Chapter 15
Truffles, Timber, Food, and Fuel: Sustainable Approaches for Multi-cropping Truffles and Economically Important Plants

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15.1 Introduction

15.1.1 What Is a Truffle?

Truffles are a polyphyletic group of fungi that produce fruiting bodies belowground or at the soil surface with spores sequestered inside (Trappe et al. 2009), and have been derived independently numerous times across the fungal tree of life (Tedersoo et al. 2010). Truffles are an important food source for forest mammals, whereas for most truffle genera (including *Tuber* P. Micheli ex F. H. Wigg.), mycophagy acts an important mode of spore dispersal (Frank et al. 2006). Many human cultures revere truffles for their gastronomic qualities, and consequently, these fungal fruiting bodies are a high-valued commodity (Mello et al. 2006).

The majority of edible truffle species are ascomycetes, and they establish mutualistic relationships with plant roots through the formation of ectomycorrhizas (EMs), benefiting the nutrition and health of their host plant (Smith and Read 2008). This includes the most economically important edible truffle species belonging to genera *Tuber*, *Terfezia*, and *Tirmania* within the order Pezizales (Hall et al. 2007) (see Chap. 2). Other edible (and putatively ectomycorrhizal) species in this order include *Choiromyces meandriformis* Vittad. (Wedén et al. 2009); *Leucangium carthusianum* (Tul. & C. Tul.) Paol. (Li 1997); *Imaia gigantea* (S. Imai) Trappe & Kovács

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(Kovacs et al. 2008); *Kalapuya brunnea* M. J. Trappe, Trappe, & Bonito (Trappe et al. 2010); *Picoa lefebvrei* (Pat.) Maire (Sbissi et al. 2010); and *Mattirolomyces terfezioides* (Mattir.) E. Fisch. (Kovacs et al. 2007).

Of all the truffle species, the European black truffle *Tuber melanosporum* Vittad. is the best studied, both in terms of its ecology and genetics (see Chap. 4). Current data indicates that truffle fruiting bodies are the sexual products of compatible haplotypes outcrossing. Although gamete fertilization is a prerequisite to fruiting, it does not ensure truffle production (Paolocci et al. 2006; Rubini et al. 2011). Environmental factors likely act as cues in initiating the sexual cycle and can influence whether initiated truffle primordia develop to maturity. The signals, receptors, and environmental cues that are involved in truffle fertilization and maturation are still unknown. Recent studies have demonstrated that *Tuber* and other Pezizales truffle fungi produce asexual spores (mitospores) (R Healy, unpublished data). Although the ecological function of these structures is still uncertain, putatively they are involved in reproduction and/or root colonization.

15.1.2 Truffles and Sustainability

Truffles are largely collected in the wild from native and naturalized habitats. Although attempts have been made to cultivate many truffle species, the majority of truffle species have so far evaded cultivation. For instance, many *Tuber* species including the European white truffle *Tuber magnatum* Pico and the North American white truffle *Tuber gibbosum* Harkn. have yet to be cultivated in a repeatable manner.

Reductions in natural truffle habitat has been implicated in the continuous decline of truffle production in Europe over the past century, yet given global trends in increases of planted area of poplar for biofuels (*Populus* sp.), and pine (*Pinus* sp.) and Douglas fir (*Pseudotsuga* sp.) for timber (FAO 2006), potential habitat for truffle species is increasing globally.

The market price for truffles varies, depending on species, geographic origin, size, quality, and the quantity harvested during the season. The European white truffle (*T. magnatum*) is the most expensive truffle species (up the price of 5,000 €/Kg). In contrast, the Chinese truffle *Tuber indicum* Cooke & Massee is quite inexpensive (about 10–50 €/Kg). Italy currently prohibits the trade and sale of *T. indicum*, because of concerns regarding its introduction into European ecosystems and potential for genetic introgression with the native economically important sister species *Tuber melanosporum* Vittad. (Murat et al. 2008). In 2006, annual revenues based on marketed European truffle species, i.e., *T. magnatum*, *T. melanosporum*, *Tuber borchii* Vittad., *Tuber aestivum* Vittad., and *Tuber brumale* Vittad., were estimated at over ¼ billion US dollars (Splivallo 2006).

The high market value and symbiotic nature of truffles make them attractive as a centerpiece of sustainability (Fig. 15.1). Truffles grow in the companionship of living host trees, so ecosystems that are producing truffles are also sequestering

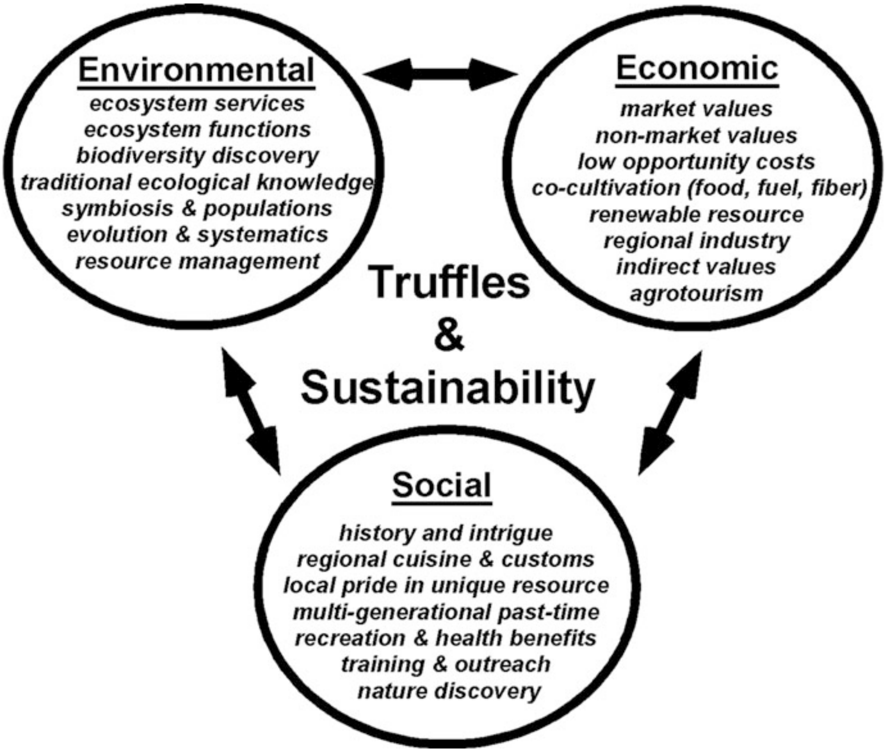

Fig. 15.1 Conceptual model of truffles as they pertain to sustainability. Truffle culture involves environmental, economic, and social components. Truffle fungi provide numerous environmental benefits, particularly to the health and function of many tree species and forest ecosystems. Economically, truffles have a high market value, but there are nonmarket and indirect values of truffles, which are harder to quantify. Socially, truffles attract interest from various social, ethnic, economic, and age groups. Truffle hunting is a healthy pastime for families and friends and can provide a sense of regional pride and uniqueness

carbon as woody biomass and in stocks of soil carbon. In many cases, economically important species of timber (Douglas-fir), nut (pecan), and biofuel (poplar and pine) trees associate with truffles, and these fungi improve in the health and growth of these plants. Truffles are also important to forest food webs and can be an important food source for diverse wildlife mammals, including flying squirrels, deer, and bear (Trappe et al. 2009). Because many truffle species are ruderal species (r-selected) and disturbance adapted, they have potential value in low-input agroforestry and reforestation applications. Truffle fungi have the ability to persist in disturbed habitats that are stressful to other fungi. Because they are directly involved in the nutrition of their host, nutrient additions are usually not needed (or conducive) for truffle production. These biological traits are adaptive to low-input agroforestry and reforestation. Finally, hunting truffles is an enjoyable pastime in many cultures, age groups, and families and is an activity that brings people to the forests. Truffles may contribute to the family diet or can be used to supplement family income. Thus, truffles have important social, ecological, and economic value.

15.2 Truffle Cultivation

Truffles are cultivated as a long-term (perennial) economic crop and in some countries as a means of land-use stability and reforestation (Bonet et al. 2006). As discussed in a previous chapter (see Chap. 9), roots of a compatible host seedling or tree must first be inoculated with truffle spores or mycelia (Hall et al. 2007; Michaels 1982) and well mycorrhized before transplanting in the field. Best practices call for simulating ecological conditions that optimize the growth of the fungus and also support growth of the host (see Chap. 10). Once truffles are produced, the truffle life cycle has been completed; however, a single genet may support annual fruiting for many years thereafter; the life span of a particular individual genet or colony is not known but could span decades given suitable edaphic and climate conditions (Garcia-Montero et al. 2006; Le Tacon et al. 1982). Most commercial truffle species benefit from climates without extreme summer heat or extreme winter cold. Primordia usually abort in conditions of low water (drought), excessive heat, and heavy frost; fruiting bodies are also vulnerable and damaged by extreme temperatures and dry conditions (Trappe et al. 2009).

Soil conditions that favor truffle production are going to vary with species. Generally, well-drained calcareous soils, with alkaline pH, commonly between 7.5 and 8.0, medium humus content, and good aeration, are desired (Granetti et al. 2005). However, soil moisture and temperature preferences vary greatly between species. Some species live in moist soil, even submerged under water for part of the year (e.g., *T. magnatum*, *Tuber macrosporum* Vittad.), and in cooler habitats almost always covered by lush vegetation. Other species (e.g., *T. melanosporum*) prefer permeable soils, never saturated with water and well heated by sunrays. Truffle species also vary in their soil preferences, and although alkaline conditions are recommended for most of the cultivated species, some species appear to tolerate soils with neutral pH values (e.g., *T. aestivum*) or lower (pH 5.0–6.0) (e.g., *T. borchii*, *T. gibbosum*) (Gardin 2005) (see Chap. 10).

Today, truffle cultivation is feasible for many species, and truffles are successfully produced in man-made truffiéres (Chevalier 1998; Hall and Yun 2002; Zambonelli et al. 2002). Most of the truffle cultivation industry is centered on the black truffle, *T. melanosporum*, but other species including *T. borchii* and *T. aestivum* are cultivated with equal and even greater success and in a broader range of hosts and habitats (Bencivenga and Baciarelli Falini 2012). Unfortunately, truffle cultivation is not yet routine for *T. magnatum* because mycorrhizas are difficult to obtain; therefore, contamination with other ectomycorrhizal species [e.g., *Tuber maculatum* Vittad., *T. borchii*, *Sphaerosporella brunnea* (Alb. & Schwein.) Svrček & Kubička, and *Pulvinula convexella* (P Karst.) Pfister (=*Pulvinula constellatio*)] is more prevalent (Bertini et al. 2006). However, in 2008 the French "Institut National de la Recherche Agronomique" requested a patent for the production of *T. magnatum* mycelium with the aim to obtain mycorrhized plants (INRA 2008). In Italy there are some old truffle orchards of *T. magnatum*, planted 20 years ago or more, that produce appreciable amounts of truffles but only in years having suitable climatic conditions (Gregori et al. 2010; Baciarelli Falini et al. 2010). There is also recent interest in the cultivation of *T. macrosporum* (Benucci et al. 2011a, 2012b) which can grow quite large in some habitats (Glamoclija et al. 1997). Seedling roots of various hardwood species are receptive to *T. macrosporum* spores (Benucci et al. 2012a; Giovannetti and Fontana 1980–1981), and attempts to cultivate this species have been reported (Vezzola 2005).

In general, truffle cultivation needs a detailed study of the plantation site, which includes soil physical–chemical analysis, climate, topography, local vegetation, etc. Besides the choice of high-quality mycorrhized plants, pre- and post-plantation farming operations such as soil amendments, plowing, weeding, irrigation, pruning, mulching, spore-inoculum addition, and pest and disease control[1] are fundamental for successful production of fruiting bodies (Bencivenga and Baciarelli Falini 2012).

Currently, truffles are cultivated as a monocrop, but as we propose below, there is considerable potential for sustainable multi-cropping of truffles with economic host plants for meeting multiple objectives of food, fuel, and fiber (Porter et al. 2009).

15.2.1 Cases of "Spontaneous" Truffle Production

In their native habitat, truffles are perennial and fruit seasonally, some years in abundance (North et al. 1997). There are a number of instances where truffles fruit spontaneously (without human inoculation) in soils being managed for particular plant crops.

For example, in Europe both *T. melanosporum* and *T. brumale* are known to naturalize and fruit within hazelnut orchards within their native range of southern Europe (Reyna 2007). On the other hand, *T. aestivum* and *T. borchii* (as well as other nonmarketable truffle species like *Tuber rufum* Pico and *Tuber excavatum* Vittad.) sometimes fruit under planted trees belonging to *Quercus* sp., *Tilia* sp., *Ostrya* sp., and *Pinus* sp. in reforestations, city parks, and street trees (GMN Benucci, personal observation). Both *T. magnatum* and *T. borchii* are known to

[1] In Europe, truffle plantations are not so often damaged by pathogens or pests and usually do not require any particular treatment. One exception however is downy mildew [*Erysiphe alphitoides* (Griffon & Maubl.) U. Braun & S. Takam.], a common pest of oaks (e.g., *Q. pubescens*) in truffle orchards in Europe and the USA. Precautionary measure should be taken when introducing exotic hosts to a nonnative habitat (Fisher et al. 2012), and as in any nursery trade, care should be taken to avoid transporting and transplanting pests, diseases, or susceptible rootstock into one's orchard. In the USA, eastern filbert blight [*Anisogramma anomala* (Peck) E. Müll.] has become a major problem in truffle orchards using susceptible hazelnut species (*C. avellana*). Resistant hybrid cultivars have been produced, but resistance varies. Similarly, a range of diseases affect pecan. These are more of a problem in humid climates but can be managed for (Sparks 2005). Emerging diseases, such as sudden oak death (*Phytophthora ramorum* Werres, de Cock, & Man in't Veld), could be disastrous if introduced to an oak orchard (Garbelotto and Pautasso 2012).

fruit in cultivated plantations of *Populus* spp. and riparian strips along watercourses in Northern Italy (Benucci et al. 2012a, b). In the United States, young Douglas-fir timber stands in the Pacific Northwest support fruitings of *Tuber oregonense* Trappe, Bonito, & P. Rawl., *T. gibbosum*, *L. carthusianum*, and *K. brunnea*, and their natural production is the basis for a cottage truffle industry (Trappe et al. 2009). In the Southern USA, pecan orchards support large fruitings of *Tuber lyonii* Butters, a phenomenon that has been noted through anecdotes for decades and more recently through molecular analyses (Bonito et al. 2011a). In China, substantial quantities (>300 tons) of *T. indicum* are harvested from regenerated forest stands (Murat et al. 2008). In all cases mentioned, the native species of truffle appears to function as a pioneer species and is tolerant to moderate disturbances. Further investigations with these and other truffles species are surely needed, and management regimes will have to be optimized, but exciting opportunities for multi-cropping truffles and trees as a sustainable approach for aiding in land and economic stability exist.

15.2.2 Hosts Preferences of Edible Truffle Species

Some truffle species show high host specificity, they grow in association with particular host species but not others (Molina and Trappe 1982). Host specificity appears to be a gradient from high (host specific) to low (host generalist). For instance, the cultivated truffle *T. melanosporum* grows and fruits well with Northern Hemisphere angiosperm (e.g., oak, hazelnut) hosts, but poorly with gymnosperm (pines) and some Southern Hemisphere taxa (e.g., *Eucalyptus* sp.). In contrast, other truffle species including *T. borchii* and *T. aestivum* are more general in terms of host and fruit in association with many species of gymnosperms (e.g., *Pinus* spp., *Picea* spp., *Cedrus* spp.) and angiosperms (e.g., *Quercus* spp., *Populus* spp.) and even form ectomycorrhizas readily with species in genera they are not normally associated with [e.g., *Carya illinoinensis* (Wangenh.) K. Koch] (Benucci et al. 2011b, 2012b).

Currently, truffles are cultivated on a narrow set of host species. Yet many unexplored possibilities for cultivating truffles on species being used for timber, biofuels, or food (nut) production exist. Host fidelity (and lack of) for particular truffle species offers novel opportunities for their cultivation in symbiosis with economically important trees.

Many edible ectomycorrhizal Pezizales truffles associate with angiosperm hosts (e.g., *T. melanosporum*, *T. magnatum*, and *Terfezia* spp.). These fungi tend to be better adapted to soils with higher pH (see Chap. 2). In contrast, the species *T. gibbosum*, *T. oregonense*, and *K. brunnea* appear to be endemic in the Pacific Northwest, USA, growing in association with Douglas-fir, an important timber species. Often, the soils under coniferous hosts are more acidic in pH and have lower nutrient availability (Binkley and Sollins 1990). Taxonomic-level host plant preferences for edible species of truffles in the Pezizales are summarized in Table 15.1. *Terfezia* and *Tirmania* are adapted to exploit two major soil types,

Table 15.1 Phylum-level host plant preferences for common edible truffle species

Truffle species	Angiosperm	Gymnosperm
Tuber aestivum Vittad.	×	×
Tuber borchii Vittad.	×	×
Tuber brumale Vittad.	×	×
Tuber canaliculatum Gilkey		×
Tuber gibbosum Harkn.		×
Tuber indicum Cooke & Massee	×	×
Tuber lyonii Butters	×	
Tuber macrosporum Vittad.	×	
Tuber magnatum Pico	×	
Tuber melanosporum Vittad.	×	
Tuber mesentericum Vittad.	×	

acid and basic. Particular fungal species tend to show high specificity toward particular hosts (either basophilous or acidophilous species) of Cistaceae (mainly *Helianthemum* spp.), Fagaceae, and Pinaceae (Díez et al. 2002). Chapter 14 provides further details on *Terfezia* sp. cultivation.

Mattirolomyces terfezioides (Pezizaceae) is another economically interesting hypogeous ascomycete closely related to *Terfezia* genus. Kovács and colleagues (2007) reported uncertain host relationships for this species. However, they suggest that *M. terfezioides* could putatively form symbiotic relationships with woody plants such as *Celtis occidentalis* L., *Crataegus monogyna* Jacq., *Ligustrum vulgare* L., and *Robinia pseudoacacia* L. and herbaceous ones such as *Muscari racemosum* (L.) Mill., *Salvia glutinosa* L., and *Viola cyanea* Čelak; alternatively, *M. terfezioides* could be functioning a saprobe.

15.2.2.1 Truffles and Pinaceae Hosts

There are many commercialized truffle species that appear exclusively with Pinaceae hosts. As previously mentioned, in the Pacific Northwest of North America, *T. gibbosum*, *T. oregonense*, *L. carthusianum*, and *K. brunnea* are endemic to habitats with Douglas-fir. These truffle species have not yet been cultivated but appear naturally and sometimes abundantly. In Eastern North America, the endemic species *Tuber canaliculatum* Gilkey is found under conifers, particularly *Pinus strobus* L. (eastern white pine, 5-needle) and *Tsuga canadensis* (L.) Carrière (eastern hemlock) but also with *Picea* spp., and EMs have been synthesized with *Pinus taeda L.* (loblolly pine) (Fig. 15.2e, f, i). There is potential that these species could be co-cropped in timber, biomass, and Christmas tree lots across the USA or in other locations.

Some truffle species are host generalists but can fruit abundantly with coniferous host plants. *Tuber aestivum*, *T. borchii*, and *T. indicum* are three other economically important truffle species that grow and fruit well in symbiosis with a wide range of coniferous hosts (in addition to angiosperm hosts). There are a multitude of opportunities for co-cropping truffles with coniferous hosts in different geographic regions. For instance, the pine genus (*Pinus* sp.) alone accounts for over eight

Fig. 15.2 Pecan, nuts, truffles, and some mycorrhization success on pine (*Pinus taeda*) and pecan (*Carya illinoinensis*). (**a**) Adult pecan branch with nuts and *T*. *lyonii* fruiting bodies collected in a commercial orchard. (**b**) *T*. *aestivum* ectomycorrhizas (EMs) on *C*. *illinoinensis* seedlings (*bar* = 500 µm). (**c**) *T*. *borchii* EMs on *C*. *illinoinensis* seedlings (*bar* = 500 µm). (**d**) *T*. *borchii* EMs on *P*. *taeda* (*bar* = 500 µm). (**e**) Mycelial net *T*. *canaliculatum* EMs on *P*. *taeda* (*bar* = 500 µm). (**f**) Growing *T*. *canaliculatum* EMs on *P*. *taeda* (*bar* = 200 µm). (**g**) Outer mantle cells of *T*. *borchii* EMs on *C*. *illinoinensis* seedlings (*bar* = 30 µm). (**h**) Cystidia of *T*. *aestivum* EMs on *C*. *illinoinensis* seedlings (*bar* = 30 µm). (**i**) Cystidia of *T*. *canaliculatum* EMs on *P*. *taeda* seedlings (*bar* = 30 µm). (**j**) Cross section of *T*. *aestivum* EM on *C*. *illinoinensis* (*bar* = 30 µm). (**k**) Cystidia of *T*. *borchii* EMs on *C*. *illinoinensis* seedlings (*bar* = 30 µm). (**l**) Outer mantle type of *T*. *aestivum* EMs on *C*. *illinoinensis* seedlings (*bar* = 30 µm)

million hectares of planted forest (FAO 2006). Other genera such *Larix* sp., *Pseudotsuga* sp., *Abies* sp., and *Picea* sp. are cultivated in many countries and at many latitudes for timber, fiber, and even Christmas trees.

15.2.2.2 Truffles and Angiosperm Hosts

A number of edible truffle species tend to associate preferentially with angiosperm hosts. As mentioned previously, the black truffle *T. melanosporum* shows strong preference for angiosperm hosts including *Quercus* and *Corylus* spp. (Murat et al. 2004). Although forming EMs with some gymnosperm species, the black truffle apparently only fruits with angiosperm hosts (Garcia-Montero et al. 2007). Both fungus and plant symbionts appear well adapted to the dry climate and karst-based soils in Europe. The prized white truffle *T. magnatum* also appears to be associated only with angiosperms. It is commonly reported as a *Populus* spp. (poplar) associate but also associates with *Corylus* (hazelnut), *Quercus* (oak) and *Ostrya* (hornbeam) species (Bencivenga and Granetti 1990; Murat et al. 2005).

In the USA, *T. lyonii* fruits under oaks and pecans in residential habitats and also naturalizes in pecan orchards (Fig. 15.2a) (Bonito et al. 2011a; Hanlin et al. 1989).

In the eastern USA, many cases have occurred where large fruiting bodies of *T. lyonii* were harvested from truffle orchards established with oak and hazelnut trees pre-inoculated with *T. melanosporum* (G Bonito, personal observation). Thus, it seems that *T. lyonii* is quite compatible with the management regime and regular disturbances associated with managed orchards and lawns (Bonito et al. 2011a). Harvesting truffles in addition to pecans is a mechanism for adding value to a farm and brings in extra revenue, particularly important in non-masting years when the nut crop yield is lower. Currently, most truffle biomass produced in pecan orchards goes uncollected, due to a lack of trained truffle dogs and a strong focus on pecan production, which is one of the major economic crops of the region.

As previously mentioned, the truffle species *T. aestivum*, *T. borchii*, and *T. indicum* are host generalists and fruit with both gymnosperms and angiosperms. These species have been inoculated (and in some cases have fruited) in association with native and exotic conifer and angiosperm host plants (Benucci et al. 2011a; Bonito et al. 2011b). For example, these species can associate with economic North American tree species including loblolly pine (*P. taeda*) (Fig. 15.2d), pecan (*C. illinoinensis*) (Fig. 15.2b, c, g, h, j–l), and poplar (*Populus trichocarpa* Torr. & A. Gray).

15.3 Multi-cropping Truffles and Trees

A number of considerations need to be made for successful truffle and tree multi-cropping. Climate and soil characteristics pose the biggest constraints. In some cases, it is feasible to amend soil characteristics such as pH and nutrient level.

Truffle species each have their own habitat preferences; success in their fruiting depends on many factors including where they are being grown. When cultivating native truffle species, the cultivator may experience a “home court advantage.” However, the growth of truffles as exotic species could benefit from a phenomenon known as “competition release” (Mitchell et al. 2006). If a dearth of compatible or competitive ectomycorrhizal competitors is present, then the introduced species is more likely to proliferate. Thus, host ecology and geography are important in the success (and failure) of truffle orchards, just as are soils (see Chap. 6). When experimenting with new hosts, special attention should be paid to the plant health and nutrition in order to survive at the pH required by the partner truffle species. Particular hosts, and even genotypes, may favor mycorrhization and fungal species performance (Courty et al. 2011).

15.3.1 Preliminary Steps in Multi-cropping Truffles and Economic Plants

From observations and knowledge about the ecology of plants and truffle species of interest, various host–fungus combinations can be envisioned (Table 15.2).

Preliminary steps to co-cropping European truffle species with the economically important North American nut tree *Carya illinoinensis* have recently been reported (Benucci et al. 2012b). Healthy levels of *T. borchii* (≈62 %) and *T. aestivum* (≈42 %) mycorrhizal colonization were obtained with spore-slurry inoculations (Fig. 15.2b, c, g, h, j–l). Preparations are being made to plant these seedlings as an experimental plot in Italy to ascertain whether the pecan host maintains colonized with these species in the field, and whether productive fruitings of these truffle species can be realized when associated with pecan. Such long-term research is unpredictable and is not very conducive for short-term funding cycles as has been expressed by Hall and Haslam in Chap. 11.

Outside of Europe, *T. melanosporum* has been inoculated onto native *Nothofagus* spp. in Chile (Pérez et al. 2007) and *Quercus* spp. (Michaels 1982) and pecan (G. Bonito, unpublished data) in North America. It is not clear, however, when outplanted into exotic soils whether *T. melanosporum* will be competitive against the resident ectomycorrhizal flora.

15.3.2 Possibilities for Intercropping with Economic Species and Co-inoculating with Multiple Truffle Species

Another possibility, probably little known by truffle farmers, is to cultivate a combination of truffle plants and other crops in the same field. For example, in central Italy single compatible host plants (e.g., *Quercus* spp.) naturally growing

Table 15.2 Tree species, major uses, and potential for multi-cropping with truffles

Plant species	Used for			Used in truffle cultivation	Potential multi-cropping
	Fruits	Fuel	Timber		
Quercus ilex L.		×		×	×
Quercus pubescens Willd.		×	×	×	×
Quercus cerris L.		×	×	×	×
Quercus robur L.			×	×	×
Quercus petraea (Mattuschka) Liebl.		×	×		×
Corylus avellana L.	×			×	×
Ostrya carpinifolia Scop.		×		×	×
Carpinus betulus L.			×	×	×
Populus sp.		×	×	×	×
Tilia platyphyllos Scop.			×	×	×
Tilia cordata Mill.			×	×	×
Pinus taeda L.		×	×		×
Pinus pinea L.	×		×	×	×
Pinus halepensis Mill.			×	×	×
Pinus nigra L.			×		×
Cedrus sp.			×	×	×
Carya illinoinensis (Wangenh.) K. Koch	×				×
Juglans regia L.	×		×		×
Castanea sp.	×		×		×
Larix sp.			×		×
Pseudotsuga sp.			×		×

between old olive (*Olea europaea* L.) trees have become mycorrhized with truffles and produce ascocarps. For this reason, some truffle plantation were established by alternating rows of mycorrhized plants (e.g., of *Quercus* spp.) and rows of olive trees (Granetti and Baciarelli Falini 1997).

A number of experiments have investigated the effects of co-inoculating multiple different truffle species on a single host (Mamoun and Olivier 1993; Donnini et al. 2010). Results indicate that multiple economic truffle species (e.g., *T. borchii*, *T. aestivum*, *T. melanosporum*) can be maintained on a single host and that they may in fact improve the growth of each other. Further, a greater proportion of the roots become mycorrhized, which is predicted to limit the opportunity of nontarget fungi to associate with the host (Kennedy et al. 2009).

15.3.3 Additional Considerations When Multi-cropping Economic Truffles and Trees

Co-cropping economic species of truffles alongside other crops such as nuts, timber, or pulpwoods is a reasonable improvement to the old concept of truffle cultivation aimed at solely producing truffles. With a dual cropping system, crop failure (e.g., nuts) could be balanced by truffle production (and/or wood products). Multi-cropping of economic tree species and truffles represents a plausible alternative for boosting rural or disadvantaged economies by providing farmers with a valuable source of income. Due to the ectomycorrhizal symbiosis with forests trees, truffle cultivation does not generally require amendments, pesticides, or fertilizers and thus could be cultivated in completely organic and sustainable manner.

15.4 In Situ Inoculation of Young Forest/Timber Stands

In situ inoculation of young forest/timber stands is one approach to truffle cultivation that still needs more study. Addition of truffle spore slurry to young growing or already mature truffle orchards of forest stands could be managed to augment mycorrhization possibilities for developing roots of host trees (Bencivenga and Baciarelli Falini 2012; Baciarelli Falini et al. 2010). This is a common strategy used in truffle orchards to maintain production, although its efficacy has not been well tested. It seems unlikely that a residential community of ectomycorrhizal fungi can be replaced by spore inoculations in the field. However, if target taxa are already present, then their abundance could be augmented through in situ inoculation with spore slurries or spores could serve as propagules for sexual fertilization. A preliminary study on field inoculation with economic truffle species onto adult hazelnuts that no longer bear nuts has been carried out (Morcillo et al. 2010). This study mixed spore inocula with hydrogels, root promoting factors, and spore germination promoting factors (following the Mycoforest Technology method). An increase in amount of truffle mycorrhizas and fruiting was documented. However, because adequate controls were not included in the study, it is unclear whether the trees are responding to the disturbance, truffle propagules already in the soil, or the experimental treatment.

15.5 Future Opportunities

Considering mycorrhization successes of pecans and pines with *T. aestivum* and *T. borchii*, we foresee new mycorrhization trials with other valuable truffle species such as *T. melanosporum*, *T. brumale*, and *T. magnatum*.

Future research will also involve other potential coniferous hosts and angiosperm hosts such as the putatively ectomycorrhizal common walnut (*Juglans regia* L.) (Wang and Qiu 2006), other *Carya* species, and species of chestnut (e.g., *Castanea sativa* Mill.). Hickory and chestnut are adapted to a range of climatic and soil conditions, overlapping in cases with those suitable for truffle growth, and they may be potential symbiotic hosts for some *Tuber* spp.

Given the expansive plantations of coniferous ectomycorrhizal hosts globally, we see great opportunities for establishing these as dual truffle and timber systems.

The European species *T. borchii* and *T. aestivum* appear to be among the best suitable for such endeavors, given their broad host range and established markets. Of course, possibilities also exist for the use of North American species such as *T. canaliculatum*, *T. lyonii*, and *T. oregonense* and other tasty but less appreciated European species such as *T. macrosporum*.

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