Acta Mycologica

ORIGINAL RESEARCH PAPER Acta Mycol 49(2):267–277 DOI: 10.5586/am.2014.024
Received: 2014-10-22 Accepted: 2014-12-05 Published electronically: 2014-12-31

Characteristic of *Tuber* spp. localities in natural stands with emphasis on plant species composition

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Abstract

Fungi belonging to the genus *Tuber* establish ectomycorrhizal symbioses with shrubs, trees and some herbaceous plants. Some *Tuber* species, for example, *T. melanosporum*, *T. magnatum*, *T. aestivum* are economically important because they produce edible fruiting bodies with a distinctive taste and flavor. Our concept of truffle ecophysiology is dominated by the symbiosis with deciduous hosts, such as: *Quercus* spp., *Fagus sylvatica*, *Castanea sativa*, *Corylus* spp., *Carpinus betulus*, *Ostrya carpinifolia*, *Betula verrucosa*, and *Tilia* spp., whereas the real range of hosts in nature seems to be much wider. Moreover, interactions between *Tuber* mycelium and plant community could be more complex than just forming the ectomycorrhizal symbiosis. Here we show our inventory of plants and soils at six truffle' sites in the southern part of Poland (Nida Basin and Przedbórz Upland). The aim of this study was to widen our understanding of ecological factors affecting *Tuber* spp., in the context of pioneering stage of research on truffles in Poland. We hope our findings will have a practical application and will help to choose suitable soils for truffle orchards.

Keywords: hypogeous fungi; truffles; forest stands, plant community; soil parameters

Introduction

Truffles are prized fungi due to their taste and aroma. They are hypogeous and belong to Pezizales, a large group of ectomycorrhizal fungi growing in symbiosis with the roots of several vascular plant species (angiosperms and gymnosperms). Among the different

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Handling Editor: Maria Rudawska

species of truffles, *Tuber magnatum* (white truffle), *Tuber melanosporum* (black truffle), *Tuber aestivum* (summer truffle) and *Tuber macrosporum* Vittad. (the smooth black truffle) are most valued and expensive.

The first two species mentioned earlier are mainly confined to Mediterranean and some South Europe countries (Bulgaria and Serbia) while *T. aestivum* is widely distributed throughout Europe [1,2]. The fungus forms ectomycorrhizal symbioses with many different species, including *Corylus avellana*, *Quercus robur*, *Fagus sylvatica*, *Tilia cordata* and *Pinus nigra*. This truffle species prefers calcareous soils with pH levels near or above 7–8, although it occurs in beech woods on lime-deficient soils in the United Kingdom [3]. According to Czerniecki [4] some species of truffles were eaten by Polish nobility in the author's time, so it seems likely that *T. aestivum* sites have existed in Poland for at least 300 years. Some reports of attempts to cultivate truffles are present in late 19th century literature [5–7]. In the last decade, new distributional data for *T. aestivum* and other truffle species have been reported from Poland [8,9] and neighboring countries, Slovakia [10], the Czech Republic [11] and Germany [12].

Tuber macrosporum is another truffle species highly appreciated as delicacies, which has been found recently in Poland [9]. This species is common in central Italy while it has been reported as very rare in the Czech Republic, France, Hungary, Romania, Serbia, Switzerland, Ukraine and the United Kingdom [13]. Two years ago the species was found in Germany where they were considered extinct [12]. *Tuber macrosporum* forms ectomycorrhizae with *C. avellana*, *C. colurna* and *Quercus* species, for example: *Q. petraea*, *Q. pubescens*, *Q. robur* and *Q. suber* [15]. Coniferous species, especially *Pinus sylvestris* can also form ectomycorrhizae with *T. macrosporum* [15].

Currently, due to growing demand for truffles, the establishment of truffle orchards in Poland is in progress. Some areas are especially conducive to truffle cultivation due to similarities of soil and/or climatic conditions to that of French and Italian localities. Truffle productivity and phenology is driven by interrelated biotic and abiotic factors, such as: climate, physiology, pedology and vegetation [14]. The data presented here have a practical application for those who are interested in truffle cultivation. The knowledge of truffles' soil requirements and the plant species sharing the same ecological niche should be useful in choosing a suitable location for truffle orchards.

Material and methods

The study was conducted in Nida Basin and Przedbórz Upland, the southern part of Poland. The environmental characteristic of the natural forest stands is given in Tab. 1.

The truffle localities at the stands were found using the trained truffle dogs in collaboration with Researchers from Agricultural University in Nitra. Inventories were made in 2012–2014. Yield of truffles is given in Tab. 2. Species of truffles were identified on the basis of microscopic features and compared to the criteria by Granetti et al. [15]. Samples of fruiting bodies were also taken for molecular identification. The DNA sequences are deposited in Genbank NCBI (accession numbers to their sequences are as follow: *T. maculatum*-KJ524540.1, *T. excavatum*-KJ524535.1, *T. macrosporum*-KJ524532.1, *T. aestivum*-KJ524527.1, *T. rufum*-KC330221).

Tab. 1 Environmental characteristic of *Tuber* spp. sites.

Plot No.	Site	Location	Bedrock	Altitude (m)	Vegetation	*Tuber* species
1	M	Nida Basin	marlstone	250–252	Broadleaved forest with *Quercus robur*, *Tilia cordata*, *Carpinus betulus*, *Fagus sylvatica* and *Corylus avellana*	*T. aestivum* *T. excavatum* *T. maculatum* *T. rufum*
2	SA	Nida Basin	marlstone	311–314	Oak forest with *Quercus petraea*, *Carpinus betulus*, *Cerasus avium* and *Corylus avellana*	*T. aestivum* *T. excavatum* *T. rufum*
3	WR	Nida Basin	marlstone	290–296	Broadleaved forest with *Quercus petraea*, *Acer pseudoplatanus*, *Carpinus betulus* and *Corylus avellana*	*T. aestivum* *T. excavatum*
4	GR	Nida Basin	gypsium	254–261	Oak forest with *Quercus robur* and *Carpinus betulus*	*T. aestivum* *T. excavatum*
5	PR	Przedbórz Upland	Jurassic limestone	312–325	Beech forest with *Fagus sylvatica*	*T. aestivum*
6	NW	Nida Basin	marly limestone	227–228	Thicket with *Carpinus betulus*, *Acer campestre* and *Populus tremula*	*T. aestivum* *T. macrosporum* *T. maculatum* *T. excavatum*

Over three years of study, the mean annual precipitation was 600 mm and the annual mean temperature for the same period was 8.0°C. At each of the six localities the sampling site (100 m^2) was established. The vegetation at each site was surveyed in order to determine whether there was any indicator species for *T. aestivum* and/or co-occurring truffle species. The host-plants as well as the plants of forest floor whose biotopic preferences coincided with those of *Tuber* spp. are summarized in Tab. 3. The soil was sampled at the central part of each site by removing the litter and vegetation layers and then collecting approximately 0.5 kg of soil down to a depth of 0–30 cm, depending on the rockiness of the soil. The soil analyses were performed by the Polish Centre for Accreditation (No. AB740).

The soil pH in water (Tab. 3) and essential nutrient contents were measured according to ISO 10390 [17] and PB-14ed.2 of 1 January 2010 [using inductively coupled argon-plasma spectrometry following mineralization in chloric (VII) acid], respectively. The percentages of N and C were analyzed according to ISO13878 [17] and ISO 10694 [18]. The soil texture was evaluated on the basis of occurrence of three particle size fractions: <2 µm (clay), 2–63 µm (silt), and 63–2000 µm (sand) [19].

Tab. 2 Yield (number of fruitbodies) of *Tuber* spp. within three years at investigated sites.

Tuber species	Site/year																	
	M			SA			WR			GR			PR			NW		
	2012	2013	2014	2012	2013	2014	2012	2013	2014	2012	2013	2014	2012	2013	2014	2012	2013	2014
T. aestivum	17	74	41	17	21	11	1	15	303	0	0	32	7	166	368	0	14	147
T. excavatum	116	265	152	0	22	13	120	97	67	0	29	20	0	0	0	0	14	46
T. macrosporum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	18	0
T. maculatum	2	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	9
T. rufum	1	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
Total	136	340	195	17	43	24	121	110	372	0	29	52	7	166	368	1	47	202

Tab. 3 Overview of the soil properties at the *Tuber aestivum* sites.

Measured parameter	Site						Mean ±*SD*	Range
	M	SA	WR	GR	PR	NW		
Soil particle size fractions (%)								
Clay	33.10	40.23	23.30	17.11	22.69	14.42	25.14 ±9.80	14.42–40.23
Silt	55.83	47.03	61.70	37.32	28.33	22.17	42.06 ±15.53	22.17–61.70
Sand	11.07	12.74	15.00	45.57	48.98	63.41	32.80 ±22.60	11.07–63.41
Chemical characteristics								
pH (H_2O)	7.2	7.1	7.0	7.3	7.2	7.5	7.22 ±0.17	7.00–7.5
$CaCO_3$ total (%)	6.02	1.17	31.52	1.81	6.04	4.04	8.43 ±11.49	1.17–31.52
Ca (%)	34.44	15.64	42.80	14.99	-	19.77	25.53 ±12.45	14.99–42.80
C total (%)	10.09	5.165	5.625	5.38	5.98	2.108	5.72 ±2.56	2.11–10.09
C organic (%)	9.364	5.024	4.791	5.16	5.25	1.623	5.20 ±2.46	1.62–9.36
N total (%)	0.764	0.356	0.41	0.46	0.371	0.138	0.42 ±0.20	0.14–0.76
C/N	13.2	14.5	13.7	11.7	16.12	15.30	14.09 ±1.58	11.70–16.12

Results

Our inventory revealed five species from *Tuber* genus: *T. aestivum*, *T. macrosporum*, *T. rufum*, *T. excavatum* and *T. maculatum*. The geographical names of the site where the species were found will be available for further research, but not for publication. Publishing site names could lead to reckless prospecting for truffles, resulting in damage to the surrounding flora. At five out of six sites *T. aestivum* was accompanied by other truffle species (Tab. 1, Tab. 2). The stands are mixed broadleaved forest, with diverse plants of forest floor. The stand PR is rather poor in forest floor vegetation, and the only host-species is *Fagus sylvatica*. Only species of *T. aestivum* were found there. Soils from the investigated localities are of Rendzic type. Their chemical properties are given in Tab. 3. According to the atlas of forest soils of Poland [21], the bedrock of the regions consists of Cretaceous marlstone, limestone, gypsum and of Miocene clays and sands. Various Quaternary deposits cover more than three quarters of the region. The analyzed soils varied from "heavy" (up to 40.23 % clay) to "light" (up to 63.41% sand) – Tab. 3.

The highest diversity of trees and shrubs at investigated sites were in the stand depicted as WR. This stand was the richest in regard to herbaceous plants and mosses. The lowest richness of forest floor plants and mosses was observed at the stand NW. Plants associated with *Tuber* species are given in Tab. 4.

Number of tree and shrub host-species differ from two to six in all stands. Coniferous species were represented by *Pinus sylvestris* and its presence was noted only at stand WR.

One hundred and five species of forest floor plants were identified, among them, three orchids' species – putative host-species of truffles. They were present in all but one stand (NW). At this site the lowest number of plants of the forest floor was observed. In the stand PR no species representing mosses were noted.

Tab. 4 Plant species recorded on the studied plots of *Tuber* spp. sites known (in bold font) and potential host plants are distinguished in boxes.

Tree and shrubs	M	SA	WR	GR	PR	NW
Abies alba		+				
Acer campestre						+
Acer platanoides		+			+	
Acer pseudoplatanus	+		+	+		
Carpinus betulus	+	+	+	+		+
Cerasus avium	+	+	+	+		
Cornus sanguinea	+	+	+		+	+
Corylus avellana	+	+	+	+		
Crataegus monogyna	+	+	+	+	+	+
Daphne mezereum		+				
Euonymus europeus	+		+	+		+
Euonymus verrucosus	+	+			+	
Fagus sylvatica	+		+	+	+	
Frangula alnus			+			
Fraxinus excelsior	+	+	+			+
Juniperus comunis						+
Ligustrum vulgare						+
Lonicera xylosteum	+	+	+			
Malus sylvestris				+		+
Padus avium				+		
Padus serotina				+		
Pinus sylvestris			+			
Populus tremula						+
Prunus spinosa			+		+	+
Pyrus communis				+	+	+
Quercus petraea	+	+	+	+		
Quercus robur	+	+	+	+		+
Quercus rubra		+				
Rosa canina	+	+	+		+	
Sambucus nigra	+	+	+	+		
Sorbus aucuparia		+	+			
Tilia cordata	+					
Ulmus glabra				+		
Ulmus minor			+			
Viburnum opulus	+	+	+	+	+	+
Host species / all species	6 / 17	4 / 18	6 / 20	5 / 16	1 / 9	3 / 14

Plants of the forest floor	M	SA	WR	GR	PR	NW
Herbaceous plants						
Actaea spicata			+			
Adoxa moschatellina	+		+			
Aegopodium podagraria	+		+	+		+
Agrostis capillaris	+	+	+	+	+	+

Tab. 4 *(continued)*

Ajuga reptans	+		+	+		+
Anemone nemorosa	+	+	+	+	+	
Asarum europaeum	+		+	+		+
Astragalus glycyphyllos	+	+	+	+	+	
Athyrium filix-femina				+		
Brachypodium sylvaticum	+		+	+	+	
Calamagrostis epigejos				+		
Campanula persicifolia			+			
Campanula rapunculoides	+	+			+	
Campanula rotundifolia					+	
Campanula trachelium				+	+	
Carex digitata		+	+		+	
Carex echinata			+		+	
Carex hirta		+				
Carex sylvatica		+	+	+		+
Carex umbrosa			+			
Cephalanthera damasonium	+	+	+		+	
Chaerophyllum aromaticum	+		+	+		
Chamaecytisus ratisbonensis			+			
Cimicifuga europaea	+		+		+	
Cirsium arvense			+			
Clinopodium vulgare			+			
Convallaria majalis	+		+	+	+	
Cruciata glabra	+	+	+			+
Cypripedium calceolus			+			
Dactylis polygama					+	
Dryopteris carthusiana			+			
Dryopteris filix-mas			+			
Epipactis helleborine	+					
Fallopia dumetorum				+		
Festuca gigantea				+		
Festuca heterophylla					+	
Fragaria vesca				+		+
Galeobdolon luteum					+	+
Galeopsis pubescens	+	+	+	+		
Galium aparine				+		
Galium boreale			+		+	
Galium mollugo				+		
Galium odoratum	+	+	+	+	+	
Galium schultesii				+	+	
Galium sylvaticum	+			+		+
Geranium robertianum	+		+		+	
Geum urbanum	+	+	+	+	+	+

Tab. 4 *(continued)*

Glechoma hederacea				+		
Hepatica nobilis					+	
Hieracium murorum			+		+	
Holcus mollis	+					
Impatiens parviflora			+			
Lapsana communis		+		+		
Laserpitium latifolium			+			
Lathyrus nigier		+			+	
Lathyrus vernus	+	+	+	+	+	
Lilium martagon	+		+	+		
Luzula pilosa			+			
Lysimachia nemorum						+
Maianthemum bifolium	+	+	+	+		
Melica nutans			+	+		
Melampyrum nemorosum	+		+			
Melittis melissophyllum	+	+	+		+	
Milium effusum	+	+	+	+		
Moehringia trinervia			+	+		
Mycelis muralis	+				+	
Oxalis acetosella			+			
Paris quadrifolia			+			
Pimpinella saxifraga					+	
Plantago major			+			
Polygonatum multiflorum	+		+	+		+
Primula elatior			+		+	
Prunella vulgaris				+		
Pulmonaria obscura	+	+	+	+	+	+
Ranunculus cassubicus	+		+	+		
Ranunculus lanuginosus	+		+			
Rubus idaeus			+			
Rubus saxatilis			+			
Rubus nessensis		+	+		+	
Rubus pedemontanus		+				
Sanicula europaea	+	+	+	+	+	+
Solidago virgaurea					+	
Sonchus oleraceus				+		
Stachys sylvatica				+		
Stellaria holostea				+		
Taraxacum officinale			+	+	+	+
Torilis japonica					+	
Urtica dioica			+			
Veronica chamaedrys			+			
Vicia sepium			+			
Vincetoxicum hirundinaria					+	
Viola mirabilis	+		+	+	+	

Tab. 4 *(continued)*

Viola reichenbachiana	+	+	+	+	+	+
Mosses						
Atrichum undulatum			+			
Brachythecium velutinum	+	+				
Eurhynchium angustiret	+					
Eurhynchium striatum			+	+		
Fissidens taxifolius						+
Hypnum cupressiforme		+				
Mnium undulatum						+
Mnium elatum			+			
Oxyrrhynchium hians	+		+			+
Pseudoscleropodium purum			+			
Rhytidiadelphus triquetrus			+			
Host species / all species	2 / 38	1 / 25	2 / 66	0 / 43	1 / 37	0 / 17

Discussion

Truffles mainly depend on mutual relationships with angiosperm hosts including *Quercus* spp., *Fagus sylvatica* and *Corylus avellana* [21,22]. However some species such as *Tuber puberulum* Berk. & Broome and *Tuber borchii* Vittad. prefer gymnosperm hosts [23]. Host specificity plays an important role but is not clearly defined for most wildlife associations [24]. Our previous inventory suggested that, in Poland, the deciduous host species are highly important to the occurrence of *Tuber* species [9]. For example, the greatest abundance of *T. aestivum* fruiting bodies was found in forests where species such as: *Quercus robur*, *Corylus avellana*, *Fagus sylvatica*, *Carpinus betulus* and *Tilia cordata* occur together [8]. Our findings presented in this work changed this opinion slightly, since at PR site the only host–species tree is beech and, moreover, we obtained here the great yield of *T. aestivum* within the last three years. Fructification of *T. astivum* at plots where *P. sylvestris* and/or *P. tremula* is present, are confirmation of findings by Stobbe et al. [12]. The authors mentioned *Picea abies*, *Abies alba*, *Ulmus* spp. and *Populus* spp. as potential hosts. Some of the above-cited studies reported the reduction of vegetation due to the presence of *Tuber* spp. According to Gryndler and co-authors [25] this could be the result of negative effect of the *Tuber* mycelium on the growth of some of the non-host plants. There can be also direct effects (both positive and negative) of the non-host plants on the *Tuber* mycelium. Thus, the interaction between *Tuber* mycelium and plant community could be more complex than just forming the ectomycorrhizal symbiosis. Physico-chemical analysis of soils on our sites revealed that the soil texture is moderately varied, ranging from silty-clay to clayey-silt and more rarely silty, silty-sandy or clayey. The analyzed soils varied from sandy clay (up to 40.23% clay) to sandy loam up to 63.41% sand. The latter soils are generally thought to be less favorable for truffle fructification, however even excessively sandy soils can support truffle development if they are sufficiently rich in calcium [26,27]. Our results showed that soils with the higher content of calcium, in the form of Ca cations and $CaCO_3$, were conducive to the higher fructification of *T. aestivum*. All investigated soils were poor in readily degradable nitrogen, a C/N ratio was above ten and varied from 13.2 to 16.12. This means that the development of mycorrhizae could not have been limited.

Development of truffles depends on pH as well. According to Chevalier and Frochot [28] truffles can grow in soils with pH range 6.8 to 8.0. However it cannot be claimed that pH 7.5 is the optimal level to maintain the highest level of mycorrhization on host plants roots [29]. Although we did not investigate the mycorrhiza structure, our observation made on truffles' fructification showed that *T. aestivum* can form fruitbodies even in soils with pH 7. The results might be really important for truffle growers, especially in countries where the area of calcareous soils is limited.

The role of soil properties other than pH and carbonate content for truffle fructification is still poorly understood, so further work is needed. Establishing of truffle orchards in our country has been started in a previous decade and is still in progress. We have to take into account the numerous, interrelated biotic and abiotic factors driving truffle productivity and phenology from a holistic perspective [14]. Otherwise, we can derive some contradicted conclusion, for example, the greatest abundance of *T. aestivum* fruiting bodies was found in forests where all of host-species occurred together [8]. However, at the site indicated as PR, where the only host-species tree is beech, we found a great abundance of *T. aestivum* fruit bodies within the last three years (2012–2014). Therefore we have to be cautious with the statement that a greater diversity of host-species occurs with a higher yield of truffles. Presently, we would rather be of the opinion that each of the investigated sites is unique. As long as we do not have the climatic, soil and vegetation data combined together our conclusions will remain preliminary.

Acknowledgments

The authors thank two anonymous reviewers for useful comments improving the manuscript. This work was due to grant financed by State Forest Holding No. OR-2717/19/11.

Authors' contributions

The following declarations about authors' contributions to the research have been made: design of the study: DH, ARG; study data interpretation, manuscript preparation, literature review: DH, ARG, HS.

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