

QUERCIRHIZA QUADRATUM: A REVISION OF THE CHARACTERS AND IDENTITY OF THE AD TYPE ECTOMYCORRHIZA

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Abstract

The well-known AD type, described first by Giraud in 1988, is considered as a competitor in black truffle (*Tuber melanosporum* Vittad.) plantations. It has been mainly observed in *T. melanosporum* and *T. magnatum* Pico plantations in France and Italy. This ectomycorrhiza has always been observed on roots of oak (*Quercus ilex* L. and *Q. faginea* Lam.) and hazelnut (*Corylus avellana* L.) plantations with “burnt” areas around the trees, even in those that do not produce black truffle sporocarps, so it can create false expectations in young plantations. The AD type has also been described in nurseries, as a competitive ectomycorrhiza on seedlings inoculated with black truffle. In Spain, AD type has been detected in black truffle plantations and natural holm oak stands in Navarra, Soria, Huesca, Zaragoza, Teruel, Castellón and Valencia. In 2005, De Román & De Miguel, suggested that AD type could be a telephoroid type due to its anatomical and morphological characters. In 2006, Baciarelli-Falini *et al.* using molecular techniques identified this type as an Ascomycotina belonging to Pezizales. The detailed anatomical, morphological and molecular study of the AD type led to a description as *Quercirhiza quadratum* (Águeda *et al.* 2008). Based on the anatomical and morphological characters, the AD type belongs to the Ascomycotina. The presence of Woronin bodies on hyphal septa, and the sometimes slightly dissolved septa, are two typical characters of this group. The DNA sequences obtained from the AD types studied showed close similarities with members of Pyronemataceae and Sarcosomataceae (Pezizales). Both taxonomic groups correspond to the same AD type as found by Baciarelli Falini *et al.*, (2006). One of the studied sequences showed a close identity (100% maximum identity, 84% coverage) with *Trichophaea woolhopeia* (Cooke & W. Phillips) Arnould, although records of this fungal species are scarce in the Iberian Peninsula.

Key words: ectomycorrhizae, ecology, population biology, taxonomy.

Introduction

The well-known AD type, described first by Giraud in 1988, is considered as a competitor in black truffle (*Tuber melanosporum* Vittad.) plantations. It has been mainly observed in *T. melanosporum* and *T. magnatum* Pico plantations in France and Italy. This ectomycorrhiza has always been observed on roots of oak (*Quercus ilex* L. and *Q. faginea* Lam.) and hazelnut (*Corylus avellana* L.) plantations with “burnt” areas around the trees, even in those that do not produce black truffle sporocarps, so it can create false expectations in young plantations. The AD type has also been described in nurseries, as a competitive ectomycorrhiza on seedlings inoculated with black truffle. In Spain, AD type has been detected in black truffle plantations and natural holm oak stands in Navarra, Soria, Huesca, Zaragoza, Teruel, Castellón and Valencia.

The aim of this work is to give the complete description of this ectomycorrhiza in order to put some light in its identity.

Material and methods

Following Agerer & Rambold (2004-2008) methods, ectomycorrhizae from five different locations in Spain were characterized and described. Reference specimen for *Quercus* ectomycorrhizae: Spain, Castilla y León, Prov. Soria, La Quiñonería, coord.: 41° 34' 36.11" N, 2° 3' 13.68" W; 06.10.2000; ecology: *Quercus ilex* stands with *Tuber melanosporum* production; soil core and myc. isol.: B. Águeda, herb. Departamento de Investigación y Experiencias Forestales de Valonsadero (JCyL), VALONSADERO – MYCORRHIZA 050 (in Soria, Spain). - Further material studied: Spain, Navarra, Oloriz, coord.: 30TXN12 (special coordinates of University); 02.04.2008; ecology: *Q. faginea* and *Q. ilex* subsp. *ballota* stands; soil core and myc. isol.: A.M. de Miguel, herb. Universidad de Navarra, PAMP-Mycorrhiza 200 (in Pamplona, Spain). - Spain, Aragón, Prov. Teruel, UTM coord.: 30T 0691507/4444998; 18.03.08; *Q. ilex* seedling from a nursery, inoculated with *Tuber melanosporum*; myc. isol.: A.M. de Miguel, herb. Universidad de Navarra, PAMP-Mycorrhiza 203 (in Pamplona, Spain). Ectomycorrhizae used for molecular analyses: Spain, Navarra, Oloriz, coord: 30TXN12; 15.06.2006; ecology: *Q. faginea* stand; soil core and myc. isol.: A.M. de Miguel, herb. Universidad de Navarra PAMP-Mycorrhiza 150 (in Pamplona, Spain), GenBank accession number: EU822505. - Spain, Navarra, Ollogoyen, coord: 30TWN72; 6.11.2007; ecology: *Q. ilex* subsp. *ballota* stands; soil core and myc. isol.: A.M. de Miguel, herb. Universidad de Navarra PAMP-Mycorrhiza 201 and PAMP-Mycorrhiza 202 (in Pamplona, Spain), GenBank accession numbers: EU822506 and EU822507, respectively.

Molecular characterization of different mycorrhiza samples was carried out by sequencing fragments of the nuclear ribosomal DNA region. DNA extraction was performed with the PowerSoil™ DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA) according to the manufacturer's instructions. Amplifications of ITS rDNA sequences were carried out with an Applied Biosystems 9700 PCR thermocycler using the primers ITS1F (5'-TCCGTAGGTGAACCTGCGG-3') (Gardes & Bruns, 1993) and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al.*, 1990). Nucleotide sequences of the amplified region were obtained with a Applied Biosystems 3730 DNA analyzer and registered in the NCBI GenBank database with the following accession numbers: EU822505, EU822506 and EU822507. Fungal identification was carried out by searching highly similar sequences in the GenBank and Unite databases.

Results

Short description of the ectomycorrhizae

This brown ectomycorrhiza is well characterized by its rectangularly ramified emanating hyphae and by a two-layered pseudoparenchymatous mantle, the outer one is covered by mounds of mostly globose cells, which are lacking on the very tip. Emanating hyphae straight close to the mantle, continuing in rather irregularly shaped branches with thick (often unevenly) cell walls, smooth or patchily covered by distinct warts, without clamps. Ends of emanating hyphae simple, ramified or tortuous, even screw-like, sometimes forming almost ball-like structures, always thin walled. Septa accompanied by Woronin bodies and, sometimes, slightly dissolved. Rhizomorphs and cystidia absent.

A complete description of *Quercirhiza quadratum* can be found in Águeda *et al.*, (2008).

Molecular characterization

The sequence EU822505 showed 100% identity with DQ402507 (84% query coverage) identified as a member of Pyronemataceae (Baciarelli-Falini *et al.*, 2006). The sequences EU822506 and EU822507 showed 87 and 89% maximum identity with DQ402506 (97 and 87% query coverage, respectively) identified as a member of Sarcosomataceae (Baciarelli Falini *et al.*, 2006).

Discussion

"*Quercirhiza quadratum*" can easily be distinguished from similar ectomycorrhizae. Most distinctive are the predominantly rectangularly branched, clampless emanating hyphae,

Woronin bodies associated septa, and a pseudoparenchymatous mantle with angular cells that is covered by mounds of globose cells. From the similar *Genea* ectomycorrhizae they differ in the presence of heaps of cells, whereas in *Genea* only solitary cells occur on the mantle surface, and *Genea* forms in addition only a few emanating scarcely ramified hyphae (Agerer, 2006; Brand, 1991a, b; Jakucs & Bratek, 1998; Jakucs *et al.*, 1998). *Tomentella* ectomycorrhizae with similar mantle types have either cystidia, and/or clamps, lack Woronin bodies and show differently ramified emanating hyphae (Agerer, 2006; Agerer & Rambold, 2004-2008). Some *Tuber* ectomycorrhizae also present mantles with angular cells, but typical awl-shaped cystidia are usually and emanating hyphae, when present, are smooth (Agerer, 2006; Agerer & Rambold, 2004-2008).

“*Quercirhiza quadratum*” belongs to the well-known AD type, described first by Giraud (1988), who chose the name, “angle droit” in French language, referring to the abundant rectangular ramification of its emanating hyphae. This ectomycorrhiza is considered as competitor in black truffle (*Tuber melanosporum* Vittad.) plantations and it had been observed mainly in France (Giraud, 1988; Sourzat *et al.*, 1993; Sourzat, 1994; Verlhac *et al.*, 1990), and also in Italy in *T. melanosporum* and in *T. magnatum* Pico plantations (Bencivenga *et al.* 1992, Bencivenga *et al.*, 1995; Granetti & Baciarelli Falini, 1997; Baciarelli Falini & Granetti, 1998). This ectomycorrhiza has always been observed on oak roots (*Q. ilex*, *Q. faginea* Lam.) and hazelnuts (*Corylus avellana* L.) that always showed “burnt-areas” around trees. The AD type has also been described in nurseries, as a fearsome competitive ectomycorrhiza on seedlings inoculated with black truffle (Bencivenga *et al.*, 1995; Di Massimo *et al.*, 1996).

In Spain, there are proofs of its presence in black truffle plantations in Navarra, Soria, Huesca, Zaragoza, Teruel, Castellón and Valencia. In fact, “*Q. quadratum*” was one of the first types identified as ectomycorrhizal competitor in black truffle plantations established in Navarra (De Miguel & Sáez, 1997, 2005; Etayo & De Miguel, 1998), and also in Soria (Águeda *et al.*, 2001) and in Castellón (Domínguez-Núñez *et al.*, 2005). De Román & De Miguel (2005), reported this type for the first time in natural holmoak (*Quercus ilex*) stands, suggesting that it was a telephoroid type due to its anatomical and morphological characters.

In 2006, Baciarelli Falini *et al.*, using molecular techniques to characterize the ectomycorrhizae of a black truffle plantation, identified their samples Ecm7 and Ecm8 (both showing AD morphotype) both as Ascomycotina belonging to Sarcosomataceae and Pyronemataceae (Pezizales), respectively. Similarly, the molecular analyses performed with “*Q. quadratum*” in the present study showed a maximum identity with both fungal families. However, our sequence EU822505 showed also a close identity (100% maximum identity, 84% coverage) with *Trichophaea woolhopeia* (Cooke & W. Phillips) Arnould, Pezizales, Pyronemataceae (GenBank sequence DQ200835). As there are only five records of *T. woolhopeia* in Iberian Peninsula, very scattered and no one in productive black truffles areas (Pando, 2000), it could not be concluded that “*Quercirhiza quadratum*” belongs to this species. Moreover, three additional species of *Trichophaea* are reported for Spain (s. below).

The members of Pezizales are a considerable proportion of the ectomycorrhizal symbionts in mature boreal deciduous and coniferous forests (Tedersoo *et al.*, 2006), with the potential to rapidly recolonize the site after a disturbance. This could be transferable to Mediterranean stands but there are few records of pezizalean taxa due to the fact that this fungi produce inconspicuous or hypogeous sporocarps that are easily overlooked unless specifically searched for. Molecular techniques could help to increase the knowledge of the role of these group of fungi under different environmental situations.

Ectomycorrhizae of the Pyronemataceae are, in general hydrophylic, of the contact or short-distance exploration type with few rough and clampless emanating hyphae (Agerer, 2006). There are only five ectomycorrhizal genera of the Pyronemataceae known until now: *Genea*, *Humaria*, *Sphaerosporella*, *Tricharina* and *Trichophaea*. The hypogeous genus *Genea* is the only member of this family known today to form pseudoparenchymatous mantles with globular cells on the surface (Agerer, 2006). *Humaria* ectomycorrhizae have also angular outer mantle layers, but epidermoid inner mantle layers and warted, thick-walled emanating hyphae (Erős-

Honti *et al.*, 2008), although all mantle layers of *H. hemisphaerica* are plectenchymatous according to Ingleby *et al.*, (1990). *Sphaerosporella brunnea* ectomycorrhizae have densely packed plectenchymatous mantles with infrequent emanating hyphae (Danielson, 1984; Meotto & Carraturo, 1988). *Tricharina* ectomycorrhizae have plectenchymatous mantles and also infrequent emanating hyphae (Ingleby *et al.*, 1990). *Trichophaea* ectomycorrhizae form pseudoparenchymatous outer mantle layers, but plectenchymatous to pseudoparenchymatous inner mantle layers, with infrequent emanating hyphae (Tederloo *et al.*, 2006). According to the molecular analysis, the sequence EU822505 would be closely related to the genus *Trichophaea*. On the other hand, no significant coincidences in the ITS sequences were found with the *Humaria* and *Genea* ectomycorrhiza characterized by Erős-Honti *et al.* (2008).

In the Iberian Peninsula there are nine records for *Genea*: *G. fragans* (Wallr.) Paoletti, *G. hispidula* Berk., *G. klotzschii* Berk. & Broome, *G. pulchra* Corda, *G. sphaerica* Tul. & C. Tul., *G. subbaetica* Moreno-Arroyo, Gómez & Calonge, *G. thaxterii* Gilkey, *G. vagans* Mattir. and *G. verrucosa* Vittad., five records for *Humaria*: *H. coccinea* (Fr.) Quélet, *H. hemisphaerica* (F.H. Wigg.: Fr.) Fuckel, *H. melaloma* Karsten, *H. sabranskyana* Bäuml., *H. superans* Bond., one for *Sphaerosporella*: *S. brunnea* (Alb. & Schwein.) Svrček & Kubička, three for *Tricharina*: *T. fibrillosa* (Currey) Yang & Korf, *T. gilva* (Bond. ex Cooke) Eckblad, and *T. praecox* (Karst.) Dennis, and four for *Trichophaea*: *T. gregaria* (Rhem.) Bond., *T. hemisphaerioides* (Mouton) Graddon, *T. paraphysinscrustata* Donadini, Torre & Calonge and *T. woolhopeia* (Pando, 2000).

References

- AGERER R., 1991 - *Characterization of ectomycorrhiza*. In: Norris JR, Read DJ, Varma AK (eds.) *Techniques for the study of mycorrhiza*. *Methods Microbiol* 23: 25-73.
- AGERER R., 2006 - *Fungal relationships and structural identity of their ectomycorrhizae*. *Mycol Progress* 5: 67-107.
- AGERER R., RAMBOLD G., 2004–2008 - [first posted on 2004-06-01; most recent update: 2008-01-04]. DEEMY – *An Information System for Characterization and Determination of Ectomycorrhizae*. www.deemy.de – München, Germany.
- ÁGUEDA B., FERNÁNDEZ-TOIRÁN L.M., DE MIGUEL A.M., 2001 - *Ectomicorrizas presentes en la plantación trufera ‘Los Quejigares’ (Soria)*. In: III Congreso Forestal Español, Granada. Tomo 1: 100-106.
- ÁGUEDA B., AGERER R., DE MIGUEL A.M., PARLADÉ J., 2008 - *Quercirhiza quadratum + Quercus ilex L. subsp. ballota (Scop.) Desf. Samp. Descr Ectomyc* 11/12
- BACIARELLI FALINI L., GRANETTI B., 1998 - *Analisi delle micorrize di Tuber melanosporum Vitt. e di altri funghi in una tartufaia coltivata a Corylus colurna L.* *Micol Ital* 1: 3-12.
- BACIARELLI FALINI L., RUBINI A., RICCIONI C., PAOLOCCI F., 2006 - *Morphological and molecular analyses of ectomycorrhizal diversity in a man-made Tuber melanosporum plantation: description of novel truffle-like morphotypes*. *Mycorrhiza* 16:475-484.
- BENCIVENGA M., DONNINI D., DI MASSIMO G., 1992 - *Analisi delle micorrize in una tartufaia coltivata di Tuber melanosporum undici anni dopo l'impianto*. *Micol Veget Medit* 7: 159–171.
- BENCIVENGA M., DI MASSIMO G., DONNINI D., TANFULLI M., 1995 - *Micorrize inquinanti frequenti nelle piante tartufigene. Nota1- Inquinanti in vivaio*. *Micol Ital* 2: 167–178.
- BRAND F., 1991a - *Ektomykorrhizen an Fagus sylvatica. Charakterisierung und Identifizierung, ökologische Kennzeichnung und unsterile Kultivierung*. *Libri Bot* vol. 2, IHW, Eching.
- BRAND F., 1991b - *Genea hispidula*. In: Agerer R (ed.) *Colour Atlas of Ectomycorrhizae*, Einhorn, Schwäbisch Gmünd, plate 57.
- DANIELSON R.M., 1984 - *Ectomycorrhiza formation by the operculate Discomycete Sphaerosporella brunnea*. *Mycologia* 76(3): 454-461.
- DE MIGUEL A.M., SÁEZ R., 1997 - *La Truficultura: reforestación indirecta de áreas residuales*. In: II Congreso Forestal Español, Pamplona. Tomo 6: 431-436.
- DE MIGUEL A.M., SÁEZ R., 2005 - *Algunas micorrizas competidoras de plantaciones truferas*. *Pub. Biol. Universidad de Navarra, Serie Botánica*, 16: 1-18.

- DE ROMÁN M., DE MIGUEL A.M., 2005 - *Post-fire, seasonal and annual dynamics of the ectomycorrhizal community in a Quercus ilex L. forest over a 3-year period*. Mycorrhiza 15: 471-482.
- DI MASSIMO G., GARCÍA-MONTERO L.G., MANJÓN J.L., DÍEZ J., 1996 - *Hongos micorrícicos competidores de Tuber nigrum Bull. (T. melanosporum Vitt.) presentes en ecosistemas naturales y de viveros del centro de España*. Bol Soc Micol Madrid 21: 189-199.
- DOMÍNGUEZ-NÚÑEZ J.A., RODRIGUEZ BARREAL J.A., SÁIZ DE OMEÑACA J.A., 2005 - *Ectomicorizas en dos plantaciones truferas de encina (Quercus ilex L. subsp. ballota (Desf.) Samp.) en Castellón*. Bol San Veg Plagas 31: 147-157.
- ERŐS-HONTI Z., KOVÁCS G.M., SZEDLAY G., JAKUCS E., 2008 - *Morphological and molecular characterization of Humaria and Genea ectomycorrhizae from Hungarian deciduous forests*. Mycorrhiza 18: 133-143.
- ETAYO M.L., DE MIGUEL A.M., 1998 - *Estudio de las ectomicorizas en una trufera cultivada situada en Olóriz (Navarra)*. Pub Biol Universidad de Navarra, Serie Botánica 11: 55-114.
- GARDES M., BRUNS T.D., 1993 - *ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts*. Mol Ecol 2: 113-118.
- GIRAUD 1988 - *Prélèvement et analyse de mycorrhizas*. In: Parra C (ed.) CTIFL – La truffe. FNPT (Paris), pp. 49-63.
- GRANETTI B., BACIARELLI FALINI L., 1997 - *Competizione tra le micorrize di Tuber melanosporum Vitt. e quelle di altri funghi in una tartufaia coltivata a Quercus ilex L.* Micol Ital 3: 45-59.
- INGLEBY K., MASON P.A., LAST F.T., FLEMING L.V., 1990 - *Identification of ectomycorrhizas*. ITE Research publication no. 5.
- JAKUCS E., BRATEK Z. 1998 - *Genea verrucosa*. In: Agerer R (ed.) Colour Atlas of Ectomycorrhizae, Einhorn, Schwäbisch Gmünd, plate 120.
- JAKUCS E, BRATEK Z., AGERER R., 1998 - *Genea verrucosa Vitt. + Quercus spec.* Descr Ectomyc 3: 19-23.
- MEOTTO F., CARRATURO P., 1988 - *Ectomicorizia di Sphaerosporella brunnea (A. & S.) Svrcek & Kubicka in piantine tartufigene*. Allionia 28: 109-116.
- PANDO F., 2000 - *Bases de datos en línea de las colecciones de criptógamas del Herbario MA*. http://www.rjb.csic.es/colecciones_herbario_cripto_consult.php. (18-06-2008).
- SOURZAT P., 1994 - *Guide pratique de Trufficulture*. Station d'experimentation sur la Truffe. Lycée Professionnel Agricole de Cahors. Le Montat.
- SOURZAT P., KULIFAJ M., MONTANT C., 1993 - *Résultats techniques sur la trufficulture à partir d'experimentations conduites dans le Lot entre 1985 et 1992*. Station d'experimentation sur la Truffe. GIS Truffe. Lycée Professionnel Agricole de Cahors. Le Montat.
- TEDERSOO L., HANSEN K., PERRY B.A., KJØLLER R., 2006 - *Molecular and morphological diversity of pezizalean ectomycorrhiza*. New Phytol 170: 581-596.
- VERLHAC A., GIRAUD M., LETEINTURIER J., 1990 - *La Truffe: guide pratique*. CTIFL. Paris.
- WHITE T.J., BRUNS T., LEE S., TAYLOR J., 1990 - *Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics*. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ. (eds.) PCR protocols. A guide to methods and applications. Academic Press. San Diego, USA, pp. 315-322.