Studies on the ecophysiology of *Tuber aestivum* populations in the Carpatho-Pannonian region

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Abstract: Various studies were carried out in order to determine the ecological requirements for summer (burgundy) truffle biotypes in the Carpatho-Pannonian region. Based on the experience gained from in situ collections and rearings, it seems that many insects, including specialist species, have a part in spreading the fungus. The data of soil analysis show that *Tuber aestivum* has limited tolerance (stenotopic) of several soil parameters, but has a broad range of tolerance (eurytopic) of other soil parameters, which may contribute to its wide distribution. Studies on the soil horizons of habitats with outstanding productivity confirm the importance of a well-balanced water supply. The secret of the best regions may be the soil profile, which could explain the excellence of the Jászság region in Europe. The analysis of phytoindication can provide useful information for the description of water supplies and the local microclimate. The summer truffle is rare or absent in dry forest types (e.g., oak forests on sandy or loess soils) and in the forests of wetlands. All in all, summer truffles appear to have a strong preference for certain plants and soil types. On the basis of the above results and of coexistence analysis, an attempt was made to establish a niche segregation model.

Zusammenfassung: Verschiedene Untersuchungen wurden durchgeführt um die ökologischen Ansprüche der Sommertrüffel-Biotypen in der Karpaten-Pannonischen Region zu erheben. Aufgrund der Erfahrung, die bei in situ-Aufsammlungen gemacht wurden, scheint es, dass viele Insekten, inklusive spezialisierter Arten, eine Rolle in der Ausbreitung des Pilzes haben. Die Bodenanalysedaten zeigen, dass *Tuber aestivum* eine begrenzte Toleranz (stenotop) für einige, jedoch eine hohe Toleranz (eurytop) für andere Bodenparameter hat, was zu ihrer weiten Verbreitung beitragen könnte. Untersuchungen des Bodenhorizonts von Habitaten mit außerordentlich hoher Produktivität bestätigen die Wichtigkeit einer ausgewogenen Wasserversorgung. Das „Geheimnis" der besten Regionen mag das Bodenprofil sein, was die herausragende Stellung der Jászság Region in Europa erklären könnte. Die Analyse der Indikatorpflanzen kann wertvolle Informationen liefern für die Beschreibung des Wasserhaushaltes und des lokalen Mikroklimas. Die Sommertrüffel ist selten oder fehlt in trockenen Waldtypen (z. B. Eichenwälder auf sandigen oder lößhaltigen Böden) und in Wäldern von Feuchtgebieten. Insgesamt scheint die Sommertrüffel eine strenge Vorliebe für bestimmte Pflanzen und Bodentypen zu haben. Auf der Basis obiger Ergebnisse und anhand von Koexistenzanalysen wurde versucht, ein Einnischungsmodell zu erstellen.

Summer (burgundy) truffles are the most widespread species of black truffles. The economic value of black truffles is well-known, and is based on their outstanding role in gastronomy. However, little is known about the ecological role of truffles in natural plant associations in Europe. This lack of knowledge has been underlined by the rapid spread of invasive fungi, such as that of winter truffles (*Tuber brumale* VITTAD.) in certain regions of Europe over the last few decades, or the identification of Chinese truffles in European truffle plantations in recent years (MURAT & al. 2008). On these grounds, many authors (GARCIA-MONTERO & al. 2008) ave drawn attention to the fact that autochthonous European truffle species, such as the burgundy truffle, are now endangered.

Research on burgundy truffles was pushed into the background for a long time due to the privileged status enjoyed by *T. melanosporum*. In Burgundy, however, serious efforts have been underway in last decades to rehabilitate burgundy truffles (CHEVALIER & al. 2005), leading to the expansion both of research on the species and of its cultivation throughout Europe. The species has been found to avoid strongly acidic soils and, unlike *T. melanosporum*, to survive on soils containing no lime (WEDÉN & DANELL 2007). In contrast to périgord truffles, it does not require loose, well-aerated soil, tending to prefer heavier soils. It is also characterised by a high humus requirement. The data obtained so far suggest that the species favours semihumid habitats (ILLYÉS & al. 2008). Acquiring information on the ecological requirements of burgundy truffles is complicated not only by the fact that wide-ranging investigations have only been carried out in a few cultivation regions in Europe, but also by the great heterogeneity of the European habitats as regards both soil types and plant associations. The present paper summarises the results obtained so far on the ecological and eco-physiological aspects of populations found in the Carpatho-Pannonian region.

Material and methods

The incidence of burgundy truffles in the Carpatho-Pannonian region was evaluated using the Microsoft Access database set up for the hypogaeous species in the region (MERÉNYI & al. 2008). This includes older herbarium data published or revised by mycologists from this region and also the data of freshly collected material. The latter is constantly being expanded and the data include the results of coenological surveys and soil analysis. Since 1992 surveys have been made of 169 burgundy truffle habitats. Based on the BRAUN-BLANQUET combined estimation method, the surveys were carried out using 10 × 10 m quadrants on the areas around truffle nests. When evaluating the phytoindication value of truffle habitats, the data for the individual plant associations were analysed both together and separately, using the ecological indicator values optimised by BORHIDI (1993) for the Carpatho-Pannonian biogeographical region. The analysis included the species composition of the herbaceous canopy (presence/absence) and the dominance/abundance relationships. The syntaxonomic ranking of each plant association was based on the coenological data, following BORHIDI (2003), SOÓ (1981) and SIMON (2000) or, in the case of Trans-sylvanian associations, following DONITA & al. (1992). The habitat analysis was complemented by chemical and physical soil analysis, and by the examination of soil stratification. The soil analyses were carried out according to the Hungarian standards at the Soil Conservation Laboratory of the Plant Health and Soil Conservation Station of Fejér County.

The soil maps compiled for Hungary at the Research Institute for Soil Science and Agricultural Chemistry of the Hungarian Academy of Sciences and for Europe (STANNERS & BOURDEAU 1995) were used in the evaluation. Co-existence analysis was based on the joint occurrence of burgundy truffles with other hypogaeous fungi within the quadrants used to collect coenological data. The maggots found in burgundy truffles were hatched into flies in moist sawdust, while beetles were collected from the fruit bodies and from the soil surrounding them.

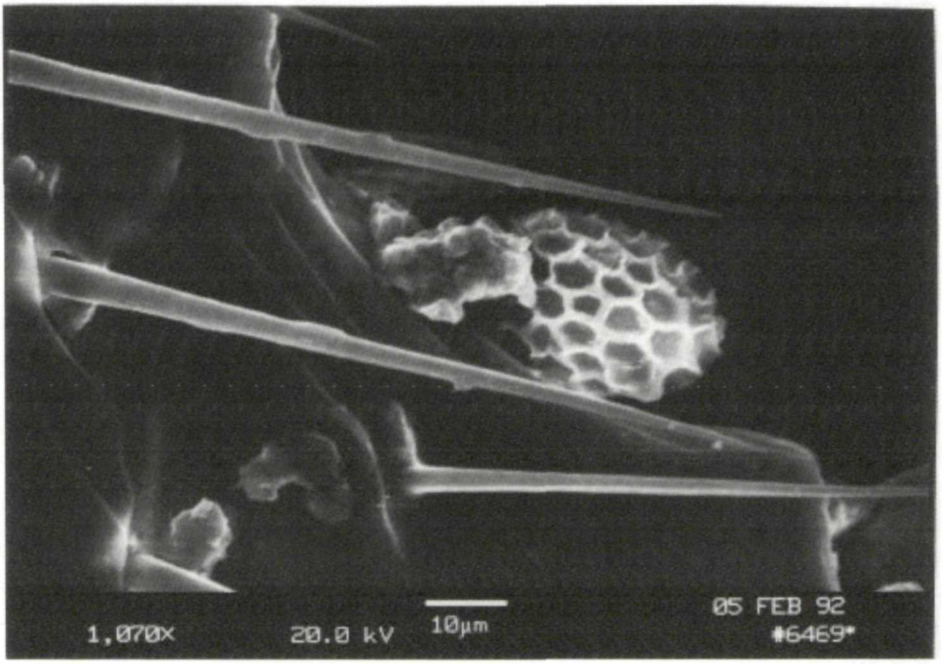

Fig. 1. Spore of *Tuber* spec. adhering to the cuticle of an adult specimen of *Leiodes cinnamomea* (SEM).

Results and discussion

Burgundy truffles are one of the most widespread truffle species in the Carpatho-Pannonian region, though there are many parts of the region where they are not found. The database compiled by the authors confirmed information provided by the burgundy truffle habitat map drawn by HOLLÓS (1911), that this fungus is generally missing from areas with acidic soil, such as acidic parts of the Carpathian range and the North Hungarian hills (Mátra, Zemplén), from areas with acidic sandy soil (Nyírség, Belső-Somogy) and from Western Transdanubia. It is also missing from sandy soils with poor water management (region between the Rivers Danube and Tisza) and from loess soils (Mezőség).

The chemical analysis of 96 soil samples (Table 1) taken from truffle-beds in various burgundy truffle habitats indicated that more than two-thirds of the *Tuber aestivum* habitats in the Carpathian Basin have heavy clay texture, a fifth are clay, while the remainder are clay loam or loam. The pH is generally weakly alkaline or neutral, or possible mildly acidic. A comparison of the phytoindication (TWR) analysis and soil pH data indicated a good degree of correlation. The humus content was high, with little deviation, indicating a balanced supply of humus. Most of the soils contained little (5-8%) or only traces of (0.1-5%) lime. The phosphate content of the tested soils exhibited great variability, with a very high content (>36 mg/100 g) in a third of the samples. The majority of the samples also had very high (>36 mg/100 g) potassium content.

In an earlier study with a much smaller sample number the soil requirements of burgundy truffles were compared with those of five other *Tuber* species (BRATEK & al. 1999). Among the seven soil parameters tested, at least two exhibited a significant difference between the requirements of burgundy truffles and other *Tuber* species, possibly reflecting the niche segregation of *Tuber* species on the basis of chemical soil properties.

The now tested soils belonged to the following types: over a third were brown forest soils with clay illuviations, a quarter were Ramann's brown forest soils and a sixth were meadow alluvial soils, while meadow soils and meadow chernozems each made up almost a tenth of the samples. It was interesting to note that the Jászság forests, where the greatest truffle yields were recorded, belonged to this last category. Samples in this category differed significantly from the other soil types only in their lower (1.3 m/m % on average) or completely lacking lime content and in their much lower soluble calcium ion content (average: 5146 ppm). A significantly ($p<0.001$) higher P_2O_5 content (around 1400 ppm) was detected in soils with outstanding productivity, compared with soils in the good, moderately good and poor categories (below 300 ppm).

The use of SYN-TAX 2000 (PODANI 2001) to compare the vegetation of the habitats only revealed groupings on the basis of woody plants, indicating three clearly distinct groups: the largest group, Group I, included habitats where *Carpinus betulus* was dominant, Group II consisted of habitats with *Quercus robur* dominance, while the smallest group, Group III, had *Quercus cerris* as the dominant species. The 169 *T. aestivum* habitats analysed in the Carpatho-Pannonian region could be divided into 23 plant associations, the largest (39%) being the montane Carici pilosae-Carpinetum association, followed by the Acer campestri-Quercetum association (11%), while the Quercetum petraeae-cerris and Melampyro bihariense-Carpinetum associations each made up over 5%.

Based on the phytoindicative traits of individual plants, the vegetation of natural *T. aestivum* habitats reveals much about the ecological requirements of the fungus. Joint analysis considering both the species composition (presence/absence) of the herbaceous canopy and the dominance/abundance relationships may provide a more accurate picture of the real ecological requirements, since the widespread presence of a species with broad ecological tolerance (e.g., *Galeobdolon luteum*, *Brachypodium sylvaticum*, *Viola sylvestris*) or tolerance of soil disturbance (e.g., *Geum urbanum*, *Rubus caesius*) may distort the analysis, while the presence of specialist plants (e.g., *Lathyrus vernus*, *Helleborus purpurascens*, *Lithospermum purpureo-coeruleum*) may provide important information about the habitat even in the case of low abundance. One of the most decisive ecological parameters proved to be the occurrence in relation to soil moisture or water table (WB: 1-12; BORHIDI 1995). Unlike the soil reaction (RB: 1-9) or the nitrogen requirement (NB: 1-9), this cannot be measured by routine soil analysis. From the soil moisture point of view, plants indicative of semi-humid habitats, under intermediate conditions (WB 5) were present in the greatest species number and abundance. In the case of abundance they were followed by plants of fresh soils (WB 6) and plants of moist soils that do not dry out and are well aerated (WB 7). Plants of semi-dry habitats (WB 4) had somewhat smaller abundance than the WB 7 plants, but had a species number similar to that of WB 6 plants.

Table 1. Results of chemical analysis of 96 samples taken from truffle-beds of various burgundy truffle habitats in the Carpatho-Pannonian region.

Parameters	Average	Standard deviation	Minimum	Maximum
$pH(H_2O)$	7.17	0.34	6.7	7.94
K_A	65.06	11.17	39.00	92.00
$CaCO_3$ m/m %	6.18	7.45	0.00	39.00
Humus m/m %	6.45 %	1.95	2.76 %	10.80 %
NO_3-NO_2-N mg/kg	15.51	11.47	1.90	53.30
P_2O_5 mg/kg	423.22	647.40	13.00	2534.20
K_2O mg/kg	498.06	234.74	139.00	1200.10
CA ppm	25208.02	27096.57	1884.60	136903.20

Based on the pedological, climatic and plant ecological results discussed above, an increasingly accurate picture can be formed of the ecological requirements of burgundy truffle populations in the Carpatho-Pannonian region, thus allowing potential growing areas to be identified. It is clear that the ecological requirements of burgundy truffles are distinct from those of many other underground fungus species, though overlapping with those of numerous hypogaeous fungi. Co-existence analysis based on the database also suggested the existence of characteristic niche preferences. For example, burgundy truffles occur most frequently in habitats where the fruit bodies of *Tuber excavatum* and *T. fulgens* are also found. In other habitats burgundy truffles tend to be associated with *T. rufum, T. brumale* or certain *Hymenogaster* species (*H. luteus, H. citrinus-H. olivaceus* aggr.).

Among the beetles that feed on the fruit bodies of burgundy truffles, *Leiodes cinnamomea* is by far the most common (72%) in the Carpatho-Pannonian region, while *Agaricophagus cephalotes* and *Colenis immunda* make up more than 10% each, and *Agaricophagus reitteri* was also found sporadically. Scanning electron microscopic (SEM, Fig. 1) studies confirmed the role of these beetle species in spreading the fungus, as large numbers of fungal spores were found adhering to the underparts and legs of adult beetles feeding on truffles. On the other hand, SEM revealed no spores on flies hatched from the fruit bodies of truffles, thus excluding their role in spreading the fungus. The most frequent species (>10% occurrence) among the 980 flies hatched in moist sawdust were: *Cheilosia scutellata*, *Suillia gigantea*, *Coboldia fuscipes*, *Sciaridae* spec. and *Lycoriella solani*.

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