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ORIGINAL RESEARCH ARTICLE

Environmental conditions that promote the occurrence of truffles (*Tuber* spp.) on historical sites in Poland

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Abstract. This article highlights historical data regarding truffles' occurrence in Poland. Along with the soil parameters the plant communities at the sites were studied. The results of the chemical soil analyses showed that the soil pH in water on 5 sites was acidic (from 4.3 to 6.1), and only in one, Wiązowna, was the pH (7.2) conductive to truffles development. Similarly, the content of calcium carbonate ($CaCO_3$) in soil samples was low (from 0 to 0.03%), except for Wiązowna, where $CaCO_3$ was 0.12%. Among the 24 reported species of trees and shrubs, 7 species were host-plants of summer truffle (*Tuber aestivum* Vitt.). Out of the 7 species, oak and hornbeam were present at four localisations. Across the sites, 31 species of ground-layer plants were identified. Among these, *Epipactis helleborine* was only one host-species of summer truffle. Our findings indicate that formation of truffles fruiting-bodies depends on specific habitat characteristics. The key factors determining this process are soil parameters, such as: texture, pH and calcium content. Our inventory showed that the sites we studied still persist as natural stands, although only one of them seems to be favorable for truffles development: this site is located in Wiązowna, where soil is of pH 7.2 and *E. helleborine*, (host species for truffles from Orchidaceae) is found, fulfills the environmental requirements of truffles.

Key words: truffle, host plants, soil parameters, habitat, historical sites

1. Introduction

Truffles are fungus characterised by underground fruiting bodies (hypogeal organism). They create mycorrhiza, a mutualistic symbiosis with many species of forest trees and bushes, for instance with oak, beech, tilia, hornbeam and hazel. The high culinary and commercial value of truffles is because of its characteristic smell of fruiting bodies (Brillant-Savarin 1825). The first mention about truffles in the Polish literature can be found in Czerniecki's book (1682). It is the first Polish cookbook in which the author wrote the recipe for 'tertofele w popiele' (truffles in ash). Other notices about cultivation and culinary properties of the valuable underground fungus can be found in some books published at the end of the 19th century and at the beginning of the 20th century (Aleksandrowicz, Błoński 1894; Gawarecki 1895; Spausta 1897; Swoboda 1928; Dąbrowska 1996).

Despite rich literature about truffles, truffle occurrence in Poland was questioned until the 1940s (Orłoś 1947). In 1953, Lubelska described sites where *Tuber aestivum* occurred. However, there are no herbarium materials available to confirm the identity of truffle fruiting bodies noticed by Lubelska. *Tuber mesentericum* is another truffle species that occurs in Poland. It was found and identified by Ławrynowicz (1999) at one site in Kraków-Częstochowa Upland.

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In 2007, fruiting bodies of *T. aestivum* Vittad. were found by Hilszczańska et al. (2008) at several sites in Poland. It is not excluded that sporocarps of the fungus occur in Poland in large numbers, for instance, fruiting bodies of *Boletus*. However, the species is not known so well due to hypogeal growth (fruiting bodies are in the soil, 10 cm under the ground) as well as due to lack of tradition of picking truffles for culinary purposes.

The aim of the research is evaluation of historical sites of *Tuber* spp. on the basis of soil parameters and the diversity of plant communities with regard to environmental conditions that are conducive to truffles' growth.

2. Materials and methods

Based on the historical data, environmental analysis of the old sites where truffles formed was performed. Collected information regards geology of the terrain, species composition, the age and history of stands. 6 localisations were chosen in the central part of Poland (Table 1). The occurrence of trees and bushes interacting with truffles in mycorrhiza symbiosis was the criterion for the selection. Geographic coordinates are not included in the publication due to the danger of excessive sites penetration. Five out of six selected sites are forest stands, managed and cultivated. Only Wiązowna site is the former truffle garden, plantation that was cultivated before the World War II. Human activity is limited at the site, but natural succession of plants influences species composition of the former plantation.

From the end of June to the mid-July 2012 hunting for truffle fruiting bodies was conducted at all sites with the help of the scent hound, Lagotto romagnolo. Then, the square test site was established of 10 m long side. The list of all tree and bush species was done, as well as the list of plants in the forest floor within the established site (100 m^2). Potential mycorrhizal partners of the summer truffle (*Tuber aestivum* Vittad.) were pointed (Table 3). From the central part of the site the forest floor and some plants were removed and the 0.5 kg soil sample (up to 30 cm depth) was taken for further research of the chemical composition and texture of the soil. Soil particle size fractions analysis was done according to standard PN ISO 11277 (2005). Soil was classified according to standard PN-EN ISO 14688-2 (2005) (Fig. 1).

Soil pH was marked on the basis of standard PN-ISO 10390 (1997). Calcium carbonate was estimated by Scheibler method (ISO 10693 1994). The percentage of N was estimated by PN-ISO 13878 (2002) method, and the percentage of C was estimated according to PN-ISO 10694 (2002). The content of basic nutrients was analysed according to the method PB-14 ed. 2 (01.01.2010). Analyses were conducted in the Polish Centre for Accreditation No. AB740. Chemical soil characteristic was compared to results obtained from five sites of *Tuber aestivum* described recently in Poland (Hilszczańska 2008).

Table 1. Characteristics of *Tuber* spp. historical sites

Plot No.	Site	Location	Type (forest/plantation)	Altitude (m)	Date of inventory	References	*Tuber* species
1	Skuły	Rawa Plateau	forest stand	189	28.06.2012	Ławrynowicz (1988)	*Tuber rufum*
2	Wiązowna	Garwolin Plain	plantation established before World War II	149	09.07.2012	Dąbrowska (1996)	*Tuber* sp.
3	Maciejowice 1	Żelechów Plateau	forest stand	143	09.07.2012	Aleksandrowicz i Błoński (1894)	*Tuber aestivum*
4	Maciejowice 2	Żelechów Plateau	forest stand	130	09.07.2012	Aleksandrowicz i Błoński (1894)	*Tuber aestivum*
5	Kępa Solecka	Chodel Basin	forest stand	127	10.07.2012	Aleksandrowicz i Błoński 1894	*Tuber aestivum*
6	Tuszyn	Łódź Plateau	forest stand	205	11.07.2012	Spausta 1897	*Tuber* sp.

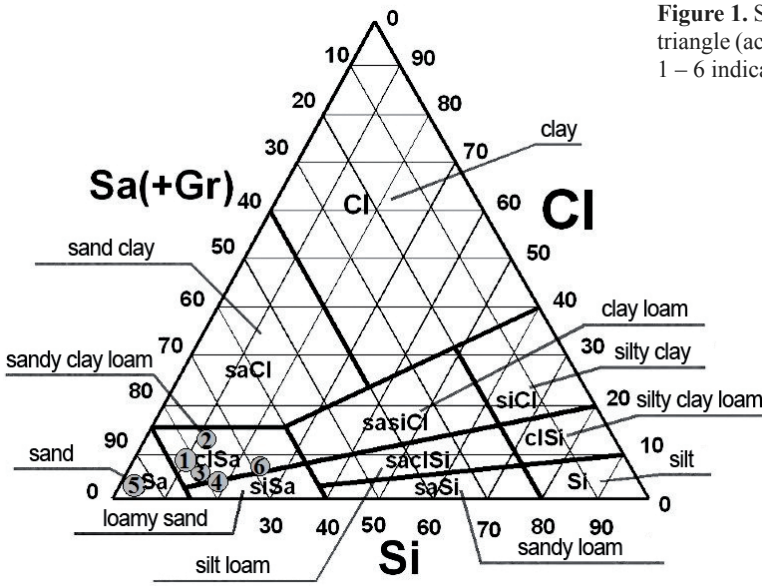

Figure 1. Soil texture diagram of 6 truffle historical sites – the triangle (according to Tarnawski et al. 2011, altered). Numbers 1 – 6 indicate location depicted in Table 1.

3. Results

Hunt with the dog gave no results and truffle fruiting bodies were not found at any site. Results of the chemical analysis and soil texture are presented in Table 2. Soils from researched stands differ significantly with regard to pH. Analyses showed that in Skuły and Kępa Solecka sites, soil pH was highly acidic, and in Maciejowice 1 site, it was acidic. In Maciejowice 2 and Tuszyn sites, analyses showed higher soil pH – 6.1 and 6.0. Only in Wiązowna site, analyses showed that soil pH was on the verge of alkaline and neutral (7.2). The content of calcium carbonate was 0–0.03% in analysed soil samples with the exception of Wiązowna site – 0.12% $CaCO_3$.

Table 2. Overview of the soil composition at the *Tuber* spp. historical sites

Measured parameter		Site					
		Skuły	Wiązowna	Maciejowice 1	Maciejowice 2	Kępa Solecka	Tuszyn
Soil particle size fractions (%)	clay	8.87	11.89	7.77	5.65	3.73	8.71
	silt	11.52	11.35	13.41	16.50	2.68	23.94
	sand	79.61	76.76	78.82	77.85	94.59	67.35
Water pH		4.3	7.2	5.1	6.1	4.4	6.0
$CaCO_3$ total (%)		0.00	0.12	0.00	0.03	0.00	0.03
Ca (%)		0.030	0.490	0.090	0.160	0.010	0.110
P (%)		0.012	0.043	0.036	0.021	0.009	0.023
Fe (%)		0.110	0.790	0.530	0.250	0.090	0.550
Mg (%)		0.070	0.150	0.060	0.050	0.010	0.110
K (%)		0.110	0.100	0.100	0.080	0.030	0.150
Ca/Mg		0.43	3.27	1.50	3.20	1.00	1.00
K/Mg		1.57	0.67	1.67	1.60	3.00	1.36
C total (%)		1.156	3.840	1.996	2.480	0.627	2.140
C organic (%)		1.156	3.826	1.996	2.476	0.627	2.136
N total (%)		0.087	0.351	0.176	0.177	0.050	0.177
C/N		13.30	10.90	11.30	14.00	12.50	14.00

The content of Ca, Mg, K and acidifiers (P and Fe) was significantly higher in the former truffle plantation than in the forest site. The content of phosphorus ions was over 0.03% only in Maciejowice 1 and Wiązowna sites. At all sites, with the exception of Kępa Solecka site, the content of potassium ions was 0.08–0.15%.

Calcium relation to magnesium in soil was from 0.43 (Skuły site) to 3.27 (Wiązowna site). Potassium relation to magnesium does not exceed 3.5. The highest result was in Kępa Solecka site – 3.00, and the lowest in Wiązowna site – 0.67.

The content of carbon and nitrogen in researched soil was the lowest in Kępa Solecka site (C – 0.627%; N – 0.087%) and Skuły site (C – 1.156%; N – 0.050%) and the highest in Wiązowna site (C 3.840%; N – 0.351%). The carbon relation to nitrogen was from 10.9 (Wiązowna site) to 14.0 (Tuszyn and Maciejowice 2 sites).

Soils were divided according to the ISO 'Krajowy' triangle. According to the triangle, soil was qualified as loamy sand (clSa) at Wiązowna, Skuły, Tuszyn and Maciejowice 1 sites; as silty sand (siSa) at Maciejowice 2 site; and as sand (Sa) at Kępa Solecka site (Fig. 1).

Among 24 tree and bush species indicated altogether at all sites, 7 species create mycorrhiza symbiosis with the summer truffle. Oak and hornbeam were the most common and were found at four sites. Hazel and tilia were at three sites (Table 3). Among the rest of tree and bush species, which are potential partners for truffles, beech and two conifer species (Scots pine and Norway spruce) were found. Thirty-one species of forest floor plants were identified. Among them, one species – broad-leaved Helleborine (*Epipactis helleborine*) – was symbiotic to truffles. In general, Skuły site (21 species) and Wiązowna (19 species) were the richest with regard

Table 3. Plant species recorded on the studied plots of *Tuber* spp. historical sites. Potential host plants are distinguished in boxes.

Species	Skuły	Wiązowna	Maciejowice 1	Maciejowice 2	Kępa Solecka	Tuszyn
			Tree and shrubs			
Abies alba						+
Acer platanoides			+			
Acer pseudoplatanus	+					
Aesculus hippocastanum			+			
Alnus glutinosa	+		+			
Betula pendula	+		+			
Carpinus betulus	⊞		⊞	⊞		⊞
Cornus sanguinea	+	+				
Corylus avellana		⊞			⊞	⊞
Crataegus monogyna		+				
Fagus sylvatica			⊞			⊞
Frangula alnus				+	+	
Fraxinus excelsior	+	+				
Padus avium	+					
Picea abies						⊞
Pinus sylvestris				⊞		⊞
Populus tremula	+	+				
Quercus robur	⊞		⊞		⊞	⊞
Ribes nigrum		+				
Salix caprea		+				
Sorbus aucuparia					+	
Tilia cordata		⊞	⊞	⊞		
Ulmus glabra	+					
Viburnum opulus	+					
Total	11	8	8	4	4	6

Species	Skuły	Wiązowna	Maciejowice 1	Maciejowice 2	Kępa Solecka	Tuszyn
Plants of the forest floor						
Aegopodium podagraria		+	+			
Anemone nemorosa	+				+	
Asarum europaeum	+					
Athyrium filix-femina			+	+		
Campanula latifolia		+				
Convallaria majalis	+			+		+
Epipactis helleborine		⊞				
Equisetum arvense		+				
Galeobdolon luteum	+		+			
Galium aparine		+				
Galium sylvaticum					+	
Geum urbanum		+				
Glechoma hederacea		+				
Hepatica nobilis						+
Hypericum perforatum					+	
Impatiens noli-tangere			+		+	+
Lathyrus sylvestris				+		
Lysimachia nummularia		+				
Maianthemum bifolium				+	+	+
Melittis melissophyllum	+			+		
Oxalis acetosella				+		+
Polygonatum odoratum			+	+		
Polygonum persicaria					+	
Pulmonaria obscura		+				
Ranunculus cassubicus	+					
Rubus sp.		+			+	+
Solidago sp.		+				
Stellaria holostea	+					
Vaccinium myrtillus						
Viola reichenbachiana	+			+		+
Viola riviniana						
Total		11	5	8	7	7

to floral diversity, while Kępa Solecka site (11 species) was the poorest.

4. Discussion

Tuber fungus growth, especially during fruiting, is still poorly examined due to the fact that it is the underground process. Fruiting bodies can be formed because of symbiosis between truffles and some plants and only in special habitat conditions (Stobbe et al. 2012). Besides flora, soil has the key role in truffles' growth. Soil texture, pH and the amount of calcium ions are the basic factors that determine the presence of truffles (Chevalier 2012).

The content of calcium was very low in soil at the researched historical sites. Only in the soil at Wiązowna site, the content of calcium was on the acceptable level for truffles' growth. Thus, one has to verify if fungus registered as truffles in the past was really *Tuber*

sp. Soil pH questions it as well. Comparison between soil pH in the researched sites and soil pH at the present sites of *T. aestivum* (Hilszczańska et al. 2008) shows that only Wiązowna site was characterised by correct soil pH (7.2). In Europe truffles' fruiting in soils of pH lower than 7 was observed only in Gotland (Wedèn et al. 2004). However, in spite of low soil pH, the content of phosphorus was high at Wiązowna site.

The content of phosphorus ions, within the range characteristic for places where summer truffles grow (Hilszczańska et al. 2008), was observed in Maciejowice 1 and Wiązowna sites. Similar results were obtained when the content of iron was analysed. Besides the aforementioned sites, the content of iron was significantly lower than the standard beneficial for *Tuber* fungus growth (Bruhn, Hall 2011). In this regard, Wiązowna site fulfils positive criteria for truffles' growth.

Soil particle size composition at natural site of *T. aestivum* is very diverse both in Poland (Hilszczańska et al., unpublished data) and in Europe (Wedèn et al. 2004; Garcia-Montero et al. 2008, 2012). In Poland, summer truffles fruiting bodies were found both in 'clay' soil, with over 55% of loam, and in 'light' soil, with over 92.5% of sand. The latter claims to be non-beneficial for truffles' growth. However, if sandy soil is rich in calcium, *T. aestivum* can be cultivated in such ground (Chevalier, Sourzat 2012). Although soil from Wiązowna site does not fulfil completely all requirements, it is still the only appropriate site for truffle culture among the researched historical sites.

Plant communities are other factors that determine truffles' growth. They comprise host plants, which create mycorrhiza symbiosis with truffles, and other plants that do not create symbiosis with the fungus. The latter as *Fraxinus excelsior* or *Crataegus* spp. form beneficial air content in soil that stimulates truffles' growth. According to Wedèn et al. (2004), fraxinus (*F. excelsior*), which has special canopy structure, has positive influence on soil temperature, whereas *Crataegus* spp. has positive influence on air content in soil. Fraxinus often accompanies species that are basic host plants for truffles, for instance, *Quercus robur*, *Corylus avellana*, *Fagus sylvatica*, *Carpinus betulus* and *Tilia cordata. T. aestivum* fruiting bodies have been often found in such neighbourhood (Gazo et al. 2005). Among the researched sites, fraxinus was found at Wiązowna and Skuły sites.

At Wiązowna site, the highest number of forest floor species (11) was registered in comparison to other sites (Table 3). *Epipactis helleborine*, an orchid in the plant community of the forest floor at the site, confirms beneficial habitat conditions for the fungus because orchid is one of the plants that create mycorrhiza symbiosis with summer truffles (Selosse et al. 2004).

5. Summary

The research conducted indicated few half-natural forest habitats that can be beneficial for *Tuber* spp. fungus growth at historical sites where fungus used to occur. Soil analyses at sites of potentially beneficial flora showed, however, that chemical composition and soil texture diverge significantly from soil characteristics known from the present sites where truffles were found. The most beneficial environmental conditions for truffles' growth are at Wiązowna site. The last information about discovering truffles fruiting bodies at the site comes from the 1940s. It is conceivable that truffles fruiting bodies can be still found there, but due to unregulated legal status of the estate, no regular research is conducted at the site.

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Contributions

A.R-G. – design the study, study data collecting, study data interpretation, manuscript preparation, literature review. D.H. – design the study, study data interpretation, manuscript preparation, literature review. H.S. – study data interpretation, manuscript preparation, literature review.

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