in other systems is the symbiosis functionality before differentiation of EM tissues in the pre-mycorrhizal phase. In this context the approach for molecular investigations of this model rather followed the second line mentioned above.

The initial SSH experiment (Krüger et al., 2004) enabled to rapidly gain access to a small bank of genes with a high probability of being symbiosis related, by starting from small amounts of material. Analyzing the identifiable clones of this bank showed its coherence, thus it could be used for a functional study on the effect of IAA (Frettinger et al., 2004).

We also tried a directed approach by looking for a specific gene group (class III chitinases), the expression of which was to expect in the pre-mycorrhizal stage (Frettinger et al., 2006b). This way is not always practicable and for example using the same approach, we failed to find a homolog for the monosaccharide transporter gene *AmMst1* of *Amanita muscaria* (Nehls et al., 1998) in our model fungus *P. croceum*. However EM relevant genes ruling the C and N exchanges are actually being characterized in Fagaceae (Heinz Renneberg, personal communication), which opens perspectives to find new symbiosis related genes in oaks and to characterize their regulation.

Apart these limited approaches, producing a broader bank via the classical way would have necessitated high amounts of homogenous material difficult to gather with a miniaturized system. The sole available oak bank was the one with 1866 ESTs corresponding to approximately 600 different genes developed by Derory et al. (2006) for out bursting buds of *Q. petrea*. Knowing that this bank was not the most adequate for our purpose, we used it for a macroarray experiment, considering that it contains genes expressed at six stages of a major developmental event that might at least partially be also regulated in the mycorrhizal interaction. Indeed we found about 11% of the genes to be expressed in pre-mycorrhizal roots. Noteworthy is that in works with EST banks specifically developed for studying EM formation in other systems similar percent of gene expression were found (Martin et al., 2001; Johansson et al., 2004; Duplessis et al., 2005). Besides the ESTs of Derory et al. (2006) our array also included the 55 ESTs gathered from our SSH experiment (Krüger et al., 2004). The array showed a clear differential regulation within the hierarchical root system (Frettinger et al., 2006a; Table 3) and only two clones of the SSH were found

expressed (Table 2). Both traits suggest that gene regulation varies dynamically between the different root types and the different stages of the interaction as also found by Duplessis et al. (2005) in eucalypts inoculated with *Pisolithus microcarpus*.

In most recent transcript studies, established arrays are used for following expression patterns along different steps of the EM formation (Duplessis et al., 2005; LeQuéré et al., 2005), to compare the regulative effects of different host plants (LeQuéré et al., 2004) or the functionality in different parts of EM fungal colonies (Wright et al., 2005). The perspective for our model will be on the basis of new oak genes revealed by a targeted research or in new extended EST banks to develop a specific array for investigating the molecular crosstalk between the fungus and different parts of the plant during the whole events sequence of the symbiosis from the pre-mycorrhizal stage until senescence. The same system will be used for precisely assess the role played by the endogenous rhythmic plant growth. We will also compare the effects of different fungal partners and trophic contexts.

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References

Alaoui-Sossé, B., Parmentier, C., Dizengremel, P., Barnola, P., 1994. Rhythmic growth and carbon allocation in *Quercus robur*. 1. Starch and sucrose. Plant Physiol. Biochem. 32, 331–339.

Arocena, J.M., Glowa, K.R., 2000. Mineral weathering in ectomycorrhizosphere of subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.) as revealed by soil solution composition. For. Ecol. Manage. 133, 61–70.

Asiegbu, F.O., Nahalkova, J., Li, G., 2005. Pathogen-inducible cDNAs from the interaction of the root rot fungus *Heterobasidion annosum* with Scots pine (*Pinus sylvestris* L.). Plant Sci. 168, 365–372.

Balasubramanian, S., Kim, S.J., Podila, G.K., 2002. Differential expression of a malate synthase gene during the preinfection stage of symbiosis in the ectomycorrhizal fungus *Laccaria bicolor*. New Phytol. 154, 517–527.

Barnola, P., Alatou, D., Lacointe, A., Lavarenne, S., 1990. Etude biologiques et biochimiques du déterminisme de le croissance rythmique du chêne pédonculé (*Quercus robur* L.). Effects de l'ablation des feuilles. Ann. Sci. For. 21, 619–631.

Belgrand, M., Dreyer, E., Joannes, H., Velter, C., Scuiller, I., 1987. A semi-automated data processing system for root growth analysis: application to a growing oak seedling. Tree Physiol. 3, 393–404.

Benjdia, M., Rikirsch, E., Muller, T., Morel, M., Corratge, C., Zimmermann, S., Chalot, M., Frommer, W.B., Wipf, D., 2006. Peptide uptake in the ectomycorrhizal fungus *Hebeloma cylindrosporum*: characterization of two di- and tripeptide transporters (HcPTR2A and B). New Phytol. 170, 401–410.

Bestel-Corre, G., Dumas-Gaudot, E., Poinsot, V., Dieu, M., Dierick, J.F., van Tuinen, D., Remacle, J., Gianinazzi-Pearson, V., Gianinazzi, S., 2002. Proteome analysis and identification of symbiosis-related proteins from *Medicago truncatula* Gaertn. by two-dimensional electrophoresis and mass spectrometry. Electrophoresis 23, 122–137.

Björkman, E., 1942. Über die Bedingungen der Mykorrhizabildung bei Kiefer und Fichte. Symb. Bot. Ups. 6, 1–191.

Boris-Lawrie, K., Roberts, T.M., Hull, S., 2001. Retroviral RNA elements integrate components of post-trasnciptional gene expression. Life Sci. 69, 2697–2709.

Bradbury, S.M., Danielson, R.M., Visser, S., 1998. Ectomycorrhizas of regenerating stands of lodgepole pine (*Pinus contorta*). Can. J. Bot. 76, 218–227.

Brand, F., 1991. Ektomykorrhizen an *Fagus sylvatica*. Charakterisierung und Identifizierung, ökologische Kennzeichnung und unsterile Kultivierung. Libri Bot. 2, 1–228.

Burgess, T., Dell, B., Malajczuk, N., 1996. *In vitro* synthesis of *Pisolithus-Eucalyptus* ectomycorrhizae: synchronization of lateral tip emergence and ectomycorrhizal development. Mycorrhiza 6, 189–196.

Buscot, F., Herrmann, S., 2004. At the frontier between basiodiomycotes and plants: reciprocal interactions between mycorrhiza formation and root development in an *in vitro* system with oaks and hymenomycetes. In: Agerer, R., Piepenbring, M., Blanz, P. (Eds.), Frontiers in Basidiomycote Mycology. IHW, Eching, Germany, pp. 361–376.

Buscot, F., Weber, G., Oberwinkler, F., 1992. Interactions between *Cydindrocarpon destructans* and ectomycorrhizas of *Picea abies* with *Laccaria laccata* and *Paxillus involutus*. Trees 6, 83–90.

Chalupa, V., 1984. *In vitro* propagation of oak (*Quercus robur* L.) and linden (*Tilia cordata* MILL.). Biol. Plant. 26, 374–377.

Champagnat, P., Barnola, P., Lavarenne, S., 1986a. Quelques modalités de la croissance rythmique endogène des tiges chez les végétaux ligneux. Naturalia Monspeliensia – No. h.s., pp. 279–302.

Champagnat, P., Payan, E., Champagnat, M., Barnola, P., Lavarenne, S., Bertholon, C., 1986b. La croissance rythmique de jeunes chênes pédonculés cultivés an conditions contrôlées et uniformes. Naturalia Monspeliensia – No. h.s., pp. 303–337.

Chen, D.M., Bastias, B.A., Taylor, A.F.S., Cairney, J.W.G., 2003. Identification of laccase-like genes in ectomycorrhizal basidiomycetes and transcriptional regulation by nitrogen in *Piloderma byssinum*. New Phytol. 157, 547–554.

Cosgrove, D.J., Chao, L., Cho, H.-T., Hoffmann-Benning, S., Moore, R.C., Blecker, D., 2002. The growing world of expansions. Plant Cell Physiol. 43, 1436–1444.

Christy, E.J., Sollins, P., Trappe, M.J., 1982. First survival of *Tsuga heterophylla* without mycorrhizae and subsequent ectomycorrhizal development on decaying logs and mineral soil. Can. J. Bot. 60, 1601–1605.

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

Derory, J., Léger, P., Garcia, V., Schaeffer, J., Hauser, M.T., Salin, F., Lushing, C., Plomion, C., Glössl, J., Kremer, A., 2006. Transcriptome analsysis of bud burst in sessile oak (*Quercus petraea*). New Phytol. 170, 723–738.

DeRuijter, N.C.A., Rook, M.B., Bisseling, T., Emons, A.M.C., 1998. Lipochitooligosaccharides re-initiate root hair tip growth in *Vicia sativa* with high calcium and spectrin-like antigen at the tip. Plant J. 13, 341–350.

Dighton, J., Mason, P.A., 1985. Mycorrhizal dynamics during forest tree development. In: Moore, D., Casselton, L.A., Wood, D.A., Frankland, J.C. (Eds.), Developmental Biology of Higher Fungi. Cambridge University Press, Cambridge, pp. 117–139.

Duplessis, S., Courty, P.E., Tagu, D., Martin, F., 2005. Transcript patterns associated with ectomycorrhiza development in *Eucalyptus globulus* and *Pisolithus microcarpus*. New Phytol. 165, 599–611.

Ekblad, A., Wallander, H., Näsholm, T., 1998. Chitin and ergosterol combined to measure total and living fungal biomass in ectomycorrhizas. New Phytol. 138, 143–149.

Erdtman, H., 1948. Corticrocin, a mycorrhiza pigment. Nature 160, 331.

Eriksson, J., Hjortstam, K., Ryvarden, L., 1981. The Corticiaceae of North Europe. Fungiflora A/S, Oslo.

Erland, S., Finlay, R.D., 1992. Effects of temperature and incubation time on the ability of three ectomycorrhizal fungi to colonize *Pinus sylvestris* roots. Mycol. Res. 96, 270–272.

Erland, S., Söderström, B., Andersson, S., 1990. Effects of liming on ectomycorrhizal fungi infecting *Pinus sylvestris* L. II. Growth rate in pure culture at different pH values compared to growth rates in symbiosis with the host plant. New Phytol. 115, 683–688.

Erland, S., Taylor, A.F.S., 1999. Resupinate ectomycorrhizal fungal genera. In: Carney, J.W.C., Chambers, S.M. (Eds.), Ectomycorrhizal Fungi: Key Genera in Profile. Springer, Berlin, Heidelberg, New York, pp. 347–363.

Favre, J.M., Juncker, B., 1987. *In vitro* growth of buds taken from seedlings and adult plant material in *Quercus robur* L. Plant Cell Tiss. Org. 8, 49–60.

Favre, J.M., Juncker, B., 1989. Variations in expression of episodic growth by *in vitro* cultured shoots of oak (*Quercus rubur* L.). Ann. Sci. For. 46, 206–210.

Frettinger, P., Derory, J., Herrmann, S., Plomion, C., Lapeyrie, F., Oelmüller, R., Martin, F., Buscot, F., 2006a. Transcriptional changes in two types of pre-mycorrhizal roots and in ectomycorrhizas of oak microcuttings inoculated with *Piloderma croceum*. Planta-2006-02-0118.R2.

Frettinger, P., Herrmann, S., Lapeyrie, F., Oelmüller, R., Buscot, F., 2006b. Diffferential expression of two class III chitinases in two types of roots of *Quercus robur* during pre-mycorrhizal interactions with *Piloderma croceum*. Mycorrhiza 16, 219–223.

Frettinger, P., Oelmüller, R., Buscot, F., Herrmann, S., 2004. Molecular investigations in ectomycorrhizae establishment in the *Quercus robur* - *Piloderma croceum* model: influence of indole-3-acetic on transcripts regulation. Endocyt. Cell Res. 15, 570–578.

Gay, G., Bernillon, J., Debaud, J.C., 1992. Comparative analysis of IAA production in ectomycorrhizal, ericoid, saprophytic fungi in pure culture. In: Read, D.J., Lewis, D.H., Fitter, A.H., Alexander, I.J. (Eds.), Mycorrhizas in Ecosystems. Cambridge University Press, Cambridge, pp. 356–366.

Gay, G., Normand, L., Marmeisse, R., Sotta, B., Debaud, J.C., 1994. Auxin overproducer mutants of *Hebeloma cylindrosporum* Romagnesi have increased mycorrhizal activity. New Phytol. 128, 645–657.

Gibson, F., Deacon, J.W., 1990. Establishment of ectomycorrhizas in aseptic culture: effects of glucose, nitrogen and phosphorus in relation to successions. Mycol. Res. 94, 166–172.

Goodman, D.M., Trofymow, J.A., 1998. Comparison of communities of ectomycorrhizal fungi in old-growth and mature stands of Douglas-fir at two sites on southern Vancouver Island. Can. J. For. Res. 28, 574–581.

Grunze, N., Willmann, M., Nehls, U., 2004. The impact of ectomycorrhiza formation on monosaccharide transporter gene expression in poplar roots. New Phytol. 164, 147–155.

Hagerberg, D., Pallon, J., Wallander, H., 2005. The element content in the mycelium of the ectomycorrhizal fungus*Piloderma* sp. during the colonization of hardened wood ash. Mycorrhiza 15, 387–392.

Hallé, F., Oldeman, R.A., Tomlinson, P.B., 1978. Tropical Trees and Forests An Architectural Analysis. Springer, Berlin, Heidelberg, New-York.

Harley, J.L., McCready, C.C., 1950. Uptake of phosphate by excised mycorrhizas of beech. New Phytol. 49, 388–397.

Harmer, R., 1990. Relation of shoot growth phases in seedling oak to development of the tap root, lateral roots and fine root tips. New. Phytol. 115, 23–27.

Herrmann, S., Munch, J.-C., Buscot, F., 1998. A gnotobiotic culture system with oak microcutiings to study specific effects of mycobionts on plant morphology before, and in the early phase of, ectomycorrhiza formation by *Paxillus involutus* and *Piloderma croceum*. New Phytol. 138, 203–212.

Herrmann, S., Oelmüller, R., Buscot, F., 2004. Manipulation of the onset of ectomycorrhiza formation by indole-3-acetic acid, activated charcoal or relative humidity in the association between oak microcuttings and *Piloderma croceum*: influence on plant development and photosynthesis. J. Plant Physiol. 161, 509–517.

Hibbett, D.S., Gilbert, L.B., Donoghue, M.J., 2000. Evolutionary instability of ectomycorrhizal symbioses in basidiomycetes. Nature 407, 506–508.

Hilbert, J.L., Costa, G., Martin, F., 1991. Ectomycorrhizin synthesis and polypeptide changes during the early stage of eucalypt mycorrhiza development. Plant Physiol. 97, 977–984.

Ho, I., Zak, B., 1979. Acid phosphatase activity of six ectomycorrhizal fungi. Can. J. Bot. 57, 1203–1205.

Hodge, A., Alexander, I.J., Gooday, G.W., Williamson, F.A., 1996. Localization of chitinolytic activities in *Fagus sylvativa* mycorrhizas. Mycorrhiza 6, 181–187.

Hutchison, L.J., Piché, Y., 1995. Effects of exogenous glucose on mycorrhizal colonization *in vitro* by early-stage and late-stage ectomycorrhizal fungi. Can. J. Bot. 73, 898–904.

Jacob, C., Courbot, M.L., Martin, F., Brun, A., Chalot, M., 2004. Transcriptomic responses to cadmium in the ectomycorrhizal fungus *Paxillus involutus*. FEBS Lett. 576, 423–427.

Javelle, A., Morel, M., Rodriquez-Pastrana, B.R., Botton, B., Andre, B., Marini, A.M., Brun, A., Chalot, M., 2003. Molecular characterization, function and regulation of ammonium transporters (Amt) and ammonium-metabolizing enzymes (GS, NADP-GDH) in the ectomycorrhizal fungus *Hebeloma cylindrosporum*. Mol. Microbiol. 47, 411–430.

Johansson, T., LeQuéré, A., Ahren, D., Söderström, B., Erlandsson, R., Lundeberg, J., Uhlen, M., Tunlid, A., 2004. Trancriptional responses of *Paxillus involutus* and *Betula pendula* during formation of ectomycorrhizal root tissue. Mol. Plant-Microbe Interact. 17, 202–215.

Jones, D.L., Darrah, P.R., 1996. Re-sorption of organic coumpounds by roots of *Zea mays* L. and its consequences in the rhizosphere. 3. Characteristics of sugar influx and efflux. Plant Soil 178, 153–160.

Juncker, B., Favre, J.M., 1994. Long-term effects of culture establishment from shoot-tip explants in micropropagating oak (*Quercus robur* L.). Ann. Sci. For. 51, 581–588.

Klebs, G., 1917. Ueber das Verhältnis von Wachstum und Ruhe bei den Pflanzen. Biol. Zbl. 37, 373–415.

Kottke, I., Guttenberger, M., Hampp, R., Oberwinkler, F., 1987. An *in vitro* method for establishing mycorrhizae on coniferous tree seedlings. Trees 1, 191–194.

Kottke, I., Oberwinkler, F., 1986. Mycorrhiza of forest trees – structure and function. Trees, 1–24.

Krüger, A., Peskan, T., Frettinger, P., Herrmann, S., Buscot, F., Oelmüller, R., 2004. Identification of pre-mycorrhiza related plant genes in the association between *Quercus robur* and *Piloderma croceum*. New Phytol. 163, 149–157.

Larsen, M.J., 1983. On *Piloderma bicolor* in North America and its relationship to *Piloderma byssinum*. Mycologia 75, 1092–1093.

Larsen, M.J., Smith, J.E., McKay, D., 1997. On *Piloderma bicolor* and the closely related *P. byssinum*, *P. croceum* and *P. fallax*.. Mycotaxon 63, 1–8.

Lavarenne, S., 1968. Croissance comparée des tiges et des racines de jeunes chênes cultivés en conditions contrôlées. C.R. Acad. Sci. Paris 266, 778–780.

LeQuéré, A., Schützendübel, A., Rajashekar, B., Canbäck, B., Hedh, J., Erland, S., Johansson, T., Tunlid, A., 2004. Divergence in gene expression related to variation in host specificity of an ectomycorrhizal fungus. Mol. Ecol. 13, 3809–3819.

LeQuéré, A., Wright, D.P., Söderström, B., Tunlid, A., Johansson, T., 2005. Global patterns of gene regulation associated with the development of ectomycorrhiza between birch (*Betula pendula* Roth.) and *Paxillus involutus* (Batsch) Fr. Mol. Plant-Microbe Interact. 18, 659–673.

Li, X.P., Björkman, O., Shih, C., Grossman, A.R., Rosenquist, M., Jansson, S., Niyogi, K.K., 2000. A pigment-binding protein essential for regulation of photosynthetic light harvesting. Nature 403, 391–395.

Lugtenberg, B.J.J., Kravchenko, L.V., Simons, M., 1999. Tomato seed and root exudate sugars: composition, utilisation by *Pseudomonas* biocontrol strains and role in rhizophere colonization. Environ. Microbiol. 1, 439–446.

Mahmood, S., Finlay, R.D., Fransson, A.M., Wallander, H., 2003. Effects of hardened wood ash on microbial activity, plant growth and nutrient uptake by ectomycorrhizal spruce seedlings. FEMS Microbiol. Ecol. 43, 121–131.

Malajczuk, N., Lapeyrie, F., Garbaye, J., 1990. Infectivity of pine and eucalypt isolates of *Pisolithus tinctorius* on roots of *Eucalyptus urophylla in vitro*. New Phytol. 114, 627–631.

Marjanovic, Z., Uehlein, N., Kahlenhoff, R., Zwiazek, J.J., Weiß, M., Hampp, R., Nehls, U., 2005. Aquaporins in poplar: What a difference a symbiont makes! Planta 222, 258–268.

Markkola, A., Kuikka, K., Rautio, P., Härmä, E., Roitto, M., Tuomi, J., 2004. Defoliation increases carbon limitations in ectomycorrhizal symbiosis of *Betula pubescens*. Oecologia 140, 234–240.

Marmeisse, R., Guidot, A., Gay, G., Lambilliotte, R., Sentenac, H., Combier, J.P., Melayah, D., Fraissinet-Tachet, L., Debaud, J.C., 2004. *Hebeloma cylindrosporum* – a model species to study ectomycorrhizal symbiosis from gene to ecosystem. New Phytol. 163, 481–498.

Martin, F., Duplessis, S., Ditengou, F., Lagrange, H., Voiblet, C., Lapeyrie, F., 2001. Developmental cross talking in the ectomycorrhizal symbiosis: signals and communication genes. New Phytol. 151, 145–154.

Martin, F., Laurent, P., Decarvalho, D., Burgess, T., Murphy, P., Nehls, U., Tagu, D., 1995. Fungal gene-expression during ectomycorrhiza formation. Can. J. Bot. 73, 541–547.

Martin, F., Tuskan, G.A., DiFazio, S.P., Lammers, P., Newcombe, G., Podila, G.K., 2004. Symbiotic sequencing for the *Populus* mesocosm. New Phytol. 161, 330–335.

Marx, D.H., 1979. Synthesis of *Pisolithus* ectomycorrhizae on White Oak seedlings in fumigated nursery soil. Forest Service Research Note, USDA, SE, 280.

Mazarei, M., Lennon, K.A., Puthoff, D.P., Rodermel, S.R., Baum, T.J., 2003. Expression of an Arabidopsis phosphoglycerate mutase homologue is localized to apical meristem regulated by hormones, and induced by sedentary plant-parasitic nematodes. Plant Mol. Biol. 53, 513–530.

McCown, B.H., 2000. Special symposium: *in vitro* plant recalcitrance. Recalcitrance of woody and herbaceous perennial plants dealing with genetic predeterminism. In vitro Cell Dev. Biol. Plant 36, 149–154.

Meier-Dinkel, A., 1987. *In vitro* propagation and *in vivo* establishment of pedunculate oak (*Quercus robur* L.) and sessile oak (*Quercus petraea* (Matt.) Liebl.). Allg. Forst. Jagdztg. 158, 199–204.

Melin, E., 1936. Methoden der experimentellen Untersuchungen mykotropher Pflanzen. In: Abderhalden, E. (Ed.), Handbuch der biologischen Arbeitsmethoden, Sect II. Urban & Schwartzenberg, Berlin, pp. 1015–1108.

Nehls, U., Mikolajewski, S., Magel, E., Hampp, R., 2001. Carbohydrate metabolism in ectomycorrhizas: gene expression, monosaccharide transport and metabolic control. New Phytol. 150, 533–541.

Nehls, U., Wiese, J., Guttenberger, M., Hampp, R., 1998. Carbon allocation in ectomycorrhizas: identification and expression analysis of an *Amanita muscaria* monosaccharide transporter. Mol. Plant-Microbe Interact. 11, 167–176.

Newton, A.C., 1991. Mineral nutrition and mycorrhizal infection of seedling oak and birch. 3 epidemiologic aspects of ectomycorrhizal infection, and the relationship to seedling growth. New Phytol. 117, 53–60.

Nylund, J.E., Unestam, T., 1982. Structure and physiology of ectomycorrhizae I. The process of mycorrhiza formation in norway spruce *in vitro*. New Phytol. 91, 63–79.

Ouziad, F., Hildebrandt, U., Schmelzer, E., Bothe, H., 2005. Differential gene expressions in arbuscular mycorrhizal-colonized tomato grown under heavy metal stress. J. Plant Physiol. 162, 634–649.

Peter, M., Courty, P.E., Kohler, A., Delaruelle, C., Martin, D., Tagu, D., Frey-Klett, P., Duplessis, S., Chalot, M., Podila, G., Martin, F., 2003. Analysis of expressed sequence tags from the ectomycorrhizal basidiomycetes*Laccaria bicolor* and *Pisolithus microcarpus*.. New Phytol. 159, 117–129.

Peterson, R.L., Chakravarty, P., 1991. Techniques in synthesizing mycorrhiza. In: Norris, J.R., Read, D.J., Varma, A.K. (Eds.), Methods of Microbiology, 23. Academic Press, London, pp. 75–114.

Raidl, S., Bonfigli, R., Agerer, R., 2005. Calibration of Quantitative Real time Taqman PCR by correlation with hyphal biomass and ITS copies in mycelia of*Piloderma Croceum*. Plant. Biol. 7, 713–717.

Ramstedt, M., Niehaus, W.G., Söderhäll, K., 1986. Mannitol metabolism in the mycorrhizal fungus *Piloderma croceum*. Exp. Mycol. 10, 9–18.

Reddy, S.M., Pandey, A.K., Melayah, D., Marmeisse, R., Gay, G., 2003. The auxin responsive gene Pp-C61 is up-regulated in *Pinus pinaster* roots following inoculation with ectomycorrhizal fungi. Plant Cell Environ. 26, 681–691.

Redecker, D., Kodner, R., Graham, L.E., 2000. *Glomalean* fungi from the ordovician. Science 289, 1920–1921.

Reich, P.B., Teskey, R.O., Johnson, P.S., Hinckley, T.M., 1980. Periodic root and shoot growth in oak. Forest Sci. 26, 590–598.

Richard, F., Moreau, P.A., Selosse, M.A., Gardes, M., 2004. Diversity and fruiting patterns of ectomycorrhizal and saprobic fungi in an old-growth Mediterranean forest dominated by *Quercus ilex* L. Can. J. Bot. 82, 1711–1729.

Riedacker, A., Belgrand, M., 1983. Morphogénèse des systèmes racinaires des semis et boutures de chêne pédonculé. Plant Soil 71, 131–146.

Roberts, W., Selitrennikoff, C.P., 1990. Zeamatin, an antifungal protein from maize with membrane-permaeabilizing activity. J. Gen. Microbiol. 136, 1771–1778.

Rosling, A., Landeweert, R., Lindhal, B.D., Larsson, K.-H., Kuyper, T.W., Taylor, A.F.S., Finlay, R.D., 2003. Vertical distribution of ectomycorrhilzal fungal taxa in a podzol soil profile. New Phytol. 159, 775–783.

Rosling, A., Lindahl, B.D., Finlay, R.D., 2004. Carbon allocation to ectomycorrhizal roots and mycelium colonising different mineral substrates. New Phytol. 162, 795–802.

Roth-Bejerano, N., Livne, D., Kagan-Zur, V., 1990. *Helianthemum-Terfezia* relations in different growth media. New Phytol. 114, 235–238.

Rudawska, M.L., Kieliszewska- Rokicka, B., 1997. Mycorrhizal formation by *Paxillus involutus* strains in relation to their IAA-synthesizing activity. New Phytol. 137, 509–517.

Rygiewicz, P.T., Andersen, C.P., 1994. Mycorrhizae alter quality and quantity of carbon allocated below ground. Nature 369, 59–60.

Salzer, P., Bonanomi, A., Beyer, K., Vögeli-Lange, R., Aeschbacher, R.A., Lange, J., Wiemken, A., Kim, D., Cook, D.R., Boller, T., 2000. Differential expression of eight chitinase genes in *Medicago trunculata* roots during mycorrhiza formation, nodulation, and pathogen infection. Mol. Plant-Microbe Interact. 13, 763–777.

Schreiner, T., Hildebrandt, U., Bothe, H., Marner, F.J., 1998. Chemical and biological characterization of corticrocin, a yellow pigment formed by the ectomycorrhizal fungus *Piloderma croceum*. Z. Naturforsch. 53, 4–8.

Seaton, G.G.R., Walker, D.A., 1990. Chlorophyll fluorescence as a measure of photosynthetic carbon assimilation. Proc. R. Soc. London 242, 29–35.

Slankis, V., 1973. Hormonal relationships in mycorrhizal development. In: Marks, G.C., Kozlowski, T.T. (Eds.), Ectomycorrhizae. Academic Press, New-York, pp. 231–298.

Smith, J.E., Molina, R., Huso, M.M.P., Larsen, M.J., 2000. Occurrence of *Piloderma fallax* in young, rotation-age, and old-growth stands of Douglas-fir (*Pseudotsuga menziesii*) in the Cascade Range of Oregon, USA. Can. J. Bot. 78, 995–1001.

Smith, S.E., Read, D.J., 1997. Mycorrhizal Symbiosis, second ed. Academic Press, Harcourt, Brace & Compagny, London.

Snedden, W.A., Fromm, H., 2001. Calmodulin as a versatile calcium signal transducer in plants. New Phytol. 151, 35–66.

Soumoy, L., Thiébaut, B., Sérey, I., 1996. Développement de quelques Fagaceae japonaises; germination, modèle architectural, et systématique. Can. J. Bot. 74, 1248–1259.

Tagu, D., Palin, B., Balestrini, R., Gelhaye, E., Lapeyrie, F., Jacquot, J.P., Sautière, P.E., Bonfante, P., Martin, F., 2003. Characterization of a symbiosis- and auxin-regulated glutathione-*S*-transferase from *Eucalyptus globulus* roots. Plant Physiol. Biochem. 41, 611–618.

Toal, M.E., Yeomans, C., Killham, K., Meharg, A.A., 2000. A review of rhizosphere carbon flow modelling. Plant Soil 222, 263–281.

Ulrich, J.M., 1960. Auxin production by mycorrhizal fungi. Physiol. Plant. 13, 429–443.

Vieitez, A.M., San-Jose, M.C., Vieitez, E., 1985. *In vitro* plantlet regeneration from juvenile and mature *Quercus robur* L. J. Hort. Sci. 60, 99–106.

Walker, J.F., Miller, O.K., Horton, J.L., 2005. Hyperdiversity of ectomycorrhizal fungus assemblages on oak seedlings in mixed forests in the southern Appalachian Mountains. Mol. Ecol. 14, 829–838.

Wallander, H., Wickman, T., Jacks, G., 1997. Apatite as a P source in mycorrhizal and non-mycorrhizal *Pinus sylvestris* seedlings. Plant Soil 196, 123–131.

Wong, K.K.Y., Fortin, J.A., 1988. A Petri dish technique for the aseptic synthesis of ectomycorrhizae. Can. J. Bot. 67, 1713–1716.

Wright, D.P., Johansson, T., LeQuéré, A., Soderstrom, B., Tunlid, A., 2005. Spatial patterns of gene expression in the extramatrical mycelium and mycorrhizal root tips formed by the ectomycorrhizal fungus *Paxillus involutus* in association with birch (*Betula pendula*) seedlings in soil microcosms. New Phytol. 167, 579–596.

Yang, T., Poovaiah, B.W., 2000. Molecular and biochemical evidence for the involvement of calcium/calmodulin in auxin action. J. Biol. Chem. 275, 3137–3143.

Sylvie Herrmann is senior scientist at the department for Biocenosis Research of the Centre for Environmental Research Leipzig-Halle, a member of the Helmholz Society (Germany). She obtained her PhD for Plant Biology at the Strasbourg University, followed by a Post Doc fellowship granted by the European Commission at the Tübingen University (Germany), before occupying different research scientist positions at the Federal Centre of Agricultural Research and the Technical University of Braunschweig and at the Universities of Jena and Leipzig (Germany). Her focus is on plant experimental morphology and *in vitro* tissue culture. She has been working on floral induction in orchids, protoplast-regeneration in potato before coming to mycorrhiza research. She developed the oak model system and has coordinated its exploitation at the morphological, physiological and molecular levels.

François Buscot is full Professor for Terrestrial Ecology at the University of Leipzig and head of the Department for Soil Ecology at the Centre for Environmental Research Leipzig-Halle, a member of the Helmholz Society (Germany). He obtained a PhD for Plant Science at the Strasbourg University and a Habilitation for Soil Biology at the Technical University of Braunschweig (Germany). After a Post-Doc as Humboldt fellow at the University of Tübingen he was research scientist at the Federal Centre of Agricultural Research of Braunschweig before occupying successive Professorships at the universities of Jena and Leipzig. He first investigated the biology and ecology of the fungal genus *Morchella,* before turning to research on mycorrhizal and soil fungi with methods ranging from field work to molecular ecology over electron microscopy. He was involved in major programs on ecosystem functioning, release of transgenic trees, functional diversity of soil microorganisms and their link to above ground diversity, cycling and accumulation of carbon in soils, mechanisms that regulate mycorrhizal associations. He is President of the German Society for Mycology and Lichenology and vice Chairman of the board for Plant Sciences at the German Science Foundation.