Chapter 10
Soils and Techniques for Cultivating *Tuber melanosporum* and *Tuber aestivum* in Europe

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10.1 Introduction

Scientific truffle cultivation began in France in 1973, with the large-scale production and planting of truffle-mycorrhized plants (Chevalier 1983). To optimise this development, J. Grente first proposed a rational method of trufficulture in 1972 outlining the details in his text "Perspectives pour une trufficulture moderne" at the same time as J. Delmas, at INRA in Bordeaux, established the first detailed studies of truffle soils (Grente and Delmas 1972–1974; Delmas 1976). Grente's method was founded on four principles:

1. The choice, and if necessary, the improvement of the *environment*
2. The choice of *tree species* best adapted to the environment at hand
3. The *inoculation* of host trees
4. The *maintenance of environmental conditions* favorable to mycorrhization and then to fruiting

This chapter focuses on the analysis of environmental conditions for truffle cultivation with particular attention to soil characteristics and cultural methods to improve the suitability of soils for truffle production of *Tuber melanosporum* Vittad. (Fig. 10.1a) and *Tuber aestivum* Vittad. (Fig. 10.1b, c). While genetic studies suggest that *T. aestivum* and *T. uncinatum* Chatin are synonyms (with *T. aestivum* having taxonomic priority) (Wedén et al. 2005; Paolocci et al. 2004), they are still marketed as different species. Most researchers consider them to be ecological forms of the same species having different maturation times (autumn for *T. uncinatum*,

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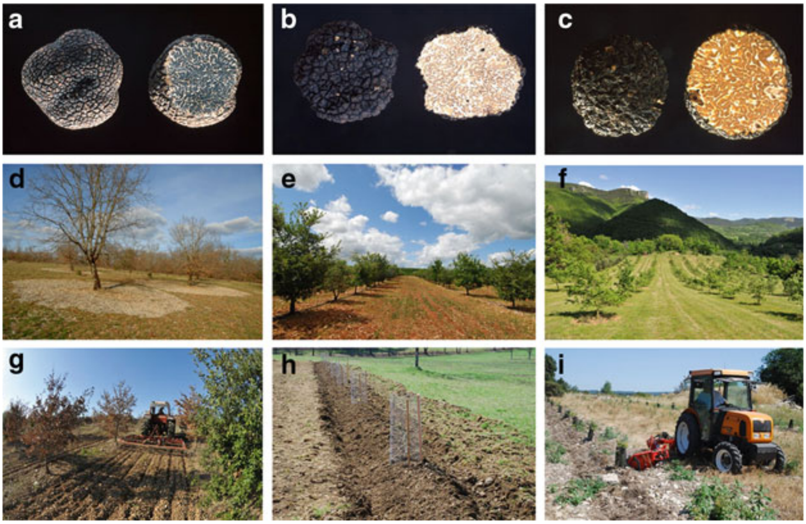

Fig. 10.1 *Tuber melanosporum* ascoma (**a**), *Tuber aestivum* f. *aestivum* ascoma (**b**), *Tuber aestivum* f. *uncinatum* ascoma (**c**), Different methods of *T. melanosporum* cultivation traditional method (**d**), truffle arboriculture (or Pallier) method (**e**), chalk grassland (or lawn ecosystem of Tanguy) method (**f**),classical method of tilling the soil (**g**), young plantation established with the J.A.AD. method (**h**), tilling the soil with the J.A.AD method (**i**)

summer for *T. aestivum*) and ecological preferences (Chevalier 1979; Chevalier et al. 1979, 1994; Chevalier 2010a; Gregori 1991, 2010; Mello et al. 2002). The form of *T. aestivum* marketed as *T. uncinatum* has a greater value in the marketplace. It matures later in the season and is characterised by a darker gleba and smaller peridium warts (Chevalier et al. 1979) and has a stronger and more pleasant aroma (Fig. 10.1b). While there is little interest in cultivating the less valuable summer form of *T. aestivum* (*T. aestivum* f. *aestivum*) (Fig. 10.1c), techniques for cultivating the autumn form of *T. aestivum* (*T. aestivum* f. *uncinatum*) have been specifically developed in Europe. Thus, the ecological requirements and cultivation techniques described in this chapter apply to *T. aestivum* f. *uncinatum* (in the text above it is simply called *T. aestivum*).

10.2 Truffle-Producing Soils

Soil physico-chemical characteristics are a determining factor in truffle production. Even when a plant is well-mycorrhized and the climate is favorable, fruiting cannot take place if certain soil characteristics are inappropriate. Delmas provided a thorough study of truffle soils in the early 1970s, and similar studies were made in Italy in the 1980s by Bencivenga and Granetti, and Lulli and Bragato

(Bencivenga et al. 1990; Bragato et al. 1992, 2001; Lulli et al. 1991, 1992, 1999). Soil conditions favorable to truffle production vary by species, and those favorable for *T. melanosporum* are not necessarily the same as for *T. aestivum*.

10.2.1 Geologic Substrate and Soil Types

10.2.1.1 Sedimentary Bedrock

French soils producing *T. melanosporum* have been characterised by Delmas (Grente and Delmas 1972–1974; Delmas et al. 1981), Callot (1999) and Sourzat (Sourzat 1989–1995–2002, 2008, 2011); those in Italy by Bencivenga et al. (1990), Lulli et al. (1991, 1992, 1995, 1999), Bragato et al. (2001) and Raglione et al. (1992, 2001, 2011); French soils producing *T. aestivum* have been characterised by Le Tacon (1997); those in Italy by Granetti et al. (2005) and Gregori (2010); those in Hungary by Bratek et al. (2010) and those in Sweden by Wedén et al. (2004). The following outline is drawn from these authors.

Delmas (Grente and Delmas 1972–1974) defines precisely the physico-chemical characteristics of French truffle soils. They extend very specifically over the extensive Jurassic, Cretaceous or Tertiary sedimentary rock deposits from the Aquitaine Basin to the Mediterranean zone. In Périgord, most truffle soils are situated on limestone of the middle or upper Jurassic period that contains bands of hard limestone. On the other hand, in Provence the banding is more varied, and in this very rugged region, the peculiar features of the climate, there is no neat relationship between the type or layer of limestone and productivity.

Truffle soils are often not very deep, and, at the extreme, truffle cultivation occurs on lithosols, represented mainly by rendzina profiles or brown calcareous soils. Briefly, they may be nearly devoid of limestone, but the complex is saturated with calcium and the pH is neutral. Nevertheless, in these calcic brown soils, the soil structure remains granular or finely polyhedral. Carbonates can lead to very different levels of truffle production depending on their abundance but also depending on their nature (hardness, solubility, particle size, composition). Other soil types can be encountered, but they are always relatively shallow and overlay fissured limestone bedrock. Besides the essential presence of limestone (or calcium), the nature of the substratum (fissuring, position of sediments, thickness and structure) has an important influence on the growth of the host tree and the ensuing mycorrhizal symbiosis.

The principal agronomic features of successful truffle production are good crumb structure, good circulation of water and gases throughout the profile (absence of hydromorphic layers engendering asphyxia), sufficient organic matter content of appropriate quality characterised by a calcic mull in equilibrium with a C/N ratio of approximately 10, sufficient resistance to surface erosion, a balanced texture (clay–silt–sand) and a mineral composition in which no essential element is markedly deficient or in excess.

Callot (1999) confirmed Delmas' work (Grente and Delmas 1972–1974), particularly the varied geologic age of sedimentary formations, and added to the list of truffle-producing regions by including the Devonian limestone of the Eastern Pyrenees to the stoney Quaternary limestone alluvia of the Rhone and Durance River valleys. He also emphasised the importance of the nature of the geologic substrate for the production of truffles and their variability.

In Italy, *T. melanosporum* truffières are essentially situated on sediments dating mainly from the Mesozoic Era and, more specifically, of Jurassic and Cretaceous periods (Mannozzi-Torini 1970; Pacioni 1986). The range of substrata seems more restricted than in France. The soils are calcareous rendzinas rich in clay with a light and friable texture that favors water penetration. They are generally superficial, well aerated and permeable. Their texture is essentially sandy, sandy–silty or sandy–clayey, whereas in central Italy, there are brown soils developed from red clays by decarbonisation of the subjacent limestone.

In contrast to *T. melanosporum*, *T. aestivum* develops in sedimentary terrain of very different geologic age, from the Paleozoic Era to Quaternary and recent alluvia. This diversity of substrata (and the fact that *T. aestivum* is less thermophilic than *T. melanosporum*) explains the vast geographic distribution of this species, from Morocco to Sweden and from Ireland to Azerbaijan (Bagi and Fekete 2010; Chevalier 2010a). In France, *T. aestivum* soils are rendzinas or brown calcareous soils.

In Italy, the celebrated "truffe de Fragno" (*T. aestivum* f. *uncinatum*) develops, in the province of Parma, on terrain derived from sedimentary rock (marly limestone) from the Mesozoic (Cretaceous) and Cenozoic Eras (Palaeocene, Eocene and Oligocene Epochs) called "flyschs".

In Hungary, *T. aestivum* soils belong to several types. More than one-third of those tested soils are brown forest soils with clay illuviations, 25 % are Ramann's brown forest soils, 17 % are meadow alluvial soils, while meadow soils and meadow chernozems each make up almost 10 % of the soils (Bratek et al. 2010).

In Sweden, where the soils date from the Paleozoic Era, the soil sites are characterised by either moraine marlstone or different kinds of gravel and sand mixtures, or a mix of the two; the bedrock can be stratified, predominantly crystalline, partly fine oolitic limestone, reef-shaped limestone, reef limestone, marly limestone, marlstone, sand limestone and lime sandstone.

10.2.1.2 Volcanic and Metamorphic Bedrock

Although the majority of favorable substrata are sedimentary, exceptions exist. Callot (1999) points out the atypical case in which productive truffières develop on acid recarbonated soils derived from crystalline rock, in the eastern Pyrenees. These truffières are, in fact, situated in colluvial zones where calcareous gravel provided the available calcium needed for truffles. An even more unusual example of very productive truffières has been recently identified on granitic sand resulting from the decomposition of alkaline granite in the eastern Pyrenees [Chevalier G, Urban A, Pla T (2009) Truffières à *Tuber melanosporum* atypiques sur granites alcalins dans le Vallespir, Pyrénées-orientales (unpublished)]. This phenomenon raises the question of the role of limestone in the fructification of *T. melanosporum*. In fact, these soils do not contain limestone, but they do contain calcium. This calcium does not come from an exogenous contribution as in the case of the recarbonated truffière soils, but rather from an endogenous contribution resulting from decomposition of the calcic feldspar (anorthite) component of the bedrock.

Callot's work (1999) has confirmed the importance of a highly fractured substrate, which facilitates development of a deep root system, and of the soil's capacity to store water reserves. However, even in a limestone region, not all situations favor truffles. In fact, the permeability of the subsoil is essential. The structure of the subsoil, which determines drainage, conditions the speed at which a truffière enters into production and the intensity of truffle production.

Callot (1999) has also brought out the importance of having a horizon in contact with the bedrock, which demonstrates a high level of fauna-induced porosity. This horizon, called "Sbio", is an excellent indicator of good drainage and of soils favorable to truffles. Soil fauna, a major factor determining the structure and aeration of the soil surrounding the ascomata, regulates the microbial activity in truffière soils (see Chap. 6). Soils with high potential as truffières are always well drained and have high levels of soil fauna activity. The most productive truffières are most often located in well-drained deep soils. Compact clayey subsoils are always unfavorable.

This information acquired during the 1990s jostles traditional dogmas, which state that "productive truffle soils are most often relatively shallow, and, at the limit, truffles can be cultivated on some lithosols" or "most of the best truffières are situated in a textural region that excludes the extremes". Excessively sandy soils can support excellent truffières, if they are sufficiently rich in calcium, just as a very stony site can produce truffles.

The quality of the subsoil is fundamental for truffle production. In truffle cultivation, analysis of the subsoil has often been neglected, in favor of physico-chemical analysis of the surface fine earth. Before installing a truffle plantation, one is well advised to excavate a soil trench (preferably down to the rock) and examine the profile, thus avoiding gross errors in planting on unsuitable terrain.

10.2.2 Physical Characteristics of "Truffle Soils"

A wide range of soil factors interplay which makes it difficult to define the ideal truffle soil. Principle component analysis of soil physico-chemical data has permitted only partial discrimination of productive truffle soils from those that do not produce brûlés or truffles. Productive soils are characterised by simultaneously relatively elevated contents of fine sand and total limestone, associated with relatively small amount of fine silt, nitrogen and organic matter (Delmas et al. 1981). Statistical analysis has, however, permitted Raglione et al. (1992) to significantly distinguish natural sites producing *T. melanosporum* from those producing

T. aestivum and *T. brumale*, which are often located in close proximity. A similar methodology was later applied by Lorenzoni et al. (1995) in studying natural *T. melanosporum*, *T. aestivum* and *T. brumale* sites. Some of the variables studied (pH, carbonates, silt and assimilable manganese) also permitted distinction of *T. melanosporum* soils from those of *T. aestivum* and of *T. brumale*.

The outcome is that analyses made of the surface soil alone are insufficient to judge the truffle-producing capacity of a soil. What is more, it is not useful to analyse all the elements. From the soil physical point of view, evaluation of stoniness and granulometric analysis of the fine soil is indispensable. From the chemical characteristics point of view, the key elements to analyse are pH (water), total limestone, exchangeable calcium, organic matter, and the C/N ratio, realising that the optimum values depend on the truffle species to be cultivated.

10.2.2.1 Soil Structure

The soil structure in *T. melanosporum* truffières is always granular. In central Italy, despite severe soil erosion, which prevents the regular development of horizons, natural truffières have similar morphological characteristics. The structure of the horizons where truffles develop is characterised predominantly by fine to medium granular and coagulated aggregates, rather than fine subangular polyhedric aggregates. The fine earth, even in the presence of abundant skeletal material (over 80 %), is never compact, but is rather well organised, supple and friable. As a result, the soil has good permeability and drainage. In addition, the soil does not develop surface cracks, even in periods of severe drought (Raglione et al. 1992).

The brûlé, which is formed by the truffle mycelium, causes a structural modification of the surface soil. It is well known that a heavy soil outside the brûlé can become light and ashy within the brûlé. The effect of calcium on soil structure is equally well known. This is not the same process as the mineral degradation by the truffle fungus that contributes to the loosening of the soil (Neel et al. 2007).

The soil structure of *T. aestivum* truffières in northeastern France consists of good to excellent aggregation, on account of the nearly constant presence of limestone and of the high organic matter content. The individual aggregates are angular and very distinctive. This excellent structure assures roots and their fungal associates' suitable aeration and compensates for clayey or clayey–silty characteristics of many of these soils. Good soil structure permits water circulation and prevents development of hydromorphic phenomena in the upper soil profile, despite a heavy texture. The constant presence of stones or blocks of limestone and fissures in the bedrock assures deep drainage.

In Italy, in the region of Parma, the structure is comparable with an aggregation moderately developed and fine. The particles are of medium size, polyhedral and angular. The macroporosity is good, comprising abundant very fine pores. Drainage is excellent, on account of the soil structure, which permits water and gas circulation that prevents hydromorphic phenomena developing in the upper profile.

10.2.2.2 Soil Texture

Truffles display a remarkable adaptability because they can fruit in a range of soil types, from sandy to stoney and with varying proportions of clay and silt. Among the most important conditions required by truffles are good aeration and water circulation. The nutrition of the fungus is assured by the mineral macro-elements and organic matter derived from the soil and roots.

The texture of soils producing *T. melanosporum* is extremely variable. Taking into consideration truffières covering nearly all of the French truffle regions, the granulometric analyses made by Delmas et al. (1981) provide the following results: 25–99 % fine soil; 7.2–45.8 % clay; 3.5–53.3 % fine silt; 2.6–36.2 % coarse silt; 4.3–63.2 % fine sand and 0.5–70 % coarse sand.

Sourzat (2008) advises against planting in soils of greater than 40 % clay. Nevertheless, he indicates that in Quercy, France, some very productive *T. melanosporum* truffières occur on soils containing up to 49 % clay, in a shallow stony rendosol. In fact, biological activity that brings about a change in the characteristics of the organic matter in the brûlé can compensate for high clay content. Likewise, significant stoniness can greatly compensate for elevated clay content (37–42 %) in certain truffle-producing soils of the southwest on hard Jurassic limestone. Truffles are capable of developing in stony environments, taking advantage of the little fine earth accumulated between the stones. In fact, the habitat is not really truly unfavorable except for its sensitivity to drought and the small proportion of fine earth with respect to the mass of constantly ventilated substrate (Sourzat 1989, 1995, 2002, 2011). On the other hand, sandy soils (up to 85 %) can support excellent truffières, contrary to the opinion that in situating truffières "marginal textures must be excluded". Silty soils can also produce truffles. The excess silt, in limestone terrain, can be rebalanced with sufficient organic matter content. The importance of stoniness needs to be taken into account in soil analysis, though this is rarely done for practical reasons. Analysis exclusively of the fine earth can lead to avoidance of truffle cultivation in soils with elevated levels of clay, while significant stoniness can compensate for this imperfection (Ricard 2003).

For *T. aestivum*, in France, the soil texture in natural truffières is also extremely variable, from silty–clay to clayey–silt, and more rarely silty, silty–sandy or clayey. Textures are therefore generally "heavy" (up to 52.8 % clay). These heavy textures permit the tree and the *Tuber* mycelium to obtain sufficient water. Very silty textures are rare, probably due to the poor structure induced by this granulation. Sandy textures are nearly absent due to the soil types, generally acidic, that one encounters with this texture, and the poor reserves of available water associated with this texture. In Hungary, more than 65 % of the habitats in the Carpathian Basin have a heavy clay texture, 20 % are clayey soils, while the remainder are either clay loam or loam. In Italy, in the region of Parma, the stoniness of the soil is light to moderate, with very small (2–6 mm) and small (6–20 mm) angular limestone gravel. The texture is variable, ranging from the balanced type ("terre franche") to the "franco-argileux" type. On the contrary, in Sweden, the soils are generally light, even tending to sandy with 10.4–32.6 % clay (averaging 19.3 %), 9.8–64.7 % silt (averaging 25.1 %) and 12.9–79.8 % sand (averaging 55.6 %). In the soil texture triangle, Italian soils are centrally situated between the more clayey French soils and the more sandy Swedish soils. These differences are explained by the diversity of bedrocks between southern Europe and Sweden, the Swedish soils having undergone the effects of the last Ice Age. The same is true for the soils of northern Europe–Denmark, northern Germany, Poland, the Baltic republics and Byelorussia. In comparison with *T. melanosporum*, *T. aestivum* is capable of fruiting in "heavy" soils.

10.2.3 Soil Chemical Characteristics

Soil physical analysis is important to clarify the functionality of the soil in terms of drainage, aeration and biological activity, but this alone is not sufficient to assess a soil for truffle production. In France, reference values for truffière production were defined in the 1970s by Delmas and Poitou at INRA's Mushroom Research Station in Bordeaux. Since then, these values are always taken into consideration by specialised laboratories when assessing the suitability of soil analyses. However, there is a need for the 1970s standards to be updated. The elements classically taken into account are pH (water), richness in total and active limestone (Drouineau Galet method), total organic matter, carbon (generally the method of Anne using sulfochromic oxidation), total nitrogen (Kjeldahl method), exchangeable calcium, potassium and magnesium (extraction by neutral ammonium acetate) and plant available phosphorus (Joret Hébert method). Sometimes extractable trace element concentrations are also taken into account.

10.2.3.1 Organic Matter

Delmas et al. (1981) highlighted the highly variable level of organic matter in French truffières that range from 0.8 % to 8.3 %. Sourzat (2008) also notes organic matter levels of between 4 % and 8 % in the southwest of France (maximum 9.7 %) and 1.5–4 % in the southeast occasionally with as low as 0.54 % in sandy soils. However, generally productive truffle soils contain a low organic matter content in the surface A horizons (Callot 1999). Truffles are therefore capable of fruiting in both soils with very low organic matter content as well as those with elevated levels.

The quality of organic matter in the surface layer of soil is also important. Before truffle production begins, the soil of the brûlé becomes clear of vegetation, and organic matter levels fall. Consequently, truffles are often harvested in soils with little organic matter, with mull humus C/N ratio of approximately 10. The quantity and the nature of the soil organic matter evolve over the course of time. The speed with which organic matter is transformed depends on microbial activity and the soil fauna, in relationship with the temperature, humidity and aeration of the soil habitat. The action of the truffle mycelium, which breaks down the organic material, must not be forgotten (Chevalier 2010b). The brûlé contains less free fresh organic matter (of rapid evolution), than of stable, highly evolved, greatly polymerized structural organic matter. In central Italy, organic matter content varies from 1.13 % to 17.4 % (averaging 4.58 %). A level of 17.4 % is abnormally high. Italian truffle-producing soils are therefore, on average, higher in organic matter than those of southeastern France. Increases in organic matter content, for example, caused by the build-up of debris from grass and weeds can lead to the appearance of non-target ectomycorrhizal species.

In French soils producing *T. aestivum*, levels of organic matter content are generally between 4.4 % and 21.0 %, and occasionally may even go over 21 %. Limestone coats the non-transformed organic matter. This protective layer prevents further breakdown of the organic matter which then accumulates in the profile and gives the calcareous mull its characteristic black color. This high level of organic matter is in part responsible for the excellent structure of these soils. In Hungary, the organic matter is almost always high ranging between 2.76 % and 10.80 % and averaging 6.45% indicating a balanced supply of humus. In Italy, the soils of zones producing *T. aestivum* are characterised by quite high levels of organic matter, both in truffle-producing locations (11–14 %) and in neighbouring nonproducing locations (8–12 %). Nevertheless, organic matter levels can also be low, as in the province of Parma where it is only 1.6 %. In Swedish truffières, organic matter content ranges from 5.96 % to 21.22 %. In summary, soil organic matter levels are elevated in French, Italian and Swedish truffle soils and are higher in *T. aestivum* than in *T. melanosporum* soils.

10.2.3.2 Carbon

In *T. melanosporum* truffières, levels of carbon range from 0.47 % to 5.0 % in France and from 0.55 % to 3.25 % (averaging 1.55 %) in Italy. In *T. aestivum* truffières, carbon levels range from 2.6 % to 12.4 % (averaging 5.6 %) in France and from 3.47 % to 12.33 % in Sweden. Thus, *T. aestivum* appears to be found in soils with higher soil C levels than *T. melanosporum*.

10.2.3.3 Total Nitrogen

In *T. melanosporum* truffières, levels of total nitrogen vary from 0.46 ‰[1] to 5.22 ‰ (averaging 2.37 ‰) in France, compared to 0.80 ‰ to 2.78 ‰ (averaging 1.5 ‰) in Italy (Bencivenga et al. 1990; Bragato et al. 1992, 2001; Lulli et al. 1991, 1992; Lulli and Primavera 1995). In *T. aestivum* truffières in northeastern France, total nitrogen levels range from 2.6 ‰ to 7.6 ‰ and in the region of Parma from 1.1 ‰

[1] % refers to parts per hundred (percent), whereas ‰ refers to parts per thousand.

to 6.1 ‰ (averaging 4.2 ‰). Thus, *T. aestivum* also appears to occur in soils with higher total nitrogen concentrations than *T. melanosporum*.

10.2.3.4 Carbon to Nitrogen Ratio

The C/N ratio provides information on the evolution of the organic matter. In France, the C/N ratio in truffières varies from 8.57 to 13.7. Sourzat (2008) points out the very low C/N ratios (e.g. 5.9) in some soils with very little organic matter but rich in limestone. In such situations, the low level of organic matter is a function of the high level of limestone which stimulates soil microbial activity and a breakdown of the organic matter. Low C/N ratios often correspond to very sandy soils; however, sandy soils can also have higher C/N ratios in areas where ligneous organic matter accumulates. High C/N ratios can also be found in natural truffières located in very stony soils with 48 % to 56 % total limestone where a highly calcareous fine black earth develops between the stones.

In *T. aestivum* truffières of northeastern France, the C/N ratio is generally between 8.9 and 12, indicative of an excellent capacity for mineralization of organic matter and a good availability of mineral nitrogen, principally in nitric form. Nevertheless, C/N can reach much higher levels (up to 20.4), well above the upper limit for *T. melanosporum*. In the region of Parma, C/N varies from 8.2 to 16.9 (averaging 10.8). Swedish soils are similar to those in France with C/N levels as high as 18.2 although the minimum is 9.7 and the average 13.

10.2.3.5 pH

French soils producing *T. melanosporum* are generally alkaline, with pH ranging between 7.7 and 8.35. However, exceptions exist with a pH 7.0 in a truffière in southwestern France on a sandy soil with 0 % total limestone but with 3.8 ‰ exchangeable calcium (CaO) (Sourzat 2008) points to. In Italy, productive truffières are also found on soils with pH as low as 7.05 although normally the pH is up to 8.25 with an average of 8.00 so that in central Italy pH is the most uniform chemical parameter.

For *T. aestivum* in France, due to the constant presence of limestone from the surface down, the pH is always above 7 and ranges between 7.1 and 8 in the A1 horizon as a function of the amount and nature of organic matter. In central Italy, owing to the limestone presence, the pH is likewise elevated (despite the high percentage of organic matter) giving values near neutrality or slightly alkaline. In the region of Parma, values range from 7.1 to 8.1 (averaging 7.5). In Hungary, the pH is generally weakly alkaline or neutral, or possibly mildly acidic (6.7–7.94; averaging 7.17). In Sweden, the pH is quite variable. It can reach as high as 7.9 or as low as 6.8 (averaging 7.5).

10.2.3.6 Total Limestone

In French *T. melanosporum* truffières, the level of total limestone can be highly variable and go from just a trace to as high as 74 % in some soils. Similarly, in central Italy, the level of total limestone can vary from just a trace to 83.9 % averaging 29.6 %. Although the majority of Italian truffle soils are calcareous, some are simply calcic.

Detailed studies of productive and unproductive sites led by Callot (1999) showed that the most productive soils generally have surface A horizons with, in addition to low organic matter content, a clear recarbonation and a strong macro porosity caused by the soil fauna (Callot 1999). Deeper, these soils exhibit a level of unconsolidated calcium carbonate accumulation (K), a horizon of strong faunal macroporosity (Sbio) and a well-drained calcareous subsoil (C) with water storage potential.

Using calcareous gravel on paths and roads can help render adjacent acidic soils suited to truffle production (Callot 1999; Sourzat 2008). Similarly, the incorporation of large amounts of limestone into acidic to very acidic soils has been practiced in the USA (Garland 2001), Australia (Malajczuk and Amaranthus 2008) and New Zealand (Hall et al., 2007). Similar techniques have also been used in France to produce *T. aestivum* and more recently *T. melanosporum* in Limousin (central France) on soils derived from metamorphic bedrock (schist) with initial pH 5.2 [Chevalier 2003; Chevalier G, Urban A, Pla T (2009) Truffières à *Tuber melanosporum* atypiques sur granites alcalins dans le Vallespir, Pyrénées-orientales (unpublished)].

Generally *T. aestivum* soils are less rich in limestone than those of *T. melanosporum*. In France, the majority of natural *T. aestivum* truffières are characterised by the presence of limestone from the surface down. The level of limestone is highly variable and ranges from 0.4 % to 52 %. The amount of total limestone has no effect on the quality of truffière soils. Only its presence at the surface is important. In Italy, the level of calcium carbonate in *T. aestivum* soils is just as variable, although generally it is relatively low and between 0.9 % and 12 %. However, in the region of Parma, it can be as high as 52.9 % with an average of 21.9 %. In Hungary most of the soils contain little (5–8 %) or only traces of lime (0.1–5 %), while in Sweden, total limestone can be from just a trace to 10.5 %.

10.2.3.7 Exchangeable Calcium

In France, reference values for exchangeable calcium are generally from 4 % to 16 ‰. But in the French eastern Pyrenees, values of 2.31 ‰ are recommended on recarbonated schist and 2.13 ‰ on alkaline granite.

In the calcareous truffières of central Spain, truffle development and ecological conditions of brûlés favor the formation of significant quantities of active limestone and exchangeable calcium (Garcia-Montero et al. 2007a, b; Chap. 6).

The quantity of active limestone is significantly higher and that of total limestone is lower within the brûlés than outside of the brûlés. But then the activity of *T. melanosporum*, its fructification and the size of the brûlés are simultaneously advantaged by high concentrations of active limestone and exchangeable calcium. This results in a feedback process, which in return favors development of truffle mycelium. This feedback explains why the development of *T. melanosporum* and the production of truffles increase with the size of the brûlé, which is itself a consequence of a successful symbiosis. This feedback loop also provides the truffle with an advantage over its competitors, especially *T. aestivum* and *T. mesentericum*, when the active limestone reaches a high concentration in *T. melanosporum* brûlés. Garcia-Montero (2007a, b; Chap. 6) deduced that the active limestone is an essential factor for fruiting, development of brûlés and the aggressiveness of the black truffle when in competition with other truffle species. These observations are also valid for central Spain, where the soils are very calcareous. However, the occurrence of truffières on crystalline bedrock in France requires a rethink of the roles of total limestone, active limestone and exchangeable calcium in truffle production.

In wild *T. aestivum* truffières of northeastern France, the level of exchangeable calcium ranges from 2.8 ‰ to 7.9 ‰. The absorbent complex is always saturated, mainly with exchangeable calcium, because limestone is always present (Chevalier G, Urban A, Pla T (2009) Truffières à *Tuber melanosporum* atypiques sur granites alcalins dans le Vallespir, Pyrénées-orientales (unpublished)). In Sweden, levels of exchangeable calcium are on average slightly higher at 3.6–10.7 ‰ (averaging 6.7 ‰). It is surprising that the minimum of 2.8 ‰ is a little higher than the value of 2.13 ‰ recently determined for *T. melanosporum* on alkaline granite.

10.2.3.8 Exchangeable Magnesium

In French *T. melanosporum* truffières, exchangeable magnesium levels are from 0.05 ‰ to 0.52 ‰, and although normal levels of exchangeable MgO considered normal is from 0.10 ‰ to 0.30 ‰, truffières on sandy soil can have much lower or higher values (as low as 0.08 ‰ or as high as 0.75 ‰) (Sourzat 2008). Sourzat has concluded that lower or higher than normal levels of magnesium do not seem to affect truffière production. In natural *T. aestivum* truffières of northeastern France, levels of exchangeable magnesium are slightly elevated but sufficient (0.05–0.41 ‰). Italian values from 0.12 ‰ to 0.59 ‰ are close to those in France. In the region of Parma, values vary from 0.07 ‰ to 0.30 ‰. In Sweden, the values are generally good, similar to those in France: 0.09–0.45 ‰ (averaging 0.19 ‰).

10.2.3.9 Exchangeable Potassium

In French *T. melanosporum* truffières, exchangeable potassium content varies from 0.04 ‰ to 0.50 ‰. Levels from 0.1 ‰ to 0.3 ‰ are considered normal. Truffle soils are not deficient in this element. In Italy, values are higher: 0.1–1.17 ‰.

In the wild *T. aestivum* truffières of northeastern France, the levels of exchangeable potassium are medium: 0.25 ‰ to 1.04 ‰ (averaging 0.59 ‰). In Italy (in the region of Parma), they range from 0.089 ‰ to 0.520 ‰ (averaging 0.306). In Hungary the majority of the studied soils have high potassium content (0.139–1.200 ‰; averaging 0.498 ‰).

10.2.3.10 Available Phosphorus

For *T. melanosporum* in France, levels of available phosphorus run from 0.006 ‰ to 0.980 ‰ with upper figure being an abnormal exception. In Italy, levels are between 0.006 ‰ and 0.025 ‰.

In soils producing *T. aestivum*, as is the case with most calcareous soils, phosphorus content is low, between 0.02 ‰ and 0.08 ‰. In Italy (in the region of Parma), values range from 0.001 ‰ to 0.018 ‰ (averaging 0.008 ‰). In Hungary, the phosphate content of the tested soils exhibits great variability, with a very high content in a third of the studied soils (0.013–2.534 ‰; averaging 0.423 ‰). The values in Sweden (0.02–0.12 ‰) are consistent with the French values. Low levels of active phosphorus in soils do not seem to affect truffle production.

10.3 Climate

The climate is also a decisive factor for truffle production that needs to be evaluated according the distribution of its rainfall and temperature variations during the truffle's growth cycle. *T. melanosporum's* climatic requirements can be summarised by relatively damp and warm springs with no late frosts, hot summers punctuated with rainstorms, relatively damp autumns without early frosts and winters without heavy frosts (which might destroy the truffles) and moderate rainfall. The elevation of southwest France's truffle grounds is generally between 100 and 400 m. In the southeast, the Mediterranean climate allows the establishment of truffle grounds above this elevation. In the Hautes-Alpes, natural truffle grounds are present up to 1,500 m on well-oriented sites and at the lower limit of the larch tree-line, while on the Larzac plateau, in the Aveyron, some exist at 800 m on sites with a sunny aspect.

In very favorable climatic conditions, the range of soils that support truffle production is wide and, for example, includes soils with trace levels of limestone or with organic matter imbalances. However, when climatic conditions are marginal, only exceptionally favorable soils permit mycorrhization and fructification.

10.4 Cultivating *Tuber melanosporum* in Europe

Over the past 40 years, considerable progress has been made in the cultivation of truffles with millions of truffle-mycorrhized trees planted in France, Italy and Spain.

10.4.1 Modern Approaches for Truffle Cultivation

10.4.1.1 Three Methods Used in France in the Recent Past

When we speak of truffle cultivation in France, we mean principally growing the Périgord black truffle, *T. melanosporum*. The method was first devised at the start of the nineteenth century when observant country dwellers in the southwest and southeast decided to sow acorns or plant oaks with this in mind. The cultivation of Burgundy truffle, *T. aestivum* f. *uncinatum*, has recently taken off in the northeast of the country, since the eighties. Among other species which are cultivated more or less privately, there is the white summer truffle, *T. aestivum* (*T. aestivum* f. *aestivum*), the winter truffle (*Tuber brumale* Vittad.) and the Bagnoli truffle (*Tuber mesentericum* Vittad.). Numerous truffle species are also found in truffle-producing areas and can cause problems. The species the most feared in truffières are *T. brumale* and *T. aestivum* (*T. aestivum* f. *aestivum*)—even if some growers tend to put them forwards as worthwhile species.

Three techniques for cultivating *Tuber melanosporum* in Europe can be distinguished, each with their variations, which have different results depending on the environment and changing times. Truffle growers do not all agree on the best method to use; and additionally each one introduces his own nuances to his preferred approach according to his soil, the climate and, above all, whether or not there is an existing family tradition of truffle cultivation.

The main methods are:

1. *Traditional method*: practised before the introduction of mycorrhized plants—whose followers are becoming fewer (Fig. 10.1d)
2. *Arboriculture* (known as the "*Pallier*" *method*): conceived from simple modern principles since the marketing of mycorrhized plants started in 1974 (Fig. 10.1e)
3. *Chalk grassland* or "*Tanguy*" *method*: from the 1990s onwards, based on the functioning of natural truffle grounds (Fig. 10.1f)

Other methods of truffle cultivation have been proposed (Rebière 1974; Fioc 1987; Ricard 2003); however, here we focus on the three main methods of truffle cultivation stated above.

10.4.1.2 Choosing a Modern Truffle Cultivation Method

The results obtained in France and Europe—and above all in countries where there are no species of truffle naturally present in the environment—lead us to consider the justification for using each method, in particular those of truffle arboriculture and chalk grassland. In Australia, where *T. brumale* as well as all other species of *Tuber* other than *melanosporum* are absent, the common hazelnut seems to maintain a perpetual liaison with *T. melanosporum*, for example, in Manjimup, Western Australia. In France, the same hazelnut tree has a strong affinity with *T. brumale*, which contaminates it in numerous regions despite a high level of mycorrhization of plants checked by the INRA and the CTIFL (Centre Technique Interprofessionnel des Fruits et Légumes—Inter-professional Technical Centre for Fruits and Vegetables). On the other hand, the Downy oak (*Quercus pubescens* Willd) and Green oak (*Quercus ilex* L.) are species perfectly adapted to *T. melanosporum* as there is strong affinity between these species. This relationship is confirmed in Australia and New Zealand.

The parallel between the "Tanguy" method of truffle cultivation in chalk grassland and the workings of natural truffle grounds in southern France is easy to demonstrate. Natural truffle grounds develop in open spaces or clearings, especially in environments resulting from the neglect of cultivated land, when these spaces become wasteland reach 10–15 years of age. Such natural truffle grounds correspond to a particular state or type of vegetation (Mesobromium, Xerobromium, etc.) during the evolution of the wasteland towards its final form (woodland) in calcareous zones. They display the characteristics of biodiversity that we attempt to develop in the most efficient grassy plantations.

Truffle arboriculture appears to give noteworthy results in the absence of pressure due to contamination; whereas truffle cultivation in chalk grassland allows us to limit such contamination. Everything seems to show that in France, the choice of a method should be governed by the principle of precaution. It is a question of choosing the route which is likely to present the fewest risks, even if one can assume that these risks are very low in certain open or lightly wooded landscapes.

Precautionary technical procedures, faced with contamination by mycorrhizal fungi present in the environment, include three stages:

Stage 1: Assure the best possible re-establishment of the plant mycorrhized with *T. melanosporum* during the first year following planting, and even the second

Stage 2: Discourage the growth of the mycorrhized plant, in order to avoid contamination by various fungi: creation of the natural environment preferred by the truffle is sought after during the formation of burnt areas

Stage 3: Improve the quantity and quality of the production while maintaining its durability, once truffle fructification has been triggered

It is possible to foresee two further stages:

Stage 4: Renovate the old plantation so as to give a second life to the production by opening up the environment

Stage 5: Rip up the old truffle wood to provide a fresh base to the truffle plantation

The method of cultivation in three stages was defined in the "Guide Pratique de Trufficulture" (Sourzat 1989–1995–2002, 2011). In this 3rd edition (2002) of the "Guide Pratique de Trufficulture", the three steps were summarised by "Planter" (Planting for the 1st step), "Entretenir" (Maintaining for the 2nd step) and "Produire" (Producing for the 3rd step). This truffle cultivation method was perfected for European countries where there are climate and soil conditions favorable for truffle growth but where there are often risks of other EM fungi contaminations. However, this method can also be adapted to other conditions.

The new methods J.A.AD J (Jeune = young in French), A (Adolescent = immature in French), AD (Adulte = adult in French) (Dessolas et al. 2007–2008, Pargney et al. 2011) and M.R.T. (the reasoned methods of trufficulture) (Chevalier 2010b) comprise also three equivalent steps (Fig. 10.1h, i). All these methods are described below.

10.4.2 Establishing a Truffle Plantation (First Step)

The objective is a perfect planting of mycorrhized trees by the end of the second year of vegetation so that the trees are then able to withstand the demands of the truffle ecosystem and of the natural conditions preferred by the truffle.

10.4.2.1 Terrain and Soil

In order to restrict the damage caused by a prolonged dry period, the chosen site should ideally have easy accessibility and preferably proximity to an existing (or created) water source to enable better water management while the trees are getting established and during the production period. The site should not be on too steep a slope or without services or access for modern machinery. Cold aspects (north, northeast, northwest) in an enclosed valley are not suitable because of the possibility of immature truffles freezing in November and December. Whenever possible, the rows of trees should be aligned north–south in order to allow the sun to warm up the soil in the afternoons during the cold winter period.

Soil must be limestone with a water pH of around 8 and have physico-chemical characteristics suited to truffle production. These characteristics should be ascertained from the start by means of a complete soil analysis, if there are no other indications as to the potential of local truffle production. Lime can be added if the content of this element is low. When soil has water pH neutral or acid, it is necessary to add lime in order to increase pH at a good level (at less 7.8), which is the case in many new countries that are cultivating black truffles, including Australia, New Zealand, USA, Chile and Argentina. Doses can be varied from 10 to 100 tonnes per ha. Soil analysis should be carried out from the start with a sample of at least 500–800 g for a full analysis. Several samples of soil should be taken from different locations at depths of between 5 and 10 cm. These extractions should be mixed up together in a bucket in order to get a representative sample. If the soil on the site is homogenous, a single sample can be sent to the laboratory for analysis. Subsoil must be permeable and allow run off of excess water. If the drainage characteristics of the subsoil are unsure, a trench can be dug with a mechanical digger and analysed by a skilled technician. When there is superficial topsoil on broken bedrock, it is not recommended to make a passage of a ripper. The use of it can transform the land into a field of big stones. In this case, a stone crusher will have to be used as far as possible before launching into tree planting.

The plantation should avoid sites of recent oak deforestation without at least 2 or 3 years of wheat or barley cultivation in between, during which time all the roots of the previous trees are removed. The purpose of carrying out this work is to limit the problem of contamination from other mycorrhizal fungi species present on the roots of the felled oaks. If an oak hedge or woodland is situated around the edge of the planting site, a gap of at least 8 m should be left between their border and the first planting. Big trees, which are already mycorrhized by other kind of fungi (especially basidiomycetes), can contaminate the young plants infected with *T. melanosporum*. Creating a trench between the bordering trees and the first row of newly planted oaks will protect the small ones from contamination of the big trees via belowground roots. Size of the field can be taken in consideration in wooded environment with oaks. Big fields (at least 1 ha) are preferred over smaller fields because they offer a larger buffer against contaminating fungi.

10.4.2.2 Preparation of the Terrain

If planting is to take place on recently cultivated land, the soil should be worked in the autumn then prepared with the aid of a plough or *vibroculteur* in the same way as for planting a fruit orchard. Once the planted area is again covered with grass or grazing, only the plant rows or even just the immediate area surrounding each tree, should be carefully worked by hand (with a fork or hoe), motorised plough or weed cutter. Working within a defined space helps to preserve the ecosystem of the limestone orchard, which is a very favorable environment for the formation of natural truffières and truffle plantations. The size of the hole to be dug should be in the order of 20–30 cm in depth and 50–80 cm in diameter. Its size depends on the suitability of the topsoil (fine soil). If the soil is loose, a hole 50–60 cm in diameter is sufficient; if it is very stony and shallow, it can be done with a mini digger and can be 60 cm–1 m in diameter. The big stones will have to be removed and the topsoil replaced around the plant. The best time to dig the hole for planting is before winter, in order to benefit from the effect of the frost on the soil, especially if plantation is done on the spring. Cut the grass very short with a weed cutter before digging the hole when the plantation is in grass, fallow land, sheep pasture or juniper. It is not necessary to remove all small shrubs (juniper, wild roses, sloe, brambles). If there are not too many, it is enough to reduce their size by appropriate pruning. Do not leave a large oak tree in the middle of the plantation in the hope that it will become truffle producing. To keep one beautiful oak tree, it is necessary to do a sanitary perimeter (8–10 m) around it in order to prevent contamination.

10.4.2.3 Tree Spacing

The trees should be planted in straight lines 4–6 m apart; the rows should be 6 or 8 m apart (400–200 plants per hectare). Spacing of 4 m on the row allows for more easy irrigation. The spacing can be decided upon according to the nature of the soil (deep topsoil: wide spacing) or according to availability of irrigation (trees with rapid growth: wider spacing more important). For example, in the case of deep topsoil with irrigation, one could plant at 6 m $\times$ 10 m (166 plants per hectare). Dense planting demands important interventions which take time and a commitment to pruning, trimming and thinning the trees. The goal of these interventions is to save *space of conquest* (available space) between the brûlés. Irrigation is necessary because the dryness of the soil is increased as the tree grows.

10.4.2.4 Recommended Tree Species

Young plants should be infected by the black truffle, *T. melanosporum* to the exclusion of all other contaminating species. These infected plants should be able to be monitored beforehand (batch sampling) by official organisations (CTIFL or INRA in France). Downy oaks (*Q. pubescens*) and holm oaks (*Q. ilex*) can be planted in the southwest of France. In the southeast, one can plant pedunculate oaks (*Quercus robur* L.) or kermes oaks (*Quercus coccifera* L.) which are adapted to dry and stony terrain. *Q. pubescens* takes longer to fruit (8–10 years) than *Q. ilex* (5–7 years) but production is generally more sustained. Hazelnut trees (*Corylus avellana* L.) can be planted by way of experiment (not more than 10 % in the plantation) in order to observe the infection potential of the planting area (presence of *T. brumale* spores). The use of *C. avellana* is always a delicate thing, although interesting results can often be observed in some parts of France and in the countries where it is not native. Root system of this species grows faster than those of *Q. pubescens* and *Q. ilex*, and may be contaminated easily. The different species can be positioned in separate rows or intermixed within the row.

10.4.2.5 Staking

In many cases trees need to be staked when planted or to help support their growth. Choose a staking method suited to protection of the plant (netting or shrub shelter). For maximum sun between rows in winter, staking along the north–south axis is preferable. Avoid staking in the direction of the slope in the case of erosion prone land.

10.4.2.6 Planting

Planting should take place in winter. *Q. ilex* is preferably planted at the end of the winter so as not to run the risk of frost damage to the young trees coming out of incubation (greenhouse). Plant in a well-drained soil, not too dry and not too sticky. Remove the plants from their packaging as soon as they are received. Place them upright in netting or plastic buckets. Take care to store the plants where they will not be liable to freezing, drying out or blanching (from lack of light).

Ensure that the soil at the bottom of the container or root ball is not too dry. If the surrounding soil is dry and powdery, immerse it in a bucket of water for one to two minutes until the bubbles stop rising to the surface (soaking the plant). After digging out the hole, open or unclip the container and delicately position the root ball in the hole and cover it with fine soil. Avoid large stones or soil containing grass or large roots coming into contact with the root ball. Do not allow the root ball to touch the wooden stake (leave 5 cm between the root ball and the stake). Fill in the hole and pack down the soil around the plant with the toe of shoe or boot. Put two or three centimetres of soil above ground level. Protect the tree with wire netting or possibly a shrub-shelter (Tubex). The use of a 60 cm high shrub-shelter (Tubex) allows mounding of earth around it which is preferable to mulching. The soil forming a mound up against the tube of the shrub-shelter increases the water retention of the soil and aids maintenance. What is more, the mound of soil, which will naturally dry out towards the top, encourages the development of the *brûlé*. When the planting is carried out with soil mounding in limestone pasture, the mound must be at least 15–20 cm high to be truly effective against drying out. It is essential that the young plants are watered during the week after planting in order to limit the stress of transplanting.

10.4.2.7 Maintenance of the Young Plantation

It is important that the saplings are protected against rabbits and roe deer by means of wire netting (or shrub-shelter). These animals eat leaves of the truffle trees and delay truffle fruiting. Where wild boars might be present in the area, an electric fence should be installed and checked at least once a fortnight. A chemical weed killer used twice a year under the fenceline increases its effectiveness. Competing weeds that appear during the summer months should be removed. Where there is little grass growth (shallow soil), removal of grass by weedeater is preferable to the use of glyphosate. Chemical foliar weedkillers (glyphosate, gluphosinate) should be used as a last resort and with great caution. Caterpillar of the butterfly bombyx *Lymantria dispar* L. damages the foliage. If need be, treat with an insecticide which does not kill bees. With powdery mildew, which provokes white spots on leaves, use liquid sulphur.

10.4.2.8 Irrigation

In the first 2 years, be sure that the plants do not suffer from water shortage. Watering can be done with containers of water or from a big tank or can be automated with drippers attached to a polyethylene pipe. Watering should commence around mid-May. The most important watering months for oak tree growth are May and June. Watering every 8–10 days is recommended during the hot months of June, July and August. Trees planted using the mounded earth method can be watered directly into the shrub-shelter (Tubex) every 10–12 days.

10.4.3 Maintenance Between Planting and Fruiting (Second Step)

The object is to provide *T. melanosporum* with the optimum conditions for production, particularly once the natural truffle has begun to form. In traditional truffle areas in an oak wooded environment, it appears that the cultivation conditions, similar to those necessary for fruit tree cultivation, present the risk of a drift in the status of the mycorrhization, and of fruiting, towards *T. brumale*. Authors of some of the first manuals on truffle cultivation in France (De Bosredon 1887; Pradel 1914) recommended to "*leave it to nature once the brûlés begin to form*".

10.4.3.1 Soil Maintenance

There are two possible options: to till the soil with manual or mechanic tools or control the growth of the native flora which form the basis of the truffle ecosystem. Both options are recommended even if the second solution seems preferable in the long run, considering the results of scientific experiments and observing the evolution of the environment once the formation of the natural or wild "truffière" has started. The first option can be used without inconvenience when there are no risks of fungi contaminations.

Work the soil as little as possible so as not to encourage the propagation of the competing fungi (*T. brumale* and *T. aestivum*), that is, a very superficial working of the soil (5 cm) once or twice a year with machinery, moving further away from the row of trees as they grow so as not to disturb the progression of the brûlé (Fig. 10.1g). The presence of some hardy plants (brambles, grass, etc.) can be favorable to the truffle in the same way as vine stock or lavender stems. It is not necessary to suppress everything. Clear between the planting rows at less once or twice a year and clear around the trees within the row with a weedeater as often as necessary to restrict the growth of the grass. Do not use chemical weedkillers other than on vigorous plants which are difficult to control. The presence of some competing young plants (to the growth of the tree) is not prejudicial to the truffle if the tree's growth is already well established. A balance has to be found between grass growth and tree growth. The growth of the grass should not compromise the growth of the tree and slow down truffle production if the tree lacks vigour. The ideal would be to work around each tree by hand with a hoe, a practice that may seem ill suited to modern agricultural concepts. Manual working of the soil around the surface of the brûlé will not upset the equilibrium between *T. melanosporum* and its companion fungi. In deep soil, mechanical tilling is less dangerous than in shallow one as there can appear fungi equilibrium under the tilled layer.

10.4.3.2 Irrigation

In wooded oak environments, do not irrigate unless there has been no rain for 3–4 weeks. It is important to control the growth of the trees as their lateral roots are susceptible to contamination. If the trees have taken well in the first year or the first 2 years, the truffle trees will easily tolerate a long period without water (one month and plus). In open areas without oak trees frequent irrigation can be done: trees grow faster and can produce earlier with the risk of a shorter duration of truffle production.

10.4.3.3 Pruning

Prune lightly to achieve a spread out shape or stake upright if the tree has a creeping or rambling tendency. Do not cut a whole stem from the base of the tree. Go rather for gradual pinching, that is, cutting one stem or branch on several different occasions. A tree gains nourishment from the leaves as much as from the roots.

10.4.3.4 Protection Methods

Look out for powdery mildew on *Q. pubescens* and treat with liquid sulphur. Treat for leaf-eating caterpillars (*L. dispar*) in May and June with ecological or chemical insecticide and look out for moths (*Acrocercops brongniardella* Fabricius) on the *Q. ilex*. The larvae of *A. brongniardella* unstick the cuticle of leaves and protect the planted area against wild boar with electric fencing.

Remove the shrub-shelters (or Tubex) once the tree is more than 50 or 60 cm high. Install individual tree protection against deer instead of the shrub-shelters, or rabbit proof fencing in addition. To protect against deer, cut 1.5 m of wire netting (Ursus) 95 cm in height to place around the tree (cost of this protection around 1 €). This protection is held in place in the soil with stones or with the aid of one or two metal spikes made from concrete reinforcing iron rods.

10.4.3.5 Fertilisation and Feeding

Once soil analysis has been performed, correct any possible deficiencies. An improvement of crushed lime is advised in soils with low levels of this element (1–2 %). A simple soil analysis (water pH and limestone level) can indicate what is needed.

10.4.4 Improving and Maintaining Truffle Production (Third Step)

Once production has started, the aim is to ensure optimum production for as long as possible.

10.4.4.1 Soil Maintenance

In working the soil, the aim is to obtain larger truffles in soil that is aerated, vital and equipped with good water reserves. With mowing or slashing of the grass, a part of the water reserve of the soil is saved. The soil must be turned at the point when the vegetation of the tree and the growth of the mycelium of *T. melanosporum* begin in the soil with the spring warmth. It used to be done by hand with the aid of a one or multi-pronged implement. It is generally done up to the end of harvesting up to mid-May when the soil is fairly dry, to a depth of 5–8 cm. When the work is done by tractor, the raking implements used are the plough or the *vibroculteur* (sometimes *Actisol* or rotary hoe); some people prefer the disc harrow (*cover crop*) which does not pull out or break the roots. Manual working with a “bigos” (a 3- or 4-pronged hand tool) is preferable on very good producing trees taking care not to cut the roots. If it is carried out without turning the soil, simply lifting it to increase the aeration, the work can be done much later, up to the end of June. When the work is done manually or lightly by machinery, it should be restricted to the area covered by the brûlé.

10.4.4.2 Irrigation

Watering should be carried out according to the life cycle of the truffle given that the most crucial period is in August and that the truffle can withstand a certain amount of dryness (20–25 days on average without climatic heat wave). The month of June is generally the time when the truffle primordia are formed. For good management of the truffle’s water requirements, rain gauges must be used and rainfalls recorded.

Agricultural irrigation systems are recommended. Use of micro-sprinklers at a low rate of flow (40–80 l per hour) and low pressure (1–2 bars) is a practical and economical solution. It requires good filtration at the water source. It is pointless watering non-productive brûlés. With a tank of water, it is possible to irrigate the best brûlés in the truffle plantation limiting the supply to 20 l per square metre, or 20 mm. In the interests of water economy, irrigate only the parts of the brûlé which are most likely to yield truffles. In July, watering can take place every 15–20 days (20–25 mm); in August, every 10–12 days (20–25 mm) and in September, every 15–20 days (20–25 mm). Covering the ground with branches prolongs the time the truffle can withstand the dry period and counteracts a weak or non-existent water supply. The soil type (sandy or clay) and the climactic conditions will determine the moisture content of the soil and the method of irrigation

10.4.4.3 Pruning and Thinning

Pruning of the truffle trees is carried out differently according to the regional conditions. A new approach has been identified in France by the Station Trufficole du Montat. It consists of maintaining *T. melanosporum*-infected tree in a state in which the truffle is stronger than the tree. In this approach, the trees that are not producing truffles are removed to allow more room for the ones that are productive. Pruning should be done gradually on the producing trees. In order to minimise the possibility of failure, it is best to prune the trees in a plantation in stages: a third in the first year and so on each year. Pruning is usually done in spring during the months of March or April. Pruning in August can help to reduce vigorous tree growth.

As truffle trees mature, orchards may need to be thinned. The aim is to keep a *space of conquest* for the brûlé which in principle grows at the rate of 10–25 cm a year in the case of a tree with good *T. melanosporum* production. Where the *space of conquest* is inclined to diminish in size, some trees should be cut down. Such thinning encourages new growth and preserves the environmental conditions favorable to *T. melanosporum*.

10.4.4.4 Complementary Inoculation

Some new experiences in France and Spain confirm old practices for improving production. They consist of bringing truffles under trees that are not yet producing. Truffles harvested at the beginning of the season are preserved in a deep freezer. In the spring, they are crushed and mixed up with vermiculite to create the inoculum. This inoculum is added in a small trench under each tree at the limit of the canopy. Doses of truffle can be between 10 and 30 g per tree. Nevertheless, with smaller dose, good results were observed. Benefits on truffle production were obtained on trees with brûlés where the fructification of the fungus has not already triggered.

10.4.4.5 Fertilisation and Additives

The mode of feeding the developing truffle fruiting body is not well understood. Once the soil is tested, adjust any possible deficiencies. Organic mineral fertilisation to improve truffle production can be experimented. Only treat one part of the brûlé, about a quarter of the whole area at most, so that a negative outcome will not affect the whole. Improving the biological activity of the soil (earthworms and insects) by adding small branches/twigs is good for truffle nutrition. The addition of topsoil on the surface of the productive brûlés can be beneficial.

10.4.4.6 Protection

Treat leaf-eating caterpillars (*L. dispar*) if there is a significant presence. Use insecticide appropriate to the extent of the infestation. Protect the site against wild boar with an electric fence. Put reinforced wire netting (a 2.4×3.6 m sheet with 20 cm squares) on the best brûlés when, despite electric fencing, wild boars are likely to come and root around, attracted by the irrigation during a dry summer.

10.4.4.7 Harvesting and Grading

Harvesting takes place from the end of November up to mid-March and is accomplished with the aid of a trained dog or pig. The truffle fly can be a useful indicator of truffles in Europe. Harvesting should be done in drained soils, which are neither frozen nor too wet.

Harvested truffles are sorted and the surrounding earth is removed. The truffles should be wrapped in a piece of cotton cloth and kept in a cool place until market day. They can be stored in the fridge, taking care to put them in an airtight container. In south of France, in winter, only high quality *T. melanosporum* is sold at the market. Black truffles that are damaged or rotten should be broken up and buried in the soil under the young trees, which are not yet producing or used as inoculum as described.

10.4.5 Improved Techniques for Truffle Cultivation: Deep Cultivation and Severe Limb Pruning with J.A.AD. and M.R.T. Methods

Many authors recognise the interest in cultivating the soil, to decompact it and to aerate it in order to obtain larger truffles in a living environment provided with an ample reserve of water. Although earlier techniques recommend shallow cultivation, so as not to damage the root system, recent methods of truffle cultivation recommend much deeper cultivation (15–20 cm) of the soil. These include the J.A.AD. (Dessolas et al. 2007–2008; Pargney et al. 2011) and M.R.T. (Chevalier 2010b), which have been described in detail in recently published works. These methods give a special attention to regenerating root systems and take into account particularly the biology of the truffle and its host, especially the different phases of the truffle cycle. Compared with earlier methods, the originality of the new methods rests on several points: different management of the zones in production (soil cultivation) and the inter-rows (revegetation), deep cultivation of the soil from the outset and severe pruning from the outset (Fig. 10.1h, i).

Deep cultivation leads to the deep formation of truffles, of better quality because they are less exposed to drought, to freezing and to parasites. What is more, evaporation from the soil is reduced. Finally, whether it is done manually or with adapted tools, it results in some (but not excessive) root pruning, in a manner that promotes root regeneration in order to feed the truffle mycelium.

Judicious cultivation of the soil, through limiting evaporation and pruning of roots, and a severe limb pruning regimen, by reducing transpiration of the tree during the summer, can contribute to an improved economy of water in truffle orchards (Chevalier and Wehrlen 2008). These practices, coupled with an increased depth of soil achieved by building raised beds (J.A.AD.), in some cases, have permitted the harvest of truffles without irrigation in 2003, a year characterised by a heat wave and extreme drought. Well-adapted cultivation methods can permit the harvest of some truffles 3–4 years after planting (Chevalier 1983).

10.5 Conclusion

A greater familiarity with the physico-chemical characteristics of soils favorable to development of *T. melanosporum* and *T. aestivum* has permitted improved techniques for working the soil that better address the needs of the host plant and the truffle fungus. It should be emphasised that the soil requirements of *T. aestivum* are less strict than those of *T. melanosporum*, but *T. aestivum* is also more demanding of water.

Before planting, unfavorable factors can be corrected using appropriate cultural practices. This may include planting on raised beds to increase the thickness of useful soil, incorporating coarse material to lighten the soil, mechanical fracturing of the subsoil, amendments of limestone and calcium, eliminating weeds and ectomycorrhizal hosts and establishing irrigation. Such physical and chemical amelioration of soils could lead to a considerable growth in the zones in Europe available for truffle cultivation.

Techniques for cultivating *T. melanosporum*, as described above, have been successfully used in France for many years (Sourzat 1989–1995–2002, 2011). These methods were perfected taking into account the pressure of contamination by ectomycorrhizal fungi in wooded environments, which are a major factor limiting the success of truffle cultivation in Europe. More recent approaches for truffle cultivation including the J.A.AD. (Dessolas et al. 2007–2008; Pargney et al. 2011) and M.R.T. (Chevalier 2010b) methods are aimed to improve truffle production. Implementation of field studies on truffle cultivation together with research on the biology, ecology and genetic of truffles should continue to contribute to improved truffle cultivation techniques.

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