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BEST PRACTICES FOR CULTIVATION OF TRUFFLES

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Abstract

Cultivation of truffles in managed orchards has become an important agricultural alternative in rural Mediterranean regions. The declines of wild ***Tuber melanosporum*** throughout its natural range, its high market value and the development of nursery and cultivation techniques have encouraged its successful cultivation during the last several decades. We present here the state of the art of black truffle cultivation, habitat requirements of the fungus, cultivation techniques and descriptions of the mycorrhizae of four important ***Tuber*** species. Additionally we provide recent research findings with special implications for truffle cultivation and a brief review of truffle culinary products. In the last section we address the developing Truffle Sector in Southwest Turkey.

1. Introduction

Truffles are the fruiting bodies of fungi that grow belowground and are best known for their unique aromas and culinary value. Several species are outstanding because they are highly prized in both regional and international cuisines. Truffles belong to the kingdom Fungi, division Eucomycota, subdivision Ascomycotina, class Ascomycetes, order Pezizales, family Tuberaceae and genus ***Tuber*** (Trappe 1979). The genus ***Tuber*** is one of the most important in the family Tuberaceae given its economic value, and the ample scientific attention it has received.

Figure 1a: *Three species of Tuber from the Mediterranean region, from left to right: T. mesentericum, T. melanosporum and T. brumale (Photo by Pere Muxí).*

The term "truffle" is often used to refer to all hypogeous or "underground" fungi, but precisely refers to those species included in the genus ***Tuber*** with at least 180 species currently known in the world (Bonito et al. 2010). They produce their spores in "sporocarps" or more specifically "ascocarps" (class Ascomycetes), which develop belowground. This life style allows the sporocarps to be more resistant to desiccation and freezing than fungi that form aboveground fruiting bodies. Truffles are the sporocarps or "spore-bearing structures" of these fungi.

The aromas emitted by the different ***Tuber*** species are highly variable, with strong and persistent characteristics, which are important for the attraction of animals and insects that consume the truffles and disperse the spores. The earthy and unique truffle aromas are the crux of their culinary popularity and contribute to the high value of fresh truffles.

Fungi in the genus ***Tuber*** are mycorrhizal. This is an essential feature for understanding truffles because mycorrhizal fungi live in symbiosis with living plants and cannot complete their life cycle without the carbohydrates derived from the association with the host plant.

The host plant or tree also benefits from the association because the mycorrhizal fungi make available key nutrients from the soil, provide protection against pathogens and serve to extend the plant root system via the network of belowground mycelia (Finlay 2008).

There are no reports of intoxication from truffle species in the genus ***Tuber,*** although most lack culinary and commercial value. However several species with exceptional organoleptic qualities are collected for their culinary value. European species of greater economic interest based on their aromatic characteristics are (Reyna 2007):

Tuber aestivum Vitt. ***(=Tuber uncinatum*** Chat***.)***
Tuber borchii Vitt. (= ***Tuber albidum*** Pico)
Tuber brumale Vitt.
Tuber macrosporum Vitt.
Tuber magnatum Pico.
Tuber melanosporum Vitt.
Tuber mesentericum Vitt.
Tuber rufum Pico.

Of the nearly 200 species of truffles, about 32 are mainly found in the Mediterranean region, and ***T. melanosporum*** is the most sought-after species. The Italian white truffle or ***T. magnatum*** is found nearly exclusively in small areas of Italy, Croatia and Romania, which together with its unique aroma and quality, make it the most expensive of the truffles. Other important culinary and commercial truffles include ***T. aestivum, T. brumale*** and ***T. borchii.***

The black truffle, ***T. melanosporum,*** is found in calcareous soils from native forests of southern Europe, mainly in France, Spain and east to north-central Italy. This species forms sporocarps (truffles) which ripen in the late autumn and winter, and are collected with the assistance of trained dogs. Occasionally, this species has also been found in woodlands of Croatia, Slovenia, Serbia, Portugal, Switzerland, Germany, Hungary, Bulgaria, Greece and Turkey (Reyna 2007).

Black truffles from natural woodlands are in decline throughout Europe. Truffle collections have suffered dramatically over the last century (Reyna 2000). In France, during the twentieth century, production of ***T. melanosporum*** dropped from 1,000 to 50 tons (Callot 1999). Despite important advances in truffle research, wild black truffle production continues to decline. The current decline in wild truffles is so significant that the most viable alternative to restore production is the improved management of cultivated black truffle plantations or orchards.

1.1 Truffle cultivation is an important agroforestry practice that promotes rural development.

Cultivation of the black truffle (***T. melanosporum***) can bring a vital source of prosperity for rural communities with suitable habitat, becoming a complementary activity to agricultural traditions, diversifying the rural economy and promoting a renewed land-use balance. Truffle orchards contribute to the reforestation of marginal farm lands with open oak woodlands that provide both ecologic and economic benefits for rural communities.

Cultivation in orchards began intensively with the development of nursery techniques to induce ***T. melanosporum*** ectomycorrhiza formation in receptive host seedlings (Chevalier and Grente 1978). Productive truffle orchards in France, Italy and Spain presently provide rural landowners with an alternative to agricultural subsidies, promote restoration of woodland habitats, recovering abandoned cereal lands with relatively low agricultural inputs (Samils et al. 2008).

Mass production of ***Tuber***-colonized host seedlings has brought the most important progress in truffle cultivation over the past 50 years. In Spain the first plantations were established in the early 1970's by importing seedlings from France, and at the onset of the 1980's the first businesses to cultivate and sell locally grown truffle-inoculated plants began to operate. Since then, the expansion of truffle plantations has created an economic boom for the tree nursery-sector specializing in the production of truffle-inoculated seedlings. With the maturation of established and well-managed truffle plantations, economic benefits to growers and their communities have been significant. Currently, the province of Teruel in Aragon, Spain, produces the majority of Spanish black truffles, distinguishing this region for having the greatest concentration of black truffle orchards worldwide and growing nearly 25% of the world's black truffle production.

Figure 1b: *Large-scale nursery production of Tuber-colonized host seedlings.*

Annual fresh weight productions from plantations varies widely with reports of 50 kg/ha from 13-14 yr.-old plantations in Italy (Bencivenga and Di Massimo 2000), 45 kg/ha reported from irrigated plantations in Spain (Carbajo 2000) and 15-50 kg/ha with exceptional yields up to 110kg/ha in 14 yr.-old plantations in France (Chevalier and Frochot 1997). These yields were reported nearly 20 years ago and the techniques for managing black truffle plantations have advanced significantly. Many truffle growers today are applying concepts learned from experimentation with managing the water needs of their trees and their truffles, enhancing the soil environment and reducing competition from herbaceous plants. Techniques vary according to the conditions of a particular site and the age of the plantation. The results are earlier onset of truffle production and increased quantities of high quality truffles. Professional truffle growers are now reporting 50 to 100 kg/ha with exceptional yields up to 200 kg/ha in good sites in Spain.

1.2 Technical Guidance for Truffle Growers

Truffle cultivation in well-managed orchards has been successful in its native European regions as well as in other countries of the world (Reyna and Garcia-Barreda 2014; Morcillo et al. 2015). Unfortunately, not all orchards are productive. The lack of success may be due to multiple factors ranging from poor site selection, inappropriate host tree, poor plant quality, lack of proper management or problems with pests and diseases.

There are numerous guides and handbooks prepared by experts to assist farmers in decision-making at many steps in the process. Here we provide guidelines developed for growing ***T. melanosporum*** in Spain that were first published in 2005 as:

> **"Technical Guide for the Cultivation of the Black Truffle"**
> "Guía técnica para el cultivo de trufa negra (***Tuber melanosporum*** Vitt.)" by Daniel Oliach, José Antonio Bonet, Christine Fischer, Antoni Olivera, Juan Martínez de Aragón, Laura M Suz, and Carlos Colinas and was edited by the Centre Tecnològic Forestal de Catalunya. ISBN: 84-689-5025-4.

This present "Best Practices For Cultivation of Truffles" expands on the 2005 Guide with additional research and observations collected over these years by many authors whose work is cited throughout the text. Many of the observations made over the last fifty years continue to provide valid insights and are applicable to many species of ***Tuber***. Additionally, the following guidelines can be used as a framework for developing technical guides for other truffle species and for other regions of the world.

We have witnessed dramatic advances in the truffle-growing sector in Spain over the last twenty years. Prior to the year 2000, the majority of black truffles were collected from naturally-occurring forests of ***Quercus ilex, Q. faginea***. and ***Q. coccifera*** and occasionally in hazelnut (***Corylus avellana***) orchards. Most truffle hunters were also dedicated to other farming or business activities.

Presently there is a growing sector of professional truffle farmers who are exclusively focused on growing and marketing black truffles in thoughtfully managed plantations. Understanding the principles for irrigation, weed control and soil management have been fundamental in advancing the success of truffle farmers.

New agricultural equipment has been designed to address the needs for managing truffle soils. Methods for monitoring soil moisture and calculating and applying irrigation have been developed. Research efforts have been directed at post-harvest technology to extend truffle shelf-life. Membership in Truffle Associations has expanded for collaboration in seeking technical assistance, pest monitoring, economic support and strategic planning for the sector from regional governments.

2. Land Suitability

The most favorable land for the establishment of a truffle plantation is determined by its geographical, climatic, geological, soil, and biotic conditions. These factors have interactive influences on the ecological niche for truffle habitat, and our understanding of its ecology is expanding as the black truffle habitat has extended beyond its native range.

2.1. Geographical conditions

The geographical location may determine the distribution of truffles, but the geographical parameters themselves are not determinant and should be considered along with the climate.

Altitude
The appropriate altitude for the establishment of a plantation is a parameter presenting discrepancies among experts because it cannot be set apart from the latitude and orientation. In Europe wild truffles are found from near sea level in France (Olivier et al. 1996) to 1,800 m in Granada, Spain (Reyna 2000). In Spain the majority of wild truffle sites are located between 600-1,200 m above sea level.

Orientation
The influence of orientation depends, in turn, on the altitude and latitude as well as exposure to dominant winds. The majority of wild truffle sites are found on south-facing slopes, but for lower latitudes in Spain, there is more of a tendency for natural truffles sites to thrive on cooler or north-facing slopes (Reyna 1992; 2000).

Slope
The majority of naturally occurring truffle sites are not found in completely level areas due to problems of poor drainage. More frequently truffles are collected from sites with slight inclines (<15%) (Delmas and Poitou 1973; Reyna 1992).

2.2. Climatic Conditions

The climate most suitable for the black truffle is characterized by marked seasonal changes, and with a period of relatively short summer aridity, interrupted by rain storms. The truffle can withstand occasional extreme weather conditions, but oceanic climates with minimal seasonal differences or continental climates with very hot summers, very cold winters and lacking in intermediate moderations are not suitable. Also, the arid Mediterranean climates with insufficient or exclusively winter rainfall, or climates with excessively long cold periods are not very suitable for black truffles.

Precipitation
Water availability is of primary importance for truffle production, especially in the spring and early summer when rainfall plays a decisive role during the growth period of the black truffle (Büntgen et al. 2015). There are no experimental studies on the combined water needs of the symbiotic organism (the mycorrhiza)

and the host tree. From naturally occurring truffle sites, the range of annual precipitation observed varies between 485 and 1,500 mm/year, with a minimum of 72 mm rainfall during the summer months (Reyna 2000; Ricard 2003).

This is quite a wide range but it is important to consider that a given precipitation which provides sufficient water needs for a plant grown in colder conditions may create water stress for the same plant in warmer conditions.

Sites with lower rainfall may be suitable for growing black truffles if other site parameters are acceptable and a reliable irrigation system can meet the water needs during dry periods.

Temperature

Truffles prefer Mediterranean climates with mild springs and relatively warm summers, autumns without early frosts that may impede the ripening of sporocarps, and winters without extreme cold (Sourzat 1997). Although truffles can withstand extreme temperatures up to 43ºC in summer and -25ºC in winter (Sáez and De Miguel 1995; Reyna 1992; Reyna 2000), it is preferable to choose sites without severe temperature extremes. Winter temperatures are particularly important as they will influence the quality of the ripening of black truffle. Winter temperatures lower than –10°C for more than five days (Olivier et al. 2002; Sourzat 2003) are excessive.

Prolonged high summer temperatures may condition the site's suitability for a plantation, but examples from outside the native European habitat have introduced new observations for this parameter. The successful black truffle orchards in Australia have demonstrated that this fungus can adapt to a climate with monthly mean maximum temperatures above 24.8ºC for a period of 4 months of the year (pers. comm. Harry Eslick, ArborCarbon Pty. Ltd. AU).

For young seedlings in the establishment phase, there is an interaction between soil temperature and water stress as demonstrated in pot studies of ***T. melanosporum*** inoculated ***Q. ilex*** seedlings grown under 4 different water potentials (Olivera et al. 2006). With very hot soil conditions (Tº of 35-40ºC) there was a diminished mycorrhizal development in seedlings compared to those grown under cooler or normal summer soil conditions (Tº of 30-33ºC); and no amount of watering compensated for mycorrhizal loss under extremely high soil temperatures. However, at normal or cooler soil temperatures, a moderate water stress was favorable because it resulted in higher mycorrhizal development. The goal is to help moderate soil temperature at times of water stress.

Mulching can reduce temperature extremes, modulating the soil temperatures in summer and in winter to depths of 10-15 cm (Michels 2003; Sourzat 2003; Olivera et al. 2014a). **Table 1a** presents the optimal temperature range described in published studies. These ranges are based on observations from productive and native black truffle habitats, but there is no experimental data that defines the upper and lower temperature limits for this species.

TABLE 1a: Proposed range of temperatures for cultivation of the black truffle (Reyna 1992; Hernández 1994; Callot and Jaillard 1996; Reyna 2000; García-Montero et al. 2001).

Mean annual temperature (°C)	8.6 – 14.8
Mean highest daily temperature of the warmest month (°C)	23 – 32
Mean temperature of the warmest month (°C)	17.4 – 23.5
Mean lowest daily temperature of the coldest month (°C)	(-2) – (-6)
Mean temperature of the coldest month (°C)	1 – 8.2
Extreme maximum temperature (°C)	43
Extreme minimum temperature (°C)	(-9) – (-25)

2.3. Geologic Conditions

The most preferred parent materials are those from the Secondary-Mesozoic era including the Triassic, Jurassic and Cretaceous periods. Soils that evolved from the late Jurassic rocks are best (Sáez and De Miguel 1995), although alluvial deposits from the Quaternary period are also excellent (Olivier et al. 2002).

2.4. Soil conditions

Truffles thrive in calcareous soils at a depth of 10-40 cm (Sourzat 1997) on Inceptisols, Entisols and Mollisols (Raglione et al. 2001). The most significant soil parameters and approximate ranges for the correct location and development of a truffle plantation are presented below.

Stoniness

The stoniness of the soil is a highly valued quality for a truffle cultivation site because it contributes to good drainage and aeration. A stone layer creates a mulching effect and helps to regulate the temperature of the topsoil (Olivera et al. 2014a). Temperature moderation during hot periods helps to maintain condensation and ensures soil fauna activity (Callot 1999). Soil stoniness is positively related to good truffle production (García-Montero et al. 2007a), but soils that are too stony, with low rainfall, or where the volume of stoniness exceeds that of fine soil, should be excluded (Sourzat 1997). A high stone content is not favorable where the clay content and organic matter are very low because these sites will remain too dry for long periods. In wild truffle sites the presence of gravels in the soils is quite variable and can range between 0.2 and 90%, creating superficial stony surfaces that completely cover the soil (Reyna 2000). Surface stoniness decreases summer evaporation and protects soil against compaction and erosion from rainfall (Reyna 2000).

Figure 2a: *Soil stoniness is a positive quality for black truffle sites.*

Soil pH

The acidity or alkalinity of a soil is represented by the pH. Values below pH 7 are considered acidic, and values above, alkaline. The pH value is one of the most important determining factors for selecting a site to cultivate the black truffle due to its preference for alkaline soils.

The recommended pH range for black truffle cultivation is between 7.5 and 8.5 (Delmas et al. 1982; Sourzat 1997; Jaillard et al. 2016), the most favorable being close to 8 (Poitou 1988; Bencivenga et al. 1990). The pH of wild truffle sites varies between 7.1 and 8.8 (Bencivenga and Granetti 1988; Sáez and de Miguel 1995; Sourzat 2001), and the most productive wild and planted truffle beds in Northern Spain and Southern France have pH (water) around of 7.9 ±0.3 (Jaillard et al. 2014).

Acidity or alkalinity

Soils are acidic or alkaline depending on the degree of activity of the hydrogen ions present in the soil solution. An alkaline soil means that there is a high saturation of exchangeable cations (potassium, calcium, magnesium and sodium) rather than anions (hydrogen and aluminum), which are more abundant in acid soils. Additionally, for the production of black truffles, the alkaline pH must be the result of an abundance of exchangeable calcium and low exchangeable sodium.

In general, sodium has a negative influence on soil structure, but sodium contributes to the alkalinity of soil pH values, so it is advisable to evaluate the distribution of the basic cations within an alkaline soil to ensure that there is not an excess of sodium. A measure based on the sum of the calcium and magnesium exchangeable cations divided by the soil exchange capacity has been proposed as more appropriate than pH to study the ability of a soil to produce black truffles (Ciesielski et al. 1997; Jaillard et al. 2014). These authors find that values between 0.6 and 4 of this ratio are most suitable for truffle production.

However, pH measurements and the relationship between bivalent cations and exchangeability are complementary, and pH values between 7.5 and 8.3 are favorable for black truffles (Jaillard et al. 2016).

Calcium
Calcium carbonate is essential for the cultivation of the black truffle, although a low soil calcium level may be compensated for by a significant presence in the parent material or in large soil particles (Reyna 2000). If it is not present in large particles or stones, soil concentration must exceed 1% and may reach up to 84% (Bencivenga and Granetti 1988; Poitou 1988; Raglione et al. 2001).

In wild truffle sites, 40% of truffle production can be explained by the presence of active calcium carbonate (Active $CaCO_3$) (García-Montero et al. 2007b). The Active $CaCO_3$ represents the fine fraction of calcium carbonate with a diameter of less than 50 microns. This constitutes an important reserve of calcium exchange cations, which together with the total calcium carbonate, regulates the pH and favors the structure of the soil by agglutinating fine soil particles. The Active $CaCO_3$ is an indicator of the balance between solubilization and leaching of the coarse limestone fractions, which is considered of great importance for truffle habitat (Callot 1999).

Texture
The optimal texture classes for truffle site establishment are loam, sandy loam and sandy clay loam (Colinas et al. 2007) although wild truffle beds are formed in almost every type of texture (Delmas et al. 1981; Bencivenga and Granetti 1988), provided that other necessary conditions are present.

Excessively sandy soils are problematic because of their poor water retention capacity, and heavy clay soils (with >46 % clay) are not suitable because of their potential for significant compaction (Raglione et al. 2001). Acceptable maximum levels of clay depend on stoniness, organic matter and the biological activity of the soil, all of which help aeration and prevent compaction.

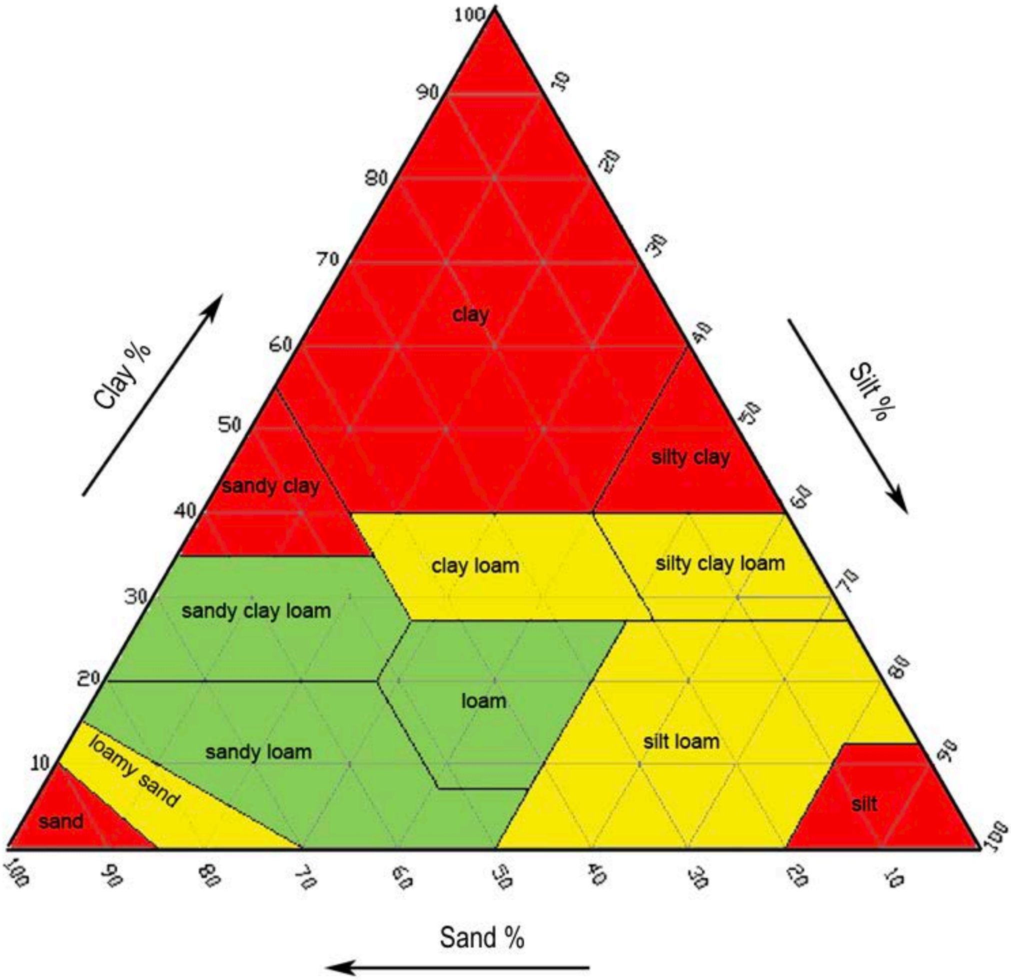

__Figure 2b:__ Soil texture triangle with indications for truffle growing aptitude. Textures highlighted in green are optimal; those in yellow are adequate, and those in red are not desirable.

Organic Matter

Organic matter in the soil improves structure, helps the formation of aggregates and increases porosity and cation exchange capacity. It also regulates the soil pH, increases water retention and stimulates biological activity. For these reasons it is an important factor to consider when choosing the site for truffle cultivation.

The amount of organic matter in truffle bed soils varies considerably with minimum and maximum absolute values at 0.8% and 37% respectively with a mean of 5% (Jaillard et al. 2016). The recommended range for truffle cultivation is from 1.5% to 8% (Delmas and Poitou 1974; Grente and Delmas 1974; Poitou 1990).

The C/N Relationship

The carbon/nitrogen (C/N) relationship reflects the amount of mineralization in the soil and gives an indication of biological activity. This should be evaluated particularly in heavy soils with high clay content where an optimal C/N is desirable (Sourzat 2001).

The recommended range of the C/N ratio for cultivation of the black truffle lies between 8 and 15 (Delmas and Poitou 1973; Delmas et al. 1981; Poitou 1988; Sourzat 1997), with optimum values near 10. The minimum and maximum absolute values observed in wild truffle sites are 0.1 and 26 respectively (Bencivenga and Granetti 1988; García-Montero et al. 2001).

Macronutrients (N, P and K)

Despite being essential nutrients, significant concentrations of nitrogen, phosphorus and potassium in the soil are not necessary for truffle production. Generally, most soils have sufficient amounts of these nutrients for maintaining both fungal and tree growth and therefore, apart from unusual circumstances, there should not be deficiency problems. The problems associated with macronutrients are often due to concentration levels having been raised too high from added fertilizers in cropped fields. Plants depend on mycorrhizal fungi for capturing soil nutrients at the typically low concentrations found in most soils. When concentrations are exceptionally high, plants can absorb nutrients without the intermediary fungus and mycorrhizal colonization rates drop, which may cause the loss of the desired fungus (***T. melanosporum***, in this case), which depends on the tree for obtaining carbon (its energy source) and maintaining its life cycle.

The recommended range of organic nitrogen content (Kjeldahl method) for the cultivation of the black truffle lies between 0.1% and 0.3% (Poitou 1987; Olivier et al. 1996; Sourzat 2001) with minimum and maximum absolute values reported from wild truffle sites at 0.05% and 0.52% (Delmas et al. 1981). The black truffle and other soil microorganisms are capable of transforming the different forms of phosphorus into an assimilated form suitable for its host (Olivier et al. 1996). The measurement of total phosphorus in truffle bed cultivation is more significant than the individual portions. The recommended range of total phosphorus is from 0.1% to 0.3% (Poitou 1987; 1990; Olivier et al. 1996).

The recommended range for soil potassium content for cultivation of the black truffle lies between 0.01% and 0.03% (Poitou 1987; Olivier et al. 1996; Sourzat 2001) with minimum and maximum absolute values of natural truffle beds at 0.0096% and 0.12% (Delmas et al. 1981).

Structure
The structure describes the way in which individual particles make up the soil and create resulting cavities or pores. The best structure for the development of the black truffle is one which permits aeration within the soil and effective water drainage through the pores thus enabling penetration of the tree roots and the truffle mycelium (Delmas and Poitou 1973; 1974; Poitou 1988). The most ideal structure for black truffle cultivation is a granular or crumbly structure with water-stable aggregates under 4 mm (Jaillard et al. 2014).

Table 1b lists the recommended ranges for the most significant soil parameters for determining suitability for the cultivation of black truffles.

TABLE 1b: Recommended range for the main soil parameters for black truffle cultivation (Delmas and Poitou 1973; Grente and Delmas 1974; Delmas et al. 1981; Delmas et al. 1982; Poitou 1987; 1988; 1990; Bencivenga and Granetti 1988; Olivier et al. 1996; Sourzat 1997; 2001; Reyna 2000; Raglione et al. 2001).

PARAMETER	RECOMMENDED RANGE
pH	7.5 – 8.5
Organic material (%)	1.5 – 8
Calcium carbonate (% $CaCO_3$)	1 – 83.7
Exchangeable Calcium (% CaO)	0.4 – 1.6
Nitrogen (Kjeldahl) (%)	0.1 – 0.3
Phosphorus (%)	0.1 – 0.3
Potassium (%)	0.01 – 0.03
Texture	Loam, sandy loam, clay loam, silt loam, sandy clay loam
Structure	Granular or crumbly
C/N ratio	8 – 15

2.5. Biological Conditions

Cultivation Land Use History
The legacy of previous crops where the plantation is established will affect the future evolution of the land. Sites where cereals, forage crops, and legumes have previously been cultivated are suitable (Reyna 2000). Vineyards and fruit orchards are also considered suitable (Sourzat 1997), and in general, previous crops which form endomycorrhyzal symbioses. In the case of woody crops, it is important to test the health of the roots. An infection caused by pathogenic fungi such as ***Armillaria*** sp. or ***Phytophthora*** sp. could seriously damage the plantation. Additionally, recently burned forest soils with no further treatment may be receptive to ***T. melanosporum*** (Martínez de Aragón et al. 2012).

Host Trees

In its native truffle habitats ***T. melanosporum*** can form mycorrhizas with evergreen holm oak (***Quercus ilex*** sp. ***ilex***, ***Q. ilex*** sp. ***ballota***), semi-deciduous and deciduous oak trees, portuguese oak, and downy oak (***Q. faginea, Q. pubescens***), Kermes oak (***Q. coccifera***), hazelnut trees (***Corylus avellana***), rockrose (***Cistus incanus***), several pine species (***Pinus pinea, P. halepensis, P. nigra***), European hop hornbeam (***Ostrya carpinifolia***), European hornbeam or ironwood (***Carpinus betulus***), and linden trees (***Tilia*** sp.) (Palenzona 1969; Manna 1992; Bencivenga et al. 1995).

2c **2d**

Figures 2c and 2d: *The most successful host trees for black truffle cultivation in Spain at present, Q. ilex, and Q. faginea respectively.*

Plants found within the burn

There are a few plants which are not affected by the phytotoxic properties of ***T. melanosporum*** which is known to cause a burn or "brûlé" around the base of the host tree. These include Mahaleb cherry (***Prunus mahaleb***), dogwood (***Cornus sanguinea***), juniper (***Juniperus oxycedrus, J. communis***), stonecrop (***Sedum altissimum***) and red fescue (***Festuca rubra***) (Sáez and De Miguel 1995; Olivier et al. 1996), and in areas with mild climates, ***Ulex parviflorus*** (Sourzat 1997).

3. Planting

In order to begin planting it is necessary to prepare the land, obtain the tree seedlings, and plant during the appropriate season, having already chosen the planting density.

3.1. Preparing the land

Preparation of the land depends, in part, on previous land usage and the condition of the land at the time prior to planting.

In order to facilitate drainage and aeration, it may be necessary to rip the soil with a sub-soiler or a chisel in order to break up a possible hardpan from previous land use. Afterwards, superficial cultivation is important for smoothing and leveling the soil with graders or cultivators. The recommended time period is during the summer and autumn months before planting. It should be done on dry ground to reduce compaction and without mixing soil horizons.

Land cultivation can also be carried out in sections of 1 meter wide strips along the planting rows. In areas with superficial soils or non-compacting (sandy textured) soils with low organic matter and minor plant cover, seedlings may be planted without pre-plantation cultivation (Ricard 2003).

If the previous crop has been woody, all the roots should be removed in order to prevent the proliferation of ***Armillaria*** sp. or other pathogenic fungi in the roots.

Figure 3a: *Oak tree infected with pathogenic Armillaria sp. (Photo by Jonàs Oliva).*

Figure 3b: *White mycelium of pathogenic Armillaria sp. at base of infected tree.*

To establish a black truffle orchard on acidic soils it is necessary to raise the pH. This should be calculated based on actual characteristics of the soil. A sample estimation would be 1 ton of a mixture of lime ($CaCO_3$) and calcium hydroxide ($Ca(OH)_2$) per hectare in order to elevate the soil pH by 0.1 in the top 20 cm of superficial soil (Hall and Brown 1989).

3.2. Acquiring the Plant

The host plant species should be chosen according to the plantation site. Inoculated seedlings of several species of deciduous and evergreen oak trees including holm oak, hazelnut tree, and occasionally rockrose may be available. Ideally, the chosen host should be the most suitable tree for the plantation site. In Spain, best results have been observed with the evergreen holm oak ***(Quercus ilex)*** and deciduous downy oak (***Q. faginea***). In contrast, observations from sites planted with hazelnut trees (***Corylus avellana***), indicate that this tree is much more receptive to a diversity of other mycorrhizal fungi including ***T. brumale*** than the holm oak and downy oaks (Ricard 2003).

Plants should have a well-developed root system with an abundance of trophic roots colonized with ***T. melanosporum*** mycorrhizae. There should be no mycorrhiza from any other ***Tuber*** species. A low percentage of mycorrhizas from fungi commonly found in greenhouse conditions is acceptable although these fungi are not desirable if they dominate the root system (Fischer and Colinas 1996). Seedling quality should comply with current regulations and established forestry standards. The plants should be correctly hardened-off, particularly if they are to be planted in autumn.

If seedlings are not certified, it is advisable to have the plants analyzed in a qualified laboratory to confirm the seedling quality and mycorrhizal status prior to outplanting. A brief description of the mycorrhizae of four important species of ***Tuber*** can be found in Section 2 of this book, “Best Practices for Cultivation of Truffles”.

Figure 3c: *Inoculated oak seedlings with label of certification ready for planting.*

3.3. Selecting Planting Density

The planting density depends on the chosen host species and the fertility of the land, which in turn is dependent on the depth of the soil as well as the content of organic matter and clay in the soil. Density should be lowest in areas of long growing seasons where higher annual tree growth is expected.

Density also varies according to the weed control model. For this reason, if one is considering cultivating the land often, it is best to space the trees accordingly. Spacings of 5x5 m are chosen to obtain densities of 400 plants/ha (Estrada and Alcántara 1990). Currently the most widely used planting grids are those which establish a density of 200-330 plants/ha. This is achieved by using spacings of 6x5, 6x6, or 7x5. Also it is useful to consider the width and capabilities of the machinery to be used and the irrigation system installed.

3.4. Planting time

According to the weather of each region, planting should be carried out from the beginning of the autumn rainy season to the end of the spring frosts. It is preferable to plant at a time when natural rainfalls will follow the planting, prior to the onset of the driest and warmest months.

3.5. Planting

The day before planting it is best to water the plants so that the root plug is intact and to reduce potential transplant shock. Plantation should not be undertaken during freezing periods or with strong winds. Planting is carried out manually. Make a hole large enough to contain the plant. Carefully place the plant so that it sits vertically with its roots well extended in such a way that the root collar is slightly underground. Next, fill the planting hole with fine soil and pack it firmly by stepping around the plant to prevent air pockets. After planting it is advisable to water each plant with 5 liters of water. Seedling shelters should be used if there is threat of animal browsing.

Figure 3d: *Manually plant the inoculated seedlings after preparing the site.*

Figure 3e: *Seedling shelters are placed at time of plantation.*

4. Maintenance

After planting, the truffle plantation must be managed properly during every season to obtain good production. For other types of crops such as fruit trees or almonds, there is a wide variety of information regarding proper management protocols, and the response to treatments is clearly observable: one can directly observe tree growth in the field and observe flowering and fruit growth. However, the objective of black truffle cultivation is to aid in the development of a fungus that develops underground and is therefore not directly observable.

The first indication of effective fungal growth is the appearance of the truffle burn between the fourth and seventh year, although this does not guarantee truffle production. The "truffle burn" (**Figures 4a and 4b**) refers to the area immediately surrounding a "truffle tree" that appears bald for the striking loss of vegetation due to the phytotoxic properties of the fungus (Streiblova et al. 2012).

The first truffles usually are not produced until 6 to 10 years after planting. Until then, in order to follow the development of the fungus, one may observe the proliferation of mycorrhizae in the tree's roots, or test the soil for the presence of ***T. melanosporum*** mycelium using a molecular analysis (Suz et al. 2006, Parladé et al. 2013).

4a **4b**

Figures 4a and 4b: *Wild truffle site where the truffle burn is visible, an area surrounding the host tree the, devoid of vegetation.* ***4b***. *Plantation site in full production for black truffles, Tuber melanosporum, collected within the burn.*

4.1. Weed control

Stage 1: From the establishment of the plantation to the appearance of truffle burns

In this stage, weed control around the young trees is independent from the vegetation management or weed control model chosen later. During the first 2-4 years it is important to keep the area around the plants free of weeds and competing vegetation. This improves seedling survival by eliminating competition for water and nutrients, while increasing survival and proliferation of ***T. melanosporum*** mycelium.

In the rows between each plant the land should be cultivated with tools that can be controlled for depth such as cultivators or disc harrowers (never a rototiller, which mixes the soil) to a depth no greater than 15-20 cm (Reyna 2000).

Figure 4c: *Cultivation tools are used to remove weeds, aerate the soil and smooth surface.*

A herbicide treatment, once a year aimed at eliminating weeds in the summer period to avoid competition for soil water may provide immediate results compared with mechanical tilling with tractors or with no intervention at all (Olivera et al. 2011). However, herbicides should be used as a last resort to avoid the incorporation of chemical phytotoxins into the soil.

An effective alternative is the use of mulching or covering the ground surrounding the young tree with materials that reduce the solar radiation reaching the soil, thus limiting the germination and photosynthetic capacity of the competing vegetation (Olivera et al. 2014a). In truffle orchards, a folded white polypropylene fabric mulching has been shown to favor the expansion of ***T. melanosporum*** in the soil when compared with other fabrics or manual or mechanical weed removal. Straw cover or black polypropylene did not work well in a study with ***T. uncinatum*** (Zambonelli et al. 2005).

Figure 4d: *Mulching with double-layer white polypropylene fabric at time of plantation establishment.*

Stage 2: Years following the appearance of truffle burns

Once the truffle burn appears, careful cultivation of the site is recommended. Because the burn is an indication of active ***T. melanosporum*** mycelium in the soil, it is important to protect the soil from aggressive interventions at this time.

At this stage, the soil should be cultivated with depth-control tines to a depth no greater than 10 cm (Sáez and de Miguel 1995; Reyna 2000), although deeper land cultivation helps to protect truffles from damage caused by frost and insects. Excessive cultivation (3-4 or more times per year) may have a negative effect on the structure and porosity of the soil because of the destruction of soil aggregates, increasing compaction and diminishing microbial activity (Ricard 2003), resulting in the opposite effect sought for in truffle cultivation. The best times are the months of March-April (Sourzat 1997).

The objectives of truffle orchard cultivation include:

- Elimination of weeds and plants that compete with truffle mycelium and tree seedlings for water and nutrients;
- Promotion of soil aeration;
- Improvement of the water-holding capacity of the soil.

Land cultivation may be altogether unnecessary in sandy or extremely loose soils where there is good natural aeration (Ricard 2003).

4.2. Irrigation

Stage 1: Establishment and Pre-production
Judicious watering is recommended for the first years until the root system is well established. In the case of a prolonged drought of 20 days or more, young seedlings should be irrigated, particularly during the first year. Three to four liters is recommended per plant every two to three weeks depending on soil type, climatic conditions and on the intensity of dry periods (Sourzat 2002).

This is a useful recommendation when one cannot estimate the water shortage, but it is problematic because the necessary water quantity depends on the type of soil. Over watering can contribute to a decrease in ***Tuber*** mycorrhizal colonization status (Bonet et al. 2006; Olivera et al. 2011), and may favor non-desirable colonization of the root system by other mycorrhizal fungi including ***T. brumale*** (Merényi et al. 2016).

The water needs of truffle plantations have been studied from two points of view: the correlation between truffle production and monthly rainfall (Le Tacon et al. 2014), and the correlation between abundance of truffle mycorrhizae and water inputs (rainfall plus irrigation) as a function of potential evapotranspiration (Bonet et al. 2006; Olivera et al. 2011). Estimated irrigation rates with a coefficient of 0.5 applied to reference evapotranspiration gave good results when applied every two or three weeks between May and July. This coefficient should be lower between the months of August and October.

To determine the irrigation rate based on potential evapotranspiration: First, estimate the evaporative demand for a given site by locating the reference evapotranspiration; then, apply a coefficient of cultivation of 0.5 to calculate the irrigation dose for April through July, and 0.3-0.5 between August and October. It is recommended to allow for at least one period of water stress during the summer (Olivera et al. 2014b). This requires factoring in water storing capacity of the soil, which will depend on the soil texture and structure, as well as subtracting the rainfall.


Figures 4e and 4f: *Irrigation needs will vary over the lifetime of a plantation and depend on multiple factors, including soil type, evapotranspiration rates and plantation age.*

The age of the plantation and the volume of the tree crowns should be considered to adjust the coefficients of cultivation for the conditions of the orchard. Since the Mediterranean-Continental climate is subject to great inter-annual variability in rainfall, it is recommended to monitor soil water content beyond the months proposed for irrigation.

The most accurate approach to estimating water needs is measuring water potential directly in the orchard. During the establishment phase there is a delicate balance between the higher water potential that would optimize the growth of the seedlings and the lower water potential that would inhibit the growth and mycorrhizal formation of other fungi that can displace ***T. melanosporum***. When the soil water potential stays below -0.4 MPa, truffle mycorrhizae develop well, while those of other fungi are limited (Olivera et al. 2006).

If the plants cannot be irrigated, they should be mulched. Mulching favors the growth of truffle mycelium (Olivera et al. 2014a) and also helps to retain soil humidity.

Stage 2: Production
According to historical recommendations, in this stage the plants should be irrigated with 50-60 l/m^2/month from May-June until August-September (Grente and Delmas 1974; Olivier et al. 1996), or 30 l/m^2 every 15-20 days (Sourzat 1997), 30 l/m^2 every 3 weeks (Fortuny and Estrada 1986) or between 30-50 l/m^2 each month based on the soil's ability for water retention (Verlhac et al. 1990). With these quantities one must subtract the actual rainfall (Estrada and Alcántara 1990; Sourzat 1997). Carbajo (1999) recommends 25 l/m^2 every 15 days during the months July, August and September.

Each truffle farmer must develop his or her own particular guidelines depending on site conditions and annual weather behavior (Olivier et al. 1996).

Sprinklers or micro sprinklers are recommended over drip irrigation. Mulching with straw or other plant materials can help prolong the humidity of the irrigation and may be kept in place for the entire summer but without covering the entire burn. Mulching material with a diameter of about 50 cm of material can be spaced out at a minimum of 60 cm intervals (Callot 1999; Ricard 2003).

Our recent data show that the highest productivity of an orchard is achieved by watering to field capacity and allowing the soil to dry, but without letting the water potential stay below -1 MPa for more than 2 weeks and never below -2 MPa for more than 1 week, before watering again throughout the growing season (Oliach et al. 2016).

4.3. Fertilization

Most soils have sufficient amounts of nutrients for black truffle development. The black truffle is a fungus adapted to living in marginally fertile soils. It is advisable to fertilize only on plantations with land exceptionally low in a particular nutrient to make up for the deficit (Olivier et al. 1996). Regular soil analysis may be indicated to monitor nutrient status in truffle soils, particularly if amendments to correct pH or nutrient deficiencies have been made.

Since most of the host tree´s fine root tips are colonized by mycorrhizal fungi, the host's nutrient demand is primarily met by these fungi. Applying fertilizers indiscriminately to the soil can potentially jeopardize the balance between the fungus and the host. With this idea, foliar fertilization applied to the host plant was tested as an alternative to stimulate the growth of ectomycorrhizal fungi. However, field results have varied from null (Bonet et al. 2006), to negative (Olivera et al. 2011) to selectively positive as observed with a foliar fertilizer high in potassium (Suz et al. 2010).

Occasionally, seedlings can develop chlorosis because of an iron (Fe) deficiency caused by Fe becoming unavailable at very high pHs. This can be solved by the foliar application of an iron chelating product.

Figure 4g: *Foliar chlorosis observed in Quercus ilex.*

4.4. Pruning

For the first years of the plantation tree pruning is carried out primarily for correcting structural defects (Sourzat 2000; 2002) and to develop the desirable tree form for creating conditions most favorable for truffle development (Ricard 2003).

Formation pruning or early training is aimed at generating a tree with a conical (Carbajo 1999) or oval shape (Grente and Delmas 1974) thereby eliminating lower branches and basal sprouts. Thus, the amount of light reaching the ground is increased, and there is room for installation of an irrigation system, and additionally, future truffle gathering is more efficient. It is also a good idea, at this stage, to leave 2 or 3 stems per tree in order to not lose the tree completely if one stem breaks in a storm.

Formation pruning may begin in the third year depending on the plant's strength and should be done with low intensity. It is recommended yearly. Later, at the

4h

4i

Figures 4h and 4i: *Tree pruning is performed to eliminate lower branches and to create conical and open crowns.*

onset of the tenth year, one should try to limit tree growth and expansion of the root system and to avoid canopy closure (Ricard 2003). During this stage, pruning may be more intense and be necessary every 2-5 years so that pruned branches have not yet developed heartwood.

In hot climates and with strong solar radiation one can prune the higher branches of the tree in order to aid aeration and conserve lower branches. This shades the ground and promotes a buffering of temperature extremes (Ricard 2003).

Some farmers are reducing costs by machine pruning hedge-style plantations where trees are planted closer in rows, with greater separations between rows.

Pruning is often recommended during the winter or the vegetative dormancy phase (Sáez and De Miguel 1995), to preserve the carbohydrates stored in the roots. However, pruning during the growing season, particularly during the second half, has the advantage of allowing for a faster and better healing of tree wounds, reducing the risk of infection by heartwood-rotting fungi.

4.5. Addition of truffle spores and soil amendments to the orchard

A common practice in many truffle plantations today is the deliberate input of truffle spores to the soils in the root zone of well-established host trees. Also known as "Spanish wells" this technique is based on the hypothesis that fenced-in truffle sites require the input of spores to mimic the natural dispersal activities of insects and animals that dig-up, consume and spread truffle spores naturally. Given that both mating types (via spores or mycelium) are required for the initiation of a new truffle (Rubini et al. 2011), it is believed that spore input is beneficial. A potting soil mixed with truffle spores is added to create small pockets of well-aerated soil where evenly formed truffles can develop.

Figure 4j: *Preparation of holes around a maturing tree for the addition of truffle spores and soil amendments.*

Positive results have been observed repeatedly by truffle farmers, especially 2 years after the application of the mixture of spores and potting soil. An experimental approach to validate the effectiveness of this technique was reported by Murat et al. (2016), confirming increased truffle production in a 7-yr. old orchard beneath trees where spore and substrate amendments were added to 8-10 cm deep holes at 50 cm from the trunk of the host tree. The number of productive trees at the site was increased, but not the productivity of truffle-producing trees.

Figure 4k: *Truffles collected from a single "Spanish well".*

Experimental data with robust significant results are scant because it is hard to control for the many variables involved in this practice: site, tree age, initial colonization status, fungal and tree genetic factors, timing of application, the number of spores applied and quality and composition of accompanying soil amendments.

There are some drawbacks to this intervention. Truffle farmers observe that a single hole may contain many small truffles where only 1 or 2 are well formed and mature, while the others have been disturbed in the collection process, and lack commercial quality. Therefore, the quantity of spores and the size of the pocket or hole are important. Another threat is the accidental introduction of undesirable spores of other truffles or of root pathogens to the orchard. Both of these threats highlight the importance of good practices, using quality sterile substrate and truffles whose identity has been confirmed by experts before making such amendments to the orchard.

4.6. Diseases

Maintaining a healthy black truffle orchard is a long-term project that requires thoughtful planning and vigilance over several decades. There are many factors that can contribute to health issues for the host tree or the developing truffle belowground. An important initial factor is the choice of tree species. When planting tree seedlings that are either non-native or come from off-site populations of a particular species, there is a risk that these trees may suffer damage from mildew, frosts, insects and drought due to poor adaptation to climate conditions, while subjected to unfamiliar watering regimes and frequent tree pruning.

Another factor is the extensive planting of monocultures over large areas of land. The ecology of hypogeous fungi involves spore dispersal by insects and mammals. There are many mycophagus organisms that consume and disperse truffle spores such as wild boars (***Sus scrofa*** L.), the truffle fly (***Helomyza tuberivora*** R.-Desv.) and truffle beetles (***Leiodes cinnamomea***), but their populations, like many other natural predators, can explode when there is an abundance of truffles. These organisms are responsible for reducing truffle production and quality, and others may threaten the health and survival of the trees.

Figure 4l: *Wild boars are one of many truffle-loving animals known to consume and disperse truffle spores.*

Figures 4m and 4n: *Insect damage to fresh truffles threaten truffle production and quality.*

A valuable source of information on phytosanitary threats to the emerging Black Truffle industry can be found in a survey made by Martín-Santafé et al. (2014) in Aragon, Spain, a region with more than 6000 ha planted in truffle plantations and with many seedlings nurseries that also suffer plant health problems. These authors have identified more than 50 health threats to their truffle sector affecting all stages of the process.

Particular thought should be given to the risk of newly introduced diseases that could spread quickly through monocultures. The European Plant Protection Organization lists organisms that could be detrimental to truffle cultivation and should be carefully monitored to eradicate any possible introduction before it is too late.

Of special concern are infections by fungi from the genus ***Phytophthora*** that can inflict serious damage to truffle orchards and have been recently detected throughout Europe (Pérez-Sierra et al. 2013; Jung et al. 2016).

In truffle cultivation management, aggressive attacks against the offending organisms are not recommended since every treatment applied may affect the truffle ecosystem. However, careful identification, monitoring and prompt interventions can promote seedling health, truffle quality and orchard longevity. The support and expertise of trained truffle cultivation specialist, agricultural and forestry extension services and truffle growers' associations can provide early detections and proper response to potential biosecurity issues.

Figure 4o: *Monitoring and reporting signs of disease can provide early detections of potentially threatening diseases in truffle plantations.*

5. Cost-Benefit Analysis

The total yield on the initial investment is calculated by obtaining the annual yields from differences in income and expenses, and discounting according to the value of the euro in the first year of establishment (Oliach et al. 2008). According to our calculations, the total yield of one hectare of ***T. melanosporum*** according to the Net Present Value (NPV) is 50,231 € which corresponds to an annual net cash flow of 2,691 €/ha over 35 years.

This cost-benefit analysis gives an orientation of potential yields, given that we assume that the price of truffles and production quantities are constant. There are various studies which discuss profits obtained from plantations in Spain, France and Italy which show Net Present Values fluctuating between 19,424 €/ha and 66,972 €/ha (Bonet and Colinas 2001, including references cited therein). According to these studies, the average yield obtained using the internal rate of return (IRR) is always greater than 9% and the investment recovery period is always greater than 10 years.

At the time these calculations were made, truffle plantations reached commercial-level productions at about 8 to 10 years, with high variability depending on the quality of the initial seedlings planted, the land chosen and overall site management. The reported truffle yields ranged between 10 and 50 kg/ha year in productive orchards (Oliach et al. 2008), although production is irregular over the lifespan of the plantation.

Improved management technology and early irrigation have shortened the pre-production phase of plantations and has increased the yields, so this cost benefit analysis should be considered conservative.

These calculations will vary depending on the cost of labor, the cost for site preparations and site maintenance which will vary by region and by country. These may include liming, fencing, weed control and installation of an irrigation system. The cost benefit outcome will also vary according to the market value of the truffle cultivated.

6. Mapping of Suitable Lands For Truffle Habitat and Cultivation

A useful tool for farmers, policy makers and regional forestry and agricultural decision makers is the development of a map that demonstrates land suitable for truffle habitat (Colinas et al. 2007; De Laurentiis and Spinelli 2009; Alonso Ponce et al. 2010; Serrano-Notivoli et al. 2016). These maps are based on soil, climate, annual rainfall and truffle production.

Figure 6a shows the land suitable for the development of truffles in Catalonia, NE Spain, based on information current in 2007. This area coincides mainly with the Pre- Pyrenees range, where both natural open oak forests and cultivated truffle orchards produce truffles with proper management today. We anticipate, that based on predictions of global climate change models, there will be a reduction of 29% of the total area suitable for the cultivation of black truffles by 2040 in Catalonia (Figure 6b).

These maps provide landscape-level guidance for areas suitable for growing ***T. melanosporum***, including areas that are considered suitable if irrigation support is available. In addition to landscape level guidance it is necessary to test the suitability of soils and geography of individual sites destined for truffle cultivation before investing in a truffle orchard establishment.

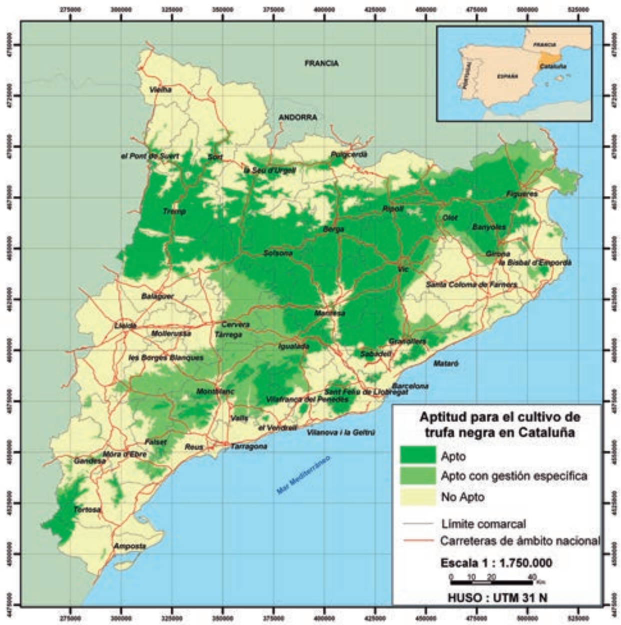

Figure 6a: *Map estimating aptitude for growing black truffles in Catalonia in 2007. Source: Colinas et al. (2007).*

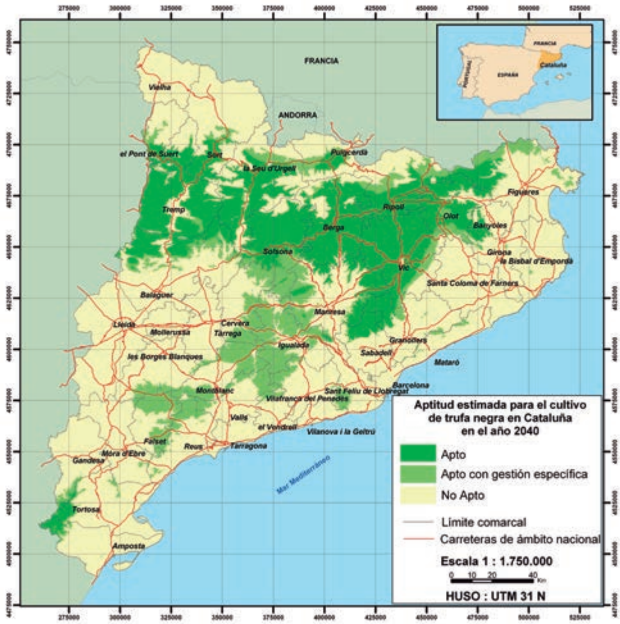

Figure 6b: *Map estimating aptitude for growing black truffles in Catalonia in 2040. Source: Colinas et al. (2007).*

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8. Description of Mycorrhizae of *Tuber melanosporum, Tuber brumale, Tuber aestivum* and *Tuber indicum*.

The characteristics of the ectomycorrhizae of different ***Tuber*** species can be observed in plant roots by examining several macroscopic and microscopic features. Although the ectomycorrhizae of many species of fungi are similar, there are some clearly observable characteristics that can be used to help identify the mycorrhizae of ***T. melanosporum, T. brumale, T aestivum*** and ***T. indicum***. Species identification can be further confirmed by the molecular techniques of sequencing and polymerase chain reaction (PCR).

The objective of this section is to present a brief description of the mycorrhizae of four species of ***Tuber*** that may be encountered in inoculated seedlings prepared for the purpose of establishing truffle orchards. These descriptions and photos can assist technicians, nursery managers, and students to recognize the mycorrhizae of these ***Tuber*** species in tree roots. The exact dimensions and branching arrangements of a well-developed ***Tuber*** mycorrhizal system will vary according to the host plant species.

The mycorrhizae of these ***Tuber*** species share a similar color and shape: golden to reddish brown to dark brown, with a cylindrical form that tends to be broader at the tip. The mantle morphology for ***T. melanosporum, T. brumale*** and ***T. indicum*** is nearly identical for the interior mantle with a tightly arranged "puzzle pattern". ***T. aestivum*** displays an internal mantle of cells arranged in a polygonal pattern.

Therefore, it is important to also observe the cystidia of the different mycorrhizae.

The differences between ***T. melanosporum*** and ***T. indicum*** are difficult to distinguish microscopically, and these two species can be identified using PCR primers designed for this purpose (Paolocci et al. 1999).

8.1 *Tuber melanosporum*

T. melanosporum ectomycorrhizae are golden to reddish brown becoming dark brown with age. An individual mycorrhizal root appears cylindrical or swollen in the tip (Figure 8a). With maturity the mycorrhizae develop multiple ramifications, whose morphology depends on the host tree. With ***Quercus ilex*** they appear as pyramidal or similar to a grape cluster Figure 8b.

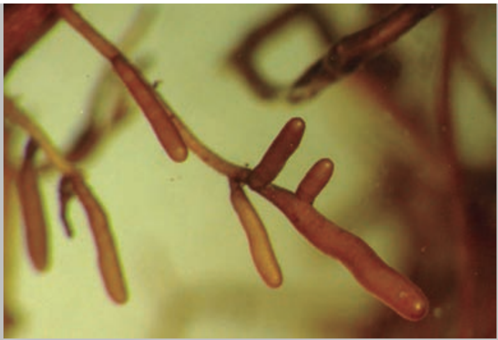
Mantle anatomy: Pseudoparenchymal epidermoid (Agerer 1987) also referred to as a "puzzle pattern". External mantle with elongated cells, from which the cystidia originate. The cells of the interior mantle are yellow-beige, with thick walls that become darker with maturity.

Cystidia: The cystidia (external hyphae emanating from the surface of the mycorrhizae) are transparent, with bifurcations at approximately a right angle.

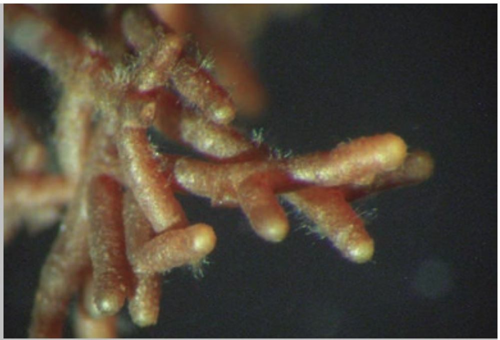
They are distributed irregularly on the surface of the mycorrhiza, sometimes absent and often very abundant. Length of 100-200 μm; diameter of 3.0-3.5 μm at the base and decreasing to 2.2-2.8 μm at the tip Figure 8d.

Further descriptions of ***T. melanosporum*** mycorrhizae can be found in: Rauscher, T., Agerer, R., Chevalier, G. (1995).

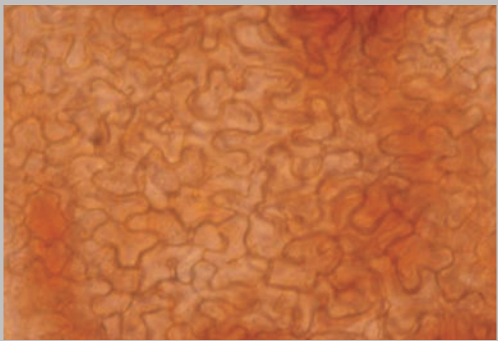
Pérez, F., Palfner, G., Brunel, N., Santelices, R. (2007).



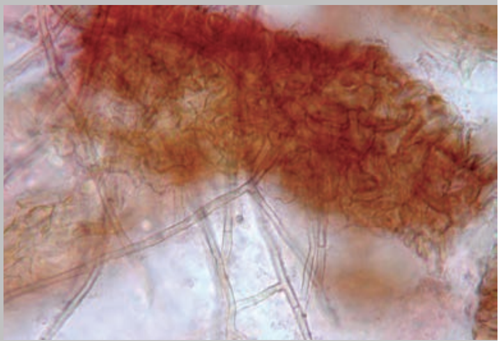
8a



8b



8c



8d

Figures 8a: *Mycorrhizae of* *<u>Tuber melanosporum</u>* *with golden brown color and smooth surface;* ***8b:*** *in bifurcating system with cystidia emanating from the surface.* ***8c:*** *<u>Tuber melanosporum</u>* *mantle with the "puzzle" design of tightly interwoven inner mantle cells, and* ***8d:*** *cystidia with the characteristic bifurcation.*

8.2 *Tuber brumale*

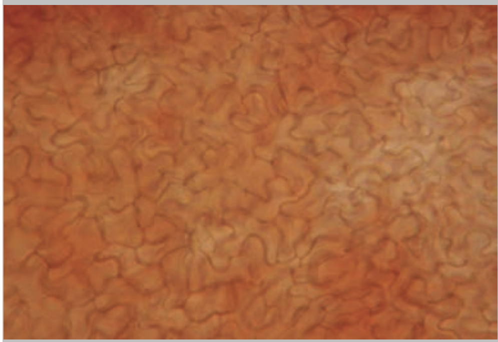
The mycorrhizae of the winter truffle, ***T. brumale***, are smooth brown, cylindrical and similarly to ***T. melanosporum***, they are broader in the tip than at the base. They can be detected by their “bottlebrush” or “halo” appearance due to the presence of many fine cystidia that emerge from the surface. These external hyphae or cystidia are transparent, generally very regular in length and evenly distributed. They have needle-shaped morphology, with a simple septum at the base.

Mantle anatomy: Pseudoparenchymal epidermoid (Agerer 1987), very similar to ***T. melanosporum***. External mantle with elongated cells, from which the cystidia originate. The cells of the interior mantle are yellow-beige with pale to dark brown thickened walls.

Cystidia: Transparent needle-shaped, straight or slightly curved and pointed at the end. Length of 50-90 μm, lacking bifurcations. Diameter of 4.5-10μm at the base and decreasing to 0.8-2.0μm at the end. The base of the cystidia is swollen and often with a basal septum.

8e

8f



8g

8h

Figures 8e and 8f: *Mycorrhizae of Tuber brumale with short, pointed evenly distributed arrangement of cystidia.* ***Fig. 8g:*** *T. brumale mantle cells, similar to T. melanosporum with “puzzle” design, and* ***Fig. 8h:*** *T. brumale cystidia, with tapering and pointed tips.*

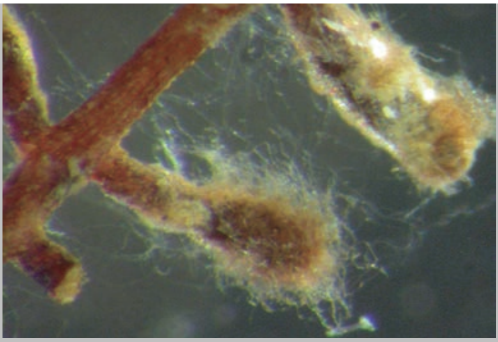
Further descriptions of ***T. brumale*** mycorrhizae can be found in: Fischer, C., Suz, L.M., Martín, M.P. and Colinas, C. (2004).

8.3 *Tuber aestivum*

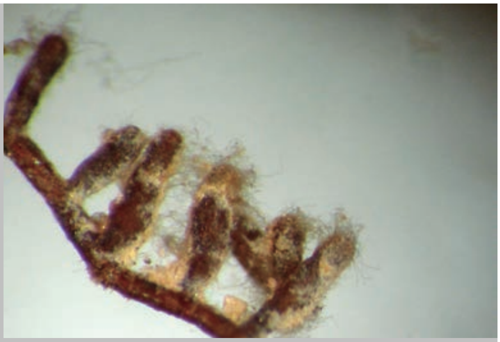
The ectomycorrhizae of the summer truffle, ***T. aestivum***, are light to dark brown, cylindrical and ramifying into systems with maturity. They can be detected by the presence of their abundant, long and curving cystidia that emerge from the surface, and which tend to trap soil particles. These cystidia are transparent, with various lengths and lacking in septae.

Mantle anatomy: Pseudoparenchymal polygonal (Agerer 1987). This polygonal pattern is tightly developed and appears in the form of rectangles, squares and triangles. The cells of the interior area of the mantle are pale beige to reddish with thick brown cell walls of 0.5-1.0 µm.

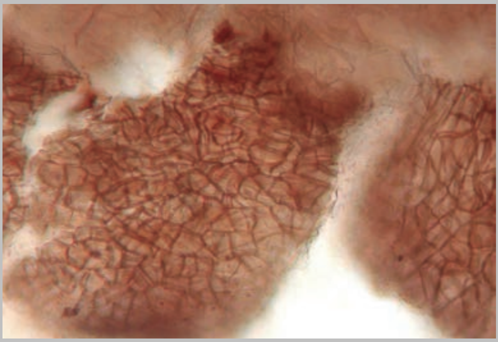
Cystidia: Transparent and without septae, very curved and with an even diameter and a tendency to be abundant and lengthy, arranged densely on the surface of the ectomycorrhizae.



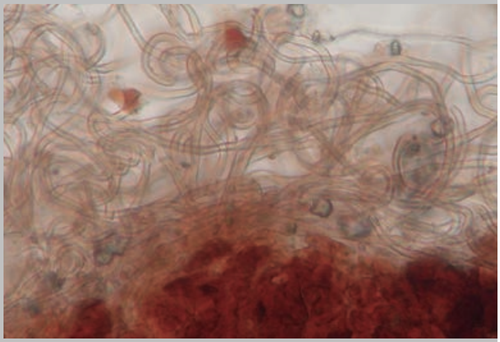
8i



8j



8k



8l

Figures 8i and 8j: *Mycorrhizae of Tuber aestivum with a very hairy appearance due to the abundance of long, curving cystidia.* ***Fig. 8k:*** *T. aestivum mantle cells, with "polygonal" design, and* ***Fig. 8l:*** *abundant even-diameter long curving cystidia.*

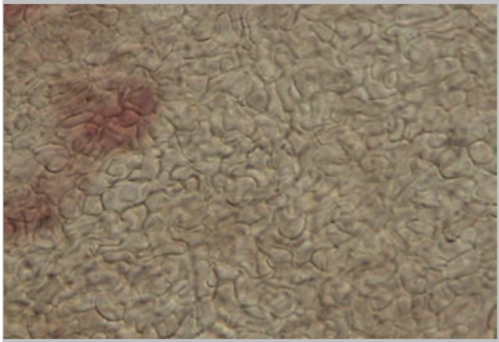
Further descriptions of ***T. aestivum*** mycorrhizae can be found in: Müller, W. R., Rauscher, T., Agerer, R., Chevalier, G. (1996).

8.4 *Tuber indicum*

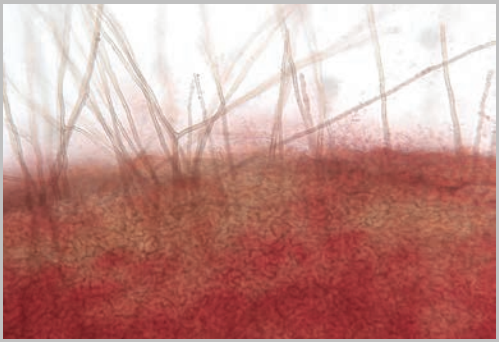
T. indicum mycorrhizae are very similar in appearance and morphology to the mycorrhizae of ***T. melanosporum***. They are light to dark reddish-brown depending on their age. Individual mycorrhizal root tips appear smooth, cylindrical and mostly swollen in the tip. With maturity the mycorrhizae develop multiple ramifications whose form will depend on the host tree. The cystidia are irregularly distributed over the surface of the mycorrhizal, and may be frequent or absent.

8m

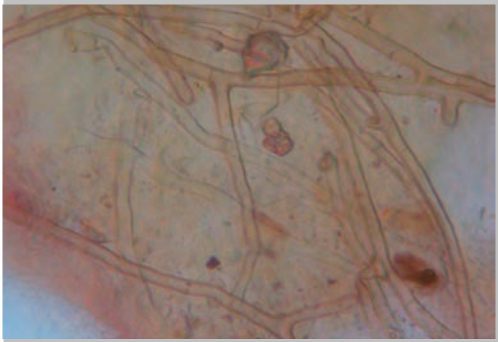
8n



8o



8p



8q

Figures 8m and 8n: *Mycorrhizae of Tuber indicum with light brown color, swollen tips and fine cystidia emanating from the surface; mycorrhizae in ramified mycorrhizal system with Q. ilex.* ***Fig. 8o:*** *Tuber indicum mantle with the "puzzle" design of tightly interwoven inner mantle cells, and* ***Fig. 8p*** *and* ***8q:*** *cystidia on mantle surface with the characteristic bifurcation at 90º.*

Mantle anatomy: Pseudoparenchymal epidermoid inner mantle, also referred to as a "puzzle pattern". External mantle with elongated cells, from which the cystidia originate. The cells of the interior mantle are tightly organized and are yellow-beige to dark brown with thin to thick walls.

Cystidia: The external hyphae or cystidia are transparent, with bifurcations at approximately a right angle. They are irregular in length with simple septae. Length typically of 100-300μm; diameter of 3.0-4.0μm at the base and decreasing in width and tapering at the end.

Further descriptions of ***T. indicum*** mycorrhizae can be found in:
García-Montero, L. G., Di Massimo, G., Manjón, J. L., García-Abril, A. (2008).
Geng, L. Y., Wang, X. H., Yu, F. Q., Deng, X. J., Tian, X. F., Shi, X. F., Shen, Y. Y. (2009).

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9. Research advances for application in truffle cultivation

9.1 How are *Tuber melanosporum* truffles formed?

In the past 10 years studies dedicated to understanding the life cycle of ***Tuber melanosporum*** have provided a new level of insight into how truffles develop belowground and reproduce. The worldwide interest in cultivation of black truffles has provided an important impetus for understanding truffle ecology, and the availability of advanced molecular tools has revolutionized the way scientists have been able to approach many questions (Kües and Martin 2011). Here we present some of the most outstanding findings that have implications for truffle cultivation, conservation of natural truffle habitats and promotion of quality truffles.

One of the most important questions is related to primordia initiation. In other words: “How are truffles born?” There have been many hypotheses regarding how a new truffle (fruit-body) is formed. For many years it was believed that truffles were self-fertilizing fungi (as opposed to outcrossing fungi), but it has been clearly demonstrated that ***T. melanosporum*** as well as ***T. magnatum*** are heterothallic. Heterothallic organisms have sexes that reside in different individuals. The truffle is the fruit of the fusion of two individual fungal hyphae which have complementary mating type genes, MAT1 -1 and MAT1-2 (Paolocci et al. 2006; Riccioni et al. 2008; Martin et al. 2010; Rubini et al. 2011a).

A mature black truffle carries millions of spores tightly enclosed in asci. Each spore possesses only one of the 2 mating types, although within a single ascus both mating types will be present (**Figure 9a**). The ectomycorrhizae (the symbiotic organ attached to the roots of the host tree, **Figure 9b**) also possess only 1 of the 2 mating types. For fertilization to occur and the formation of new truffles, it is believed that hyphae from the “maternal mycelium”, linked to a living tree through the mycorrhizae, must fuse with ***T. melanosporum*** hyphae, “paternal mycelium”, of the opposite mating type which must also be present in the soil (Rubini et al. 2014). The mystery that still challenges scientists is the source of the “paternal mycelium”. It may come from several possible, but not clearly demonstrated, sources: mycelium from ectomycorrhizae of a neighboring tree, germinating spores (**Figure 9c**) of truffles that have remained in the soil, spores dispersed through the activities of animals and insects which feed on truffles, and possibly a free-living ***T. melanosporum*** spermatocyst (Healy et al. 2013; Selosse et al. 2013).

Figure 9a: Tuber melanosporum ascus encasing 4 spiny-ornamented spores.

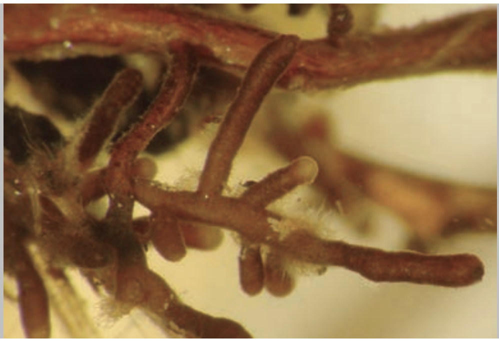

Figure 9b: Tuber melanosporum ectomycorrhizae.

Figure 9c: Germinating spore of Tuber melanosporum.

This recognition that both mating type genes must be present for the initiation of truffles has prompted field trials to examine the distribution of mating types found in ectomycorrhizae and in the mycelia from natural truffle beds, and in productive and non-productive truffle sites (Rubini et al. 2011b; Zampieri et al. 2012). It appears that there is a tendency for the ***T. melanosporum*** mycorrhizae of a given tree and neighboring trees within a truffle site to be dominated by a single mating type and a similar genotype (Murat et al. 2013), thereby requiring genetic input from beyond the mycorrhizae present in the host tree to provide the opposite mating type. Both mating types have been observed on a single tree (Linde and Selmes 2012), suggesting that this may be an important but not the only limiting factor to successful truffle production.

This research on mating types underscores the importance of the dispersion of spores by animals and insects as has been described in the biology of truffles (Trappe and Claridge 2010). The natural habitat for truffles includes natural perturbations of the soils and interactions with other organisms. Through careful and deliberate site management involving soil cultivation and the possible introduction of spores, the initiation of new truffles can be promoted in truffle orchards.

9.2 ***Tuber melanosporum*** **genome studies confirm its symbiotic lifestyle**.

In 2010 the results of sequencing the entire genome of ***T. melanosporum*** were published representing a major accomplishment in research on fungal genetics through the collaborative effort of many scientists (Martin et al. 2010). This work was critical to the identification of the mating-type genes and has provided new information on how symbiotic ectomycorrhizal fungi interact with their host plants. The work reveals the relevance of the evolution of mutualism between plants and fungi over millions of years. Although many truffle handbooks describe a “saprotrophic phase” for black truffles, the latest research indicates that ***T. melanosporum*** requires a living host tree for most of its carbon to complete its life cycle, confirming that this fungus is clearly a symbiotic organism (Zeller et al. 2008). Although the developing truffle has limited capabilities to absorb some nutrients and water from the soil, the black truffle does not have the genetic make-up to live as a saprotrophic fungus.

With the use of carbon isotope labelling (^{13}CO), Le Tacon et al. (2013) addressed the transfer of carbon from a host hazelnut tree to the developing black truffle mycorrhizae and fruit-bodies belowground. They demonstrated that black truffles rely on carbon from the host tree throughout their entire development and maturation. The carbon detected in the truffles had been assimilated through photosynthesis during the tree’s growing season.

These results highlight the importance of a healthy host tree with good photosynthetic capacities and carbon storage for the production of truffles. Additionally the connectivity of the truffle belowground to the mycorrhizae attached to the roots of the host tree is crucial for the transfer of carbon through maturation. Truffle cultivation activities such as weed control and hoeing that disturb the roots and soil should be planned in accordance with protecting this connectivity.

9.3 **Proliferation and habitat dominance are characteristic of well-established *Tuber melanosporum* as observed in the truffle burn.**

Another area of research has examined the truffle burn. This phenomenon of phytotoxic activity results in the development of bald spots where grasses and other plants are reduced or eliminated by the activity of the black truffle mycelia. The burn provides the first visible signal that the mycelia of ***T. melanosporum*** is proliferating belowground. By studying the DNA of fungi taken from soil within and outside the burn area, scientists have been able to observe the growth pattern of ***T. melanosporum***, and to identify the diversity of fungi in this zone. Findings indicate that this fungus tends to dominate the fungal community, reducing overall fungal diversity (Napoli et al. 2010; Belfiori et al. 2012) and reaching its maximum mycelia quantities within 5-7 years after establishment (Liu et al. 2014).

These findings highlight the competitive strength of this fungus once it becomes successfully established. Therefore, when planning for a truffle site, the establishment phase is extremely important. Providing conditions that are optimal for the successful colonization and expansion of ***T. melanosporum*** can have significant long-term consequences.

9.4 Truffle Aromas and Volatile Organic Compounds are important for belowground signaling and for truffle culinary quality.

The recent research on the aromatic compounds produced by truffles has opened a very exciting vision of how this fungus interacts belowground with the many other organisms in its community. More than 200 volatile organic compounds (VOC) have been identified which truffles produce over the course of their lifecycle (Culleré et al. 2010; Splivallo et al. 2011). It has been hypothesized that the VOCs act as chemical signals to interact with plants, bacteria, and other fungi and to attract insects and animals involved in spore dispersal. These aromas vary among different truffle species, and according to the life stage of the fungus and even across different genotypes of the same species (Splivallo et al. 2012).

The aromas in maturing ***T. melanosporum*** truffles change with age and temperature, making it crucial to monitor these factors for the control of culinary quality. The quality and value of fresh truffles depends very much on the aromas. Identification and conservation of desirable aromas can be an important aspect of truffle cultivation and marketing. For example, with the use of gas chromatography-olfactometry, ***T. indicum*** can be distinguished from ***T. melanosporum*** based on the aromas produced (Culleré et al. 2013) as a means of preventing fraud in black truffle marketing.

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10. Truffle Products

The truffle is a perishable and seasonal product. The harvesting season for black truffles, ***T. melanosporum***, is from mid-November to mid-March in the northern hemisphere, but it varies with regions, and the truffle maturation is staggered over the winter season. Truffle production is variable over the 3-month season and quantities and qualities depend on weather conditions of the entire previous year.

Truffles are sold fresh, canned or frozen. In addition, truffles serve as important ingredients in more elaborate products such as soups, pâtés and sauces.

10.1 Whole fresh truffles:

This is the product with the greatest economic value. For this product there are different prices depending on the category. Categories are based on characteristics of size, aromas and appearance. For example, truffles from 5 to 15-gram size or truffles with knobby shapes may be sold at a lower price to a truffle products manufacturing company, while the larger, intact and more uniformly shaped quality fresh truffles will be sold to restaurants at higher prices.

10.2 Truffle products:

Products made with black truffles are marketed worldwide in preparations such as extra virgin olive oil with truffle, rice with black truffles, truffle foie gras and pâtés, cheeses and truffle liqueurs.

Canned

Until recently this was the only way to preserve truffles. Canned truffles are prepared in controlled high temperature and pressure autoclaves, and placed in jars with salt-water.

Frozen

Freezing is considered the best process for conserving truffles, when it is carried out in fast freezing tunnels. The drawback is the high cost of the equipment, so it is primarily the large companies, engaged in truffles and other mushrooms year-round, which are able to provide fast frozen preserved truffles.

Products derived from fresh truffles

Juice: There are different qualities of truffle juices, depending on the density (with more or less amounts of salt water for processing); quality and prices depend on the density.

Truffle pieces: These are fresh truffles that have been broken for various reasons such as during the washing process, transport or even at the time of harvest. France allows for sale of fresh ***T. melanosporum*** and ***T. brumale***, whole and in pieces. The advantage (compared to peelings) is that you can see what you are buying. With regard to taste and aroma, pieces should not differ from whole fresh truffles. The drawback compared to whole truffles is their appearance.

<u>Truffle peelings or trim less than 15 grams:</u> These are from truffles that have had unacceptable or rotten pieces removed due to insect damage, freezing, immaturity and/or small pieces that have broken-off in the process cleaning. This is the least expensive of truffle products and is used as a preserved product rather than served fresh. It is used directly in cooking, or in preparing sauces, cheeses and sausage made with truffles. One problem is traceability and possibilities of being mixed with lower quality truffles.

The species most commonly used in Spain for conservation are ***T. melanosporum, T. aestivum*** and ***T. indicum.***

11. The Developing Truffle Sector in Turkey

11.1 Truffle species in Turkey

Turkey is known to have a rich botanical flora due to its unique location that encompasses 3 distinct phyto-geographical regions within its borders: Euro-Siberian, Mediterranean, and Irano-Turanian. Turkey has a wide range of arid to wet lowlands with expansive forested mountains and alpine zones, contributing to highly diverse forest types and habitats and a wealth of plant and fungal species (Castellano and Türkoğlu 2012).

Mycological studies in Turkey can be found as early as 1915 with more than 2000 taxa recorded (Sesli and Denchev 2009), including 26 truffle or truffle-like fungi. Many of these species are appreciated for their gastronomic qualities, but a comprehensive list of truffles that are consumed in different regions of Turkey is not available. According to the ***Truffle Promotion and Research Association*** from the Mugla region of Turkey (Trüf Mantarı Tanıtım ve Araştırma Derneği), the truffle species that have been reported from the southwest region of Turkey are the following:

Tuber aestivum Vitt.
Tuber borchii Vitt.***(= Tuber albidum*** Pico***)***
Tuber brumale Vitt.
Tuber magnatum Pico**.**
Tuber melanosporum Vitt**.**
Tuber mesentericum Vitt**.**
Tuber rufum Pico.
Tuber uncinatum Chat**.**

11.2 Collecting and cultivating *Tuber aestivum* (syn. *Tuber uncinatum*) in southwest Turkey

The most important culinary truffle from southwest Turkey currently is ***T. aestivum***, which is collected from native oak and mixed oak- pine forests (***Quercus ithaburensis*** subsp. ***macrolepis***, ***Q. robur***, ***Q. infectoria***, ***Q. coccifera***, ***Pinus nigra***, ***P. brutia***) from May through December. Truffles are collected with trained dogs and the truffles with gastronomic quality are sold primarily to local restaurants.

T. aestivum is the most widely distributed culinary truffle collected in Europe, with a high genetic diversity from natural woodlands (Molinier et al. 2016). This truffle is known to thrive in both Mediterranean and continental climates, with a geographic range from Morocco to Sweden and from Portugal to Russia. Its altitudinal range may vary from 50 m to 1600 m asl (Stobbe et al. 2013). This truffle may fruit continuously under adequate moisture and temperature conditions, and there are reports of ***T. aestivum*** production throughout the entire year from Germany (Stobbe et al. 2012). However, mature ***T. aestivum*** ascocarps can be limited by summer drought in its Mediterranean environments and by winter frost in areas of higher elevation and more northerly latitudes (Le Tacon 2016).

T. aestivum (Summer truffle) and ***T. uncinatum***, (Burgundy truffle) were once considered separate species based on their ecological and morphological traits, with ***T. aestivum***

(with a pale white interior) maturing in the summer, and ***T. uncinatum*** (with a beige to brown interior) maturing in the autumn. Both are quality culinary truffles with international markets. Today they are considered to belong to a single monophyletic group (Wedén et al.2005; Molinier et al. 2013), with many subpopulations and ecotypes. Important differences among subpopulations have been identified with respect to aroma profiles (Splivallo et al. 2012) and adaptations to particular environmental conditions (Wedén et al. 2004). Selecting truffle inoculum from a successful ecotype could be an ecologically sound approach for truffle cultivation within a given climatic region.

11.3 Opportunities for increasing the commercial production of naturally occurring *T. aestivum* in southwest Turkey

Mapping the exiting habitat

The extent of the potential habitat for summer truffles in southwest Turkey has not been thoroughly explored. A well-planned mapping of existing natural ***T. aestivum*** sites could provide valuable ecological and economic benefits for the developing truffle sector. By employing trained truffle dogs and expert taxonomists the areas where ***T. aestivum*** grows naturally could be identified. This mapping would enable the truffle sector to estimate the potential areas for further exploration, management and protection.

An initial step is the identification of habitat characteristics, similar to those defined in Section 1 of this handbook for ***T. melanosporum***, with parameters for geography, geology, soil texture, soil structure, pH, climate factors including annual rainfall, patterns of rainfall, and annual temperature ranges. Additional research from Stobbe et al. (2013), and Chapters 3, 10 and 13 from "***True Truffle (Tuber*** spp.) ***in the World***" (Zambonelli, Iotti, Murat, eds. 2016) and Angelini et al. (2016) can provide documented habitat information, and provide ascocarp characteristics of this truffle and others of the genus ***Tuber.***

Identification of host tree species

Another important task is the identification of the different species of trees that host the mycorrhizal symbiosis with ***T. aestivum*** in the most productive natural truffle sites. A wide range of hosts has been observed including species from the genera ***Quercus, Fagus, Tilia, Pinus, Ostrya*** and ***Corylus*** (Stobbe et al. 2013), but these vary throughout the natural range of ***T. aestivum***. The most favorable tree species will be those whose habitat requirements best match the region. It is possible that different tree species may support different subpopulations of this truffle, which could be useful information for selection of inoculum for truffle cultivation projects.

Management and protection of natural habitat

When areas with good natural truffle production are located, management activities may be indicated to help reduce effects of summer drought that often limit both quality and quantity of mature truffles. These could include mulching, reduction of tree density, tree pruning, soil cultivation to improve soil moisture retention, and elimination of herbaceous competition.

It is important to protect natural truffle areas from disturbance or development projects that would disrupt the life cycle of the truffle. Proper timing and methods for collection can be employed to optimize the aromatic qualities of the truffles and contribute to a more desirable final product.

11.4 Opportunities for cultivation of *T. aestivum* in southwest Turkey

Truffle cultivation in planned orchards: Establishment phase

Successful cultivation of truffles in orchards today is based on the introduction of seedlings colonized with the desired ***Tuber*** species. During the establishment phase the orchard sites are selected to correspond with the ecological parameters and soil conditions described for the desired truffle. Necessary pre-plantation activities such as sub-soiling, liming, installation of irrigation systems and seedling selection must be undertaken. Successful truffle collection of ***T. aestivum*** from orchards using inoculated seedlings has been reported (Salerni et al. 2014; Benucci et al. 2011), although there is limited information on management and production of summer or burgundy truffles in established orchards (Stobbe et al. 2013).

Quality seedlings inoculated with T. aestivum

A critical first step in the orchard establishment is selection of quality seedlings with good colonization levels by mycorrhizae of ***T. aestivum*** throughout the tree root system. When purchasing the truffle seedlings, untrained farmers cannot visualize or inspect the root systems for the presence of the truffle because microscopic and molecular tools are necessary to confirm the identity of the mycorrhizae present. Programs and protocols to ensure that inoculated seedlings meet standards for truffle cultivation have been developed to promote successful establishment of new orchards (Bencivenga et al. 1987; Donnini et al. 2014; Fischer and Colinas 1996, 2014).

Both seedling quality (healthy root structure, plant vigor) and mycorrhizal status are important factors that contribute to successful orchard establishment. Seedlings with root deformities such a “J-root” or seedlings lacking in truffle mycorrhizae can lead to orchard failure and loss of important investments in time and in economic resources. To meet the quality standards for truffle seedlings, plants should be evaluated by independent experts.

Presently there are four nurseries that specialize in the production of seedlings inoculated with ***T. aestivum*** for truffle cultivation in Southwest Turkey:

- 1 private nursery: Demirsoy Tarım Hayvancılık LTD ŞTİ **(Figure 11a)**.
- 1 university nursery: Muğla Sıtkı Koçman University Truffle Application and Research Centre.
- 2 General Directorate of Forestry Regional Directorates:
  Denizli Regional Directorate of Forestry Nursery.
  Muğla Regional Directorate of Forestry Nursery.

Figure 11a: *Nursery production of Quercus robur seedlings inoculated with Tuber aestivum*

Truffle cultivation in planned orchards: Management phase

The technology of growing truffles is a relatively new endeavor compared to crops such as corn and wheat or almonds and olives. Crops that grow aboveground can be seen and monitored. But with truffles, farmers must learn to care for the trees that support a crop that cannot be directly observed. Therefore guidance from experts, monitoring programs and well-designed studies are essential when applying new technologies.

Truffle cultivation is a long-term investment and can provide significant ecological and economic benefits to rural communities (Samils et al. 2008). These benefits cannot be realized until there is commercial production, which begins 8-10 years after establishment. With the many experimental sites and studies dedicated to cultivating ***T. melanosporum*** there are three outstanding management factors that have been critical to short term and long term success: weed management, soil management and water management.

Orchard management principles will vary over the lifespan of the orchard and vary with respect to the site conditions and host tree selected for the truffle orchard. For example, timing and quantities for irrigation will depend on the weather conditions, the soil characteristics and on the stage of the orchard: newly established, pre-production or full production stage. Successful truffle production requires an understanding of these principles at each stage of the process.

An agricultural extension service or specialized agroforestry program to help local farmers and truffle growers is extremely valuable for this new crop that grows belowground. This specialized service could help anticipate needs and challenges for particular sites and help address on-going problems. Agricultural extension experts in truffle cultivation could significantly advance the truffle-growing sector, and prevent losses on investments related to errors from lack of understanding of the biology of truffles.

11.5 Recommendations: Truffle cultivation for long-term success

Ultimately the success of a truffle sector is the production and marketing of quality truffles that provide economic benefits to individual producers and their communities. This involves marketing of fresh and preserved truffle products and an understanding of local, regional, national and international markets. It may also extend to ecotourism and gastronomic collaborations.

Developing a new agroforestry sector requires collaboration among all stakeholders involved: foresters, land management planners, seedling nurseries, research institutions and regional development leaders. Timely economic assistance, technical assistance, and marketing strategies can promote the success of this new sector.

Here we provide recommendations for the next steps in promoting the production and marketing of quality truffles in Southwest Turkey. These recommendations are based on the Diagnosis of the truffle sectors in Spain and Southwest Turkey and the interactive workshops held in Fethiye, Turkey and in Solsona Spain during the TRUMAP program Actions: A.1, A.2, and A.4:

1. Develop a program to certify seedling quality and mycorrhizal status provided by trained technicians to ensure that new truffle orchards are established with quality seedlings.
2. Educate a team of truffle experts who can provide technical support and supervise research sites to respond to the technical questions from truffle growers and collectors.
3. Create a program of economic support available to truffle growers who complete a basic course on truffle cultivation.
4. Develop a map of aptitude for ***T. aestivum*** and ***T. melanosporum*** in the southwest region of Turkey to define the ideal habitats and limitations for cultivation of these species.

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12. . Stakeholders in the Truffle Sector of Southwest Turkey

- Demirsoy Tarım Hayvancılık LTD ŞTİ
- Yaşar GÜNEY
- Tuncer TUNÇBİLEK
- Set LTD ŞTİ
- Fatih SAYGIN
- Hasan Hüseyin BOZKURT
- Ziyafet ARSLAN
- Gürol GEZER
- Denizli Regional Directorate of Forestry (Denizli Orman Bölge Müdürlüğü)
- Truffle Implementation and Research Center (Trüf Uygulama ve Araştırma Merkezi)
- Western Mediterranean Forestry Research Center (Batı Akdeniz Ormancılık Araştırma Enstitüsü)
- General Directorate of Forestry (Orman Genel Müdürlüğü) Kybele Fine Foods A.Ş.
- Sultan Pastaneleri
- Truffles Promotion and Research Association (Trüf Mantarı Tanıtım ve Araştırma Derneği)