
Truffles: What we know and what should we know?

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Truffles are a polyphyletic group of fungi that produce fruiting bodies belowground or at the soil surface with spores sequestered inside. 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. Of all the truffle species, the European black truffle *Tuber melanosporum* is the best studied, both in terms of its ecology and genetics. The market price for truffles varies, depending on species, geographic origin, size, quality, and the quantity harvested during the season. The high market value and symbiotic nature of truffles make them attractive as a centerpiece of sustainability. The cultivation of EEMMs has made considerable progress over the past 200 years. This research has concentrated on the truffles, the most expensive of the EEMMs. A few species of truffles, mostly in the genus *Tuber*, have been cultivated commercially. Some new technologies have been developed for the management of EMM plantations, in order to maximize their production. Some useful findings have come from recent molecular studies of EM fungi, including sequencing the *T. melanosporum* genome. This genome sequence will serve as a valuable resource for future studies regarding the biology and ecology of *Tuber*. Modern technologies involving the use of molecular approaches for truffle genome studies have provided better understanding of the biology and plant-fungus symbioses of EMMs. Cultivation and management of EMMs is in progress although cultivation on some mycorrhizal mushrooms remains challenging.

Introduction

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.*

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2007). Of all the truffle species, the European black truffle *Tuber melanosporum* is the best studied, both in terms of its ecology and genetics. 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 (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). Although the ecological function of these structures is still uncertain, putatively they are involved in reproduction and/or root colonization.

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* and the North American white truffle *Tuber gibbosum* have yet to be cultivated in a repeatable manner. 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* is quite inexpensive (about 10–50 €/Kg). In 2006, annual revenues based on marketed European truffle species, i.e., *T. magnatum*, *T. melanosporum*, *Tuber borchii*, *Tuber aestivum* and *Tuber brumale* 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. 1). Truffles grow in the companionship of living host trees, so ecosystems that are producing truffles are also sequestering 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).

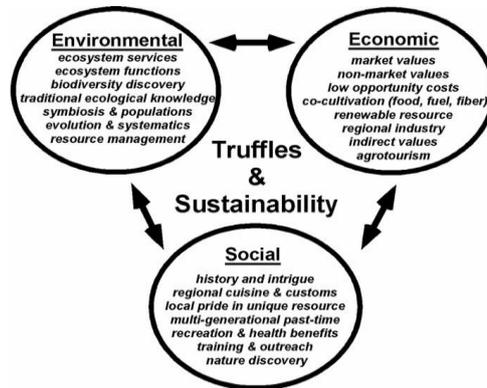


Fig. 1- Conceptual model of truffles as they pertain to sustainability.

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.

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.

Life Cycle of the Truffles

As early as the 1950s, croziers had been observed by Lillian Hawker and Dave Minter in truffles (Fig. 2; Hall *et al.* 2010), and in some species of truffle, asci containing eight spores were known (Montecchi and Lazzari, 1993). From this it should have been apparent that truffle spores were almost certainly produced sexually like other genera in the Pezizales, and the vast majority of ascomycetes for which the full sexual life cycle has been observed (Alexopoulos *et al.* 1996).

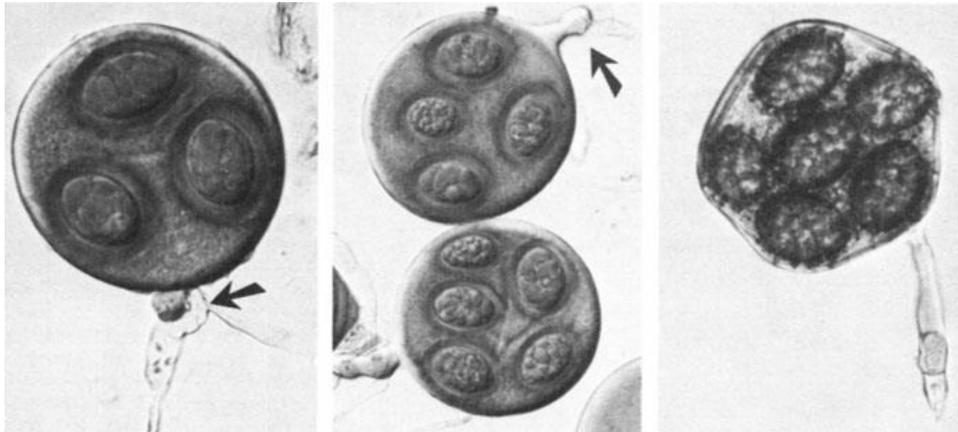


Fig. 2- Truffle croziers, evidence of sexual reproduction, photographed by Dave Minter in 1985.

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). Roots of a compatible host seedling or tree must first be inoculated with truffle spores or mycelia (Hall *et al.* 2007) and well mycorrhized before transplanting in the field. 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* and *T. macrosporum*), 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).

Today, truffle cultivation is feasible for many species, and truffles are successfully produced in man-made truffières (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. 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 are fundamental for successful production of fruiting bodies (Bencivenga and Baciarelli Falini, 2012).

Future Remarks

EMMs are not only a gourmet food but also significant sources of livelihood. Recent advances in genetics and molecular biological techniques in EMMs have provided better understanding of biology for an enhanced production of EMM. Molecular methods, such as PCR with specific primers and multiplex PCR and sequencing, have potential in verifying inoculum and mycorrhizal trees. Molecular technologies also provide a new approach for understanding mycorrhizal associations and therefore have implications for cultivation and management of EMM plantations (Visnovsky *et al.* 2010). The cultivation of EEMMs has made considerable progress over the past 200 years. This research has concentrated on the truffles, the most expensive of the EEMMs. As with much agricultural science, research on the cultivation of edible mycorrhizal mushrooms can be a slow process. For example, in New Zealand, it took a decade to produce the first black truffle and another decade to produce significant harvests in Australia. A few species of truffles, mostly in the genus *Tuber*, have been cultivated commercially. Techniques have been extended and developed for the cultivation of epigeous species. The last decade's environmental deterioration has become a worldwide problem due to varied reasons. Protection and management of EMM resources has become urgent matter, particularly in developing countries. Some new technologies have been developed for the management of EMM plantations, in order to maximize their production. Some useful findings have come from recent molecular studies of EM fungi, including sequencing the *T. melanosporum* genome. This genome sequence will serve as a valuable resource for future studies regarding the biology and ecology of *Tuber*. Phylogenetic information such as single-based polymorphisms has been used to design species-specific primers for white (Mello *et al.* 1999) and black truffles (Mabru *et al.* 2001). PCR-RFLP using a SNP on the mitochondrial LSU-rDNA is an easy method to differentiate *Tuber melanosporum* from other truffle species like *T. aestivum*, *T. brumale* or *T. indicum* (Mabru *et al.* 2004). It is anticipated that metagenomics and comparative genomics of other edible EEMMs will stimulate new frontiers of activity.

We urge that this is done in concert with more long-term research projects focused on the cultivation of EEMMs. Modern technologies involving the use of molecular approaches for truffle genome studies have provided better understanding of the biology and plant-fungus symbioses of EEMMs. Cultivation and management of EEMMs is in progress although cultivation on some mycorrhizal mushrooms remains challenging.

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