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Entry

The Mysteries of the White Truffle: Its Biology, Ecology and Cultivation

Simone Graziosi [1], Ian Robert Hall [2] and Alessandra Zambonelli [1,*]

[1] Department of Agricultural and Food Sciences, University of Bologna, Viale G. Fanin 44, 40127 Bologna, Italy
[2] Truffles & Mushrooms (Consulting) Limited, P.O. Box 268, Dunedin 9054, New Zealand
* Correspondence: alessandr.zambonelli@unibo.it

Definition: *Tuber magnatum* Picco is the most expensive of the truffles and a great deal of research has been carried out in an attempt to solve the mysteries of its ecology and biology. However, considerable work remains to be done particularly on those secrets of its life cycle that remain a mystery. It is known that *T. magnatum* is heterothallic, but it has yet to be determined how fertilization occurs between the two strains of different mating types. It is also known that the white truffle is an ectomycorrhizal fungus, and its mycorrhizas can be produced in greenhouses, but then they seem to disappear in the field. The role of other soil microorganisms, fungi and bacteria, on its soil mycelial development and fructification is intriguing but is far from being completely understood. All these uncertainties have made the cultivation of *T. magnatum* extremely difficult and only recently have we had the scientific proofs that it is possible. Even so, many questions remain unanswered and the management practices of *T. magnatum* plantations are still to be better defined to also enable the taming of this truffle.

Keywords: *Tuber magnatum*; biological and ecological aspects; aroma; microbiome; mycorrhizas

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1. Introduction

Truffles are fleshy ascomycetous fungi that generally fruit underground. The spores are sequestered in the fruiting body (the ascoma or ascocarp), which consists of a more or less subspherical mass. Many researchers prefer to consider as "true truffles" only the Ascomycota in the genus *Tuber* belonging to the Tuberaceae family and in the order Pezizales [1]. There are more than 180 species, all characterized by an ectomycorrhizal habit and naturally are mostly restricted to the Northern Hemisphere [2]. The phylogeny of the genus has been studied by many authors [3–5] with the most recent by Bonito et al., 2013 [2], which provided a complete phylogenetic analysis of the genus and its recognized 11 main clades: Aestivum, Rufum, Excavatum, Gibbosum, Japonicum, Puberulum, Macrosporum, Maculatum, Multimaculatum, Melanosporum and Gennadii. Although all the *Tuber* species are edible, only four are considered culinary delicacies and command really high prices: *Tuber melanosporum* Vittad. (Périgord black truffle) in the Melanosporum clade, *Tuber borchii* Vittad. (Bianchetto truffle) in the Puberulum clade, *Tuber magnatum* Picco (Italian white truffle) and *Tuber aestivum* Vittad. (Burgundy truffle) which are both included in the Aestivum clade.

Undoubtedly, *T. magnatum* ranks above the others because of its intense aroma and unique flavor. As a consequence, Italian retail prices for *T. magnatum* are higher than for any other truffle and make it one of the world's most expensive foods [6], with prices ranging from 1000 to 6000 EUR kg^{-1} [7] and a business of about 0.9 billion EUR $year^{-1}$ [8].

In poor production years such as 2007 and 2021 [9], when dry summers reduced production, prices reached 7000 EUR kg^{-1}, and outside of Italy in upmarket stores such as Harrods in London, prices reached dizzying heights. In this entry, the distinctive morphological characters of this truffle are described as well as its biology and ecology. The current status of its cultivation is reported, which emphasizes the gaps in our knowledge that actually limit the possibility of taming this truffle in contrast to the other European species of *Tuber*.

2. Morphology

Tuber magnatum is a "white truffle" because as well as several other *Tuber* spp. (*Tuber borchii*, *Tuber dryophilum* Tul. & C. Tul. and *Tuber puberulum* Berk. & Broome) it is characterized by smooth, pale-colored ascomata. In contrast, the majority of other edible truffles (e.g., *T. melanosporum*, *T. aestivum* and *Tuber macrosporum* Vittad.) are called "black truffles" on account of their brown, black and warty surfaces. The ascomata of *T. magnatum* are generally smaller than those of European black truffles (*T. melanosporum*, *T. aestivum* and *T. brumale* Vittad.) but somewhat larger than those of other white truffles (*T. borchii*, *T. maculatum* Vittad. and others). They weigh from just a few grams to, in rare cases, more than a kilogram. They are distinguished by smooth to suede-like surfaces and their color varies from pale yellowish brown to yellow ochre, olivaceous or greenish grey (Figure 1a). Sometimes, they have black or brownish spots but can be tinged pink (Figure 1b,c) which Amicucci et al. (2018) [10] identified as corresponding to some carotenoid-producing bacteria of the genera *Microbacterium* and *Chryseobacterium*. On the other hand, Ratti et al. (2016) [11] hypothesized that the brown spots may be caused by mycoviruses included in the Endornavirus genus. The peridium is 250–1250 μm thick and its thickness seems to be related to the origin of the ascoma [12]. It is formed of small subspherical cells measuring (3–) 9–15 μm by (3–) 6–12 (–13) μm.

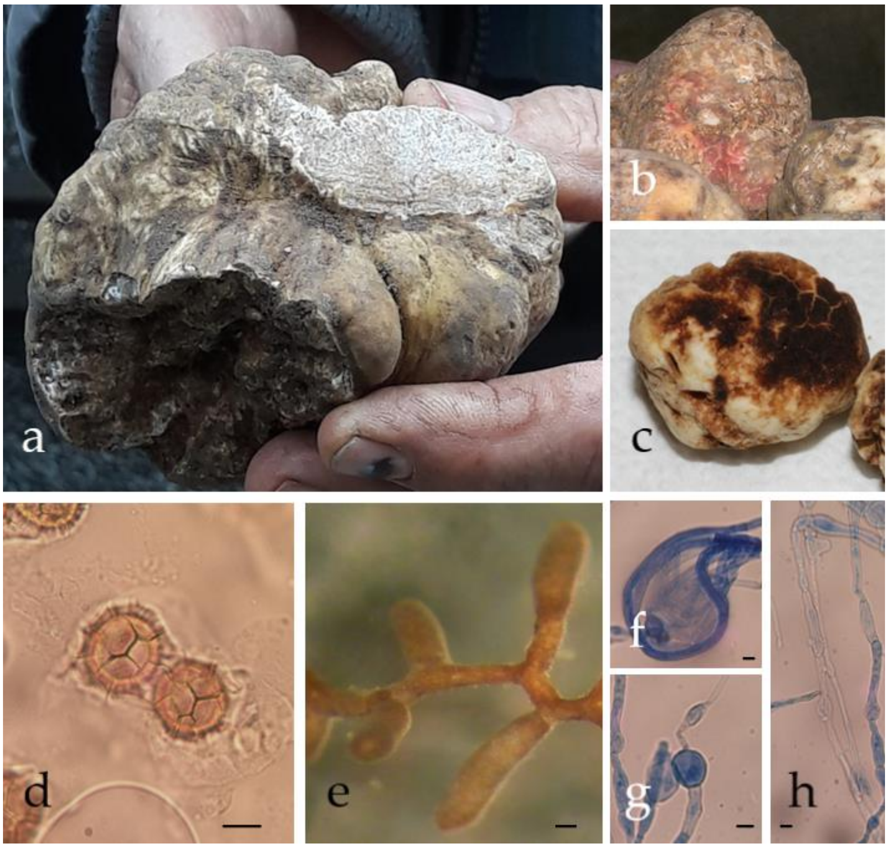

Figure 1. Morphology of *T. magnatum* ascomata (**a**–**c**), spores (bar = 10 μm) (**d**), mycorrhizas (bar = 100 μm) (**e**) and mycelium-colored with blue cotton (bar = 10 μm) (**f**–**h**). (**a**) *T. magnatum* broken ascoma, (**b**) reddish patches, (**c**) brown spots, (**d**) reticulated spores, (**e**) *T. magnatum* mycorrhizas, (**f**) hyphal aggregates, (**g**) vesicles and (**h**) anastomosis.

The gleba contains, fine, clear veins, which are light-hazel-colored at maturity. In the case of *T. magnatum,* one to three (rarely four) spores are enclosed in each ascus. The spores are round or broadly elliptical, yellowish or yellow-brown measuring (20–) 25–32 (–37) µm by (15–) 20–30 (–35) µm (without ornamentation). The surface of the spores is characterized by a special ornamentation, a reticulum that reaches 3 to 5 µm in height, with wide polygonal meshes, with two to four along the greatest diameter (Figure 1d) [13].

The mycelium of *T. magnatum* was first described by Fontana (1968) [14] after examining just a few hyphae originating from the glebal tissue. Later, Mischiati and Fontana (1993) [15] reported to have isolated *T. magnatum* mycelium from mycorrhizas. However, a few years later, the same authors showed by genetic analyses that this mycelium did not belong to *T. magnatum* but to other white truffles [16]. Our recent morphological analyses (data not shown) [17] confirmed that *T. magnatum* mycelium showed the morphological structures typical of the mycelia of *Tuber* species such as numerous vesicles, rare anastomoses and hyphal aggregates (Figure 1f–h) [18].

Tuber magnatum mycorrhizae are similar to ectomycorrhizas formed by other *Tuber* spp. They show monopodial ramifications, range between simple and irregular pinnate and are single or in small groups [16]. The color is paler than in other *Tuber* mycorrhizas, especially in the young ones which are yellow-grey or amber, often with darker markings due to tannin deposits in the innermost mantle layer adjacent to root cells (Figure 1e). The surface may be smooth or may protrude emanating hyphae and very frequently hyaline, long, ramified cystidia, which are characteristic of *T. magnatum* mycorrhizas. The mantle surface is pseudoparenchymatous and epidermoid and consists of cells with an irregularly sinuous outline or tending to polyhedral. Longitudinal and cross sections reveal that the pseudoparenchymatous mantle is arranged in 5–6 layers of cells, 20–30 µm thick. It is possible to distinguish the inner layer, because it has a cytoplasmic content, while the outer one is empty. The Hartig net infects only the outer epidermal cell layer [16,19].

3. Aroma

Tuber magnatum, like all truffles, has the spores sequestered in the hypogeous ascoma. This makes it impossible for the truffle to actively discharge its spores and to disseminate them by wind as so many other members of the Pezizaceae family rely on. Instead, they produce strong aromas, which attract mycophagous animals (mammals, arthropods and mollusks) [20,21] which disseminate their spores. The aroma of the white truffle is characteristic and dominated by the volatile component 2,4-Dithiapentane also known as bis(methylthio)methane. More than 60 additional volatile organic compounds (VOCs) add to the complexity of the white truffle aroma, although only 11 of them are sufficiently concentrated to be perceivable by the human nose [22,23]. Among them is a 3-(methylthio) propanal with a potato-like aroma, a 2- and 3-methylbutanal with a malty aroma, a 2,3-butanedione with buttery tones, in addition to 2,4-dithiapentane which has sulphury and garlic-like aromas. Among the nonvolatile compounds is an amine-like compound with a sperm-like aroma identified as 3,4-dihydro-2(*H*) pyrrol (1-pyrroline) [22]. Regrettably all the commercial white-truffle-flavored products are added with an inappropriate and excessive quantity of synthetic 2,4-dithiapentane and these do not present the complexity and quality of natural truffle aromas [24,25]. In the future, natural truffle aroma volatiles could be potential alternatives to replace the existing synthetic aromas [26], but these have yet to be commercially applied.

Environmental factors can also influence the range of organic volatiles, so harvesting location and picking season are involved in the composition of aromas [23,27–29]. Furthermore, the complex aroma of *T. magnatum* does not depend only on its native compounds, but also on the microorganisms, in particular yeast and bacteria, that live within the ascocarps [30,31]. Although the origins of 2,4-dithiapentane are currently unknown, one possibility is that it may be produced by Betaproteobacteria inside *T. magnatum* fruiting bodies [31], similar to the one of the thiophene derivatives which originate from bacteria inhabiting *T. borchii* [32]. Moreover, during prolonged storage of *T. magnatum* fruiting bodies, there are changes in the microbial communities and with them, volatile profiles, due the formation of spoilage volatile compounds [33] which reduce quality. Postharvest storage conditions may also bring about changes in the truffle aroma [34].

4. Biology

The life cycles of the truffles are not fully understood. Many authors have studied the process that could lead to ascocarp formation [35–38]. Until 2005, *T. magnatum* was assumed to be self-compatible or a highly inbred species. This was supported by genotyping using codominant markers, since no heterozygous ascocarps were detected [39,40]. However, later, extensive genetic exchange was detected in the species populations [41], in contrast with previous ideas. Moreover, the SSR genotyping of gleba and asci separately [42] suggested the hypothesis that the sterile hyphae of the gleba, which represent the majority of *T. magnatum* ascocarps were composed of haploid maternal material, while ascospores contained both paternal and maternal information. Eventually the genome sequence confirmed that *T. magnatum,* like other *Tuber* spp., was heterothallic [43]. *T. melanosporum* and *T. borchii* mating-type studies with SSR genotyping revealed that ectomycorrhizas and gleba were both formed by a haploid mycelium of the same maternal mating type, instead of spores containing several different genotypes [38,44,45]. However, one question remains unanswered: what is the origin of the male genotype?

Field studies have shown the formation of mycelial mats in *T. borchii* producing areas producing hyaline and obovate mitospores (conidia) having holoblastic origin in repeatedly branched conidiophores [46,47]. These mitospores seem unable to germinate so it has been suggested they may have a reproductive role and, as in some other ascomycetes, act as spermatia [47]. Similarly, newly productive New Zealand *T. borchii* truffières and one *T. borchii* truffière in eastern Tibet also appear to show the early stages of fruiting are also associated with strange mats of hyphae [48], similar to those described by Healey [47]. Although these "conidia" have not been found associated with other economically important European species of *Tuber* [17], we cannot exclude that they are present because they are simply too difficult to detect in soil. Similar structures have also been found in the in vitro mycelium of *Tuber japonicum* Hir. Sasaki, A. Kinosh & Nara [49], but never on *T. borchii* mycelium in vitro conditions [17].

Other postulates might be advanced regarding the identity of the paternal partner: they are mycelia from nearby ectomycorrhizas [50,51], free living mycelia in soil [51], germinating ascospores [20,38,52] or mycelia that exist as endophytes in nonhost plants [53]. This last hypothesis has been rejected for *T. aestivum* and *T. melanosporum* [54].

5. Distribution and Ecology

Tuber magnatum has long been considered an almost exclusive Italian truffle restricted to the north and center of the country with smaller patches, in Istria, Croatia southeastern France and the Ticino Canton of Switzerland [13]. However, recent studies have also found it in Sicily, the Geneva Canton of Switzerland [55], Hungary and several Balkan regions [56]. Surprisingly it has also been found in Thailand [57]. It can also be found in Italy in Summer (from July) but generally these truffles do not mature until early autumn (September–October) [58]. These early truffles are generally more superficial (5–10 cm deep) and for this reason and the high temperatures they are exposed to, they often suffer from bacterial rot and insect damage and hence are of low quality. During the height of the season (October to December) the ascomata are usually deeper (10–30 cm) and in some cases, for example under small landsides, they can also be found as deep as 80 cm under the soil surface.

Truffles are mycorrhizal hypogeous fungi, so the host plant and the pedoclimatic characteristics are the most important and most studied ecological factors that determine their development. *Tuber magnatum* grows in well-drained and well-structured marls, without gravels, but seems better adapted than the other truffles to clay soils. The most suitable soil composition is more or less equal proportions of sand and clay. The optimal pH varies from neutral to moderately alkaline and characterized by a high degree of base saturation and sufficient water throughout the year [59,60]. This species tends to be found where the summer temperatures are about 20 °C [60]. Soils suitable for *T. magnatum* mainly belongs to Calcaric Fluvisols, Colluvic Calcaric Regosols and Fluvic Eutric Cambisols [61]. It is also associated with stagnant surface water, very soft soils and more than 15 % of the soil volume occupied by interconnected macropores. Ideal places are the banks of rivers or other waterways. Other areas include forest margins and where the soil is disturbed or under isolated trees. The constant presence of water is obligatory and encourages dense canopy cover [61].

T. magnatum is primarily associated with *Alnus cordata* Desf. (Italian alder), *Corylus avellana* L. (hazelnut), *Ostrya carpinifolia* Scop., *Populus alba* L. (white poplar), *P. tremula* L. (European trembling aspen), *P. nigra* L. (Lombardy poplar), *Quercus cerris* L. (Turkish oak), *Q. ilex* L. (holm oak), *Q. pubescens* Willd. (downy oak), *Q. robur* L. (English or common oak), *Salix alba* L. (white willow), *S. caprea* L. (pussy willow), *Tilia cordata* Mill. (small-leaved lime), *T. platyphyllos* Scop. (large-leaved lime) [13] and *Fagus sylvatica* L. [55]. It is also, though rarely, found under *Pinus* spp. [17] but the ectomycorrhizal association with conifers has never been verified using molecular tools.

Other nonectomycorrhizal plants and in particular scrubby species such as *Cornus sanguinea* L., *Rubus ulmifolius* Schott., *Rosa canina* L., *Crataegus monogina* J acq. and *Juniperus communis* L. [62] seem to play a key role in *T. magnatum* ecology. These plants perhaps modify the soil microclimate creating favorable conditions for the development of *T. magnatum* mycelium. Moreover, these plants may also host *T. magnatum* mycelium as endophyte preserving it. While this has yet to be verified for *T. magnatum*, several other *Tuber* species such as *T. melanosporum, T. aestivum* and *T. borchii* have been shown to form symbiotic associations with nonmycorrhizal plants including orchids [54,63–67]. The *Tuber* mycelium association with ectomycorrhizal plants and traditionally considered nonhost plants form a common mycorrhizal network which could play important roles in nutrient and signal transportation between a range of plants [68]. Strangely, *T. magnatum* ascoma production occurs in areas where its mycorrhizas are rare or even absent [69,70], as well as in productive patches [70].

Thanks to qPCR and its optimization for *T. magnatum* DNA quantification [71], the amount of white truffle mycelium in soil was investigated as well as its distribution in the soil. These studies revealed that *T. magnatum* mycelium was present from within 100 m of the nearest productive tree [72] and its amount was related to depth. In fact, at 20–30 cm, the mycelial density was significantly higher than at 10–20 cm [60]. Although, it has not been investigated, *T. magnatum* mycelium may grow at deeper than 30 cm considering that its ascomata were collected as deep as 80 cm [73]. At lower depths, the soil layers are less disturbed and at such depths may protect the mycelium from environmental stress such as high temperatures or drought and may reduce competition with other organisms. This idea is supported by the elevated sensitivity of *T. magnatum* to environmental stresses occurring during hard winters and dry summers [74]. The mycelium reaches the best growth rate in spring [75]. In that period, temperatures and water availability increase and the host plant sprouts out and restarts its activity after winter [76]. A temperature of 20 °C and a water content near saturation are the best parameters for the white truffle mycelium growth [60]. Marjanović et al. (2015) [59] defined *T. magnatum* as a species that needed a high level of water availability and was hypo-oxygenation-tolerant.

The microbiome and in particular bacteria seem to have not only a key role in *T. magnatum* aroma biosynthesis (see Section 3) but also in its mycelium soil development, mycorrhiza formation and fruiting body formation and nutrition [77,78].

A diverse microbial population has been found in *T. magnatum* ascomata [23,79–82]. Microorganisms associated with the truffle ascocarps seem to have a potential role in nitrogen fixation as shown in studies carried out on several *Tuber* species. Barbieri et al. (2010) [81] identified the nitrogenase gene *nifH* belonged to *Bradyrhizobium* spp. inside the ascocarps, and the nitrogen fixation yield was shown to be comparable with early nodules of legumes associated with specific nitrogen-fixing bacteria [81,83,84]. Bacteria can colonize the ascoma at the primordium stage [85] and penetrate from the soil. Then, they can be selected by the fungus, reducing their biodiversity into the fruiting bodies irrespective of the soil microbial population. Proteobacteria (Alphaproteobacteria and Gammaproteobacteria) seem to be the most relevant group of truffle microorganisms followed by Actinobacteria and Firmicutes [33,85–87]. *Bradyrhizobium* is the most abundant genus which is linked to its nitrogen-fixation activity. Actinobacteria may also be associated with the truffle's nutritional requirements. Isolates with sequence similarities to *Curtobacterium flaccumfaciens* and *Rhodococcus* sp. were isolated from ascomata of *T. magnatum*; they can solubilize $Ca_3(PO_4)_2$ in a way that is dependent on the nitrogen and carbon sources present. Furthermore, they can produce chelating compounds, perform ammonification and degrade β-glucan. These bacteria metabolize different substances and can support the white truffle in its development: *C. flaccumfaciens* decomposes chitin, pectin, lipids and proteins, while *Rhodococcus* sp. exhibits urease activity. The nutrition function is not the only one: *Rhodococcus* isolates inhibited *Verticillium leptobactrum*. In this way, it can protect the fungus from degrading organisms [88].

Giorgio et al. (2022) [89] showed that although soil type affected the taxonomic structure of the soil microbial communities in productive patches, *T. magnatum* ascomata had a common core of microbiota. However, inside the ascomata, there were also variable bacterial taxa which were associated with specific environmental conditions [82]. In the future, this variable fraction of bacterial communities may be used to track the geographic origin of *T. magnatum* ascomata [78].

Tuber magnatum is also associated with specific fungal communities. Leonardi et al. (2013) [70] found that the ectomycorrhizas formed by fungi of the Thelephoraceae, Sebacinaceae and Inocybaceae families were the most abundant within the *T. magnatum* ascoma productive points. In contrast, the mycorrhizas formed by Russulaceae were more common in unproductive locations [70]. The presence of specific fungal species may be a bioindicator of suitable habitats for *T. magnatum*. For example, Lalli et al., 2015 [90] found that fruiting bodies of *Amanita stenospora* Contu, *Cortinarius aprinus* Melot, *Hebeloma quercetorum* Quadr. and *Hygrophorus arbustivus* var. *quercetorum* Bon & Chevassut were commonly present in *T. magnatum* productive areas. On the other hand, Mello and colleagues [91] found that the mycelium of *Mortierella* sp. and *Fusarium* sp. was more abundant in *T. magnatum* productive niches than in unproductive ones. Moreover, near the picking point of white truffle ascomata, the fruiting bodies and the ectomycorrhizas of other truffle species were often found together, such as *T. rufum* and *T. maculatum* [69,70].

The copresence with *T. magnatum* of even more *Tuber* species was shown by examining the soil DNA at the fruiting points using specific primers [7,92]. Whereas the DNA of *T. borchii*, *T. dryophilum*, *T. rufum*, *T. maculatum*, *T. brumale*, and *T. macrosporum* were detected together with that of *T. magnatum*, the DNA of *T. macrosporum* and *T. aestivum* was never amplified, showing different ecological niches and or/competitive exclusion [7].

6. White Truffle Production and Harvesting in Natural Sites

Tuber magnatum is the truffle which has the longest tradition of being harvested by wild truffle hunters. Truffle hunting and extraction in Italy was inscribed in 2021 [93] in the Representative List of the Intangible Cultural Heritage of Humanity by UNESCO. That is a set of knowledge and practices that has been passed down from father to son, generation after generation [13]. The truffle hunters use trained dogs that are able to detect the truffle in the soil thanks to their highly developed sense of smell and use of special spades to extract the truffles without disturbing the soil conditions. Today, white truffle hunting has an important socioeconomic role, especially in the mountainous and hilly marginal areas of the Italian peninsula. In the Italian region of Molise, truffle hunters reach 1.47% of the total population [94]. The mystic of truffle hunting has created a truffle culture and access to a new truffle tourism, where a truffle hunter together with his dog will lead tourists through the woods looking for white truffle [95]. This interest in truffle hunting comes at a time when the rural economies of mountainous areas are struggling with high unemployment and with securing sustainable agricultural income [96].

Truffle hunting is traditionally practiced while respecting the environment and plant biodiversity, ensuring the seasonal regeneration of the truffle. However, white truffle natural production in Italy is endangered by several factors including the truffle hunting pressure in some Italian regions. The number of truffle hunters is constantly increasing, and their official number is now more than 70,000 [94] in Italy. Moreover, climate change and forest abandonment threaten the production of this truffle. Although some forest managements such as soil tillage, irrigation and selective thinning in production areas can help truffle production and *T. magnatum* mycelium development in the soil [97], they are difficult to realize in the mountainous areas of the Apennines where *T. magnatum* is widespread [13,98]. More specific studies for the improvement of natural white truffle production areas through the adoption of innovative silvicultural techniques are in progress in Italy [94].

7. Cultivation

Modern truffle cultivation started in the 1970s. It consists of producing mycorrhizal plants in a greenhouse and then out-planting them into areas with suitable soils and climate [13]. Using this technique, *T. melanosporum*, *T. aestivum*, *T. borchii* and other less valuable *Tuber* spp. are now successfully cultivated not only in Europe but also in countries well outside their natural distribution such as Australia, Chile, Israel, New Zealand, South Africa and the UK [13,17,99].

Because of the price commanded by *T. magnatum*, it was natural that research towards the end of the 1990s would focus on this species in Italy [100]. However, after a dozen years, the unreliability of production of the first plantations induced a degree of skepticism towards white truffle cultivation [101] and questions were raised whether the right inoculum had been used to begin with. Bullini et al., 1994 [102] then demonstrated, using isoenzymes, that none of the commercial plants that they had tested had been mycorrhized with *T. magnatum* but with other *Tuber* species such as *T. borchii* and *T. maculatum.* Mello et al., 2001 [16] and Rubini et al., 2001 [19], using a molecular analysis, subsequently showed that the ectomycorrhizal morphology of *T. magnatum* described in Italy in 1978 was incorrect and most of the plants produced at that time were contaminated with less valuable species of truffle—a consequence of the spore inoculation technique that had been used [13,103]. Plants that had been used to establish truffières sometimes were also contaminated with common greenhouse contaminants such as *Sphaerosporella brunnea* (Alb. & Schwein.) Svrček & Kubička and *Pulvinula convexella* (P. Karst.) Pfister [104–106]. Fortunately, some *T. magnatum* cultivation attempts were successful albeit limited to just a few ascomata [107]. Donnini et al., 2000 [107] and Gregori et al., 2008 [101] reported that different *T. magnatum* orchards established with *Tilia* spp., *Quercus* spp., *Populus* spp., *Salix caprea* L. and *Ostrya carpinifolia* Scop. mycorrhized with *T. magnatum* between 1986 and 1993 and started to produce from 6 to 10 years after planting. In the first years, these orchards produced large whitish ascocarps molecularly identified as *T. borchii* [17] and only later was *T. magnatum* [101] produced. The medium annual amount of *T. magnatum* ascocarps harvested was between a few hectograms and a few kilograms per hectare [101]. Similarly, a small truffière covering 4000 m^2 located in Emilia Romagna produced only *T. borchii* from the 3rd year to the 12th year after planting and then *T. magnatum* from the 13th year after planting, which completely replaced *T. borchii* (Figure 2) [108]. This behavior of several Italian *T. magnatum* truffle grounds could be explained as a species succession from an early-stage fungus (*T. borchii*) [109] and a later-stage fungus (*T. magnatum*), which tends to fruit under adult plants. The coexistence of the mycelia of different *Tuber* species in *T. magnatum* productive sites is a common feature as previously reported (see Section 5) [7,92]. Probably, these other *Tuber* spp. do not compete with *T. magnatum* as thought in the past but share the same ecological niche establishing neutral or even synergistic relationships. However, in these productive first *T. magnatum* plantations, we cannot exclude that the production was a spontaneous event and not the consequence of planting, at that time, uncertain *T. magnatum* mycorrhized plants. These plantations were in fact all realized in areas where *T. magnatum* naturally occurred. A spontaneous mycorrhization often occurs in poplar planted in the fresh, deep calcareous soils along the channels of the Padania plain in Italy. Thus, in order to increase *T. magnatum* production the member of the local truffle hunting association used to plant poplar cuttings renting the soil from public administration [17].

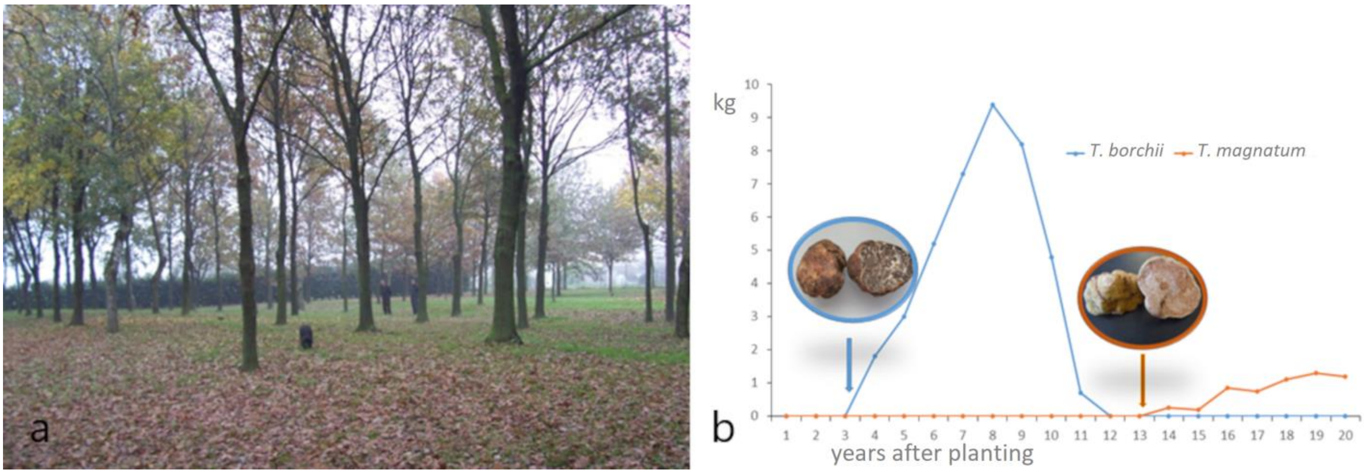

Figure 2. Old *T. magnatum* cultivation. (**a**) *T. magnatum* orchard of *Q. robur* mycorrhized plants; (**b**) ascocarp production trend during the first 20 years after plantation.

Nowadays, *T. magnatum* cultivation has received a new impetus [110]. With the development of specific primers, *T. magnatum* mycorrhizas and mycelium can now be unequivocally identified [71,98,111] and several nurseries have begun to produce certified *T. magnatum* mycorrhized plants both in Italy and France [17,112]. However, the prices of these plants are higher than for those inoculated with other species of truffle because of the high cost of the inoculum and the still present difficulties in producing plants well colonized with *T. magnatum*.

In 2019 and 2020, it was demonstrated in France that it was possible to cultivate *T. magnatum* just like other species of truffles, and moreover, outside the natural *T. magnatum* area [112], and only 4.5 years after planting, similar to *T. melanosporum* and *T. aestivum* orchards [113,114]. This is particularly exciting, but field studies are still necessary to optimize *T. magnatum* cultivation practices.

8. Conclusions and Prospects

Tuber magnatum is undoubtedly the most sought-after and valuable of the truffles. Although many studies have been devoted to this species over the past thirty years, numerous aspects of its biology and ecology are still to be unraveled. A Google scholar search covering the past 22 years produced 2440 results from 1990 to today (retrieved on 21 September 2022). Moreover, its peculiar environmental characteristics and its harvesting culture contribute to create an aura of mystery around this truffle and to increase its commercial value.

After numerous failures and uncertain results, *T. magnatum* cultivation has now been deemed possible inside and outside Italy [112]. It opens up new perspectives to increase its production, which has actually been declining due to several factors including climate changes and overharvesting. Planning actions to protect the white truffle's natural habitat and to preserve this species and the plantation of experimental plantations are needed. However, an optimization of the management strategies of *T. magnatum* cultivation is necessary to definitely tame this precious truffle. New molecular tools perfected in the last years (such as qPCR) for the detection and quantification of *T. magnatum* mycelium in the soil could assist in this indispensable research processes.

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References

1. Mello, A.; Murat, C.; Bonfante, P. Truffles: Much More Than a Prized and Local Fungal Delicacy. *FEMS Microbiol. Lett.* **2006**, *260*, 1–8. [CrossRef] [PubMed]
2. Bonito, G.; Smith, M.E.; Nowak, M.; Healy, R.A.; Guevara, G.; Cázares, E.; Kinoshita, A.; Nouhra, E.R.; Domínguez, L.S.; Tedersoo, L.; et al. Historical Biogeography and Diversification of Truffles in the Tuberaceae and Their Newly Identified Southern Hemisphere Sister Lineage. *PLoS ONE* **2013**, *8*, e52765. [CrossRef]
3. Percudani, R.; Trevisi, A.; Zambonelli, A.; Ottonello, S. Molecular Phylogeny of Truffles (Pezizales: Terfeziaceae, Tuberaceae) Derived from Nuclear rDNA Sequence Analysis. *Mol. Phylogenet. Evol.* **1999**, *13*, 169–180. [CrossRef] [PubMed]
4. Jeandroz, S.; Murat, C.; Wang, Y.; Bonfante, P.; le Tacon, F. Molecular Phylogeny and Historical Biogeography of the Genus *Tuber*, the 'True Truffles. *J. Biogeogr.* **2008**, *35*, 815–829. [CrossRef]
5. Bonito, G.; Trappe, J.M.; Rawlinson, P.; Vilgalys, R. Improved Resolution of Major Clades within *Tuber* and Taxonomy of Species within the *Tuber gibbosum* Complex. *Mycologia* **2010**, *102*, 1042–1057. [CrossRef]
6. The Most Expensive Food in The World: 13 Costly Food Items. Available online: https://luxurycolumnist.com/the-most-expensive-food-in-the-world/ (accessed on 20 September 2022).
7. Leonardi, P.; Baroni, R.; Puliga, F.; Iotti, M.; Salerni, E.; Perini, C.; Zambonelli, A. Co-Occurrence of True Truffle Mycelia in *Tuber magnatum* Fruiting Sites. *Mycorrhiza* **2021**, *31*, 389–394. [CrossRef]
8. Heinonen, I. The Soil Bacteria Associated with *Tuber magnatum* Productive Sites. Master's Thesis, University of Helsinki, Helsinki, Finland, 2021.
9. Urbani, O.; (Truffleland s.r.l, S. Anatolia di Narco, Perugia, Italy). Personal communication, 2022.
10. Amicucci, A.; Barbieri, E.; Sparvoli, V.; Gioacchini, A.M.; Calcabrini, C.; Palma, F.; Stocchi, V.; Zambonelli, A. Microbial and Pigment Profile of the Reddish Patch Occurring within *Tuber magnatum* Ascomata. *Fungal Biol* **2018**, *122*, 1134–1141. [CrossRef]
11. Ratti, C.; Iotti, M.; Zambonelli, A.; Terlizzi, F. Mycoviruses Infecting True Truffles. In *True Truffle (Tuber spp.) in the World*, 1st ed.; Zambonelli, A., Iotti, M., Murat, C., Eds.; Springer: Berlin/Heidelberg, Germany, 2016; Volume 47, pp. 333–349. [CrossRef]
12. Monaco, P.; Naclerio, G.; Bucci, A.; Mello, A. Determination of the Peridium Thickness of *Tuber magnatum* Ascomata from Molise Region. *Ital. J. Mycol.* **2021**, *50*, 92–98. [CrossRef]
13. Hall, I.R.; Brown, G.T.; Zambonelli, A. *Taming the Truffle. The History Lore and Science of the Ultimate Mushroom*, 1st ed.; Timber Press: Portland, OR, USA, 2007; p. 304.
14. Fontana, A. Miceli Di Funghi Ipogei in Coltura Pura. In Proceedings of the 1st International Truffle Conference, Spoleto, Italy, 24–25 May 1968; pp. 127–134.
15. Mischiati, P.; Fontana, A. In Vitro Culture of *Tuber magnatum* Mycelium Isolated from Mycorrhizas. *Mycol. Res.* **1993**, *97*, 40–44. [CrossRef]
16. Mello, A.; Fontana, A.; Meotto, F.; Comandini, O.; Bonfante, P. Molecular and Morphological Characterization of *Tuber magnatum* Mycorrhizas in a Long-Term Survey. *Microbiol. Res.* **2001**, *155*, 279–284. [CrossRef]
17. Zambonelli, A.; (University of Bologna, Bologna, Italy). Personal communication, 2022.
18. Iotti, M.; Amicucci, A.; Stocchi, V.; Zambonelli, A. Morphological and Molecular Characterization of Mycelia of some *Tuber* Species in Pure Culture. *New Phytol.* **2002**, *155*, 499–505. [CrossRef] [PubMed]
19. Rubini, A.; Paolocci, F.; Granetti, B.; Arcioni, S. Morphological Characterization of Molecular-Typed *Tuber magnatum* Ectomycorrhizae. *Mycorrhiza* **2001**, *11*, 179–185. [CrossRef]
20. Ori, F.; Menotta, M.; Leonardi, M.; Amicucci, A.; Zambonelli, A.; Covès, H.; Selosse, M.A.; Schneider-Maunoury, L.; Pacioni, G.; Iotti, M. Effect of Slug Mycophagy on *Tuber aestivum* Spores. *Fungal Biol.* **2021**, *125*, 796–805. [CrossRef] [PubMed]
21. Elliott, T.F.; Truong, C.; Jackson, S.; Zúñiga, C.L.; Trappe, J.M.; Vernes, K. Mammalian Mycophagy: A Global Review of Ecosystem Interactions between Mammals and Fungi. *FUSE* **2022**, *9*, 99–159. [CrossRef]
22. Schmidberger, P.C.; Schieberle, P. Characterization of the Key Aroma Compounds in White Alba Truffle (*Tuber magnatum* Pico) and Burgundy Truffle (*Tuber uncinatum*) by Means of the Sensomics Approach. *J. Agric. Food Chem.* **2017**, *65*, 9287–9296. [CrossRef]
23. Niimi, J.; Deveau, A.; Splivallo, R. Geographical-Based Variations in White Truffle *Tuber magnatum* Aroma Is Explained by Quantitative Differences in Key Volatile Compounds. *New Phytol.* **2021**, *230*, 1623–1638. [CrossRef]

24. Splivallo, R.; Ottonello, S.; Mello, A.; Karlovsky, P. Truffle Volatiles: From Chemical Ecology to Aroma Biosynthesis. *New Phytol.* **2011**, *189*, 688–699. [CrossRef]
25. Wernig, F.; Buegger, F.; Pritsch, K.; Splivallo, R. Composition and Authentication of Commercial and Home-Made White Truffle-Flavored Oils. *Food Control.* **2018**, *87*, 9–16. [CrossRef]
26. Phong, W.N.; Gibberd, M.R.; Payne, A.D.; Dykes, G.A.; Coorey, R. Methods Used for Extraction of Plant Volatiles Have Potential to Preserve Truffle Aroma: A Review. *Compr. Rev. Food Sci. Food Saf.* **2022**, *21*, 1677–1701. [CrossRef]
27. Gioacchini, A.M.; Menotta, M.; Guescini, M.; Saltarelli, R.; Ceccaroli, P.; Amicucci, A.; Barbieri, E.; Giomaro, G.; Stocchi, V. Geographical Traceability of Italian White Truffle (*Tuber magnatum* Pico) by the Analysis of Volatile Organic Compounds. *Rapid Commun. Mass Spectrom.* **2008**, *22*, 3147–3153. [CrossRef]
28. Strojnik, L.; Grebenc, T.; Ogrinc, N. Species and Geographic Variability in Truffle Aromas. *Food Chem. Toxicol.* **2020**, *142*, 111434. [CrossRef] [PubMed]
29. Vita, F.; Giuntoli, B.; Bertolini, E.; Taiti, C.; Marone, E.; D'Ambrosio, C.; Trovato, E.; Sciarrone, D.; Zoccali, M.; Balestrini, R.; et al. Tuberomics: A Molecular Profiling for the Adaption of Edible Fungi (*Tuber magnatum* Pico) to Different Natural Environments. *BMC Genom.* **2020**, *21*, 90. [CrossRef]
30. Buzzini, P.; Gasparetti, C.; Turchetti, B.; Cramarossa, M.R.; Vaughan-Martini, A.; Martini, A.; Pagnoni, U.M.; Forti, L. Production of Volatile Organic Compounds (VOCs) by Yeasts Isolated from the Ascocarps of Black (*Tuber melanosporum* Vitt.) and White (*Tuber magnatum* Pico) Truffles. *Arch. Microbiol.* **2005**, *184*, 187–193. [CrossRef] [PubMed]
31. Vahdatzadeh, M.; Deveau, A.; Splivallo, R. The Role of the Microbiome of Truffles in Aroma Formation: A Meta-Analysis Approach. *Appl. Environ. Microbiol.* **2015**, *81*, 6946–6952. [CrossRef] [PubMed]
32. Splivallo, R.; Deveau, A.; Valdez, N.; Kirchhoff, N.; Frey-Klett, P.; Karlovsky, P. Bacteria Associated with Truffle-Fruiting Bodies Contribute to Truffle Aroma. *Environ. Microbiol.* **2015**, *17*, 2647–2660. [CrossRef]
33. Niimi, J.; Deveau, A.; Splivallo, R. Aroma and Bacterial Communities Dramatically Change with Storage of Fresh White Truffle *Tuber magnatum*. *LWT* **2021**, *151*, 112125. [CrossRef]
34. Pennazza, G.; Fanali, C.; Santonico, M.; Dugo, L.; Cucchiarini, L.; Dachà, M.; D'Amico, A.; Costa, R.; Dugo, P.; Mondello, L. Electronic Nose and GC–MS Analysis of Volatile Compounds in *Tuber magnatum* Pico: Evaluation of Different Storage Conditions. *Food Chem.* **2013**, *136*, 668–674. [CrossRef]
35. Payen, T.; Murat, C.; Bonito, G. Truffle Phylogenomics: New Insights into Truffle Evolution and Truffle Life Cycle. *Adv. Bot. Res.* **2014**, *70*, 211–234. [CrossRef]
36. Belfiori, B.; Riccioni, C.; Paolocci, F.; Rubini, A. Characterization of the Reproductive Mode and Life Cycle of the Whitish Truffle *T. borchii*. *Mycorrhiza* **2016**, *26*, 515–527. [CrossRef]
37. Selosse, M.A.; Schneider-Maunoury, L.; Taschen, E.; Rousset, F.; Richard, F. Black Truffle, a Hermaphrodite with Forced Unisexual Behaviour. *Trends Microbiol.* **2017**, *25*, 784–787. [CrossRef]
38. Leonardi, P.; Murat, C.; Puliga, F.; Iotti, M.; Zambonelli, A. Ascoma Genotyping and Mating Type Analyses of Mycorrhizas and Soil Mycelia of *Tuber borchii* in a Truffle Orchard Established by Mycelial Inoculated Plants. *Environ. Microbiol.* **2020**, *22*, 964–975. [CrossRef] [PubMed]
39. Frizzi, G.; Lalli, G.; Miranda, M.; Pacioni, G. Intraspecific Isozyme Variability in Italian Populations of the White Truffle *Tuber magnatum*. *Mycol. Res.* **2001**, *105*, 365–369. [CrossRef]
40. Mello, A.; Murat, C.; Vizzini, A.; Gavazza, V.; Bonfante, P. *Tuber magnatum* Pico, a Species of Limited Geographical Distribution: Its Genetic Diversity inside and Outside a Truffle Ground. *Environ. Microbiol.* **2005**, *7*, 55–65. [CrossRef] [PubMed]
41. Rubini, A.; Paolocci, F.; Riccioni, C.; Vendramin, G.G.; Arcioni, S. Genetic and Phylogeographic Structures of the Symbiotic Fungus *Tuber magnatum*. *Appl. Environ. Microbiol.* **2005**, *71*, 6584–6589. [CrossRef] [PubMed]
42. Paolocci, F.; Rubini, A.; Riccioni, C.; Arcioni, S. Reevaluation of the Life Cycle of *Tuber magnatum*. *Appl. Environ. Microbiol.* **2006**, *72*, 2390–2393. [CrossRef] [PubMed]
43. Murat, C.; Payen, T.; Noel, B.; Kuo, A.; Morin, E.; Chen, J.; Kohler, A.; Krizsán, K.; Balestrini, R.; da Silva, C.; et al. Pezizomycetes Genomes Reveal the Molecular Basis of Ectomycorrhizal Truffle Lifestyle. *Nat. Ecol. Evol.* **2018**, *2*, 1956–1965. [CrossRef]
44. Rubini, A.; Belfiori, B.; Riccioni, C.; Arcioni, S.; Martin, F.; Paolocci, F. *Tuber melanosporum*: Mating Type Distribution in a Natural Plantation and Dynamics of Strains of Different Mating Types on the Roots of Nursery-Inoculated Host Plants. *New Phytol.* **2011**, *189*, 723–735. [CrossRef]
45. Murat, C.; Rubini, A.; Riccioni, C.; de la Varga, H.; Akroume, E.; Belfiori, B.; Guaragno, M.; le Tacon, F.; Robin, C.; Halkett, F.; et al. Fine-Scale Spatial Genetic Structure of the Black Truffle (*Tuber melanosporum*) Investigated with Neutral Microsatellites and Functional Mating Type Genes. *New Phytol.* **2013**, *199*, 176–187. [CrossRef]
46. Urban, A.; Neuner-Plattner, I.; Krisai-Greilhuber, I.; Haselwandter, K. Molecular Studies on Terricolous Microfungi Reveal Novel Anamorphs of Two *Tuber* Species. *Mycol. Res.* **2004**, *108*, 749–758. [CrossRef]
47. Healy, R.A.; Smith, M.E.; Bonito, G.M.; Pfister, D.H.; Ge, Z.W.; Guevara, G.G.; Williams, G.; Stafford, K.; Kumar, L.; Lee, T.; et al. High Diversity and Widespread Occurrence of Mitotic Spore Mats in Ectomycorrhizal Pezizales. *Mol. Ecol.* **2013**, *22*, 1717–1732. [CrossRef]
48. Hall, I.R.; (Truffles & Mushrooms (Consulting) Limited, Dunedin, New Zealand). Personal communication, 2022.
49. Nakano, S.; Obase, K.; Nakamura, N.; Kinoshita, A.; Kuroda, K.; Yamanaka, T. Mitospore Formation on Pure Cultures of *Tuber japonicum* (Tuberaceae, Pezizales) in Vitro. *Mycorrhiza* **2022**, *32*, 353–360. [CrossRef] [PubMed]

50. Taschen, E.; Rousset, F.; Sauve, M.; Benoit, L.; Dubois, M.P.; Richard, F.; Selosse, M.A. How the Truffle Got Its Mate: Insights from Genetic Structure in Spontaneous and Planted Mediterranean Populations of *Tuber melanosporum*. *Mol. Ecol.* **2016**, *25*, 5611–5627. [CrossRef] [PubMed]
51. de la Varga, H.; le Tacon, F.; Lagoguet, M.; Todesco, F.; Varga, T.; Miquel, I.; Barry-Etienne, D.; Robin, C.; Halkett, F.; Martin, F.; et al. Five Years Investigation of Female and Male Genotypes in Périgord Black Truffle (*Tuber melanosporum* Vittad.) Revealed Contrasted Reproduction Strategies. *Environ. Microbiol* **2017**, *19*, 2604–2615. [CrossRef] [PubMed]
52. Staubli, F.; Imola, L.; Dauphin, B.; Molinier, V.; Pfister, S.; Piñuela, Y.; Schürz, L.; Sproll, L.; Steidinger, B.S.; Stobbe, U.; et al. Hidden Fairy Rings and Males—Genetic Patterns of Natural Burgundy Truffle (*Tuber aestivum* Vittad.) Populations Reveal New Insights into Its Life Cycle. *Environ. Microbiol* **2022**, *1*, 1–16. [CrossRef]
53. le Tacon, F.; Rubini, A.; Murat, C.; Riccioni, C.; Robin, C.; Belfiori, B.; Zeller, B.; de la Varga, H.; Akroume, E.; Deveau, A.; et al. Certainties and Uncertainties about the Life Cycle of the Périgord Black Truffle (*Tuber melanosporum* Vittad.). *Ann. For. Sci.* **2016**, *73*, 105–117. [CrossRef]
54. Schneider-Maunoury, L.; Deveau, A.; Moreno, M.; Todesco, F.; Belmondo, S.; Murat, C.; Courty, P.E.; Jąkalski, M.; Selosse, M.A. Two Ectomycorrhizal Truffles, *Tuber melanosporum* and *T. aestivum*, Endophytically Colonise Roots of Non-Ectomycorrhizal Plants in Natural Environments. *New Phytol.* **2020**, *225*, 2542–2556. [CrossRef]
55. Büntgen, U.; Lendorff, H.; Lendorff, A.; Leuchtmann, A.; Peter, M.; Bagi, I.; Egli, S. Truffles on the Move. *Front. Ecol. Environ.* **2019**, *17*, 200–202. [CrossRef]
56. Bratek, Z.; Gógán, A.; Halász, K.; Bagi, I.; Erdei, V.; Bujàki, G. The Northernmost Habitats of *Tuber magnatum* Known from Hungary. In Proceedings of the 1st Hypogean Mushroom Conference, Rabat, Malta, 6–8 April 2004; pp. 6–8.
57. Suwannarach, N.; Kumla, J.; Meerak, J.; Lumyong, S. *Tuber magnatum* in Thailand, a First Report from Asia. *Mycotaxon* **2017**, *132*, 635–642. [CrossRef]
58. Gregori, G.L. Problems and Expectations with the Cultivation of *Tuber magnatum*. In Proceedings of the 2nd International Conference on Edible Mycorrhizal Mushrooms, Cristchurch, New Zealand, 3–6 July 2001; pp. 1–9.
59. Marjanović, Z.; Glišić, A.; Mutavdžić, D.; Saljnikov, E.; Bragato, G. Ecosystems Supporting *Tuber magnatum* Pico Production in Serbia Experience Specific Soil Environment Seasonality That May Facilitate Truffle Lifecycle Completion. *Appl. Soil Ecol.* **2015**, *95*, 179–190. [CrossRef]
60. Iotti, M.; Leonardi, P.; Vitali, G.; Zambonelli, A. Effect of Summer Soil Moisture and Temperature on the Vertical Distribution of *Tuber magnatum* Mycelium in Soil. *Biol. Fertil. Soils* **2018**, *54*, 707–716. [CrossRef]
61. Bragato, G.; Marjanović, Ž.S. Soil Characteristics for *Tuber magnatum*. In *True Truffle (Tuber spp.) in the World*, 1st ed.; Zambonelli, A., Iotti, M., Murat, C., Eds.; Springer: Berlin/Heidelberg, Germany, 2016; Volume 47, pp. 191–209. [CrossRef]
62. Bencivenga, M.; Granetti, B. Flora, Vegetazione e Natura Dei Terreni Di Alcune Tartufaie Naturali Di *Tuber magnatum* Pico Dell'Italia Centrale. In Proceedings of the 2nd International Truffle Conference, Spoleto, Italy, 24–27 November 1988; pp. 415–431.
63. Julou, T.; Burghardt, B.; Gebauer, G.; Berveiller, D.; Damesin, C.; Selosse, M.A. Mixotrophy in Orchids: Insights from a Comparative Study of Green Individuals and Nonphotosynthetic Individuals of *Cephalanthera damasonium*. *New Phytol.* **2005**, *166*, 639–653. [CrossRef] [PubMed]
64. Ouanphanivanh, N.; Merényi, Z.; Orczán, K.; Bratek, Z.; Szigeti, Z.; Illyés, Z. Could Orchids Indicate Truffle Habitats?: Mycorrhizal Association between Orchids and Truffles. *Acta Biol. Szeged.* **2008**, *52*, 229–232. Available online: https://abs.bibl.u-szeged.hu/index.php/abs/article/view/2630 (accessed on 22 September 2022).
65. Illyés, Z.; Ouanphanivanh, N.; Rudnóy, S.; Orczán, Á.K.; Bratek, Z. The Most Recent Results on Orchid Mycorrhizal Fungi in Hungary. *Acta Biol. Hung.* **2011**, *61*, 68–76. [CrossRef] [PubMed]
66. Teššitelová, T.; Teššitel, J.; Jersáková, J.; Říhová, G.; Selosse, M.A. Symbiotic Germination Capability of Four *Epipactis* Species (Orchidaceae) Is Broader than Expected from Adult Ecology. *Am. J. Bot.* **2012**, *99*, 1020–1032. [CrossRef] [PubMed]
67. Graziosi, S.; Leonardi, P.; Zambonelli, A. Symbiotic Interations between Orchids and *Tuber borchii*. *Ital. J. Mycol.* **2022**, *51*, 58–65. [CrossRef]
68. Wang, Y.; He, X.; Yu, F. Non-Host Plants: Are They Mycorrhizal Networks Players? *Plant. Divers.* **2022**, *44*, 127–134. [CrossRef]
69. Murat, C.; Vizzini, A.; Bonfante, P.; Mello, A. Morphological and Molecular Typing of the Below-Ground Fungal Community in a Natural *Tuber magnatum* Truffle-Ground. *FEMS Microbiol. Lett.* **2005**, *245*, 307–313. [CrossRef]
70. Leonardi, M.; Iotti, M.; Oddis, M.; Lalli, G.; Pacioni, G.; Leonardi, P.; Maccherini, S.; Perini, C.; Salerni, E.; Zambonelli, A. Assessment of Ectomycorrhizal Fungal Communities in the Natural Habitats of *Tuber magnatum* (Ascomycota, Pezizales). *Mycorrhiza* **2013**, *23*, 349–358. [CrossRef]
71. Iotti, M.; Leonardi, M.; Oddis, M.; Salerni, E.; Baraldi, E.; Zambonelli, A. Development and Validation of a Real-Time PCR Assay for Detection and Quantification of *Tuber magnatum* in Soil. *BMC Microbiol* **2012**, *12*, 93. [CrossRef]
72. Zampieri, E.; Murat, C.; Cagnasso, M.; Bonfante, P.; Mello, A. Soil Analysis Reveals the Presence of an Extended Mycelial Network in a *Tuber magnatum* Truffle-ground. *FEMS Microbiol. Ecol.* **2010**, *71*, 43–49. [CrossRef]
73. Ceruti, A.; Fontana, A.; Nosenzo, C. *European Species of the Genus Tuber. An. Historical Revision.*, 1st ed.; Museo Regionale di Scienze Naturali: Torino, Italy, 2003; p. 467.
74. Le Tacon, F. Influence of Climate on Natural Distribution of Species and Truffle Production. In *True Truffle (Tuber spp.) in the World*, 1st ed.; Zambonelli, A., Iotti, M., Murat, C., Eds.; Springer: Berlin/Heidelberg, Germany, 2016; Volume 47, pp. 153–167. [CrossRef]

75. Iotti, M.; Leonardi, M.; Lancellotti, E.; Salerni, E.; Oddis, M.; Leonardi, P.; Perini, C.; Pacioni, G.; Zambonelli, A. Spatio-Temporal Dynamic of *Tuber magnatum* Mycelium in Natural Truffle Grounds. *PLoS ONE* **2014**, *9*, e115921. [CrossRef] [PubMed]
76. Ekblad, A.; Wallander, H.; Godbold, D.L.; Cruz, C.; Johnson, D.; Baldrian, P.; Björk, R.G.; Epron, D.; Kieliszewska-Rokicka, B.; Kjøller, R.; et al. The Production and Turnover of Extramatrical Mycelium of Ectomycorrhizal Fungi in Forest Soils: Role in Carbon Cycling. *Plant Soil* **2013**, *366*, 1–27. [CrossRef]
77. Frey-Klett, P.; Garbaye, J.; Tarkka, M. The Mycorrhiza Helper Bacteria Revisited. *New Phytol.* **2007**, *176*, 22–36. [CrossRef]
78. Monaco, P.; Naclerio, G.; Mello, A.; Bucci, A. Role and Potentialities of Bacteria Associated with *Tuber magnatum*: A Mini-Review. *Front. Microbiol.* **2022**, *13*, 1–7. [CrossRef]
79. Citterio, B.; Cardoni, P.; Potenza, L.; Amicucci, A.; Stocchi, V.; Gola, G.; Nuti, M. Isolation of Bacteria from Sporocarps of *Tuber magnatum* Pico, *Tuber borchii* Vitt. and *Tuber maculatum* Vitt. In *Biotechnology of Ectomycorrhizae*; Springer: Berlin/Heidelberg, Germany, 1995; pp. 241–248. [CrossRef]
80. Barbieri, E.; Guidi, C.; Bertaux, J.; Frey-Klett, P.; Garbaye, J.; Ceccaroli, P.; Saltarelli, R.; Zambonelli, A.; Stocchi, V. Occur-rence and Diversity of Bacterial Communities in *Tuber magnatum* during Truffle Maturation. *Environ. Microbiol.* **2007**, *9*, 2234–2246. [CrossRef]
81. Barbieri, E.; Ceccaroli, P.; Saltarelli, R.; Guidi, C.; Potenza, L.; Basaglia, M.; Fontana, F.; Baldan, E.; Casella, S.; Ryahi, O.; et al. New Evidence for Nitrogen Fixation within the Italian White Truffle *Tuber magnatum*. *Fungal Biol.* **2010**, *114*, 936–942. [CrossRef]
82. Monaco, P.; Bucci, A.; Naclerio, G.; Mello, A. Heterogeneity of the White Truffle *Tuber magnatum* in a Limited Geographic Area of Central-Southern Italy. *Environ. Microbiol. Rep.* **2021**, *13*, 591–599. [CrossRef]
83. Il Tartufo: Una Simbiosi Multipla. Available online: https://www.georgofili.it/ (accessed on 21 September 2022).
84. Barbieri, E.; Ceccaroli, P.; Palma, F.; Agostini, D.; Stocchi, V. Ectomycorrhizal Helper Bacteria: The Third Partner in the Symbiosis. In *Edible Ectomycorrhizal Mushrooms*, 1st ed.; Zambonelli, A., Bonito, G.M., Eds.; Springer: Berlin/Heidelberg, Germany, 2012; Volume 34, pp. 125–141. [CrossRef]
85. Antony-Babu, S.; Deveau, A.; van Nostrand, J.D.; Zhou, J.; le Tacon, F.; Robin, C.; Frey-Klett, P.; Uroz, S. Black Truffle-Associated Bacterial Communities during the Development and Maturation of *Tuber melanosporum* Ascocarps and Putative Functional Roles. *Environ. Microbiol.* **2014**, *16*, 2831–2847. [CrossRef]
86. Benucci, G.M.N.; Bonito, G.M. The Truffle Microbiome: Species and Geography Effects on Bacteria Associated with Fruiting Bodies of Hypogeous Pezizales. *Microb. Ecol.* **2016**, *72*, 4–8. [CrossRef]
87. Sillo, F.; Vergine, M.; Luvisi, A.; Calvo, A.; Petruzzelli, G.; Balestrini, R.; Mancuso, S.; de Bellis, L.; Vita, F. Bacterial Communities in the Fruiting Bodies and Background Soils of the White Truffle *Tuber magnatum*. *Front. Microbiol.* **2022**, *13*, 1288. [CrossRef]
88. Pavić, A.; Stanković, S.; Saljnikov, E.; Krüger, D.; Buscot, F.; Tarkka, M.; Marjanović, Ž. Actinobacteria May Influence White Truffle (*Tuber magnatum* Pico) Nutrition, Ascocarp Degradation and Interactions with Other Soil Fungi. *Fungal Ecol.* **2013**, *6*, 527–538. [CrossRef]
89. Giorgio, M.; Niccolò, B.G.M.; Benedetta, T.; Luisa, M.; Leonardo, B.F.; Gregory, B.; Pietro, B.; Alberto, A.; Domizia, D.; Emidio, A. Fungal and Bacterial Diversity in the *Tuber magnatum* Ecosystem and Microbiome. *Microb. Ecol.* **2022**, *1*, 1–14. [CrossRef] [PubMed]
90. Lalli, G.; Leonardi, M.; Oddis, M.; Pacioni, G.; Salerni, E.; Iotti, M.; Zambonelli, A. Expanding the Understanding of a Forest Ectomycorrhizal Community by Combining Root Tips and Fruiting Bodies: A Case Study of *Tuber magnatum* Stands. *Turk. J. Botany* **2015**, *39*, 527–534. [CrossRef]
91. Mello, A.; Miozzi, L.; Vizzini, A.; Napoli, C.; Kowalchuk, G.; Bonfante, P. Bacterial and Fungal Communities Associated with *Tuber magnatum*-productive Niches. *Plant. Biosyst.* **2010**, *144*, 323–332. [CrossRef]
92. Zampieri, E.; Mello, A.; Bonfante, P.; Murat, C. PCR Primers Specific for the Genus *Tuber* reveal the Presence of Several Truffle Species in a Truffle-Ground. *FEMS Microbiol. Lett.* **2009**, *297*, 67–72. [CrossRef]
93. Truffle Hunting and Extraction in Italy, Traditional Knowledge and Practice-Intangible Heritage-Culture Sector–UNESCO. Available online: https://ich.unesco.org/en/RL/truffle-hunting-and-extraction-in-italy-traditional-knowledge-and-practice-01395 (accessed on 22 September 2022).
94. Mipaaf-Tavolo Tecnico Settore Tartufo. Available online: https://www.politicheagricole.it/flex/cm/pages/ServeBLOB.php/L/IT/IDPagina/11100 (accessed on 22 September 2022).
95. Truffle Turism. I Sapori Della Collina Di Torino. Available online: https://isaporidellacollinaditorino.com/en/truffle-turism-2/ (accessed on 22 September 2022).
96. Águeda, B.; Zambonelli, A.; Molina, R. *Tuber* 2013: Scientific Advances in Sustainable Truffle Culture. *Mycorrhiza* **2014**, *24*, 1–4. [CrossRef]
97. Salerni, E.; Iotti, M.; Leonardi, P.; Gardin, L.; D'Aguanno, M.; Perini, C.; Pacioni, P.; Zambonelli, A. Effects of Soil Tillage on *Tuber magnatum* Development in Natural Truffières. *Mycorrhiza* **2014**, *24*, 79–87. [CrossRef]
98. Laruccia, N.; Marletto, V.; Leonardi, P.; Puliga, F.; Zambonelli, A. Map of Suitability for the Spontaneous Growth of *Tuber magnatum* in Emilia-Romagna (Italy). *Ital. J. Mycol.* **2020**, *49*, 38–53. [CrossRef]
99. Reyna, S.; Garcia-Barreda, S. Black Truffle Cultivation: A Global Reality. *For. Syst.* **2014**, *23*, 317–328. [CrossRef]
100. Giovannetti, G. Prima Produzione Di Carpofori Di *Tuber magnatum* Pico Da Piante Fornite Da Vivai Specializzati. In Proceedings of the 2nd International Truffle Conference, Spoleto, Italy, 24–27 November 1988; pp. 297–302.

101. Gregori, G.L.; Donnini, D.; Bencivenga, M. *Tuber magnatum*: Alcuni Esempi Produttivi Di Tartufaie Coltivate in Italia. In Proceedings of the 3rd International Truffle Conference, Spoleto, Italy, 25–28 November 2008; pp. 741–749.
102. Bullini, L.; Biocca, E.; Chevalier, G.; Dupré, C.; Ferrara, A.M.; Palenzona, M.; Urbanelli, S. Use of Genetic Markers in the Study of Mycorrhizae and Mycelia of the Genus *Tuber*. In Proceedings of the International Symposium on Biotechnology of Ectomycorrhizae: Molecular approaches, Urbino, Italy, 9–11 November 1994; pp. 9–11.
103. Iotti, M.; Piattoni, F.; Zambonelli, A. Techniques for Host Plant Inoculation with Truffles and Other Edible Ectomycorrhizal Mushrooms. In *Edible Ectomycorrhizal Mushrooms*, 1st ed.; Zambonelli, A., Bonito, G.M., Eds.; Springer: Berlin/Heidelberg, Germany, 2012; pp. 145–161. [CrossRef]
104. Donnini, D.; Bencivenga, M. Micorrize Inquinanti Frequenti Nelle Piante Tartufigene. *Micol. Ital.* **1995**, *2*, 185–207.
105. Amicucci, A.; Zambonelli, A.; Guidi, C.; Stocchi, V. Morphological and Molecular Characterisation of *Pulvinula constellatio* Ectomycorrhizae. *FEMS Microbiol. Lett.* **2001**, *194*, 121–125. [CrossRef] [PubMed]
106. Donnini, D.; di Massimo, G.; Baciarelli Falini, L.; Bencivenga, M. Le (ECM) Delle Tartufaie Naturali e Coltivate in Umbria. In Proceedings of the 100th Congress of Italian Botany Society, Rome, Italy, 20–23 September 2005; pp. 846–847.
107. Donnini, D.; Bencivenga, M.; Baciarelli Falini, L. Risultati Di Esperienze Pluriennali Nella Coltivazione Di *Tuber magnatum* Pico in Umbria. *Micol. Ital.* **2000**, *29*, 33–39.
108. Previati, A.; (Ferrara, Italy). Personal communication, 2022.
109. Zambonelli, A.; Lotti, M.; Giomaro, G.; Hall, I.; Stocchi, V.T. *borchii* Cultivation: An Interesting Perspective. In Proceedings of the 2nd International Conference on Edible Mycorrhizal Mushrooms, Crop & Food Research, Christcurch, New Zealand, 3–6 July 2002; pp. 1–7.
110. Fleming, N. Fungal Findings Excite Truffle Researchers and Gastronomes. *Nature* **2022**. [CrossRef] [PubMed]
111. Amicucci, A.; Zambonelli, A.; Giomaro, G.; Potenza, L. Identification of Ectomycorrhizal Fungi of the Genus *Tuber* by Species-specific ITS Primers. *Mol. Ecol.* **1998**, *7*, 273–277. [CrossRef]
112. Bach, C.; Beacco, P.; Cammaletti, P.; Babel-Chen, Z.; Levesque, E.; Todesco, F.; Cotton, C.; Robin, B.; Murat, C. First Production of Italian White Truffle (*Tuber magnatum* Pico) Ascocarps in an Orchard Outside Its Natural Range Distribution in France. *Mycorrhiza* **2021**, *31*, 383–388. [CrossRef]
113. Olivier, J.M.; Savignac, J.C.; Sourzat, P. *Truffe et Trufficulture*, 3rd ed.; Fanlac: Perigueux, France, 2018; p. 352.
114. Todesco, F.; Belmondo, S.; Guignet, Y.; Laurent, L.; Fizzala, S.; le Tacon, F.; Murat, C. Soil Temperature and Hydric Potential Influences the Monthly Variations of Soil *Tuber aestivum* DNA in a Highly Productive Orchard. *Sci. Rep.* **2019**, *9*, 12964. [CrossRef]