Chapter 14
European Black Truffle: Its Potential Role in Agroforestry Development in the Marginal Lands of Mediterranean Calcareous Mountains

S. Reyna-Domenech* **and S. García-Barreda**

Abstract The European black truffle is the highly valued fruiting body of the ectomycorrhizal fungus *Tuber melanosporum* Vitt. Despite the technical advances achieved during the 19th and the 20th centuries, truffle production has suffered a pronounced decline. At present, the ecological requirements of this fungus are relatively well-known and cultural practices have been developed to meet these requirements; nevertheless, plantation yields remain highly unpredictable. Black truffles are naturally found in many Mediterranean calcareous mountains with limited agricultural and forestry potential. An agroforestry approach, integrating management of truffle-producing forests and cultivation of the fungus in marginal agricultural land could contribute to sustainable rural development of these less favoured areas, thanks to the socio-economic and environmental implications of the black truffle cultivation.

Keywords Non-timber forest products,sustainable development, truffle cultivation, *Tuber melanosporum* Vitt., truffle silviculture

Introduction

The European black truffle (*Tuber melanosporum* Vitt. or *T. nigrum* Bull.) is an ectomycorrhizal fungus from the class Ascomycetes. Its fruiting body (sporocarp) is found underground and is highly valued in international *haute cuisine*, because of its refined and pervasive flavor. Current black truffle production comes almost exclusively from France, Spain and Italy. These countries comprise most of the worldwide black-truffle natural distribution area (Fig. 14.1).

Fundación Centro de Estudios Ambientales del Mediterráneo (Fundación CEAM), C/ Charles R Darwin 14, Parque Tecnológico, 46980 Paterna, Valencia, Spain

E.T.S. Agronomos y Montes, Universidad Politécnica de Valencia Camino de Vera sn Valencia 46022 Spain

*Corresponding author: e-mail: sreyna@agf.upv.es

A. Rigueiro-Rodríguez et al. (eds.), *Agroforestry in Europe: Current Status and Future Prospects*.

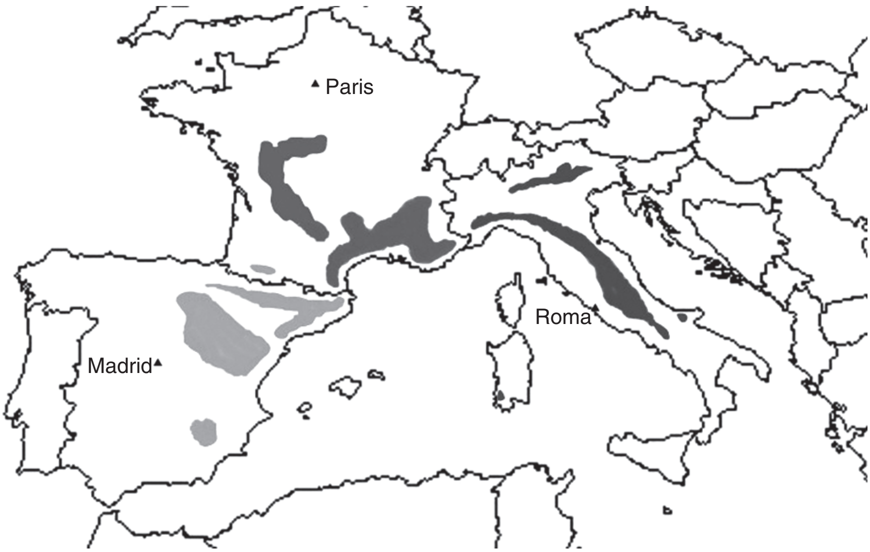

Fig. 14.1 Main natural distribution areas for *Tuber melanosporum* (French area is taken from Delmas 1983, Italian from Gregori 2007 and Spanish is modified from Reyna 2000)

Historical Evolution of Truffle Production

The definitive rise in truffle use in French and Italian cooking began in the 16th century and was driven by kings, princes and other aristocrats. Increased culinary use led to technical advances in the management of naturally occurring *truffières* (non-planted truffle-producing stands) and expanded truffle harvesting in France and Italy during the 19th century, which is known as the golden age of black truffle production. In 1868, French production was estimated to be around 1,588 t (Chatin 1869).

At the beginning of the 19th century, a French farmer named Joseph Talon made an important finding on the relation between truffles and oaks, which he summed up in the sentence "If you want truffles, sow acorns". Thousands of hectares of oak trees were planted thanks to this simple idea.

The dread plague *Phylloxera* also played a role in this golden age, since truffle cultivation was used as an alternative to vineyards (Olivier et al. 1996). The French reforestation laws of 1860 and 1882 contributed to the expansion of truffle-producing trees, like the evergreen holm oak (*Quercus ilex* L.), in calcareous mountains such as the Luberon and Mont Ventoux (Diette and Lauriac 2005). In Italy, the reforestation activities driven by Mattirolo and Francolini were outstanding (Granetti 2005a).

In the 20th century, however, French truffle production declined spectacularly, and the current annual production in this country is between 10–50 t (Fig. 14.2). It is commonly accepted that this decline was a consequence of rural depopulation

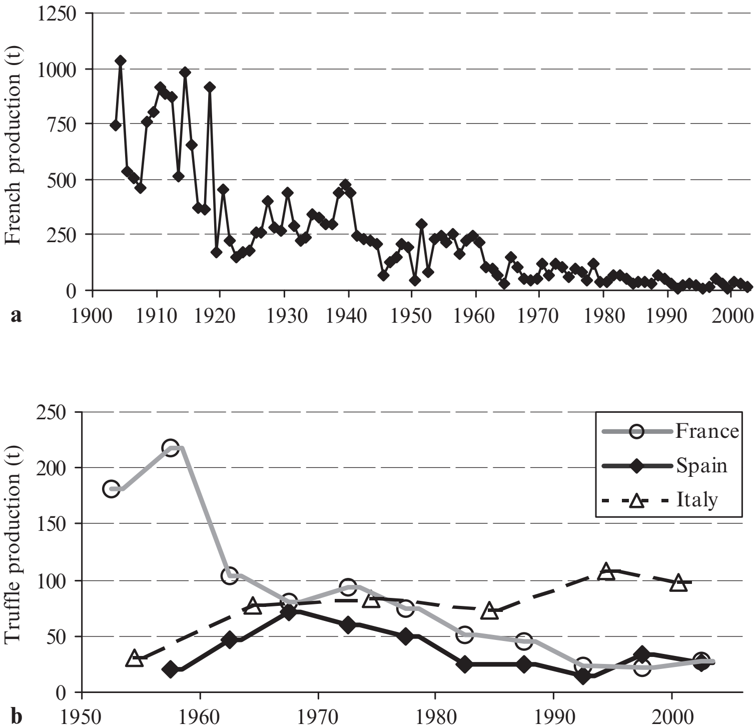

Fig. 14.2 Evolution of black truffle production in France during the 20th century **(a)** and comparison of production trends in France, Spain and Italy from 1950 to 2005 **(b)**. In Italy, all harvested species are considered (French production is taken from Diette and Lauriac 2005, Spanish from Reyna 2000 and Italian from Pettenella et al. 2004)

caused by two World Wars and by the rural to urban drift. This rural abandonment resulted in the practical disappearance of firewood cutting, charcoal production, lands dedicated to marginal agricultural activities and forest grazing by the once-vast flocks of sheep. As a consequence, forest stand density increased greatly. In addition, much of the farmers' empirical knowledge on truffle management was lost (Blondel and Aronson 1995; Olivier et al. 1996; Bye and Chazoule 1998; Sourzat 2001).

In Italy, the decline in production was smaller and limited to the first half of the century (Manna 1990). The main reasons for this decline were deforestation during the war periods 1914–1918 and 1939–1945 (truffle trees were cut for firewood) and the traditional problems of poaching and over-harvesting, which are related to Italian property laws. Despite this, Italian truffle production seems to have increased since the 1950s (Pettenella et al. 2004) (Fig. 14.2).

In contrast to France and Italy, Spain has only recently incorporated truffles into its popular gastronomy. And although Chatin (1892) had already mentioned a small Spanish truffle production (the first harvesters in Spain were probably Frenchmen searching for truffles in the Catalan Pyrenees), it was not until the 1950s that the systematic collection of naturally occurring Spanish truffles began. By the 1970s, most of the naturally occurring Spanish *truffières* had been found and exploited. The oldest known truffle plantation in Spain dates from 1968 and is located in Castelló (eastern Spain). It followed methods similar to those used in traditional French and Italian plantations.

An alarming reduction in Spanish truffle production was observed in the 1970s (Fig. 14.2). This can be explained by the increasing rural depopulation, the collapse of many traditional forest uses and the intensive harvesting practices that prevented the natural reinoculation of short roots by means of spores. Additional reasons for the decline could be that reforestation activities using conifers were carried out in truffle-producing stands, and that, according to wild fauna data, there was a spectacular increase in the number of wild boars. Although truffle spores are dispersed when eaten by wild boars, this species has the potential to damage the roots and the soil of the *truffières* through its extensive and deep rooting activity (Reyna 2000).

In the 1970s, another relevant discovery was made in France and Italy. Mycorrhizal seedling production was developed by researchers from the INRA (French National Institute for Agricultural Research) and the IPLA (Piedmont Institute for Trees and the Environment), on the basis of the experiments by Mannozzi-Torini (Chevalier 2001b). The introduction of mycorrhizal-infected seedlings on the market greatly increased plantation activity in France and Italy, in an attempt to mitigate the worldwide scarcity of truffles.

Moreover, truffle growers in France began to form associations at the end of the 1960s, and the French government established subsidies for truffle plantations on agricultural lands in the 1970s. Public technical assistance and training activities for agriculturalists were also developed (Bye and Chazoule 1998).

It is estimated that French truffle agriculturalists planted 1,000 ha year^{-1} between 1990 and 2005 (Sourzat 2005). At present, it is estimated that there are about 8,000 ha of young mycorrhizal-infected plantations (less than 10 years old) and about 10,000 ha with ages ranging from 10 to 30 years (Escafre and Roussel 2006). The main tree species used in these plantations are downy oak (*Quercus humilis* Mill. or *Q. pubescens* Willd.), evergreen holm oak and hazel (*Corylus avellana* L.). The most active regions (with the highest production levels and plantation rates) are located in the southeast (Vaucluse, Drôme and Gard) and the southwest (Lot and Dordogne).

The most active regions in Italy at present are located in the centre of the country (Marche, Umbria and Abruzzo). Detailed data on the area occupied by truffle plantations are not available, but considering all the plantations of the different truffle species (including *Tuber magnatum* Pico, *T. aestivum* Vitt., *T. borchii* Vitt., *T. brumale* Vitt., etc., which are a minority in comparison with *T. melanosporum*), it is estimated that there are about 5,000 ha (Gregori 2007). In *T. melanosporum* plantations, the most commonly used host tree is downy oak, but hazel, European hop-hornbeam (*Ostrya carpinifolia* Scop.) and evergreen holm oak are also common.

In 1971, the world's largest truffle plantation belonging to a single landowner was planted in Soria (northern Spain). It consists of 600 ha mainly planted with evergreen holm oak, 250 ha of which are irrigated (Reyna et al. 2007). Despite the presence of this huge plantation, Spanish truffle plantations did not spread until the end of the 1980s, when mycorrhizal-infected plants were first made available on Spanish markets (Rodríguez-Barreal et al. 1989). At present, it is estimated that there are more than 4,000 ha of truffle plantations in Spain. The current annual rate of planting is estimated between 250 and 500 ha year^{-1}. The most active regions are located in the east (Teruel, Castelló) and the northeast (Soria, Huesca). The most planted tree species is evergreen holm oak, although hazel, downy oak and Portuguese oak (*Quercus faginea* Lam.) are also common (Reyna et al. 2005).

These privately initiated plantations have succeeded in stopping the decline in truffle production in France and Spain, but a real recovery in national productions has not yet been observed (Sourzat 2005; Reyna et al. 2005). As a result, the current production of black truffles in Europe has two clearly differentiated provenances: naturally occurring *truffières* or truffle plantations. There are no official data on this, but opinion surveys among dealers and experts suggest that fewer than 10% of French black truffles and about 70% of Spanish ones come from natural *truffières*. In Italy, considering all truffle species together, about 50% come from natural *truffières* (Gregori 2007; Sourzat 2007).

Truffle research has been promoted in France and Italy by the five International Congresses on Truffles held since 1968. In Spain, it was not until the 1990s that research activity increased. Most current research groups in the three countries are limited by modest staff, poor financial resources and little collaboration with the private sector. The experience of the experimental stations in Le Montat (Lot) and Sant' Angelo (Marche), in which there is close collaboration between researchers and truffle growers is especially interesting.

Ecology

Life Cycle

Since it is a symbiotic fungus, the black truffle depends mainly on living host trees as a carbohydrate source. Its symbiotic phase is formed by ectomycorrhizae (ECM) and their attached mycelium. Nevertheless, *T. melanosporum* mycelium conserves some pathogenic and saprophytic capabilities: it can infect the roots of some weeds and grasses producing necrosis in the root cortices and it forms stromata on the roots of host trees (Plattner and Hall 1995; Pargney et al. 2001a). These capabilities and/or the toxic substances produced by the fungus (Bonfante et al. 1971; Pacioni 1991; Papa et al. 1992) seem to be responsible for the formation of the *brûlé* (burn). The *brûlé* is the area where most of the sporocarps appear; it is characterised by its scarce vegetation. Other interesting vegetative structures are the latent hyphae found in old, non-active ECM by Pargney et al. (2001b).

In relation to the sexual stage, the environmental and biological determinants of the fruiting process have not yet been discovered. A strictly self-fertilizing reproductive system was proposed by Bertault et al. (1998), but recent research on *T. magnatum* suggests that this truffle outcrosses (Paolocci et al. 2006). The morphology of the fertilization process also remains unknown (Poma et al. 2006). Callot et al. (1999) described *T. melanosporum* ascogonium, but the antheridia of this species have not been identified.

A great number of truffle primordia can be found in the soil of productive *brûlés* in June (Olivier et al. 1996; Callot et al. 1999; Di Massimo et al. 2006). Most of them die without achieving maturity. The sporocarp is supposed to grow autonomously, without any attachment to the host tree, from the initial stages of development (Barry et al. 1994; Callot et al. 1999). From December to March, the mature sporocarps produce their particular smell and attract various mammals and insects, which disperse their spores.

Symbiont Plants

T. melanosporum has a relatively wide range of host species (Ceruti et al. 2003). The most widespread truffle-producing plants are evergreen holm oak, downy oak and hazel. Locally, other species can also sustain good truffle production, depending on the climate and the soil: English oak (*Quercus robur* L.), kermes oak (*Q. coccifera* L.), Portuguese oak, European hop-hornbeam, European hornbeam (*Carpinus betulus* L.), Oriental hornbeam (*C. orientalis* Mill.), etc.

The root system of each plant species develops in a different way and this affects *T. melanosporum* ECM numbers. In 3-year-old plantations, Olivera (2005) found that hazel seedlings had a more extensive root system and a higher percentage of ectomycorrhzial short roots colonized by *T. melanosporum* than evergreen holm oak and Portuguese oak. In an older plantation (7 years), however, Etayo (2001) found a higher number of soil-resident fungi colonizing short roots of hazel than those of evergreen holm oak, suggesting that hazel short roots are more readily colonized by *T. melanosporum*, but also by soil-resident fungi.

Climate

Most truffle-producing regions are located in transition zones between Mediterranean-type climates and temperate ones. They usually experience a summer arid period (the mean temperature in degree celsius being more than twice the amount of rainfall in millimeter), but this is not as marked as in the typical Mediterranean-type climate (with an arid period of 3 or more months). The mean annual temperature in the truffle-producing regions is 8.6–14.8°C, the mean temperature of the warmest month is 16.5–23°C, the mean temperature of the coldest month is 1–8°C and the mean annual rainfall is 450–900 mm, although areas with up to 1,500 mm have been cited in France (Pacioni 1987; Reyna 2000; Ricard et al. 2003).

Summer rainfall (July and August) is a highly unpredictable climatic factor in most of the *T. melanosporum* distribution area, and it is the climatic factor with the greatest influence on sporocarp yield in productive *brûlés* (Callot et al. 1999; Ricard et al. 2003). In this period the sporocarp is likely to grow autonomously, and it seems to be sensitive to low soil moisture.

Since there is considerable variability between regions, analyses of climatic data do not show any clear summer rainfall threshold for yield reduction, but Ricard et al. (2003) point out that August rainfall in years of maximum production ranges from three to four times the mean temperature. The number of summer days without rain which the sporocarps can withstand depends on soil characteristics and maximum temperatures, but the experience of truffle growers and climatic analyses suggest that it is around 30–35 days (Roux 2001).

Soil

Black truffle typically inhabits calcareous soils, with pH 7.5–8.5 and some content of calcium carbonate in soil gravels, fine mineral particles or clay-humus complex. These soils are never saline (conductivity lower than 0.35 mmhos cm^{-1}, measured in solution 1:5). Their organic matter is usually 1–8%, the C/N ratio is usually 5–15 and they lack leaf litter (Bencivenga et al. 1990; Sourzat 1997; Reyna 2000). Sand-size organic matter and microbial activity are lower than in surrounding soil (Callot et al. 1999; Ricard et al. 2003). They are well-aerated and well-drained soils, with a well-developed structure and no soil crust and are never hydromorph soils (Lulli et al. 1999; Callot et al. 1999). Truffle-producing soils are usually found on low south-facing slopes (5–30%).

Soil biology (especially microflora and macrofauna) might also be important, but present knowledge is limited (Mamoun and Olivier 1992; Callot et al. 1999; Mello et al. 2006).

Stand Vegetation

Truffle-producing stands show a convergent vegetation physiognomy. Most of the best naturally occurring *truffières* locate in areas with open vegetation (canopy cover lower than 30%, Hart-Becking index higher than 1.5, scarce shrub cover) and receive direct sunlight on their soil surface (Fig. 14.3). Most of the new *truffières* appear in such a situation (Olivier et al. 1996; Reyna et al. 2004; Sourzat 2004; Granetti 2005b).

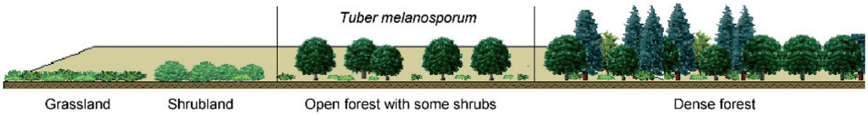

Fig. 14.3 Plant succession in abandoned agricultural land, showing the stage at which *T. melanosporum* is usually found

The Hart-Becking index is calculated as the ratio between the average spacing of trees and their dominant height. It is a practical index for assessing the stand density of the forest (i.e. tree cover on an area).

Competitor Ectomycorrhizal Fungi

The soil contains propagules from a high diversity of ectomycorrhizal fungi. The soil inoculum potential accounts for all the propagules that can effectively colonize fine roots. It depends on land use: soils that have not supported ectomycorrhizal plants (oaks, pines, etc.) for a long time show a lower ectomycorrhizal inoculum potential. Therefore, if seedlings inoculated with *T. melanosporum* are planted on these soils, their roots are less likely to be contaminated with soil-resident fungi (Fig. 14.4, Frochot et al. 1990). *T. melanosporum* competes poorly with many soil-resident fungi, and this seems to be related to the low level of genetic diversity of the black truffle and its narrow tolerance to environmental variation (Bertault et al. 1998).

The use of mycorrhizal-infected seedlings reduces root colonization by soil-resident ectomycorrhizal fungi when compared to non-mycorrhized control seedlings (Frochot et al. 1990; Reyna et al. 2006). The quality of seedling mycorrhization (number and percentage of colonized roots) is consequently an important component in black truffle cultivation. Bourrières et al. (2005) found that the percentage of short roots colonized by *T. melanosporum* in the nursery and 4 years later in the field were correlated.

The most important competitor in many truffle orchards is *T. brumale*, especially in France. This truffle shows similar ecological requirements to *T. melanosporum*, but has wider ecological amplitude (Mamoun and Olivier 1993; Callot et al. 1999; Raglione et al. 2001). It is commonly accepted that the main problems appear in plantations where the cultural practices have not taken into account the ecological requirements of *T. melanosporum* (low pH, lack of soil aeration, excessive irrigation, shade, fertilization or organic matter). Linear mechanised tilling helps to expand the propagules of this species (Sourzat 2004).

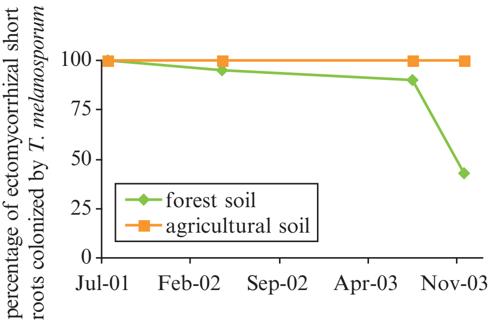

Fig. 14.4 Land use affects persistence of *T. melanosporum* mycorrhizae on roots of commercially inoculated evergreen holm oak seedlings. Roots grown in forest soil show more colonization from soil-resident fungi. Differences between land uses were significant for the three measurements (From Reyna et al. 2006)

Harvesting and Improvement of Naturally Occurring *Truffières*

Truffle Silviculture

In many black truffle-producing forests and old truffle orchards in France, Italy and Spain, the stand density is higher than advisable (canopy cover higher than 30%, Hart-Becking index lower than 1.5). Truffle silviculture models have been designed and experimentally applied in Spain (Hernández et al. 2001; Reyna et al. 2004) and Italy (Gregori et al. 2001; Tagliaferro 2001) to combat this situation. They consist of opening the vegetation and eliminating the non-productive, competitor trees and their associated ECM. Trees and shrubs from species that do not produce black truffles are clearcut, whereas trees from black truffle-producing species are pruned, until a canopy cover lower than 30% is achieved around the *brûlés*. Other cultural practices can also be applied when necessary: weeding, soil tillage, liming, slash burning, etc.

With clearcutting, pruning and weeding, Gregori et al. (2001) immediately regenerated truffle production (around 5 kg ha^{-1}) in an old truffle orchard in Marche. Previously, the orchard hardly produced black truffles, and after the treatment no black truffles were found in the corresponding unmanaged control plot.

In natural *truffières* in Castelló, Reyna and Garcia (2005b) monitored the evolution of ECM and sporocarp yields after truffle silviculture (clearcutting pines, pruning of *Quercus* and removing shrubs) was applied in 2000–2001 (Fig. 14.5). A slight but non-significant increase in the percentage of ectomycorrhizal short roots colonized by *T. melanosporum* was observed (Fig. 14.6). In relation to sporocarp yield, given that no unmanaged control plots existed, the evolution of the production was assessed through comparison to yield data previous to the treatment. Given that sporocarp yield is highly variable from year to year, mainly for climatic reasons, an index for the meteorological suitability of the year was incorporated (i.e. black truffle production in Spain). In this way, it seems that the *truffières* responded positively to the silvicultural treatments (Fig. 14.6), but only after 3 years (in 2003–2004, when the ratio between sporocarp yield and the suitability index was for the first time higher than in 1997–1998, before the management treatment was imposed).

Fig. 14.5 Application of truffle silviculture in a mixed pine-oak forest with a low density of *brûlés* (Reyna et al. 2004). Clearcutting of pines and pruning of oaks are carried out only around currently producing *brûlés* (*brûlés* in white)

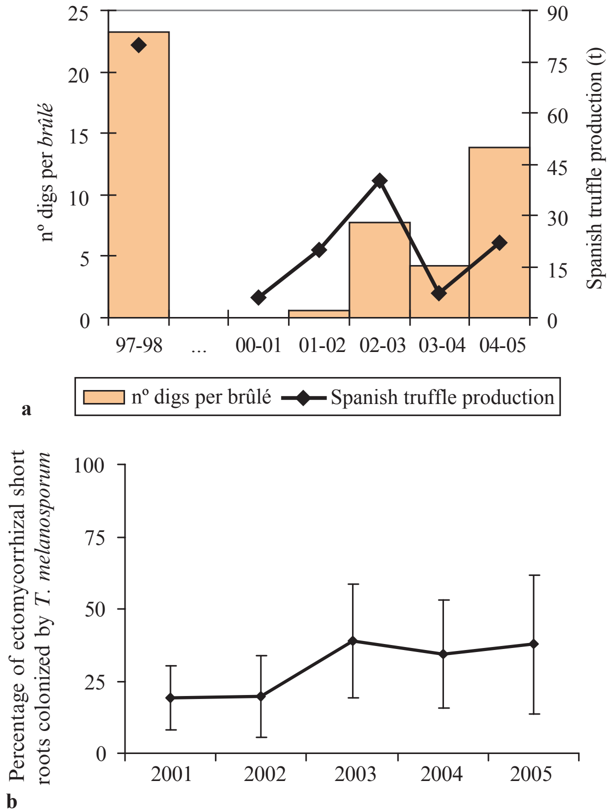

Fig. 14.6 Response of *T. melanosporum* to truffle silviculture: **(a)** sporocarp production in the managed *truffières* (estimated on the basis of the number of digs), with respect to the suitability of meteorological conditions in the region (estimated on the basis of truffle production in Spain), **(b)** percentage of ectomycorrhizal short roots colonized by *T. melanosporum*. The silvicultural treatments were carried out in 2000–2001. Digs are the holes made for harvesting truffles (Figures taken from Reyna and Garcia 2005b)

Property Conflicts and Harvesting Regulation

In Spain and France, truffles belong to the landowner by right of accession (Omaggio 2001; Trull 2007). In Italy, however, truffles are considered "*res nullius*" or ownerless property (i.e. they are not the object of rights of any specific subject and therefore they belong to the first taker) if the soil is not cultivated (De Angelis 2005).

Due to the high market price of *T. melanosporum,* over-harvesting is common. Because the spores of the truffle are completely encased in the sporocarps, systematic and intensive harvesting prevents reinoculation of the exploited *truffières* and colonization of new ones.

Over-harvesting and the use of destructive harvesting techniques are more likely to occur when the legal property rights of mycological resources are not properly defined or when a private exploitation system does not exist (common property resources). Sustainability should be guaranteed through reinoculation of the *truffières* and/or harvesting regulation (Gil et al. 2001).

Cultivation of the Black Truffle

Cultural Practices

Truffle cultivation consists of three clearly differentiated stages: the plantation and colonization period (the first 4–7 years, in which the plant adapts to field conditions and the fungus spreads), the consolidation period (until the 10th–15th year: the *brûlé* is developed and the first truffles appear) and the exploitative period (i.e. the productive stage, from the moment the orchard attains full production).

As the requirements of the cultivated fungus are slightly different at each stage, the cultural practices must also be specifically applied. Cultural practices also differ depending on local climate, soil conditions and previous experience of the agriculturalist. Thus, a variety of trufficulture models exist (e.g. in France: Pallier and Tanguy methods) (Olivier et al. 1996; Callot et al. 1999).

Most available experimental data on truffle cultivation focus on its effect on *T. melanosporum* ECM, which is mainly useful for the pre-productive period, not the exploitative period. Unfortunately, experiments on cultivation technique effects on sporocarp yield are scarce, lack scientific design and are highly influenced by interannual meteorological variability. Despite this, some remarks are provided below regarding present experiments on this subject, because of their practical interest to managers and researchers.

First of all, the establishment of the trees is critical, since plants with quicker growth are the first to form the *brûlé* and to produce sporocarps (Shaw et al. 1996; Granetti and Baciarelli 1997; Lulli et al. 1999; Letizi et al. 2001). Widespread fertilization is recommended by some authors (Chevalier and Poitou 1989). Bonet et al. (2006) found no detrimental effect from low-level foliar fertilization (fertilizer: 12% w w^{-1} nitrogen, 7% w w^{-1} phosphoric anhydride and 7% w w^{-1} potassium oxide, diluted in water to 1.2%) on *T. melanosporum* ECM in the short term (18 months); however, a meta-analysis of mycorrhizal response to phosphorus and nitrogen showed some potential detrimental effects of fertilization on mycorrhizal abundance (Treseder 2004).

Quality of the seedlings (morphology and physiological status of plants) and previous preparation of the ground also play a role in successful establishment. Tree shelters can be helpful as well: Olivera (2005) found that they increased shoot growth in *Q. ilex* L. seedlings without any effect either on root biomass or on *T. melanosporum* ECM.

Soil management of the plantation influences soil moisture, evolution of soil organic matter and soil biology. A choice must be made between chemical control of weeds, mechanical soil tilling and grassing (i.e. maintaining a spontaneous or seeded grass cover between the rows of trees).

Pesticides are not generally recommended because their long-term effects on *T. melanosporum*, soil fauna and soil microflora are unknown; only glyphosate and ammonium gluphosinate (herbicides) have been scientifically tested, and they seem harmless in the short term (Garvey and Cooper 2001; Bonet et al. 2006). Presence of the grass *Festuca ovina* L. (which is common in productive *brûlés* in France) has a detrimental effect both on 1-year-old hazel survival and on *T. melanosporum* ECM (Mamoun and Olivier 1997); thus, the close proximity of grasses to young seedlings should be avoided. Bonet et al. (2006) also found that chemical weed control increased seedling survival during the 1st year, as compared to mechanical tilling and unmanaged control.

Mechanical soil tilling is also used during the exploitative period to increase sporocarp production. No conclusive data exist on its effectiveness, but Ricard et al. (2003) suggest that it increases sporocarp size and depth and yields more rounded sporocarps.

In relation to soil moisture management, excessive irrigation should be avoided. Bonet et al. (2006) found that high levels of summer irrigation (totally compensating for the water deficit) during the establishment period reduced the number of *Q. ilex* short roots and *T. melanosporum* ECM when compared to moderate irrigation (compensating for half the water deficit) and unmanaged control. Mamoun and Olivier (1990) found that a high level of irrigation (soil moisture 31%) increased root growth in hazel, reduced the number of *T. melanosporum* ECM and increased root colonization by other ectomycorrhizal fungi, when compared to more reduced irrigation levels (soil moisture 12% and 21%).

During the exploitative period, summer irrigation is commonly used to increase sporocarp production, especially in dry summers. No conclusive data exist on its long-term effects, the optimum dose or the frequency required, but the available results (Ricard et al. 2003; Hernández et al. 2005) suggest that it increases the total weight of the truffles produced, but not their mean size. Hernández et al. (2005) performed two waterings of 5–15 mm per summer (water interval: 15–25 days), and Ricard et al. (2003) carried out four waterings of 20 mm (water interval: 20 days) per summer.

Mulching usually consists of calcareous stones, shrub branches or cereal straw. It aims to prevent soil water evaporation (Horton et al. 1996), but it can also have side effects on ECM. In a young plantation, Etayo (2001) found that permanent mulching with cereal straw reduced short root colonization by *T. melanosporum* and increased the number of other ectomycorrhizal species colonizing the short roots. Zambonelli et al. (2005) found the same effect in a *T. uncinatum* plantation. In productive *truffières*, no conclusive data are available, but Hernández et al. (2005) found that mulching with calcareous stones increased weight yield of sporocarps. In a productive truffle orchard in southeastern France, soil stoniness appeared as the main ecological factor accounting for spatial variability in sporocarp production (Oliach et al. 2005).

Finally, tree management (pruning, choice of planting distance) is mainly related to stand density and becomes important when the plantation ages. The trees are usually pruned from the 3rd–5th year (when the trees reach an approximate height of 1 m) and an inverted cone-shaped crown is sought. The most common planting distances range from 4 × 5 to 8 × 8 m for oaks, hazel and hop-hornbeam.

Sporocarp Yield and Economic Evaluation

In contrast to saprobic species, which can be easily grown, ectomycorrhizal fungi are far more difficult to cultivate. Some black truffle plantations produce more than 20 kg ha^{-1} $year^{-1}$ at the age of 15 while others, at the same age, have not started to produce (Olivier et al. 1996; Bye and Chazoule 1998; Callot et al. 1999; Bencivenga and Di Massimo 2000; Reyna et al. 2007). Technical assessment would be necessary to find out if the reasons that account for these differences are environmental (soil, climate), biological, or due to management practices (Sourzat et al. 2001). The economic yield of a plantation will depend on the age it attains full production and the percentage of trees producing sporocarps. Duration of the exploitative period is unknown, but it seems to be at least 25–30 years, since plantations of this age range are currently in production and show no signs of depletion.

In spite of these uncertain factors, economic evaluations have been attempted in France, Italy and Spain. Bonet and Colinas (2001) reviewed some of them and found that the internal rate of return- IRR (the discount rate that makes the net present value of cash flow of a project equal to zero, that is, the interest yield expected from the investment) was always above 9%, although the investment return time was longer than 10 years.

As an example, an economic evaluation is presented which attempts to deal with two of the most uncertain factors in the truffle plantation project: sporocarp yield and truffle price. The price of European black truffles varies greatly from year to year and its future level is likely to depend on the evolution of global black truffle production. The estimated IRR for the chosen assumptions is shown to vary from

Table 14.1 Estimated internal rate of return for a truffle plantation when considering different assumptions related to sporocarp yield, irrigation and truffle price. Establishment and exploitation costs have been calculated for eastern Spain [a]. It is assumed that the plantation starts producing at the age of 8 and reaches full production at the age of 15. Lifetime of the project: 25 years

	Black truffle price (euros kg^{-1})		
Sporocarp yield at full production, irrigation	200	400	800
5 kg ha^{-1} – unirrigated	4	8	12
10 kg ha^{-1} – unirrigated	5	10	15
15 kg ha^{-1} – unirrigated	10	15	21
20 kg ha^{-1} – irrigated	10	15	21
30 kg ha^{-1} – irrigated	13	19	25
60 kg ha^{-1} – irrigated	18	25	33

[a]Cropland price: 4,500 euros per hectare, 250 seedlings per hectare are planted at a price of 6 euros each, establishment and exploitation cost: 25,000 euros for non-irrigated plantations and 52,000 euros for irrigated plantations

4–12% in the lower yield scenarios to 18–33% in the higher yield ones (Table 14.1). Public subsidies, which are common in many regions, are not included in these calculations.

Socio-economic and Environmental Impact of Black Truffle

Economic Importance: Estimated Production and Prices

Making a correct evaluation of the total truffle production is difficult because of the level of secrecy that generally surrounds the sector. In recent years, however, information has become more transparent and accessible, especially since plantations are beginning to predominate. According to the GET (European Tuber Group), from 1990 to 2002 the mean annual European production of *T. melanosporum* was 59 t (approximately 40% France, 40% Spain and 20% Italy). Annual variability in truffle production remains high because it is strongly correlated with summer rainfall (annual production varied between 10–50 t in France, 5–80 t in Spain and 2–30 t in Italy). An undetermined amount of truffles are sold unofficially and are not quantified in the statistics, e.g., Gregori (2007) suggests that current Italian production is about 50–80 t). The GET estimates that international markets could absorb a truffle supply ten times higher.

Prices are also highly variable. From 1991 to 2005, the mean black truffle price paid by wholesalers to truffle growers in France varied between 200–650 euros kg^{-1} (Sourzat 2007), whereas in Spain it varied between 150–520 euros kg^{-1} from 1995 to 2006 (prices measured in constant 2005 euros). The prices paid by wholesale dealers to truffle growers depend on the annual production (law of supply and demand); the quality, size and degree of impurities of the truffles (truffles from plantations are usually higher valued) and the country where they are sold (yearly

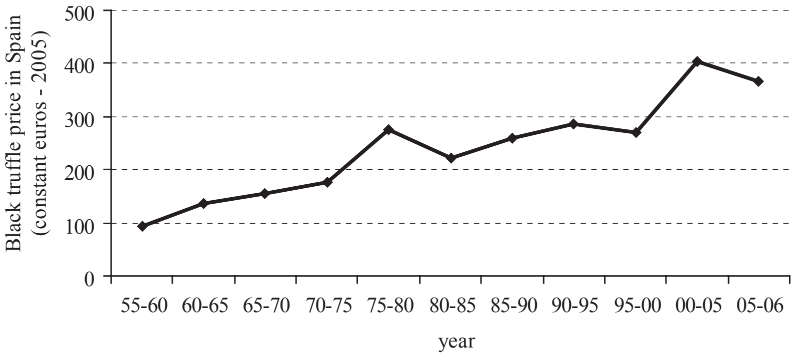

Fig. 14.7 Evolution of mean black truffle prices in Spain (paid by wholesalers to truffle harvesters), measured as constant euros of 2005 (Reyna et al. 2005)

prices paid to French truffle growers are about 40% higher than those received by their Spanish counterparts). Apart from these variations, the price of the black truffle in Spain is estimated to have increased 4% each year over the last 50 years (Fig. 14.7). In France, a similar tendency emerges from the 1991-to-2005 data, and, in addition, truffle growers are increasingly using retail e-commerce to sell their truffles (Sourzat 2007).

Consumer prices can be much higher (e.g. the price of fresh black truffles in Paris reached 2500 euros kg^{-1} in 2005).

The average annual value of black truffle production in France (revenues for truffle growers) is estimated to be 20 million euros, while the total economic impact of trufficulture is estimated to be 70 million euros (Escafre and Roussel 2006). In Italy, the value of the production of all species of truffles was estimated to be 18 million euros for the year 1999 (Pettenella et al. 2004). In Spain, an annual value of 2.4–9.7 million euros is estimated for black truffle production (years 2000–2005) on the basis of the estimated production and the mean price paid to truffle growers at the Vic market (northeastern Spain) (Reyna et al. 2005).

Social Implications: Rural Development

In many regions of the *T. melanosporum* distribution area, but especially in the southernmost ones, ecological conditions limit the agricultural potential and yields of traditional Mediterranean crops. For instance, in the Iberian Mountain Range (northeastern Spain) agricultural activity is usually centred on the cultivation of cereals; however, barley production levels rarely exceed 2,000 kg ha^{-1}, which is at the very limit of positive economic returns. Other unirrigated crops such as almond, carob and olive trees are not feasible because of inadequate temperature conditions, and vineyards are almost always outside the areas protected by official guarantees of origin and quality. Moreover, due to poor accessibility, these areas also show little industrial development and, as a result, they have suffered significant depopulation and ageing.

At present, trufficulture offers a viable development alternative and is helping to stop rural depopulation in these regions. Black truffle is also having indirect implications on agricultural land prices. According to Samils et al. (2003), land prices in Sarrión (a town in Teruel where truffle orchards are quickly spreading over former cropland and wasteland) increased 300% from 1995 to 1999, while unirrigated cropland prices increased 75% and 14% in the neighbouring provinces of Teruel and Zaragoza, respectively.

For most farmers in France, Italy and Spain, truffles are not the main source of income, but they provide economic diversification and extra incomes. In France it is estimated that there are 15,000 truffle growers, while in Italy it is estimated that there are about 200,000 truffle harvesters, although only 5% are professional harvesters or growers (Pettenella et al. 2004; Escafre and Roussel 2006).

Apart from agricultural activities and direct retail of fresh truffles, the fungus is also responsible for a variety of new activities such as mycorrhizal plant nurseries, preparation of canned truffles, retail of manufactured food products, organization of truffle fairs, development of local mycological gastronomy and agrotourism (guided visits to truffle orchards, visits to traditional truffle markets, ecomuseums in Sorges, Dordogne, and Metauten, northern Spain, etc.). In Italy, the National Association *Città del Tartufo* (Towns of Truffles) was founded for the promotion of truffles and the organization of shows.

Environmental Value: Sustainability and Multifunctionality

By providing many renewable resources, Mediterranean forests have historically played an essential role in the livelihood of Mediterranean peoples. However, on the northern shore the objectives of forest management have shifted in the last decades from production of material products (firewood, charcoal, livestock, etc.) to production of non-material goods and services (leisure, landscape, erosion and water-cycle control, biodiversity, etc.), due to social changes and low timber yields. As a result, the link between rural populations and their environment has in part been lost.

In this situation, revenues from the black truffle (or other non-timber forest products like game management) maintain this link and contribute to the conservation of natural formations of Mediterranean oaks. They also contribute to oak extension through new plantations.

Many truffle orchards can easily be considered as organic crops as they are grown without pesticides and synthetic fertilizers, produce quality and healthy food products and safeguard infiltrated water quality (IFOAM 2006). In addition, trufficulture keeps many small and marginal field plots cultivated and thus limits soil erosion risk and increases water infiltration rates.

Fig. 14.8 Truffle plantations could potentially be used in fuelbreaks as a means of controlling the spread of spontaneous vegetation and enhancing the sustainability of wildfire hazard reduction structures

In summary, in Mediterranean mountainous counties, truffle harvesting has an added value in that it falls fully within the concept of sustainable development, due to the ecological conditions in which it is produced and the socio-economic environment benefiting from its production.

In relation to other possible forest products and services, forest management for truffle production is largely compatible with wildlife and game (except for excessive populations of wild boar), extensive livestock production and wildfire hazard reduction.

Fuelbreaks are the basis of wildfire hazard reduction. Their target vegetation structure is very similar to that of truffle silviculture; thus, the biocidal effects of truffles can be used in fuelbreaks as a means of reducing maintenance work and promoting a multifunctional and more sustainable management (Fig. 14.8). In fact, productive truffle orchards constitute excellent fuelbreaks, due both to *brûlés* and tillage (Reyna and Garcia 2005a).

Future Prospects

Black truffle is naturally found in many Mediterranean calcareous mountains with limited agricultural and forestry potential. An agroforestry approach that integrates the management of truffle-producing forests with truffle cultivation in marginal agricultural lands could contribute to the sustainable rural development of these less favoured areas, thanks to the socio-economic and environmental implications of the black truffle. However, some legal, institutional, cultural, ecological and technical issues must be taken into account.

Future prospects for naturally growing *truffières* are poor unless adequate silvicultural measures are applied. If this does not take place, these *truffières* will continue to thicken, thus preventing the permanence of the truffle and, more importantly, the appearance of new *truffières*. The present decline has also been caused by abusive practices that are gradually disappearing, though, unfortunately, they are still being applied in certain areas. There are only two ways to eliminate these unprofessional practices: by developing legal regulations that address the reality of the situation and, especially, by developing agrarian extension activities for the people who are directly implicated.

In contrast, from the point of view of plantation trufficulture, the potential is high, due to the extension of both calcareous soils and climatically appropriate areas. One serious problem is the risk of accidentally introducing Asiatic truffles in

European plantations; these include *Tuber himalayense* Zhang et Minter, *T. indicum* Cook et Massee, *T. pseudohimalyense* Moreno, Manjón, Diez et García-Montero and *T. pseudoexcavatum* Wang, Moreno, Riousset et Manjón, all of which lack commercial value and can be found in European truffle batches. The confusion or deceit that Spanish, French and Italian nurserymen may be subjected to by those who sell low-value truffle species for inoculation purposes constitutes a grave threat for the sector, not only because of the economic repercussions involved for the agriculturalist, but also because of the serious ecological problems derived from the uncontrolled introduction of very invasive exotic species.

In the last years, specific protocols for rapid molecular identification of *Tuber* spp. ECM are being developed (Paolocci et al. 1999; Douet et al. 2004). Molecular methods could assure a more accurate monitoring of nursery-inoculated seedlings, but they can also be used to prevent the fraudulent use of sporocarps of species other than *T. melanosporum*, as well as for ecological studies, e.g., studies on intraspecific genetic variability, reconstruction of the past history of truffles, characterization of the ectomycorrhizal community, detection of *T. melanosporum* mycelia in soil, etc. (Mabru et al. 2004; Richard et al. 2005; Mello et al. 2006; Suz et al. 2006).

Currently, there are no official directives regulating either the production in nurseries of seedlings inoculated with European black truffle or the certification of their quality and purity. A European-scale truffle certification protocol is needed to serve as a reference for European truffle controllers.

At present, truffle plantations in some regions receive subsidies from the Common Agricultural Policy (CAP) for the afforestation of agricultural land. These subsidies have two key aspects that should be emphasized. First of all, the plantations that receive subsidies from the CAP are granted the legal consideration of forest areas, and this could make their management difficult in the medium term if unaccompanied by complementary legislative measures, because legal limitations on cultural practices obviously differ between agricultural and forest land.

Secondly, if the cost of the land is excluded, CAP subsidies mean no cost for the farmer. In similar situations, subsidies higher than 80% have shown little effectiveness, because in many cases the aim shifts from producing the product to simply accessing the subsidy, which becomes a business in itself. Obviously, this is not the case in regions with a tradition of trufficulture, where these subsidies are likely to work; however, in some other regions plantations established thanks to subsidies will be bad examples of truffle cultivation.

An interesting opportunity arises from the new European Union Regulation on Rural Development (EAFRD 2005); its measure "First establishment of agroforestry systems on agricultural land" could be exploited by Member States for truffle plantations, e.g., Hungary intends to include mycorrhizal plantations aimed at producing *T. aestivum*, *T. magnatum*, *T. macrosporum Vitt*. and *Mattirolomyces terfezioides* (Mattir.) E. Fisher (FVM 2007). The French government and the French Federation of Truffle Growers are also looking for a way that truffle plantations can receive single farm payments (SPF), by deriving from the European definition of permanent woodland a French-specific definition that excludes truffle plantations (MAP 2007).

Professional associations of truffle harvesters and growers are well-developed in France and Italy, while they are more recent in Spain. The national federations have recently formed the GET. Thus, the various public administrations now have valid interlocutors to help them to both organise the markets and establish co-financing mechanisms for the producing sector. A result of this association is the joint trufficulture project being developed by France, Italy and Spain with the aim of producing quality truffles, in large amounts, and with long production periods (GET 2004).

The general ecological requirements of the black truffle are relatively well-known, but research is still needed regarding its sexual reproduction, environmental determinants of fruiting and influence of soil microorganisms. Yields from truffle plantations remain highly unpredictable; thus, research effort must also be addressed at improving trufficulture models that are suitable for local situations. Some researchers are also studying whether the genetics of the tree plays a role in its production of truffles (Callot et al. 1999; Chevalier 2001a).

Acknowledgements We gratefully acknowledge the support of the General Directorate of Forest Resources, Environmental Ministry of the Valencia Autonomous Region, and its Territorial Services in Castelló. The Foundation CEAM is financed by the Valencian Regional Government and Bancaixa.

References

Barry D, Staunton S, Callot G (1994) Mode of absorption of water and nutrients by ascocarps of *Tuber melanosporum* and *Tuber aestivum*: a radioactive tracer technique. Can J Bot 72:317–322

Bencivenga M, Di Massimo G (2000) Risultati produttivi di tartufaie coltivate di *Tuber melanosporum* Vitt. in Umbria. Micol Ital 2:38–45

Bencivenga M, Calandra R, Granetti B (1990) Ricerche sui terreni e sulla la flora delle tartufai naturale di *Tuber melanosporum* Vitt. delle Italia centrale. In: Atti del secondo congresso internazionale sul tartufo, Università degli Studi di Perugia, Spoleto, Italy, 24–27 November 1988

Bertault G, Raymond M, Berthomieu A, Callot G, Fernández D (1998) Trifling variation in truffles. Nature 394:734–374

Blondel J, Aronson J (1995) Biodiversity and ecosystem function in the Mediterranean Basin: human and non-human determinants. In: Davis GW, Richardson DM (eds.) Mediterranean-type ecosystems. The function of diversity. Springer, Berlin/Heidelberg, Germany/New York

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

Bonet JA, Fischer CR, Colinas C (2006) Cultivation of black truffle to promote reforestation and land use stability. Agron Sustain Dev 26:69–76

Bonfante P, Fontana A, Montacchini F (1971) Studi sull'ecologia del *Tuber melanosporum*. I Dimostrazione di un effetto fitotossico. Allionia 17:47–54

Bourrières D, Coves H, Tixier R, Ricard JM (2005) Effects of the initial level of mycorrhization of young plants inoculated with Tuber melanosporum. In: Abstracts of the IV International Workshop on Edible Mycorrhizal Mushrooms, Universidad de Murcia, 28 November–2 December 2005

Bye P, Chazoule C (1998) Production, protection et profession truffières. Cah Écon Sociol Rural 46–47:132–152

Callot G, Bye P, Raymond M, Fernández D, Pargney JC, Parguey-Leduc A, Janex-Favre MC, Moussa R, Pagès L (1999) La truffe, la terre, la vie. INRA, Paris
Ceruti A, Fontana A, Nosezo C (2003) Le specie europee del genere *Tuber*. Una revisione storica. Regione Piemonte, Torino, Italy
Chatin A (1869) La truffe. Etude des conditions générales de la production. Lacour, Nîmes, France
Chatin A (1892) La truffe. Botanique de la truffe et des plantes truffières. Ed JB Baillière et Fils, Paris
Chevalier G (2001a) Du congrès de Spoleto a celui d'Aix-en-Provence: les avances en matiere de recherches sur la truffe et la trufficulture en France. In: V Congrès International Science et Culture de la Truffe, Centre des Congrès, Aix-en-Provence, 4–6 March 1999
Chevalier G (2001b) Le plant mycorhizé INRA: passé, present, avenir. In: V Congrès International Science et Culture de la Truffe, Centre des Congrès, Aix-en-Provence, 4–6 March 1999
Chevalier G, Poitou N (1989) Study of important factors affecting the mycorrhizal development of the truffle fungus in the field using plants inoculated in nurseries. Agric Ecosyst Environ 28:75–77
De Angelis A (2005) Normativa siu tartufi. In: Granetti B, De Angelis A, Materozzi G (eds.) Umbria, terra di tartufi. Regione Umbria, Gruppo Micologico Ternano, Terni, Italy
Delmas J (1983) La truffe et sa culture, 2nd edn. INRA, Bordeaux, France
Diette S, Lauriac A (2005) La sylviculture truffière: aperçus historiques, apports techniques et enjeux pour la région méditerranéene. For Méditerr 26(2):157–168
Di Massimo G, Baciarelli L, Donnini D, Bencivenga M (2006) Ascocarps density in a truffle plantation. In: IV International Workshop on Edible Mycorrhizal Mushrooms. Universidad de Murcia, 29 November–2 December 2005
Douet JP, Castroviejo M, Mabru D, Chevalier G, Dupré C, Bergougnoux F, Ricard JM, Médina B (2004) Rapid molecular typing of *Tuber melanosporum*, *T. brumale* and *T. indicum* from tree seedlings and canned truffles. Chem Mater Sci 379:668–673
EAFRD (European Agency for Rural Development) (2005) Council Regulation (EC) n°1698/2005 of 20 September 2005 on support for rural development. http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2005:277:0001:0040:EN:PDF. Cited 29 Apr 2007
Escafre A, Roussel F (2006) Rapport relatif au développement de la trufficulture française. http://www.agriculture.gouv.fr/spip/IMG/pdf/developpement_truffi_franc.pdf. Cited 29 Apr 2007
Etayo ML (2001) Seguimiento del estado de micorrización de una parcela de cultivo de trufa negra. Valoración del simbionte arbóreo y efecto del tratamiento de acolchado. Estudio preliminar de las micorrizas de un área trufera colindante. Dissertation, Universidad de Navarra
Frochot H, Chevalier G, Bardet MC, Aubin JP (1990) Effet de la désinfection du sol et des antécedents culturaux sur l'évolution de la mycorhization avec *Tuber melanosporum* sur noisetier. In: Atti del secondo congresso internazionale sul tartufo, Università degli Studi di Perugia, Spoleto, Italy, 24–27 November 1988
FVM (Földmüvelésügyi és Vidékfejlesztési Minisztérium) (2007) New Hungary Rural Development Programme 2007–2013. http://www.fvm.hu/download.php?ctag=download&docID = 2715. Cited 29 Apr 2007
Garvey DC, Cooper PB (2001) French black truffle - establishment and production in Tasmania. Rural Industries Research and Development Corporation, Australian Government. http://www.rirdc.gov.au/reports/NPP/01–084.pdf. Cited 25 Sep 2006
GET (Groupement Européen Tuber) (2004) Programme de developpement de la trufficulture et de reconstitution de l'espace truffier en Europe. http://www.fft-tuber.org/GET.pdf. Cited 25 Sep 2006
Gil R, Bados R, Medraño P, Gómez A (2001) Regulación del aprovechamiento de la trufa (*Tuber melanosporum* Vitt.) en bosques mediterráneos. In: Actas del III Congreso Forestal Español, Palacio de Exposiciones y Congresos, Granada, Spain 25–28 September 2001
Granetti B (2005a) L'evoluzione delle ricerche sui tartufi. In: Granetti B, De Angelis A, Materozzi G (eds.) Umbria, terra di tartufi. Regione Umbria, Gruppo Micologico Ternano, Terni, Italy

Granetti B (2005b) Miglioramento e rinnovamento delle tartufaie. In: Granetti B, De Angelis A, Materozzi G (eds.) Umbria, terra di tartufi. Regione Umbria, Gruppo Micologico Ternano, Terni, Italy

Granetti B, Baciarelli L (1997) Competizione tra le micorrize di *Tuber melanosporum* Vitt. e quelle di altri funghi in una tartufaia coltivata a *Quercus ilex* L. Micol Ital 26(1):45–59

Gregori G (2007) Principales aspectos de la trufa y la truficultura en Italia. In: Reyna S (ed) Truficultura. Fundamentos y técnicas. Mundi-Prensa, Madrid

Gregori G, Elisei S, Pasquini L, Sacchi A, Spezi D (2001) Rigenerazione di una vecchia tartufaia coltivata di *Tuber melanosporum* Vitt. In: V Congrès International Science et Culture de la Truffe, Centre des Congrès, Aix-en-Provence, 4–6 March 1999

Hernández A, Reyna S, Giner M, Folch L, Tagliaferro F (2001) El Proyecto LIFE-Medio Ambiente "Revalorización de bosques productores de trufa: un ejemplo de gestión sostenible". In: III Congreso Forestal Español, Palacio de Exposiciones y Congresos, Granada, Spain, 25–28 September 2001

Hernández A, Giner M, Gil R (2005) Proyecto de demostración LIFE-Medio Ambiente Revalorización de bosques productores de trufa: un ejemplo de gestión sostenible. Rev for Esp 37:4–10

Horton R, Bristow KL, Kluitenberg GJ, Sauer TJ (1996) Crop residue effects on surface radiation and energy balance – a review. Theor Appl Climatol 54(1–2):27–37

IFOAM (International Federation of Organic Agriculture Movements) (2006) http://www.ifoam.org. Cited 25 Sep 2006

Letizi HC, Marchetti A, Rinaldini E (2001) Il contributo della Associazione Nazionale Conduttori Tartufaie (ACT) di Acqualagna (PS) allo sviluppo della tartuficoltura. In: V Congrès International Science et Culture de la Truffe, Centre des Congrès, Aix-en-Provence, 4–6 March 1999

Lulli L, Bragato G, Gardin, L (1999) Occurrence of *Tuber melanosporum* in relation to soil surface layer properties and soil differentiation. Plant Soil 214:85–92

Mabru D, Dupré C, Douet JP, Leroy P, Ravel C, Ricard JM, Médina B, Castroviejo M, Chevalier G (2004) Rapid molecular typing method for the reliable detection of Asiatic black truffle (*Tuber indicum*) in commercialized products: fruiting bodies and mycorrhizal seedlings. Mycorrhiza 11(2):89–94

Mamoun M, Olivier JM (1990) Dynamique des populations fongiques et bactériennes de la rhixosphère des noisetiers truffiers. III Effet du régime hydrique sur la mycorhization et la microflore associée. Agronomie 10:77–84

Mamoun M, Olivier JM (1992) Effect of soil Pseudomonads on colonization of hazel roots by the ectomycorrhizal species *Tuber melanosporum* and its competitors. Plant Soil 139:265–273

Mamoun M, Olivier JM (1993) Competition between *Tuber melanosporum* and other ectomycorrhizal fungi under two irrigation regimes. I Competition with *Tuber brumale*. Plant Soil 149:211–218

Mamoun M, Olivier JM (1997) Mycorrhizal inoculation of cloned hazels by *Tuber melanosporum*: effect of soil disinfestation and co-culture with *Festuca ovina*. Plant Soil 188:221–226

Manna D (1990) La tartuficoltura nello spoletino. In: Atti del secondo congresso internazionale sul tartufo, Università degli Studi di Perugia, Spoleto, Italy, 24–27 November 1988

MAP (Ministère de l'Agriculture et de la Pêche) (2007) Protocole 2007–2013 relatif au développement de la production de truffes. http://www.agriculture.gouv.fr/spip/IMG/pdf/truffes_protocole_070122.pdf. Cited 29 Apr 2007

Mello A, Murat C, Bonfante P (2006) Truffles: much more than a prized and local fungal delicacy. FEMS Microbiol Lett 260(1):1–8

Oliach D, Barrière P, Ruiz G, Souche G, Jaillard B (2005) Ecophysiological and pedological factors and black truffle (*Tuber melanosporum* Vitt.) production in an evergreen holm oak (*Quercus ilex* L.) truffle orchard. In: Abstracts of the IV International Workshop on Edible Mycorrhizal Mushrooms, Universidad de Murcia, 28 November–2 December 2005

Olivera A (2005) Efectos de la modificación de parámetros del cultivo de la trufa sobre la proliferación de micorrizas de *Tuber melanosporum* Vitt. Dissertation, Universitat de Lleida

Olivier JM, Savignac JC, Sourzat P (1996) Truffe et trufficulture. Fanlac, Perigueux, France
Omaggio JP (2001) Le vol de truffes: légistation, lutte et position des juridiction répressives en France. In: V Congrès International Science et Culture de la Truffe, Centre des Congrès, Aix-en-Provence, 4–6 March 1999
Pacioni G (1987) El cultivo moderno y rentable de la trufa. De Vechi, Barcelona, Spain
Pacioni G (1991) Effects of *Tuber* metabolites on the rizospheric environment. Mycol Res 95:1355–1358
Paolocci F, Rubini A, Granetti B, Arcioni S (1999) Rapid molecular approach for a reliable identification of *Tuber spp* ectomycorrhizae. FEMS Microbiol Ecol 28(1):23–30
Paolocci F, Rubini A, Riccioni C, Arcioni S (2006) Reevaluation of the life cycle of *Tuber magnatum*. Appl Environ Micobiol 72(4):2390–2393
Papa G, Bortolo R, Cassani G (1992) Componenti di *Tuber melanosporum* con attività fitotossica. Micol Veget Medit 7(1):109–110
Pargney JC, Chevalier G, Dupré C, Genet P, Jalade M (2001a) Étude des stromas fongiques se développant sur les racines des plants mycorhizés par la truffe. In: V Congrès International Science et Culture de la Truffe, Centre des Congrès, Aix-en-Provence, 4–6 March 1999
Pargney JC, Genet P, Chevalier G (2001b) Localisation d'hyphes latentes dans les mycorhizes âgées de truffe (*Tuber melanosporum* Vitt.): étude ultrastructural, cytochimique et microanalytique. In: V Congrès International Science et Culture de la Truffe, Centre des Congrès, Aix-en-Provence, 4–6 March 1999
Pettenella D, Klöhn S, Brun F, Carbone F, Venzi L, Cesaro L, Ciccarese L (2004) Cost Action E30 Economic integration of urban consumers' demand and rural forestry production. Italy's country report. http://www.apat.gov.it/site/_files/English_documents/Italy-Report.pdf. Cited 29 Apr 2007
Plattner I, Hall IR (1995) Parasitism of non-host plants by the mycorrhizal fungus *Tuber melanosporum*. Mycol Res 99(11):1367–1370
Poma A, Limongi T, Pacioni G (2006) Current state and perspectives of truffle genetics and sustainable biotechnology. Appl Microbiol Biotechnol 72:437–441
Raglione M, Spadoni M, Cavelli S, Lorenzoni P, De Simone C (2001) Les sols des truffières naturelles de *Tuber melanosporum* Vitt dans l'Apennin central (Italie). In: Actes du Ve Congrès International Science et culture de la Truffe, Centre des Congrès, Aix-en-Provence, 4–6 March 1999
Reyna S (2000) Trufa, truficultura y selvicultura trufera. Mundi-Prensa, Madrid
Reyna S, Garcia S (2005a) La truficultura y la selvicultura trufera en la defensa contra incendios forestales. In: II International Conference on Prevention Strategies of Fires in Southern Europe. Auditori La Pedrera, Barcelona, Spain, 9–11 May 2005
Reyna S, Garcia S (2005b) Effect of truffle sylviculture treatents on the mycorrhization and tree regeneration of a mixed forest with spontaneous Périgord black truffles. In: IV International Workshop on Edible Mycorrhizal Mushrooms. Universidad de Murcia, 29 November–2 December 2005
Reyna S, Garcia S, Folch L, Pérez-Badia R, Galiana F, Rodríguez-Barreal JA, Domínguez-Núñez JA, Saiz de Omeñaca JA, Zazo J (2004) Selvicultura trufera en montes mediterráneos. In: Vallejo R, Alloza JA (eds.) Avances en el estudio de la gestión del monte mediterránea. Fundación CEAM, València, Spain
Reyna S, De Miguel A, Palazón C, Hernández A (2005) Spanish trufficulture. In: IV International Workshop on Edible Mycorrhizal Mushrooms. Universidad de Murcia, 29 November–2 December 2005
Reyna S, Garcia S, Folch L (2006) Influencia del uso del suelo sobre el potencial de inóculo ectomicorrícico y la competitividad de las ectomicorrizas de *Tuber melanosporum*: evaluación mediante bioensayos en invernadero. Invest Agrar: Sist Recur For 15(3):208–220
Reyna S, Colinas C, De Miguel A, Saez R, Garcia S, Hernández A (2007) Experiencias en plantaciones truferas españolas. In: Reyna S (ed) Truficultura. Fundamentos y técnicas. Mundi-Prensa, Madrid, pp 277–330

Ricard JM, Bergougnoux F, Callot R, Chevalier G, Olivier JM, Pargney JC, Sourzat P (2003) La truffe. Guide technique de trufficulture. Ctifl, Paris

Richard F, Millot S, Gardes M, Selosse MA (2005) Diversity and specificity of ectomycorrhizal fungi retrieved from an old-growth Mediterranean forest dominated by *Quercus ilex*. New Phytol 166(3):1011–1023

Rodríguez-Barreal JA, Saiz de Omeñaca JA, Zazo J (1989) Efectos positivos de la micorrización controlada con el hongo *Tuber melanosporum* Vitt. de la especie forestal *Corylus avellana* L obtenido mediante reproducción vegetativa. Bol Veg Plagas 15:207–214

Roux JJ (2001) Les relations pluie-récolte sur 17 années dans le Var: *T. melanosporum* 1982–1998. In: V Congrès International Science et Culture de la Truffe, Centre des Congrès, Aix-en-Provence, 4–6 March 1999

Samils N, Olivera A, Danell E, Alexander S, Colinas C (2003) Aportación de la truficultura al desarrollo económico. Vida Rural 10(181):54–60

Shaw PJA, Lankey K, Jourdan A (1996) Factors affecting yield of *Tuber melanosporum* in a *Quercus* plantation in southern France. Mycol Res 100(10):1176–1178

Sourzat P (1997) Guide practique de trufficulture. Station d'Expérimentations sur la Truffe, Le Montat, France

Sourzat P (2001) Evolutions technologiques et écologiques de la trufficulture en France et notamment dans le département du Lot. In: V Congrès International Science et Culture de la Truffe, Centre des Congrès, Aix-en-Provence, 4–6 March 1999

Sourzat P (2004) Questions d'ecologie appliquées à la trufficulture. Station d'Expérimentation sur la Truffe, Le Montat, France

Sourzat P (2005) Approach of the French truffle cultivation. In: IV International Workshop on Edible Mycorrhizal Mushrooms. Universidad de Murcia, 29 November–2 December 2005

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

Sourzat P, Sánchez A, Ourzick A, Roux JJ (2001) Analyse du statut mycorhizien de *brûlés* stériles dans trois régions de France: Midi-Pyrénées (Lot), PACA (Var), Charente-Poitou (Vienne). In: V Congrès International Science et Culture de la Truffe, Centre des Congrès, Aix-en-Provence, 4–6 March 1999

Suz L, Martín MP, Colinas C (2006) Detection of *Tuber melanosporum* DNA in soil. FEMS Microbiol Lett 254:251–257

Tagliaferro F (2001) Manuale de tartuficoltura. IPLA, Torino, Italy

Treseder KK (2004) A meta-analysis of mycorrhizal responses to nitrogen, phosphorus and atmospheric CO2 in field studies. New Phytol 164:1–9

Trull J (2007) Marco legislativo de la trufa. In: Reyna S (ed) Truficultura. Fundamentos y técnicas. Mundi-Prensa, Madrid

Zambonelli A, Iotti M, Zinoni F, Dallavalle E, Hall IR (2005) Effect of mulching on Tuber uncinatum ectomycorrhizas in an experimental truffière. N Z J Crop Hortic Sci 33(1):65–73