SZENT ISTVÁN UNIVERSITY

Studies on cultivation possibilities of summer truffle (*Tuber aestivum* Vittad.) and smooth black truffle (*Tuber macrosporum* Vittad.) in Hungary

PHD THESIS

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Gödöllő

2011

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BACKGROUND AND OBJECTIVES

According to the Biblical knowledge, consumption of truffles originates from ancient times and in certain areas of the Mediterranean region. From medieval centuries use of black truffles gained ground in other European countries, based primarily on French and Hungarian cookbooks of the period. Truffles have been originated from its natural forests from hundreds of years, until TALON reached rapid success on truffle cultivation in the 19th century. The two World Wars caused serious decline in French truffle business. A boom started again thanks to newly established associations in the 50's and cultivation research success in the 60's. As a result of improvements in cultivation practices, truffle cultivation spread both in European and non-European countries in recent decades. One of the most important species of Europe is white truffle (*Tuber magnatum*), being sold on truffle auctions each year. The species proved to be common in Italy, Croatia, Serbia and in some habitats it can also be found in Hungary. Researchers have been studying its cultivation for decades, and although some Italian plantations have reached some success, technology is still not revealed and the secret of the species kept unknown. The most common truffle species is perigord black truffle (*T. melanosporum*), produced in France, Italy and Spain in great quantities. Due to its Mediterranean characteristics, cultivation of the species in Hungary can be challenging. The third most common truffle species is summer truffle (*T. aestivum*), well distributed in Hungary and being the target species of decade-long Hungarian cultivation attempts. Besides the above mentioned species, smooth black truffle (*T. macrosporum*) and bianchetto (*T. borchii*) are also popular in European truffle cuisine (ZAMBONELLI et al. 2000, ZAMBONELLI et IOTTI 2005*).* *T. macrosporum* considered widespread in Hungary while populations of *T. borchii* are less known by truffle collectors.

The two main target species of my research were summer truffle (*Tuber aestivum*) and smooth black truffle (*T. macrosporum*). Due to its wide ecological tolerance of the first one and its distribution in Hungarian habitats, cultivation possibilities have been considered established. My studies of *T. aestivum* have focused on the following:

- Development of truffle seedling cultivation methodology as a basis for truffle cultivation
- Study on truffle-receptivity of the most common host species of European nurseries and Hungarian natural habitats
- Studies on international and Hungarian truffle seedling quality control systems and development of an accurate and time-effective seedling mycorrhiza control method
- Establishment and monitoring of truffle plantations.

Despite its outstanding organoleptic quality low quantity of *Tuber macrosporum* is available on the European market and only few articles focus on its ecology. However, studies revealed its suitability for cultivation in Italy. The facts of being less studied in spite of its high gastronomic values motivated me to carry out research with the above mentioned species, focusing on description of soil and vegetation of its natural habitats. During my studies I seized the opportunity of combining international truffle habitat ecology research methods with Hungarian forest site descriptions. One of my objectives in the topic was to develop an effective and comprehensive method for natural truffle site description and apply this method when describing *T. macrosporum* Hungarian natural habitats and to determine cultivation criteria for *T. macrosporum* based on my findings.

MATERIALS AND METHODS

Studies on summer truffle mycorrhiza formation

Research trials were conducted in the Horticultural Research Unit supervised by the Institute of Horticultural Technologies of the Szent István University between 2005 and 2010 in an unheated plastic tunnel greenhouse of 100 m^2 surface. Substrate and suspension based inoculation techniques have been compared on Turkish oak (*Quercus cerris*) seedlings. 18 months after inoculation summer truffle mycorrhiza level of sampled seedlings have been examined based on FISCHER et COLINAS (2006) methodology. Further experiments have been conducted to study host plant receptivity of the most common plant species of European nurseries and Hungarian natural truffle habitats. Besides these experiments studies on international and Hungarian seedling quality control methodology was conducted in order to develop an accurate and effective technique. Vitalizing effect of summer truffle was also been studied from 2007 on Turkish oak (*Quercus cerris*), English oak (*Q. robur*), hazel (*Corylus avellana*) and large-leaved lime (*Tilia platyphyllos*) seedlings produced in non-heated greenhouse and Turkish oak (*Quercus cerris*), English oak (*Q. robur*) and hazel (*Corylus avellana*) seedlings originated from plantations. Phenological state and photosynthetic activity was compared to mycorrhiza level. Phenological state was determined by measuring height and stem diameter, photosynthetic activity was documented by Konica-Minolta SPAD 502 Plus tool.

Monitoring of summer truffle (*Tuber aestivum*) orchards

Soil and mycorrhiza analysis was carried out two times from 2003 on an extensive truffle orchard (Hőgyész) established in 1999-2000. Besides the contribution of establishment of three plantations (Eger1, Eger2 and Eger3), monitoring was also carried out on them. Eger1 and Eger3 is an extensive orchard based on hazel (*Corylus avellana*) and Turkish hazel (*C. colurna*) seedlings, while Eger2 plantation was established with intensive English oak (*Quercus robur*) and Turkish oak (*Q. cerris*) seedlings. Soil sampling has been carried out according to MSZ-08-0202-1977 standard; later samples have been taken from the close environment of roots. Soils have been analyzed in accredited soil laboratory. As fields of plantations were used as garden, plough-land or pasture, soil analysis result were evaluated according to Hungarian cropland evaluation system (ANTAL 2000, BUZÁS 1983). Root sampling for mycorrhiza analysis was carried according to VERLHAC et al. (1990), while FISCHER et COLINAS (2006) method was used for mycorrhiza check.

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Methodology for *Tuber macrosporum* natural habitat research

During the research habitats of stable presence of *Tuber macrosporum* were selected. According to the results of a five-year-long pre-monitoring of habitats, locations in three areas have been selected for detailed soil, vegetation and mycorrhiza community analysis. 5 habitats of three locations in Bükk region, 5 habitats of three locations in South Transdanubia and 3 habitats of two locations in the Great Plain were examined (Figure 1.)

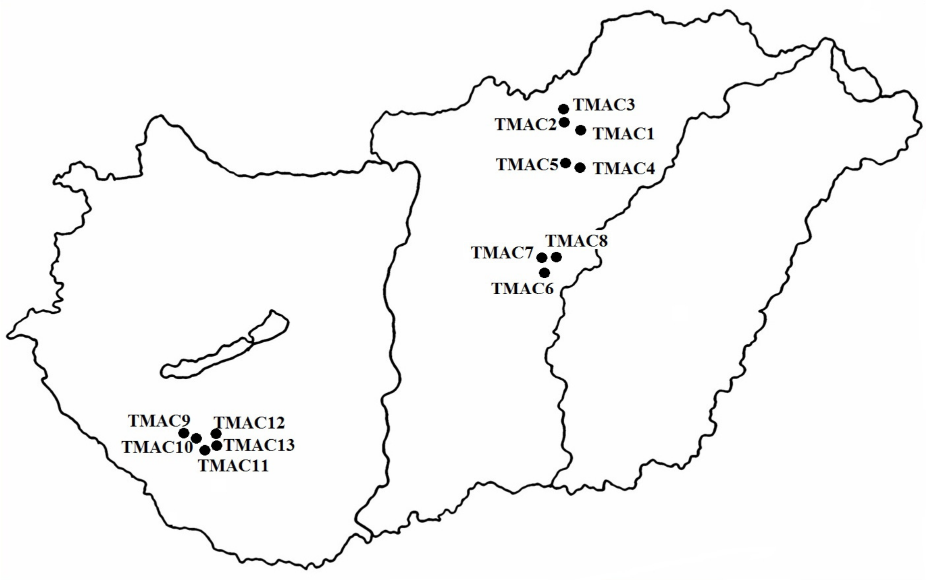


Figure 1. Locations of Tuber macrosporum sampling sites

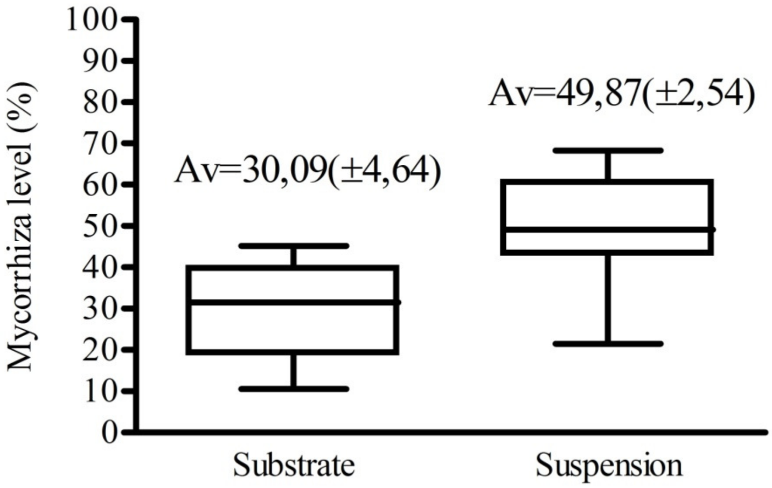
Soil sampling was carried out by using Pürckhauer soil sampler (FINNERN 1994). Analyzed parameters have been determined based on ÁESZ (2001). Soils have been analyzed according to current standards. Plant nomenclature was used according to SIMON (2000), while vegetation description was carried out based on BRAUN-BLANQUET (1964) methodology using exact percentages instead of plant coverage categories. Ecological indicators and naturalness values were evaluated based on methods of BORHIDI (1993). As most of the habitats considered to be forestry land of planned establishment, canopy level considered less informative, therefore herbaceous level indicator values were used for phytocoenology analysis. Root sampling for ectomycorrhiza community analysis was also carried out taking into account geographical representativeness.

RESULTS

Results of seedling mycorrhization

Comparative study of inoculation methods

Significant difference has been revealed between inoculation methods (substrate and suspension based) using Turkish oak (*Quercus cerris*) seedlings (P=0,0014) (Figure 2.).



Figue 2. Comparison of summer truffle (Tuber aestivum) mycorrhization methods (n=30)

Host tree receptivity to summer truffle (Tuber aestivum) mycorrhiza

Mycorrhiza level of Turkish oak (*Quercus cerris*), English oak (*Q. robur*), hazel (*Corylus avellana*) and large-leaved lime (*Tilia platyphyllos*) seedlings mycorrhized by summer truffle (*Tuber aestivum*) was compared during the experiments. Neither analysis of variances (P=0,1165, R^2=0,1242) nor post test (P>0,05 in both cases) showed difference among host plant receptivity to summer truffle (*Tuber aestivum*) mycorrhiza resulting in being all suitable for summer truffle (*Tuber aestivum*) truffle cultivation (Figure 3.)

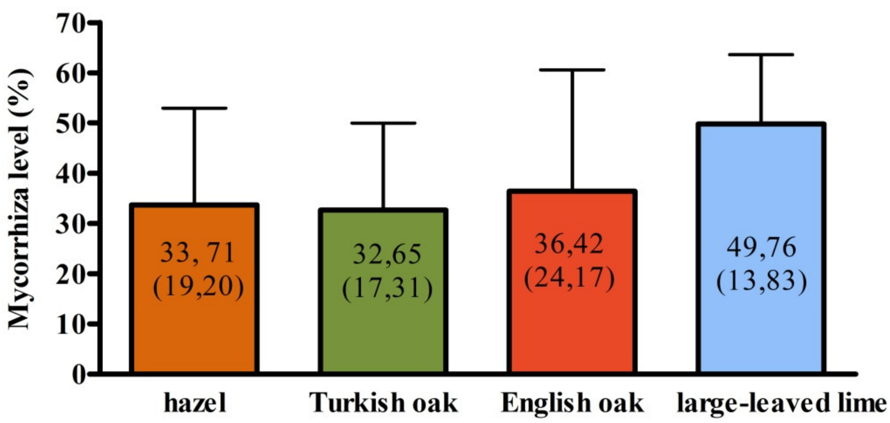


Figure 3. Average mycorrhiza level of different host plants mycorrhized by summer truffle (Tuber aestivum) Average (SD) (n=12/plant species)

Comparative study of mycorrhized seedling quality control methods

Analysis of variations has resulted in non-detectable difference among four mycorrhized seedling quality check methods (P=0,6633), nor post test showed difference from real mycorrhiza level (P>0,05 in both cases). Method of CHEVALIER et GRENTE (1978) was not comparable by statistical analysis due to lack of mycorrhiza levels in percentages: only mycorrhization categories could have been estimated.

Table 1: Comparison of different mycorrhized seedling check methods (green: underestimation, red: overestimation, bold numbers: highest difference from real mycorrhiza level)

Method/Number of seedlings	**1.**	**2.**	**3.**	**4.**	**5.**	**6.**	**7.**	**8.**	**9.**	**10.**	**11.**	**12.**
CHEVALIER et GRENTE (1978)	3	4	4	5	4	2	4	3	2	4	4	3
BACH et al. (2008)	2	2	2	3	3	1	3	2	1	2	2	2
	32%	***57%***	*40%*	*88%*	*80%*	*22%*	*80%*	*62%*	*20%*	*70%*	*76%*	*50%*
PALAZÓN et al. (1997, 1999)	*36%*	*68%*	***72%***	*91%*	***82%***	***20%***	*65%*	*83%*	*34%*	*58%*	*76%*	***66%***
FISCHER et COLINAS (2006)	*35%*	***57%***	*54%*	***93%***	*80%*	*43%*	*74%*	*76%*	*35%*	***77%***	***78%***	*58%*
BENCIVENGA et al. (1995)	***46%***	***57%***	*47%*	*89%*	*71%*	*51%*	***60%***	***87%***	***51%***	*73%*	*76%*	*62%*
Total root examination	*27%*	*76%*	*54%*	*87%*	*56%*	*41%*	*72%*	*71%*	*21%*	*65%*	*64%*	*48%*

The trial has revealed that seedling check methods generally (71%) overestimated real mycorrhiza levels (Table 1.). Highest differences from real mycorrhiza levels are not connected to a certain method although in most of the cases (41,6%) BENCIVENGA et al. (1995) estimated less accurately (Table 1.).

Development of a mycorrhized seedling quality check and control method

After comparing international and Hungarian mycorrhized seedling quality check methods the following technique has been developed: the first step is a qualification of plants according to Hungarian forest plant material propagation regulations (regulation 110/2003. (X.21.) FVM). Criteria include health parameters and dimensions according to plant age, recommended to complete by the fact that – unlike traditional forest plant propagation techniques – truffle mycorrhized seedlings are produced in containers which affects root development. The second step of seedling check is analysis of mycorrhiza characteristics. According to the results methods based on counting of root tips are less dependent on experience of examining person while methods of estimation require certain routine. As mycorrhiza density can differ in the upper and lower part of the root system it is recommended to divide roots into two parts, counting 100-100 root tips in each part and determine them as mycorrhized, non-mycorrhized and colonized by contaminant species. Mycorrhiza level should be calculated taking into account all counted root tips. Lot qualification should be based on the following:

- other *Tuber* species is not present on the seedlings
- no seedling without mycorrhiza
- target species mycorrhiza level is >30%
- contaminant species mycorrhiza level is <30%

Trials on truffle vitalizing effect

In most of the treatments statistical analysis revealed no correlation between mycorrhiza level and seedling development (height, stem diameter) or photosynthetic activity (SPAD values) except in the case of greenhouse-grown Turkish oak (*Quercus cerris*) seedlings where slight correlation could been detected by PEARSON correlation between mycorrhiza level and photosynthetic activity (R^2=0,4001) (Figure 4).

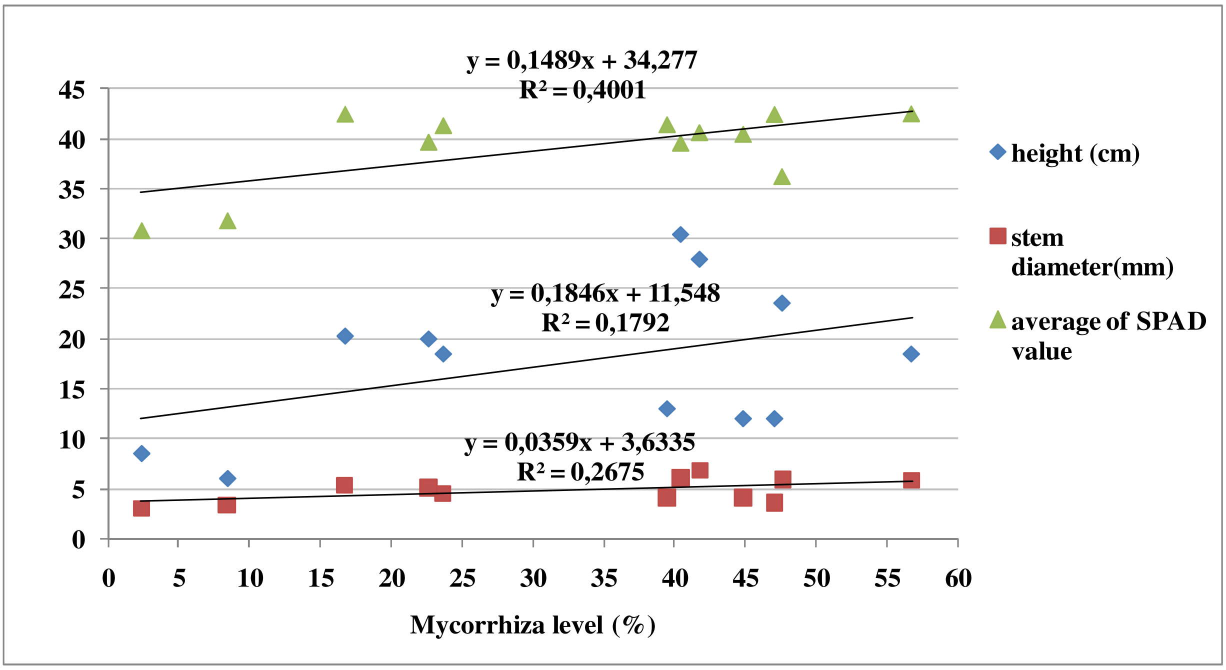


Figure 4. Correlation between seedling development (height, stem diameter), photosynthetic activity (SPAD values) and mycorrhiza level of greenhouse-grown Turkish oak (Quercus cerris) seedlings

Results of truffle plantation monitoring

Results of the soil analysis

Results of pH analysis – compared to soils of natural truffle habitats – revealed suitability of the truffle grounds to cultivate summer truffle (*Tuber aestivum*). According to the literature lime content was very variable on the natural habitats of summer truffle (*Tuber aestivum*) and all the examined plantations fall into the range. Sticky point by Arany is only an indication concerning Hungarian truffle beds, not being limiting factor due to its high variability. Organic matter content of truffle plantations is below to natural habitats' values as truffle beds are located in forests where intensive organic matter deposition occurs. However, organic matter content is expected to be raise with time. Wide tolerance of summer truffle (*Tuber aestivum*) toward macroelements does not exclude plantations, while exchangeable calcium can be a limiting factor according to the literature as soils of all the plantations contain low concentration of exchangeable calcium (below 275,8 ppm). However, II. and VII. parcels of Hőgyész and Eger2 plantation approximate summer truffle (*Tuber aestivum*) requirements of the element. Exchangeable magnesium falls within the range cited by the literature (5,14-45 ppm) for all the plantations.

Mycorrhiza monitoring of truffle plantations

Results of mycorrhiza examination reflect different applied technologies of the studied four plantations. On roots of seedlings in Hőgyész plantation, established by extensive, forestry methods species diverse, but low level of mycorrhization were found. Mycorrhiza level of target species (summer truffle (*Tuber aestivum*), winter truffle [*T. brumale*] and smooth black truffle [*T. macrosporum*]) proved to be low on average (0-16,66% and 0,73-8,8%) in both times of examination (2004 and 2006). Contaminant species neither showed high percentage of mycorrhization (0,17-26,43% and 0,7%-20,27%, respectively).

Mycorrhiza examination results of an extensive orchard with hazel (*Corylus avellana*) seedlings (Eger1 plantation) were in accordance with the technology and maintenance practice: medium, but balanced mycorrhiza levels (30,43-48,88%, 42,42% on average, n=12, SD=6,59%) were detected without contaminant fungi.

Eger2 plantation was established using mycorrhized seedlings of Turkish oak (*Quercus cerris*) and English oak (*Q. robur*) (intensive method). Mycorrhiza level changed differently on the two species: summer truffle (*Tuber aestivum*) mycorrhiza on Turkish oak slightly declined (from 39,98% to 34,2%), while mycorrhiza of English oak notably increased (from 57,7% to 71%). On English oak seedling roots only one was colonized by contaminants which interfered with summer truffle (*Tuber aestivum*) mycorrhiza.

On Eger3 plantation sampling proved to be difficult due to undeveloped surface root system of Turkish hazel (*Corylus colurna*) seedlings resulted in failure of mycorrhiza check. Lack of irrigation can be blamed for poor root surface development also causing the drying out of numerous trees. The above described conditions affected heavily summer truffle (*Tuber aestivum*) mycorrhiza formation: low mycorrhiza levels were detected (0-6,17%) with high level of contaminants (0-70,17%). Due to lack of irrigation significant number of trees dried out. Mycorrhiza check of seedlings planted out in 2008 showed similar results: many of the root samples were not evaluable; on the small amount of healthy roots low level of summer truffle (*Tuber aestivum*) mycorrhiza (0-1,32%) and considerable amount of contaminant mycorrhiza (0-28,57%) were detected.

Development of a system for description of smooth black truffle (*Tuber macrosporum*) natural habitats

International and Hungarian literature on truffle natural habitat research mainly focus on climatic, relief and pedological factors; aspect of forest environment are less emphasized. Hungarian forest site description research has more than a half century long history; results of this research should be taken into account. A new methodology for smooth black truffle (*Tuber macrosporum*) habitat description has been developed considering the above mentioned factors.

First step of the general description of a natural truffle site is determining climatic factors as follows: annual rainfall, rainfall in vegetation period, annual mean temperature, mean temperature of the hottest and coldest months, number of frosty days and climatic category based on forest site description methodology. Elevation, orientation and micro-relief should be included in relief parameters. International truffle literature less emphasizes on hydrology and water management characteristics, although these factors considered vital. Detailed description of soil of the habitat is essential, being responsible also for water management characteristics. Recommended factors are: soil type, type of mother rock, depth of organic layer and depth of soil layers. Besides these parameters it is recommended to examine soil color (wet Munsell color), granulometry, organic matter content (estimation based on soil color), structure, compacted areas, quantity of roots, secondary soil formations, aggregations and estimate lime content and alkalinity for each soil layer. Laboratory chemical analysis parameters should be defined according to truffle ecological requirements/tolerance. Basis parameters are: pH (both (H_2O and KCl), lime content, organic matter content, concentration of available macroelements, exchangeable calcium and magnesium. Besides these parameters further analysis can be justified for example in the case of saline (total soil content, exchangeable sodium) or alkaline (microelement content) soils. Vegetation studies of truffle habitats play a vital role in truffle ecological research. Diversity, species composition and richness can confirm soil analysis results and can describe characteristics of the truffle site. Canopy, shrub level and herbaceous level description (species composition and coverage) are recommended to estimate plant species frequency and abundance; to determine vegetation association type or to analyze phytocoenology parameters such as ecological indicators, social behaviour and naturalness.

Description and evaluation of smooth black truffle (Tuber macrosporum) Hungarian habitats

During the studies 13 Hungarian habitats of smooth black truffle (*Tuber macrosporum*) was examined focusing on site description, vegetation and ectomycorrhiza community survey. Examined truffle habitats occur on low elevation (83-380 m above see level) with variable macro relief. However, flat micro relief patterns are dominant in all except one case on bottom of wide valleys, hill bases, floodplains or terraces. Mountain region habitats belong to carpino-querco climatic category while habitats in the Great Plain belong to steppe category according to annual rainfall; however, microclimatic characteristics are affected by water presence. Concerning hydrology soil moisture is complemented by waters of other origin (subsurface water, flooding, etc.) on half of the habitats. Hydrology affects water management as all of the habitats belong to moist-semi wet-wet categories. Habitat description revealed significant antropogenous or other effect (forest animals' activities) on soils causing disturbance. Soil genetic types included chernozem (from Great Plain habitats), luvisols and planosols (hilly regions). Compacted layers were typical in 30-60 cm depth but in some cases they occurred close to the surface (5-10 cm). Due to compacted layers and the presence of water gleys and ferric precipitations are frequent. Physical analysis of soil samples from 0-30 cm depth revealed that soils belong to loamy silt (50%), loamy (15%), sandy-loamy silt (15%), sandy silt (10%) and silt (10%). Soil granulometry resulted in indicating uniform soils without extreme textures which explains preference of smooth black truffle (*Tuber macrosporum*) toward medium heavy soils of balanced water management.

New scientific results

1. Based on comparative study of different inoculation techniques of summer truffle (*Tuber aestivum*) mycorrhized seedling production suspension based inoculation method proved to be more effective than substrate based one.
2. Trials focusing on the vitalizing effect of summer truffle with different host plants [Turkish oak (*Quercus cerris*), English oak (*Q. robur*), hazel (*Corylus avellana*) and large-leaved lime (*Tilia platyphyllos*) seedlings produced in non-heated greenhouse and Turkish oak (*Quercus cerris*), English oak (*Q. robur*) and hazel (*Corylus avellana*) seedlings originated from plantations] confirm literature findings of controversial results depending heavily on environmental and biological factors.
3. Different international and Hungarian mycorrhized seedling quality control standards were compared tin order to develop an accurate and time-effective method.
4. Smooth black truffle (*Tuber macrosporum*) habitat description methodology was developed and used.
5. Ecological demand of smooth black truffle (*Tuber macrosporum*) was determined as follows:
    - micro relief
    - water management
    - hydrology
    - anthropogenic effect, disturbance
    - soil texture
    - pH
    - organic matter content
    - phosphorus content

Based on the above listed factors smooth black truffle (*Tuber macrosporum*) requires habitats of plain micro relief (valley bottoms, terraces, hill bottoms or floodplains) with temporary water surplus and moist, semi-wet or wet water management. Medium heavy soils are disturbed, human affected with neutral or slightly alkaline pH, high organic matter and phosphorus content.

CONCLUSIONS AND RECOMMENDATIONS

Mycorrhization experiments with summer truffle (*Tuber aestivum*) revealed the importance of use of quality plant and fungi material in order to achieve high mycorrhiza levels. Suspension based inoculation proved to be significantly more successful in mycorrhization than substrate based inoculation. Nevertheless, further trials are necessary to refine the method and to standardize inocula quantity.

Comparative trials on summer truffle (*Tuber aestivum*) mycorrhiza receptivity of the most common host species used in European nurseries and present in natural truffle habitats [Turkish oak (*Quercus cerris*), English oak (*Q. robur*), hazel (*Corylus avellana*) and large-leaved lime (*Tilia platyphyllos*)] resulted in the suitability of all examined species for mycorrhized seedling production and summer truffle cultivation. Difference on mycorrhiza levels was not detected by statistical analysis; however, some other factor should taken into consideration when establishing a plantation. Turkish oak (*Quercus cerris*) and English oak (*Q. robur*) have slow growing, therefore 6-8 is necessary to harvest the first truffles while hazel can produce truffles 4-6 years after planting. The above mentioned fact also has a disadvantage: fast root development can give ground to contaminant mycorrhiza fungi. Besides producing truffles, hazel (*Corylus avellana*) and lime (*Tilia platyphyllos*) can be used in double cropping producing fruits and flowers (respectively).

Success on truffle cultivation is heavily affected by seedling quality based on mycorrhizas on the root system. Comparative study of 5 international and Hungarian seedling quality check method has not revealed significant differences in accuracy, however, in some cases results depended on the experience of examining person [for example BACH et al (2008) and CHEVALIER et GRENTE (1978)] and time-efficiency of the methods was also different. Seedling check method generally overestimated real mycorrhiza levels but extreme differences were not detected. To summarize results, although mycorrhized seedling quality control methods basically differ from each other, significant difference in accuracy has not been detected. Nevertheless routine of the examiner person and time-effectiveness is a crucial question when examining large quantities.

Literature cites different outcomes on the vitalizing effect of truffle species. Phenological development (height and stem diameter), photosynthetic activity (SPAD-values) and mycorrhiza level of summer truffle mycorrhized Turkish oak (*Quercus cerris*), English oak (*Q. robur*), hazel (*Corylus avellana*) and large-leaved lime (*Tilia platyphyllos*) seedlings produced in non-heated greenhouse and Turkish oak (*Quercus cerris*), English oak (*Q. robur*) and hazel (*Corylus avellana*) seedlings originated from plantations were examined in order to reveal correlation. Results were in harmony of previous findings on host plant depending (BRATEK 2008, CHEVALIER et GRENTE 1974, ZAMBONELLI et GOVI 1990), partial (BRATEK 2008) or environment affected vitalizing effect (BENCIVENGA et VENANZI 1990). Therefore plant growth indicated mycorrhiza level is non-applicable for pre-selection of plants in nurseries or truffle plantations.

One of the basic factors of truffle plantation establishment is to choose suitable field. Despite that in some cases summer truffle (*Tuber aestivum*) requirements were not fulfilled - studies carried out on truffle plantations (Hőgyész, Eger1, Eger2 and Eger3) revealed their ecological suitability for truffle cultivation. Mycorrhiza monitoring has also been carried out on the plantation based on international practice with the following results:

I) Mycorrhiza levels of seedlings in Hőgyész plantation reflect the extensive/forest cultivation technology. Low general level of mycorrhization was detected, mycorrhiza level of summer truffle (*Tuber aestivum*) was variable and it frequently occurred with contaminant species, similarly to natural habitats. Although not all the parcels were suitable for truffle cultivation due to orientation, the plantation proved to be successful, producing truffles from 2007 in growing quantities.

II) Extensive Eger1 plantation with hazel (*Corylus avellana*) seedlings can be considered to be promising thanks to well balanced and medium high mycorrhiza levels. Reasons for the above results can be explained by well-chosen pre-crop (vegetables with endomycorrhizas) and strict observance of cultivation technology instructions.

III) Mycorrhiza levels of mycorrhized seedlings prove the advantage of quality controlled material: mycorrhiza level of summer truffle (*Tuber aestivum*) was the highest of all the plantations. Nevertheless, as an effect of a close Austrian pine (*Pinus nigra*) forest contaminants can be traced in some seedlings.

IV) Eger3 plantation proves the importance of technological specifications: despite soil suitability development of seedlings and truffle mycorrhiza is not satisfactory. Lack of irrigation caused the dry off of half of the seedlings and development is not guaranteed without regular watering.

As the examined plantations differ from technology, host plant species and fungi material, comparison was not aimed. However, general conclusion can be made on the importance of site selection and strict observance of cultivation technology instructions. Vital points of the previous one are orientation, soil parameters (medium heavy, neutral or weakly basic soils with good water management) and regular irrigation concerning technology.

Cultivation technology of a truffle species is based on its ecological requirements. International literature cites climatic parameters, relief and soil physical and chemical factors as important. However, as truffle host species form forests, application of forest site description methods when describing truffle natural habitats is justified.

Besides the most important hypogeous fungus of Hungary, summer truffle (*Tuber aestivum*), other truffle species of economic value are also present (winter truffle [*Tuber brumale*], sand truffle [*Mattirolomyces terfezioides*] and smooth black truffle [*T. macrosporum*]). The latest one, despite its outstanding organoleptic characteristics, is less known in the market and cultivation of the species is not widespread due to lack of knowledge on ecological demand. Research was conducted on natural sites of *Tuber macrosporum* describing ecological parameters such as climatic factors, relief, water management, soil parameters and vegetation. Macro relief parameters of the examined habitats is variable, however, micro relief is similar: plain surface in most of the cases such as valley bottoms, floodplains or terraces. These factors have an effect on hydrology and water management: water has a significant role in all the habitats, including climate as most of the examined places occurs in carpino-querco climatic category while habitats in the Great Plain belong to steppe category modified by the presence of water. Therefore it is stated that well balanced water conditions are essential for smooth black truffle (*Tuber macrosporum*), being a strict criteria for future plantations. This finding was confirmed by soil texture analysis where silt and silty clay soils of good water management were found. Other common characteristic of the examined sites is the disturbance caused by humans or other factors (water or animals). Chemical analysis of soils revealed the dominance of neutral or slightly basic soils of variable, but mainly medium lime content, high organic matter and phosphorus content. The later is in opposition with some findings of the literature where high phosphorus content declared as an unfavourable soil parameter (MORCILLO et al. 2007, RICARD 2003). The high phosphorus concentration can be explained by micro relief patterns as terraces where subsoil of high phosphorus content arrives from slopes or floodplains where regular inundations of rivers. Botanical investigations confirmed soil analysis findings on the preference of smooth black truffle (*Tuber macrosporum*) toward high and well balanced soil moisture, neutral or slightly basic pH and disturbed soil and habitat conditions.

Therefore basic cultivation requirements of smooth black truffle (*Tuber macrosporum*) can be summarized as follows:

- plain microrelief
- soils of moist, semi-wet of wet water management
- soils where moisture is complemented by other resources (flood or leaking water from hillside) or irrigated
- soils of good water management (mainly loamy silt)
- soils of neutral or basic pH, low or medium lime with high organic matter and phosphorus.

Hungary is well suitable for the cultivation of different truffle species, including smooth black truffle (*Tuber macrosporum*). The species has an outstanding organoleptic value which could help in spreading in the international and Hungarian market. Low quantities of the truffle in current market hinder its success which could be resolved by establishment of plantations.

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