First attempt towards cultivation of *Tuber aestivum* in Poland

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Accepted 11. 3. 2010

Key words: *Tuber aestivum*, *Quercus robur* – Truffle cultivation.

Abstract: *Tuber aestivum* has been recently recorded from Poland. A selection of local inocula of *T. aestivum* collected from fruiting bodies found in Poland in 2007 was used to inoculate 1-year-old seedlings of *Quercus robur*. The ectomycorrhizal structure was monitored for six months in greenhouse; the colonization rate was very high and 100% of the investigated seedlings possessed ectomycorrhizae of *T. aestivum*. In October 2008, 130 seedlings were planted to a site with rendzic soil. With 96% the survival rate of seedlings after one year in the field was really high. *Tuber aestivum* mycorrhizae dominated (75%) on the roots. The ectomycorrhizae of adventive fungi could also be observed, mainly *Thelephora* and *Hebeloma*. Through this research project, we want to create an incentive for restoration of some endangered species by establishing oak plantations.

Zusammenfassung: *Tuber aestivum* wurde kürzlich in Polen gefunden. Eine Auswahl von Inokula aus 2007 in Polen gesammelten *T. aestivum*-Fruchtkörpern wurde für die Inokulation einjähriger *Quercus robur* Sämlinge verwendet. Die Zusammensetzung der Ektomykorrhizen wurde sechs Monate im Glashaus beobachtet. Die Besiedlungsrate war sehr hoch und 100% der untersuchten Sämlinge hatten *T. aestivum*-Ektomykorrhizen. Im Oktober 2008, wurden 130 Sämlinge an einem Standort mit Rendzina gepflanzt. Mit 96% war die Überlebensrate der Sämlinge nach einem Jahr im Feld ausgesprochen hoch. *Tuber aestivum* Mykorrhizen dominierten (75%) auf den Wurzeln. Ektomykorrhizen adventiver Pilze konnten auch beobachtet werden, hauptsächlich *Thelephora* und *Hebeloma*. Durch dieses Forschungsprojekt möchten wir eine Initialzündung für die Wiederansiedlung einiger gefährdeter Arten mithilfe von Eichenpflanzungen bewirken.

Over the last few decades, considerable progress has been made in the controlled infection of forest trees with selected ectomycorrhizal fungi (PEREZ & al. 2007). For the ecosystems these ectomycorrhizal fungi are of considerable importance because of the benefits of the mutualistic association they provide to host plants (PACIONI & COMANDINI 1999). Of particular interest are those fungi that form edible fruiting bodies. The most important in terms of international demand and high culinary value are some species of truffles, including *Tuber aestivum* VITT. (HALL & al. 2007).

Tuber aestivum grows in an ectomycorrhizal symbiosis with many different tree or shrub species, e.g., *Quercus robur* and *Corylus avellana* (CHEVALIER & FROCHOT 1997). According to PALENZONA & al. (1972) *Carpinus*, *Fagus*, *Populus*, and *Tilia* are the usual hosts of *Tuber* species. *Tuber aestivum* prefers calcareous soils with a pH

close to 7-8 or higher, although in the United Kingdom, it may be found in beech woods on lime-deficient soils (PEGLER & al. 1993).

The summer truffle *Tuber aestivum* was considered to be rare in Poland, since it was only recorded by LUBELSKA (1953). However, due to lack of any herbarium specimens, this record could not be confirmed. Recently, new data on the distribution of *T. aestivum* and other species of truffles have been reported from Poland (HILSZCZAŃSKA & al. 2008, ŁAWRYNOWICZ & al. 2008). The aim of our study was to investigate the survival of truffle mycorrhizae in a greenhouse experiment, as well as in the experimental plantation one year after planting.

Material and methods

In April 2008, 1-year old seedlings of *Quercus robur* obtained from Polish bare root nursery were inoculated with spores of *Tuber aestivum* fruiting bodies found in Poland in 2007 (HILSZCZAŃSKA & al. 2008). The seedlings were potted in a previously sterilized mixture of peat moss and vermiculite (4 : 4, v:v; approx. pH 7.2) and inoculated with spores of frozen *T. aestivum* fruiting bodies. About 1.5 g of the spore mixture was placed close to the roots of the planted seedlings. After inoculation seedlings were kept for six months in a greenhouse and watered every two days, according to the temperature and substrate condition. Six months after inoculation, ten plants were monitored for mycorrhizae formation. Some mycorrhizal tips from all treatments were taken to DNA analysis. Amplification by PCR and sequencing was done with the pair of primers ITS5 and ITS7 (BERTINI & al. 1999).

Soil pH and contents of the basic nutrients were analysed in the site where the seedlings have been planted (ISO 1994; 1995 a, b, c; Table 1).

In October 2008, the seedlings were outplanted to a site with rendzic soil (pH 7.54). Seedlings were planted with 3 m between seedlings within the row and rows were planted 4.5 m apart. In September 2009, ten seedlings were monitored for mycorrhizae formation. Samples of roots were taken with a metal corer. All fine root tips were screened in water under a stereo microscope (Zeiss, Stemi-2000 C). Twenty-two samples of mycrorrhizae were checked by DNA sequencing of the ITS.

Results

Tuber aestivum mycorrhizae were found in all of the ten investigated samplings of roots. Some of these mycorrhizae were in an early stage of development and some were senescent. The DNA analysis of *Tuber* mycorrhiza confirmed the identification using microscopy. ITS from chosen samples was compared to the sequence from *T. aestivum* fruiting bodies that were used as inoculum (Table 2). Percentage of *T. aestivum* mycorrhizae on roots of oaks was 40% after six months of growth in the greenhouse. After one year of growing in the field the percentage of these mycorrhizae was 75% (Table 3). Colonization of roots by adventive ectomycorrhizal fungi at the first stage after inoculation, as well as after one year in the field was observed. The adventive fungi, which were indentified according to the characteristic features, belonged probably to *Thelephora* (20%) and *Hebeloma* (5%).

Survival rate of the oak seedlings was high, only 2% died in the greenhouse and 4% in the field (Table 3).

Discussion

The fact that mycorrhizae of *T. aestivum* survived the first year in the experimental plantation is promising for future cultivation. The soil parameters in our plantation were favourable for *T. aestivum* mycorrhizae since the fungus prefers soils poor in readily degradable nitrogen (WEDEN & al. 2004, CHEVALIER & FROCHOT 1997). C/N

ratio of soils was above ten that is known to be an indication that the soil has not been N-fertilised (ERIKSSON & al. 1997). A low phosphorus concentration and a high Ca/Mg ratio (Table 1) were also favourable for the development of *T. aestivum* mycorrhizae. The K/Mg ratio was below two at all sites (Table 1), which indicates that the plant uptake of magnesium was not negatively affected.

Table 1. Soil parameters of experimental site (data are average for five samples taken in different parts of the site) where seedlings inoculated with *Tuber aestivum* are planted. Value of P, Fe, Ca, Mg, and K are given as $g \times kg^{-1}$; C, N, and $CaCO_3$ as total per cent

pH_{H20}	pH_{KCl}	P	Fe	Ca	Mg	K	Ca/Mg	K/Mg	C	N	$CaCO_3$	C/N
7.54	6.71	0.17	6.29	38.1	1.52	2.3	10.9	1.6	1.77	0.08	7.99	19.24

Table 2. Identification of the fungal symbiont on root tips of *Qercus robur* seedlings

Fungus	Length of sequenced ITS (bp) or brief description	Accession no. of most similar ITS sequence in GenBank	Identities
Tuber aestivum	690	EU326689	100%

Table 3. Survival, mycorrhization, colonization, and contamination rates of *Tuber aestivum* on *Quercus robur* inoculated with *Tuber aestivum*

	6 months greenhouse	1 year plantation
Total seedlings	134	130
Surviving seedlings	131 (98%)	125 (96%)
Total sampled seedlings	10	10
Sampled seedlings colonized by *T. aestivum*	10 (100%)	10 (100%)
Root tips with *T. aestivum*	40%	75%
Root tips with other fungi	54 %	25%
Non-mycorrhized tips	6%	-

It seems that really the high survival rate of the seedlings was due to the time of planting. A lot of irrigation during the first months after outplanting is needed to assure seedling survival. Our seedlings were planted in the end of October, which worked out to be successful.

Tuber aestivum mycorrhizae were not easily displaced in the establishment phase in the field. All investigated seedlings possessed ectomycorrhizae of *T. aestivum* and their share was growing with the age of the seedlings. However, problems with fungi contaminating truffle-inoculated plants are still present. The most common competitors are species of *Thelephora* and *Scleroderma* (HALL & al. 2007). In our seedlings mycorrhizae of *Thelephora* and *Hebeloma* were quite abundant. *Hebeloma* was also common in inoculated seedlings of *Quercus ilex* (PEREZ & al. 2007). Mycorrhizae of *Hebeloma* are present on roots even if the substrate used for growing is sterilized (GRANETTI & al. 2005).

References

BERTINI, L., AMICUCCI, A., AGOSTINI, D., POLIDORI, E., POTENZA, L., GUIDI, C., STOCCHI, V., 1999: A new pair of primers designed for amplification of the ITS region in *Tuber* species. – FEMS Microbiol. Letters **173**: 239-245.

CHEVALIER, G., FROCHOT, H., 1997: La truffe de Bourgogne. – Levallois-Perret: Pétrarque.

ERIKSSON, J., ANDERSSON, A., ANDERSSON, R., 1997: Tillståndet i Svensk Åkermark. Naturvårdsverket Rapport No. 4778. – Stockholm: Naturvårdsverket Förlag.

GRANETTI, B., DE ANGELIS, A., MATEROZZI, G., 2005: Umbria terra di tartufi, Regione Umbria, Italia. – Terni: Umbriagraf.

HALL, I., BROWN, G., ZAMBONELLI, A., 2007: Taming the truffle. The history, lore, and science of the ultimate mushroom. – Portland, Oregon: Timber press.

HILSZCZAŃSKA, D., SIEROTA, Z., PALENZONA, M., 2008: New *Tuber* species found in Poland. – Mycorrhiza **18**(4): 223-226.

ISO 1994: 10390. Soil quality. Determination of pH. – Genf: International Organization for Standardization.

ISO 1995 a: 11261. Soil quality. Determination of total nitrogen. Modified Kjeldahl method. – Genf: International Organization for Standardization.

ISO 1995 b: 11466. Soil quality. Extraction of trace elements soluble in aqua regia. – Genf: International Organization for Standardization.

ISO 1995 c: 10694. Soil quality. Determination of organic and total carbon after dry combustion (elementary analysis). – Genf: International Organization for Standardization.

LUBELSKA, B., 1953: O występowaniu trufli (*Tuber* MICH. i *Choiromyces* VITT.) w Polsce. – Fragm. Flor. Geobot. **1**: 87-96 [In Polish].

ŁAWRYNOWICZ, M., KRZYSZCZYK, T., FAŁDZIŃSKI, M., 2008: Occurrence of black truffles in Poland. – Acta Mycol **43**(2): 143-151.

PACIONI, G., COMANDINI, O., 1999: *Tuber*. – In CAIRNEY, J. W. G., CHAMBERS, S. M., (Eds): Ectomycorrhizal fungi. Key genera in profile, pp. 163-186. – Berlin, Heidelberg: Springer.

PALENZONA, M., CHEVALIER, G., FONTANA, A., 1972: Sintesi micorrizica tra i miceli in coltura di *Tuber brumale*, *T. melanosporum*, *T. rufum* e semenzali di conifere e latifoglie. – Allionia **18**: 41-52.

PEGLER, D. N., SPOONER, B. M., YOUNG, T. W. K., 1993: British truffles. A revision of British hypogeous fungi. – Kew: Royal Botanical Gardens.

PEREZ, F., PALFNER, G., BRUNEL, N., SANTELICES, R., 2007: Synthesis and the establishment of *Tuber melanosporum* VITT. ectomycorrhizae on two *Nothofagus* species in Chile. – Mycorrhiza **17**(7): 627-632.

WEDEN, C., CHEVALIER, G., DANELL, E., 2004: *Tuber aestivum* (syn. *T. uncinatum*) biotopes and their history on Gotland, Sweden. – Mycol. Res. **108**(3): 304-310.