Chapter 14
Enhancing White Truffle (*Tuber magnatum* Picco and *T. borchii* Vittad.) Cultivation Through Biotechnology Innovation

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Abstract *Tuber magnatum* Picco, the Italian white truffle, and *T. borchii* Vittad., the bianchetto truffle, are two European white truffles that command high prices because of their excellent gastronomic properties and unique aromas. Indeed, *T. magnatum* is one of the most expensive culinary delicacies. Although cultivation of *T. borchii* began nearly 200 years after the Périgord black truffle (*Tuber melanosporum* Vittad.), it has rapidly met with market approval and is now cultivated not only in Europe but in countries outside of Europe where this truffle does not grow naturally. In contrast, reliable methods for cultivating *T. magnatum* have only just been developed which hopefully will quickly lead to its widespread cultivation. In this chapter, we present an overview of the distribution, economic importance, a taxonomic appraisal, genetic resource characterization and conservation, and review current cultivation practices. We also review recent biotechnology developments and their potential application for cultivation and domestication of *T. magnatum*. The future prospects of genetic engineering for improving truffle strain characteristics are also addressed.

Keywords Bianchetto truffle · Biology · Cultivation · Italian white truffle · Mycelial cultures · Strain genetic selection

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14.1 Introduction

A hypogenous life cycle is common in nearly every major group of fleshy fungi within the phyla Ascomycota, Basidiomycota and Mucoromycotina (Tedersoo et al. 2010). Underground fruiting structures are formed in most of the Ascomycetes families (Trappe et al. 2009) but *true truffles* are considered to be only those species in the genus *Tuber*, family Tuberaceae, order Pezizales (Zambonelli et al. 2016). The origin of the genus *Tuber* is estimated to be in the early Cretaceous, around 142 Mya (Bonito et al. 2010; Jeandroz et al. 2008) evolving from an epigeous ancestor (Bonito et al. 2010). Spores are sequestered inside the fruiting bodies and generally are dispersed by mycophagous animals that are attracted by a wide range of powerful aromas (Zambonelli et al. 2017). Most truffles live in ectomycorrhizal (ECM) relationships with shrubs and trees in temperate forests mostly in the Northern Hemisphere although the desert truffles inhabit semi deserts of the northern Mediterranean and Africa (Bonito et al. 2013).

The genus *Tuber* includes around 200 species (Bonito et al. 2010) but only a handful have economic value, with the European species the most valuable, such as the white truffles *T. magnatum* Picco (Italian white truffle) and *T. borchii* Vittad. (bianchetto truffle) and the black truffles *T. melanosporum* Vittad. (Perigord black truffle) and *T. aestivum* Vittad. (summer truffle). Other edible black truffles that are locally commercialized in Europe are *T. brumale* Vittad. (winter truffle), *T. mesentericum* Vittad. (truffe mèsentèrique) and *T. macrosporum* Vittad. (smooth black truffle) (Hall et al. 2007). Several other *Tuber* species from China, such us the Chinese black truffles, *T. indicum* Cooke and Massee, *T. sinoaestivum* J.P. Zhang and P.G. Liu, the Chinese white truffle *T. panzhihuanense* X.J. Deng and Y. Wang (Deng et al. 2013; Wang 2012; Zhang et al. 2012) and white North American truffles such as *T. oregonense* Trappe, Bonito and P. Rawl., *T. gibbosum* Harkn. and *T. lyonii* Butters (Lefevre 2012) are locally consumed or exported to Europe where they are often passed-off as their more expensive European cousins.

In this chapter, the new biotechnological approaches for improving cultivation techniques and for germplasm conservation of the European white truffles *Tuber magnatum* and *T. borchii* are detailed and their very different lifestyles contrasted.

14.2 *Tuber magnatum* and *T. borchii* Characteristics

14.2.1 Morphology

Truffles present different morphological features during the three different phases of their life cycles: vegetative as free-living mycelium, symbiotic as ectomycorrhizas, and reproductive ascomata (the fruiting bodies).

The hyphae of all *Tuber* spp. are 3–8 μm in diameter, septate, hyaline and simply branched, showing frequent anastomoses, rare coils and vesicles (Fig. 14.1a) (Iotti

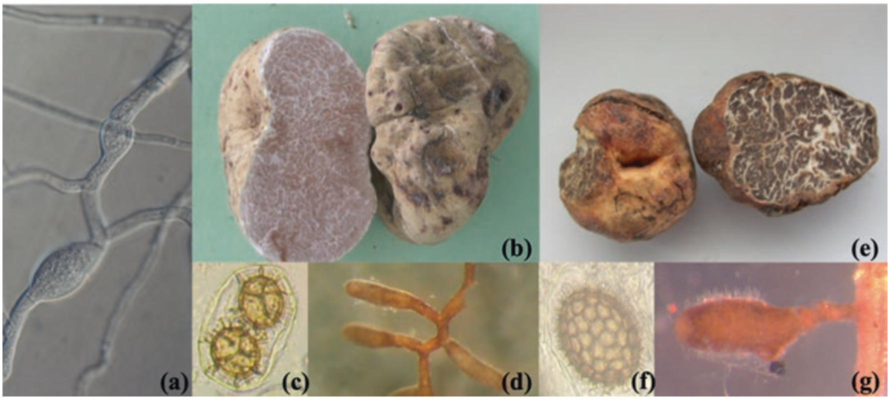

Fig. 14.1 Morphological features of the *Tuber magnatum* and *T. borchii*: (**a**) *T. borchii* mycelium, (**b**) *T. magnatum* ascoma, (**c**) *T. magnatum* spores, (**d**) *T. magnatum* mycorrhizas, (**e**) *T. borchii* ascomata, (**f**) *T. borchii* spore, (**g**) *T. borchii* mycorrhiza. (Figure constructed by A. Zambonelli)

et al. 2002). In *T. borchii* vesicles are more numerous, assuming the characteristics of chlamydospores in unfavorable conditions such as in the presence of adverse bacteria (Barbieri et al. 2005), when cultures age (Iotti et al. 2002) or when iron is deficient (Picceri et al. 2018). The mycelial colonies are regular, whitish in color with most of the biomass growing into the agar or completely submerged when in liquid culture. In contrast, the fruiting bodies and the mycorrhizas differ greatly and are characteristic for each species.

Despite the often very dark to black patches on the surfaces of *Tuber borchii* and *T. magnatum* both are regarded as white truffles because both have ascomata with a smooth peridium and are generally straw-colored. In contrast, the black truffles have a brown to black peridium ornamented by warts, for example, *T. melanosporum, T. aestivum, T. mesentericum* and *T. brumale*. The peridium of *T. magnatum* is smooth, olive-yellow in color; the gleba at maturity is ocher to pale brown marbled with numerous white thin sterile veins (Fig. 14.1b). Occasionally, *T. magnatum* ascomata show pink to red patches on the surface and within the gleba, probably due to bacterial infection by *Microbacterium* and/or *Chryseobacterium* (Amicucci et al. 2018). However, these do not appear to affect the aromatic characteristics of the ascomata. The spores are subglobose to broadly ellipsoid, light-yellow, yellow-ocher at maturity, ornamented with an irregular reticulum (2–) 3–5 (−7) μm high, with 2–4 meshes across the width of the spores. (Fig. 14.1c). In contrast, the peridium of *T. borchii* is minutely pubescent caused by a layer of yellow reddish-brown colored hyphae with setose hairs and the gleba at maturity is dark brown with wide, white veins (Fig. 14.1e). The spores are ellipsoid to broadly ellipsoid, ocher-brown at maturity, ornamented with a regular reticulum with meshes 3–7 (−10) μm high, 3–10 across the width of spores (Fig. 14.1f) (Zambonelli et al. 2000a).

The mycorrhizas of both species are macroscopically similar, simple or ramified with whitish-ocher, fulvous colors, in *Tuber borchii,* and ocher to grayish-white in *T. magnatum* (Mello et al. 2001; Zambonelli et al. 1993). Microscopically the mantles are similar, composed of epidermoid cells ornamented by awl-shaped hyaline cystidia. These are never ramified in *Tuber borchii* ectomycorrhizas and 70–140 µm long (Fig. 14.1g), whereas they are often branched in *T. magnatum* and up 480 µm long (exceptionally 580 µm) (Giomaro et al. 2000; Mello et al. 2001; Riccioni et al. 2016) (Fig. 14.1d).

14.2.2 Distribution

Until the turn of the millennium *Tuber magnatum* was considered an almost exclusive Italian truffle restricted to the north and center of the country with smaller patches, in Istria, Croatia southeastern France and the Ticino Canton of Switzerland (Hall et al. 1998). However, recent studies have found it in Sicily (Vasquez et al. 2014), the Geneva Canton of Switzerland (Büntgen et al. 2019), Hungary and several Balkan regions (Bratek et al. 2007; Riccioni et al. 2016). Intriguingly, is has also been found in Thailand (Suwannarach et al. 2017). In contrast, *T. borchii* has one of the widest distributions of any truffle being found throughout Europe, from southern Finland to Sicily and from Ireland to Hungary and Poland (Hall et al. 2007; Shamekh et al. 2009) (Fig. 14.2).

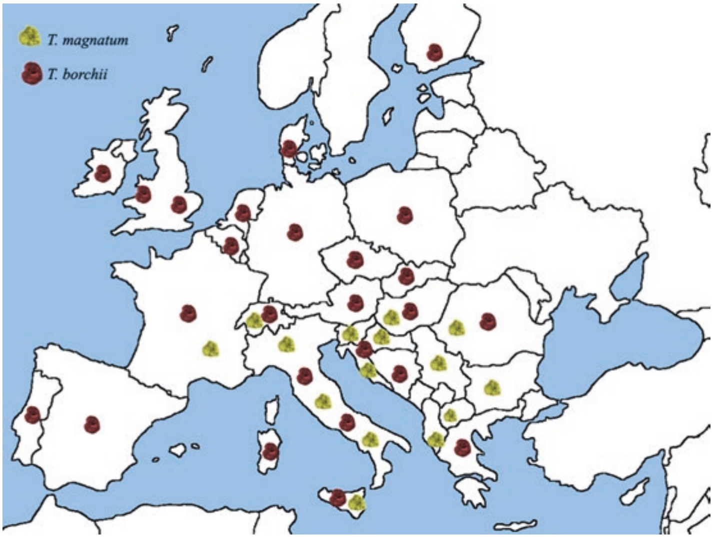

Fig. 14.2 Geographical distribution of *Tuber magnatum* (green symbols) and *T. borchii* (brown symbols). (Sources: Gogan-Csorbai et al. 2018; Hall et al. 2007)

14.2.3 Economic Importance

The high economic value of truffles is due to their unique aromas and flavors, which make them one of the most expensive food delicacies. *Tuber magnatum* is the truffle that commands the highest prices although this varies depending on the size of the ascoma and from season to season. In poor harvest years such as 2017, the retail prices in Italy were very high ranging between 3500 EUR/kg and 5000 EUR/kg. The production in 2018 was greater and prices fell to 1000–2100 EUR/kg (https://www.termometropolitico.it/1332538_2019-05-02-prezzo-tartufo-bianco.html). Outside Italy even higher prices can be paid, for example, *T. magnatum* was sold in Harrods, London, for GBP 6500/kg on 25 September 2010 (Badalyan and Zambonelli 2019; Hall, personal communication). *Tuber borchii* commands lower prices than *T. magnatum* and *T. melanosporum*, although recently it has become more appreciated and prices have risen somewhat. In 2019 retail prices in Italy were 105–305 EUR/kg depending on the size (https://www.andareatartufi.com/wordpress/quotazioni-tartufo-bianchetto/rom). In contrast, the off-season price of *T. borchii* produced in New Zealand that are not mixed with inferior species of white truffle can fetch much higher prices that hover around NZD 3000/kg (about 1700 EUR/kg (http://www.trufflesandmushrooms.co.nz/Tuber%20borchii%20web.pdf). Unlike black truffles, white truffles are used either uncooked or added to dishes just after cooking in order to retain the delicate, volatile aromas such as when flavoring pasta or salads.

14.2.4 Ecology

Tuber magnatum has specific ecological requirements, which limit its distribution in Europe. It is generally found below 600 m elevation, but in Southern and Central Italy it can be found up to 900 m elevation. It develops in woods with more or less closed canopies, as well as areas with relatively sparse vegetation. It often fruits in floodplains or around stream beds probably because its mycelium displays a high demand for water for growth in soil (Iotti et al. 2018; Marjanović et al. 2015). The host plants are: *Alnus cordata* Desf. (Italian alder), *Corylus avellana* L. (hazelnut), *Ostrya carpinifolia* Scop., *Populus alba* L. (white poplar), *P. tremula* L. (European trembling aspen), *P. nigra* L. (Lombardy poplar), *Quercus cerris* L. (Turkish oak), *Q. ilex* L. (holm oak), *Q. pubescens* Willd. (downy oak), *Q. robur* L. (English or common oak), *Salix alba* L. (white willow), *S. caprea* L. (pussy willow), *Tilia cordata* Mill. (small-leaved lime), *T. platyphyllos* Scop. (large-leaved lime) (Hall et al. 2007) and *Fagus sylvatica* L. (Büntgen et al. 2019). It is also, though rarely, found under *Pinus* spp. (Zambonelli, personal communication) but the ectomycorrhizal association with conifers has never been verified with molecular tools. *Tuber magnatum* requires well-drained soils without gravels, having a preference for neutral to extremely alkaline soils (pH 7–8.5), with abundant active $CaCO_3$ (Hall et al. 1998).

The peculiar characteristics of *T. magnatum* soils are good aeration and very soft consistency due to its elevated volume (around 15%) occupied by interconnected macropores (Bragato and Marjanović 2016). The climatic requirements include a winter temperature range of 2–8 °C (in January) and summer temperatures of 18–26 °C (in June). Ideal precipitation ranges between 500–2000 mm and spread more or less evenly throughout the year, although in summer this tends to be in the form of thunderstorms (Hall et al. 2007). The ascomata are sensitive to winter frost and summer drought which helps explain their preponderance in riparian habitats (Iotti et al. 2018; Le Tacon 2016).

Tuber borchii has broad ecological requirements and is found from sea level to 1100 m elevation (Tanfulli et al. 1999). It forms ectomycorrhizal associations with a wide range of host plants including several species of conifers including pines and cedars (Table 14.1). It is most commonly found on *Pinus* spp. particularly *P. pinea* and *P. pinaster* in the coastal areas of the Mediterranean Sea. Recently it was found to form mycorrhizas with commercially interesting fruit plants such as the strawberry tree (*Arbutus unedo*, Lancellotti et al. 2014) and pecan (*Carya illinoinensis,* Benucci et al. 2012). Surprisingly, it can also form mycorrhizas with orchids (Tešitelová et al. 2012) confirming its capacity to form symbiotic associations with herbaceous plants, as predicted by Mannozzi-Torini (1988).

Tuber borchii grows in moderate to strongly calcareous, very sandy to silty soils (Fig. 14.3) such as those in coastal areas of Italy, as well as in the same alkaline soils where the Italian white truffles are harvested in the Apennines. In Italy it is also found in neutral and slightly acidic soils where the pH ranges from 6–7. Occasionally the pH can be as low as 5.2 (Gardin 2005) but such soils are more likely to be the home of inferior white truffles such as *Tuber maculatum* Vittad. (Hall, personal communication). The climate of areas where bianchetto is found in Italy and New Zealand ranges from cool temperate to Mediterranean, with annual rainfall of 600–1600 mm.

14.2.5 Aroma Characteristics

The aroma of truffles is due to the volatile organic compounds (VOCs) which are produced during ascoma formation and maturation. Aroma production is a biological strategy used by truffles to attract mycophagous animals which disseminate their spores (Zambonelli et al. 2017). Although around 300 VOCs have been reported in truffles (Splivallo et al. 2011) only 10–20 odorants per species are responsible for the typical truffle odors perceived by the human nose (Schmidberger and Schieberle 2017). Some of these are common to several truffle species particularly 2- and 3-methylbutanal, 2- and 3-methylbutan-1-ol and oct-1-en-3-ol. Others are species-specific such as 2,4-dithiapentane in *Tuber magnatum* and thiophene derivatives in *T. borchii* (Fiecchi et al. 1967; Gioacchini et al. 2005; Splivallo and Ebeler 2015; Splivallo et al. 2011).

Table 14.1 Putative host plants of the white truffles

Family	Species	*Tuber borchii*	*T. magnatum*
Betulaceae	*Alnus cordata*	*	*
	Corylus avellana	*	*
	Ostrya carpinifolia	*	*
Fagaceae	*Fagus sylvatica*	*	*
	Quercus cerris	*	*
	Q. ilex	*	*
	Q. petraea	*	*
	Q. pubescens	*	*
	Q. robur	*	*
Tiliaceae	*Tilia americana*	*	
	T. cordata	*	*
	T. × *europaea*	*	*
	T. platyphyllos	*	*
Juglandaceae	*Carya illinoinensis*	*	
Pinaceae	*Abies alba*		*
	Cedrus atlantica	*	*
	C. deodara		
	Larix spp.	*	
	Picea excelsa	*	
	Pinus brutia	*	
	P. nigra ssp. *nigra*, *P. nigra* ssp. *nigricans*	*	
	P. pinaster var. *atlantica*	*	
	P. pinea	*	*
	P. strobus	*	
	P. sylvestris	*	
Salicaceae	*Populus alba*	*	*
	P. nigra	*	*
	P. tremula		*
	Salix alba	*	*
	S. caprea	*	*
Ericaceae	*Arbutus unedo*	*	
Cistaceae	*Cistus albidus*	*	
	C. incanus		
	C. monspeliensis		
	C. salviaefolius and other *Cistus* spp.		
Orchideaceae	*Epipactis* spp.	*	

Sources: Benucci et al. (2012), Büntgen et al. (2019), Hall et al. (2007), Lancellotti et al. (2014) and Tešitelová et al. (2012)

Truffle products aromatized using synthetic scents are quite different and generally less appealing than fresh ascomata, primarily because they contain a markedly higher quantity of 2,4-dithiapentane and two VOCs, dimethyl sulfoxide and dimethyl sulfone, which are absent from ascomata (Wernig et al. 2018).

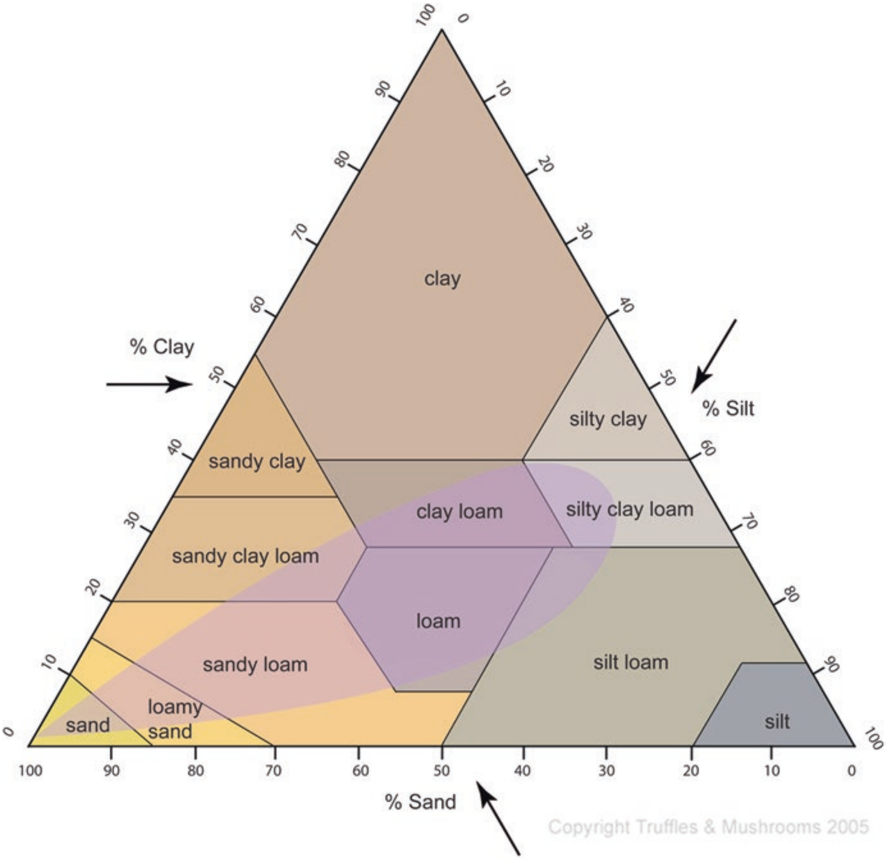

Fig. 14.3 Soil textural triangle for the bianchetto truffle. The area marked in violet is suitable for this truffle. The arrows indicate the direction the reader needs to read the graph. (Source: Hall et al. 2007)

Not only does genotype affect aroma (Splivallo et al. 2012) but also associated bacteria, yeasts and other fungi that also produce VOCs and contribute to truffle aromas (Buzzini et al. 2005; Pacioni and Leonardi 2016; Splivallo and Ebeler 2015; Splivallo et al. 2014; Vahdatzadeh et al. 2015). Truffle aroma is also influenced by other environmental factors such as soil composition, climate and the host plant (Splivallo and Culleré 2016). It is therefore not surprising that as ascoma maturity, methods for storing, changes in microbiome composition, season and geographical location, all affect VOCs profiles and perceived quality (Gioacchini et al. 2008; Pennazza et al. 2013; Vahdatzadeh et al. 2019; Vita et al. 2015; Zeppa et al. 2004). Mycelial fermentation techniques have been used to produce natural truffle aromas but these were found to be less complex (Splivallo et al. 2007; Tirillini et al. 2000). For example, 3-methyl-4,5-(2 H)thiophene is produced only during the sexual stage of *Tuber borchii* fruiting body formation. However, the VOCs composition of *Tuber* mycelium can be improved by supplying axenic cultures of truffle mycelium with leucine, isoleucine, phenylalanine and methionine inducing the synthesis of the VOCs derived from amino acid catabolism (Ehrlich pathway) (Li et al. 2012; Liu et al. 2013; Splivallo and Maier 2016; Vahdatzadeh and Splivallo 2018; Xiao et al. 2015). It is hoped that strain selection may be used to improve truffle mycelium VOC production (Vahdatzadeh and Splivallo 2018) as well as repeated freeze-thaw cycles (Xiao et al. 2015).

This capacity of *Tuber* mycelium to produce VOCs may be commercially exploitable and lead to the production of natural flavor with a higher consumer acceptance of truffle-flavored food products (i.e. truffle-flavored olive oil) that currently predominantly contain synthetic flavors (Splivallo and Maier 2016).

14.3 Cultivation

14.3.1 Traditional Cultivation Practices

The first techniques for cultivating truffles were developed for *Tuber melanosporum*, possibly independently by Josef Talon and Pierre Mauléon in the nineteenth century (Hall and Zambonelli 2012). They simply sowed acorns collected under oaks that were producing truffles and then transplanted these into adjacent soils. Only in the 1970s was modern truffle cultivation introduced into France and Italy after the discovery of the mycorrhizal nature of truffles. This consists of producing mycorrhizal plants in the greenhouse and then out planting them into areas with suitable soils and climate. Using these techniques, *T. aestivum*, *T. borchii*, and *T. brumale* are now cultivated as well as *T. melanosporum,* although the latter is still the most extensively cultivated, not only in Europe but in countries outside of Europe (Hall et al. 2017; Reyna and Garcia-Barreda 2014). In recent years *T. borchii* cultivation has become more widespread in Italy (Zambonelli et al. 2015), Spain, Portugal (https://micofora.com/en/growing-tuber-borchii/) and in France. *Tuber borchii* is also successfully cultivated in New Zealand, Australia the USA (Zambonelli et al. 2015) and China (Wang Yun, personal communication).

The production of *Tuber* mycorrhizal plants represents the first important step in modern truffle cultivation. The plants are preferably colonized with a single *Tuber* species of interest and are preferably free of other contaminating ectomycorrhizal fungi. Generally, the quantification of mycorrhizal formation is assessed morphologically. Under a stereomicroscope the percentage of root tips colonized by *Tuber* spp. is counted or estimated (Andrés-Alpuente et al. 2014) although the use of this method requires trained personnel (Sisti et al. 2010).

Although, the link between the level of mycorrhizal formation on inoculated seedlings prior to planting has not been conclusively demonstrated, planting seedlings with high degrees of truffle mycorrhizal colonization is recommended to improve the chance of truffle production in plantations (Murat 2015). For this reason in Italy and France *Tuber* mycorrhizal plants can be commercialized only if the degree of mycorrhization (number of root tips colonized with *Tuber*/total number of tips ×100) is above 30% (Donnini et al. 2014; Govi et al. 1997; Regione Emilia-Romagna 2018). *Tuber borchii* commercialized plants usually exceed this level of mycorrhizal infection. In contrast, the mycorrhizas of *T. magnatum* can be difficult to obtain and only a company in France is actually selling *T. magnatum* mycorrhizal plants at prices 5–10 times higher than *T. borchii* or black truffle mycorrhizal plants. Morphological and molecular methods have now been perfected in order to identify and quantify mycorrhizal formation on root systems (Alvarado and Manjon 2013; Andrés-Alpuente et al. 2014; Fischer and Colinas 1996; Mello et al. 2006; Rocchi et al. 1999; Zambonelli et al. 1993, 2012a). In contrast, to ectomycorrhizas of black truffles (*T. aestivum*, *T. brumale* and *T. melanosporum*) (Zambonelli et al. 1993), the morphological characters of all white truffles are similar, and in particular the mycorrhizas of *T. maculatum*, *T. oligospermum*, *T. dryophilum* and *T. borchii* (Boutahir et al. 2013; Zambonelli et al. 1999) which require the use of molecular techniques to be sure. Species specific primers for *T. borchii* and *T. magnatum* were designed to be used in simple, multiplex or quantitative PCR (Amicucci et al. 1998, 2000; Iotti et al. 2012a; Mello et al. 1999; Rubini et al. 2001) (Table 14.2). Spore inoculation is used by the vast majority of companies that produce *Tuber* mycorrhizal plants because it is simple and effective. First, fresh, dry or frozen truffles are ground in water to create a spore suspension which is then used to inoculate sterile seedling or cuttings a few months old. However, with spore inoculation techniques, plants can become contaminated with undesirable truffle species if the truffles are not carefully selected and identified. Moreover, pests and pathogens can be introduced with the inoculum. Furthermore, because ascospores are produced sexually, each plant is potentially colonized by a different suite of fungal genotypes from every other with unknown characteristics.

In order to achieve ascoma production the infected plants have to be planted in the field in environmental conditions (soil, climate) that suit both the host plant and the truffle. *Tuber borchii* tends to fruit more quickly than *T. melanosporum.* In experimental *Pinus pinea* plantations the first ascomata were found only 3–4 years after planting (Hall, personal communication; Zambonelli et al. 2000b). Although, *T. borchii* cultivation is becoming more common, no specific research on how to manage *T. borchii* cultivation has been carried out and no handbook on *T. borchii* cultivation is available in the market, although a booklet is available from Ian Hall (2017) that reviews what are thought to be the ideal soil and climatic conditions. However, farmers tend to copy guidelines for *T. melanosporum* cultivation which in low pH soils can result in excessive applications of lime and depressed soil trace element concentrations.

Some success has recently been reported by Bach et al. (2021) in the production of *T. magnatum* in France and it is to be hoped that this will lead to its routine cultivation rather than the erratic positive results obtained in Italian experiments of the 1990s and 2000s (Bencivenga et al. 2009).

Table 14.2 Molecular markers used for genetic characterization in *Tuber borchii* and *T. magnatum*

Tuber species	Marker type	Sequence (5′–3′)/repeat motif [1]	Primer name/ locus name[1]	Reference
Tuber borchii	ITS-rDNA	TGTATGGGATGCCCTATCGGACT	TboI (fwd)	Amicucci et al. (1998)
		CTATTACCACGGTCAACTTC	TboII (rev)	
	ITS-rDNA	TGCCCTATCGGACTCCCAAG	TBA (fwd)	Mello et al. (1999)
		GCTCAGAACATGACTTGGAG	TBB (rev)	
	ITS-rDNA	GAAGTTGACCGTGGTAATAG	rTboII (fwd)	Amicucci et al. (2000)
		TCCTCCGCTTATTGATATGC	ITS4 (rev)	
	SSR	$(TATTTT)_{10}$	Tb1	Leonardi et al. (2019)
		$(AGGC)_{8}$	Tb11	
		$(AAC)_{8}$	Tb151	
		$(GGA)_{12}$	Tb155	
		$(GAG)_{8}$	Tb156	
		$(TTTAGA)_{5}$	Tb17	
		$(CCTT)_{8}$	Tb206	
		$(GAGGGA)_{6}$	Tb244	
		$(AGAAGG)_{5}$	Tb293	
		$(CTTTT)_{5}$	Tb43	
		$(TACC)_{8}$	Tb43bis	
		$(AGA)_{9}$	Tb46	
		$(AAAG)_{8}$	Tb704	
		$(GACT)_{8}$	Tb83	
Tuber magnatum	ITS-rDNA	GGATGCGTCTCCGAATCCTGAAT	Tmag1[a] (fwd)	Amicucci et al. (1998)
		CGGGCCCTTTCTCAGACTGCTG	Tmag2 (rev)	
	ITS-rDNA	TCCTACCAGCAGTCTGAGAAAGGGC	P7 (fwd)	Mello et al. (1999)
		TGAGGTCTACCCAGTTGGGCAGTGG	M3[b] (rev)	
	ITS-rDNA	TCCTCCGCTTATTGATATGC	ITS4 (rev)	Amicucci et al. (2000)
	ITS-rDNA	GTCACTGAAAACCCACTCACG	TSMAGN (fwd)	Rubini et al. (2001)
		TGAGGTCAACCCAGTTGGACAGT	ITSBACK3 (rev)	
	β-tubulin	CCTCCCAATTTGCAATACAC	tubmagnf (fwd)	Zampieri et al. (2009)
		AAAGACGAAGTTATCTGGCCTGA	elytubr (rev)	
	ITS-rDNA	GCGTCTCCGAATCCTGAATA	TmgITS1for (fwd)	Iotti et al. (2012a)
		ACAGTAGTTTTTGGGACTGTGC	TmgITS1rev (rev)	
		TGTACCATGCCATGTTGCTT	TmgITS1prob	
	SSR	$(AC)_{20}(TC)_{18}$	MA2	Rubini et al. (2004)
		$(GA)_{17}$	MA4	
		$(AC)_{18}(TC)_{10}$	MA5	
		$(GT)_{16}$	MA7	
		$(CT)_{16}(T)_{14}$	MA12	
		$(TC)_{16}/(TC)_{7}$	MA13	
		$(GA)_{30}$	MA14	
		$(TG)_{15}$	MA19	

(continued)

Table 14.2 (continued)

Sources: Parladé et al. (2016) with modifications; Zambonelli et al. (2012a)

[1] Sequence and primer name are referred to internal transcribed spacer (ITS) of nuclear ribosomal DNA (rDNA), i.e. ITS-rDNA. β-tubulin markers, repeat motif and locus name for short sequence repeat (SSR) markers

[a] In multiplex PCR with ITS4 as reverse primer

[b] In multiplex PCR with ITS1 as forward primer

14.3.2 New Biotechnologies for Cultivating Tuber borchii

Tuber borchii is considered the model species for genetic and biological studies on truffles because its mycelium can be cultured in vitro more easily than other species of truffle. In contrast, difficulties in making pure cultures of *T. magnatum* have always hindered insights into its biology.

The first mycorrhizal plants with *Tuber borchii* mycelium were produced in greenhouses on *Pinus strobus* and poplar by the Italian researchers Fontana and Palenzona (1969). Later a technique to produce *Tuber* mycorrhizal plants under in vitro conditions on *Populus alba* micropropagated plantlets was perfected by Zambonelli et al. (1989), and later on with *Tilia platyphyllos* (Sisti et al. 1998). The method involves raising micropropagated plantlets in a medium with a low concentration of auxin (0.2 mg/l of NAA) to allow the differentiation of roots with multiple secondary roots with limited growth. The mycelium of *T. borchii* is then isolated onto PDA agar in Petri dishes and then bulked up in MMN liquid medium. The mycorrhizal synthesis is performed in vermiculite moistened with MS/2 liquid medium at pH 6.3 (Giomaro et al. 2005). Subsequently, *Tilia platyphyllos-Tuber borchii* mycorrhizal plants became a model to study plant/fungus interactions (Menotta et al. 2004; Polidori et al. 2002, 2007; Zeppa et al. 2002).

Tuber borchii mycorrhizal plants inoculated with mycelia have never been produced commercially for a variety of reasons. Spore inoculation is easier and gives good results with *T. borchii*. Moreover, nurseries producing *Tuber* mycorrhizal plants are usually not equipped with laboratories for isolating and cultivating *Tuber* mycelium in vitro. Although *T. borchii* mycelium generally grows more quickly than other *Tuber* spp. (Iotti et al. 2002) it is still more difficult to manipulate compared with other mycorrhizal mushrooms. It was not until a few years ago that there was evidence that plants inoculated with mycelial-pure cultures could produce truffles. In fact, Paolocci et al. (2006) hypothesized, and later confirmed by Martin et al. (2010), that when the *T. melanosporum* genome was sequenced and the mating type idiomorphs characterized, that truffles were heterothallic and that two strains carrying different mating types would be needed to mate in order to produce the fruiting bodies (de la Varga et al. 2017; Rubini et al. 2014).

Iotti et al. (2016) reported that the first experimental *Tuber borchii* truffle orchard inoculated with 5 different *T. borchii* strains individually or mixed had started to produce and 99 ascomata were eventually collected between February and April with a total fresh weight of 722 g. This demonstrated for the first time that it was possible to produce truffles with mycorrhizal plants inoculated with pure cultures.

Subsequently, a study conducted on this truffière by Leonardi et al. (2019) showed that the paternal genotypes originated by recombination of the inoculated strains and only in a few cases from alien genotypes. Furthermore, 4 of the 5 inoculated strains persisted 9 years after establishing the plantation and remained confined to their inoculated plants without migrating into the neighboring strain areas.

The isolation and genetic characterization of different strains from geographical regions with varying soil and climatic conditions could lead to strains that produce ascomata with enhanced organoleptic characteristics and suited to particular environmental conditions. Recent work conducted by Vahdatzadeh and Splivallo (2018) on the mycelium of nine *Tuber borchii* strains has highlighted how genetically-different strains produce different aromas. Other work conducted by Leonardi et al. (2017) has shown how *T. borchii* strains differ in their tolerance to heat stress, demonstrating that it should be possible, in this regard at least, to select the most suitable strains for a specific cultivation area.

14.4 Genetic Diversity and Germplasm Conservation

14.4.1 Genetic Diversity

A proper understanding of genetic variation within and among *Tuber* species and populations, as well as their phylogeography, is indispensable for efficient utilization of a valuable truffle.

The first studies on the genetic diversity of *Tuber* species involved the analysis of random amplified polymorphic DNA (RAPD) and sequence characterized amplified region (SCAR) markers (Amicucci et al. 1997; Bertini et al. 1998; Gandeboeuf et al. 1997a, b; Lanfranco et al. 1993; Potenza et al. 1994; Rossi et al. 2000). However, ITS regions of nuclear rDNA, alone or in combination with other loci, quickly become the elective markers to explore the genetic diversity within *Tuber* species and to infer their phylogenetic relationships (Amicucci et al. 1996; Bonito et al. 2010; Bonuso et al. 2010; Guillemaud et al. 1996; Halász et al. 2005; Henrion et al. 1994; Huang et al. 2009; Iotti et al. 2007; Mello et al. 1996; Merényi et al. 2014; Murat et al. 2004, 2005; Paolocci et al. 1995, 2004; Qiao et al. 2018; Riccioni et al. 2019; Sica et al. 2007; Wang et al. 2006; Wedén et al. 2005). Microsatellite markers were first investigated for truffle typing (Amicucci et al. 2002; Longato and Bonfante 1997) but they were found more useful for studying population genetics (Bertault et al. 2001; Figliuolo et al. 2013; Leonardi et al. 2019; Linde and Selmes 2012; Molinier et al. 2015, 2016a, b; Murat et al. 2013; Riccioni et al. 2008; Rubini et al. 2005; Taschen et al. 2016).

The first studies of *Tuber magnatum* and *T. borchii* have surprisingly deduced that the two species fall into two distinct clades of the genus *Tuber* (Percudani et al. 1999). *Tuber magnatum* is co-located inside the Aestivum clade which includes species that are morphologically quite different: *T. aestivum, T. mesentericum* and

T. sinoaestivum, three species of black truffle characterized by a warty peridium and reticulate ascospores, and *T. panniferum* which is a unique truffle with a wooly peridium and very spiny ascospores (Bonito and Smith 2016; Bonito et al. 2013; Jeandroz et al. 2008). In contrast, *T. borchii* is co-located inside the Puberulum clade, which includes at least 63 morphologically-similar species distributed across Europe, Asia, North America, South America and North Africa (Bonito et al. 2013; Lancellotti et al. 2016).

Several studies on *Tuber magnatum* genetic intraspecific variability have been carried out to find molecular markers that may define its geographic origin. The first studies, carried out by RAPD (Amicucci et al. 1997; Gandeboeuf et al. 1997a; Lanfranco et al. 1993; Pomarico et al. 2007; Potenza et al. 1994) showed a very low intraspecific polymorphism in *T. magnatum* with respect to other *Tuber* spp. This low genetic diversity in *T. magnatum* was later confirmed by several researchers (Iotti et al. 2012a; Mello et al. 2005; Pomarico et al. 2007) who analyzed ITS regions and other genetic markers. Only simple sequence repeat loci (SSR) proved to be suitable as markers for population genetic studies of *T. magnatum*. Rubini et al. (2004) first selected 8 polymorphic SSRs and used them to analyze 370 ascomata from 5 main areas. They defined 28 populations, one of which was from the Istria region of Croatia and 4, 12, 10 and 1 from the north, center, center-south and south of Italy, respectively.

Recently a new transcriptome assembly of the Italian white truffle has allowed the identification of 2581 gene-based SSR markers (Vita et al. 2018). In addition, the *T. magnatum* genome has now been sequenced (Murat et al. 2018a) and a bioinformatics workflow was applied in order to mine for microsatellite sequences. As a result, 11,189 microsatellite markers specific to *T. magnatum* were found with 3377 marker loci matching with *T. magnatum* proteins (Uncu and Uncu 2019). This will provide some important new tools to explore *T. magnatum* genetic diversity and to find genetic markers that might define the genetic origin of the ascoma.

After the first studies on the genetic diversity of *Tuber borchii* using RAPD (Bertini et al. 1998; Gandeboeuf et al. 1997a) and IGS (Ciarmela et al. 2002), a multigene phylogeny was carried out on 61 representative specimens with a broad distribution throughout Italy (Bonuso et al. 2010). In this study two cryptic species of *T. borchii* were identified.

Recently the *Tuber borchii* genome has also been sequenced (Murat et al. 2018b) and showed 1111 SSRs. Among them 14 SSRs were highly polymorphic and were able to differentiate single genotypes after testing on 50 ascomata harvested in Italy and Hungary (Leonardi et al. 2019).

14.4.2 Genetic Resources Conservation Approaches

The conservation of truffle genetic resources is becoming more important than ever because of the increasing threat from deforestation, overharvesting, climate warming and changing agricultural practices.

Truffle cultivation could be an efficient method to ex situ conserve *Tuber* genetic resources (Varese et al. 2011). To achieve this goal, it would be important to use only spore inoculation methods from locally-harvested truffles in order to secure their genetic diversity. Although truffles growing in natural areas are assumed to be genetically adapted to the site's climatic and edaphic conditions, it is also possible that they represent the vestiges of a distribution when conditions may have been quite different. Regardless, the creation of a germplasm bank of *Tuber* spp. is an imperative in the future once mycelial inoculation becomes the method of choice for nurseries producing *Tuber* mycorrhizal plants for cultivation.

14.4.2.1 In Vitro Conservation of *Tuber* spp. Genetic Resources

In the conservation of mycelial pure cultures in vitro it is very important to ensure that their purity, vitality and genetic integrity is maintained over time (Smith 2004; Voyron et al. 2007). Currently, many conservation methods are available (Nakasone et al. 2004) and the choice of the most appropriate method should depend on the species under consideration and on the purpose of conservation.

The short-term conservation of *Tuber* mycelial subcultures, obtained by taking small plugs of mycelium from the edge of a colony, is easiest and inexpensive, but the risk of nutrient medium contamination is high. Furthermore, *Tuber* mycelium is known to grow very slowly in pure culture (Iotti et al. 2002, 2012b) and repeated transfers may lead to the loss of infectivity, viability and induce mutation or changes in gene expression (Coughlan and Piché 2005; Smith 2012).

Another preservation technique is cryopreservation in liquid nitrogen, which consists of preserving the biological material at ultra-low temperature after the addition of several cryoprotectants. In this process, the material is gradually cooled down to a temperature close to −196 °C, and then stored at that temperature. In this condition all biological activity, including biochemical reactions that would lead to cell death, are blocked and prevents any cellular and genetic damage (Smith et al. 2001). Moreover, cryopreservation reduces work load and saves space needed for preservation on agar, ensures the continued infectivity of strains, and streamlines the accessibility of the collection. The greatest disadvantages of this technique are the large initial investment and ongoing operating costs primarily for the continuous supply of liquid nitrogen which needs to be constantly controlled and maintained. Cryopreservation of *Tuber borchii* mycelium was tested for the first time by Stielow et al. (2012) although the survival ratio on charcoal filter paper strips after thawing was low. In 2017, Piattoni et al. (2017) used a different cryopreservation protocol, employing sorbitol, sucrose and dimethyl sulfoxide as cryoprotectans which allowed cryopreservation of *T. borchii* mycelium without reducing its infectivity.

A similar technique to that of cryopreservation, with the high survival rate of biological material, is ultra-freezing (Kitamoto et al. 2002). This differs from cryopreservation at a higher storage temperature, ranging from −80 to −130 °C and the use of an ultra-freezer instead of liquid nitrogen. This technique, applied to various ectomycorrhizal mushrooms, has shown differing results depending on the cryoprotectans used and the protocol applied (Crahay et al. 2013; Kitamoto et al. 2002; Obase et al. 2011). A recent protocol, applied to the medicinal mushroom *Ganoderma lucidum* (Curtis) P. Karst., developed by Leonardi et al. (2018) also showed very promising results with *Tuber* spp. and currently tests are underway to verify the survival of *Tuber* spp. mycelia at ultra-freezing temperatures.

14.4.2.2 In Situ Conservation of *Tuber magnatum*

Tuber magnatum has yet to be successfully cultivated so the only way to commercially exploit it without damaging natural production is to conserve it in situ. Intensive harvesting of truffles has been shown to cause decreases in truffle production in Europe and China as a consequence of physical damage to *Tuber* ectomycorrhizas, the soil mycelium and habitat, and perhaps suppressing fertilization. In order to protect this important resource, it is essential to enforce strict rules during harvest to protect their unique environment, to ban the harvesting of unripe ascomata by, for example, raking and to stop overharvesting in some areas allowing spore diffusion.

Regretably, fungi seldom receive legal protection and examples of in situ conservation are rare (Venturella et al. 2011) despite the Italian national law (L. 752/1985, https://www.gazzettaufficiale.it/eli/id/1985/12/21/085U0752/sg) that covers the most important rules for harvesting, cultivation, conservation and marketing of truffles. Some positive aspects of this law are, for example, that immature truffles are protected, dogs (rather than pigs) must be used to locate truffles, a special small trowel must be used to excavate the truffles and excavations are to be covered immediately after a truffle is harvested to avoid damage to the mycorrhizas. However, the biggest concern for *T. magnatum* in situ conservation is the ever-increasing number of truffle harvesters, which currently stands at around 45,000 in Italy (Ministry of Agriculture, Food and Forestry Policies and Tourisms https://www.politicheagricole.it/flex/cm/pages/ServeBLOB.php/L/IT/IDPagina/11100). Sadly, these collectors have open access to extensive oak forests and woodlands and other lands not under cultivation and occupied by *T. magnatum*. Furthermore, there is a lack of political motivation to reduce the number of collectors or to create truffle reserves or ecological corridors, where truffle harvesting is forbidden.

The preservation and plantation of *Tuber magnatum* host plants and the protection of the specific truffle environments are the only effective conservation measures proposed in some parts of Italy. In some municipalities of Tuscany, for example, the owners of *T. magnatum* productive trees receive a reward if they do not cut the trees. In some regions, the local government can give landowners the exclusive right to collect truffles but only after it has been verified that their productive forest has been significantly improved through an adequate thinning of the undergrowth.

Until recently, traditional cultural practices have often been applied in an attempt to improve *Tuber magnatum* areas without knowledge of their subsequent effects. This is because *T. magnatum* ectomycorrhizas are rarely detected in the field (Leonardi et al. 2013; Murat et al. 2005) and so cannot be used as an indicator of its diffusion in soil, and the production of ascomata is too scattered and variable to follow in short-term experiments.

However, a molecular monitoring method based on quantitative PCR has been perfected (Iotti et al. 2012a) in a recent Italian regional project (MAGNATUM) that allows the quantification of the mycelium of *Tuber magnatum* in soil. For the first time we can now develop a simulation model to predict the mycelial dynamics of *T. magnatum* at varying soil temperatures and moisture conditions (Iotti et al. 2018) and measure the effects of managing summer irrigation. This also provides the first management guidelines aimed at maintaining and improving the environmental conditions we believe are suited to *T. magnatum*, such as soil tillage to relieve soil compaction (Salerni et al. 2014) and the selective removal or thinning of invading shrubs like brambles (*Rubus* spp.) (Zambonelli et al. 2012b).

14.5 Conclusion and Prospects

Tuber magnatum and *T. borchii* are two morphologically-similar white truffles which are traditionally important natural economic resources in Italy and a few other European countries. However, the less valuable *T. borchii* has become increasingly popular over the past 20 years following its successful cultivation first in Italy, followed by New Zealand. It has a very wide ecological range and can be easy cultivated. In contrast, *T. magnatum* has defied routine cultivation but there are few signs that it will be commercially cultivated anytime soon.

In addition to traditional cultivation methods, new techniques are also available for *Tuber borchii*. In particular, a technique for inoculation of plants with pure cultures has been perfected that opens up the possibility of selecting genotypes with particular characteristics, for example, specific climatic or edaphic zones.

The genome sequencing of *Tuber borchii* should help reveal the set of genes that control the adaptability of *T. borchii* to different environmental conditions and its productivity and quality. Genetic transformation of *Tuber* mycelia has been scarcely investigated (Grimaldi et al. 2005; Poma et al. 2005) and the proposed techniques not further developed. However, the discovery of the CRISPR/Cas9 immune system of bacteria and archaea and their repurposing for genome editing has elicited a new era in genetic engineering for filamentous fungi (Kwon et al. 2019) that may be applied to improve the characteristics of isolated *Tuber* strains increasing their ability to produce aromas and/or their adaptability to extreme climatic conditions. This might increase the cultivation area and help to overcome the risks of declining truffle production in the southern parts of Europe caused by predicted global warming (Thomas and Büntgen 2017). Obviously, to better exploit its genetic variability, the creation of a germplasm bank to preserve *T. borchii* genetic resources is mandatory. Cryopreservation techniques of mycelia have been shown to be an efficient tool for long-term preservation of truffle strains without affecting their viability and infectivity.

The cultivation of *Tuber magnatum* may receive a boost in the new genomic era. For example, comparative genomics could help to identify those genes which control *T. magnatum* mycorrhiza formation and nutrition, and to find conditions that favor its mycorrhiza development and in vitro cultivation of mycelia.

Appendix: White Truffle Research Institutions

Institution name	Specialization and research activities	Address	Contact person and website
University of Bologna	Truffle cultivation and biology	Via Fanin 44,40127 Bologna, Italy	Alessandra Zambonelli alessandr.zambonelli@unibo.it https://www.unibo.it/sitoweb/alessandr.zambonelli
University of L'Aquila	Truffle phylogeny	Via Vetoio, 67100 Coppito – L'Aquila, Italy	Mirco Iotti https://mesva.univaq.it/?q=docenti/scheda/Iotti%20Mirco
Truffles and Mushrooms (Consulting) Ltd.	Edible ecomycorrhizal fungi cultivation	P.O. Box 268, Dunedin 9054, New Zealand	Ian R. Hall truffle@trufflesandmushrooms.co.nz https://trufflesandmushrooms.co.nz/
INRA- Nancy (France)	Truffle genomics and cultivation	54280 Champenoux France	Claude Murat claude.murat@inra.fr http://www.nancy.inra.fr/
Ispr. CNR	Truffle ecology and metagenomics	Strada delle Cacce 73, 10135 Torino, Italy	Antonietta Mello antonietta.mello@ipsp.cnr.it http://www.ipsp.cnr.it/
Goethe-Universität Frankfurt am Main	Truffle aroma	Gebäudeteil N100, Raum 2.08 Max-von-Laue-Str. 960438 Frankfurt am Main, Germany	Richard Splivallo splivallo@bio.uni-frankfurt.de https://www.bio.uni-frankfurt.de/43967846/Abt__Splivallo
University of Urbino	Truffle aroma and gene expression	Via A. Saffi, 2 – 61029 Urbino (PU), Italy	Antonella Amicucci antonella.amicucci@uniurb.it https://www.uniurb.it/persone/antonella-amicucci
University of Perugia	Truffle cultivation	Borgo XX giugno, 74, 06121 Perugia, Italy	Domizia Donnini domizia.donnini@unipg.it http://www.agr.unipg.it

Institution name	Specialization and research activities	Address	Contact person and website
Michigan State University	Truffle biology	1066 Bogue Street – Rm 286A, East Lansing, 48824 USA	Gregory Bonito bonito@msu.edu https://bonito.psm.msu.edu/

References

Alvarado P, Manjon J (2013) A quantitative and molecular examination of *Tuber melanosporum* mycorrhizae in *Quercus ilex* seedlings from different suppliers in Spain. For Syst 22(2):159–169. https://doi.org/10.5424/fs/2013222-03559

Amicucci A, Rossi I, Potenza L et al (1996) Identification of ectomycorrhizae from *Tuber* species by RFLP analysis of the ITS region. Biotechnol Lett 18(7):821–826. https://doi.org/10.1007/BF00127896

Amicucci A, Rossi I, Potenza L et al (1997) Use of sequence characterised amplified region and RAPD markers in the identification of the white truffle *Tuber magnatum* Pico. Biotechnol Tech 11(3):149–154. https://doi.org/10.1023/A:1018493111804

Amicucci A, Zambonelli A, Giomaro G et al (1998) Identification of ectomycorrhizal fungi of the genus *Tuber* by species-specific ITS primers. Mol Ecol 7(3):273–277. https://doi.org/10.1046/j.1365-294X.1998.00357.x

Amicucci A, Guidi C, Zambonelli A et al (2000) Multiplex PCR for the identification of white *Tuber* species. FEMS Microbiol Lett 189(2):265–269. https://doi.org/10.1111/j.1574-6968.2000.tb09241.x

Amicucci A, Guidi C, Potenza L et al (2002) Microsatellite primed-PCR to select molecular markers for *Tuber* species. Biotechnol Lett 24(4):263–267. https://doi.org/10.1023/A:1014080527613

Amicucci A, Barbieri E, Sparvoli V et al (2018) Microbial and pigment profile of the reddish patch occurring within *Tuber magnatum* ascomata. Fungal Biol 122(12):1134–1141. https://doi.org/10.1016/j.funbio.2018.07.007

Andrés-Alpuente A, Sánchez S, Martín M et al (2014) Comparative analysis of different methods for evaluating evergreen oaks mycorrhized with black truffle. Mycorrhiza 24(1):29–37

Bach C, Beacco P, Cammaletti P, Babel-Chen Z, Levesque E, Todesco F, Cotton C, Robin B, Murat C (2021) First production of Italian white truffle (*Tuber magnatum* Pico) ascocarps in an orchard outside its natural range distribution in France. Mycorrhiza. https://doi.org/10.1007/s00572-020-01013-2

Badalyan SM, Zambonelli A (2019) Biotechnological exploitation of macrofungi for the production of food, pharmaceuticals and cosmeceuticals. In: Sridhar KR, Deshmukh SK (eds) Advances in macrofungi: diversity, ecology and biotechnology. CRC Press, Boca Raton, pp 199–230

Barbieri E, Gioacchini AM, Zambonelli A et al (2005) Determination of microbial VOCs from *Staphylococcus pasteuri* against *Tuber borchii* using SPME and gas chromatography/ion trap mass spectrometry. Rapid Commun Mass Spectrom 19:3411–3415

Bencivenga M, Di Massimo G, Donnini D et al (2009) The cultivation of truffles in Italy. Plant Divers 31(16):21–28

Benucci GMN, Bonito G, BaciarelliFalini L (2012) Mycorrhizal inoculation of pecan seedlings with some marketable truffles. Acta Mycol 47(2):179–184

Bertault G, Rousset F, Fernandez D et al (2001) Population genetics and dynamics of the black truffle in a man-made truffle field. Hered 86(4):451–458. https://doi.org/10.1046/j.1365-2540.2001.00855.x

Bertini L, Agostini D, Potenza L et al (1998) Molecular markers for the identification of the ectomycorrhizal fungus *Tuber borchii*. New Phytol 139(3):565–570. https://doi.org/10.1046/J.1469-8137.1998.00203.X

Bonito GM, Smith ME (2016) General systematic position of the truffles: evolutionary theories. In: Zambonelli A, Iotti M, Murat C (eds) True truffle (*Tuber* spp.) in the world: soil ecology, systematics and biochemistry. Soil biology, vol 47. Springer, Cham, pp 3–18

Bonito GM, Gryganskyi AP, Trappe JM et al (2010) A global meta-analysis of *Tuber* ITS rDNA sequences: species diversity, host associations and long-distance dispersal. Mol Ecol 19(22):4994–5008. https://doi.org/10.1111/j.1365-294X.2010.04855.x

Bonito GM, Smith ME, Nowak M et al (2013) Historical biogeography and diversification of truffles in the Tuberaceae and their newly identified southern hemisphere sister lineage. PLoS One 8(1):e52765. https://doi.org/10.1371/journal.pone.0052765

Bonuso E, Zambonelli A, Bergemann SE et al (2010) Multilocus phylogenetic and coalescent analyses identify two cryptic species in the Italian bianchetto truffle, *Tuber borchii* Vittad. Conserv Genet 11(4):1453–1466. https://doi.org/10.1007/s10592-009-9972-3

Boutahir S, Iotti M, Piattoni F et al (2013) Morphological and molecular characterization of *Tuber oligospermum* mycorrhizas. Afr J Agric Res 8(29):4081–4087

Bragato G, Marjanović ŽS (2016) Soil characeristics for *Tuber magnatum*. In: Zambonelli A, Iotti M, Murat C (eds) True truffle (*Tuber* spp.) in the world: soil ecology, systematics and biochemistry. Soil biology, vol 47. Springer, Cham, pp 191–209

Bratek Z, Gógán A, Halász K et al (2007) The northernmost habitats of *Tuber magnatum* known from Hungary. In: First hypogean mushroom conference, Rabat, Morocco, 6–8 April 2004, pp 6–8

Büntgen U, LendorfH LA et al (2019) Truffles on the move. Front Ecol Environ 17(4):200–202. https://doi.org/10.1002/fee.2033

Buzzini P, Gasparetti C, Turchetti B et al (2005) Production of volatile organic compounds (VOCs) by yeasts isolated from the ascocarps of black (*Tuber melanosporum* Vitt.) and white (*Tuber magnatum* Pico) truffles. Arch Microbiol 184(3):187–193. https://doi.org/10.1007/s00203-005-0043-y

Ciarmela P, Potenza L, Cucchiarini L et al (2002) PCR amplification and polymorphism analysis of the intergenic spacer region of ribosomal DNA in *Tuber borchii*. Microbiol Res 157(1):69–74. https://doi.org/10.1078/0944-5013-00132

Coughlan AP, Piché Y (2005) *Cistus incanus* root organ cultures: a valuable tool for studying mycorrhizal associations. In: Declerck S, Fortin JA, Strullu DG (eds) In vitro culture of mycorrhizas. Springer, Berlin, pp 235–252

Crahay C, Declerck S, Colpaert JV et al (2013) Viability of ectomycorrhizal fungi following cryopreservation. Fungal Biol 117(2):103–111. https://doi.org/10.1016/j.funbio.2012.12.003

De la Varga H, Le Tacon F, Lagoguet M et al (2017) Five years investigation of female and male genotypes in périgord black truffle (*Tuber melanosporum* Vittad.) revealed contrasted reproduction strategies. Environ Microbiol 19(7):2604–2615. https://doi.org/10.1111/1462-2920.13735

Deng XJ, Liu PG, Liu CY et al (2013) A new white truffle species, *Tuber panzhihuanense* from China. Mycol Prog 12(3):557–561. https://doi.org/10.1007/s11557-012-0862-6

Donnini D, Benucci GMN, Bencivenga M et al (2014) Quality assessment of truffle-inoculated seedlings in Italy: proposing revised parameters for certification. For Syst 2:385–393. https://doi.org/10.5424/fs/2014232-05029

Fiecchi A, Kienle M, Scala A et al (1967) Bis-methylthiomethane, an odorous substance from white truffle, *Tuber magnatum* Pico. Tetrahedron Lett 8(18):1681–1682

Figliuolo G, Trupo G, Mang S (2013) A realized *Tuber magnatum* niche in the upper Sinni area (South Italy). Open J Genet 3(2):102–110. https://doi.org/10.4236/ojgen.2013.32013

Fischer C, Colinas C (1996) Methodology for the certification of *Quercus ilex* seedlings inoculated with *Tuber melanosporum* for commercial application. In: First international conference on mycorrhiza, Berkeley, CA, 4–9 August 1996

Fontana A, Palenzona M (1969) Sintesi micorrizica di *Tuber albidum* in coltura pura, con *Pinus strobus* e pioppo euroamericano. Allionia 15:99–104

Gandeboeuf D, Dupré C, Roecket-Drevel P et al (1997a) Grouping and identification of *Tuber* species using RAPD markers. Can J Bot 75(1):36–45. https://doi.org/10.1139/b97-005

Gandeboeuf D, Dupre C, Chevalier G et al (1997b) Typing *Tuber* ectomycorrhizae by polymerase chain amplification of the internal transcribed spacer of rDNA and the sequence characterized amplified region markers. Can J Microbiol 43(8):723–728. https://doi.org/10.1139/m97-104

Gardin L (2005) I tartufi minori in Toscana: gli ambienti di crescita dei tartufi marzuolo e scorzone. ARSIA, Firenze

Gioacchini AM, Menotta M, Bertini L et al (2005) Solid-phase microextraction gas chromatography/mass spectrometry: a new method for species identification of truffles. Rapid Commun Mass Spectrom 19(17):2365–2370. https://doi.org/10.1002/rcm.2031

Gioacchini AM, Menotta M, Guescini M et al (2008) Geographical traceability of Italian white truffle (*Tuber magnatum* Pico) by the analysis of volatile organic compounds. Rapid Commun Mass Spectrom 22(20):3147–3153. https://doi.org/10.1002/rcm.3714

Giomaro G, Zambonelli A, Sisti D et al (2000) Anatomical and morphological characterization of mycorrhizas of five strains of *Tuber borchii* Vittad. Mycorrhiza 10(3):107–114. https://doi.org/10.1007/s005720000065

Giomaro G, Sisti D, Zambonelli A (2005) Cultivation of edible ectomycorrhizal fungi by in vitro mycorrhizal synthesis. In: Declerck S, Strullu DG, Fortin JA (eds) In vitro culture of mycorrhizas. Soil biology, vol 4. Springer, Berlin, pp 253–267

Gogan-Csorbai A, Posta K, Morcillo M et al (2018) Preliminary results on *Tuber borchii* Vitt. ecological studies. In: TAUESG Vísby conference. https://doi.org/10.13140/RG.2.2.24298.57289

Govi G, Bencivenga M, Granetti B et al (1997) Metodo basato sulla caratterizzazione morfologica delle micorrize. In: Il tartufo-Regione Toscana, Spoleto, Italy

Grimaldi B, de Raaf MA, Filetici P et al (2005) *Agrobacterium*-mediated gene transfer and enhanced green fluorescent protein visualization in the mycorrhizal ascomycete *Tuber borchii*: a first step towards truffle genetics. Curr Genet 48:69–74. https://doi.org/10.1007/s00294-005-0579-z

Guillemaud T, Raymond M, Callot G et al (1996) Variability of nuclear and mitochondrial ribosomal DNA of a truffle species (*Tuber aestivum*). Mycol Res 100(5):547–550. https://doi.org/10.1016/S0953-7562(96)80007-4

Halász K, Bratek Z, Szegő D et al (2005) Tests of species concepts of the small, white, European group of *Tuber* spp. based on morphology and rDNA ITS sequences with special reference to *Tuber rapaeodorum*. Mycol Prog 4(4):281–290. https://doi.org/10.1007/s11557-006-0132-6

Hall IR (2017) Bianchetto truffle, *Tuber borchii*. Truffles and Mushrooms Consulting Ltd, Dunedin

Hall I, Zambonelli A (2012) Laying the foundations. In: Zambonelli A, Bonito GM (eds) Edible ectomycorrhizal mushrooms. Soil biology, vol 34. Springer, Berlin, pp 3–16

Hall IR, Zambonelli A, Primavera F (1998) Ectomycorrhizal fungi with edible fruiting bodies. *Tuber magnatum*, Tuberaceae. Econ Bot 52(2):192–200. https://doi.org/10.1007/978-3-319-31436-5_1

Hall IR, Brown GT, Zambonelli A (2007) Taming the truffle: the history, lore, and science of the ultimate mushroom. Timber Press, Portland

Hall IR, Fitzpatrick N, Miros P et al (2017) Counter-season cultivation of truffles in the southern hemisphere: an update. Ital J Mycol 46:21–36. https://doi.org/10.6092/issn.2531-7342/6794

Henrion B, Chevalier G, Martin F (1994) Typing truffle species by PCR amplification of the ribosomal DNA spacers. Mycol Res 98(1):37–43. https://doi.org/10.1016/S0953-7562(09)80333-X

Huang JY, Hu HT, Shen WC (2009) Phylogenetic study of two truffles, *Tuber formosanum* and *Tuber furfuraceum*, identified from Taiwan. FEMS Microbiol Lett 294(2):157–171. https://doi.org/10.1111/j.1574-6968.2009.01571.x

Iotti M, Amicucci A, Stocchi V et al (2002) Morphological and molecular characterization of mycelia of some *Tuber* species in pure culture. New Phytol 155(3):499–505. https://doi.org/10.1046/j.1469-8137.2002.00486.x

Iotti M, Amicucci A, Bonito G et al (2007) Selection of a set of specific primers for the identification of *Tuber rufum*: a truffle species with high genetic variability. FEMS Microbiol Lett 277:223–231. https://doi.org/10.1046/j.1469-8137.2002.00486.x

Iotti M, Leonardi M, Oddis M et al (2012a) Development and validation of a real-time PCR assay for detection and quantification of *Tuber magnatum* in soil. BMC Microbiol 12(1):93. https://doi.org/10.1186/1471-2180-12-93

Iotti M, Piattoni F, Zambonelli A (2012b) Techniques for host plant inoculation with truffles and other edible ectomycorrhizal mushrooms. In: Zambonelli A, Bonito GM (eds) Edible ectomycorrhizal mushrooms. Soil biology, vol 34. Springer, Berlin, pp 145–161

Iotti M, Piattoni F, Hall IR et al (2016) First evidence for truffle production from plants inoculated with mycelial pure cultures. Mycorrhiza 26(7):793–798. https://doi.org/10.1007/s00572-016-0703-6

Iotti M, Leonardi P, Vitali G et al (2018) Effect of summer soil moisture and temperature on the vertical distribution of *Tuber magnatum* mycelium in soil. Biol Fertil Soils 54(6):707–716. https://doi.org/10.1007/s00374-018-1296-3

Jeandroz S, Murat C, Wang YJ et al (2008) Molecular phylogeny and historical biogeography of the genus *Tuber*, the ‘true truffles’. J Biogeogr 35(5):815–829. https://doi.org/10.1111/j.1365-2699.2007.01851.x

Kitamoto Y, Suzuki A, Shimada S et al (2002) A new method for the preservation of fungus stock cultures by deep-freezing. Mycoscience 43(2):143–149. https://doi.org/10.1007/s102670200021

Kwon MJ, Schütze T, Spohner S et al (2019) Practical guidance for the implementation of the CRISPR genome editing tool in filamentous fungi. Fungal Biol Biotechnol 6:15. https://doi.org/10.1186/s40694-019-0079-46, 20

Lancellotti E, Iotti M, Zambonelli A et al (2014) Characterization of *Tuber borchii* and *Arbutus unedo* mycorrhizas. Mycorrhiza 24(6):481–486. https://doi.org/10.1007/s00572-014-0564-9

Lancellotti E, Iotti M, Zambonelli A et al (2016) The Puberulum group sensu lato (whitish truffles). In: Zambonelli A, Iotti M, Murat C (eds) True truffle (*Tuber* spp.) in the world: soil ecology, systematics and biochemistry. Soil biology, vol 47. Springer, Cham, pp 105–124

Lanfranco L, Wyss P, Marzachí C et al (1993) DNA probes for identification of the ectomycorrhizal fungus *Tuber magnatum* Pico. FEMS Microbiol Lett 114(3):245–251. https://doi.org/10.1111/j.1574-6968.1993.tb06581.x

Le Tacon F (2016) Influence of climate on natural distribution of *Tuber* species and truffle production. In: Zambonelli A, Iotti M, Murat C (eds) True truffle (*Tuber* spp.) in the world: soil ecology, systematics and biochemistry. Soil biology, vol 47. Springer, Cham, pp 153–167

Lefevre C (2012) Native and cultivated truffles of North America. In: Zambonelli A, Bonito G (eds) Edible ectomycorrhizal mushrooms. Soil biology, vol 34. Springer, Heidelberg, pp 209–226

Leonardi M, Iotti M, Oddis M et al (2013) Assessment of ectomycorrhizal fungal communities in the natural habitats of *Tuber magnatum* (Ascomycota, Pezizales). Mycorrhiza 23(5):349–358. https://doi.org/10.1007/s00572-012-0474-7

Leonardi P, Iotti M, Zeppa SD et al (2017) Morphological and functional changes in mycelium and mycorrhizas of *Tuber borchii* due to heat stress. Fungal Ecol 29:20–29. https://doi.org/10.1016/j.funeco.2017.05.003

Leonardi P, Puliga F, Iotti M et al (2018) Ultra-low freezing to preserve the lingzhi or reishi medicinal mushroom *Ganoderma lucidum* (Agaricomycetes). Int J Med Mushroom 20(7). https://doi.org/10.1615/IntJMedMushrooms.2018026535

Leonardi P, Murat C, Puliga F et al (2019) Ascoma genotyping and mating type analyses of mycorrhizas and soil mycelia of *Tuber borchii* in a truffle orchard established by mycelial inoculated plants. Environ Microbiol. https://doi.org/10.1111/1462-2920.14777

Li YY, Wang G, Li HM et al (2012) Volatile organic compounds from a *Tuber melanosporum* fermentation system. Food Chem 135(4):2628–2637. https://doi.org/10.1016/j.foodchem.2012.07.013

Linde CC, Selmes H (2012) Genetic diversity and mating type distribution of *Tuber melanosporum* and their significance to truffle cultivation in artificially planted truffières in Australia. Appl Environ Microbiol 78(18):6534–6539. https://doi.org/10.1128/AEM.01558-12

Liu RS, Zhou H, Li HM et al (2013) Metabolism of L-methionine linked to the biosynthesis of volatile organic sulfur-containing compounds during the submerged fermentation of *Tuber melanosporum*. Appl Microbiol Biotechnol 97(23):9981–9992. https://doi.org/10.1007/s00253-013-5224-z

Longato S, Bonfante P (1997) Molecular identification of mycorrhizal fungi by direct amplification of microsatellite regions. Mycol Res 101(4):425–432. https://doi.org/10.1017/S0953756296002766

Mannozzi-Torini L (1988) Il tartufo e la sua coltivazione. Edagricole, Bologna

Marjanović Ž, Glišić A, Mutavdžić D et al (2015) Ecosystems supporting *Tuber magnatum* Pico production in Serbia experience specific soil environment seasonality that may facilitate truffle life cycle completion. Appl Soil Ecol 95:179–190. https://doi.org/10.1016/j.apsoil.2015.05.007

Martin F, Kohler A, Murat C et al (2010) Périgord black truffle genome uncovers evolutionary origins and mechanisms of symbiosis. Nature 464(7291):1033–1038. https://doi.org/10.1038/nature08867

Mello A, Nosenzo C, Meotto F et al (1996) Rapid typing of truffle mycorrhizal roots by PCR amplification of the ribosomal DNA spacers. Mycorrhiza 6(5):417–421. https://doi.org/10.1007/s005720050141

Mello A, Garnero L, Bonfante P (1999) Specific PCR-primers as a reliable tool for the detection of white truffles in mycorrhizal roots. New Phytol 14:511–516. https://doi.org/10.1046/j.1469-8137.1999.00356.x

Mello A, Fontana A, Meotto F et al (2001) Molecular and morphological characterization of *T. magnatum* mycorrhizas in a long-term survey. Microbiol Res 155(4):279–284. https://doi.org/10.1016/S0944-5013(01)80005-7

Mello A, Murat C, Vizzini A et al (2005) *Tuber magnatum* Pico, a species of limited geographical distribution: its genetic diversity inside and outside a truffle ground. Environ Microbiol 7(1):55–65. https://doi.org/10.1111/j.1462-2920.2004.00678.x

Mello A, Murat C, Bonfante P (2006) Truffles: much more than a prized and local fungal delicacy. FEMS Microbiol Lett 260(1):1–8. https://doi.org/10.1111/j.1574-6968.2006.00252.x

Menotta M, Gioacchini AM, Amicucci A et al (2004) Headspace solid-phase microextraction with gas chromatography and mass spectrometry in the investigation of volatile organic compounds in an ectomycorrhizae synthesis system. Rapid Commun Mass Spectrom 18(2):206–210. https://doi.org/10.1002/rcm.1314

Merényi Z, Varga T, Geml J et al (2014) Phylogeny and phylogeography of the *Tuber brumale* aggr. Mycorrhiza 24(1):101–113. https://doi.org/10.1007/s00572-014-0566-7

Molinier V, Murat C, Frochot H et al (2015) Fine-scale spatial genetic structure analysis of the black truffle *Tuber aestivum* and its link to aroma variability. Environ Microbiol 17(8):3039–3050. https://doi.org/10.1111/1462-2920.12910

Molinier V, Murat C, Peter M et al (2016a) SSR-based identification of genetic groups within European populations of *Tuber aestivum* Vittad. Mycorrhiza 26(2):99–110. https://doi.org/10.1007/s00572-015-0649-0

Molinier V, Murat C, Baltensweiler A et al (2016b) Fine-scale genetic structure of natural *Tuber aestivum* sites in southern Germany. Mycorrhiza 26(8):895–907. https://doi.org/10.1007/s00572-016-0719-y

Murat C (2015) Forty years of inoculating seedlings with truffle fungi: past and future perspectives. Mycorrhiza 25(1):77–81. https://doi.org/10.1007/s00572-014-0593-4

Murat C, Díez J, Luis P et al (2004) Polymorphism at the ribosomal DNA ITS and its relation to postglacial re-colonization routes of the Perigord truffle *Tuber melanosporum*. New Phytol 164(2):401–411. https://doi.org/10.1111/j.1469-8137.2004.01189.x

Murat C, Vizzini A, Bonfante P et al (2005) Morphological and molecular typing of the below-ground fungal community in natural *Tuber magnatum* truffle-ground. FEMS Microbiol Lett 245(2):307–313. https://doi.org/10.1016/j.femsle.2005.03.019

Murat C, Rubini A, Riccioni C et al (2013) Fine-scale spatial genetic structure of the black truffle (*Tuber melanosporum*) investigated with neutral microsatellites and functional mating type genes. New Phytol 199(1):176–187. https://doi.org/10.1111/nph.12264

Murat C, Pyen T, Noel B et al (2018a) Pezizomycetes genomes reveal the molecular basis of ectomycorrhizal truffle lifestyle. Nat Ecol Evol 2(12):1956–1965. https://doi.org/10.1038/s41559-018-0710-4

Murat C, Kuo A, Barry WW et al (2018b) Draft genome sequence of *Tuber borchii* Vittad., a whitish edible truffle. Genome Announc 6(25):e00537–e00518. https://doi.org/10.1128/genomeA.00537-18

Nakasone KK, Peterson SW, Jong SC (2004) Preservation and distribution of fungal cultures. In: Bills GF, Mueller GM, Foster MS (eds) Biodiversity of fungi: inventory and monitoring methods. Elsevier Academic Press, Amsterdam, pp 37–47

Obase K, Lee SY, Chun KW et al (2011) Regeneration of ectomycorrhizal fungal isolates following deep freezer storage. Mycobiology 39(2):133–136. https://doi.org/10.4489/MYCO.2011.39.2.133

Pacioni G, Leonardi M (2016) Truffle-inhabiting fungi. In: Zambonelli A, Iotti M, Murat C (eds) True truffle (*Tuber* spp.) in the world: soil ecology, systematics and biochemistry. Soil biology, vol 47. Springer, Cham, pp 283–299

Paolocci F, Cristofari E, Angelini P et al (1995) The polymorphism of the rDNA region in typing Ascocarps and Ectomycorrhizae of truffle species. In: Stocchi V, Bonfante P, Nuti M (eds) Biotechnology of ectomycorrhizae. Springer, Boston, pp 171–184

Paolocci F, Rubini A, Riccioni C et al (2004) *Tuber aestivum* and *Tuber uncinatum*: two morphotypes or two species? FEMS Microbiol Lett 235(1):109–115. https://doi.org/10.1111/j.1574-6968.2004.tb09574.x

Paolocci F, Rubini A, Riccioni C et al (2006) Reevaluation of the life cycle of *Tuber magnatum*. Appl Environ Microbiol 72(4):2390–2393. https://doi.org/10.1128/AEM.72.4.2390-2393.2006

Parladé J, De la Varga H, Pera J (2016) Tools to trace truffles in soil. In: Zambonelli A, Iotti M, Murat C (eds) True truffle (*Tuber* spp.) in the world: soil ecology, systematics and biochemistry. Soil biology, vol 47. Springer, Cham, pp 249–266

Pennazza G, Fanali C, Santonico M et al (2013) Electronic nose and GC-MS analysis of volatile compounds in *Tuber magnatum* Pico: evaluation of different storage conditions. Food Chem 136(2):668–674. https://doi.org/10.1016/j.foodchem.2012.08.086

Percudani R, Trevisi A, Zambonelli A et al (1999) Molecular phylogeny of truffles (Pezizales: Terfeziaceae, Tuberaceae) derived from nuclear rDNA sequence analysis. Mol Phylogenet Evol 13(1):169–180. https://doi.org/10.1006/mpev.1999.0638

Piattoni F, Leonardi P, Siham B et al (2017) Viability and infectivity of *Tuber borchii* after cryopreservation. CryoLetters 38(1):58–64

Picceri GG, Leonardi P, Iotti M et al (2018) Bacteria-produced ferric exopolysaccharide nanoparticles as iron delivery system for truffles (*Tuber borchii*). Appl Microbiol Biotechnol 102(3):1429–1441. https://doi.org/10.1007/s00253-017-8615-8

Polidori E, Agostini D, Zeppa S et al (2002) Identification of differentially expressed cDNA clones in *Tilia platyphyllos-Tuber borchii* ectomycorrhizae using a differential screening approach. Mol Genet Genomics 266(5):858–864. https://doi.org/10.1007/s00438-001-0607-8

Polidori E, Ceccaroli P, Saltarelli R et al (2007) Hexose uptake in the plant symbiotic ascomycete *Tuber borchii* Vittadini: biochemical features and expression pattern of the transporter TBHXT1. Fungal Genet Biol 44(3):187–198. https://doi.org/10.1016/j.fgb.2006.08.001

Poma A, Colafarina S, Limongi T, Pacioni G (2005) *Tuber borchii* mycelial protoplasts isolation, characterization and functional delivery of liposome content, a new step towards truffles biotechnology. FEMS Microbiol Lett 253:331–337. https://doi.org/10.1016/j.femsle.2005.10.003

Pomarico M, Figliuolo G, Rana GL (2007) *Tuber* spp. biodiversity in one of the southernmost European distribution areas. Biodivers Conserv 16(12):3447–3461. https://doi.org/10.1007/s10531-006-9013-1

Potenza L, Amicucci A, Rossi I et al (1994) Identification of *Tuber magnatum* Pico DNA markers by RAPD analysis. Biotechnol Tech 8(2):93–98. https://doi.org/10.1007/BF00152847

Qiao P, Tian W, Liu P et al (2018) Phylogeography and population genetic analyses reveal the speciation of the *Tuber indicum* complex. Fungal Genet Biol 113:14–23. https://doi.org/10.1016/j.fgb.2018.02.001

Regione Emilia-Romagna (2018) Disciplinare per la produzione e certificazione di piante micorrizate con tartufo. http://agricoltura.regione.emilia-romagna.it/fitosanitario/doc/Autorizzazioni/piante-micorrizate. Accessed 16 Oct 2019

Reyna S, Garcia-Barreda S (2014) Black truffle cultivation: a global reality. For Syst 23(2):317–328. https://doi.org/10.5424/fs/2014232-04771

Riccioni C, Belfiori B, Rubini A et al (2008) *Tuber melanosporum* outcrosses: analysis of the genetic diversity within and among its natural populations under this new scenario. New Phytol 180(2):466–478. https://doi.org/10.1111/j.1469-8137.2008.02560.x

Riccioni C, Rubini A, Belfiori B et al (2016) *Tuber magnatum*: the special one. What makes it so different from the other *Tuber* spp.? In: Zambonelli A, Iotti M, Murat C (eds) True truffle (*Tuber* spp.) in the world: soil ecology, systematics and biochemistry. Soil biology, vol 47. Springer, Cham, pp 87–103

Riccioni C, Rubini A, Türkoğlu A et al (2019) Ribosomal DNA polymorphisms reveal genetic structure and a phylogeographic pattern in the Burgundy truffle *Tuber aestivum* Vittad. Mycologia 111(1):26–39. https://doi.org/10.1080/00275514.2018.1543508

Rocchi MBL, Sisti D, Giomaro G et al (1999) Metodi statistici applicati ad un modello di micorrizazione in vitro (*Tilia platyphillos*Scop. X *Tuber borchii* Vittad.). Mic Ital 2:38–43

Rossi I, Bartolacci B, Potenza L et al (2000) Identification of white truffle species using RAPD markers. Plant Soil 219(1–2):127–133. https://doi.org/10.1023/A:1004792528164

Rubini A, Paolocci F, Granetti B et al (2001) Morphological characterization of molecular-typed *Tuber magnatum* ectomycorrhizae. Mycorrhiza 11(4):179–185. https://doi.org/10.1007/s005720100116

Rubini A, Topini F, Riccioni C et al (2004) Isolation and characterization of polymorphic microsatellite loci in white truffle (*Tuber magnatum*). Mol Ecol Notes 4(1):116–118. https://doi.org/10.1111/j.1471-8286.2004.00587.x

Rubini A, Paolocci F, Riccioni C et al (2005) Genetic and phylogeographic structures of the symbiotic fungus *Tuber magnatum*. Appl Environ Microbiol 71(11):6584–6589. https://doi.org/10.1128/aem.71.11.6584-6589.2005

Rubini A, Riccioni C, Belfiori B et al (2014) Impact of the competition between mating types on the cultivation of *Tuber melanosporum*: Romeo and Juliet and the matter of space and time. Mycorrhiza 24(1):19–27. https://doi.org/10.1007/s00572-013-0551-6

Salerni E, Iotti M, Leonardi P et al (2014) Effects of soil tillage on *Tuber magnatum* development in natural truffières. Mycorrhiza 24(1):79–87. https://doi.org/10.1007/s00572-013-0543-6

Schmidberger PC, Schieberle P (2017) Characterization of the key aroma compounds in white Alba Truffle (*Tuber magnatum* Pico) and Burgundy Truffle (*Tuber uncinatum*) by means of the sensomics approach. J Agric Food Chem 65(42):9287–9296. https://doi.org/10.1021/acs.jafc.7b04073

Shamekh S, Donnini D, Zambonelli A et al (2009) Wild Finnish truffles. Plant Divers 31(16):69–71

Sica M, Gaudio L, Aceto S (2007) Genetic structure of *Tuber mesentericum* Vitt. based on polymorphisms at the ribosomal DNA ITS. Mycorrhiza 17(5):405–414. https://doi.org/10.1007/s00572-007-0115-8

Sisti D, Giomaro G, Zambonelli A et al (1998) In vitro mycorrhizal synthesis of micropropagated *Tilia platyphyllos* Scop. plantlets with *Tuber borchii* Vittad. mycelium in pure culture. Acta Hort 457:379–387. https://doi.org/10.17660/ActaHortic.1998.457.47

Sisti D, Giomaro G, Rocchi M et al (2010) Nuove prospettive nel controllo delle piante micorrizate con tartufo. In: Donnini D (ed) Proceedings of the third international conference on truffles. Comunità Montana dei Monti Martani Serano e Subasio, Spoleto, pp 679–687

Smith D (2004) Fungal genetic resource centres and the genomic challenge. Mycol Res 108(12):1351–1362. https://doi.org/10.1017/S0953756204001650

Smith D (2012) Culture collections. In: Sariaslani S, Gadd GM (eds) Advances in applied microbiology, vol 79. Academic, San Diego, pp 73–118

Smith D, Ryan MJ, Day JG (2001) The UK National Culture Collection (UKNCC) biological resource: properties, maintenance and management. UKNCC, Surrey

Splivallo R, Culleré L (2016) The smell of truffles: from aroma biosynthesis to product quality. In: Zambonelli A, Iotti M, Murat C (eds) True truffle (*Tuber* spp.) in the world: soil ecology, systematics and biochemistry. Soil biology, vol 47. Springer, Cham, pp 393–407

Splivallo R, Ebeler SE (2015) Sulfur volatiles of microbial origin are key contributors to human-sensed truffle aroma. Appl Microbiol Biotech 99(6):2583–2592. https://doi.org/10.1007/s00253-014-6360-9

Splivallo R, Maier C (2016) Production of natural truffle flavours from truffle mycelium. US Patent 9,277,760, 8 Mar 2016

Splivallo R, Bossi S, Maffei M et al (2007) Discrimination of truffle fruiting body versus mycelial aromas by stir bar sorptive extraction. Phytochemistry 68(20):2584–2598. https://doi.org/10.1016/j.phytochem.2007.03.030

Splivallo R, Ottonello S, Mello A et al (2011) Truffle volatiles: from chemical ecology to aroma biosynthesis. New Phytol 189(3):688–699. https://doi.org/10.1111/j.1469-8137.2010.03523.x

Splivallo R, Valdez N, Kirchhoff N et al (2012) Intraspecific genotypic variability determines concentrations of key truffle volatiles. New Phytol 194(3):823–835. https://doi.org/10.1111/j.1469-8137.2012.04077.x

Splivallo R, Deveau A, Valdez N et al (2014) Bacteria associated with truffle-fruiting bodies contribute to truffle aroma. Environ Microbiol 17(8):2647–2660. https://doi.org/10.1111/1462-2920.12521

Stielow JB, Vaas LA, Göker M et al (2012) Charcoal filter paper improves the viability of cryopreserved filamentous ectomycorrhizal and saprotrophic Basidiomycota and Ascomycota. Mycologia 104(1):324–330. https://doi.org/10.3852/11-155

Suwannarach N, Kumla J, Meerak J et al (2017) *Tuber magnatum* in Thailand, a first report from Asia. Mycotaxon 132:635–642. https://doi.org/10.5248/132.635

Tanfulli M, Giovannotti E, Donnini D et al (1999) Analisi della micorrizazione in tartufaie coltivate di *Tuber aestivum* Vitt. e *Tuber borchii* Vitt. impiantate da oltre 12 anni in ambienti pedoclimatici diversi. In: Actes V Congrès International Science et Culture de la Truffe, Aix en Provence, France, 4–6 March 1999, pp 480–484

Taschen E, Rousset F, Sauve M et al (2016) How the truffle got its mate: insights from genetic structure in spontaneous and planted Mediterranean populations of *Tuber melanosporum*. Mol Ecol 25(22):5611–5627. https://doi.org/10.1111/mec.13864

Tedersoo L, May TW, Smith ME (2010) Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. Mycorrhiza 20(4):217–263. https://doi.org/10.1007/s00572-009-0274-x

Tešitelová T, Tešitel J, Jersáková J et al (2012) Symbiotic germination capability of four *Epipactis* species (Orchidaceae) is broader than expected from adult ecology. Am J Bot 99(6):1020–1032. https://doi.org/10.3732/ajb.1100503

Thomas P, Büntgen U (2017) First harvest of Périgord black truffle in the UK as a result of climate change. Clim Res 74:67–70. https://doi.org/10.3354/cr01494

Tirillini B, Verdelli G, Paolocci F et al (2000) The volatile organic compounds from the mycelium of *Tuber borchii* Vitt. Phytochemistry 55(8):983–985. https://doi.org/10.1016/S0031-9422(00)00308-3

Trappe J, Molina R, Luoma D et al (2009) Diversity, ecology, and conservation of truffle fungi in forests of the Pacific Northwest. Gen. Tech. Rep. PNW-GTR-772. Department of Agriculture, Forest Service, Pacific Northwest Research Station 194, Portland, OR

Uncu A, Uncu A (2019) Genome-wide identification and annotation of microsatellite markers in white truffle (*Tuber magnatum*). Mediterr Agric Sci 32(1):31–34. https://doi.org/10.29136/mediterranean.487250

Vahdatzadeh M, Splivallo R (2018) Improving truffle mycelium flavour through strain selection targeting volatiles of the Ehrlich pathway. Sci Rep 8(1):9304. https://doi.org/10.1038/s41598-018-27620-w

Vahdatzadeh M, Deveau A, Splivallo R (2015) The role of the microbiome of truffles in aroma formation: a meta-analysis approach. Appl Environ Microbiol 81(20):6946–6952. https://doi.org/10.1128/AEM.01098-15

Vahdatzadeh M, Deveau A, Splivallo R (2019) Are bacteria responsible for aroma deterioration upon storage of the black truffle *Tuber aestivum*: a microbiome and volatilome study. Food Microbiol 103251. https://doi.org/10.1016/j.fm.2019.103251

Varese GC, Angelini P, Bencivenga M et al (2011) Ex situ conservation and exploitation of fungi in Italy. Plant Biosyst 145(4):997–1005. https://doi.org/10.1080/11263504.2011.633119

Vasquez G, Venturella G, Gargano ML et al (2014) New distributive and ecological data on *Tuber magnatum* (Tuberaceae) in Italy. Flora Mediterr 24:239–245. https://doi.org/10.7320/FlMedit24.239

Venturella G, Altobelli E, Bernicchia A et al (2011) Fungal biodiversity and in situ conservation in Italy. Plant Biosyst 145(4):950–957. https://doi.org/10.1080/11263504.2011.633115

Vita F, Taiti C, Pompeiano A et al (2015) Volatile organic compounds in truffle (*Tuber magnatum* Pico): comparison of samples from different regions of Italy and from different seasons. Sci Rep 5:12629. https://doi.org/10.1038/srep12629

Vita F, Alpi A, Bertolini E (2018) De novo transcriptome assembly of the Italian white truffle (*Tuber magnatum* Pico). Bio Rxiv 461483. https://doi.org/10.1101/461483

Voyron S, Roussel S, Munaut F et al (2007) Basidiomycetes long-term preservation by different protocols of cryopreservation and lyophilisation. In: de Medio Ambiente C, de Andalucia J (eds) 1st world conference on the conservation and sustainable use of wild fungi, Cordoba, Spain, 10–16 Dec 2007, pp 129–131

Wang X (2012) Truffle cultivation in China. In: Zambonelli A, Bonito GM (eds) Edible ectomycorrhizal mushrooms. Soil biology, vol 34. Springer, Berlin, pp 227–240

Wang Y, Tan ZM, Zhang DC et al (2006) Phylogenetic relationships between *Tuber pseudoexcavatum*, a Chinese truffle, and other *Tuber* species based on parsimony and distance analysis of four different gene sequences. FEMS Microbiol Lett 259(2):269–281. https://doi.org/10.1111/j.1574-6968.2006.00283.x

Wedén C, Danell E, Tibell L (2005) Species recognition in the truffle genus *Tuber* – the synonyms *Tuber aestivum* and *Tuber uncinatum*. Environ Microbiol 7(10):1535–1546. https://doi.org/10.1111/j.1462-2920.2005.00837.x

Wernig F, Buegger F, Pritsch K et al (2018) Composition and authentication of commercial and home-made white truffle-flavored oils. Food Control 87:9–16. https://doi.org/10.1016/j.foodcont.2017.11.045

Xiao DR, Liu RS, He L et al (2015) Aroma improvement by repeated freeze-thaw treatment during *Tuber melanosporum* fermentation. Sci Rep 5:17120. https://doi.org/10.1038/srep17120

Zambonelli A, Govi G, Previati A (1989) Micorrizazione in vitro di piantine micropropagate di *Populus alba* con micelio di *Tuber albidum* in coltura pura. Micol Ital 3:105–111

Zambonelli A, Salomoni S, Pisi A (1993) Caratterizzazione anatomo-morfologica delle micorrize di *Tuber* spp. su *Quercus pubescens*. Willd. Micol Ital 3:73–90

Zambonelli A, Iotti M, Amicucci A et al (1999) Caratterizzazione anatomo morfologica delle micorrize di *Tuber maculatum* Vittad. su *Ostrya carpinifolia* Scop. Micol Ital 3:82–88

Zambonelli A, Rivetti C, Percudani R et al (2000a) TuberKey: a delta-based tool for the description and interactive identification of truffles. Mycotaxon 74(1):57–76

Zambonelli A, Iotti M, Rossi I et al (2000b) Interaction between *Tuber borchii* and other ectomycorrhizal fungi in a field plantation. Mycol Res 104(6):698–702. https://doi.org/10.1017/S0953756299001811

Zambonelli A, Iotti M, Boutahir S et al (2012a) Ectomycorrhizal fungal communities of edible ectomycorrhizal mushrooms. In: Zambonelli A, Bonito GM (eds) Edible ectomycorrhizal mushrooms. Soil biology, vol 34. Springer, Berlin, pp 105–124

Zambonelli A, Perini C, Pacioni G (2012b) Progetto MAGNATUM. Monitoraggio delle Attività di Gestione delle Tartufaie Naturali di *Tuber magnatum*. Risultati e consigli pratici. Alimat Edizioni, Cesena

Zambonelli A, Iotti M, Hall IR (2015) Current status of truffle cultivation: recent results and future perspectives. Ital J Mycol 44(1):31–40. https://doi.org/10.6092/issn.2465-311X/5593

Zambonelli A, Iotti M, Murat C (eds) (2016) True truffle (*Tuber* spp.) in the world. Springer, Cham

Zambonelli A, Ori F, Hall I (2017) Mycophagy and spore dispersal by vertebrates. In: Dighton J, White JF (eds) The fungal community: its organization and role in the ecosystem, 4th edn. CRC, Boca Raton, pp 347–358

Zampieri E, Murat C, Cagnasso M et al (2009) Soil analysis reveals the presence of an extended mycelial network in a *Tuber magnatum* truffle-ground. FEMS Microbiol Ecol 71(1):43–49. https://doi.org/10.1111/j.1574-6941.2009.00783.x

Zeppa S, Guidi C, Zambonelli A et al (2002) Identification of putative genes involved in the development of *Tuber borchii* fruit body by mRNA differential display in agarose gel. Curr Genet 42(3):161–168. https://doi.org/10.1007/s00294-002-0343-6

Zeppa S, Gioacchini AM, Guidi C et al (2004) Determination of specific volatile organic compounds synthesised during *Tuber borchii* fruit body development by solid-phase microextraction and gas chromatography/mass spectrometry. Rapid Comm Mass Spectrom 18(2):199–205. https://doi.org/10.1002/rcm.1313

Zhang JP, Liu PG, Chen J (2012) *Tuber sinoaestivum* sp. nov., an edible truffle from southwestern China. Mycotaxon 122(1):73–82. https://doi.org/10.5248/122.73