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Tuber aestivum – hypogeous fungus neglected in the Czech Republic. A review

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The summer truffle, *Tuber aestivum*, is the most common European truffle species with environmental and economic values yet unexplored in the Czech Republic. Background information on general features of this protected hypogeous ascomycete, its history and finds in our country as well as trends in *T. aestivum* cultivation and research in central Europe are summarized.

Key words: *Tuber aestivum*, summer truffle, *Tuber* spp., cultivation, central Europe.

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Lanýž letní – *Tuber aestivum* – je nejběžnější evropský druh lanýžů, jehož environmentální a ekonomické hodnoty pro Českou republiku nejsou dosud oceněny. Jsou shrnuty hlavní znaky tohoto chráněného hypogeického askomycetu, jeho historie a nálezy v ČR i trendy v kultivaci a výzkumu *T. aestivum* ve střední Evropě.

INTRODUCTION

Tuber spp. are ascomycetes that mosty grow in calcareous soils in ectomycorrhizal association with a relatively wide range of deciduous woody plants, including oak (*Quercus* spp.), hazel (*Corylus* spp.), poplar (*Populus* spp.), beech (*Fagus* spp.) and cistus (*Cistus* spp.). They produce hypogeous ascocarps known as truffles. On ripening, every ascocarp emits its distinct species-specific smell which can be located by dogs, pigs or insects (*Suillia* sp.) and then picked up by truffle experts. *Tuber* spp. show relevant differences in their geographical distribution. More than 70 species have been recorded in the world and 32 species have been listed in Europe (Ceruti et al. 2003).

Two species, *Tuber melanosporum* Vitt., the Périgord black truffle, and *Tuber magnatum* Pico, the Italian white truffle, are of great economic value due to the unique flavor and assumed aphrosidiacal power of their ascocarps. In the international haute cuisine both fungi (together with caviar) are the most praised and most expensive delicacies. Two years ago, the black truffle *T. melanosporum* was sold in the market at around 300 to 400 Euros /kg and the white truffle *T. magnatum* at around 3000 to 4000 Euros/kg (El Karkouri et al. 2007) and the price is still increasing.

The summer truffle *Tuber aestivum* Vittadini (1831), synonym *T. uncinatum* (Wedén et al. 2005), the Burgundy truffle, does not have as strong aroma or taste as the two above-mentioned species. The selling price varies between 75–155 Euros/kg in traditional countries. *T. aestivum* belongs to truffle species commonly found in European truffle-growing regions such as Italy (tartufo scorzone), France (truffe d'été), Spain (trufa de Verano), England (English or summer truffle) and Gotland in Sweden (bourgognetryffel). Until last decades, the summer truffle has been frequently overlooked in most other European countries. Since then it has been rediscovered in dictinct habitats all over Europe (Chevalier and Frochot 1997, Montecchi and Sarasini 2000) and considered as the most common European truffle with increasing commercial value.

CHARACTERISTICS OF *T. AESTIVUM*

In central Europe T. *aestivum* prefers calcareous, lime-rich soils, in the layer of humus, usually 10–20 mm, in autumn 150–200 mm deep (Miko and Gažo, personal communication). Like other mycorrhizal species, the summer truffle grows most successfully in association with roots of host trees such as *Quercus robur*, *Corylus avellana*, *Carpinus betulus*, *Fagus silvatica* and *Tilia cordata* (e.g. Hilszczańska et al. 2008). Isolated finds can occur in urbanized localities (Pál-Fám 2001).

Fig. 1. *Tuber aestivum* – fruit body is blackish brown, covered with pyramidal warts. Original size: 30 mm in diameter. Photograph by H. Gryndlerová.

Fig. 2. 128: *Tuber bohemicum* Corda (1854) – (1) section of the fruitbody, (2) polygonal pyramidal warts of the peridium, (3) pyramidal wart with distinct peaks, (4, 5) sections of fertile tissue, (6, 7, 8, 9, 10, 11, 12) asci in various developmental stages, (13) three spores.
129: *Tuber aestivum* Tulasne (1) section of fertile tissue, (2, 3) asci with spores, (4) ascospores.
130: *Tuber brumale*.
All drawings by A. C. J. Corda (1854).

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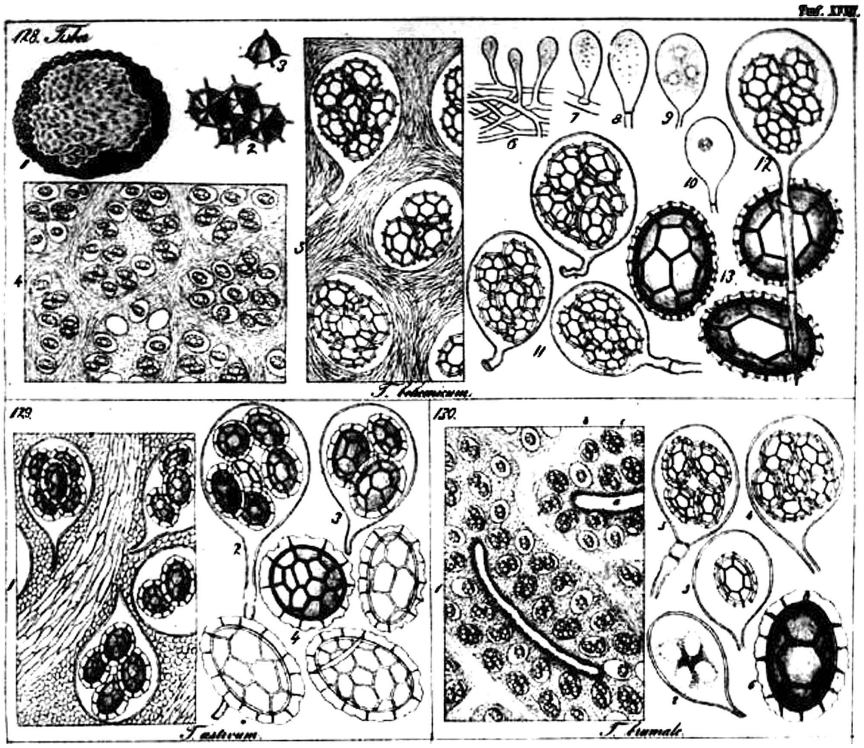

The subterranean ascocarp (Fig. 1) is usually roundish, the peridium is blackish brown with pyramidal warts and the gleba is white when immature, then yellowish and yellow grayish or hazel, marbled at maturity. It can reach the size of potato and weigh up to 0.5 kg, 20–100 mm in diameter. Asci 60–100 × 50–80 µm, globose or pyriform with a short stipe; ascus with 2–6, seldom a single spore. Spores ellipsoid, with a network of ridges, with honeycomb-like reticulations, initially hyaline, mature yellow-brown, 24–50 × 14–32 µm (Montecchi and Saracini 2000). To unequivocal advantages of the summer truffle belong a broad geographical distribution (Riousset et al. 2001), reasonable tolerance to temperature, relative resistance to humidity loss, tolerance to altitudes up to 1000 m above sea level, local soil structure and texture, soil chemistry and vegetation (Wedén et al. 2004, Bratek et al. 2001, Gažo et al. 2005). The fungus is dormant through the winter and has a comparatively higher tolerance to frost (Berthaud et al. 1998). These features are amended by a higher degree of genetic heterogeneity and apparently better survival strategy as compared with *T. melanosporum* (Gandeboeuf et al. 1997, Urbanelli et al. 1998, Berthaud et al. 1998, Mello et al. 2002, Paolocci et al. 2004). Genetic variations of *T. aestivum* suggest the sexual reproduction mode (Wedén 2004) with a presumed chromosome number 5 or 2n = 10 (Poma et al. 1998, Poma et al. 2006).

CONTROLLED TRUFFLE PRODUCTION

Over the years, the occurrence of wild truffles in traditional natural sites has dramatically declined due to climatic changes and incompetent human interventions. In the late 70's, this situation has triggered large-scale science-based programs in France and Italy to improve the situation. The most successful approach focused on establishment of artificial truffle plantations known in French as trufficulture. This approach is based mainly on nursery techniques to induce mycorrhization of receptive tree seedlings (*Quercus* spp., *Coryllus* sp. etc.) under controlled conditions with *T. melanosporum*, *T. magnatum* (Chevalier and Grente 1979, Mello et al. 2006, Hall et al. 2003) and a small percentage (10–15 %) with *T. aestivum*. Infected seedlings are planted out in orchards much like those for fruits. Occasionally plantations begin producing truffles within 3 years but typically production begins 6–10 years after establishment of seedling orchards. Truffle orchards consisting of maintained and irrigated mycorrhized trees are increasingly seen as the only way to ensure future mass production of truffles (Luard and Heseltine 2006). The continued progress in the field has made it possible to establish plantations with ecologically adapted *Tuber* strains (such as *T. aestivum*) in places where truffles have not previously grown (Hall et al. 1994, Wedén et al. 2001). In recent times, outside Europe research and artificial truffle cultivation

are in advancement in countries such as Israel, New Zealand, North America and Australia (Hall et al. 2003), to name the most important places.

RECORDS OF *T. AESTIVUM* IN THE CZECH REPUBLIC

Tuber aestivum (under the synonym *T. cibarium* Pers.) was first reported in our territory in the early 19th century by Krombholz (1821). Later, in the famous work „Icones fungorum hucusque cognitorum" Corda (1854) has described two imminent *Tuber* varieties: var. a – *T. bohemicum* (Corda), an indigenous species harvested currently in Bohemian wooded areas and sold in Prague as an important commodity, and, var. b – *T. aestivum* (Tulasne), very closely related to the former variety. Towards the end of the 19th century the summer truffle was supposed to be the only known *Tuber* species found in Bohemia (Vilhelm 1899, Velenovský 1920–1922).

Since the days of Corda up to the twenties of the 20th century, verified scientific data on truffle species are scanty. In the period 1926–1927, significant contributions to the knowledge of truffles in the former Czechoslovakia were published in Czech journals by Klika (1927), within 1947–1951 by Vacek (e.g. 1950) and by Šebek (1992). Thereafter, the genus *Tuber* has not been studied in this area in detail up to these days.

In terms of territorial distribution, finds of *T. aestivum* were reported in literature in Bohemia from Křivoklátsko-UNESCO Biosphere Reservation and Protected Landscape Area, Karlštejn National Nature Reserve, Kokořínsko Protected Landscape Area (Valda 2006), Pardubicko and Poděbradsko districts (Velenovský 1920–1922, Klika 1927), from Kladno area (Pilát 1965), Polabí lowland (Šebek 1992), Džbán highland (Houda 1994) and Teplice region (Skála 2001). In Moravia, the summer truffle was recorded in the environs of Brno, Drahany Highland (Drahanská Vysočina), Moravian Karst (Moravský kras) Protected Landscape Area, Chřiby hills and foothills of Carpathian Mountains (Macků 1914, Klika 1927, Kříž 1952, Macků 1957). Records monitoring wild-growing *T. aestivum* localities are accidentally reported on internet.

As early as 1913, there were first attempts to create artificial truffle growing conditions in our territory that can be judged as the first realization of controlled truffle production in central Europe (Macků 1914, 1957). Between the two World Wars around 14 fenced truffle fields (size 60 ares) with around 500 French and indigenous oak seedlings inoculated with truffle slices have been successfully planted, managed and recorded in middle and southern Moravia in *T. aestivum* localities. Arrangement of experiments, their development and fate as well as their implication in forestration and land development were presented in the pioneering work „Essai avec les truffières en Moravie et son evaluation dans l'économie

forestrière“ (Macků 1914). Unfortunately, the acquired knowledge has been completely lost due to inconvenient conditions during the Second World War and extreme frost damages in 1929 and 1941, with temperatures falling to –40 and –45 °C (Macků 1957).

TRENDS OF *T. AESTIVUM* CULTIVATION IN CENTRAL EUROPE

Soil and climatic requirements of *T. aestivum* can be met in several natural localities of central Europe. In addition to the distinct general advantages mentioned above, this fungus is the easiest of all truffles for commercial cultivation (Chevalier 2001, Belloli et al. 2001) and the only species with fruitbodies ripening advantageously from late May through September.

In the Czech Republic the summer truffle is listed among critically endangered species protected by the Decree for implementation, No. 395/1992 Coll. Acts., vers. 175/2006 Coll. Acts (see also Šebek 1985; Kotlaba 1992; Kotlaba 1994, 1995; Gryndler et al. 2004). *T. aestivum* is not listed in the Red list of fungi (macromycetes) of the Czech Republic (Holec and Beran 2006) for a simple reason – no hypogeous fungi were elaborated for this publication. Although all legal steps have not yet been taken in every country of central Europe, extinct, critically endangered and endangered fungi are also regulated by national laws in the Slovak Republic (Lizoň 1995, 2001; Kotlaba 1995), Hungary (Rimóczi et al. 1999; Benedek et al. 2002, 2003) and Poland (Wojewoda and Lawrynowizc 2006, Lawrynowicz et al. 2008).

In the Slovak Republic, truffles were mentioned and traditionally collected in natural habitats as early as in the 16th century. Their consumption, gastronomic knowledge and market increased continuously, culminating under the Austro-Hungarian Empire. In the last decades, however, their occurrence in the nature declined and consequently they were classified as disappearing or not longer present (Lizoň 1993). The rediscovery started within 2005–2006 with the inventory research of black truffles in Carpathian localities by enthusiastic scientists (Gažo et al. 2005, Miko et al. 2006) and initiatives taken by the First Slovak Truffle Association (Anonymus 1). *T. aestivum* (*T. uncinatum*) was found to be the most suitable indigenously growing black truffle species for further experimentations. Current interest is focused on in situ natural habitat protection and biodiversity conservation of the summer truffle and ex situ preservation of its authentic gene pool in a Slovak gene bank (Gažo and Miko 2005). For further commercial application, research is continuously developing on certification of native oak seedlings and levels of mycorrhization by well adapted *T. aestivum* ecotypes to refer to national technical standards (Miko and Gažo 2007 a, b; Gažo and Miko 2005, 2007).

In Hungary, truffles were mentioned first around 1540. From that time, they have been increasingly collected from the nature and appreciated as a culinary delicacy. The first important scientific study on hypogeous fungi of the Austro-Hungarian territory with a key map of *T. aestivum* and *Choiromyces meandriformis* records in whole Europe were presented in Hungarian by Hollós (1911). After the Second World War the interest in subterraneous fungi was stimulated by mycological exploration of the Carpathian-Pannonian region by Szemere (1965). His work published in Hungarian and German extended the general knowledge of truffle habitats and geographical distribution in Hungary, making it accessible to international experts. After overall attenuation during two oppressive periods (1914–1918 and 1948–1989), interest in truffles revived in the middle of 1990's and led to the foundation of the First Hungarian Truffle Association in 1997. Among several truffle species growing in quantities in Hungary, *T. aestivum* (*T. uncinatum*) is the most appreciated truffle yielding 4–9 t per year from its natural habitats with unlimited selling possibilities for fresh truffles on the international market (Gógán and Dimény 2003). Its selling price is presently 300 Euros/kg (Miko and Gažo, personal communication). The technology of *T. aestivum* culturing and production is considered as managed (Bratek and Halász 2005; Gógán et al. 2006; Csorbainé Gógán et al. 2007 a, b). More recently work has begun with some other *Tuber* species. Around 25 farms in Hungary have now started to cultivate truffles with perspectives for future truffle production (Anonymus 2).

In the Czech Republic, *T. aestivum* is apparently an integral component of most calcareous ecosystems with typical thermophilous vegetation as predicted by Macků (1914, 1957), Klika (1927) and Šebek (1985, 1992) and confirmed by current internet records. Like neighbouring countries, Czech Republic is in a process of land consolidation, improvement and rural development. This effort is supported by the Czech Republic's National Strategic Plan for the period 2007–2013 issued by the EU. This plan focuses primarily on protection of nature, environment and natural resources as well as on biodiversity conservation and development and diversification of rural life. Consequently, *T. aestivum* attracts increasing interest as an alternative technology for agriculture and forestry as proposed long time ago by Macků (1914, 1957).

The objective of this article is to offer background information on *T. aestivum* in the Czech Republic in order to encourage a systematic research on this truffle in our country. For a basic inventory, long-term investigations are indispensable to begin with Czech protected landscape areas (national parks, protected landscape areas, nature reserves etc.) where soil and climatic requirements of the fungus can be met. In this context, a close cooperation of researchers with a wide network of Czech amateur mycologists and authorised persons (Valda 2006) could advance the acquisition of relevant distribution data referring to defined localities. Research may take great advantage of studies of pure cultures of *T. aestivum*

in the laboratory. The mycelium can be isolated from ascocarps or mycorrhizae, identified by morphological and molecular methods and cultivated under defined conditions (e.g. Iotti et al. 2002, our unpublished results). We were able to isolate the mycelium of indigenous *T. aestivum* from an ascocarp and confirmed its identity by comparison of ITS regions of rDNA cassette with records in GenBank database (unpublished data). Our strain can be easier cultivated in the laboratory than some other *Tuber* spp. suggesting great advantages of this fungus for experimental projects.

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