Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)
Available online at www.inia.es/forestsystems
doi: http://dx.doi.org/10.5424/fs/2014232-04771

Forest Systems 2014 23(2): 317-328
ISSN: 2171-5068
eISSN: 2171-9845

Black truffle cultivation: a global reality

Santiago Reyna[1]* and Sergi Garcia-Barreda[1, 2]

[1] ETS Ingeniería Agronómica y del Medio Natural. Universidad Politécnica de Valencia. Cno. de Vera, s/n. 46022 Valencia, Spain. [2] Fundación Centro de Estudios Ambientales del Mediterráneo (CEAM). C/ Charles Darwin, 14. Parque Tecnológico. 46980 Paterna (Valencia), Spain

Abstract

Aim of study: In recent decades the cultivation of the black truffle *Tuber melanosporum* has expanded across all the Mediterranean-climate regions, but also to other regions outside the European standard for the species. We aim to describe the current extent of *T. melanosporum* cultivation.

Area of study: *Tuber melanosporum* plantations in Europe, the Mediterranean basin, Australia, New Zealand, China, America and South Africa.

Material and methods: The socioeconomic impact of *T. melanosporum* cultivation, the way in which the current situation has been achieved and the knowledge needed for its progress are reviewed.

Research highlights: *T. melanosporum* has been successfully cultivated in several countries outside its natural area, but many practices are still empirical and thus yields cannot be guaranteed. The recent advances in molecular techniques and genome science are helping to overcome some of the difficulties traditionally constraining truffle research. The role of truffles as a transitional element between agricultural and forestry activities makes its cultivation a paradigm of sustainable rural development.

Key words: *Tuber melanosporum*; Europe; Australia; New Zealand; Chile; USA.

Introduction

Wild edible fungi have traditionally been used as food or medicine worldwide (Boa, 2004). Some of them —mostly saprotrophic— are cultivated (*e.g. Agaricus bisporus*), while many others are exclusively harvested in forests. Truffles are one of the few cultivated mycorrhizal fungi: they make part of the rural European culture, and nowadays they are widely used in international haute cuisine.

The term "truffle" is sometimes used to name all hypogeous mushrooms in general, but it specifically refers to the genus *Tuber*. Bonito *et al.* (2010) report at least 180 species of *Tuber* around the world, although only about 13 have commercial interest (Bonito *et al.*, 2009). The quintessential truffles are the European black truffle (*Tuber melanosporum* Vittad.) and the Italian white truffle (*Tuber magnatum* Pico).

T. melanosporum was first cultivated in France during the 19th century (Olivier *et al.*, 1996) and it is currently cultivated worldwide (mainly in regions with Mediterranean-like climate). Despite research efforts cultivation is not completely domesticated. More recently, other *Tuber* species have also been successfully cultivated, such as *Tuber aestivum* Vittad. and its form *uncinatum*, *Tuber borchii* Vittad. and the *Tuber indicum* complex (Wang *et al.*, 2006) (Chevalier and Frochot, 1997; Zambonelli *et al.*, 2000; Hu *et al.*, 2005), whereas the attempts to grow the native North American *Tuber* species are in their first stages (Lefevre, 2012). Much effort has been devoted to *T. magnatum*, although without success (Gregori, 2007; Bencivenga *et al.*, 2009).

This review focuses on the current extent of *T. melanosporum* cultivation, which is by far the most widespread. We describe its potential socioeconomic impact, the way in which the current situation has been achieved and finally the knowledge needed for its progress.

Socioeconomic and environmental values

Economic value

In France, Italy, Spain and Australia truffles are currently a multi-million euro industry. In the first

* Corresponding author: sreyna@agf.upv.es
Received: 23-07-13. Accepted: 12-12-13.

three countries, truffles are harvested not only in planted truffle orchards (cultivated truffles) but also in natural forests (wild truffles). The value of the *T. melanosporum* production is estimated to be around 20 million euro per year in France (Escafre and Roussel, 2006), 7.5 million euro in Spain (estimated for the past decade on the basis of the mean prices at Vic market, in north-eastern Spain), and 4 million euro in Australia in 2012 (Duell, 2012). In Italy the production value of all species of *Tuber* together was estimated to be 18 million euro in 1999 (Pettenella *et al.*, 2004).

The price that farmers receive for *T. melanosporum* in Europe ranged in the past decade from less than 150 to more than 800 euros kg^{-1}. The price for retail customers can be much higher: *e.g.* in Paris and London prices higher than 2000-4000 euros kg^{-1} can be achieved. This fact has encouraged truffle growers to sell directly to restaurants and to use retail e-commerce. In Australia the mean price in 2012 for the higher quality class was about 950 euros kg^{-1} (Duell, 2012).

The high price of *T. melanosporum* makes its cultivation an interesting enterprise for farmers wherever suitable environmental conditions exist. A review of economic evaluations of truffle plantation in Europe by Bonet and Colinas (2001) showed that the internal rate of return (*i.e.* the interest yield expected from the investment) was always above 9%, although the return time of the investment was longer than 10 years. The GET (2002) estimated that markets would be able to absorb around 1,000 t of *T. melanosporum*, which represents more than ten times the current production. In Spain its current discounted value has increased at an annual rate of 3% over the past 50 years (Reyna, 2007b).

The total economic impact includes not only fresh truffles sold by farmers, but also agritourism, local mycological gastronomy, production of value-added truffle products, truffle fairs and retail markets, increase in agricultural land price in truffle-growing regions, production of mycorrhizal seedlings in nurseries, dog training, consumption of agricultural supplies by truffle growers, technical assessment services, etc. The total economic impact of *T. melanosporum* was estimated to be around 70 million euros per year in France (Escafre and Roussel, 2006). In Italy, the total economic impact of all *Tuber* species together was estimated to be more than 100 million euros (Gregori, 2013).

Social value: rural development

In many *T. melanosporum*-producing regions of the Mediterranean basin the environmental conditions and the small size of plots limit the yield of traditional agricultural crops (Pinto-Correia, 1993). Truffle cultivation represents an alternative for their agriculture: an economic diversification and an extra income. When non-agricultural activities are brought into play (*e.g.* agritourism) truffle fulfils a more robust role in rural development. Besides, maintaining marginal agricultural lands cultivated helps to preserve the traditional Mediterranean agroforestry landscapes and rural population.

The harvesting of wild *T. melanosporum* also plays a role in the rural economy of Italy and Spain. The value that truffles add to forests is particularly interesting in Mediterranean forests: given their low productivity (Domínguez-Torres and Plana, 2002) it could promote involvement of the rural communities in forest protection and management.

In Italy and France truffles are a part of the rural cultural heritage and a reason for pride. Truffle has encouraged the development of partnerships and associations of growers, municipalities (such as the Italian *Città del Tartufo*) and gourmets. Ecomuseums such as those in Sorges (France), San Giovanni d'Asso (Italy) and Metauten (Spain) introduce the public to the various aspects of truffles.

Environmental value

In its natural area, *T. melanosporum* can be grown with low environmental impact: the use of machinery and chemicals is limited, so it can be easily considered organic farming. Soil tilling of these plots increases water infiltration rates. The maintenance of soil conservation structures limits soil erosion risk in steep slopes. The use of native *Quercus* as host plants contributes to the conservation of natural forests. In the fire-prone Mediterranean landscapes, *T. melanosporum* plantations constitute excellent firebreaks due to low plantation densities, soil tilling and the herbicidal effect of the fungus.

The history of truffle cultivation

Hypogeous fungi (most probably desert truffles of the genera *Terfezia* and *Tirmania*) were consumed in the Antiquity by Mesopotamics, Egyptians, Greeks and Romans. During the Middle Ages they are scarcely cited in Europe, but in the Renaissance truffles gained a reputation in Italy and France, and truffle consumption spread among wealthy people (Reyna, 2007a). Manna (2005) reports harvest regulations in Italy during this period. Ceccarelli wrote a monograph on the management of wild *truffières* in 1564 (Granetti, 2005).

During the 18th and 19th centuries the consumption of truffles increased thanks to gourmets such as Brillat-Savarin. This encouraged the spreading of truffle harvesting and the management of wild *truffières*. A major advance occurred in the early 19th century when the French farmer Joseph Talon had the idea of sowing acorns from truffle-producing trees near or inside the *truffières*. Auguste Rousseau disseminated the technique and thousands of hectares of oaks were planted thanks to this idea, boosted by the French reforestation laws of 1860 and 1882 (Diette and Lauriac, 2005) and the expansion of available agricultural land due to the widespread vineyard destruction by the *Phylloxera* plague (Olivier *et al.*, 1996). This resulted in the golden age for truffle production, with estimated productions of 1,588 t in 1868, and 2,000 t in 1892. In Italy, Mattirolo and Francolini also promoted reforestations to increase truffle production (Granetti, 2005). In those decades De Bosredon, Chatin and Pradel wrote monographs on truffle ecology and cultivation. During the same period Carlo Vittadini provided the first detailed morphological descriptions of many *Tuber* sporocarps and a classification system (Trappe *et al.*, 2009).

In the 20th century French production sharply declined to the current 10-60 t per year, due to the rural depopulation caused by the two World Wars and by the rural to urban drift. The forest stand density increased and much of farmers' empirical knowledge was lost (Olivier *et al.*, 1996). In Italy the decline was not so severe and it is limited to the first half of the century (Manna, 1990). In the late 1960s the progress in truffle cultivation seemed arrested despite the works of Rebière and Mannozzi-Torini.

A breakthrough occurred in the early 1970s, when researchers from the IPLA and the INRA developed the inoculation of seedlings with *Tuber* in the nursery (Chevalier, 2001). Almost 90 years before, Frank had coined the term mycorrhiza and postulated that this structure involved a symbiotic relationship (Trappe *et al.*, 2009). *T. melanosporum*-inoculated seedlings were released to the market in France in 1973 (Chevalier, 2001), greatly increasing plantation activity. On the other hand, in Italy it gained momentum from 1982 (Bencivenga, 2001).

At that time French truffle growers had already began to associate, and the government had established public aids for plantation establishment (Olivier *et al.*, 1996). Italy organised the first International Truffle Congress in 1968. Up to the 1980s the intensive cultivation techniques (Pallier method) were dominant in plantations, whereas from the 1990s more extensive models (Tanguy method) were proposed (Olivier *et al.*, 1996).

Spain only became involved in the international truffle market in the 1960s, when wild *truffières* across the country began to be systematically searched and exploited. An alarming decrease in production was observed from the 1980s, after 20-30 years of intensive harvesting in a context of rural depopulation and increasing forest stand density. Except for the Arotz estate (around 600 ha planted in the 1970s), the plantation of mycorrhizal seedlings was minimal until the 1990s. In that decade truffle growers began to associate, and regional governments established public assistance for the establishment of plantations.

Current state in France, Italy and Spain

The annual European production of *T. melanosporum* was an average of around 58 t for the period 2003-2012, although highly variable from year to year (Table 1, Fig. 1).

In France, the production seems steady since the 1990s (Fig. 1), despite the rate of plantation (Table 1). Our estimation of mean yield in mature plantations is very low (Table 1), but it is consistent with the 0.5-3 kg ha^{-1} $year^{-1}$ cited by Escafre and Roussel (2006).

French growers benefit from a high domestic demand (it consumes most of the French and Spanish production), the support from public agencies to plantation establishment (Sourzat, 2007), and an extensive network of scientists and specialists (although much of their work is not published in scientific journals) (Table 1). The experience of experimental stations in which researchers and growers closely collaborate, such as that of Le Montat (Lot), seems especially interesting.

In Italy, we estimate that the mean yield of mature plantations is similar to that of France (Table 1). The strengths of *T. melanosporum* cultivation are also

Table 1. Main features of *T. melanosporum* production and associate activities in France, Italy and Spain

	France	Italy	Spain
Mean production between seasons 2003-2004 and 2012-2013 (t year^{-1})[1]	31.3	11.0	15.9
% truffles produced in plantations vs harvested in the wild[2]	90%-10%	50%-50%	60%-40%
Plantation surface (ha)[3]	24,000	7,500	10,000
Recent rate of plantation (ha year^{-1})[3]	800	400	1,000
Mean yield of mature plantations (kg ha^{-1} year^{-1})[4]	1.5	1.2	3.2
Main productive regions[2]	Drôme (>6,500 ha), Lot, Vaucluse, Dordogne, Gard	Marche (aprox. 5,000 ha), Umbria, Abruzzo	Teruel (>4,000 ha), Soria, Huesca, Castelló
Hosts in plantations[5]	Qh, Qi, Qr, Ca	Qh, Qi, Oc, Ca	Qi, Ca, Qf, Qc
No of nurseries[6]	27	8	27
Price of seedlings (euros)[6]	5-19	8-14	4-8
No of growers/harvesters[7]	20,000	180,000 [7]	10,000
No of growers/harvesters associations[7]	36	50	20
No of truffle fairs and retail markets[8]	129	68 in Umbria, Piemont, Toscana and Abruzzo	15
No of research articles (and No of citations) on truffles (2008-2012)[9]	13 (179)	43 (209)	32 (118)

[1] According to the European Group for Truffles, Oliach (pers. comm.) and Gregori (pers. comm.).
[2] According to Gregori (2007) and Sourzat (2007).
[3] Estimated from Escafre and Roussel (2006) and Gregori (2007).
[4] We estimated the mean yield of mature plantations (1) taking into account the proportion of wild production, (2) assuming that young plantations (less than seven years old) do not have a quantitatively meaningful production, and (3) estimating the surface of young plantations from the recent plantation rate.
[5] Qh: *Quercus humilis* Mill. (= *Q. pubescens*), Qi: *Quercus ilex* L, Qr: *Quercus robur* L, Ca: *Corylus avellana* L, Oc: *Ostrya carpinifolia* Scop., Qf: *Quercus faginea* Lam., Qc: *Quercus coccifera* L.
[6] According to Cocina *et al.* (2013).
[7] According to GET (2002). Pettenella *et al.* (2004) estimated that only 5% of the Italian harvesters are professionals.
[8] Including events dedicated to any *Tuber* species. According to Cena (2000), Materozzi (2005), FFT (2011), Marone (2011) and FFT (2012).
[9] According to Web of Science. Only articles in which the first author works in a research centre of the country are considered. Articles on all species of *Tuber* are considered.

similar, with a greater emphasis on exportation (Galluzzo, 2013). The introduction of exotic species such as *T. indicum* s.l. and *Tuber sinoaestivum* Zhang et Liu (Zhang *et al.*, 2012) in Italian orchards may become a serious problem (Murat *et al.*, 2008; Zambonelli *et al.*, 2012).

In Spain, plantations have made up for the collapse of wild production over the past few decades (Fig. 1). The mean yield of mature plantations is somewhat higher than in France and Italy (Table 1), despite the fact that the mean age of plantations is lower (plantation establishment started later). In the Arotz plantation the yield in the late 1990s was lower than 2 kg ha^{-1} year^{-1} in non-irrigated areas, and up to 45 kg ha^{-1} year^{-1} in the irrigated areas (Carbajo, 1999).

Domestic consumption of truffles in Spain is less than 10%, and promotion activities such as fairs are still limited (Table 1). In some regions the high plantation rates during the 2000s were mainly due to grants covering 100% of the cost of plantation establishment (excluding land purchase), thus making planting a business in itself. Some experts argue that a part of these plantations will not be carefully managed and will be less likely to succeed.

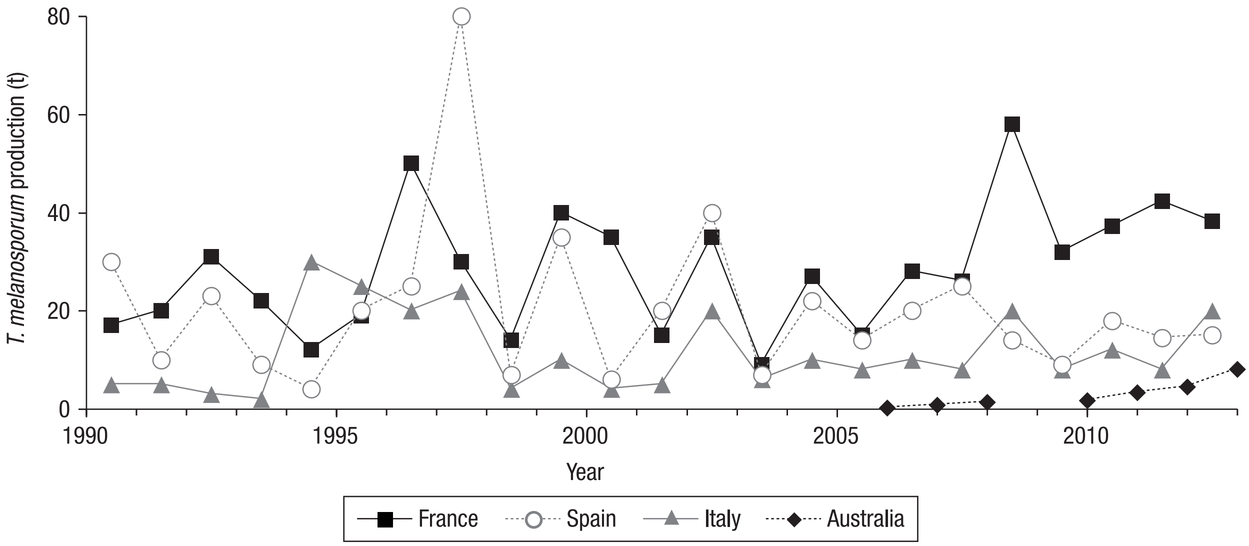

Figure 1. Estimates for *T. melanosporum* production from season 1990-1991 in France, Italy, Spain (according to the European Group for Truffles for the data up to 2009-2010, and to Oliach, pers. comm. and Gregori pers. comm. for the recent ones) and Australia (Duell, 2012).

Cultivation outside the native range

North America

The first *T. melanosporum*-inoculated plantations were established in 1979 in North Carolina and 1980 in California, and the first sporocarps were harvested in 1987 in northern California (Renowden, 2005; Lefevre, 2012). Nowadays there are plantations from California to southern British Columbia (Canada) and from central Texas to North Carolina (Renowden, 2005; Lefevre, 2012). Most of them were planted after 2003 (Pilz *et al.*, 2009). Sporocarp production has been reported in California, North Carolina, Tennessee, Texas, Oregon and British Columbia (Lefevre, 2012; Berch, 2013). Soils are usually limed to raise the pH. *C. avellana*, *Q. robur* and *Q. ilex* are commonly used as host plants.

These plantations have usually spread without any coordinated initiative or technical assessment. Furthermore, in some cases the sites are far from the climatic requirements of *T. melanosporum* (Lefevre, 2012). The overall yields are far from optimum (Table 2).

Australia and New Zealand

New Zealand was the first country in the Southern Hemisphere to establish *T. melanosporum* plantations (in 1987) and to harvest sporocarps (in 1993). The initiative was led by a scientist (Ian Hall), supported by the government (Hall and Haslam, 2012). Its development was based on climatic and edaphic studies, production of mycorrhizal seedlings in the country and quality control of mycorrhizal seedlings. A variety of field essays were established to determine the best management practices (Guerin-Laguette *et al.*, 2009). However, most information remains unpublished due to confidentiality (Hall and Haslam, 2012). *C. avellana* and *Q. robur* were initially used as host plants, and *Q. ilex* was introduced later (Zambonelli *et al.*, 2009).

Australia began to plant some years later and benefited from New Zealand experience, important subsidies for plantation establishment and a more continued government support for research (Hall and Haslam, 2012), thus being reflected in the planted surface (Table 2). Plantations are currently common in Western Australia (notably around Manjimup and Pemberton), Tasmania, New South Wales and Victoria (Lee, 2008). The agricultural practices have been adapted to the environmental conditions: they are more intensive than in Europe and plantation densities are higher (Zambonelli *et al.*, 2009).

The productive results in both countries are contrasting (Fig. 2): we estimate that the mean yield of mature plantations is 0.5 kg ha^{-1} $year^{-1}$ for New Zealand and 9.2 kg ha^{-1} $year^{-1}$ for Australia. The latter can

Table 2. Main features of *T. melanosporum* plantations outside its natural distribution area

	USA	New Zealand	Australia	Chile	South Africa	Argentina
Date of first plantation	1979	1987	1993	2003	2008	2010
Production (kg year^{-1})[1]	40	<50	4,500	7	0	0
Plantation surface (ha)[2]	120	100	700	200	30	40
Recent rate of plantation (ha year^{-1})[3]	20	Very low	30	35	10	20
No of nurseries[4]	4	4	6	4	2	2
Price of seedlings (euros)[4]	11-19	28-33	15-46	10-15	11	12

[1] According to Lefevre (2010), Duell (2012), Guerin-Laguette *et al.* (2013) and Henríquez (pers. comm.)
[2] According to Pilz *et al.* (2009), Zambonelli *et al.* (2009), Hall and Haslam (2012), Henríquez (pers. comm.) and Miros (pers. comm.)
[3] According to Treloar (2013); to the comparison of current surfaces to those 5-7 years ago (Ramírez *et al.* 2007; Lee 2008); and in the case of the USA to seedling production of the most important nurseries (Cocina *et al.* 2013) and common plantation densities.
[4] According to Cocina *et al.* (2013).

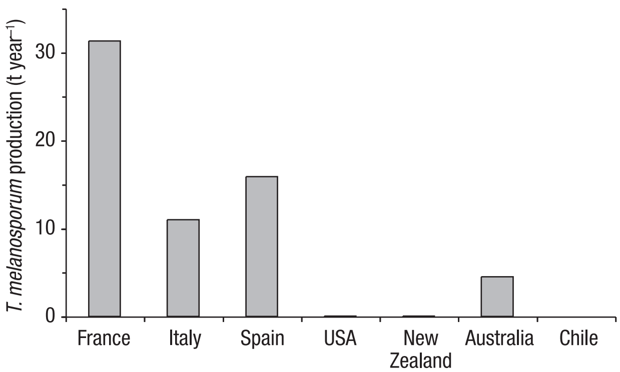

Figure 2. Estimation of current production of *T. melanosporum*.

be considered an important success, although the differences in productivity among plots are high and most production comes from a limited number of plantations (Hall and Haslam, 2012).

One important advantage for these countries is that forests are dominated by endomycorrhizal plants or ectomycorrhizal fungi very different from the European ones (Zambonelli *et al.*, 2009). As calcareous soils are scarce, liming the soil is a common practice, like in North America, and rising of the pH can also reduce the competitiveness of the scarce ectomycorrhizal fungi (Lefevre and Hall, 2000). Australia and New Zealand produce in counter-season to the main producers and consumers, and this helps their exports given that the truffles are mostly consumed fresh. In addition, their domestic demand is rapidly increasing: truffle fairs are organised to spread its culinary use, and about 800 kg were consumed in Australia in 2012 (Duell, 2012), with the bulk exported.

The success of some plantations is threatened by the accidental introduction of *T. brumale* (through the imported sporocarps used as inoculum) in both countries (Linde and Selmes, 2012; Guerin-Laguette *et al.*, 2013).

China

The first *T. indicum* s.l. plantation was established in Taiwan in 1989, and the first sporocarps were harvested in 1997 (Hu *et al.*, 2005). In continental China, the first *T. indicum* s.l. sporocarps and the first *T. melanosporum* sporocarps were harvested in neighbouring plantations in Guizhou (Wang, 2012). Another *T. melanosporum* plantation was established in Hunan (Wang, 2012), despite the excessive summer temperatures and low winter temperatures (Hall, 2013). More *T. indicum* s.l. plantations have been established in recent years in Guizhou, Hunan, Sichuan and Yunnan using native *Quercus*, *Pinus* and *Castanea* as hosts (Wang, 2012).

In most cases these activities were supported by universities and implied the development of nursery techniques. However, quality control of seedlings is not common and many plantations are established on forest soils (Wang, 2012). Most truffles are exported and the domestic demand is low.

Europe and the Mediterranean basin

T. melanosporum cultivation is being essayed across Europe and the Mediterranean basin. However, these plantations are still young and sparse, and in many cases the sites do not fulfil *T. melanosporum* climatic requirements. Some plantations have already produced sporocarps in Israel, Morocco and Sweden (Khabar 2007; Turgeman *et al.*, 2012; Wedén *et al.*, 2013).

The interest on *T. aestivum*/*T. uncinatum* cultivation is also recent and widespread. These plantations are more likely to succeed given the wider ecological requirements of this species (Stobbe *et al.*, 2013). Israel is an illustrating example (Turgeman *et al.*, 2012). In 1994 *T. melanosporum*-inoculated seedlings were planted, and in 1999 a sporocarp was harvested. But in 2002 it was impossible to find *T. melanosporum* mycorrhizas in the plantation. In 1999 more seedlings were planted, but *T. aestivum*-inoculated seedlings were non-intentionally introduced. In 2010 about 15 kg ha^{-1} of *T. aestivum* were harvested and it has continued to fruit.

South America and South Africa

Following New Zealand's example, the Universidad Católica del Maule started a project to cultivate *T. melanosporum* in Chile with government support (Fundación para la Innovación Agraria). The first plantation was established in 2003 and the first truffles were harvested in 2009 (Table 2). Most plantations are located between the regions of Valparaíso and Los Ríos. Soils are usually limed to raise pH. *Q. robur*, *Q. ilex* and *C. avellana* are the most used host plants (Henríquez, pers. comm., www.trufaschile.cl).

The initiative was based on a climatic and edaphic study of Chile, and the development of nursery techniques to produce mycorrhizal seedlings. More recently, the public initiative is addressed to establish field essays aimed at determining the best management practices and the environmental factors that enhance fruiting. Public efforts have been also launched to promote the association of growers, to train farmers and to develop commercial strategies. Being in the Southern Hemisphere, Chile has the opportunity to produce counter-season to Europe. In contrast, their domestic demand is still very low, and their farmers need technical assessment on quality of mycorrhizal seedlings and management practices.

In 2008 a nursery was established in Argentina and the first seedlings were planted in 2010 (Table 2). The first plantations are located in south Buenos Aires region and Río Negro (Henríquez, pers. comm.). People in neighbouring countries like Uruguay, Peru and Brazil have recently shown interest in starting truffle cultivation projects (Henríquez, pers. comm.).

In South Africa, *T. melanosporum* plantations were initiated in 2008 through a joint venture and the establishment of several nurseries (Table 2). *Q. robur, C. avellana* and *Q. ilex* are the most used host plants (Miros, pers. comm., http://woodfordtruffles.co.za/).

Basic research

The cultivation of *T. melanosporum* is not completely domesticated, as the uncertainties around the mating process remain (Selosse *et al.*, 2013). Basic research on truffles has been constricted by the difficulties to observe their development: the symbiotic phase is microscopic and the growth of the mycelium in pure culture is slow, whereas the sporocarp grows underground and over a period of several months.

In recent years molecular techniques are allowing a great progress: they are used to identify sporocarps, mycorrhizas and mycelia; to recognise genetically identical individuals (genets); and to determine the molecular bases of truffle biology. The genome of *T. melanosporum* has been recently sequenced by the *Tuber* Genome Consortium, opening the possibilities of genomics to truffle research (Martin *et al.*, 2010). These tools support novel approaches to important research gaps in the mechanisms regulating symbiosis, the trophic state of the fungus, population genetics and the mechanisms involved in fruiting. Linking gene functions and interactions with the biology and ecology of the fungus will improve the design and management of plantations.

An important breakthrough has been the confirmation that *T. melanosporum* is heterothallic (Riccioni *et al.*, 2008), the identification of two mating types, and the characterisation of the genes involved (Rubini *et al.*, 2011a). However, the sex organs are still largely unknown: only ascogonia have been rarely reported (Callot, 1999). Le Tacon *et al.* (2013) hypothesised that the ascogonium connects the mycorrhiza to the sporocarp during all its development.

The understanding of the ectomycorrhizal relation is being improved by recent studies on the signalling pathways to its establishment, the mechanisms of the fungus to inhibit the defensive response of the plant, the mechanisms of the partners to control each other, the role of nutrients transfer in the maintenance of the relation, the ability of the fungus to cleave sucrose

(Plett and Martin, 2011) or the role of truffle volatiles in the first contact (Splivallo *et al.*, 2011).

Although *T. melanosporum* retains some genes encoding enzymes responsible for degrading plant living cells, it has less degradative enzymes than saprotrophic fungi, thus indicating a lower ability to degrade organic tissues (Plett and Martin, 2011). This makes the fungus highly dependent on its host: Le Tacon *et al.* (2013) found that even in the late stages of the sporocarp development most carbon was supplied by the plant. The analysis of the transcription factors can help to understand the variations in the trophic behaviour of the fungus in each developmental stage (Montanini *et al.*, 2011).

The methods for quantifying mycelium of *T. melanosporum* in the soil have allowed Liu *et al.* (2014) to monitor its spread and increase of density in young plantations. Suz *et al.* (2008) showed the relation between the abundance of mycelium and the formation of the *brûlé*. Liu *et al.* (2014) also hypothesised the existence of a mycelium-carrying capacity of the soil, and it would be interesting to understand the relation of this with the abundance of volatiles.

Murat *et al.* (2013) found that *T. melanosporum* genets in young plantations were mostly small (diameter lower than 1 m), and few of them were found from year to year. Rubini *et al.* (2011b) showed that in the nursery genets compete each other for root tips, leading to the exclusion of most genets in 18 month-old seedlings. In the field intraspecific competition causes a spatial separation between mating types that can affect fruiting potential (Rubini *et al.*, 2011b). Selosse *et al.* (2013) hypothesised that the mating-type genes could also be controlling vegetative incompatibility, but Iotti *et al.* (2012) did not find genes related to mating-type heterokaryon incompatibility in *T. melanosporum*.

The understanding of the mechanisms triggering fruiting is being improved by the studies on the regulome involved in developmental shifts from the symbiotic to the reproductive phase, on carbon transfer from the plant to the sporocarp, and on mating types. But it is also essential to know which factors of the soil environment are involved: Pacioni *et al.* (2014) suggested that variations in soil temperature and water content in spring are key, and proposed to use ground penetrating radars to monitor the sporocarps without disturbing their environment.

The translation of basic research to agricultural practices can be obscured by the interaction between *T. melanosporum* and other soil organisms (*e.g.* fungal competitors, bacteria modulating the mycorrhizal relation, or interacting with the fruiting). Metagenomics makes the study of soil microbial communities easier.

Cultivation challenges

Nowadays many of the practices in *T. melanosporum* cultivation are still empirical. The success of plantations cannot be guaranteed: plantations largely differ in the sporocarp yield, the percentage of productive trees and the age at which they start producing (productive onsets at age 4 and later than year 15 have been reported).

The quality of some nursery seedlings is a problem in countries without mandatory certification systems. It can be the cause of failure for some plantations and the way that exotic species are introduced. The production of seedlings with known mating types is a challenge for the future, since they are currently inoculated with spores (Rubini *et al.*, 2011b).

The experience in North America and the Southern Hemisphere proved that it is possible to grow truffles in naturally acidic soils after liming. But if not enough lime is added to stabilise the pH, it decreases in a few years, making periodic liming necessary.

Sourzat (2008, 2010) drew attention to other soil-related issues jeopardising the success of plantations in France: the chemicals applied in the previous land use and the high pressure of ectomycorrhizal competitors (particularly *T. brumale* and *T. aestivum*) in landscapes dominated by forest patches. The competition of soil-borne fungi points to the need to understand better the factors determining the structure of ectomycorrhizal communities and their dynamics, including the possible role of truffle volatiles (Splivallo *et al.*, 2011).

In contrast, in Spain the plantations are usually located in extensive agricultural landscapes, so these problems are less frequent. However, temperatures are higher and rainfall is more scarce and irregular. This reduces the survival of the sporocarps during the summer, making research on irrigation and soil water conservation a priority.

Other challenges in French plantations (Sourzat, 2008, 2010) are the short productive life of some young plantations and the high stand densities in mature plantations. The latter draws attention to the importance of pruning and thinning. Old plantations (planted before the 1970s) which are not producing currently are seen as an opportunity for spreading truffle production by forest management.

Large concentrations of plantations pose new threats: *e.g.* the truffle growers in Sarrión (Teruel) complain that in recent years the damages of insects on sporocarps are increasing, especially in dry and hot autumns.

Outside France and Italy most plantations are still young and management techniques are not adapted to the local conditions (soil water and temperature regimes, aeration, organic matter, host plants, soil-borne ectomycorrhizal community, etc.) yet. There is not a unique management system that works in all the environmental conditions suitable for *T. melanosporum* growth. Monitoring mycorrhizas and mycelium, tree growth and physiology, and soil microclimate in these plantations is highly recommendable. In this way, it could be assured that they remain potentially successful, and that management practices could be quickly improved.

A major problem in Australia is the abundance of sporocarps growing near the soil surface in irrigated plantations. These are more frequently damaged by pests, diseases, desiccation and frosts. The first studies on the matter point that reducing the irrigation and increasing the irrigation interval could help to decrease the damages (Eslick and Dell, 2013).

In countries with a meaningful wild production (Spain and Italy) the conservation of this production is an important concern: this involves sustainable harvesting and habitat improvement (Garcia-Barreda and Reyna, 2013).

Finally, if climate change predictions for the Mediterranean basin (decrease in summer rainfall, increase of temperatures and increase in interannual variability of both rainfall and temperature) are confirmed, the suitability of many native *T. melanosporum* areas for cultivation (specially the warmest ones) will be reduced (Colinas *et al.*, 2007). A planning effort will be needed to assess the future suitability of the current native range and to adapt the cultural practices to the new situation.

Acknowledgements

This work was partially funded by the INIA project PET 2007-13-C07-04. We would like to thank Luz Cocina for her help with the text in English.

References

Bencivenga M, 2001. La tartuficoltura in Italia: problematiche e prospettive. Actes du V[e] Congrès International Science et culture de la truffe, Aix-en-Provence (France), March 4-6 1999. pp: 27-29.

Bencivenga M, Di Massimo G, Donnini D, Baciarelli-Falini L, 2009. The cultivation of truffles in Italy. Acta Bot Yunnan 31(S16): 21-28.

Berch SM, 2013. Truffle cultivation and commercially harvested native truffles. Proceeding International Symposium on Forest Mushroom, Korea Forest Research Institute, August. 6 2013 pp: 85-97.

Boa ER, 2004. Wild edible fungi: a global overview of their use and importance to people. FAO, Rome.

Bonet JA, Colinas C, 2001. Cultivo de *Tuber melanosporum* Vitt. Condiciones y rentabilidad. Forestalia 5: 38-45.

Bonito G, Trappe JM, Vilgalys R, 2009. North American truffles in the *Tuberaceae*: molecular and morphological perspectives. Acta Bot Yunnan 31(S16): 39-51.

Bonito G, Gryganskyi AP, Trappe JM, Vilgalys R, 2010. A global meta-analysis of *Tuber* ITS rDNA sequences: species diversity, host associations and long-distance dispersal. Mol Ecol 19: 4994-5008.

Callot G (coord), 1999. La truffe, la terre, la vie. INRA, Paris.

Carbajo P, 1999. Plantación de encinas micorrizadas para la producción de trufas (*Tuber melanosporum*) en la provincia de Soria. In: Micorrización en áreas mediterráneas de la Península Ibérica (Vázquez FM, Rincón S, Ramos S, Doncel E, eds). Junta de Extremadura, Mérida, Spain. pp: 107-111.

Cena C (coord), 2000. Tutto tartufo Il tartufo in Piemonte. Fabiano Ed, Canelli, Italy.

Chevalier G, 2001. Le plant mycorhizé INRA: passé, present, avenir. Actes du V[e] Congrès International Science et culture de la truffe, Aix-en-Provence (France), March 4-6 1999. pp: 300-303.

Chevalier G, Frochot H, 1997. La truffe de Bourgogne. Ed Pétrarque, Levallois-Perret, France.

Cocina L, Barriuso J, Martín M, Sánchez S, 2013. A review of nurseries producing mycorrhizal plants in Spain and the world. 1st International Congress of Trufficulture Tuber 2013, Teruel (Spain), March 5-8.

Colinas C, Capdevila JM, Oliach D, Fischer CR, Bonet JA, 2007. Mapa de aptitud para el cultivo de la trufa negra (*Tuber melanosporum* Vitt.) en Catalunya. Centre Tecnològic Forestal de Catalunya, Solsona, Spain.

Diette S, Lauriac A, 2005. La sylviculture truffière: aperçus historiques, apports techniques et enjeux pour la région méditerranéene. For Méditerr 26: 157-168.

Domínguez-Torres G, Plana E, 2002. The paradox of Mediterranean forests: between economic profitability and social demands. The Catalan case. Proc 2002-02 Forest and Nature Conservation Policy Group "Nature Forest in society. The changing role of forestry in Europe: perspectives for rural development", Wageningen (Netherlands), November 11-14 2001. pp: 133-145.

Duell G, 2012. The President's Report. [online]. National Conference of the Australian Truffle Growers Association. Available in http://www.trufflegrowers.com.au/wp-content/uploads/2012/09/2012-Presidents-Report.pdf. [13 July 2013].
Escafre A, Roussel F, 2006 Rapport relatif au développement de la trufficulture française. [online]. Available in http://agriculture.gouv.fr/IMG/pdf/developpement_truffi_franc.pdf. [13 July 2013].
Eslick H, Dell B, 2013. The role of irrigation in the rot of truffles in Western Australia. 1st International Congress of Trufficulture Tuber 2013, Teruel (Spain), March 5-8.
Fédération Française des Trufficulteurs, 2011. Où acheter la truffe? Marchés de détails, contrôlés par les organisations de trufficulteurs, de fin 2011 au printemps 2012. [online]. Available in http://www.fft-tuber.org/0186_001.pdf. [13 July 2013].
Fédération Française des Trufficulteurs, 2012. Marchés aux truffes en été et automne 2012. [online]. Available in http://www.fft-tuber.org/marche.pdf. [13 July 2013].
Galluzzo N, 2013. Italian truffle cultivation and its commercial trend in the European Union market. IJASR 3: 47-58.
Garcia-Barreda S, Reyna S, 2013. Response of *Tuber melanosporum* fruiting to canopy opening in a *Pinus*-*Quercus* forest. Ecol Eng 53, 54-60.
Granetti B, 2005. L'evoluzione delle richerche sui tartufi. In: Umbria, terra di tartufi (Granetti B, De Angelis A, Materozzi G, eds). Regione Umbria - Gruppo Micologico Ternano, Terni, Italy. pp: 7-15.
Gregori GL, 2007. Principales aspectos de la trufa y la truficultura en Italia. In: Truficultura Fundamentos y técnicas (Reyna S, ed). Mundi-Prensa, Madrid. pp: 433-464.
Gregori GL, 2013. Truffle and truffle cultivation in Italy: main aspects and news. 1st International Congress of Trufficulture Tuber 2013, Teruel (Spain), March 5-8.
Groupement Européen Tuber, 2002. Programme de developpement de la trufficulture et de reconstitution de l'espace truffier en Europe. [online]. Available in http://www.fft-tuber.org/GET.pdf. [13 July 2013].
Guerin-Laguette A, Hesom-Williams N, Parmenter G, Strong G, Wang Y, 2009. Field research and cultivation of truffles in New Zealand: an update. Acta Bot Yunnan 31(S16): 90-93.
Guerin-Laguette A, Cummings N, Hesom-Williams N, Butler R, Wang Y, 2013. Mycorrhiza analyses in New Zealand *truffières* reveal frequent but variable persistence of *Tuber melanosporum* in co-existence with other truffle species. Mycor 23: 87-98.
Hall IR, 2013. Areas of China with suitable January and July air temperatures for the Périgord black truffle. Available at: http://www.trufflesandmushrooms.co.nz/Areas%20of%20China%20with%20suitable%20air%20temperatures%20for%20the%20Perigord%20black%20truffle.pdf
Hall IR, Haslam W, 2012. Truffle cultivation in the Southern Hemisphere. In: Edible ectomycorrhizal mushrooms Current knowledge and future prospects (Zambonelli A, Bonito GM, eds). Springer, Berlin. pp: 191-208.
Hu HT, Wang Y, Hu BY, 2005. Cultivation of *Tuber formosanum* on limed soil in Taiwan. New Zeal J Crop Hort 33: 363-366.
Iotti M, Rubini A, Tisserant E, Kholer A, Paolocci F, Zambonelli A, 2012. Self/nonself recognition in Tuber melanosporum is not mediated by a heterokaryon incompatibility system. Fungal Biol 116: 261-275.
Khabar L, 2007. Truffes au Maroc: état actuel du *Tuber oligospermum* et *T. melanosporum*. Technical presentations and posters 1st World Conference on the Conservation and Sustainable Use of Wild Fungi, Córdoba (Spain), December 10-16. pp: 224-226.
Le Tacon F, Zeller B, Plain C, Hossann C, Bréchet C, Robin C, 2013. Carbon transfer from the host to *Tuber melanosporum* mycorrhizas and ascocarps followed using a 13C pulse-labeling technique. PloS ONE 8(5): e64626.
Lee B, 2008. Taking Stock of the Australian Truffle Industry. [online]. Rural Industries Research and Development Corporation. Available in http://www.trufflegrowers.com.au/wp-content/uploads/2006/09/08-124-Barry-Lees-Final-Report.pdf. [13 July 2013].
Lefevre C, 2010. Truffles and truffle cultivation in North America. Atti 3º Congresso Internazionale sul Tartufo, Spoleto (Italy), November 25-28 2008. p: 64.
Lefevre C, 2012. Native and cultivated truffles of North America. In: Edible ectomycorrhizal mushrooms Current knowledge and future prospects (Zambonelli A, Bonito GM, eds). Springer, Berlin. pp: 209-226.
Lefevre C, Hall I, 2000. The status of truffle cultivation: a global perspective. Acta Hortic 556, 513-520.
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 Microb 78: 6534-6539.
Liu B, Fischer C, Bonet JA, Olivera A, Inchusta A, Colinas C, 2014. Pattern of *Tuber melanosporum* extramatrical mycelium expansion over a 20-year chronosequence in *Quercus ilex* truffle orchards. Mycor 24 (Suppl 1): S47-S54.
Manna D, 1990. La tartuficoltura nello spoletino. Atti del secondo congresso internazionale sul tartufo, Spoleto (Italy), November 24-27 1998. pp: 579-590.
Manna D, 2005. Le vicende del tartufo in Umbria. In: Umbria, terra di tartufi (Granetti B, De Angelis A, Materozzi G, eds). Regione Umbria - Gruppo Micologico Ternano, Terni, Italy. pp: 209-219.
Marone E (coord), 2011. La filiera del tartufo e la sua valorizzazione in Toscana e Abruzzo. Firenze University Press, Firenze, Italy.
Martin F, Kohler A, Murat C, Balestrini R, Coutinho PM, Jaillon O *et al.,* 2010. Périgord black truffle genome uncovers evolutionary origins and mechanisms of symbiosis. Nature 464: 1033-1038.
Materozzi G, 2005. Fiere e mostre mercato del tartufo in Umbria. In: Umbria, terra di tartufi (Granetti B, De An-

gelis A, Materozzi G, eds). Regione Umbria - Gruppo Micologico Ternano, Terni, Italy. pp: 254-255.

Montanini B, Levati E, Bolchi A, Kohler A, Morin E, Tisserant E *et al.,* 2011. Genome-wide search and functional identification of transcription factors in the mycorrhizal fungus *Tuber melanosporum*. New Phytol 189: 736-750.

Murat C, Zampieri E, Vizzini A, Bonfante P, 2008. Is the Perigord black truffle threatened by an invasive species? We dreaded it and it has happened! New Phytol 178: 699-702.

Murat C, Rubini A, Riccioni C, De la Varga H, Akroume E, Belfiori B *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: 176-187.

Olivier JM, Savignac JC, Sourzat P, 1996. Truffe et trufficulture. Ed Fanlac, Perigueux, France.

Pacioni G, Leonardi M, Di Carlo P, De Laurentis G, 2014. Instrumental monitoring of the birth and development of truffles in a *Tuber melanosporum* orchard. Mycor 24 (Suppl 1): S65-S72.

Pettenella D, Klöhn S, Brun F, Carbone F, Venzi L, Cesaro L, Ciccarese L, 2004. Italy's country report. [online]. Cost Action E30 Economic integration of urban consumers' demand and rural forestry production. Available in http://www.isprambiente.gov.it/files/atmosfera/italy-report.pdf. [13 July 2013].

Pilz D, Lefevre C, Scott L, Julian J, 2009. Oregon culinary truffles: an emergent industry for forestry, agriculture and culinary tourism. [online]. Available in http://www.oregontruffles.org/truffles_feasibility_final.pdf. [13 July 2013].

Pinto-Correia T, 1993. Land abandonment: Changes in the land use patterns around the Mediterranean basin. In: Etat de l'Agriculture en Méditerranée. Les sols dans la région méditerranéenne: utilisation, gestion et perspectives d'évolution. CIHEAM, Zaragoza, Spain pp: 97-112.

Plett JM, Martin F, 2011. Blurred boundaries: lifestyle lessons from ectomycorrhizal fungal genomes. Trends Genet 27: 14-22.

Ramírez R, Henríquez R, Reyna S, Suárez R, 2007. Cultivo de la trufa negra en Chile. In: Truficultura Fundamentos y técnicas (Reyna S, ed). Mundi-Prensa, Madrid. pp: 483-492.

Renowden G, 2005. The truffle book. Limestone Hills Publ, Amberley, New Zealand.

Reyna S, 2007a. Introducción: Historia y perspectivas de la truficultura. In: Truficultura Fundamentos y técnicas (Reyna S, ed). Mundi-Prensa, Madrid. pp: 27-49.

Reyna S, 2007b. Sostenibilidad de la truficultura: aspectos ecológicos, económicos y sociales. In: Truficultura Fundamentos y técnicas (Reyna S, ed). Mundi-Prensa, Madrid. pp: 51-73.

Riccioni C, Belfiori B, Rubini A, Passeri V, Arcioni S, Paolocci F, 2008. *Tuber melanosporum* outcrosses: analysis of the genetic diversity within and among its natural populations under this new scenario. New Phytol 180: 466-478.

Rubini A, Belfiori B, Riccioni C, Tisserant E, Arcioni S, Martin F, Paolocci F, 2011a. Isolation and characterization of MAT genes in the symbiotic ascomycete *Tuber melanosporum*. New Phytol 189: 710-722.

Rubini A, Belfiori B, Riccioni C, Arcioni S, Martin F, Paolocci F, 2011b. *Tuber melanosporum*: mating type distribution in a natural plantation and dynamics of strains of different mating types on the roots of nursery-inoculated host plants. New Phytol 189: 723-735.

Selosse, MA, Taschen E, Giraud T, 2013. Do black truffles avoid sexual harassment by linking mating type and vegetative incompatibility? New Phytol 199: 10-13.

Sourzat P, 2007. La truficultura en Francia. In: Truficultura Fundamentos y técnicas (Reyna S, ed). Mundi-Prensa, Madrid. pp: 399-431.

Sourzat P (coord), 2008. Principe de précaution en trufficulture. Station Trufficulture du Montat, Le Montat, France.

Sourzat P (coord), 2010. L'environnement truffier: contraintes et gestion. Station Trufficulture du Montat, Le Montat, France.

Splivallo R, Ottonello S, Mello A, Karlovsky P, 2011. Truffle volatiles: from chemical ecology to aroma biosynthesis. New Phytol 189: 688-699.

Stobbe U, Egli S, Tegel W, Peter M, Sproll L, Büntgen U, 2013. Potential and limitations of Burgundy truffle cultivation. Appl Microbiol Biot 97: 5215-5224.

Suz LM, Martín MP, Oliach D, Fischer CR, Colinas C, 2008. Mycelial abundance and other factors related to truffle productivity in *Tuber melanosporum-Quercus ilex* orchards. FEMS Microbiol Lett 285: 72-78.

Trappe JM, Molina R, Luoma DL, Cázares E, Pilz D, Smith JE *et al.,* 2009. Diversity, ecology, and conservation of truffle fungi in forests of the Pacific Northwest. USDA General Technical Report PNW-GTR-772.

Treloar I, 2013. New Zealand Truffle Association Overview. [online]. National Conference of the Australian Truffle Growers Association. Available in http://www.trufflegrowers.com.au/wp-content/uploads/2012/09/New-Zealand-Truffle-Association-Overview.pdf. [13 July 2013].

Turgeman T, Sitrit Y, Danai O, Luzzati Y, Bustan A, Roth-Bejerano N *et al.,* 2012. Introduced *Tuber aestivum* replacing introduced *Tuber melanosporum*: a case study. Agroforest Syst 84: 337-343.

Wang X, 2012. Truffle cultivation in China. In: Edible ectomycorrhizal mushrooms Current knowledge and future prospects (Zambonelli A, Bonito GM, eds). Springer, Berlin. pp: 227-240.

Wang YJ, Tan ZM, Zhang DC, Murat C, Jeandroz S, Le Tacon F, 2006. Phylogenetic and populational study of the *Tuber indicum* complex. Mycol Res 110: 1034-1045.

Wedén C, Chevalier G, Backlund A, 2013. The first production results of *Tuber melanosporum* in Sweden. 1st International Congress of Trufficulture Tuber 2013, Teruel (Spain), March 5-8.

Zambonelli A, Iotti M, Rossi I, Hall I, 2000. Interactions between *Tuber borchii* and other ectomycorrhizal fungi in a field plantation. Mycol Res 104: 698-702.

Zambonelli A, Hall IR, Fitzpatrick N, 2009. Lecture de l'approche de la trufficulture dans l'Hemisphère Austral à la lumière de l'expérience italienne. In: Les nouvelles techniques de culture de la truffe (Olivier JM, Vilatte C, coord). MGD Imprimeurs, Sarlat, France. pp: 68-77.

Zambonelli A, Iotti M, Piattoni F, 2012. Chinese *Tuber aestivum* sensu lato in Europe. Open Mycol J 6: 22-26.

Zhang JP, Liu PG, Chen J, 2012. *Tuber sinoaestivum* sp. nov., an edible truffle from southwestern China. Mycotaxon 122: 73-82.