A database and its application for the development of truffle cultivation methods

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Abstract: The cultivation of *Tuber aestivum* may be influenced by many factors, hence to establish a highly productive truffle-orchard it is important to know what are the optimal ecological demands of host plant and the truffle. In the Carpatho-Pannonian region more than 100 years old research of hypogeous fungi has provided a lot of data of habitats, mainly in the last 20 years. To prepare a comprehensive analysis the following data were integrated: nearly 6000 herbarial data (with earlier herbarium), nearly 1000 coenological tables, and more than 2000 pedological surveys. In the case of *Tuber aestivum* the integrated database contains 389 herbarial data, 166 coenological tables and detailed soil analysis of 217 truffle-bed. The database has been used successfully for answering several questions on truffle ecology and truffle cultivation. Results of this project may help the characterization of Central European *T. aestivum* biotypes and also may promote distinctive site selection and maintenance of truffle orchards in different geographical microregions of Carpatho-Pannonian region.

Zusammenfassung: Die Kultur der Sommertrüffel kann durch viele Faktoren beeinflusst sein. Daher ist es für die Errichtung einer hochproduktiven Trüffelkultur wichtig die optimalen ökologischen Bedingungen des Wirtes und der Trüffel zu kennen. Im Karpaten-Pannonischen Gebiet erbrachte die mehr als 100jährige Erforschung hypogäischer Pilze viele Daten über Habitate, besonders in den letzten 20 Jahren. Beinahe 6000 Herbardaten, fast 1000 coenologische Tabellen und mehr als 2000 bodenbiologische Befunde wurden in die umfassende Analyse integriert. Für *Tuber aestivum* enthält die Datenbank 389 Herbardaten, 166 coenologische Tabellen and detaillierte Bodenanalysen von 217 Trüffelfundstellen. Die Datenbank wurde erfolgreich für die Beantwortung etlicher Fragen der Trüffelökologie und -kultur herangezogen. Die Ergebnisse dieses Projektes könnten zur Charakterisierung mitteleuropäischer *T. aestivum*-Biotypen und zur gezielten Geländeauswahl und Erhaltung von Trüffelkulturen in Kleinregionen des Karpaten-Pannonischen Gebiets beitragen.

There are still remaining some lesser known factors in the field of truffle habitat ecology (HALL & al. 2008), in spite of that the first truffle orchards were established almost 200 years ago (OLIVIER & al. 2002) and recently more than 10000 hectares produce truffle year by year (REYNA 2007). In the case of *Tuber aestivum*, which was investigated less intensively, the smattering of cultivation is more significant compared to *Tuber melanosporum*. *Tuber melanosporum* could not spread in the area ranging eastward from the Alps (LAWRYNOWICZ 1992). On the other hand *T. aestivum* is widespread crossover in Europe. Therefore it is very important to get knowledge on *T. aes-*

tivum and its cultivation in Central Europe and in Hungary as well. LÁSZLÓ HOLLÓS (1911) the world-famous Hungarian scientist of underground mushrooms submitted the first map of some hypogeous fungi. Some decades later LÁSZLÓ SZEMERE (1970) made an attempt to establish a truffle plantation. After that the studies were focussed on creating databases and monitoring of macromycetes, fungal ecology and coenology (KONECSNI 1981, RIMÓCZI & PRAJCZER 1996, LÁZÁR & PÁL-FÁM 2005).

Restarting the truffle hunting in the 1990's in Hungary facilitated the monitoring, the pedological and botanical research of other hypogeous fungi and the establishment of mycothecas. Subsequently, the number and the complexity of data made it necessary to manage them by database programs. Especially from the experimental plots of the cooperation between Eötvös University and the French National Agricultural Research Institute (INRA, BRATEK 2008) a lot of data are produced year by year, which have to be managed.

Material and methods

Databases were created with Microsoft Access. The database of hypogeous fungi consists mainly of the data of hypogeous fungi collected in the last two decades in Hungary and of mycothecas from the Carpatho-Pannonian region. Data of collections, micro- and macromorphology, coenology and plant syntaxonomy, and pedology are included in different worksheets.

The database of the Hungarian experimental plantations includes the data of the plants, which were examinated at the age of three years determining their phenological and mycorrhizal features. Furthermore, it contains the results of the botanical and pedological examinations in the fields.

Results and discussion

Query of hypogeae data

To determine the productive area of *T. aestivum* in the Carpatho-Pannonian region, its habitats were queried from herbarial worksheets of the database of hypogeous fungi.

Further information can be gained on the altitudes of habitats (82.5% of habitats located below 400 m s. m.), on the ripening period of the life cycle (from June to January) from these worksheets. The data queried of the number of collections from the last decades normalised by the total number of the collections seem to follow the annual precipitation values.

Multiple queries of hypogeae and coenological data

Plant species with frequent occurrence in *Tuber aestivum* habitats of Carpatho-Pannonian region can be queried as well. Among these species, the host plants and the indicative plants can be found beside the non-ectomycorrhiza-forming and indifferent species.

The phytoindication, which is characteristic for habitats, can be determined by analysis of TWR-values of the local herbs (BORHIDI 1993). Table 1 presents the TWR analysis of the coenological tables of 166 *T. aestivum* habitats. The comparison of these results with the TWR analysis of plantations makes possible to draw some conclusions if the area is suitable for the cultivation of a certain truffle species on a certain host plant. This method can impress better the impact of certain environmental factors, despite of being just an estimating method based on long time botanical experiences, in front of a specific measurement carried out in precise time of the particular year. Richness in nutrients and too wet character of the soils of the two plantations (Table 1) were unfavourable for mycorrhization of particular host plants, mainly for *Pinus nigra*

and *Quercus petraea*. The mycorrhization levels of Szilvásvárad orchard remain below the ones of the other plantations probably due to the unfavourably high soil moisture level for *T. aestivum* (CHEVALIER & al. 2005).

Table 1. TWR values determined in two plantations compared with TWR-values of host-plants (HORVÁTH & al. 1995) and with the TWR values of natural truffle sites. Highlighted with grey the TWR values which are significantly different from the calculated average values of the particular plantation.

Host plants	TB-scale			WB-scale			RB-scale			NB-scale			LB-scale			sampling (No.)	Average mycorrhization (%)	Average contamination (%)
Truffle plantation in Szilvásvárad	Horváth et al 1995	orchard		Horváth et al 1995	orchard		Horváth et al 1995	orchard		Horváth et al 1995	orchard		Horváth et al 1995	orchard				
		med	s-med		med	s-med		med	s-med		med	s-med		med	s-med			
Carpinus betulus	6	5	5	5	5	9 (6)	6	6	6 (7)	5	5	5	5	7	7	21	58,7	1,2
Corylus avellana	5	5	5	5	5	9 (6)	6	6	6 (7)	4	5	5	5	7	7	8	30	0
Pinus nigra	5	5	5	4	5	9 (6)	5	6	6 (7)	4	5	5	7	7	7	13	34,1	16,9
Quercus petraea	6	5	5	5	5	9 (6)	5	6	6 (7)	5	5	5	8	7	7	9	12,6	0
Quercus robur	6	5	5	6	5	9 (6)	6	6	6 (7)	4	5	5	6	7	7	15	48	0,1
Tuber aestivum	5	5	5	5	5	9 (6)	6 (7)	6	6 (7)	5	5	5	6	7	7			
Truffle plantation in Kiskunfélegyháza 2																		
Carpinus betulus	6	5 (6)	ND	6	4 (7)	ND	6	7 (6)	ND	5	7 (4)	ND	6	7	ND	24	70,2	0
Corylus avellana	5	5 (6)	ND	5	4 (7)	ND	6	7 (6)	ND	7	7 (4)	ND	5	7	ND	18	59,3	0
Pinus nigra	5	5 (6)	ND	4	4 (7)	ND	5	7 (6)	ND	5	7 (4)	ND	7	7	ND	24	78	1,1
Quercus petraea	6	5 (6)	ND	5	4 (7)	ND	5	7 (6)	ND	5	7 (4)	ND	6	7	ND	10	62,5	0
Quercus robur	6	5 (6)	ND	6	4 (7)	ND	6	7 (6)	ND	4	7 (4)	ND	6	7	ND	29	56	0
Tuber aestivum	5	5 (6)	ND	5	4 (7)	ND	6 (7)	7 (6)	ND	5	7 (4)	ND	5	7	ND			

The total average mycorrhization and contamination values (Fig. 1) of the host plants from experimental plantations were compared with the constancy values of these host plants in natural truffle sites of the region. The constancy of a plant here means how often this plant can be found together with *T. aestivum* and counted as the percentage of its appearance in the coenological tables. *Carpinus betulus* had the highest constancy (63.3%) in natural habitats. The highest mycorrhizal frequency of the plantations (61.67%) was detected also in the case of *Carpinus betulus*. *Pinus nigra* shows quite good mycorrhization in plantations, but its contamination is also quite high. In the region just few sporadic data of *T. aestivum* associated with *Pinus nigra* were reported. This is probably partly due to the special preference of truffle hunters and partly due to forestal application of this introduced species in reforestation of extremely dry sites in sandy and mountainous areas. The mycorrhization in plantations and occurrence in natural habitats of *Corylus avellana* are also at a medium level, but its average contamination remains relatively low, which is in contrast to the French experiences (CHEVALIER & al. 2005). Conversely the contamination of oaks is higher than expected.

Multiple queries of hypogeae and soil surveys

It is possible to compare the results of soil analysis, pH(H_2O), pH(KCl), SPA (Sticky point by Arany), salt, $CaCO_3$, humus, NO_3-NO_2-N, P_2O_5, K_2O, Ca, Mg, Mn, Na, Zn, Cu, Fe, Mo, B, SO_4-S, Al, As, Cd, Co, Cr, Hg, Ni, Pb, of plantations with the data of natural truffle sites measured with the same method. The productivity of natural *T. aestivum* habitats were estimated by the quantity of harvested truffles at a certain site and the

habitats were categorised into four groups: weak, medium, good, excellent. It gives the possibility to analyse and compare the soil characters of the habitats of different productivity.

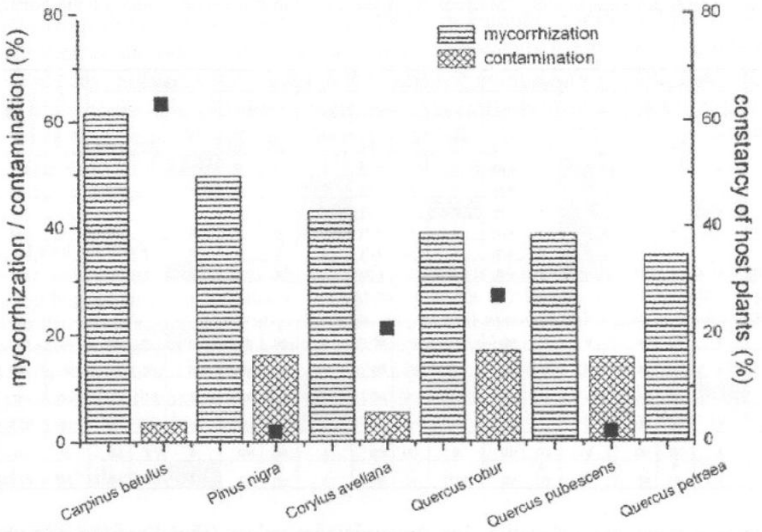

Fig. 1. Constancy values of certain *Tuber aestivum* host plants and average values of mycorrhization and contamination in the INRA-ELTE experimental plantations.

Other queries

The co-occurrences of different hypogeous species in the same area (10 × 10 m) or in the same truffle bed were recognized. These data also reveal that there are hypogeous species which are usually living together (HALL & al. 2003, LUKÁCS & al. 1993). Mostly *T. excavatum* and *T. aestivum* can be found together in the same habitats (Fig. 2). This may be due to their similar ecological demands. The co-occurrence of *Tuber* species is more frequent as it is with species of other genera. Probably this phenomenon is due to the truffle consumer insects. Up to now the contaminant mycorrhizal fungi identified by their ITS sequences are *Tuber maculatum*, *Tomentella* spec., *Suillus granulatus*, *S. collinitus*, *Scleroderma bovista*, and *S. areolatum*. Among them the only hypogeous species is *Tuber maculatum*, but as seen in Fig. 2, this species was not found in the natural habitats of *T. aestivum*. The contaminant fungi alternate during the time. So other hypogeous species can be found often in *T. aestivum* natural habitats like *Tuber excavatum*, *T. brumale* or *Hymenogaster*, appearing in the plantations just later and jeopardizing especially the older plantations (SOURZAT 2004).

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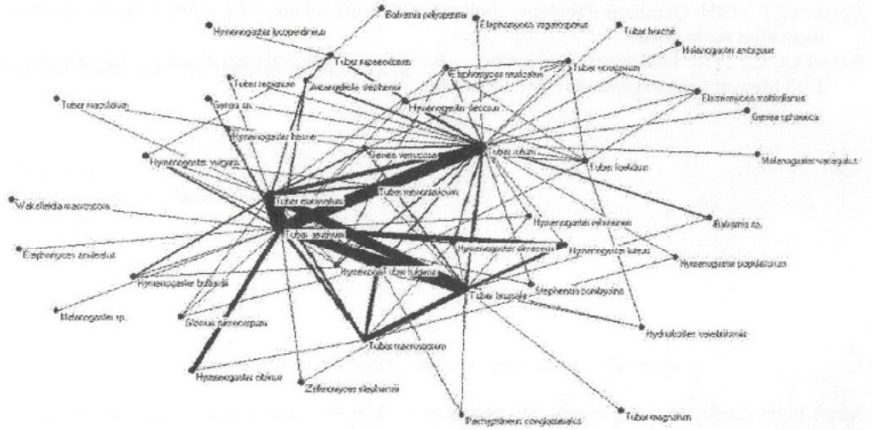

Fig. 2. Association of several hypogeous fungi: thickness of lines marks the frequency of co-occurring.

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