Scientific Papers. Series E. Land Reclamation, Earth Observation & Surveying, Environmental Engineering. Vol. X, 2021
Print ISSN 2285-6064, CD-ROM ISSN 2285-6072, Online ISSN 2393-5138, ISSN-L 2285-6064

STUDY OF THE SUCCESSFUL APPROACH TO TRUFFLE GROWING IN EUROPE - REVIEW

Veselka VLAHOVA

Agricultural University - Plovdiv, 12 Mendeleev Blvd, Plovdiv, Bulgaria

Corresponding author email: vvlahova179@abv.bg

Abstract

Truffle cultivation is an original agroforestry system. Historical data and distribution of truffle species, their characteristics and with which tree species are in symbiosis are indicated. Harvesting truffles is natural and artificial and has specifics in their hunting around the world. Italian entrepreneurs offer successful agriculture, which is related to science, and this is what they rely on in Romania. Truffle plantations on the Balkan Peninsula are an excellent form of alternative high-yield agriculture. The main accents when growing truffles are careful selection of field with appropriate soil and pH, exposure, area, climatic features and quality seedlings. In truffle plantations, yields are planned and quality is guaranteed. The facts about the rich chemical content of truffles and their beneficial effect on human health are objective. This paper aims at turning attention to the species distribution of truffles and the contemporary approach to their cultivation in Europe. As ectomycorrhizal fungi, truffle species also perform many important ecosystem functions, including organic matter decomposition, nutrient cycling and retention, soil aggregation, and transferring energy through soil food webs.

Key words*: agroforestry; ecosystem functions, natural and artificial collection; truffle.*

INTRODUCTION

The genus *Tuber* F.H. Wigg. (Ascomycota, Pezizales, Tuberaceae) is globally famous and historically appreciated for the production of hypogeous ascomata known as 'truffles' (Polemis et al., 2019). Truffles are ectomycorrhizal fungi characterized by hypogeous fruitbodies (Bonito et al., 2010; Lacheva, 2012). Ecologically, Tuber spp. form obligate mycorrhizal symbiosis with the roots of trees, such as oak, poplar, willow, hazel (Harley & Smith, 1983), pecan (Bonito et al., 2013), hornbeam (Ceruti et al., 2003), and some shrubs, such as Cistus (Mello et al., 2006), play important roles in forest functioning and biogeochemical cycles (Smith & Read, 2008; Todesco et al., 2019). In 1881 Carlo Vittadini gave a more elaborate description and was the first to recognize the importance of mycorrhizae to the nutrition of the host tree species (Trappe et al., 2009).

Albert B. Frank first recognized the mycorrhizal symbiosis in 1885 when he showed that fungi in the truffle-forming genus Tuber were found growing on the roots of living plants (Frank, 2005). The survival of truffles is based on their ability to form mycorrhizae from the roots of host trees, and in return the trees receive the nutrients that are important for their survival (Siachoono et al., 2016), as they are therefore in a symbiotic relationship (Trappe et al., 2009). Bonito et al. (2010) point to Paolocci et al. (2006) and Riccioni et al. (2008) who have intensively studied the ecology and host associations of a few commercialized European Tuber spp.

As ectomycorrhizal fungi, truffle species also perform many important ecosystem functions, including organic matter decomposition, nutrient cycling and retention, soil aggregation, and transferring energy through soil food webs. These functions contribute to the overall health, resiliency, sustainability of forest ecosystems (Trappe et al., 2009) and contributes to a stable bioeconomy in rural areas (Hilszczańska et al., 2019).

The protection of forest ecosystems is vital for the hypogeous fungi, such as valuable truffles' conservation (Kaounas, 2020).

This paper aims at turning attention to the species distribution of truffles and the contemporary approach to their cultivation in Europe.

MATERIALS AND METHODS

Recent years have witnessed an upsurge of interest in truffles in particular, thus resulting in information collected from recognized specialists representing the historical development of truffles; their ecosystem functions; as well as that they form obligate mycorrhizal symbiosis with the different ligneous species (pines, oaks, poplars, orchids, and commercially important trees, such as hazelnut, and pecan). Special attention is paid to the species considered most valuable to Europe, such as *Tuber melanosporum* Vittad, *Tuber magnatum* Pico, and *Tuber aestivum* Vittad., their preferences for pH, soils, and reporting the ways for their cultivation and harvesting from natural habitats.

RESULTS AND DISCUSSIONS

The very first uses of truffles date back to 6,000 years ago when ancients believed that truffles had magical powers aphrodisiacs (Ştefan, 2016). The first recorded account of truffles is by the ancient Greeks who used to love and appreciate them. Aristotle declared them an aphrodisiac and Pythagoras agreed, while for the Romans they were simply 'earth fruit'. During the Renaissance they returned to the tables of the wealthy (http://www.trufflegrowers.com.au).

References to edible hypogeous fungi exist in many ancient texts and it seems that the Mesopotamians, Egyptians, Greeks and Romans greatly appreciated truffles (Kaounas, 2020). Francis I of France was the first to bring truffles to rank culinary delicacy, demanding that at its sumptuous banquets he mandatory seasoned with truffles to be the most important preparations. Also, in France Louis XIV commissioned the first scientific research dedicated to cultivating truffles (Ştefan, 2016). Mello et al. (2006) cite Ciccarelli (1564) who points out that the first paper devoted to the nature of truffles appeared in 1564. After the Second World War, due to changes in the social character and forest management, the knowledge about truffles was lost (Hilszczańska et al. 2019)

The genus Tuber is present globally in temperate areas including over 200 species (Bonito et al., 2013; Todesco et al., 2019). The variety of truffles is significant- about 180 species (Bonito et al., 2010), although only about 13 have a commercial interest (Reyna & Garcia-Barreda, 2008; Bonito et al., 2009; Reyna & Garcia-Barreda, 2014), while Ştefan (2016) reports that many of them have only scientific value. Siachoono et al. (2016) reports that 50 genera of hypogeous fungi are recorded for Europe, only two being endemic (Marjanović et al., 2010, 2012). Montecchi & Sarasini (2000) describe 22 European species that belong to the genus Tuber, and Ceruti et al. (2003) lists 32 species from Europe (Iberian and Apennine Peninsulas and Southern France) as regions with the greatest diversity of hypogenic Ascomycota, and especially Tuberaceae.

Truffles in recent years have become increasingly popular around the world. Many truffle species are found in Italy, France, Spain, Australi, and Romania (Ştefan, 2016). Polemis et al. (2019) cite the authors Jeandroz et al. (2008) who point out that the variety of truffles is well documented in Europe, but until recently the Balkan Peninsula was poorly investigated. The geographic distribution of known truffle species (about 100) mainly covers the temperate zones of the northern hemisphere, with at least three differentiation areas: Europe, South-East Asia, and North America (Gajos & Hilszczańska, 2013). Pieroni (2016) cites authors (Mandeel & Al-Laith, 2007; Samils et al., 2008) who point out that truffles have been the focus of very few ethnobiological studies, mainly in desert areas. Trappe et al. (2009) reported that Helen Gilkey specialized in the "true" truffles, i.e., the ascomycetes, whereas Sanford Zeller worked with the basidiomycetes, sometimes called the "false" truffles.

The literature points out that the number of truffles found in nature decreases as a result of environmental pollution and massive deforestation. Truffles mostly grow at various altitudes - from a few meters above sea level to between 800 and 1000 m (Pacioni & Comandini, 1999), as strong ecological relationships exist between *Tuber* spp., host plants and soil type (Lulli et al., 1999; Gajos & Hilszczańska, 2013). Truffles grow best mainly in Rendzic soils (Hilszczańska et al., 2019).

Truffle species present common ecological features, such as a relatively wide range of host species and the need for calcareous soils with a sub-alkaline pH (7 to 8) (Pacioni & Comandini, 1999), except *Tuber borchii* tolerating slightly acidic soils (Mello et al., 2006). Gajos & Hilszczańska (2013) cite author Granetti (1994) who point out that well-aerated and well-drained soils with a good amount of Ca, K and S are suitable for truffles.

Truffles are seasonal wild fungi growing after rain at a depth of 5 to 15 cm under the ground (Rana et al., 2020) or at a depth of 4 to 40 cm (Ştefan, 2016). And because they grow up to 30 cm deep into the soil, they need to be found with the help of trained dogs or pigs (Hilszczańska et al., 2019). In order to find truffles after maturation, the acute sense of smell of certain animals is required. The fine sense of smell of the pig makes it very efficient, but it requires constant vigilance, because of the risk of truffles being damaged, or even swallowed. Moreover, the animal gets tired rather quickly and its transport is difficult. Unlike pigs, dogs are not naturally interested in truffles and can be trained to indicate with their paws the place where the fragrant mushrooms are located. Truffles can also be localized by fly species (genus *Suillia*), which often hover above the place where a truffle is hidden (Talou et al., 1990). The role of the dog in the search for truffles is considered crucial, because the animal works in many cases like a 'translator' for nature, providing essential information for the trifulau, thanks to its keen senses and extensive training (Pieroni, 2016). Polemis et al. (2019) cite authors Hanson et al. (2003), Trappe & Claridge (2010) pointing out that truffles serve as a source of nutrition for the soil micro-fauna and several mammals.

Rana et al. (2020) cite authors (Rota et al., 2008; González & Marioli, 2010) indicating that the weight of truffles is ranges from 30 to 300 g. Ştefan (2016) points out that those truffles are called 'the earth diamonds' due to their high prices when sold on the market, and several of them are highly prized due to their unique aroma (Bonito et al., 2010) and culinary identity (Siachoono et al., 2016; Polemis et al., 2019). Some species, such as *T. magnatum* Pico, and *T. melanosporum* Vittad., are in great demand by the food market in many countries because of their special taste and smell, resulting from a blend of hundreds of volatile compounds (Bellesia et al., 1998; Gioacchini et al., 2005; Mello et al., 2006). The analyzes have proven to contain 9% protein, 13% starchy materials, 1% fat, vitamin B_2, that is rich in vitamin A. It also contains a quantity of nitrogen in addition to carbon, oxygen and hydrogen, which makes the composition similar to the composition of meat (Rana et al., 2020). Truffles are used in the food industry in the development of the luxury gastronomic culture, the pharmaceutical and cosmetic industries (Ştefan, 2016).

There are important differences in the geographical distribution of truffles (Mello et al., 2006). Todesco et al. (2019) point out that the most valuable truffle species are *Tuber melanosporum* Vittad., *Tuber magnatum* Pico, and *Tuber aestivum* Vittad.

The edible fruiting body of *Tuber melanosporum* Vittad. is commonly known as Périgord truffle, 'black truffle' (Kaffsack, 2006) and also locally known as "sweet" truffle (Pieroni, 2016). The Périgord truffle has a much smaller ecological range (Delmas, 1978; Čejka et al., 2020) and is collected in the South and West Europe - Italy, France, and Spain (Mello et al., 2006; Barchfield, 2008; Santelices & Palfner, 2010). The migration of Périgord truffles into higher latitudes in the north of the European Alps (Thomas & Büntgen, 2017; Büntgen, et al., 2019), and the recently documented harvest has declined in the species' southern European habitats (Büntgen et al., 2012), being attributed to global warming (Thomas & Büntgen, 2019; Čejka et al., 2020). Todesco et al. (2019) cites Reyna & Garcia-Barreda (2014) saying that *T. melanosporum* plantations are found not only in the Mediterranean area but also outside their native range, including Australia, South-America, and West USA. Black truffles are grown in symbiosis, especially with oaks, and during its limited harvesting season, mainly winter, one finds them in several regions of southern Europe (Talou et al., 1990). Stobbe et al. (2013) points out that, besides harvests from natural habitats, *T. melanosporum* plantations across the Mediterranean have gained importance over the past decades (Bonet et al., 2009), especially following the application of

the technique for artificial mycorrhization of host plants developed in the 1970s, which soon became the standard for truffle cultivation (Hall et al., 2007). The demand for black truffle has stimulated studies on species (Rosa-Gruszecka et al., 2017). *T. melanosporum* was first cultivated in France during the 19th century (Olivier et al., 1996). It is currently cultivated worldwide (mainly in regions with a Mediterranean-like climate) (Reyna & Garcia-Barreda, 2014) and is highly regarded for its culinary properties (Thomas et al., 2016). Thomas (2014) presents a very detailed analysis of the climatic parameters required for growing *T. melanosporum*, including data from Africa, Asia, Australia, Europe, North America, and South America. This study provides information about countries that have a natural population of *T. melanosporum* and those that do not have a history of natural populations but are in the suitable climatic ranges of this species. The author points out that truffle cultivation is successful in areas that have lower temperature and higher rainfall levels than expected. The data compiled are extremely valuable and useful for cultivators in choosing suitable locations for a plantation. Büntgen et al. (2019) cite the authors (Thomas & Büntgen, 2019) reporting that the quality and quantity of truffles are affected by more frequent Mediterranean summer droughts, although many plantations are grown under irrigated conditions.

Tuber magnatum Pico, commonly known as the Italian and the 'white truffle' fruiting bodies have so far been collected in Italy and in East Europe- Croatia, Slovenia, and Hungary- resulting in a limited availability (Mello et al., 2006; Rosa-Gruszecka et al., 2017). According to Murat et al. (2005), for *T. magnatum* there is still a lack of cultivation methodology, although *T. melanosporum*, *T. brumale* and *T. aestivum* have been grown on plantations (Rosa-Gruszecka et al., 2017).

Tuber aestivum Vittad., commonly known as the summer and Burgundy truffle, is an ectomycorrhizal Ascomycete associated with numerous trees and shrubs. Its life cycle occurs in the soil, and thus soil parameters could influence it, such as temperature and water availability (Todesco et al., 2019). Gajos & Hilszczańska (2013) state that according to Chevalier & Frochot (1997) the natural geographical distribution of *T. aestivum* ranges from North Africa to Sweden and from Ireland to Russia. The burgundy truffle is a species with a huge market and a wide geographic spread (https://plantationsystems.com). It is often reported that France, Italy and Spain are home to the Burgundy truffle, and nowadays much of the supply comes from an ancient woodland in a country like Romania. In Romania truffles have spawned a niche in rural tourism. Truffle tourism has a future in Romania because it has environmental management and provides jobs for rural areas (Ştefan, 2016). Currently growing in much of Europe (Stobbe et al., 2013), the Burgundy truffle is expected to offer a great potential to be cultivated in new regions as climate change progresses (Stobbe et al., 2012; Čejka et al., 2020). Romania has large tracts of native woodland that become heavy with truffle collectors from across Europe hunting these fertile grounds. Indeed, Transylvania produces some of the best summer truffles on the market (https://plantationsystems.com).

Kaiser & Ernst (2016) specify that the most highly valued truffles species are native to Europe: the Périgord 'black truffle' and the Italian 'white truffle' dominate the market, and the Burgundy truffle has a lower value.

A new species of truffle- *Tuber pulchrosporum*, was described in Greece and Bulgaria (Polemis et al., 2019) and is not recommended due to its rarity collection (Kaounas, 2020). While *T. borchii* and *T. maculatum* are found throughout Europe (Riousset et al., 2001; Mello et al., 2006), Csorbainé et al. (2008) indicate that *T. macrosporum* mainly occurs on water-affected river banks, temporarily flooded river valleys or in deep and shaded valleys in the Czech Republic, Hungary, Romania, and Ukraine. Gregory et al. (2003) points out that *T. macrosporum* can also have a wider tolerance to drought. Mandić et al. (2018) points out that in personal communication with Ivan Ratoša it is understood that in the '70s the traditionally illegal truffle market, mainly held by Slovenian smugglers, was the only route for selling truffles from Serbia. The author points out that in the early 2000s M. Milenković organized courses and sold written instructions on truffle hunting, as a result of which the

number of truffle hunters in Serbia grew rapidly. Truffles currently are gaining attention in Poland, mainly due to the establishment of truffle orchards as a source of benefits in agroforestry, as the grow and collection of truffles stimulates planting oak and hazel (main host-plant species).

Truffles were first found in Bulgaria 15 years ago in Ludogorie, the Shumen Plateau and Strandzha Mountain. History shows that during Socialism there were plenty of planted species of oak, pine tree, and beech, and the development of truffles was furthered by the fact that some nursery gardens had micelle grown in truffles and sapling roots were infected. In the 90s of the XX century Patricio Panfili discovered the first black truffle in the area between Ugarchin and Lovech. There are now also Bulgarian companies offering saplings infected with micelle, as suitable trees here are hazelnut tree, oak, and lime tree. The Italians and the French recommend cultivation on lime tree, oak, and pine tree, as the hazelnut tree is the main tree species used for this purpose in Bulgaria (https://www.duma.bg).

Truffle agroforestry is the original and practical utilization of uncultivated lands with truffle mycorrhizal saplings of the fruit tree Truffle mycorrhizal saplings or forest-ornamental tree species, which form a more stable ecosystem, e.g. truffières. In Bulgaria there is hazelnut truffière, oak and hazelnut truffière, poplar truffière (http://www.kipro-bg.com). A truffle farm near the Balchik village of Obrochishte is one of the newest tourist destinations for tourists in Bulgaria (https://bgtourism.bg).

Plantations of trees where truffles are cultivated, as well as natural truffle forests, are known as truffières (Kaiser & Ernst, 2016). A research on truffle cultivation began in the mid 1800's. However, it was not until the late 1970's that truffles were harvested in French and Italian truffle orchards that had been established with artificially inoculated seedlings. Despite this success, the majority of black truffles and all other species of truffles have been collected over the years from natural areas rather than from artificial truffières. The truffle species most commonly and successfully cultivated is *T. melanosporum*, the famous "French" black truffle. Its hosts include many tree species, but the trees most frequently inoculated are *C. avellana, O. carpinifolia* and *Quercus* spp. (Lefevre & Hall, 2001). In order to cultivate *T. melanosporum*, tree species are inoculated with the fungi and once the mycohrriza is established, these young tree saplings are planted into carefully controlled field sites (Thomas, 2014). Besides forest ecosystems, ectomycorrhizal trees were also implanted in agroforestry ecosystems and in dedicated orchards for producing non-wood products such as edible fungi. The inoculation of tree seedlings with selected ectomycorrhizal fungi in nurseries (i.e., controlled mycorrhization) started a hundred years ago, as this technique has been used extensively since the 1970s to grow truffles (Murat, 2015). Todesco et al. (2019) report that according to the authors Reyna & Garcia-Barreda (2014), Murat, (2015) since the first commercialization of seedlings inoculated with *T. aestivum* and *T. melanosporum* in 1973, considerable progress has been made to improve the quality of the inoculated plants. Santelices & Palfner (2010) point out that according to Ramírez et al. (2004) previous studies carried out in Chile have demonstrated the feasibility of producing mycorrhizal plants of *C. avellana* inoculated with black truffle, matching the highest quality standards demanded on the European markets.

Ştefan (2016), analyzes the different methods of micorrhization used in the truffle culture and their advantages for encouraging the cultivation of truffles. Reyna et al. (2001) have reported attempts to inoculate adult trees with *Tuber* spp. conducted in Italy by Lo Bue et al. (1990), given the extremely painstaking effort required and the costs of their method, it is generally considered impractical and unlikely to have any commercial potential. However, Reyna (1992) have posed the possibility of generating receptive root systems and then inoculating them with a sporal suspension while Chevalier & Frochot (1997) have reported French experiments to replace *T. brumale* infections with *T. aestivum* by surface sporal injection. The experiments reported herein by us have investigated the effects of inoculating established *Q. faginea* and *Q. ilex* with *T. melanosporum* over three years (Reyna et al., 2001). Reyna et al. (2001) point out that nursery inoculation of plants and their cultivation under controlled conditions have

resulted in the development of more or less refined techniques. The authors point out that there are many bibliographic references in this direction, some of which describe the most extensively used and practical inoculation techniques (Honrubia et al., 1992), while others analyze the advantages and disadvantages of some of the techniques specifically targeted at *T. melanosporum* (Palazón et al., 1999).

T. melanosporum fruiting bodies are consumed by animals and the spores are dispersed in their excrement. However, when truffles are harvested by man and completely removed from the natural habitat there is little or no opportunity for new trees to become infected by spores. It is possible that this may partly account for decreases in the productivity of natural *T. melanosporum* truffières. Another possible reason for the decline in production is the excessive plant density now found in natural *T. melanosporum* truffières that 100 years ago were kept open by domesticated grazing animals and the collection of firewood (Reyna, 2000; Reyna et al., 2001).

The invasive biology of *Tuber* is of interest, because some of the economically important species are being intentionally introduced into ecosystems around the world (Hall et al., 2007; Bonito, 2009), yet the phenomena of human-mediated long-distance dispersal of mycorrhizal fungi are not well understood (Bonito et al., 2010). Reyna & Garcia-Barreda (2014) cites authors who indicate that *T. aestivum* Vittad., *T. borchii* Vittad. and the *T. indicum* complex have been successfully cultivated (Zambonelli et al., 2000; Hu et al., 2005), whereas without success though by *T. magnatum* (Gregori, 2007; Bencivenga et al., 2009). With a flavor that varies through the season, early summer varieties are often mildly flavored and quite clean on the palate, while those harvested from late summer onwards can be really quite intense - in a similar vein to the Perigord truffle (https://plantationsystems.com).

CONCLUSIONS

Truffles are the icons of the fungal world. Their strong aromatic scent gives them a unique culinary identity, thus becoming in recent years increasingly popular worldwide. The most highly valued truffle species are native to Europe - the Périgord black truffle, the Italian white truffle, and the Burgundy truffle that mainly grows under deciduous trees with host tree species being oaks, hazelnuts, hornbeam, alder, willows, lindens, and poplars.

In recent years a decrease has been observed in the number of truffles found in nature as a result of environmental pollution and massive deforestation. That is why attention is paid to the plantations of trees where truffles are cultivated (truffières) which **is a successful approach to truffle growing in Europe**. Truffle species also perform many important ecosystem functions, including organic matter decomposition, nutrient cycling and retention, soil aggregation, and transferring energy through soil food webs.

REFERENCES

Barchfield, J. (2008). Europe's truffle shortage blamed on global warming. *USA Today. Available at* http://www. usatoday.com/weather/climate/globalwarming/2008-02-25-europe-truffles_N.htm

Bellesia, F., Pinetti, A., Bianchi, A., & Tirillini, B. (1998). The volatile organic compounds of black truffles (*T. melanosporum* Vitt.) from Middle Italy. *Flav. Fragr. J., (13)*, 56- 58.

Bencivenga, M., Di Massimo, G., Donnini, D., & Baciarelli-Falini L. (2009). The cultivation of truffles in Italy. *Acta Bot Yunnan*., *(31*), 21-28.

Bonet, J.A, Oliach, D., Fischer, C., Olivera, A., Martinez, de Aragon J., & Colinas, C. (2009) Cultivation methods of the black truffle, the most profitable Mediterranean non-wood forest product; a state of the art review. *In: Palahi M, Birot Y, Bravo F, Gorriz E (eds.) modelling, valuing and managing Mediterranean forest ecosystems for non-timber goods and services. EFI Proceedings. (57).* 57-71.

Bonito, G. (2009). Fast DNA- based identification of the black truffe *Tuber melanosporum* with direct PCR and species- specific primers. *FEMS Microbiology Letters*, *(301)*, 171-175.

Bonito, G., Gryganskyi, A.P., Trappe, J. M. & Vilgalys, R. (2010). A global meta-analysis of Tuber ITSrDNA sequences: species diversity, host associations and long-distance dispersal. *Mol. Ecol*., *(19)*, 4994- 5008.

Bonito, G., Smith, M.E., Nowak, M., Healy, R.A., Guevara, G., Cázares, E., Kinoshita, A., Nouhra, E.R., Domínguez, L.S., Tederso, L., Murat, C., Wang, Y., Moreno, B.A., Pfister, D. H., Nara, K., Zambonelli, Al., Trappe, J.M., & Vilgalys, R. (2013). Historical Biogeography and Diversification of Truffles in the Tuberaceae and Their Newly Identified Southern Hemisphere Sister Lineage. *PLoS ONE*., *(8)*, 52765.

Bonito, G., Trappe J.M. & Vilgalys R. (2009). North American Truffle in the Tuberaceae: Molecular and Morphological Perspectives. *Acta Botanica Yunnanica*, *(16)*, 39-51.

Büntgen, U. et al. (2012). Drought-induced decline in Mediterranean truffle harvest. *Nat. Clim. Chang.*, *(2),* 827-829. doi.org/10.1038/nclim ate17 33.

Büntgen, U., Oliach, D., Martínez-Peña, F., Latorre, J., Egli, S., & Krusic, P.J. (2019). Black truffle winter production depends on Mediterranean summer precipitation. *Environ. Res. Lett*., *(14),* 074004. doi.org/10.1088/1748-9326/ab1880.

Čejka, T., Trnka, M., Krusic, P.J., Stobbe, U., Oliach, D., Václavík, T., Tege, T., & Büntgen, U. (2020). Predicted climate change will increase the truffle cultivation potential in central Europe. *(10)*. 21281, www.nature.com/scientificreports

Ceruti, A., Fontana, A., & Nosenzo, C. (2003). Le Specie Europee del genere Tuber- Una Revisione Storica. *Museo Regionale de Scienze Naturali*, Torino

Chevalier, G., & Frochot, H. (1997). La truffe de Bourgogne (*Tuber uncinatum* Chatin). *Petrarque, Levallois-Perret*, France

Ciccarelli, A. (1564). Opusculum de Tuberibus. Pavia.

Csorbainé, A.G., Bratek, Z., Illyés, Z., & Dimény, J. (2008). Studies on *Tuber macrosporum* Vittad. Natural Truffle Habitats in the Carpatho- pannon Region. 3rd Congresso Internazionale de Spoleto sul Tartufo, At Spoleto.

Delmas, J. (1978). *Tuber* spp. in the biology and cultivation of edible mushrooms (*eds. Chang, S. T. & Hayes, W. A.) Academic Press*., 645- 681.

Frank, J.L. (2005). Complex mutualism in an Oregon white oak woodland: hypogeous fungi, mycorrhizas and small mammal mycophagy. Ashland, *OR: Southern Oregon University*. 112 M.S. thesis.

Gajos, M., & Hilszczańska, D. (2013). Research on truffles: Scientific journals analysis, *8*(38), 1837-1847. doi 10.5897/SRE2013.5620, ISSN 1992-2248.

Gioacchini, A.M, Menotta, M., Bertini, L., Rossi, I., Zeppa, S., Zambonelli, A., Piccoli, G., & Stocchi, V. (2005). Solid-phase microextraction gas chromatography/mass spectrometry: a new method for species identification of truffles. *Rapid Communications in Mass Spectrometry*., *(19).* 2365-2370.

González, M.J., & Marioli J.M. (2010). Antibacterial activity of water extracts and essential oils of various aromatic plants against Paenibacillus larvae, the causative agent of American foulbrood. *J Invertebr Pathol*., *(104),* 209-13.

Granetti, B. (1994). I Tartufi: biologia a tecniche di coltivazione. *Micol Italiana*., *(2),* 63-68

Gregori, G.L., (2007). Principales aspectos de la trufa y la truficultura en Italia. *In: Truficultura Fundamentos y técnicas (Reyna S, ed*). Mundi-Prensa, Madrid., 433- 464.

Gregory, L.G., Bond, P.L., Richardson, D.J., & Spiro, S. (2003). Characterization of a nitraterespiring bacterial community using the nitrate reductase gene (narG) as a functional marker. *Microbiology (149),* 229-237. doi 10.1099/mic.0.25849-0.

Hall, I., Brown, G.T., & Zambonelli, A. (2007). Taming the truffle. Timber, Portland

Hanson. A.M., Hodge, K., & Porter, L.M. (2003), Mycophagy among primates. *Mycologist*., *(17),* 6-10. doi.org/10.1017/S0269915X0300106X.

Harley, J.L., & Smith, S.E. (1983). Mycorrhizal Symbiosis. Academic Press, Toronto.

Hilszczańska, D., Rosa-Gruszecka, Al., Kosel, B., Horak, J., & Siebyła, M. (2019). A Survey of the Knowledge of Trufes among Polish Foresters and Implications for Environmental Education, 365. doi:10.3390/f10050365.

Honrubia, M., Cano, A., & Molina-Niñirola, C. (1992) Hypogeous fungi from southem Spanish semi-arid lands. *Persoonia*, *(14)*, 647-653.

Hu, H.T., Wang, Y., & Hu, B.Y. (2005). Cultivation of *Tuber formosanum* on limed soil in Taiwan. *NZ J Crop Hortic Sci*., (33), 363-366.

Jeandroz, S., Murat, C., Wang, Y., Bonfante, P. & Le Tacon, F. (2008). Molecular phylogeny and historical biogeography of the genus Tuber, the 'true truffles'. *J Biogeogr*., *(35)*, 815-829.

Kaffsack, H.J. (2006). "Schwarze Diamanten" werden rar. Manager Magazin. Available at http://www.managermagazin.de/life/genuss/0,2828,407027,00.html

Kaiser, Ch., & Ernst, M. (2016). Truffles & Other Edible Mycorrhizal Mushrooms. Center for Crop Diversification Crop Profile. *University of Kentucky College of Agriculture, Food and Environment*. CCD-CP-83.

Kaounas, V. (2020). Edible below ground fungal fruitbodies in Greece. Innovation Network for Cork, Resin & Edibles, *FACTSHEET*, p. 4.

Lacheva, M. (2012) New records for hypogeous ascomycetes in Bulgaria. Science and Technologies. (2), 30-34.

Lefevre, Ch., & Hall, I.R. (2001). The status of truffle cultivation: A global perspective. Project: Cultivation of truffles and other edible mycorrhizal mushrooms. *Acta Horticulturae*., *(556),* 513-520. doi: 10.17660/ActaHortic.

Lo Bue, G., Gregori, G.L., Pasquini, L., & Maggiorotto, G., (1990). Síntesi micorizica in campo fra piante adulte e tartufi pregiati mediante frammenti radicali. *In: Becivanga M, Granetti B (ed). Atti del Secon. Cong. Inter. Sul Tartufo. Spoleto*., 459- 466.

Lulli, L., Bragato, G., & Gardin, L. (1999) Occurrence of *Tuber melanosporum* in relation to soil surface layer properties and soil differenciation. *Plant Soil*., *(214),* 85-92.

Mandeel, Q.A, & Al- Laith, A. (2007). Ethnomycological aspects of the desert truffle among native Bahraini and non-Bahraini peoples of the Kingdom of Bahrain. *Journal of Ethnopharmacology*. *110(1,* 118-29. doi: 10.1016/j.jep.2006.09.014.

Mandić, R., Adžemović, M., & Marjanović, Ž. (2018). Conservation and trade of wild edible mushrooms of Serbia- history, state of the art and perspectives. *Nature Conservation*., *(25),* 31-53.

Marjanović, Ž., Grebenc, T., Marković, M., Glišić, Al., & Milenković, M. (2010). Ecological specificities and molecular diversity of truffles (genus Tuber)

originating from mid-west of the Balkan Peninsula. *SciVie - Slovenian Forestry Institute*. ISSN: 0082-0598.

Marjanović, Ž., Saljnikov, E., Milenković, M., & Grebenc, T. (2012). Ecological Features of Tuber Magnatum Pico in the Conditions of West Balkans-Soil Characterization. https://www.researchgate.net/publication/291256109

Mello, A., Murat, C. & Bonfante, P. (2006). Truffles: much more than a prized and local fungal delicacy. *FEMS Microbiol. Lett*., *260*(1), 1-8.

Montecchi, A., & Sarasini, M. (2000). Funghi Ipogei d'Europa., *A M B Fondazione, Trento*, Italy. 138-141.

Murat, C. (2015). Forty years of inoculating seedlings with truffle fungi: past and future perspectives. *Mycorrhiza, (25)*, 77- 81.

Murat, C., Vizzini, A., Bonfante, P., & Mello, A. (2005). Morphological and molecular typing of the below-ground fungal community in a natural Tuber magnatum truffle-ground. *FEMS Microbiol Lett*., *245(2),*307-13.

Olivier, J.M., Savignac, J.C., & Sourzat, P., (1996). Truffe et trufficulture. *Ed Fanlac, Perigueux, France.*

Pacioni, G., & Comandini, O. (1999). Tuber. Ectomycorrhizal Fungi: *Key Genera in Profile*., 163-186.

Palazón, C., Delgado, I., Cartié, G., Delgado, I, Barriuso J., & Esteban, H. (1999). Propuesta de una metodología de evaluación y control de calidad de planta (*Quercus* spp.) micorrizada con T. *melanosporum* para la obtención en España de de la etiqueta de calidad. *International Science Culture de la Truffe. Aix en Provence.*

Paolocci, F., Rubini, A., Riccioni, C., & Arcioni, S. (2006). Reevaluation of the life cycle of *Tuber magnatum*. *Applied and Environmental Microbiology*., *(72),* 2390-2393.

Pieroni, A. (2016). The changing ethnoecological cobweb of white truffle (*Tuber magnatum* Pico) gatherers in South Piedmont, NW Italy. *Journal of Ethnobiology and Ethnomedicine*, *(12),* 11. doi 10.1186/s13002-016-0088-9.

Polemis, E., Konstantinidis, G., Fryssouli, V., Slavova M., Tsampazis, T., Nakkas, V., Assyov, B., Kaounas, V., & Zervakis, G.I. (2019). *Tuber pulchrosporum* sp. nov., a black truffle of the Aestivum clade (Tuberaceae, Pezizales) from the Balkan peninsula. *MycoKeys*. *(47),* 35-51. doi: 10.3897/mycokeys.47.32085.

Ramírez, R., Reyna, S., Santelices, R., Palfner, G., García, S., & Henríquez, R. (2004). Cultivo de trufa negra (*Tuber melanosporum* Vitt.) en Chile. Una alternativa productiva sustentable bajo un sistema de manejo agro silvícola. *Segundo Congreso Forestal Chileno, Valdivia. Universidad Austral de Chile, Valdivia, Chile.*

Rana, D.S., Dawood, Al., Al-Bahadily, Ch., Chaloob, R., Hassan, F., Shari, H., & AL-Salman. N.K. (2020). Estimation of Truffles extracts and study the biological efficacy of Ethyl 6-methyl-2-oxo-4-(2-thienyl)-1,2,3,4- tetrahydropyrimidine-5-carboxylate (EMOTTC) as one of the extracts against ophthalmic bacteria. *Sys Rev Pharm*.,*11(12*), 78-87.

Reyna, S. & Garcia-Barreda, S. (2014). Black truffle cultivation: a global reality. *For. Syst., (23)*, 317-328

Reyna, S. (1992). La trufa. Madrid, Mundi-prensa.

Reyna, S. (2000) Trufa, trufícultura y selvicultura trufera. Mundi-Prensa, Madrid

Reyna, S., & Garcia-Barreda, S., (2008). European black truffle: its potential role in agroforestry development in the marginal lands of Mediterranean calcareous mountains. *In: RigueiroRodríguez A, McAdam J, Mosquera-Losada MR (eds) Agroforestry in Europe Current status and future prospects. Springer*., 295-317.

Reyna, S., Barreal, J.A. R., Folch, L., Pérez-Badía, R., Domínguez, A., Saiz-De-Omecaña, J., & Zazo, A. (2001). Techniques for inoculating mature trees with *Tuber melanosporum* Vitt. Edible Mycorrhizal Mushrooms and Their Cultivation

Riccioni, C., Belfiori, B., Rubini, A., Passeri, V., Arcioni, S., & Paolocci, F., (2008). Tuber melanosporum outcrosses: analysis of the genetic diversity within and among its natural populations under this new scenario. *New Phytol*., *(180)*, 466-478.

Riousset, G., Riousset, L., Chevalier, G. & Bardet, M.C. (2001). Truffe d'Europe et de Chine, INRA, Paris.

Rosa-Gruszecka, Al., Hilszczańska, D., Gil, W., & Kosel, B. (2017). Truffle renaissance in Poland-history, present and prospects. *Journal of Ethnobiology and Ethnomedicine.* doi 10.1186/s13002-017-0163-x

Rota, M.C., Herrera, A., Martínez, R.M., Sotomayor, J.A., & Jordán, M.J. (2008). Antimicrobial activity and chemical composition of Thymus vulgaris, Thymus zygis and Thymus hyemalis essential oils. *Food Control*, *(19),* 681-7.

Samils, N., Olivera, A., Danell, E., Alexander, S. J., Fischer, C., & Colinas, C. (2008). The socioeconomic impact of truffle cultivation in rural Spain. Economic Botany, *62*(3), 331- 340. http://doi.org/10.1007/s12231-008-9030-y.

Santelices, R., & Palfner, G. (2010). Controlled Rhizogenesis and Mycorrhization of Hazelnut (*Corylus avellana* l.) Cuttings with Black Truffle (*Tuber melanosporum* Vitt.) *Chilean Journal of Agricultural Research. 70,(2),* 204-212.

Siachoono, S.M., Shakachite, O., Muyenga, Al. M. & Bwalya, J. (2016). Under Ground Treasure: A Preliminary Inquiry into the Ecology and Distribution of Zambian Truffles. *International Journal of Biology. 8,(2).* ISSN 1916-9671 E-ISSN 1916-968X

Smith, S. E. & Read, D. J. (2008). Mycorrhizal symbiosis. *3rd edn, Academic Press*.

Ştefan, M. (2016). The Process of Obtaining Truffles through Different Methods of Mycorrhization in the Organic System. International Conference on Competitiveness of Agro-food and Environmental Economy Proceedings., *(5),* 211-215.

Stobbe, U. et al. (2012). Spatial distribution and ecological variation of re-discovered German truffle habitats. *Fungal Ecol*. (5). 591-599. doi.org/10.1016/j.funec o.2012.02.001.

Stobbe, U. et al. (2013). Potential and limitations of Burgundy truffle cultivation. *Appl. Microbiol.*

Biotechnol., *(97),* 5215-5224. doi. org/10.1007/s0025 3-013-4956-0.

Stobbe, U., Egli, S., Tegel, W., Peter, M., Sproll, L., & Büntgen, U. (2013). Potential and limitations of Burgundy truffle cultivation. *Appl Microbiol Biotechnol*. *(97),*5215- 5224. doi 10.1007/s00253-013-4956-0.

Talou, T., Gaset, A., Delmas, M., Kulifaj, M., & Montant, C. (1990). Dimethyl sulphide: the secret for black truffle hunting by animals? *Mycological Research*. *94, (2),* 277- 278.

Thomas, P. & Büntgen, U. (2017). First harvest of Périgord black truffle in the UK as a result of climate change. *Clim. Res*., *(74),* 67-70. doi.org/10.3354/cr014 94.

Thomas, P. & Büntgen, U. (2019). A risk assessment of Europe's black truffle sector under predicted climate change. *Sci. Total Environ*. *(655),* 27-34. doi.org/10.1016/j.scito tenv.2018.11.252.

Thomas, P., & Büntgen, U. (2019). A risk assessment of Europe's black truffle sector under predicted climate change Sci. *Total Environ*., *(655)*, 27-34.

Thomas, Y., Taschen, E., & Richard, F. (2016). Taming the Black Truffle (*Tuber melanosporum*). Safeguarding Mediterranean food and ecological webs. *In book: The Mediterranean Region Under Climate Change.* doi: 10.4000/books.irdeditions.23895

Todesco, F., Belmondo, S., Guignet, Y., Laurent, L., Fizzala, S., Le Tacon, F.& Murat, C. (2019). Soil temperature and hydric potential influences the monthly variations of soil *Tuber aestivum* DNA in a highly productive orchard. *Scientific Reports*., (*9)*, 12964. doi: org/10.1038/s41598-019-49602-2 10.

Trappe, J.M, & Claridge, A.W. (2010). The Hidden Life of Truffles. *Scientific American*., *302(4*). 78-84. doi: 10.1038/scientificamerican0410-78.

Trappe, J.M, Molina, R., Daniel, L. Cázares, E., Pilz, D., Smith, J.E., Castellano, M.A., Miller, S.L.; & Trappe, M.J. (2009). Diversity, ecology, and conservation of truffle fungi in forests of the Pacific Northwest. *Gen. Tech. Rep. PNW-GTR-772. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station.,* p.194.

Zambonelli, A., Iotti, M., Rossi, I., Hall, I., (2000). Interactions between *Tuber borchii* and other ectomycorrhizal fungi in a field plantation. *Mycol Res*. (104), 698-702.

http://www.kipro-bg.com

http://www.trufflegrowers.com.au/wp-content/uploads/2017/01/truffle_history2.pdf).

https://bgtourism.bg.

https://plantationsystems.com/romania-the-truffle-basket-of-europe.

https://www.duma.bg

http://www.trufflegrowers.com.au/wp-content/uploads/2017/01 /truffle_history2.pdf The myth and magic of truffles (2017).