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Abstracts

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Organisers: Alexander Urban, Tony Pla, Orsolya Fodor

A. ORAL PRESENTATIONS

The truffle of Europe (*Tuber aestivum* Vitt.): ecology and possibility of cultivation

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Key words: Europe, truffle, *Tuber aestivum*, ecology, calcium, cultivation.

T. aestivum (*uncinatum* morphotype), known as "Burgundy truffle" in France or "Truffle of Fragno" in Italy, is the truffle species most widespread in Europe since it is native in 26 out of the 27 European countries (probably except Finland). This species has also been recorded in Morocco and China. In Europe and North Africa, *T. aestivum* is known from 10° of West longitude (Ireland) to 37° of East longitude and from 33° (Morocco) to 58° (Sweden) of North latitude. Different factors can explain this wide distribution:

First: The potential hosts are present in all the Europe: i.e. hazels, oaks, hornbeams, beechs, lime trees, poplars, birchs, fir trees, spruces, pines, cedars.

Second: *T. aestivum* is able to grow in soils exhibiting various physico-chemical characteristics: sandy ("light" soils) to clayey ("heavy" soils) or loamy textures; chemical composition not different from cultivated soils (nitrogen, phosphorus and/or potassium even rich); ability to grow in soil rich in organic matter. However, a minimum amount of calcium, depending of the soil texture, is necessary. On the contrary, the presence of lime (calcium carbonate) is not necessary.

Third: *T. aestivum* requires an oceanic, semi continental or continental climate with sufficient rains in summer and not too much low temperature in autumn. This species requires more water than *T. melanosporum* (black Perigord truffle, truffle of Norcia or Spoleto).

Fourth: The intraspecific genetic variability of *T. aestivum* is important allowing this species to adapt at various ecosystems and climates.

In France *T. aestivum* is cultivated since exactly 30 years and actually it is cultivated in various countries such as Italy, Spain, Sweden, Hungary and Austria. In Europe the potential surface to cultivate this species is immense especially because we were able to cultivate *T. aestivum* in unfavourable soils (even very acid) by liming the soils.

The cultivation of *T. aestivum* is often different from the one of *T. melanosporum*: i.e. a more important plantation density is necessary, pruning of the tree must be less important and irrigation more important. But the technique of soil management have still to be improved. In France the host species giving the best results are: hazels (*Corylus avellana* and *C. colurna*), hornbeams (*Carpinus betulus* and *Ostrya carpinifolia*), oaks (*Quercus robur*, *Q. petraea*, *Q. pubescens* and *Q. cerris*), pines (*Pinus nigra austriaca*) and cedar (*Cedrus atlantica*). Actually with the global changes (more dry and hot temperatures) the conifers give better results than deciduous species mainly because they are more sparing in water.

The most important limiting factors for the development of *T. aestivum* cultivation in Europe is the climate. In the Northern countries the production may be limited to the summer period with truffles of medium quality (morphotype *T. aestivum* in the sense of French) instead of the autumnal truffles characterized by a dark gleba and an optimal aroma.

What makes a good truffle infected tree?

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Key words: inoculum, quality control, Flouricene-diacetate, vital stain.

Modern truffle cultivation is based on planting *Tuber* infected plants in suitable sites. The first step is the production of truffle infected plants in greenhouses, generally using spore inoculation techniques. Other less common methods include the inoculating plants with pure cultures and the mother plant techniques.

Regardless of the inoculation technique used, it is advisable to check the geographic origin of the inoculum, selecting the most suitable one for the climatic conditions of the site where the plants are to be planted. It is also important to verify the quality of the inoculum before inoculating the plants. In particular, when using spore inoculation techniques, the ascomata used for making the spore suspension should undergo morphological observation and molecular testing to verify the absence of less valuable *Tuber* species. It is also advisable to assess the presence of mature spores and their vitality particularly when the inoculum is not produced from fresh truffle.

Initial studies have shown that vital staining techniques alone (Flouricene-diacetate (FDA), or in combination with non vital ones (Evans blue), can be used to this end.

Recently, Paolocci *et al.*, (2006) demonstrated that *Tuber* is heterothallic and the *T. melanosporum* genome sequencing, that is currently in progress, has recently revealed the presence of mating-type genes (Murat & Martin, 2008). So, when mycelial cultures are used as inoculum, it is important to select for the plantation, plants carrying both the mating types. In fact, the capacity to form the ascomata in the field, is dependent on the presence of both mating. Where mother plant techniques are used it is also essential that not only two mating types are present but also that the mother plant is free of contaminating ectomycorrhizal fungi.

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Sex, croziers, truffles and variation

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Key words: truffle, lifecycle, haploid, dikaryotic, hetrokaryotic, crozier, meiosis, antheridium, oogonium, variability, trigger, fruiting.

We have gone back through the literature on truffles and sought out what is known of their lifecycles. This primarily morphological information was then interpreted in the light of molecular research carried out over the past decade.
Most of the Ascomycota are primarily haploid but a dikaryotic state is formed prior to a short sexual phase. This is followed by the formation of the crozier apparatus, nuclear fusion, pairing of the chromosomes and meiosis leading to recombinant variation. The apparent absence of croziers in truffle ascoma and fewer than 8 ascospores in asci has led to the belief by some that truffles may have aberrant life cycles. However, the evidence is that *Tuber* ascospores produce a haploid thallus, these form haploid mycorrhizas, the haploid hyphae produce antheridia and oogonia just prior to ascoma production, and then crozier formation inside the truffles leads to meiosis, recombinant variation and the production of haploid spores.

There is now sufficient evidence that variation in truffles is anything but trifling. We postulate that this variability may be responsible for poor production in truffières containing plants inoculated with truffles ill adapted to climatic conditions at outplant sites.

Experience of *Tuber aestivum* Vittad. cultivation in Central Italy

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Key words: truffle orchards, production results, ecology, field experimentation.

The cultivation of *Tuber aestivum* Vittad. began later than *T. melanosporum* Vittad., thus there is little experience of it. However many productive truffle orchards were planted in the last decades in areas not suitable for *T. melanosporum*, following increased demand from farmers. These recent plantations were realized in agreement with the most recent knowledge of *T. aestivum* ecology up to date, choosing the suitable areas for this species and tending to its specific requirements. The aim of this contribution is to present some cases of truffle orchards. The production results are very satisfactory, moreover is very important how long it takes for the first production: it varies from the 4^{th} to 11^{th} year after planting. The goal of future research is to understand all different ecological situations and to define guidelines for a rational choice of plantation sites to obtain a good continuous production.

First results of studies in experimental truffle-orchards established in INRA-ELTE cooperation in Hungary

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In the framework of INRA-ELTE scientific cooperation 11 experimental truffle orchards were established between 2004-2006 in Carpatho-Pannon Region. Controlled quality seedlings were produces by Robin Pépinières (France). Seedlings of 4 host plant species were inoculated by spores of T. aestivum biotypes from several European regions. Morphological and molecular examinations were carried out on mycorrhiza of randomly selected seedling from 3-year-old parcels. Our work focused on determination of mycorrhizal frequency, description of host plant phenology, definition of contaminant mycorrhizal types and their identification based on their hole ITS sequences. The results of the above mycorrhizal research were evaluated in relation to the data of previous studies on plant physiology, pedology, meteorology, and phytoindication in order to find the answers on the following questions:
- How does the efficiency of mycorrhization of biotypes depend on the ecological features of Central-European habitats?
- How does the host plant mycorrhization depend on the local biotic and abiotic factors?
- Which ecological factors may provide advantages for early stage mycorrhiza-substitution?

Comparing the truffle orchards with the outstanding mycorrhization and the most productive natural truffières the consistent ecological factors were revealed. Comparative evaluation of the databases of the natural habitats and the truffle orchards could promote the faster development of the truffle cultivation in Carpatho-Pannon Region.

RESEARCH FROM THE UK TRUFFLE CULTIVATION PROJECT: TREE PERFORMANCE IN RELATION TO ABIOTIC FACTORS, LESSONS FROM LIMING AND DETAILED BRULE RECORDINGS

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Key words: *Tuber aestivum/uncinatum*, truffle cultivation, tree growth, climate, soil, pH, liming, UK, plantations, in-field inoculation.

The UK has a long history of the utilisation of wild summer truffles (*Tuber aestivum/uncinatum*). However, cultivation of these truffles within the UK is a relatively new industry, creating a need for local research-led advice for growers.

Wild harvests of the summer truffle have occurred over a wide range of areas in the UK, from the milder climates of southern England to northern territories including Lothian in Scotland, spanning a broad climatic profile of temperature and rainfall patterns. Population density and geological factors are strongly linked to wild truffle finds in the UK, more so than climatic variables.

Young truffle-inoculated trees, including *Quercus robur* and *Corylus avellana* have been planted in a number of sites across the UK, allowing truffle-tree growth and their development to be monitored in relation to climatic and edaphic factors as well as management systems. With a large sample size, here we report influential factors on the speed and growth of young truffle-trees.

A second characteristic of truffle-cultivation sites in the UK is a general need for significant pH adjustment. Here we report results from a number of different sites with differing pH profiles and the results of adjustment using differing techniques.

At one time the UK was a major producer of the summer truffle and it has a suitable climate for their cultivation. Through the application of novel technology and cultivation methods, our goal is to utilise research-led systems to re-establish the once thriving UK truffle industry.

Mycorrhization experiments with summer truffle (*Tuber aestivum* Vitt.)

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Key words: summer truffle (*Tuber aestivum*), mycorrhization, seedlings.

Truffles are hypogeous ascomata fungi forming mycorrhiza with forest trees of the temperate zone. Truffle ascocarp develops under the soil surface and has an extraordinary fragrance and taste, therefore they were considered as precious gastronomic delicacies from the ancient times.

Truffles – as other, valued mushrooms like chanterelle and porcini – require a plant partner to develop. To establish plantations it is indispensable to count with these plant partners. In the case of Hungary's most common truffle, the summer truffle (*Tuber aestivum* Vittad.), collected in great quantities, the most frequent host trees are the following: hornbeam (*Carpinus betulus* L.), English oak (*Quercus robur* L.), Turkey oak (*Quercus cerris* L.), common oak (*Quercus petreae* Liebl.), hazel (*Corylus avellana* L.), Turkish hazel (*Corylus colurna* L.) small-leaved linden (*Tilia cordata* Mill.), large-leaved linden (*Tilia platyphyllos* Scop.) and Austrian Pine (*Pinus nigra* Arn.).

The objective of the current experiment was to produce seedlings mycorrhized with summer truffle under controlled conditions with different host plant species as the following: *Q. cerris, Q. robur, T. platyphyllos, C. avellana*. Mycorrhiza level and phenological sate of seedlings were examined and correlation between mycorrhiza intensity and shoot development (height and stem diameter) were measured. Photosynthetic activity and chlorophyll content of the leaves were also measured.

Preliminary results show low correlation between mycorrhiza intensity and seedling shoot development. Results can be explained by close-to-optimal greenhouse conditions and/or lesser dependence of angiosperms on fungal symbionts.

Tuber aestivum orchards in Finland

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Key words: *T. aestivum*, mycorrhiza, oak, seedlings, root system.

Truffles are the most expensive edible fungi in the world. Truffles are hypogenous gourmet fungi; grow naturally and in orchards in symbiosis with certain trees such as oak, hazel and pine around the world. Truffles do not belong to the traditional Finnish kitchen nor are they a common topic in the media. Establishment of truffle orchards in Finland has started recently by the Laboratory of Bioprocess Engineering, Helsinki University of Technology. Finland is well known for its long winter and low temperatures which is not favoured by truffles. French oak seedlings infected with *Tuber aestivum* were planted in summer 2006 in north east Finland. Different protection systems were applied to protect the seedlings and their truffle mycorrhizae during frost seasons. The planted seedlings in 2006 developed very well during the last years despite the long winters and low temperatures. *Tuber aestivum* mycorrhizae had survived well in the root system of the seedlings whereas both old and new mycorrhizae were seen in all studied root samples after 3 winters from the cultivation. Finnish oak seedlings were also produced within the project and inoculated with *Tuber aestivum* spores this year. The oak seeds growing medium (substrate) had a significant effect on the rootlet system development of the produced seedlings.

Allelopathic activity of the methanol extract and its fraction of *Tuber aestivum* Vittad.

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Key words: bioassays, minimum inhibitory concentration (MIC), total phenol compounds.

Allelopathy is a complex interaction among plants, as well as among plants and microorganisms, through biochemicals released into the environment either actively (e.g. exudates) or passively (e.g. leachates or decay). The goal of allelopathy research is to maximize the utilization of locally available natural resources in agriculture and forestry, thereby reducing agrochemical inputs, while maintaining economic productivity without degrading the environment, leading to sustainable agricultural systems (Mallik and Williams, 2009). The allelopathic effect of volatile substances released by *Tuber melanosporum* Vittad., *T. borchii* Vittad. and *T. indicum* Cooke & Masee Grevillea on *Arabidopsis thaliana* Heinh. were investigated by Splivallo *et al.* (2007). Tirillini and Granetti (1995) and Tirillini and Stoppini (1996) reported the presence of phenolic derivatives in methanolic extracts of truffles. Since Rice (1984) pointed out that a large number of phenolics are allelopathic agents that play a significant role in several ecological phenomena such as seed dormancy, plant succession, vegetational patterning, plant-microbe and microbe-microbe interactions (Vokou, 1992), the aim of this research was to study the allelopathic activity of methanolic extract of *T. aestivum* Vittad. on plant species (*Hieracium pilosella* L., *Lotus corniculatus* L., *Melica ciliata* L., *Silene vulgaris* (Moench) Garcke,) and *Streptomyces* spp.. The allelopathic effect of the *T. aestivum* extract on plant species and *Streptomyces* spp. was evaluated using a Petri dish assay and by the broth microdilution method, respectively (Angelini *et al.*, 2009). The methanol extract of the *T. aestivum* inhibited the growth of roots and shoots of bird's-foot-trefoils (*Lotus corniculatus* L.), cowbell (*Silene vulgaris* (Moench) Garcke), silky spike melic grass (*Melica ciliata* L.) and mouse-ear hawkweed (*Hieracium pilosella* L.), and showed a significant dose-dependent inhibitory effect on *Streptomyces* spp.. The results of this research (financed by Fondazione Cassa di Risparmio di Perugia, Italy, project code 2009.020.0094. Ricerca Scientifica e Tecnologica) suggest that *T. aestivum* may have substances with allelopathic activity, and may be potentially useful for weed management. The methanol extract of *T. aestivum* was divided into n-hexane, chlorophorme and ethyl-acetate fraction to evaluate the active fraction.

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Studies on ecophysiology of *Tuber aestivum* populations in Carpatho-pannon region

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Different studies were carried out in order to recognize the ecological requirements for summer (burgundy) truffle biotypes of Carpatho-Pannon Region. Based on the experiences of in situ collections and rearings, many insects including specialists take part in spreading of the species. Data of soil analysis shows that T. aestivum has a limited tolerance (stenotopic) on several soil parameters hence it has a broad range of tolerance (eurytopic) on other soil parameters, which ones may probably contribute to its wide distribution. Studies on soil horizons of habitats with outstanding productivity strengthen the importance of the good balanced water supply. The secret of the best regions may be hidden in the soil profile, as it can be the situation in the case of the excellent region of Jászság in Europe. Analysis of phytoindication can provide useful informations to the description of water supply and local microclimate. The summer truffle is rare or absent in dry forest types (e. g. oak forests on sandy or loess soils) and in the forests of wetlands. All in all a strong preference of summer truffle can be detected on certain plant and certain genetic soil types as well. On the basis of the above results and the coexistence analysis a trial is made for the establishment a modell of niche-segregation.

Contribution to the study of the ecology of *Tuber uncinatum/T. aestivum* in Morocco: phytosociological and geological aspects

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Key words: Truffles, *Tuber uncinatum, Tuber oestivum*; ecology; climate; Middle Atlas; Jaaba forest; Morocco.

Morocco counts less than ten species of "Terfess" (*Terfezia, Delastria, Picoa* and *Tirmania* genera) and five species of truffles (genus *Tuber*). The terfess are harvested usually in semi-arid or arid climate in sandy-silty light soils. The forest of Mamora in the North West of Morocco, the plains of the highlands to the east and the Sahara in the South and South East are very favourable environments for the presence of these kinds of truffles. On the other hand three truffle species (*Tuber rufum* Pico, *T. excavatum* Vitt. and *T.uncinatum/T. aestivum* Vitt.) were collected fortuitously by Malençon in 1960 in the Middle Atlas between 1600 and 2000 meters above sea level under sub-humid and humid climate and in calcareous soils. In addition to the chain of the Middle Atlas, many other areas in Morocco, namely the High Atlas chain in central Morocco, the Rif mountains in the north chain of the uplands and the massive Debdou in eastern Morocco are areas potentially suitable for truffles given their climate (humid and sub humid) and the nature of their land (generally calcareous). The presence of truffles (genus *Tuber*) in these areas is likely and planting truffle seedlings seems promising. By the way the results from the first outplanting of oaks mycorrhized with *Tuber melanosporum* in the region of Debdou were positive.
The aim of this work is to characterise the phyto-ecological conditions of the middle Atlas and surrounding areas where *Tuber uncinatum / T. aestivum* was harvested by Malençon in 1960. Malençons records were analysed together with lithological, geological and phyto-sociological data established from 1954 till today, in these regions.

These analyses showed that the climate, vegetation and soil type are still favourable for the exploration and plantation of *Tuber uncinatum / T. aestivum* in these regions.

Tuber aestivum Vitt. in Balkan Penninsula

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Key words: Truffle, ecology, soil conditions, molecular determination.

Tuber aestivum Vitt, has been recently reported from Balkan Peninsula. The amount of data on this truffle has been increasing, and at this point the characteristics of ecosystems that are supporting fructification of summer truffle are to some extend described. We provide first data on *T. aestivum* ecological characterization in the conditions of western part of Balkan Peninsula. Molecular determination, as a result of analyzed ITS regions, of specimens originating from investigated regions are provided as well.

Tuber aestivum/T. uncinatum in Ireland

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Key words: Ireland, history, *Fagus*, truffière.

There are records for six modern species of the Genus *Tuber* from the island of Ireland, following the taxonomy of Riousset *et al.* 2001. Finds were recorded between 1772 and 2009. *Tuber aestivum* is the most frequently reported species with 15 published and six grey-literature or recent unpublished records. No records have been made of wild *Tuber uncinatum* although *Tuber aestivum* as recorded in Ireland has a long fruiting season, from July to January. *Tuber aestivum* sites generally feature an underlying geology of Lower Carboniferous limestone with Quaternary glacial drift on top. *Fagus sylvatica* is the most common host tree while other recorded hosts are *Quercus* spp. and *Betula pendula*. Historic sites are being resurveyed with truffle hounds as part of Forest Fungi – a research project into the geographic distributions of edible forest fungi in Ireland.

A recent development has been the importation of truffle-inoculated saplings from France and Britain. A trial truffière was established in 2007 using French-sourced *Quercus robur* and *Corylus avellana* stock. These trees were inoculated with

French *Tuber uncinatum.* 250 trees were planted in six rows in what was previously a field of grass in Co. Limerick. A few truffle trees were planted through an adjacent 15-year old broadleaved plantation. This trial is monitored under the Farm Fungi research project and, as with Forest Fungi, it is funded by COFORD, the Irish Forestry Research Agency. The mycorrhizae of introduced truffle trees are being checked for the presence of *Tuber brumale* and other detrimental alien "weed" species unrecorded in Ireland to date.

Cryptic Species in the *Terfezia boudieri* Complex

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Key words: Desert truffles, Phylogenetic species, RFLP, AFLP.

Early phylogenetic analyses revealed three internal transcribed spacer (ITS) Types in *Terfezia boudieri* isolates and have emphasized the divergence of Type 2 from Types 1 and 3. The results of further analyses of the three Types are described below.

The molecular markers used included sequences taken from the: 1 - 5' end of the ribosomal large subunit gene, 2 - a chitin synthase gene partial sequence and 3 - b-tubulin partial sequence, as well as amplified fragment length polymorphism (AFLP)-based markers. Following initial sequencing of a single PCR amplified sample for each Type, mass analysis of specimens relied on RFLP (Restriction Fragment Length Polymorphism) differences between the Types. Over 100 fruit bodies, 30 or more specimens for each ITS Type, were tested with each of the markers. The markers analysis divided the isolates into distinct three groups, each correlated to a specific ITS Type. A study of physiological traits was also undertaken. Two of the examined traits: mycelial proliferation and mycorrhiza formation, consistently showed responses paralleling the ITS Types; the data obtained suggest that *T. boudieri* is comprised of three cryptic species. These species are found in close proximity in the wild. The question whether these are true species will be discussed.

Can truffles adapt to extreme weather by morphological changes?

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Key words: truffle, morphology, ecology, climate change, Tuber uncinatum.

Over the last 10 years, the weather characteristics have changed dramatically in the UK. From a very changeable weather, with light rain and winds, on and off, we now experience storms with thunder, rain downpours with local flooding, gale force winds alternating between long dry spells. As a result, truffles now face a different ecological environment from their natural surroundings in damp earth. From the tree tops to below ground, the ecology is changing. Since 2003, truffles, mainly Tuber uncinatum, were harvested at any stages of their life cycle, from 2mm to 12 cm in varying soil structures in waterlogged and extremely dry conditions. I will illustrate these changes taking examples of species at each level of the complex environment. The truffle morphology change over time shows an innate 'intelligence' a proof of sophisticated adaptation. Some unusual truffle examples and mainly tree behaviour will be explained as a reaction for the survival of the species.

Proposal for new directions and policy reform in the truffle business

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Key words: fresh truffle, tuber aestivum, quality control, trade rules, certified mycorrhized plants.

During the past ten years a new generation of truffle enthusiasts engaged themselves to work with truffles on a daily basis, bringing a different approach in doing business in almost all levels of this industry. Young scientist, producers and businessman together are aware of the old customs and methods of the truffle trade that was based on secrecy, false marketing, lack of origin traceability etc. Truffle quality has been solely determined by its origin, and not after quality criteria. On the other hand truffle cultivators has been poised with low quality or non-mycorrhized "truffle plants", that ultimately produced no results.

Our presentation would focus on those major problems of the truffle industry that led to unsafe and dishonest commercial customs, and would propose new directions for the newly emerged participants of the industry on how to deal with truffle on the 21st century.

From landscape history to genetic diversity - conservation strategies for *Tuber aestivum*

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With the cultivation of *Tuber aestivum* still being in its infancy, harvest from wild populations still provide the main supply fort he market. Current levels of harvest are maintained thanks to the continuous exploration of new regions. However, it is doubtful if current exploitation is sustainable. While in certain countries of Europe *Tuber aestivum* is harvested in large quantities, this fungus is considered a red list species in other countries and harvest is prohibited by law.

For *Tuber melanosporum*, a dramatic decrease of harvests from natural populations in France has been recorded since the beginning of the 20th century. Populations of *Tuber aestivum* in the only well documented truffle area in Austria, the *Pinus nigra* forests of the southern part of the Vienna Basin, appear to have followed a similar trend. The reason for this decline is poorly understood. Potential explanations include climatic changes and anthropogenic changes in hydrology, land-use and forestry, as well as nitrogen immissions. Possibly, the decline of the truffle populations is also due to a stagnation of forest expansion in that region in the 20th century, since it might be the case that Tuber aestivum is limited to a certain successional state of these forests.

Currently, the most promising strategy against the decline of truffle populations is the establishment of truffle plantations. However, this kind of action needs to be complemented by a better understanding of the processes that have led to the decline of truffle productivity in many regions.

Another important aspect is the intraspecific genetic diversity, which is known to be considerable in *Tuber aestivum*. Conservation efforts should aim to maintain this diversity as an important potential resource of resilience against ongoing climatic change. Truffle populations in more natural vegetation types should be protected as potential source populations for the recolonisation of anthropogenically influenced habitats and for the mycorrhization of tree seedlings. Sporophore production in natural habitats needs to be sufficiently high to allow effective spore dispersal by animal vectors. Natural processes which create suitable habitats for Tuber aestivum should be facilitated.

Morphological and molecular diversity of the fungal ectomycorrhizal community present in a Tuber aestivum Vittad. truffle orchard

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Key words: *Tuber aestivum*, ITS, Ectomycorrhizal, Hazel, Truffle orchard.

Ectomycorrhizal (ECM) fungi play a key role in the ecology of many ecosystems through they mutualistic relationship with roots of forest trees and shrubs. ECM fungi in the genus *Tuber* produce subterranean ascocarps with superb flavours and fragrances, called truffles. These are currently largely cultivated in orchards because of the rapid decline of wild production throughout Europe. Truffle cultivation consists on transplanting mycorrhized plants on a suitable field according to ecological specific requirements for the host-fungus combination.

Tuber and other ECM species are able to co-occur and form high species-rich assemblages in the same area by occupying different ecological niches: this occur both in wild as in cultivated sites. In this situation, to better understand the conditions that promote truffle production, the investigation of the below ground fungal ECM communities is fundamental.

The main objective of this work was to study the ECM diversity and community structure associated with hazels (*Corylus avellana* L.) and european hop-hornbeams (*Ostrya carpinifolia* Scop.) in a 25 years old *Tuber aestivum* Vittad. cultivated truffle ground both with morphological and molecular approach.

In the north part of the orchard 4 area with same pedological condition and omogenous trend of production of truffles were selected. In particular 2 of these were characterized by exclusive presence of hazel trees while in the other two there were only hop-hornbeams. For each area, 10 samples were randomly taken as follow: 3 cores were taken at 3 different points each distant 1 m from the base of the tree (depth of 25 cm) and merged into one single sample; a total 40 samples were then obtained.

Ectomycorrhizas were sorted in different morphotypes and described in detail based on standard morphological and anatomical traits. Single ECM root tips, selected from each morphotype, was then used for molecular analyses in order assign it to a *taxon*. Internal transcribed spacer (ITS) of the 25S rDNA was PCR-amplified and sequenced. Similarities with known sequences were searched in the National Center for Biotechnology Information (NCBI) database using BLASTN application.

Results try to shed light on below ground composition, distribution, richness, genetic and morphologic diversity of ECM fungi associated with cultivated *T. aestivum* truffle plants of different species.

The Burgundy truffle: from the genetic and organoleptic diversity to the optimisation of production

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Key words: Burgundy truffle, genetic diversity, organoleptic properties, competition.

In France, *Tuber uncinatum* (Burgundy truffle) occurs naturally in Eastern regions. However, human activities have lead to a reduction in natural populations and despite truffle plantations which have been established in the last 30 years, the demand is still not satisfied. Producers actually encounter problems in truffle cultivation because of heterogeneity in production between different plantations, between trees within plantations and from year to year. This could be linked to mycorrhization heterogeneity in inoculated plants, competition in the field with other ectomycorrhizal fungi and a poor knowledge of the impact of soil physico-chemical properties and cultural practices on the life cycle of *T. uncinatum*.
Another problem for the producers is the fact that *T. uncinatum* and *T. aestivum* are now considered to belong to the same species. This has lead to the harvest of immature truffles during summer time in the East of France and fraudulent sell on the market. It would be therefore important to compare the genetics and ecology of *T. uncinatum* and *T. aestivum* in greater details. Interestingly, *T. uncinatum* has a great variability in its organoleptic properties which can be due to genetic, soil and environmental factors. Unfortunately until now this diversity has not been exploited for the selection of truffle isolates.
In order to stimulate the production of Burgundy truffle of high quality, it is essential to better understand the genetic and organoleptic diversity and ecology of *T. uncinatum/ T. aestivum*.
The aim of our research project is to:

- better characterise the genetic and organoleptic diversity of *T. uncinatum* and *T. aestivum* via molecular-based techniques using microsatellite markers and gas chromatography,
- isolate and prepare mycelial cultures from truffles with interesting organoleptic properties,
- optimise the mycorrhization of young plants,
- study the persistence and production of *T. uncinatum* and its competition with other ectomycorrhizal fungi in plantations using rDNA-based PCR techniques.

FIRST ATTEMPT TOWARDS CULTIVATION OF *TUBER AESTIVUM* VITT. IN POLAND

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Key words: *T. aestivum, Quercus robur,* truffle cultivation.

The research with *Tuber aestivum* truffle began in 2008. 1-year-old seedlings of *Quercus robur* were inoculated in greenhouse with spores of *Tuber aestivum* which had been collected from fruiting bodies found in Poland in 2007 [Mycorrhiza 18 (4), 2008]. Ectomycorrhizal structure was monitored for 6 months; the colonization rate was very high and 100 % of investigated seedlings possessed ectomycorrhizae of *T. aestivum*. In October 2008, 130 seedlings have been outplanted to the site that represents Rendzic type of soil. After one year the survival rate of seedlings was high - 4% of oaks seedlings died only. *T. aestivum* mycorrhizas dominated (share 75%) on the roots. The ectomycorrhizas of adventive fungi could be also observed, mainly *Thelephora* sp. and *Hebeloma* sp.

Trough this research project, we want to create an incentive for restoration of some endangered species by establishing of oak plantations.

Recent collections of *Tuber aestivum* in Poland

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Key words: hypogeous fungi, truffles, ecology, distribution.

Black truffles have been of increasing interest in the recent years in Poland. In course of systematical research of hypogeous fungi, *Tuber mesentericum* Vittad. has been discovered in calcareous soils in two localities on the Częstochowa Upland. The localities was monitored during more than twenty years (Ławrynowicz 1988, 1999, Ławrynowicz et al. 2008).

Recently, the investigations using, in some cases, trained dog were undertaken in other calcareous areas in the south part of the country. As the result several localities of *T. aestivum* Vittad. has been discovered in three regions: Wyżyna Krakowsko-Częstochowska Upland, Wyżyna Przedborska Upland and Wyżyna Kielecko-Sandomierska Upland. *Tuber aestivum* was found in a variety of forest ecosystems, mostly in ectomycorrhizal association with: *Fagus sylvatica, Quercus petraea, Q. robur, Carpinus betulus* and *Corylus avellana.*

The examination of carpophores indicated that a part of them do not reach the maturity during the whole vegetation season. On the other hand the increasing production of black truffles carpophores is to be observed in the recent years.

The discovery of a significant number of localities of *T. aestivum* enforces the increasing to belief that cultivation of that truffle would be possible in Poland. Some efforts in this direction are already undertaken (Hilszczańska et al. 2008).

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Quality assessment of truffles by aroma analysis using solid phase micro extraction (SPME) coupled with GC and multiple detection systems

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Six years ago a plantation of *T. aestivum* in eastern Austria was established and the first cultivated truffle could be harvested in autumn 2008, a real breakthrough in these latitudes. The aim of this preliminary study was to evaluate the combination of different detection systems for the characterization of truffles. Various factors influence the organoleptic properties of truffles, in particular soil characteristics ("terroir"), season of harvest, colonisation with diverse microbia, and, potentially, intraspecific variation. Before testing the influence of complex environmental or genetic parameters it is necessary to measure variations in the aroma profiles of individual truffles in dependence of freshness.

Normally the first perception of truffles is linked to sulfur compounds, given by very low odor thresholds of these compounds. An aroma active compound is considered as clearly identified if experimental and database (SKAF, flavornet) RI values, the mass spectrum (Wiley NIST05) and the olfactory perception match.

As *T. aestivum* was overripe at its harvest only a few substances were detected by GC/MS and other detection methods (FID and FPD), with 2-butanone being the most abundant one, known to be found in French and Italian samples always in highest amounts. From SNIF analysis (*Fig.2*) it can be inferred that there are more substances with low odor thresholds composing the overall aroma.

Although truffles have been found in Austria for centuries no reference is found in literature regarding the characterization of respective aroma profiles; our future aim will be to compare wild and cultivated Austrian T. *aestivum* correlating the results with the occurrence of specific microorganism in the fruiting bodies. These analyses are essential not only for consumers but especially for a promising cultivation of truffles in "new" regions.

DEVELOPING OFFICIAL METHOD TO CONTROL THE QUALITY OF MYCORRHIZED FORESTRY MATERIAL IN HUNGARY

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Summary: The interest to produce mycorrhized forestry material was increased in the Hungarian nurseries in this decade. The demand to certificate mycorrhized plants was arisen from nurserymen. The aim of our work was to establish official method to certificate mycorrhized propagation materials.
The Hungarian certification system checks the identity of the mycorrhiza, and the rate of mycorrhized roots like in the other European countries. The identification of mycorrhiza takes place in the base of morphological characteristics and using DNA based methods as well. The adaptation of PCR protocols to identify summer black truffle (*Tuber aestivum*), winter truffle (*Tuber brumale*) and weeping bolete (*Suillus granulatus*) were proved successfully. Strong corelation were found between the results of morphological and PCR based identifications at these species. The two way of the identification were complement each other well.
There is a (0-3) scale developed to quantify the rate of the mycorrhized roots. The quantity of the sample for certification is 10 plants out of 3000. The plants for sample are chosen randomly before marketing the lot.

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