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Molecular identification of *Tuber magnatum* ectomycorrhizae in the field

Luana Bertini[a], Ismaela Rossi[a], Alessandra Zambonelli[b], Antonella Amicucci[a], Achille Sacchi[c], Matteo Cecchini[d], Gianluigi Gregori[c], Vilberto Stocchi[a,*]

[a]*Istituto di Chimica Biologica Giorgio Fornaini, Università degli Studi di Urbino, Carlo Bo, Via Saffi 2, 61029 Urbino (PU), Italy*
[b]*Dipartimento di Protezione e Valorizzazione Agroalimentare, Università di Bologna, Via Fanin 46, 40127 Bologna, Italy*
[c]*Centro Sperimentale di Tartuficoltura, Regione Marche, Via Macina 2, 61048 Sant'Angelo in Vado (PU), Italy*
[d]*Istituto e Orto Botanico, Cattedra di Botanica, Università degli Studi di Urbino, Via Bramante 28, 61029 Urbino (PU), Italy.*

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Summary

Tuber ectomycorrhizae in a *Tuber magnatum* "truffière", located in Central Italy, were studied using molecular methods. Specifically, RFLP–ITS analyses, ITS sequencing and specific probes hybridization were used to identify 335 *Tuber*-like ectomycorrhizal morphotypes.

Molecular identification was possible even when distinct morphological characteristics were lacking.

For the first time, *T. magnatum* ectomycorrhizae and other coexisting *Tuber* species collected in the field were analysed using molecular tools for unambiguous identification. Although the "truffière" under investigation yields good harvests of *T. magnatum* fruiting bodies, the percentage of *T. magnatum* ectomycorrhizae found was very low (less than 4.4% of the 335 root tips analysed), whereas the percentages of *Tuber maculatum* and *Tuber rufum* were considerably higher (48.9% and 19.0%, respectively).

*Corresponding author. Tel.: +39 0722 305262; fax: +39 0722 320188.
E-mail address: v.stocchi@uniurb.it (V. Stocchi).

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Introduction

Truffles are the fruiting bodies of ascomycetous fungi belonging to the genus *Tuber*, which form ectomycorrhizae on the roots of angiosperms and gymnosperms. Some *Tuber* species, such as *Tuber magnatum*, produce edible fruiting bodies. *T. magnatum* is the most highly regarded truffle, with a market demand far exceeding the quantities that can be harvested from its natural wild habitats, primarily in Italy and Istria (Croatia) (Hall et al. 1998).

Unlike *Tuber melanosporum*, *Tuber aestivum* and *Tuber borchii*, *T. magnatum* has not been successfully cultivated in truffle beds (Tibiletti and Zambonelli 1999).

This is partly because it is difficult to obtain plants infected with *T. magnatum* that are uncontaminated by other species of white truffle, such as *T. maculatum* and *T. borchii*, or *Sphaerosporella brunnea* (Danielson 1984) and *Pulvinula constellatio* (Amicucci et al. 2001). *T. maculatum*, *T. borchii* and other *Tuber* species also seem to actively compete with *T. magnatum* when artificially infected plants are planted in ''truffières'' (Donnini et al. 2000).

In order to improve *T. magnatum* cultivation and to identify the specific requirements of this fungus, many ecological studies have been carried out in natural ''truffières'' (Hall et al. 1998). However, very few studies have been conducted on the ectomycorrhizal communities of these productive areas. In all previous investigations mycorrhizae were identified using morphological methods, which are imprecise and do not allow us to unequivocally distinguish *T. magnatum* mycorrhizae from those of *T. maculatum, T. dryophilum* and *T. borchii* (Gregori 2002).

The present study uses molecular methods to investigate the ectomycorrhizal communities of different *Tuber* species in a productive *T. magnatum* ''truffière''.

Materials and methods

Geographical location and ecological characteristics of the natural ''truffière

The ''truffière'' under investigation is located in Central Italy in the Marches region, in an area in the province of Pesaro-Urbino known as ''Il Colle'', situated 270 m a.s.l., at 43°46′43″ latitude and 12°45′20″ longitude. Covering about 6 ha, the ''truffière'' lies in a cool shady valley, exposed to the east. The climate is sub-Mediterranean and rainfall usually exceeds 900 mm per year mainly falling around the spring and autumn equinoxes with a high peak in the autumn and a second lower peak in the spring typical of Adriatic inland areas (Bocci 1982).

The physical–chemical characteristics of the soil in the ''truffière'' are reported in Table 1. The ''truffière'' is almost completely covered by vegetation (about 95%), consisting of herbaceous (60%), shrub (18%) and arboreal species (22%). The *T. magnatum* host plants are oaks, poplars, willows

Table 1. Physical–chemical characteristics of soil

Parameters	Average	Maximum values	Minimum values	Standard deviation
Sand (g/kg)	354.0	505.0	134.0	116.0
Mud (g/kg)	470.7	589.0	380.0	77.7
Clay (g/kg)	175.3	298.0	105.0	63.8
pH (H_2O)	8.0	8.5	7.6	0.2
pH (KCl)	7.6	8.1	7.3	0.2
Total carbonate (g/kg)	201.5	280.0	160.0	2.8
Active calcium carbonate (g/kg)	45.4	101.0	24.0	24.8
Organic matter (g/kg)	27.4	46.7	15.7	11.6
Total nitrogen (g/kg)	1.6	2.5	1.1	0.5
Phosphate (assimilable) (mg/kg)	4.0	8.0	1.0	2.2
Potassium (exchangeable) (mg/kg)	271.6	520.0	160.0	106.1
Magnesium (exchangeable) (mg/kg)	392.7	504.0	321.0	69.0
Cation exchange capacity (meq/100 g)	27.4	34.8	21.1	4.7
Iron (assimilable) (mg/kg)	22.8	41.5	10.4	9.1
Manganese (assimilable) (mg/kg)	19.4	26.6	11.1	6.1
Zinc (assimilable) (mg/kg)	2.7	4.6	1.9	0.9
Copper (assimilable) (mg/kg)	2.5	3.5	1.5	0.7