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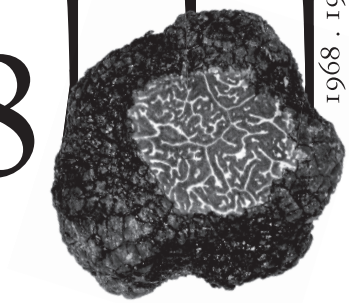

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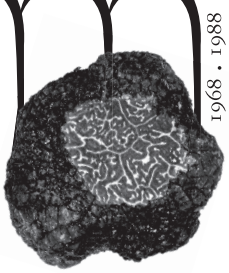

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THE MAIN AREAS OF TRUFFLE RESEARCH SINCE THE CONGRESS IN AIX-EN-PROVENCE

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KEY WORDS: *Tuber*, cultivation, systematic, biology.

Research on truffles has progressed in numerous sectors since the congress in Aix-en-Provence (1999). In the field of truffle cultivation, work is being done by Henri Dessolas to develop a new methodology known as "Formula J.A.AD." that will overcome some of the shortcomings of the Pallier and Tanguy methods. Attempts are also being made to improve the mycorrhized plants by using new mycorrhizing techniques. Particular attention has been given to the physiological relationship between the root apex and the truffle mycelium when the two come into contact with each other. The formation of ectomycorrhizae, an important phase in the truffle life cycle, has been analysed in-depth using molecular methods. Contrary to what has been previously hypothesized, the haploid mycelium of spore origin generates the mycorrhizae and the ascomes are made up primarily of haploid hyphae. Dikaryotization probably occurs inside the young ascocarps which contain the sporophytic apparatus. In the field of systematics, molecular analysis has been used to identify and characterize already known truffle species as well as to identify new ones. Molecular analyses has also been used to recognize mycorrhizae produced by different fungal species which is useful when evaluating *Tuber* – mycorrhized plants. Isoenzymes, monoclonal antibodies and aroma composition have also been used for this purpose. Also worthy of note is the work "Le specie europee del genere *Tuber* – Una revisione storica" by A. Ceruti, A. Fontana and C. Nosenso (2003) which has put a number of synonyms into order. A number of studies have investigated the behaviour of the symbiotic plants and the evolution of the ectomycorrhizae in the root apparatus after being planted out in the field under very diverse soil and climatic conditions. Molecular analyses have shown various degrees of affinity between the European and Chinese truffle species which suggests a common ancestral origin. A study on the genetic variability of *T. melanosporum* has allowed the path of recolonization in the French territory around the central massif after the last glaciation to be reconstructed. Some studies have been conducted on the intraspecific variability of economically important species and others have used molecular analyses to identify fraud in both fresh and preserved truffles. Several publications report the chemical composition of fruiting bodies, particularly the aromas. There has been a notable increase in the number of studies on truffle physiology (the reference species is usually *T. borchii*) in all of the life cycle phases (mycelium, mycorrhiza, young and mature ascocarp) including the identification, characterization and gene expression. Finally, the sequencing of the *Tuber* genome was recently completed. This complex work was the initiative of Prof. F. Martin of INRA in Nancy who acted as the coordinator of several research groups.

TRUFFLE RESEARCH AND CULTIVATION IN CHINA

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KEY WORDS: China, truffles, cultivation.

Research on truffles and their cultivation on commercial scale have expanded rapidly in China since the 2nd International Congress of Truffles in Spoleto, in 1988. Truffles were not recorded in ancient Chinese books and only 8 *Tuber* species were recorded before 1989. Truffles were not known in China until Chinese black truffles, the *T. indicum* complex, was scientifically described in 1989 and exploited commercially in the 1990s. Two genera (*Paradoxa* and *Tuber*), 24 species of *Tuber* and one species in *Paradoxa* have been found in China in the last 20 years, half of which are new species. A few desert truffle species has also been recorded. These results may reveal that China has rich diversity of truffles and might be one of centres of truffle phylogeny and distribution in the world. Whilst not formally described until the late 1980s, Chinese black truffles have been collected for consumption and trad locally for many years. However, Chinese truffles were virtually unknown internationally until the early 1990s when increasing quantities were exported to international markets annually. This has created concern about risk of biopollution of European endemic truffle populations and passing off of cheap Chinese black truffles as Périgord black truffle (*T. melanosporum*) because they are cheaper and look very much like *T. melanosporum.* Their aroma and flavour, however, are much less intense. Recently *T. indicum* was detected in one of Italian black Périgord black truffle plantation. Harvesting and trading in Chinese black truffles are becoming a multi-million dollar industry that has brought good income to the local economy and farmers. The total export of Chinese black truffles was over 800 tonnes worth over US$ 20 million in 2006. Unfortunately, forests in which truffles grow naturally have been damaged from the lack of regulation of harvesting. Until now the total production of Chinese black truffles is still increasing because new regions of production are continually being found and exploited. However, local production is declining sharply due to destructive harvesting methods. Careful management, regulation and conservation of truffles in China are urgently needed. The first truffle plantation was established in Taiwan in 1989 and produced truffles in 1996. Two other truffle plantations were established in Hunan and Guizhou Province recently but have not produced yet. The production of truffle-infected trees and establishment of truffle plantations are underway and have considerable potential in China. China is still well behind the Western world in terms of both research on and cultivation of truffles.

TRUFFLES AND TRUFFLE CULTIVATION IN NORTH AMERICA

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KEY WORDS: Oregon truffles, *Tuber gibbosum*, *T. oregonenis*, *Leucangium carthusianum*, *T. melanosporum*, Cultivation.

North America is home to considerable diversity of hypogeous ascomycetes, including three species celebrated in gastronomy. These include *Tuber oregonense*, the Oregon winter white truffle, *T. gibbosum*, the Oregon spring white truffle, and *Leucangium carthusianum*, known locally as the Oregon black truffle. Other common species have culinary potential, most notably *T. lyonii*, the pecan truffle. New species of truffles continue to be discovered in North America, such as the recently described black truffle from Mexico, *T. regimontanum*, which resembles *T. melanosporum* and *T. indicum*. In the genus *Tuber* alone, it is estimated that over twenty North American species still await description. The geographic range of the gastronomically important Oregon truffles largely corresponds with that of their principal host, *Pseudotsuga menziesii* var. *menzisii*, paralleling the Pacific Ocean from San Francisco, California to Vancouver, British Columbia. Unlike other ectomycorrhizal mushrooms commercially harvested throughout the region, the primary habitat of these three truffle species is typically near populated areas. While they do exist in natural forests, they are most abundant in afforested *P. menzisii* var *menziesii* plantations 15 to 30 years old established on former pasture or farm land. As early successional colonizers of anthropogenic habitats, these species would appear to be good candidates for agricultural domestication, although to-date, there have been no serious attempts. This is due, in part, to the fact that the Oregon truffles are harvested by raking, which produces predominantly immature truffles that lack aroma and have little culinary or commercial value. However, like other truffle species, when allowed to mature, the Oregon truffles develop powerful, attractive aromas. A switch from rakes to trained dogs may therefore significantly improve the general perception of Oregon's native truffles. The first *T. melanosporum* fruiting outside of Europe occurred in 1991 in California, but despite nearly continuous orchard establishment across the U.S. since that time, fewer than ten of these orchards have begun production. Nevertheless, orchard establishment has increased rapidly with the addition of a second seedling producer in 2001, and a current total of seven North American truffle tree nurseries. However, annual *T. melanosporum* yields to-date likely remain below 40 kg, most of which is from one young orchard in Tennessee and the original orchard in California. Success is likely to improve with recent increases in English language truffle cultivation literature, and with educational workshops offered through the Oregon Truffle Festival. Other European truffle species introduced in North America include *T. aestivum*, *T. borchii* and *T. magnatum*, although none have fruited to-date.

TRUFFLES AND TRUFFLE CULTIVATION IN EUROPE

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KEY WORDS: Europe, truffle, *T. melanosporum*, *T. uncinatum*, cultivation.

In Europe the cultivation of *Tuber melanosporum* ("Perigord truffle", "truffle of Norcia and Spoleto") is now limited to the 50th parallel in the North (Verdun area in France) and to Spain and Italy in the South.

T. uncinatum (syn. *T. aestivum*?) ("truffle of Burgundy – Lorraine – Champagne – French-County", "truffle of Fragno") has an European geographic distribution. It is probably native in the 27 countries of Europe, even in Finland (Aland island). Outside of Europe it is native in Turkey and in Morocco. Its wide distribution is due to several climatic factors (it is very much less thermophilous than *T. melanosporum* but more water requiring) and edaphic ones (it endures very variable texture soils, from sandy to very clayey, little calcareous or even not calcareous, organic matter rich. Thanks to the mycorrhizal seedlings and eventually to the provision of calcareous enrichment, the area of production of *T. melanosporum* was considerably enlarged: Australia, Israel, New-Zealand, U.S.A. and recently Morocco.

In Europe the potential area of cultivation of *T. uncinatum* is very important. The number of host-trees is high. Apart the oaks and the common hazels, Turkish hazel (*Corylus colurna*), common (*Carpinus betulus*) and -hop (*Ostrya carpinifolia*) hornbeams, Austrian black pine (*Pinus nigra austriaca*), Atlas cedar (*Cedrus atlantica*) have given good results in France. In Europe many soils are favourable without modification or after calcareous enrichment. The most important limiting factor is the climate (cold).

The cultivation of *T. uncinatum* has been introduced in Hungary, New-Zealand, Sweden. Experimentations are being carried out in Germany, Austria, Great-Britain, Finland, Slovakia, Slovenia, Serbia, Switzerland, U.S.A. The cultivation of *T. uncinatum* is different from the one of *T. melanosporum*. The keypoints are: high density plantation (1000 plants/ha); minimal pruning of the aerial part; fast creation of a shaded environment, eventually plantation of precious deciduous trees (wildservice trees, wildcherry trees, service trees) between the truffle mycorrhizal trees or of endomycorrhizal shrubs. As for *T. melanosporum*, it is necessary to uncompact the soil and to carry out a "root pruning" but at a different period of the year. Irrigation is still more important than for *T. melanosporum*: frequency and importance of the irrigations have to be improved.

GENOMIC AND CELL BIOLOGY SESSION
25TH NOVEMBER 2008

THE GENOME OF THE PERIGORD TRUFFLE (*TUBER MELANOSPORUM*) REVEALS EVOLUTIONARY INSIGHTS INTO MYCORRHIZAL SYMBIOSIS

F. Martin & Tuber *Genome Consortium*

UMR Interactions Arbres/Microorganismes, INRA-Nancy, France

The Perigord truffle (*Tuber melanosporum* Vittad.) is a 'national, cult-food', one of the worldwide recognized icone of the European gastronomy and culture, for which genomic information could act as a knowledge platform to improve its production and environmental persistence. The fruiting body of *T. melanosporum* is an edible truffle, which is a highly appreciated delicacy for its delicate taste and perfumes. There is a pressing need to develop a thorough understanding of *Tuber* genetics so that new controlled mycorrhization procedures can be established and a better understanding of the population biology of this species acquired. Here, I will present the main features of the genome sequence from *T. melanosporum* and I will highlight gene sets involved in symbiosis and fruit body formation. This 120-million-base genome assembly contains ~10,000 predicted protein-encoding genes, only 50% showing significant similarity to sequences in protein databases, particularly those from other Ascomycetes. We detected unexpected genomic features most notably a very large number of transposons and repeated sequences, and a restricted number of gene families. In contrast to the ectomycorrhizal basidiomycete *L. bicolor*, the evolution toward mycorrhizal symbiosis in *T. melanosporum* led to the loss of gene families as it is often observed in obligatory symbiosis. Given the ability of *T. melanosporum* to efficiently colonize root tissues and the long standing belief that its fruiting body is able to survive without plant links, this truffle unexpectedly showed a very limited repertoire of hydrolytic enzymes involved in degradation of plant cell wall polysaccharides. Thus, *T. melanosporum* seems to be poorly adapted for efficient degradation of carbon-rich lignocellulose, which may reflect a reliance on host-supplied sucrose. The mating type genes have been identified, confirming that *T. melanosporum* is heterothallic. The analysis of these genes will allow a better understanding of the *T. melanosporum* lifecycle and the formation of ascocarps. A genome survey identified a large set of polymorphic microsatellite repeats providing new molecular markers to analyse the natural and inoculated populations. In addition, these polymorphic molecular markers will contribute to the development of forensics to control serious frauds caused by less valuable truffles.

The predicted gene inventory of *T. melanosporum* genome, therefore, points to previously unknown mechanisms of symbiosis operating in biotrophic mycorrhizal fungi.

Acknowledgements. We would like to thank the Génoscope teams for their outstanding efforts in sequencing, assembling and annotating the T. melanosporum *genome. We would also like to acknowledge the members of the Tuber Genome Consortium for their dedication.*

AN INTEGRATED CELL AND MOLECULAR VIEW POINT OF TRUFFLE BIOLOGY

Raffaella Balestrini*, Elisa Zampieri, Simona Abbà, Antonella Faccio, Robert Roberson [1], Paola Bonfante*

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KEY WORDS: genomics and cell biology.

Specific experiments aimed to assign a biological function to the DNA sequences found in the genome are crucial in a post-annotation phase. In order to archive this aim, mRNAs and gene products localization can be very informative and can confirm the putative function deduced by bioinformatics tools. In the past we have used several cell biology approaches, i.e. *in situ* hybridization and immunolabelling (immunofluorescence and immunogold) on truffles. These approaches have already permitted the localization of transcripts and several proteins in *Tuber borchii* hyphae during various step of its life cycle. Having the full genome, we suggest that these approaches would be useful to identify the specific roles for genes belonging to a complex gene family. The use of antibodies and the localization of antigens may surely suggest functions when other approaches (i.e. transformants, RNA interference, etc...) cannot be faced. LMD (Laser MicroDissection) is a powerful tool for isolating specific tissues and cell types from sectioned biological specimens, allowing a cell specific extraction of RNA, DNA, or proteins. Preliminary experiments performed on ectomycorrhizae will be presented. We propose the LMD technology to identify genes specifically activated in each of the two fungal compartments (mantle and Hartig net) forming an ectomycorhiza. We have already been applied this technique for gene expression studies in arbuscular mycorrhizae, where the cell specificity for plant and fungal genes involved in phosphate transport has been successfully demonstrated (Balestrini *et al.*, 2007 – MPMI 20: 1055-1062). In addition, an accurate analysis at transmission electron microscopy could be also considered a valid support to integrate the molecular analyses, providing information about the presence of specific organelles/structures in fungal hyphae. We are using plunge-freezing procedure followed by freeze substitution to examine and describe the hyphal tip in *Tuber* species. These observations, together with the sequences annotated in the genome and the results derived from the other cell and molecular approaches, could highlight the mechanism at the basis of the hyphal growth in this fungus.

NEW INSIGHTS INTO TRUFFLE LIFE CYCLE AND REPRODUCTIVE MODE: FROM BASIC RESEARCH TO THE FIELD

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KEY WORDS: *Tuber*, life cycle, SSR markers, SNP, reproductive mode, cell biology.

The difficulties to grow and impossibility to mate *Tuber* spp. under controlled conditions have prevented researchers to gain direct insight into the reproductive mode of these fungi. Until now, however, the dogma was that these ascomycetes strict self because co-dominant markers never detected heterozygotic profiles in the putative diploid/dikaryiotc hyphae of ascocarps and mycorrhizas. Here we tested the hypothesis that truffles outcross and that their life cycle is prevalently haploid.

To this end, highly informative, codominant molecular markers such as SSR (Simple Sequence Repeat) and ITS/SNP (Internal Transcribed Spacer, Single Nucleotide Polymorphism) were used to genotype separately the DNA specifically contributed by the gleba and by the spores within each given *Tuber magnatum* and *T. melanosporum* ascocarps and to fingerprint single apical root-tips resulting from controlled inoculation of host plants with genotyped fruit bodies. These approaches led us to prove that the majority of the asci examined showed additional SSR and/or ITS/SNP alleles to those present in the surrounding gleba and that ectomycorrhizas always displayed only a single SSR or ITS/SNP allele. We interpreted the presence of additional alleles in the asci as a result of a mating event between two genetically distinctive truffle strains. More specifically, we envision that *Tuber* fruit bodies result from a mating event between two haploid mycelia and the fertilization step precedes and promotes the maternally-driven development of the gleba.

In conclusion, our data unequivocally show that *T. magnatum* and *T. melanosporum* can outcross, that their life cycle is prevalently haploid, and that *Tuber* ectomycorrhizas are haploid structures. Now we aim to verify whether these fungi are obligate or facultative outcrossing spp.

This study substantially deepens our understanding of the biology of *Tuber* spp. and calls for new cultivation strategies aimed at promoting the mating between strains to optimize truffle production.

TRACKING THE ORIGIN OF *TUBER MAGNATUM* AND *T. MELANOSPORUM* TRUFFLES BY MOLECULAR MARKERS

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KEY WORDS: microsatellites, AFLP, ITS, population biology, phylogeographic structure.

The quality and market price of truffles depend upon the species and, traditionally, the place of origin. The premium species *Tuber magnatum* Pico and *T. melanosporum* Vittad have restricted geographic distribution and show an apparent low level of intraspecific genetic polymorphism. Likely, these features stem from a population bottleneck suffered by these two species during the last glacial age. Consequently, it has been proposed that the different phenotypic and organoleptic traits exhibited by truffles of different origin were exclusively due to environmental factors. To test whether or not natural populations of *T. magnatum* and *T. melanosporum* were indeed genetically undifferentiated, we used highly informative molecular markers to genotype *T. melanosporum* and *T. magnatum* truffles that were harvested throughout the species distributional range. Polymorphic microsatellites (Simple Sequence Repeats – SSR) were used to evaluate 316 *T. magnatum* specimens grouped into 26 populations, whereas SSR, ITS-SNP (Internal Transcribed Spacer, Single Nucleotide Polymorphism) and AFLP (Amplified Fragment Length Polymorphism) markers were employed to fingerprinting 206 *T. melanosporum* specimens, grouped into 13 geographical populations. SSR data clearly showed that *T. magnatum* populations are both genetically and phylogeographically structured, with the southernmost and the north-westernmost populations significantly differentiated from all others. Our SSR data suggests that the *T. magnatum* postglacial expansion patterns moved from Central Italy both northward and southward. Likewise, also *T. melanosporum* populations turned out to be genetically structured, with the southernmost populations showing the highest values of allelic richness. We provide the first genetic evidence in support of the hypothesis that the potential *T. melanosporum* refugia were located in the Italian and Spanish peninsulas and after the glaciation the species range expanded northward. Thus, the presence of a genetic structure in *T. magnatum* and *T. melanosporum* will allow us to track truffle populations according to their geographic origin. Our findings are of major relevance for marketing strategies and to preserve the ecological biodiversity within naturally productive truffle areas.

THE MICROBIAL COMPLEXITY OF *TUBER MELANOSPORUM* TRUFFLEGROUNDS

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KEY WORDS: ecology and population biology, environmental genomics, truffles.

Over the last years environmental genomics has been developed as an effective tool for the discovery of microbial communities, and for a better understanding of their interactions in complex habitats, like soil.
We applied such approaches to characterize the microbial populations occurring in the rhizosphere of plants mycorrhized by *Tuber melanosporum*.
In particular, we refer to a peculiar phenomenon that is closely related to this truffle species, and is called *brulé* (burnt area). It consists in a zone around or near the host mycorrhizal tree, where plant cover is very scarce and inside which truffles are usually collected. The reasons of such a phenomenon are still unclear, likely involving phytotoxic effects of truffle's metabolites, direct competition for nutrients and water, and parasitism on the roots of the herbaceous plants.
We hypothesized an active direct or indirect role of *T. melanosporum* on the microbial populations and a relationship between the scanty plant cover and changes in biodiversity of microbial communities: with this goal, we compared fungal and bacterial microorganisms living inside and outside the burnt area.
Soil samples were collected at Cahors, France, in natural and inoculated *T. melanosporum* trufflegrounds showing the peculiar burnt areas. Denaturing gradient gel electrophoresis (DGGE) was used to produce ribosomal rDNA fingerprintings of the fungal and bacterial organisms. As a complementary approach, some samples were also processed by molecular cloning of the PCR products in order to compare the efficiency of the two techniques and to identify the fungal taxa.
DGGE and cloning results showed the same dynamics in fungal populations: increase of *T. melanosporum,* reduction of ectomycorrhizal Basidiomycota and increase of saprotrophic Zygomycota within the burnt area. From molecular cloning, AM fungi seemed not to be affected by the presence of *T. melanosporum* fruitbodies. At the mean time, bacterial DGGE showed that the principal populations remained unchanged inside and outside the burnt area. Taken in their whole, the results suggest that *T. melanosporum* may act as a powerful driver of microbial biodiversity in a truffe ground.

IDENTIFICATION OF MICROSATELLITES IN THE *TUBER MELANOSPORUM* VITTAD. GENOME

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KEY WORDS: Ecology and population biology, genome, *Tuber melanosporum*, microsatellites.

Since several years, we investigated the genetic diversity of *T. melanosporum* by sequencing some genomic regions (ITS, SCARs), analyzing few microsatellites (SSR) or applying multi-loci approaches (RAPD, AFLP). However, due to the apparent low level of *T. melanosporum* genetic diversity it was difficult to isolate polymorphic markers and all the analysis have been realized with a limited number of markers. Thanks to the genoscope and the *Tuber* Consortium, the genome of *T. melanosporum* is now available. A first screening indicated that a large number of repeat sequences, such as microsatellites, are presents in this genome. The aim of this study was (1) to identify the SSRs in the first *T. melanosporum* assembly and (2) to characterize the local populations of this truffle using these polymorphic markers.
In the *T. melanosporum* genome 22,425 SSRs (mono-, di-, tri-, tetra-, penta- hexa-nucleotides) have been identified. A subset of around 200 SSRs (di-, tri-, tetra-, penta- hexa-nucleotides) was selected for further investigations. The first results suggested that at least 50% of the SSRs tested were polymorphs with a high numbers of alleles for some of them. This important level of genetic diversity was unexpected and is in contradiction with previous analyses. On the other hand these polymorphic SSRs have been analyzed for a large number of *T. melanosporum* ascocarps coming from Italy, France and Spain. This analysis allowed us to characterize the genetic structure and the gene flow through the natural populations of this species.

We would like to thank the sequencing team of the genoscope and all colleagues from Italy, France and Spain that provided us *T. melanosporum* ascocarps.

INFLUENCE OF HOST PLANT AND DIFFERENT SUPPLIES ON MORPHOLOGY AND GENE EXPRESSION IN *TUBER BORCHII*

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KEY WORDS: genetic and cell biology, hyphal growth, carbohydrates.

During the different phases of the ectomycorrhizal fungi life cycle, marked morpho-functional, metabolic and gene expression modifications of the two symbionts occur. The complex molecular and biochemical processes involved in the different phases of the ectomycorrhizal fungi life cycle are influenced by several environmental factors. For example root exudates (which also contain some sugars) produce molecular signals that induce profound morphogenetic changes in fungal mycelium.

Since little is known about the molecular basis of morphological changes taking place in *Tuber*, our research aimed to broaden understanding of these complex processes. In particular *T. borchii* mycelial cultures grown in glucose, maltose and sucrose were investigated by carrying out morphological and molecular analyses, with the aim to identify possible modifications related to the carbohydrate substrates used in the culture media.

Morphological analyses, performed using a set of parameters (hyphal growing index, angle hyphal branching, distance between septa, hyphal diameter), highlighted an increased growth of *Tuber* mycelia in the presence of glucose, while the hyphae appeared thinner and less branched in the presence of sucrose and maltose. These data were confirmed by gene expression analyses, performed by macroarray technique and real time RT PCR experiments, which highlighted an up-regulation of the genes involved in apical growth and general metabolism. Furthermore, since glucose is also present in root plant exudates, we may speculate that it acts as a signal for the fungus to indicate the presence of the plant, and to induce the complex symbiotic process. These mechanisms can lead to morphological modifications, among which an increase branching, necessary for the fungus to get closer to the plant roots and to establish a contact.

In conclusion these studies represent a starting point for the identification and characterization of genes and proteins involved in morphological, genetic and metabolic changes that occur in *Tuber* life cycle. In addition, the knowledge of the molecular mechanisms underlying the morphogenesis may provide the tools to develop new biotechnological methodologies in forestry and ecology, in order to optimize the *in vivo* mycorrhization process with precious truffles, such as *Tuber magnatum*, the most prized among truffles, which cultivation till present numerous difficulties.

USE OF β-TUBULINE AS A DNA BARCODE TO IDENTIFY *TUBER* SP.

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KEY WORDS: *Tuber* genus, β-tubulin, specific primers, *T. magnatum*, nested PCR, soil, barcode, ecology and population biology.

The DNA barcoding is a technique that uses short DNA sequences from a standardized region of the genome as a diagnostic "biomarker" for species identification. The Internal Transcribed Spacer of the rDNA (ITS) is the most exploited genomic region to design specific primers/oligonucleotide barcodes when a single truffle species has to be detected. By contrast, primers which amplify exclusively all the *Tuber* species are not yet available. Since β-tubulin gene has been employed as an interspecific marker in different phylogenetic *Tuber* analyses, we wanted to check the efficiency of the β-tubulin gene for a quick and reliable identification of the *Tuber* genus.
To reach the aim, we designed two new primers, which amplified genomic DNA from 14 *Tuber* species and not that from fungi, which were phylogenetically related and not to *Tuber*. The primers were successfully used on soil DNA and mycorrhizas. As a second step we wanted to check the gene diagnostic power to amplify *Tuber magnatum* directly in the soil. A new forward primer was designed to amplify only DNA from *T. magnatum* and a nested protocol was then set up. Using serial dilution of a *T. magnatum* genomic DNA, we evaluated that 2-4 fg can be amplified with this approach. This result allowed us to follow the distribution of *T. magnatum* in the soil of a selected truffle-ground. Ten soil samples out of 13 gave a positive signal which sequence confirmed belonging to the expected fungus.
In conclusion, this is the first report of primers which are specific to *Tuber* genus. On the other hand, we set up a powerful protocol to detect 2-4 fg of *T. magnatum* DNA and we monitored its distribution in a truffle-ground. The β-tubulin gene is therefore a good candidate to be used as a DNA barcode for truffles.

GENE EXPRESSION DURING *TUBER BORCHII* FRUIT BODY MATURATION

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KEY WORDS: Genomic and cell biology, ectomycorrhizal fungi, *Tuber borchii*, fruit body maturation, gene expression analysis.

Truffles are hypogeous ectomycorrhizal fungi belonging to the Ascomycetes, within the species of the genera *Tuber* which have a great economical importance for the organoleptic properties of their fruit bodies. Morphological descriptions of truffle ascomata development are limited due to their hypogeous habitat: in fact only the mature ascomata produce the typical aroma recognized by trained dogs while the collection of immature truffles is fortuitous. Some species of truffle, including *Tuber borchii*, can be grown in pure cultures even if they grow slowly [1]. Symbiotic relationships with specific host plants are required for the development of the truffle fruit bodies, which cannot be produced "in vitro". Furthermore, the formation of truffle fruit body depends on nutritional and environmental status. Only some biochemical and molecular studies regarding *T. borchii* fruit body development and maturation have been published [2-6] suggesting that amino acid and isoprenoid biosynthesis, the glyoxylate cycle pathway, cell wall synthesis and mitochondrial division are regulated during fruit body morphogenesis. Despite this, our understanding on the molecular mechanisms leading to differentiation and ascospore production remain elusive.
We reported the characterization of the *T. borchii Tbz1*, a single copy gene in *T. borchii* genome, which is expressed in the early stage of ascomata maturation and absent in the symbiotic tissue. The deduced protein sequence of *Tbz1* has transmembrane helices and a signal peptide indicating a membrane localization of this protein. The homologous genes in *Tuber puberulum* (*Tpz1*), *Tuber aestivum* (*Taz1*) and *Tuber brumale* (*Tbrz1*) have been also identified. Sequence analysis showed that these genes are identical which strongly suggests that the function of the coded protein has been conserved during the evolution of the truffles.

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A FUNGAL GENE POTENTIALLY SPECIFIC TO MYCORRHIZA ESTABLISHMENT BETWEEN *TERFEZIA B0UDIERI* ISOLATES AND *CISTUS INCANUS* HAIRY ROOTS

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KEY WORDS: Cell biology; Mycorrhiza specific genes.

Four fungus-hairy root clone combinations were cultivated under two sets of conditions, in which the root and the fungus were separated by a cellophane sheet or were allowed physical contact. One of the combinations produced endomycorrhizas, the other three solely ectomycorrhizas. Fragments isolated by cDNA-AFLP analysis from cellophane-separated cultures (pre-infection) were used to identify differentially expressed genes by reverse Northern analysis.

Genes showing no homology to known sequences constituted the largest group under both growth conditions. Some fungal genes were expressed transiently, while others exhibited altered expression patterns as conditions changed from individually growing through the pre-infection stage to mycorrhizas.

Genes expressed exclusively under combinations allowing either ectomycorrhiza or endomycorrhiza under a particular condition were detected. Four of these were further analyzed. The four showed, initially, no homology to known sequences. After extension to full length – GenBank matches were found for three.

The fourth, expressed under pre-infection conditions, was a short peptide with no match in the databases. In the isolate incapable of forming endomycorrhiza under our study conditions this peptide was two amino acids shorter than in the isolate capable of such associations.

The significance of this finding will be discussed.

ECOLOGY AND POPULATION BIOLOGY SESSION
26TH NOVEMBER 2008

SOILS SUITABLE FOR THE WHITE TRUFFLE

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KEY WORD: cultivation.

Soil suitability evaluation for truffle species is aimed at recognizing places where they reproduce on the basis of two essential conditions: the presence of a host plant and a suitable soil environment. Truffle species propagate whenever they meet a host, but they will produce truffles if and only if the right soil environment is present. In the case of *T. magnatum*, right conditions are met in well-drained soils that have a moderately alkaline pH, a high amount of calcium carbonate – its fine-sized fraction, in particular – and enough water all over the year. Further compulsory characteristics are the lack of water stagnation in the system, a very soft consistence, and more than 20% of the soil volume occupied by interconnected macropores. Taken individually, these conditions are present in a large range of soil environments, but it is the way they combine to make a soil suitable for *T. magnatum*. From the pedogeographical point of view, the right combination is in fact fulfilled only in valley bottoms and hill slopes where geomorphological processes rejuvenate soils, originating a disordered distribution of particles due to: i) frequent depositions of alluvial and colluvial materials originated by fluvial dynamics; or, ii) accumulation of soil aggregates transported along slopes by mass movement. These requirements are so specific that limited changes in neighbouring soils influenced by the same soil forming processes drastically decrease white truffle production. The knowledge gained in soils for white truffle is essential to delineate and manage areas for *T. magnatum* cultivation. Well-trained pedologists can help truffle growers in selecting the more suitable areas for white truffle. Even a geostatistical approach combined with soil survey has been successfully tested to determine the productive potential of valley bottoms. Unfortunately, we are now in the paradoxical situation of knowing where to place truffle orchards without having seedlings reliably mycorrhized with *T. magnatum*: it worth noting that the few truffle orchards which have come into production are located in areas where environmental conditions suitable for white truffle – high shadowing, soft and drained soil, etc. – are fulfilled. Waiting for more effective nursery mycorrhization, white truffle production can be improved only in the wild, adopting forestry practices capable to preserve the environmental conditions suitable for *T. magnatum* growth. Trickier are soil tillage practices. In the long run, they could be counter-productive, increasing rather than decreasing soil compaction.

SOILS SUITABLE FOR THE BLACK TRUFFLES – A CONTRIBUTION TO THE CHARACTERIZATION OF THE SOILS SUITABLE FOR *TUBER MELANOSPORUM* AND *TUBER AESTIVUM* GROWTH THROUGH MINERALOGICAL ANALYSIS OF THE CLAY FRACTION

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KEY WORDS: soil, truffle ecology, clay mineralogy.

A mineralogical analysis of the clay fraction of 30 and 26 soil samples coming respectively from epipedons of natural truffle beds of *Tuber melanosporum* and *Tuber aestivum* was carried out. The aim was to investigate on possible relationship between the type of mineralogical clay and the granulometric clay content. The results of X-ray diffraction patterns indicate that the main types of mineralogical clays found in soils suitable for *Tuber melanosporum* and *Tuber aestivum* growth are represented by kaolinite, illite-mica minerals, smectites, vermiculites and hydroxyl interlayered vermiculite (HIV). The results evidence that generally kaolinite and smectites are mutually exclusive and kaolinite is associated with HIV. Since the various families of mineralogical clays presents different physical and chemical properties we have correlated the mineralogical analysis of the clay fraction with the percentage of granulometric clay of the fine earth (<2 mm fraction) taking also into account the bulk presence of rock fragments. This comparison indicates that, in the case of *Tuber melanosporum*, expansible clays (smectites) are dominant when the granulometric clay content does not exceed 30% in the fine earth and rock fragments are abundant. If the fine earth represents the whole bulk of soil, the prevalence of smectites is associated with low or very low content of granulometric clay. On the contrary, the dominance of non dynamic clays, kaolinite plus HIV and illite corresponds to high content of granulometric clay (>30%) with the rocks fragments resulting from frequent to abundant. With regard to the soils of *Tuber aestivum* the prevalence of smectites can be associated with a decidedly clayey texture (granulometric clay content >40%) even if the dominance of kaolinite corresponds usually to very high content of clay (50-85%). In summary we can affirm that for both *Tuber melanosporum* and *Tuber aestivum* there is a common trend which combines the increase of granulometric clay content with the prevalence of kaolinite plus HIV on the smectites, but that the percentage of granulometric clay, in correspondence of which this change of the type of mineralogical clay occur, is lower in the soils of *Tuber melanosporum* with reference to ones of *Tuber aestivum*. The results of the mineralogical analysis of the clay fraction confirm so the field observations, where the presence of *Tuber aestivum* is usually associated with soil characteristics indicating physical dynamicity. This is evidenced by tendency of the soil to hardening and compaction due to drying, by great plasticity in moist state and by the prevalence of medium blocky structures with respect to granular and crumby ones.

STUDY OF THE ABOVE AND BELOW GROUND ECOLOGY (VASCULAR AND MYCORRHIZAL FLORA) IN TRUFFLE BRÛLES IN NAVARRA

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KEY WORDS: truffle ecology, *brûle*, truffiére, ectomycorrhiza, vascular plants.

In spite of the continuous advances in trufficulture there are still many questions about truffle growing, hence the big importance of studing the characteristics of the environment in which it develops best.

In this study we have analysed the main ecological characteristics of the vascular flora growing in 24 holm oak *brûles.* This flora is special, very influenced by the alelopatic substances produced by the micelium of the fungus, modifying the composition and the characteristics of the flora. As a result, plants grow smaller and with a lower covering. Sometimes they even present changes in their life-cycle. Therophytes typical of disturbed areas are dominant in plantations. Xerophile plants are also common. Besides, the species found in truffiers are proper of shaken, removed and generally sunny, dry and stony places, and many of them weeds. This corroborates the fact that the brûle is a very disturbed environment, both by the micelium of the fungus and by the human interventions.

On the other hand, monitoring the mycorrhizae is very important in truffle growing since it allows us to know if the plantations are developing well or if truffle mycorrhizae have been replaced by other more competitive fungi. That is why we have carried out a below-ground study of the mycorrhizae appearing in the roots of the same holm-oaks, to check the presence of truffle mycorrhizae and to know which other mycorrhizal fungi are colonizing the roots.

ECTOMYCORRHIZAL ASSOCIATIONS OF PECAN TREES *CARYA ILLINOINENSIS* GROWN UNDER MONOCULTURE AND NATURALIZED BY TRUFFLES (*TUBER LYONII*)

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KEY WORDS: truffle ecology, mycorrhizal communities, *Tuber lyonii*.

Pecan trees *Carya illinoinensis* are an economically important nut tree in the southeastern US. They are cultivated in monocrop and Georgia is the largest producer of pecans with approximately 150,000 acres in production. In the mid-1980's, the spiney-spored truffle species *Tuber lyonii* (=*T. texense*) was found fruiting abundantly across many pecan orchards in southern Georgia, USA. In North America, *Tuber lyonii* also naturalizes in black truffle orchards that have been established with *Tuber melanosporum* on *Corylus* and *Quercus* hosts. *Tuber lyonii* has a broad geographic distribution (relative to other *Tuber* species) that ranges from Quebec, Canada to Victoria, Mexico. Although not as pungent in aroma as the prized European *Tuber* species, a commercial market has developed around *Tuber lyonii* in North America. In recent years, fruiting levels at formly productive sites in Georgia have declined, even where they were not actively harvested. The goal of this study was to determine whether *T. lyonii* mycorrhizae were still present in pecan orchards that have been known to produce truffles, and to describe the mycorrhizal communities associated with *Carya illinoinensis* in both truffle producing and non-producing orchards. We sampled ten trees at five sites (50 trees total) and from each tree three individual ectomycorrhizae were sequenced (ITS-LSU). We found that the dominant members of the mycorrhizal community were hypogeous taxa (e.g. *Tuber, Scleroderma, Hymenogaster*). *Tuber lyonii* (17%) and an undescribed species of *Tuber* (~20%) were the most recovered ectomycorrhizal species, and were found in both producing and non-producing sites. In this talk, the diversity of spiney-spored *Tuber* (Rufum clade) in North America will also be highlighted.

HYPOGEOUS FUNGI IN THE ANTHROPOGENIC SITES IN POLAND

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KEY WORDS: ecology and population biology, *Tuber*, human made habitat.

Taxonomy, ecology and chorology of hypogeous fungi are a matter of increasing interest in Poland. Extensive observations of hypogeous fungi in field conditions aiming in recognition of their environmental requirements are carried out by professional mycologists, amatours and recently also commercial producers of edible fungi. Special attention is paid to truffles because of their culinary and commercial value.

The following species of *Tuber* have been confirmed to occur in Poland so far: *T. aestivum, T. mesentericum, T. nitidum, T. rufum, T. dryophilum, T. puberulum, T. borchii, T. rapeodorum, T. maculatum.* It is significant that two species of edible truflles (*T. aestivum* and *T. mesentericum*) were found in anthropogenic sites: in oak forest planted 40 years ago on communal pasture and on tourist trail in oak-horn beam forest, which is continuously used over the whole year.

Mycological study conducted in the area of Łódź city indicated that urban habitats are also suitable for several species of hypogeous fungi. The examples will be given in the presentation.

The study was supported by the Polish State Committee for Scientific Research no. 3 PO4G07426.

ECOLOGICAL CHARACTERIZATION OF NATURAL SITES WITH *TUBER MELANOSPORUM* VITTAD. AND *TUBER MAGNATUM* PICO IN THE ABRUZZO REGION (CENTRAL ITALY)

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KEY WORDS: *Tuber melanosporum, Tuber magnatum,* Truffle, Bioclimate, Soil, Vegetation.

The results of a study carried out between 2006 and 2008 dealing with the ecological characterization of natural sites with *Tuber melanosporum* e *Tuber magnatum* are presented. The study consisted of three different analysis: bioclimatic, pedological and vegetational.
The bioclimatic analysys highlights a relatively wide ecological value of the two species, which were found in several bioclimatic horizons. However, the values of the Rivas-Martinez Thermicity and Mitrakos Montly Cold Stress indices show *T. melanosporum* to be mostly correlated with the Lower Supratemperate horizon, and *T. magnatum* with the Lower Mesoteperate horizon. The *Tuber magnatum* sites are characterized by more abundant summer precipitations and a shorter dry period; this is highlighted by the vaules of the Mitrakos Montly Dry Stress index.
The pedological analysis shows a good separation between the two truffles sites. Texiture, organic matter and total carbonates resulted to be the best parameters to describe the different ecological requirements by the two species: *T. melanosporum* requires more sand, organic matter and total carbonates (and also scheleton); *T. magnatum* more silt and clay.
The phytosociological analysis of vegetation highlights the clear cenological autonomy of the vegetations related to the two truffle species. Dry open *Quercus pubescens* formations referred to *Roso sempervirentis-Quercetum pubescentis* and *Cytiso sessilifolii-Quercetum pubescentis* associations are present in the sites of *T. melanosporum*. The vegetation of *T. magnatum* sites is highly heterogeneous, i.e. sub-mountain semi-mesophilous *Quercus pubescens woods, Ostrya carpinifolia* or *Quercus cerris* dominated mixed woods, *Corylus avellana, Pyrus pyraster, Populus tremula* or *Ulmus minor* pre-woods, igrophilous *Populus alba* or *Populus canadensis* woods. Its phytosociological placement is consequently various. In all cases it reguards fresh formations on deep soils, as underlined by the constant presence of mesophilous and meso-igrophilous species characterizing the *Erythronio dentis-canis-Carpinion betuli* alliance and the *Fagetalia sylvaticae* order; in several cases, also the igrophilous species of the *Salici purpureae-Populetea nigrae* class were found to be abundant.

Note: Research carried out within the "Carta della vocazionalità tartuficola della Regione Abruzzo" project, co-financied by Regione Abruzzo with the three-yearly 2001-2003 and 2004-2006 "Interventi di forestazione e valorizzazione ambientale" Programs and by ARSSA with the 2001-2002-2004-2005 Technic Assistance Programs.

STUDIES ON *TUBER MACROSPORUM* NATURAL TRUFFLE HABITATS IN THE CARPATHO-PANNON REGION

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KEY WORDS: ecology, *Tuber macrosporum*, soil characteristics, phytocoenology.

Literature considers *Tuber macrosporum* as a truffle distributed between 41° and 51° latitude north in Europe on soils generally limy and heavy in habitats similar to *Tuber magnatum* concerning pedoclimatic characteristics.
In Central Europe it is a common species reported from Czech Republic, Hungary, Romania, Serbia and Ukraine.
This species is mainly occurs on water affected river banks, temporarily flooded river valleys or in deep and shaded valleys.
During our study soil samples were collected in truffle beds and detailed analysis was carried out. Phytoindication method of Borhidi was used for habitat characterization.
Studies carried out on natural habitats of Hungary and Transylvania (Romania) revealed that *Tuber macrosporum* soils are heavy, clayey and loamy, from lightly acidic to lightly basic concerning pH with variable lime content and high humus level. Nitrogen, phosphorous and potassium content is also very variable from very low to very high.
Phytocoenology revealed majority of mesophyl and submontane broad-leaved mesophyl forests concerning temperature, semi-humid and intermediate moisture conditions with high plant cover with the majority of plants of neutral soils but tolerant to pH.
Coenological research resulted that from the 45 investigated habitats 23 belong to *Melampyro bihariensi-Carpinetum* association, 6 belong to *Carici pilosae-Carpinetum* association, and 5 to *Carici brizoidis-Quercetum* association.
Study was funded by project number OMFB-00938-43/2005.

LOCATION AND ECOLOGICAL CHARACTERIZATION OF NATURAL TRUFFLE GROUNDS IN THE REGION OF MURCIA (South East of SPAIN)

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KEY WORDS: ecology, vegetation, mycelium, *Tuber melanosporum*.

The Region of Murcia is located in the South East of the Iberian Peninsula, with a typical semi-arid Mediterranean climate that determinates the presence of a Termo- or Mesomediterranean vegetation. Only in the highest mountains, such as "Sierra Espuña" and "Revolcadores complex mountains", the precipitation reaches averages over 350 mm/year.

Both, the Regional Park of Sierra Espuña and Revolcadores mountains, are considered as ASCI (Areas of Special Conservation Interest) and are included in the Natura 2000 network. These areas present calcareous soils with Meso-humid, Supra- or even Oromediterranean vegetation. *Quercus ilex* subsp. *rotundifolia* natural woody formations predominate alone or mixed with *Pinus halepensis* plantations up to 800-900 m altitude or with *Pinus nigra* subsp. *salzmannii* from 900 to 1600 m at the sea level.

On the basis of the Spanish Forest Map and using the ARCMAP 9.2 and ARCVIEW-GIS 3.3 modelling and mapping software tools, we developed a cartography on the potential distribution of the black truffle (*Tuber melanosporum* Vittad.) in the Region of Murcia. By the help of trained truffle-hunter-dogs we located several ascomata of this appreciated truffle in three natural truffle grounds in Sierra Espuña Regional Park and one in Revolcadores mountains.

The flora and the mycobiota of the truffle grounds in Murcia were studied and soil analyses were carried out. The most significant results show that the vegetation coverage, sand percentage, organic matter and C/N relation are higher in these zones than in other truffle grounds or orchards from other parts of Spain, France or Italy. Smell and taste of Murcian black truffle ascomata also vary with respect to those from black truffles growing in other latitudes.

Molecular studies, based on the total soil DNA isolation followed by PCR amplification with *T. melanosporum*-specific primers are currently carried out to detect the mycelium of the black truffle in soil.

We can consider the Murcian populations of black truffle as ones of the most Southerly located in Spain, living at the ecological edge for this *Tuber* species. This, together with its phylogenetic importance, according to the glacial refugia hypothesis, make it necessary to develop a conservation program for the black truffle.

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ECOLOGICAL FEATURES OF *TUBER MAGNATUM* PICO IN THE CONDITIONS OF WEST BALKANS – SOIL CHARACTERIZATION

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KEY WORDS: truffle ecology, soil.

The precious white autumn truffle (often called Piedmont truffle), *Tuber magnatum* Pico has been reported from Serbia as late as 1998, much later then in Italy or Istria (Croatia). Since then it has been recognized as a significant economic potential, but there has been no detailed ecological analysis of its growing and fruiting characteristics in the conditions of Balkan Peninsula. Quite recently, this truffle was scientifically confirmed from Slovenia as well, and the ambitious project was settled between these two countries in order to describe *T. magnatum* habitats in the Balkans. As the simple habitat overview revealed significant differences between north Istrian and Karst (Slovenian) habitats and Serbian habitats, vegetation and soil analysis were set as the first goal of the project.

Here we report surprising differences between Slovenian (that can be assumed as Istrian due to geographical closeness) and Serbian soils and ecosystems that support white truffle production. Results clearly indicate wider ecological limits of T. magnatum then previously reported.

In addition, analysis of rDNA ITS sequences of samples from the experimental sites in both countries revealed 100% sequence identity, as well as high similarity to other sequences deposited in Gene Bank. Although the differentiation between Balkan and Istrian truffles could exist at the ecological (adaptation) level, at the level of commonly assumed eukaryotic genetic diversity (ITS regions) there were no differences. General discussion will be on soils supporting *T. magnatum* in West Balkans in comparison to Italian soils.

ECOLOGY OF TUBER MAGNATUM PICO IN THE HIGH VALLEY OF CHIASCIO (CENTRAL ITALY)

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KEY WORDS: *Tuber magnatum*, Ecology, Mycorrhizae, DNA.

Tuber magnatum Pico is the rarest and more expensive species belonging to the *Tuber* genus that fructifies in the central Italy and whose production is decreasing very fast.
Typical environments where it is common to collect white truffles are represented by valley areas characterized by particular pedoclimatic conditions as described by several authors.
In the "Alto Chiascio" area, white truffles are also harvested on hillsides and hilltops.
A complete ecological study on selected productive areas was performed. In particular, soil samples for chemical, physical and molecular analysis, root samples for mycorrhizal analysis were collected in sites where fruit bodies were harvested.
A morphological and molecular characterization of unknown ectomycorrhizae frequently found in fruit bodies collection sites was also carried out.
The results show the presence of *T. magnatum* in two plant associations: Turkey oak (*Quercus cerris*) woodland. Association: *Aceri obtusati-Quercetum cerris*, and Turkey oak (*Quercus cerris*) and hop-hombean (*Ostrya carpinifolia*) woodland. Association: *Aceri obtusati-Quercetum cerris* variant *Ostrya carpinifolia*; soils with homogeneous chemical and physical features composed by marl-sandstone formations; the absence of *T. magnatum* mycorrhizae; the precence of mycorrhizae that belong to other *Tuber* species such as *T. aestivum* Vittad., *T. macrosporum* Vittad, *T. brumale* Vittad, *T. borchii* Vittad. and *T. rufum* Vittad.
The molecular analysis of soil samples also show the presence of white truffle DNA. The molecular and morphological characterization of unknown mycorrhizae showed that they belong to the *Tomentella* genus.
In conclusion: soil represent the ecological limit for the production of fruit bodies of *T. magnatum*. White truffle DNA was found in soils even if they contained no trace of mycorrhizae of the species. Further researches are needed for a deeper characterization of the ecology and biology of this precious fungus, necessary to safeguard the natural growing environment and plan the future cultivation.

RELATIONSHIP BETWEEN THE MANAGEMENT OF FOREST VEGETATION AND THE PRODUCTION OF *TUBER AESTIVUM* VITTAD. IN A NATURAL TRUFFLE GROUND ON MONTE AMIATA (TUSCANY, ITALY): PRELIMINARY RESULTS

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KEY WORDS: ecology, silviculture, *Tuber aestivum*, *Quercus cerris*.

We report the preliminary results of an experimental project aimed at clarifying the relationship between tree and shrub cover and the production of *Tuber aestivum* fruit-bodies. The thinning carried out in a high forest of *Quercus cerris* in the municipality of Castell'Azzara (Grosseto) was preceded by classification of the area from the points of view of climate, pedology, forestry and vegetation, exploring the possibility of applying the BACI statistical method (Before-After-Control-Impact). Following this, 10 plots of a surface area of 1,000 m^2 each were selected, in half of which approximately 30% of the tree and shrub cover had been removed by thinning. In each plot a qualitative and quantitative survey of truffles was performed, using a specially designed data registration form. Collections were made every 10 days in the period indicated on the harvesting calendar. The fructification processes of *Tuber aestivum* were compared to the climatic and pedological data, shedding more light on the ecology of the species: in particular, it emerged that the production of fruit-bodies does not seem to be hampered by suboptimal climatic conditions, considering that the greatest productivity was recorded in 2007, a year in which temperatures were relatively high and rainfall low. An increase in both the number and weight of fruit-bodies was observed following thinning. Interestingly, fruit-bodies were also found in plots that had been recorded as unproductive at the beginning of the project. Although preliminary, this result could be connected to the silvicultural operations carried out.

THE SWEDISH POPULATIONS OF *TUBER AESTIVUM*

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KEY WORDS: Ecology, population biology, *Tuber aestivum*, biogeography, genetics, chemical profiling.

The truffle *Tuber aestivum* (syn. *T. uncinatum*) [1] grows naturally on the Swedish islands of Gotland and Öland in the Baltic Sea. The climate on Gotland is drier and colder than Central Europe (*e.g.* Burgundy) and Gotland is the most northerly reported locality for *T. aestivum* [2].

As yet *T. aestivum* has not been found on the Swedish mainland. In previous studies [3, 4] the populations on Gotland have been shown to consist of closely related genotypes, implicating a single introduction or repeated introductions from the same origin. The date of divergence for the samples from Gotland investigated is in accordance with the hypothesis that *T. aestivum* was introduced to the island shortly after the establishment of hazel trees after the last ice age, some 9000 years ago [4].

Despite the genetic homogeneity, local truffle hunters have reported differences in the organoleptic properties of the truffles between different sites. In order to test if chemical differences could be site specific or correlated to genetic traits, we have initiated an ongoing study analyzing correlations between chemical (LC-MS) and genetic variation (ITS sequences) among *T. aestivum* specimens from 15 different localities on Gotland.

Soil analyzes from natural populations of *T. aestivum* on Gotland have shown great variations in soil chemistry and soil particle size, ranging from sand to silt soils, with ranges from 80% sand to 65% silt respectively [2].

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TAXONOMY, BIOLOGY AND ECTOMYCORRHIZAE SESSION 26TH NOVEMBER 2008

TAXONOMICAL AND COMMERCIAL PROBLEMS IN THE VALUABLE TRUFFLE *T. B0RCHII*

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KEY WORDS: *Tuber borchii*, bianchetto, taxonomy, commerce, genetic variability

Tuber borchii Vittad. is an edible truffle with excellent culinary qualities commonly called *bianchetto* (whitish truffle). However, it is often regarded as one of the lesser truffles because it can be sold together with morphologically similar species in the Puberulum clade (Jeandroz *et al.,* 2008) such as *Tuber maculatum* Vittad.*, Tuber dryophilum* Tul. & C. Tul. and *Tuber puberulum* Berk. & Broome which have poor culinary characteristics. This is understandable because it is not a simple task to separate these species just on their external morphological features. Moreover some of the sequences of *T. borchii* reported in Genebank database are incorrect due to the incorrect identification of the sequenced specimens. This has created considerable confusion between mycologists who have assumed that sequences deposited in Genbank are correct which in turn has led to incorrect conclusions when an incorrect species has been used in ecological and phylogenetic studies (Hall *et al.,* 2007).

In this paper we define the characteristics of the commercial species *T. borchii* describing its morphological and its genetic features. The genetic variability of this truffle using different molecular markers (ITS and IGS rDNA regions, β-tubulin and PKC genes) was also analyzed.

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IS TYROSINASE EXPRESSION DURING *TUBER MELANOSPORUM* DEVELOPMENT DUE TO ENZYME INHIBITION OR GENE SWITCHING OFF?

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KEY WORDS: Truffle; tyrosinase; development; cell biology.

We have previously found that during *Tuber melanosporum* development tyrosinase activity decreases reaching a minimum at the sporal stages (5 and 6) as biochemically and histochemically detected (Miranda M. *et al.* 1996 Plant Science 120, 29-36.); moreover tyrosinase activity was found to be inhibited *in vitro* by truffle flavours such as dimethyl sulfide and bis (methyl thio)methane (Miranda M. *et al.* 1997 Pigment Cell Res. 10, 46-53). To make clear if the decreasing tyrosinase activity during truffle development is due to enzyme inhibition or gene expression repression we investigate here by real-time RT-PCR the levels of tyrosinase mRNA during *Tuber melanosporum* development. The histochemical control of tyrosinase activity has been also done. The real-time RT-PCR shows that the *Tuber melanosporum* tyrosinase mRNA levels decrease as the ascocarp ripens and also the histochemical control supports a decrease of tyrosinase activity (DOPA oxidase) with ascocarp ripening. The findings at this stage suggest that tyrosinase gene expression or pre-mRNA processing are involved in the control of tyrosinase levels during *Tuber melanosporum* development.

QUERCIRHIZA QUADRATUM: A REVISION OF THE CHARACTERS AND IDENTITY OF THE AD TYPE ECTOMYCORRHIZA

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KEY WORDS: ectomycorrhizae, ecology, population biology, taxonomy

The well-known AD type, described first by GIRAUD in 1988, is considered as a competitor in black truffle (*Tuber melanosporum* Vitt.) plantations. It has been mainly observed in *T. melanosporum* and *T. magnatum* Pico plantations in France and Italy. This ectomycorrhiza has always been observed on roots of oak (*Quercus ilex* L. and *Q. faginea* Lam.) and hazelnut (*Corylus avellana* L.) plantations with "burnt" areas around the trees, even in those that do not produce black truffle sporocarps, so it can create false expectations in young plantations. The AD type has also been described in nurseries, as a competitive ectomycorrhiza on seedlings inoculated with black truffle. In Spain, AD type has been detected in black truffle plantations and natural holm oak stands in Navarra, Soria, Huesca, Zaragoza, Teruel, Castellón and Valencia.

In 2005, De Román & De Miguel (Mycorrhiza 15: 471-482), suggested that AD type could be a telephoroid type due to its anatomical and morphological characters. In 2006, Baciarelli-Falini *et al.* (Mycorrhiza 16: 475-484) using molecular techniques identified this type as an Ascomycotina belonging to Pezizales.

The detailed anatomical, morphological and molecular study of the AD type led to a description as *Quercirhiza quadratum* (Águeda *et al.* 2008, Descriptions of ectomycorrhizae 11/12). Based on the anatomical and morphological characters, the AD type belongs to the Ascomycotina. The presence of Woronin bodies on hyphal septa, and the sometimes slightly dissolved septa, are two typical characters of this group.

The DNA sequences obtained from the AD types studied showed close similarities with members of Pyronemataceae and Sarcosomataceae (Pezizales). Both taxonomic groups correspond to the same AD type as found by Baciarelli-Falini *et al.* (2006). One of the studied sequences showed a close identity (100% maximum identity, 84% coverage) with *Trichophaea woolhopeia* (Cooke & W. Phillips) Arnould, although records of this fungal species are scarce in the Iberian Peninsula.

BIOLOGICAL ACTIVITIES OF METHANOLIC EXTRACT FROM *TUBER* SPECIES

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KEY WORDS*:* allelopathic activity, disk diffusion assay, DPPH test, ecology, truffle extracts.

Natural products, involved in inhibitory and stimulatory biochemical interactions between all types of plants including microrganisms (allelochemicals), are an important potential source for alternative agrochemicals and pharmaceuticals that could be used to solve the many problems that have arisen due to inadequate cultivation practices and abuse of synthetic herbicides.
The allelopathic effect of volatile substances released by truffles on *Arabidopsis thaliana* were investigated from Splivallo *et al*. (2007). Pacioni (1991) reported that several species of *Tuber* produce a burned area around their symbiotic plants as a result of a phytotoxic action of truffle volatiles. Tirillini and Granetti (1995) and Tirillini and Stoppini (1996) reported the presence of phenolic derivatives in methanolic (MeOH) extracts of truffle.
In this study, the potential biological activities of MeOH extracts of *Tuber magnatum* Pico, *T. borchii* Vittad., *T. melanosporum* Vittad., *T. aestivum* Vittad. and *T. brumale* Vittad. forma *moschatum* (Ferry) Ceruti were investigated with respect to the inherent allelochemical, antimicrobial and antiradical properties.
The allelopathic effect of the truffle extracts was evaluated using a Petri dish assay. Twenty seeds of each plant species (*Melica ciliata* L., *Lotus corniculatus* L., *Silene vulgaris* (Moench) Garcke, *Hieracium pilosella* L., *Sanguisorba minor* Scop.) were sown on filter paper in a Petri dish with 4.5 ml of water plus various doses of the MeOH extracts. The Petri dishes were incubated at 25 °C in a greenhouse with 10 h of artificial light (250 µmol photons m^{-2} s^{-1}) daily. The root length and seedling height of the tested plant species were measured after 21 d.
The antibacterial activity of the truffle MeOH extract against *Streptomyces* species was determined using a disk diffusion assay.
The MeOH extracts of truffle had a dose-dependent inibitory effect on plant height and root length and showed significant antibacterial activity against *Streptomyces* species.
The antiradical activity of the methanolic extracts was tested with the DPPH test. The results showed that between 38.7 and 19.2 mg were necessary to reduce 50% of the DPPH coloration. There was no linear correlation between antiradical activities and the polyphenol content.

DISTRIBUTION OF *TUBER MELANOSPORUM* MYCORRHIZAS ON ROOTSTOCKS OF HOLM-OAKS (*QUERCUS ILEX*) IN PRODUCTION

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KEY WORDS: Cultivation, ecology, *Quercus*, molecular diagnosis, mycorrhiza, Polymerase Chain Reaction, root architecture, *Tuber*.

Alcina is currently developing molecular diagnosis based on PCR technology to determine the potential of a tree to produce truffles, using either roots (mycorrhiza DNA) or soil (spore or mycelia DNA) samples. Concerning the production potential of a tree, an optimal mycorrhiza sampling protocol should be established to ensure efficient and reproducible data. The aim of the present study was to localize an area around the oak tree in which fine roots bearing mainly *Tuber* mycorrhizas would be particularly abundant. The study involves three 13 years-holm-oak trees (*Q. ilex*) that are producers of black truffles (*T. melanosporum*). For each tree, 3 soil profiles were carried out on opposite side of the trunk at the following distances: 20 cm, 50 cm (corresponding to the burned area) and 120 cm. The profiles were 100-120 cm deep and 100 cm wide. The trees were localized in the middle of the profile width. All cardinal orientations were studied. For each profile the number of fine (diameter<2 mm) and intermediary (diameter>2 mm) roots carrying mycorrhizas was counted at 20 cm intervals in the vertical axis. The presence from 3 *Tuber* species (*T. melanosporum*, *T. aestivum*, et *T. brumale*) was analyzed using PCR multiplex. The results showed that root density does not depend on the cardinal orientation. The 3 trees revealed similar rooting profiles for both fine and intermediary roots. There were more fine roots between 20 and 40 cm depth. Fine root density profile below 40 cm depth was the same for all trees and root density decreased with the depth. Two trees produced fine roots below 100cm depth. *Tuber* mycorrhizas were scarce within the first 20 cm whatever the distance from the trunk. At 120 cm from the trunk, almost all mycorrhizas were found below 40 cm depth. At the burned limit, *T. melanosporum* was detected in 70% of the analyzed samples that were performed between 20 and 40 cm depth. Near the trunk, *T. brumale* mycorrhizas were detected near the surface (0-40 cm). No *T. aestivum* mycorrhiza was found. Most of the analyzed roots found below 60 cm depth were mycorrhized with *T. melanosporum*. *T. melanosporum* could even be found below 120 cm in depth. *T. melanosporum* mycorrhizas were found throughout the depth of the studied profiles, for all distances from the trunk and all orientations. However, the present results proved that to ensure the most efficient *T. melanosporum* production diagnosis by PCR technology, sampling must be done at the burn area limit and between 20-40 cm depth which corresponds to the richest area for both fine roots and mycorrhizas belonging to this *Tuber* species.

ROLE OF *TUBER BORCHII* MANNITOL DEHYDROGENASE IN KEY ENVIRONMENTAL AND METABOLIC RESPONSES

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KEY WORDS: genomic and cell biology, mannitol metabolism, mannitol dehydrogenase, *Tuber borchii*.

In fungi, mannitol is the most important polyol found in large quantities in spores, fruiting bodies, sclerotia and mycelia [1]. Mannitol has different roles such as reserve carbon source, storage of reducing power, osmoprotection and carbon translocation of carbohydrates in mycorrhiza. It has been suggested that in plant-fungus interaction, mannitol quenches reactive oxygen species (ROS) produced by the plant as defence responses [2]. In *Tuber borchii*, mannitol is synthesized and catabolised by NADP-dependent mannitol dehydrogenase [3], and more recently, we report the identification and characterization of the mannitol dehydrogenase (TbMDH) from *T. borchii* mycelium [4]. In this work we reported the regulation pattern of MDH in different stress conditions, nutrient supply and during *Tuber* life cycle. In osmotic stress conditions, the expression data showed that *Tbmdh* messenger decreased following transfer on NaCl medium for 24 h and such down-regulation is still evident in a NaCl treatment prolonged to 48 h. In these experimental conditions a drastic decrease in MDH protein and activity was also observed, whereas the amount of mannitol remained constant. The same down-regulation response is also evident in temperature stressed mycelia. In fact, the mycelium transferred at 4 °C for 72 h shows the *Tbmdh* messenger transcript seven fold lower than the control growth at 24 °C. In addition to salt, *T. borchii* MDH activity is also influenced by carbon source. In fact, *Tbmdh* gene responds positively to mannitol and fructose, whereas gene expression remains at the basal levels in mycelia shifted in glucose or mannose. The response to mannitol is accompanied by parallel variation of TbMDH protein and enzyme activity levels. Mannitol plays an important role also in spore germination. After the mycorrhizal association between *Tuber* mycelium and its host plant, there is the organization of hypogeous fruitbody with asci and ascospores. Expression studies were conducted in ectomycorrhizae and in ascocarps with different degree of maturation on the basis of the percentage of asci containing mature spores. The results obtained highlighted that *Tbmdh* gene is more expressed in fruitbody with 50% of asci and in ectomycorrhizal structure. On the basis of these data, *T. borchii* MDH seem to have a complex regulation pattern which needs further investigations.

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HYPOGEOUS FUNGI FROM SLOVENIA

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KEY WORDS: truffle taxonomy.

The tradition of truffles in Slovenia exists fo a longer time. It is undoubtedly documented since 18th century when J.A. Scopoli in his 2nd edition of Flora Carniolica (Vienna, 1762) under the name *Lycoperdon gulosorum* described a truffle with black interior and dark brown surface, being excavated by patiently trained dogs. The first attempt to look at hypogeous fungi from Slovenia and some neighbour countries was made only recently. It gives the insight into the locally poorly known fungal world and widens the knowledge about them. Special emphasis was put on truffles (*Tuber*). Among them we found 16 taxa from Slovenia. These are: *Tuber aestivum* incl. *T. uncinatum*, *T. borchii, T. brumale, T. brumale* f. *moschatum, T. hiemalbum, T. excavatum, T. excavatum* var. *sulphureum, T. fulgens, T. macrosporum, T. magnatum, T. melanosporum, T. mesentericum, T. nitidum, Tuber puberulum, T. rufum and T. rufum* sensu lato. The group of *Tuber rufum* was found difficult to determine because of its morphological as well as ecological diversity and further attemps will be needed for that.

CHEMICAL CHARACTERISATION AND ANTIBACTERIAL ACTIVITY OF SOME *TERFEZIA B0UDIERI* EXTRACTS

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KEY WORDS: Valorisation, desert truffles, chemical characterization, antibacterial activity.

In traditional medicine, the aqueous extracts of desert truffles are used to treat some ocular infections such as blepharits and conjunctivitis. This property has prompted us to extract natural substances from carpophores of *Terfezia boudieri* Chatin., and to characterize some chemical families from these extracts and finally to test, *in vitro*, their inhibit activities on referential bacterial strains that caused certain eyes infection. Extracts were made from fresh and freeze-dried fruitbodies. The preliminary characterization tests of a few chemical families on several extracts showed the presence of tannins, saponosids, free quinons, amino acids, anthraquinons and phenolic compounds. The spectrum of activities shows a good antibacterial activity of aqueous and methanolic extracts against most studied strains (*Staphylococcus aureus, Salmonella arizonae, Pseudomonas aeriginosa*, *Escherichia coli, Enterococcus faecalis*). This activity may be due to the presence of major compounds identified in the aqueous extracts especially tannins, heterosids, phenolic compounds and saponosids, but no formal conclusion can be advanced except that dealing with the responsible agent for this activity which is a polar compound.

MYCORRHIZATION BETWEEN *TERFEZIA BOUDIERI* CHATIN AND *HELIANTHEMUM SESSILIFLORUM* DESF. IN TUNISIA

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KEY WORDS: *Terfezia boudieri*, *Helianthemum*, mycorrhization, cultivation Tunisia.

The objective of this work is to study the mycorrhization between *Terfezia boudieri* Chatin and *Helianthemum sessiliflorum* Desf. Samples of fungal specie and seed were taking from a truffle area situated in South of Tunisian. This mycorrhization was done in three conditions: inoculation of plants by *Terfezia boudieri* ascosporous; transplantation of plants on an experimental field and a seedling and inoculation of *Helianthemum sessilifloum* directly in the field.
The survey of the shape and type of mycorrhiza under binocular and under optic microscopic (after coloration by the acidic Fuschine according to the protocol of Phlippe and Hayman (1970)) showed the presence of no branched and short mycorrhiza grouped generally and localized at the secondary roots. These mycorrhizas are colourless when they are young and become dark when they are aged. All plant tested contain endomycorrhiza VA.

IN SITU MICRO- AND ULTRASTRUCTURAL STUDY OF ASCOCARP DEVELOPMENT IN *TUBER MESENTERICUM*

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KEYS WORDS: *Tuber mesentericum*, fruit body development, microscopy, morphology, soil structure, earthworm activity

Ascocarp development of *Tuber mesentericum* in its natural soil environment was studied, using light and electron scanning microscopy.
The microenvironment and the soil interface are of great importance for the ascocarp. A continuum was found between the soil and the truffle with the development of many hyphae forming the peripheral tufts, during the ascocarp's spring growth phase. In autumn, the hyphae of the peripheral tufts were no longer differentiated and the truffle had reached maturity.
During the growth phase of the ascocarp a high earthworm activity was found in the environmental soil, leading to the formation of aggregates in which truffle spores could be included. These aggregates, as well as the formation of many channels contributed to the increased microporosity of the soil thus facilitating the growth of the truffle. In addition to physical function, the aggregates participate in the nutrition of the ascocarps via the hyphae (in the tufts colonizing the aggregates).

COMPETITION BETWEEN *PISOLITHUS ARHIZUS* (SCOP.) RAUSCHERT AND *SCLERODERMA VERRUCOSUM* (BULL.) PERS. AND DIFFERENT SPECIES OF *TUBER*

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KEY WORDS: mycorrhization, fungal competition, inoculums substrate.

Two species of fungi, *Pisolithus arhizus* (Scop.) Rauschert and *Scleroderma verrucosum* (Bull.) Pers., are often present in cultivated truffle beds and seem to obstruct mycorrhization with *Tuber* species. The present work aims to verify whether *P. arhizus* and *S. verrucosum* compete for mycorrhization with other *Tuber* species in the soil.
In spring 2004 some young plants of *Quercus pubescens* Willd. were inoculated with *P. arhizus* and *S. verrucosum* alone and/or with different species of *Tuber* and in all possible combinations. 22 different test inoculums were inoculated in the same conditions and using the same quantity of inoculating material. Two types of substrates were used to raise the plants. The first (To) was made up of artificial components available on the market, namely agriperlite, vermiculite and peat in equal proportions. The second substrate (Te) was made up of a mixture of natural soil and sand in equal proportions. In fall 2004, spring 2005 and spring 2006, the percentage of mycorrhization for each of the species of fungi involved was assessed in 32% of the plants for each test inoculum. Root tips were counted and percentages were calculated for mycorrhizae of each species.
The presence of mycorrhizae was quite low some months after inoculation, especially in the artificial substrate (To). In 2005 and 2006 almost all test inoculums were mycorrhized, mainly with *Tuber* species, in both the natural and artificial substrates. *P. arhizus* shows a positive trend during three years in To, whereas in Te the opposite occurs. The presence of *S. verrucosum* was low and constant at 7.7%, but it doubled in To during the last analysis (2006); the two species are not, therefore, very competitive. The presence of other *Tuber* species is more significant: the trend is always positive. *T. borchii* Vittad. and *T. brumale* Vittad., in Te and To respectively, are very competitive. The process of mycorrhization with *T. melanosporum* Vittad. and *T. aestivum* Vittad. is rather slow, furthermore the best performance is in Te. This slowness means that, at first, mycorrhization with other competitive fungi is favoured.

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CONSERVATION, COMMERCE AND VALORIZATION SESSION 27TH NOVEMBER 2008

UMBRIA REGION: ACTIONS FOR THE REGIONAL TRUFFLE RESOURCE CONSERVATION AND DEVELOPMENT

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This presentation highlights the economical, social and environmental importance of truffles in the territory of Umbria, where all the truffle species admitted on the Italian market are well represented.
The effort of the Umbria Region to protect and develop the presence of spontaneous truffles, and to promote their cultivation, is reported.
The following aspects are shown, following also an historical analysis:

– available data, relative to this area;
– the Regional Truffle Plan;
– funds for truffle cultivation;
– the compulsory certification for mycorrhized plants produced and marketed in Umbria;
– the cartographic identification of the most suitable areas for truffle cultivation, and the following identification of truffle plantations exclusively within these areas;
– the funded research programs and projects;
– promotional and divulgative activities.

The talk ends with the presentation of a document mapping the most suitable local sites for truffle cultivation, carried out by the "Comunità Montana Alto Chiascio".

FROM COLLECTION TO PRESERVATION: THE COMMITMENT OF PIEDMONT REGION IN THE TRUFFLE CHAIN – A NATURAL AND ECONOMICAL HERITAGE PIEDMONT STACKS TO CREATE AN OPPORTUNITY OF DEVELOPMENT UNDER EQUITY AND SUSTAINABILITY CRITERIA

Piedmont Region – Mrs Bruna SIBILLE (or her representative),
Councillor for mountain and forest development, public works, soil conservation

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KEY WORDS: research, improvement and preservation of Piedmont truffle heritage.

Below the main activities promoted in the last ten years, listed in chronological order.
Verchamp Project was completed in 2006 and carried out in collaboration with Hautes Alpes Développement and Chambre d'Agriculture des Hautes Alpes. The project allowed to map the truffle tendencies in the Piedmont territory and to create many experimental truffle plantations by bedding certified mycorrhizated bedders. The project was carried out with the technical scientific support by Institute of Wood Plants and Environment (IPLA). Realization of economical studies to assess investment profitability of truffle growing in collaboration with Department of Economics and Agriculture, Forest and Environment Engineering from Turin University (DEIAFA).
Recovery of many truffle plantations in four vocated provinces, in collaboration with National Centre for Truffle Studies (CNST), to increase the production of *Tuber magnatum* Pico, the precious white truffle.
In-depth studies aimed at analysing the rhizosphere components in *Tuber magnatum* Pico plantations, carried out with Italian National Research Council (CNR) – Plant Protection Institute (IPP) from Turin.
Realization of an accurate monitoring aimed at the preservation of *Tuber melanosporum* Piedmont truffle plantations, protecting them against *Tuber indicum*. The project was carried out with Department of Vegetal Biology (DBV) from Turin University in collaboration with CNR – IPP.
Passage of the regional law no. 16 "Regulations about truffle hunt and cultivation, and improvement of regional truffle heritage" in June 2008. The regulations are aimed both at the improvement of truffle and at streamlining the procedures for truffle hunt and cultivation, and at environmental protection of vocated territories.
"The Packaging for *Tuber Magnatum* Pico" international contest in collaboration with Torino 2008 World Design Capital and National Centre for Truffle Studies from Alba. The contest was aimed at designing and creating a package suitable for *Tuber magnatum* Pico preservation and enhancement.

Further information: www.regione.piemonte.it/montagna

TRUFFLES AND TRUFFLE CULTIVATION IN "COMUNITÀ MONTANA DEI MONTI MARTANI E DEL SERANO"

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KEY WORDS: experimentation, green house sector, publication, administrative activity.

The Comunità Montana dei Monti Martani e del Serano is Local Agency founded in 1972 following up disposed law with final aim to promote the social and economical development of mountain territories. Since the beginning of its activity, it is interested at truffle problems, being a product economically very important inside the District.
Since its institution the Agency was also delegated to manage the administrative functions related to the truffle.
During 1976, it actuated, inside own green house, the production of mycorrized plants with *Tuber melanosporum* Vittad., with *Tuber aestivum* Vittad., with *Tuber brumale* Vittad. and with *Tuber uncinatum* Chatin. The green house production is still in operation towards a productive line which favours the local provenience of both symbiosis species.
During 1983, in co-operation with the actual Department of Applied Biology of University of Perugia, the Agency began the study and the research on the ecology of main black truffle species and the experimentation of agronomical techniques to use in cultivated truffle fields, realizing in a few years about 30 hectares of new truffle cultivations. This collaboration is still in progress.
In the town of Spoleto were organized two big International Conventions of Truffle: the first one, absolutely the first as international level, was organized in 1968 by Municipality of Spoleto in co-operation with Università degli Studi of Perugia and during 1988, after 20 years, was structured the second one promoted by Comunità Montana as well the one is going to be prepared during these days.
Recently, at Sportello Unico del Cittadino, front-office of information for citizen housed c/o Comunità Montana, was created a permanent service of technical assistance for truffle seekers and truffle farmers, in order to institutionalize a service that, all along, the Agency provides to ones are interested to truffles and to truffle cultivation.

THE WILLINGNESS TO PAY OF THE CONSUMER FOR FOOD QUALITY APPROACH AS THE TRUFFLE WITH AN APPLICATION OF A CHOICE MODEL

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KEY WORDS: conservation, commerce and valorization.

The willingness to pay is a very important information for the farmers because it is able to point out the ability of the consumer to compensate the economic costs stood by the farms in different actions to place in high-level markets the quality food; in this case there are a lot of costs to valorize the production and to certificate the rural productions as the truffle. The analysis on the willingness to pay have been carried out only for specific quality food put on consolidated agroindustrial systems, as extra virgin olive oil, wine, meats, producing some interesting results to define, in a short time, a lot of marketing strategies very helpful for the farms of rural areas.
The aim of this analysis is to describe the results obtained during a survey using questionnaire about the willingness to pay for a food quality certification of the truffle in an inner rural area, as the Rieti province very famous for significant production of truffle, and the economic outlook of quality certification for this area.
The results of the questionnaire have treated with the application of statistical choice models, like Probit and Logit, to define the willingness of consumer to pay a premium price for the truffle, linked to a lot of different variable as sex of person interviewed, advertising campaigns for the promotion of the product and personal income. Moreover, during the consumer survey it has been estimated the role of the new information communication technologies could have both for the purchase behaviour of the consumers and for the farms, in particular for the truffle pickers and truffle firms, that operate on the rural territory of Rieti; even if, the statistical data has shown a significant results with regard to the role of Internet to advertise the truffle and the rural areas for the firms but not to increase the direct sale of the truffle. In fact the questionnaire has shown as the consumers prefer to buy the truffle directly in the farms using the traditional sales channels. The variable personal income and sex of person interviewed have had a fundamental role on the willingness to pay a premium price for the quality certification of the truffle; in fact the value of premium price and the percentage of price in addition of a base price are not be able to cover completely the costs for the quality certification.

TOLERANCE OF FRESH SUMMER TRUFFLES (*TUBER AESTIVUM*) TO DIFFERENT LEVELS OF O_2 AND CO_2

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KEY WORDS: *Tuber aestivum*, conservation, modified atmospheres, packaging.

Truffles belonging to the species *Tuber melanosporum* (black truffles) and *Tuber aestivum* (summer truffles) are highly appreciated and in great demand due to their special organoleptic properties. In order to satisfy the consumer demand, it is necessary to increase the production of these ascocarps and to optimize their storage methods. Modified atmosphere packaging (MAP) can be very useful in extending the shelf life of truffles by preserving their organoleptic properties. The selection of O_2 and CO_2 concentrations that prolong the shelf life of a given fresh product is essential to the design of a successful MAP system. Thus the objectives of this work were to; 1) determine the effect of different levels of O_2 and CO_2 on the microbiological and quality characteristics of truffles and; 2) to establish the optimum ranges of O_2 and CO_2 to store this product.

Fresh summer truffles were obtained from the Spanish region of Aragon, transported to the laboratory in cooled, insulated boxes and processed within a few hours. Truffles were placed in glass jars of 1-liter capacity connected to the specific atmosphere (CO_2-O_2-N_2) and stored at 4 °C for 26 days. The gas flow rate to each jar was 100 $mL.min^{-1}$. Five atmospheres were tested: 0-5, 10-10, 5-20, 20-20 and 50-20% v/v CO_2 and O_2 balanced with N_2 and compared to air (0-21). The beneficial effect of the atmosphere was established based on two main criteria; 1) the microbial analysis (microbial counts of mesophilic aerobic bacteria, G° *Pseudomonas*, *Enterobacteriacea* family, moulds and yeasts as well as *Listeria monocytogenes* investigation) and; 2) the quality characterization (surface and flesh colour, texture, pH, humidity and water activity) of the truffles. These parameters were evaluated periodically.

Results showed the effectiveness of the five modified atmospheres studied on decreasing microbial populations. The lowest counts for mesophilic bacteria, *Enterobacteriaceae* family*, Pseudomonas* spp*.,* moulds and yeasts after 26 days were obtained in truffles stored on 10%CO_2-10%O_2. The atmosphere in which the texture (hardness, cohesiveness and chewiness) of the truffles remained closest to the initial data was the 5%CO_2-20%O_2. However, pH, humidity, surface colour and flesh colour (L* value) were better preserved in 10%CO_2-10%O_2 atmosphere.

Based on these results we can conclude that low O_2 as well as high CO_2 atmospheres are beneficial to fresh summer truffles, with lower microbial population and longer preservation of quality characteristics. Moreover atmospheres with 10% CO_2-10 O_2% are relatively easy to achieve selecting the adequate film over-wrap, therefore they are promising for future development of MAP storage of truffles.

THE GROWING UP OF OREGON TRUFFLES (*TUBER OREGONENSE, TUBER GIBBOSUM, LEUCANGIUM CARTHUSIANUM)*

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KEY WORDS: Oregon, raking, history, conservation, commerce, valorization.

Oregon Truffles were introduced to the market beginning in 1983, but failed to impress. The best that could be said about them was that they were "chameleons" – that is, every once-in-a-while, one got a good one. But no one knew why.

Their inability to garner culinary respect was a result of the lack of respect exercised by their excavators and traders. Twenty years of such disrespect earned Oregon Truffles the title of cheap, "faux" truffles, and created a culture of excavation of almost universally site-degrading thievery.

Then, one handler, believing that to be a needless tragedy, began an investigation into what could be done about it. Drawing an unprecedented sharing of secrets by the handful of wild harvesters who had great respect and tastes for Oregon Truffles, and were the most observant, he designed experiments quickly yielding previously undiscovered keys to presenting to the market reliably excellent Oregon Truffles.

As well, one of those trufflers had proven that the commercial harvest of Oregon Truffles could be done without risk of site degradation. Thus, a passionate crusade began to save the Oregon Truffle from its headlong rush toward the compost pile of failed culinary dreams, elevating it instead to a rightful status as a world-class culinary gem.

Discovering of the techniques by this handler was partially facilitated by the fact that he had no experience with European truffles, for, as it turns out, Oregon Truffles are as different as they are similar to their European cousins. Those differences extend to their culinary uses as well, both requiring chefs to learn new truffle tricks and allowing Oregon Truffles to enter the culinary stage without displacing any other members of the cast, broadening the reach of truffle cuisine, enriching its lore and appeal. As a *Tuber melanosporum* would say to a *T. magnatum*: "Viva la difference!"

For Oregon Truffles to finish growing up, however, they must learn from the experiences of their European elders. Wholesale excavation must be replaced by the use of dogs for selective excavation, grading standards adopted, and methods developed to reliably establish plantations.

Although Dr. LeFevre's Oregon Truffle Festival, launched relying on this new supply, has caused a sensation, the requisite fiscal and regulatory support is not yet forthcoming from government. Substantial private investment will likely be required.

CULTIVATION SESSION
27TH NOVEMBER 2008

IMPLICATIONS OF A 40-YEARS RESEARCH ON TRUFFLES: FUTURE PROSPECTS

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KEY WORDS: truffle, *Tuber*, biological cycle, mycorrhized plant, cultivation.

All the phases of truffle cycle (spore germination, mycelium development, mycorrhization, fructiferous induction, fruiting bodies development and maturation) were studied in a rather unequal way. Controlled mycorrhization has been correctly mastered, except for *Tuber magnatum.*

The most important progress in truffle cultivation resulted from massive use of mycorrhized seedlings, which made possible the introduction of *T. melanosporum* into countries, such as Australia, Israel, New Zealand, U.S.A. and recently Morocco, where it didn't exist.

Mycorrhized plants are in France under improvement by means of different combinations of high-performance tree clones and truffles from different geographical sources, in respect of plant-fungi biodiversity.

According to some new molecular biology works, truffle is heterothallic, but this character doesn't affect plant suitability to truffle production as they are inoculated by a combination of spores.

The knowledge of the nutritional process of mycelium and ascocarp is very important to be well studied, as cultivation depends on it. *T. melanosporum* has a symbiotic behaviour on new roots, but on old roots it's more saprophytic or even a weakness parasite.

Nutritional mechanism of ascocarp is still under discussion, as the theory of fruiting body independence has been brought into question. The implications on truffle fertilization are relevant.

The mechanism of burnt zones formation is badly known: we only know that truffle "prepares its nest" by emitting volatile herbicide substances and strongly degrading minerals and soil organic matter.

In opposition to mycorrhized plant, truffle culture techniques are little developed but news are now proposed. They take more into account fungal biology and ecology: indirect control of the root system by tree pruning, root pruning, management of root system growth, soil decompaction, stimulation of burnt zones formation, organic fertilization.

Future research will face a better knowledge of crucial points in fungal biology and ecology: intraspecific variability, saprophytic nutrition, burnt zones formation, mechanism of fructiferous induction, influence of hydric and thermic stress, soil eutrophysation by nitrogen, in order to improve cultivation practices.

TRUFFLE ORCHARDS IN FINLAND

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KEY WORDS: cultivation, truffles, ectomycorrhiza.

Truffles are neither part of the traditional Finnish kitchen nor are they a common topic in the national media. Truffles are expensive fungi that form ectomycorrhizal association with forest trees or with annual plants. Truffles form edible fruit bodies below ground. *Tuber borchii* is the only truffle species with commercial value found naturally in Finland so far (Kosonen 2002). Several truffle species were found naturally growing in Finnish forests in 2006 including *Tuber scruposum, T. maculatum* and *T. foetidum*. The first truffle orchards in Finland were established in summer 2006 in Juva, Rantasalmi and Mikkeli. Sixteen experimental plots (75 m×50 m) are located on private agricultural farms. Oak, *Corylus avellana* and *Tilia cordata* seedlings inoculated with truffle (*Tuber aestivum* Vittad.=*T. uncinatum* Chatin) were imported from France and planted (80-100 seedlings per each plot) together with four Finnish control non-inoculated oak (*Q. robur*) seedlings. In addition new oak seedlings of Finnish origin inoculated with the same truffle species were planted in this summer in all plots. The seedlings were protected during the winter season against frost. Soil temperature was followed throughout the year and the lowest temperature recorded was –2.4 and –7 in 2006 and 2007 respectively. Survival of the seedlings after the winter 2006 and 2007 were evaluated and root samples were collected in September 2007 and 2008 and the existing of *Tuber aestivum* were microscopically evaluated. Molecular PCR-DGGE method will be applied in the near future.

LONGTERM REGIONAL LAND PLANNING TO PROMOTE TRUFFLE CULTIVATION BASED ON SOIL AND WEATHER MODELS IN CATALONIA, NE SPAIN

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KEY WORDS: Land planning, GIS, truffle cultivation habitat.

Black truffle (*Tuber melanosporum* Vittad.) cultivation through the establishment of orchards with truffle-inoculated trees continues to be a promising economic alternative in areas with suitable truffle habitat. The continuing increases in demand for black truffles and their high market value provide real incentives in Spain, where presently there are about 7,500 ha dedicated to truffle orchards. To provide a basis for long term planning for the Autonomous Community of Catalonia, in northeastern Spain, we have identified areas with suitable truffle habitat based on present climate conditions and for conditions predicted for 2040. The parameters chosen to determine suitable habitat are the following: mean annual precipitation, summer precipitation, mean annual temperature, mean temperature of the coldest month, mean temperature of the hottest month, soil pH and texture. Our estimated variation of temperature and rainfall for 2040 is based the Intergovernmental Panel on Climate Change (IPCC) report for the Iberian Peninsula. We have categorized geographical regions within Catalonia according to their suitability for black truffle cultivation according to The Digital Climate Atlas for Catalonia, an Earth Digital Model and soil analyses. Our results indicate a total of 1,582,662 ha are presently suitable for truffle cultivation, of which 506,804 ha would require additional irrigation or applications of lime to maintain adequate pH. Within this total area defined, 383,758 ha are presently dedicated to dryland farming activities that require subsidies from the European Union CAP in order to remain competitive, or lands which have suffered forest fires. The estimated climate variation expected by 2040 will reduce suitable truffle lands by 14%, from the present 49% to 35% of the total land surface of Catalonia.

TRUFFLE GROWING ACTIVITY IN NAVARRA. QUESTIONS WITHOUT AN ANSWER

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KEY WORDS: truffle growing, cultivation techniques, truffle aptitude.

First plantations were carried out in 1989. The ignorance about truffle and trufficulture was total. Almost everything about truffle was unknown, almost even its existence, but as a consequence of the permanent search for new crops that contribute to diversify and improve agricultural productions of the region, it was decided with great interest to carry out the first plantations, and find information, mainly in France and Italy.
Almost twenty years have passed. Technical and scientific advances have taken place but there are still many doubts. The expectations raised at first have not been reached in many cases. In spite of that, trufficulture is a reality, that covers important areas in those zones suitable for trufficulture, both in Navarra and in Spain, and it is still increasing.

In Navarra, with the experience acquired, a few points can be made:

– Mycorrhized plant production has evolve positively in Spain, with an improvement of mycorrhization techniques, which guarantees one of the most important factors in the reasonable trufficulture, together with land selection, climate and cultivation techniques, among others.
– The soil: lands selected for new plantations suit truffle needs. In the last two years it has been planted more than in the preceding 15. They are bigger plots, which is favourable to the correct management of plantation.
– Irrigation: in new trufficulture the irrigation studies and practice have become very necessary. The experimentation in this subject is necessary to cover crop needs.
– Spreading and training: promoting trufficulture as a diversification alternative in rural areas has been the aim reached through courses, trips, conferences, festivals, commercial exhibitions, competitions..., which has culminate with the creation of the first Truffle Museum in Spain.

AN INNOVATIVE TECHNIQUE FOR SOIL INOCULUM DETERMINATION APPLIED TO TRUFFLE CULTIVATION

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KEY WORDS: spore extraction from soil, mycorrhiza, Polymerase Chain Reaction, *Tuber*, truffle cultivation, experimentation.

The recent advances in Genetics and Molecular Biology have allowed emergence of new tools that are both reliable and low cost and can be applied to several domains. For example in our case, PCR permitted the specific detection of the *Tuber* species under different biological forms: spores, mycelia, fruiting-body and mycorrhiza. However, these new tools are essentially used by Research Laboratories.
For the past two years, the Alcina company has developed a series of tests for the detection in the soil of inoculum (spore and mycorrhiza) suitable for truffle cultivation and experimentation.
This battery of tests is genuinely innovative, one of the most original aspects of this series consists in a technique to isolate specifically *Tuber* spores from agricultural or forest soils. The sample of spore obtained is pure and ready for PCR analysis.
After sample collection (spore, mycorrhiza), DNA is extracted, amplified by PCR multiplex. The migration of DNA fragments in agarose gel is evaluated by visually monitoring migration.
We developed this battery of tests for three species: *Tuber melanosporum, T. aestivum* and *T. brumale* and offers truffle producers a double interest:

– Soil assessment is of key importance before plantation of a truffle orchard: spore analysis of the soil determines the presence or not of a natural inoculum and provides refined evaluation of the production potential.
– In the specific context of ruffle orchards management, mycorrhiza analysis enables accurate evaluation of the fungus dynamics (whether the fungus is stable, has disappeared or suffers from species competition) and consequently permits to better adapt culture practices to truffle cultivation.

These tests are also adequate in the context of experimental programs as they permit precise monitoring of fungus dynamics before and after orchard plantation or actions in natural production areas (clearing).
A short-term evolution of these techniques will be mycelia analysis and the development of the tests to other *Tuber* species and competitors.

TRUFFLE CULTIVATION IN FRANCE AND ITALY

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KEY WORDS: *Tuber melanosporum* Vitt., hoeing, irrigation, pruning.

The truffle cultivation for the production of *Tuber melanosporum* in France and Italy presents some common points and differences in relationship with the agricultural history and environment evolution. With the example of the truffle cultivation practiced or used by Angellozzi brothers (Roccafluvionne, Marches), we have tried to understand what are the elements which can contribute to improve the methods applied in the two countries. After to have defined the actual conceptions of the truffle cultivation in France and Italy, we have studied the conditions, the technical characteristics and the way of thinking which do the worth at this example. Conditions concerns agricultural history; technical characteristics aims early working of the soil, pruning the 2nd or 3rd years, irrigation in the phase of production; way of thinking regards the balance between environment and cultivation techniques. It results of the comparison of French and Italian models with Angellozzi example that the management of the host tree, in its aerial parts and subterranean one in deep soil, has two objectives: 1) maintain the root system in juvenile stage, 2) favour a lasting truffles production with born and growth of many fruit buddies. In getting out of the classical models of cultivation, Angellozzi's method is characterized by 2 steps: 1) the first one corresponds at the planting of perfectly mycorrhized trees settled in apparently very good conditions of environment with a management limited to hoeing and pruning; 2) the second one consists mainly to deeply hoe the soil, irrigate frequently in small quantity, control the growth of trees by a hard pruning. Angellozzi's method can improve French and Italian truffle cultivation, especially on the way of thinking the control of the host tree growth regarding the lasting of the truffle production.

CAN BURNT FOREST LANDS BE USED FOR BLACK TRUFFLE CULTIVATION?

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KEY WORDS: forest fire, *Tuber melanosporum,* reforestation, ECM competitiveness.

Forest fires stop the flow of carbohydrates from the host plants to the ectomycorrhizal (ECM) fungi colonizing their roots, causing potential decrease or loss of the fungi in the soil community. The viability of reforestation with *Tuber melanosporum*-inoculated seedlings in burnt forestlands where soil and climatic characteristics are suitable for truffle cultivation depends partly on the competition of native ECM in the soil after fire. We evaluated the biotic aptitude of burnt forestlands for *T. melanosporum* by examining the survival and competitiveness of this fungus compared with the native ECM community when out planted with *Quercus ilex* (holm oaks) seedling. We also evaluated the role of the ECM host plants, which resprout after a forest fire, in the maintenance and dynamic processes of the ECM fungal community. The study followed a factorial design with two levels: 1) *T. melanosporum*-inoculated and non-inoculated *Q. ilex* seedlings and 2) presence and absence of ECM host plants capable of resprouting after the fire. We established 10 experimental plots where 360 holm oaks were planted. Four and a half years after plantation, the truffle-inoculated holm oaks maintained 36% of their root tips colonized with *T. melanosporum*, and 8 years after plantations (2008), 45% of the inoculated holm oaks displayed a *burn* area. Additionally, the inoculated oaks presented greater survival rates and greater root and aerial development, compared to the non-inoculated oaks. A greater ECM morphotype richness was associated with seedlings planted in plots with the presence of ECM host plants, whereas the *T. melanosporum* mycorrhizal ratio was constant. These results suggest that reforestation with *T. melanosporum*-inoculated seedlings could be successful following forest fires and highlight the competitiveness of this fungus within the ECM community in these soils.

RESEARCH RESULTS IN TRUFFLE BEDS OF THE UMBRIA REGION

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KEY WORDS: Cultivation, *Tuber*, mycorrhization.

In 1980-1981 two Regional laws were issued by the Region Umbria, with the aim of regulating the truffle picking and promoting its cultivation. Based on these laws, a Regional Truffle Plan began for the years 1983-1986, to let farmers know about this new type of agricolture, and to test cultivation techniques applicable to truffle cultivation.
Thanks to this Plan, 59 experimental truffle plantations were completed, comprising 115 hectares spread across the Region, characterized by different pedoclimatic properties.
In the year 1997, the Umbria Region signed an agreement with the Department of Plant Biology, now Dept. of Applied Biology, of the University of Perugia, to investigate the state of art of its truffle plantations and to test some cultivation techniques.
The research work carried out over the years 1997-2000 (in 14 year-old truffle plantations) investigated the truffle plantation conditions and their degree of mycorrhization. Among the 59 truffle plantations, only 7 appeared to have completely failed; in the remaining 52, the analysis of the mycorrhization level showed that mycorrhizae of the cultivated truffle were maintained, expecially in species of the genus *Quercus*. In the years 2001-2004 (in 18 year-old truffle plantations) the research work focused on the cultivation techniques applicable to the truffle-producing plants, to favour fruit body formation and their growth. The experimental work involved 13 truffle plantations: soil tilling, watering, plant pruning, truffle spore inoculum soil supply and soil corrections were mainly tested. During the year 2008, the first results have been recorded. In particular, spore inoculum soil supply, $CaCO_3$ soil supply and watering showed to be beneficial; while pruning and soil tilling had a limited effect, maybe due to the truffle bed age.
The analyses have also shown that all the investigated Umbrian truffle plantations were productive. Truffle production level was variable due to different pedoclimatic conditions, and in some instances the truffles produced were different from those cultivated. *Tuber magnatum* Pico truffle plantations were found productive (a limited production of 2-4 kg per hectare) and economically interesting.
Unfortunately, in Italy the truffle does not belong to the land owner, and it is often therefore subject to a deleterious picking. It follows that truffle production levels recorded in the Regional plantations are underestimated and not realistic.

AUXOMETRY OF THE *TUBER AESTIVUM/UNCINATUM* CARPOPHORE

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KEY WORDS: Truffle cultivation; growth curve, carpophores, *Tuber aestivum/Tuber uncinatum*.

The *Tuber aestivum* is a type of truffle that is being used more and more in truffle cultivation, especially as a substitute for *Tuber malanosporum*, because of its greater adaptability to the most varied growing environments. Despite its widespread use there are few studies on the development and macroscopic growth of the carpophore. Such studies would be very useful, in particular in order to define the handling of cultures and the timing of intervention as a function of the growth requirements of the carpophores. The Authors monitored the growth of several *Tuber aestivum/uncinatum* carpophores in a truffle cultivation consisting of 28 European hophornbeams (*Ostrya carpinifolia*), mycorrhized with *Tuber aestivum/Tuber uncinatum*, planted in 1992 on land located in the province of Vicenza (Altavilla Vicentina). Maximum measurements in two perpendicular directions were taken to estimate the surface area of the carpophores and the way they develop. Such measurements of the carpophores were taken as they came up from the ground every week for two consecutive years (2007 and 2008) starting in the month of January through the month of June. Hence the growth curve was studied as a function of the date of the measurement and several climatic variables such as maximum, average and minimum temperatures, precipitation and wind. Once each carpophore reached maturity, it was collected and weighed. Using a multiple regression model with the stepwise elimination of the predictive variables the degree of dependence of the dependent variable (carpophore growth) on the climatic predictive variables was quantified. In addition, the growth curve was parametrized using appropriate singmoidal functions. This study, though preliminary, sheds light on the last stages of growth of the carpophores and allows us cultures more rationally.

A QUARTER CENTURY OF TRUFFLE CULTIVATION IN NEW ZEALAND – SUCCESSES AND PROBLEMS

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KEY WORDS: *Tuber melanosporum*, *Tuber borchii*, *Tuber aestivum*, truffle cultivation, Southern Hemisphere, New Zealand, management, problems, successes.

At the 1988 Spoleto congress Ian Hall briefly outlined his plans for cultivating truffles in New Zealand, and in particular *Tuber melanosporum*, to cater for out-of-season Northern Hemisphere markets. The first *T. melanosporum* truffles were found in 1993 on a truffière owned by Alan Hall on the East Coast of the North Island at 38° 35' S. However, it was not until 1997 that Alessandra Zambonelli unearthed a 250 g truffle in the same truffière that was to be the first truffle of the first commercial harvest in the Southern Hemisphere. Truffles were later harvested in truffières between 38°S and 44°S. Truffle cultivation subsequently spread first to Australia in 1992 followed by Chile and South Africa.

Research became increasingly difficult following the establishment of the Crown Research Institutes in New Zealand in 1992. After this funding for truffle research was reduced and ultimately discontinued in 1996. The Edible Fungi Research Programme lost all of its remaining funds in 1998, the year after the first commercial harvest was made. Scientists and technicians were only able to keep their jobs and continue research by generating revenue from commercial activities and conducting mycorrhizal research in other areas.

The purpose of our presentation will be to outline the scientific and other problems that were encountered in New Zealand's truffle research programme, how they were overcome, the successful commercial cultivation of first *Tuber melanosporum* and very recently *Tuber borchii*. Research on the production of truffles and other edible ectomycorrhizal mushrooms in plantation forests will also be outlined.

FEASIBILITY OF TRUFFLE PLANTATIONS IN FIREBREAKS

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KEY WORDS: *Tuber melanosporum*, firebreak, truffle cultivation, bioassay, land use, lime, fire.

Firebreaks are strips of open vegetation inside forested areas, constructed to fight against wildfire. They are frequently affected by controversy concerning high maintenance costs, environmental impact and land use conflicts. On the other hand, Perigord black truffle is mainly produced in open forests that have a structure similar to that sought for firebreaks, and the biocidal effects of the truffle could help to maintain this structure. In addition, truffle ascocarps command high prices on the market. The successful establishment of truffle plantations in firebreaks would represent a multifunctional use which could increase the economic and social sustainability of wildfire prevention. Nevertheless, this establishment is conditioned by several factors: 1) black truffle requires specific soil and climatic conditions, and 2) firebreaks are usually constructed on forest soils, whereas trufficulture is a technique developed for use on agricultural land.

This study aims, first, to identify the main obstacles in establishing young inoculated seedlings on forest soils and, then, to select techniques to overcome these obstacles. Laboratory bioassays were used as pre-feasibility studies. Initial results showed that the ectomycorrhizal inoculum potential was much higher in forest soils than in agricultural soils, with the effect that previously mycorrhized plants were contaminated with native soil fungi and more than half of the truffle mycorrhizas were replaced in less than two years. To reduce the soil inoculum potential, several physical and chemical treatments were assayed. Both overliming and high temperature reduced the inoculum potential, but some doses also affected root growth. Therefore, proper site preparation of the planting hole could be a useful tool for establishing truffle plantations in firebreaks. If no site preparation is performed, there is a serious risk that native soil ectomycorrhizas will replace the truffle ones in the short term.

THE PROJECT "DESARROLLO INTEGRAL DE LA TRUFICULTURA DE TERUEL (SPAIN)"

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KEY WORDS: Truffle cultivation, trufficulture research, Plan Específico para Teruel (PET).

We present this research Project named "Desarrollo Integral de la Truficultura de Teruel" included in the "Plan de Actuación Específico para Teruel" to impulse the development of some strategical activities for Teruel province as trufficulture with more than 3000 ha of cropping surface.
The institutional support of the Spanish State through "Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria" (INIA) lies in the finantial of scientific research and technical development carried by the main trufficulture R+D Spanish teams with the aim of studying the less known aspects that are delaying the development of this activity.
This project is multidisciplinary based, it counts with a finantial support near to one million euros distributed for 3 years (2008-2010) and insists on the need of a knowledge flow among the members of the trufficulture sector and the participant research teams in order to convert Teruel into a Reference Laboratory of European trufficulture.

THE TERRITORIAL ANALYSIS OF THE TRUFFLE PRODUCTION: A PREREQUISITE IN THE BIOTECHNICAL EXPERIMENTATION

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KEY WORDS: Sustainable development, Truffle Cultivation, forestry truffle, Lot (France).

The part of the program of PSDR (for and on the regional development) research "INGEDICO" (Instruments of management and collective devices of conservation and recovery of naturals and renewable ressources) deals with a concerted management of limestone hills with environmental and landscape devices of ecosystems and associated practices.
The purpose of this communication is to expose the first results of the research in the natural Park of limestone hills the region of Quercy (Lot, France).
The analysis of environmental issues of those territories favorable for truffle production (issues of fire risks and biodiversity, issues of erosion risks and water) is essential before establishing a mapping of experimental areas. This delimitation has been obtained with a multi-criteria analysis with the Electre III method built with different types of data: the pedo-climatic truffle potential, the right location of environmental areas, the analysis of mobilized and mobilizable management instruments for truffles and the analysis of human dynamics and practices connected to the concerned networks of actors. Eighty interviews of truffles growers and managers of expert data (Agricultural, forestry, and environmental) reinforce those first results allowing an assessment of practices of truffles growers and take advantage of existing expertise and tools linked to each environmental issue, while implying local actors to the research approach. Concretely, this territorial approach of truffle practices makes it possible to:

– to study the organization of actors and whether or not they contribute the preservation of these natural resources with their practices;
– to propose mobilizable instruments for a concerted management of limestone hills and ecosystems of truffles according to present environmental issues;
– to implement experimental bio-technological protocols to promote environmental practices.

TRUFFICULTURE AND FOREST MANAGEMENT IN TERUEL

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KEY WORDS: *Tuber melanosporum*, Teruel, truffle silviculture, firebreak, truffle cultivation, charcoal kiln, ecology.

In the framework of the Socioeconomic Development Plan for the province of Teruel (Spain), a coordinated trufficulture research project is currently being carried out. Perigord black truffle has been a significant agroforestry resource in this region since 1950, when people began collecting naturally growing forest truffles. The main goals of this research project are related to the management of truffle plantations, which have spread very quickly since 1990. However, there are also other goals related to the management of forests that spontaneously produce black truffles and whose productivity is currently experiencing a pronounced decline. In this subproject, the concrete aims are: 1) to adapt management techniques to the ecological conditions in Teruel and disseminate said techniques among forest managers and owners, 2) to evaluate the feasibility of establishing truffle plantations in firebreaks, and 3) to improve the understanding of the ecological relationship between former charcoal kilns and the spontaneous formation of *truffières*.

With respect to the first aim, several truffle silviculture pilot projects have been proposed for two forests with naturally occurring truffles. The ecological conditions in the *truffières* and the factors limiting their productivity have been analyzed and improvement actions have been proposed. Regarding the second aim, three firebreaks have been experimentally planted for demonstrative purposes. They have also been designed to evaluate the efficacy of several types of site preparation. Finally, with regard to the third aim, the effect of ancient charcoal kilns on current soil properties and on their potential for truffle cultivation are to be studied. In addition, two experimental charcoal kilns will be set up to evaluate their short-term effect on soil and ectomycorrhizal fungi.

The research project for the "Integral Development of Trufficulture in Teruel" is not intended to establish definitive conclusions on many of these research questions during its duration (2007-2010), since the life cycle of the truffle is much more prolonged. Nevertheless, this project has already been successful in encouraging collaboration between researchers, public administrators and truffle growers and in opening communication channels for scientific dissemination and exchanges of empirical experiences.

INOCULATION OF ADULT HAZELNUT GROVES WITH *TUBER BRUMALE* AND *TUBER MELANOSPORUM*

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KEY WORDS: Truffle cultivation, *Tuber melanosporum, Tuber brumale, Corylus avellana*, hazels, field inoculation, *Micoforest Technology®*.

The hazelnut tree has a great social an economic value in the NE of Spain, nowadays sunk in economic crisis. 15.000 Hectares of these groves lay on potential truffle producing areas. *Tuber melanosporum* and *Tuber brumale* fruit in some of them naturally. The ecology and soil characteristics of these natural truffieres are discussed. We found no significant differences related to the particle size (50-200 µm versus <50 µm) of the organic matter between *T. brumale* and *T. melanosporum* natural truffieres.

Our aim is to develop inoculation techniques in mature groves and later to coordinate the truffle and hazelnut cultivation, trying to get an added value to the traditional crop. In this work large inoculations using the *Micoforest Technology®* have been carried out in mature hazels with *Tuber brumale* on 3.230 hazels, and *Tuber melanosporum* on 1.300 hazels, in a total area of 11,3 hectares. They were carried out two inoculations with sporal inoculum in all the trial fields, spring-fall within the same year or spring-spring with one year delay. Percentage of trees that got mycorrhizae of *Tuber melanosporum* one year after the first inoculation are between 28,6% and 45,2% of the inoculated hazels. The hazels that present mycorrhizae of *Tuber brumale* are between 24,1% and 56,2% after the first inoculation.

IMPACT OF THE CULTURAL PRATICES THE TREE FIRST YEARS OF THE PLANTATION ON THE TRUFFLE-PRODUCING POTENTIAL

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KEY WORDS: *Tuber melanosporum* Vittad., soil, burnt areas, mycorrhizal status.

Setting up truffle plantations needs to take account of different factors to be sure of satisfactory results when they enter into the production phase. The parameters generally taken into consideration are the environment, previous cultivation, the orientation, the topography and the nature of the soil. Using plantations aided by grants from local authorities in Midi-Pyrenees, this study aims to determine the effect on the truffle-producing potential of data recorded before planting and of cultivation methods.

We chose to study a certain number of plantations in the département of the Lot on the basis of environmental and farming factors. The results concern the development of the trees, the formation of burnt areas, the mycorrhizal status and the general state of cultivation. The mycorrhizal status was checked on a sample of trees.

The initial results of this study show that working the soil under young trees planted on former uncultivated land gives the best presages of future production. In the best situations burnt zones are seen from the third year onwards under ever green and downy oaks. The mycorrhizal status (with excellent indices) of these trees is dominated by *Tuber melanosporum*.

CULTIVATION SESSION
28TH NOVEMBER 2008

TRUFFLE CULTIVATION IN SPAIN

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KEY WORDS: Cultivation, *Tuber melanosporum*, ecology, sustainable development.

In Spain truffle cultivation takes on a special value because it fits fully within the concept of sustainable development due to the ecological conditions in which it is produced and the economic and social environment that profits from its production. In Spain truffles have two clearly differentiated provenances: they are either produced naturally in spontaneous truffières or they are produced in the numerous truffle plantations established during the last 25 years with mychorrized plants, many of which have already entered in production.

The fundamental change in Spanish trufficulture is considered to have occurred in the decade of the 1970s, when an alarming descent in natural truffle production was first observed. This coincided with increasing rural depopulation.

At present, it is estimated that there are more than 4000 ha of truffle plantations in Spain. The current annual rate of planting is estimated between 250 and 500 ha a year. The most active regions are located in the east (Teruel, Castelló) and the northeast (Soria, Huesca). The most planted tree species is evergreen holm oak, although hazel, downy oak and Portuguese oak (*Quercus faginea* Lam.), Especially notable is the 600 ha Arotz plantation in Soria, which produces 2500 kg of truffles a year

In this paper we describe the typical truffle plantation in Spain: ecology, cultivation techniques, pruning, irrigation systems, nurseries of mycorrhized plants etc,

The extension and public promotion of trufficulture in Spain is widely supported from the technical and scientific fields with the collaboration of the various truffle-collectors associations.

This paper includes also information on production, prices, truffle distribution, markets and trade, plantations, research activities and publications. Finally we do a diagnosis of future perspectives for the truffle sector in Spain.

ECOLOGY OF TRUFFLE PLANTATIONS IN TERUEL (SPAIN): SAMPLING METHOD FOR ITS CHARACTERIZATION

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KEY WORDS: ecology, truffle cultivation, parameter, sampling.

Even today there are many unknown factors related to the ecology of black truffle plantations that could provide useful information to improve sporocarps production. In addition, nowadays there are a lot of truffle plantations over 10-years-old which produce or not black truffles that could be studied.

With the aim of going deeply into the ecology of the holmoak (*Quercus ilex* L.) inoculated with *Tuber melanosporum* Vitt. plantations in Teruel (central-east Spain), the method described below was set out. This action fits within a broader plan called "Development of truficulture in Teruel", which deals with many other aspects of truffle cultivation, development and conservation. Specifically, our goal is to combine the information obtained from soil characteristics, presence/absence of mycorrhiza and sporocarps and host trees biometrics to get the optimal parameters for the establishment of new plantations and those that reject its use.

Plantations over 10-years-old are studied, both producing and non-producing black truffle sporocarps and distributed throughout the Teruel province. Near 100 plots are included in this study to cover as much as possible different places. Before sampling, it is necessary to get the owners information about the history of the plantation, the sporocarps production and the cultural treatments carried out in their plantations.

Field study, conducted in spring, focuses on a representative spot of every plantation as regards the characteristics of soil, vegetation and production of black truffles. This sampling point has nine host trees with variable area depending on the used frameworks for planting. Three soil extraction points are randomly selected in the area, near the line of glass. In each point, two adjoining samples are collected in the South-East of every tree for soil and ectomycorrhizae analysis. South-East orientation was chosen for being this which *T. melanosporum* ectomycorrhizae prefers, under previous experiences in inner Iberian Peninsula sites. A total of 6-cylinder, 4 cm in diameter and 20 cm deep, are extracted in each plantation. In order to estimate the apparent density, outside the burnt area, is made an additional hole, 30×30 cm and 20 cm deep. Also, measurements of physiographical and tree parameters are taken.

A full analysis of soil and presence/absence *T. melanosporum* ectomycorrhiza detection is doing in laboratory. Multivariate analysis techniques to detect potential patterns of behaviour of the parameters with the production and/or mycorrhization of black truffle will be done.

The limited sampling period and the large number of plots and parameters to study makes this task will be extended by more than two years and now is conducted at just 30%.

THE PRINCIPLE OF PRECAUTION IN TRUFFLE CULTIVATION

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KEY WORDS: *Tuber melanosporum* Vittad., *Tuber brumale* Vittad., contamination pressure, preceding crop, biodiversity.

Truffle cultivation started in France at the beginning of the 19th century with exceptional results at the end of that century and the beginning of the next. The fall in truffle production of *Tuber melanosporum* which started after the first or second world wars – according to location – motivated a programme of revitalization from the 1970's onwards. Mycorrhizal inoculation of plants starting in 1974 plus the experimental work of the 1980's slowed the decline and saved truffle production in France. Nevertheless, truffle cultivation comes up against some difficulties which impede the complete success of its revival. We have identified the strong and weak points by comparing production conditions and the results obtained in different regions of France and other countries visited in Europe and the rest of the world. Amongst the adverse points, we have noted so far which seem to be unfavourable to truffle production: 1) wooded environments which create pressure by contamination with forest habitat fungi (*Tuber brumale*), 2) previous intensive cultivation using chemical products (pesticides and fertilizers), 3) poor management of the growth and density of mycorrhiza–inoculated trees with regard to the nature of the soil, 4) working the soil with drawn cultivators, 5) using herbicidal products which decrease biodiversity. Amongst the favourable points are: 1) the use of tested mycorrhiza–inoculated plants, 2) light cultivation techniques which respect biodiversity identical to that of natural truffle grounds, 3) planting in open, lightly-wooded countryside. In southern Italy and Spain, where the industrialisation of agriculture arrived later than in France, the difficulties of truffle production seem to be less limitative. In Australia, truffle production seems to be easier with the accompanying arboriculture because the environment is free from other contaminants of the genus *Tuber*. These observations lead us to propose that truffle production in France should be subjected to certain precautions. These precautionary measures for truffle production imply giving special attention to the following points: 1) the choice of previous methods of culture corresponding to fallow land, 2) a technical cycle which favours a phase of installation of the fungus after taking control of the tree, 3) an upkeep of the soil which maintains a certain biodiversity, 4) regulation of water for the fungus without stimulating the growth of the trees, 5) pruning the trees according to the extent of the burnt zones and the space available for the fungus to occupy, 6) introducing spores and various other practices. Work is in hand relating especially to the usefulness of the presence of certain animals within the boundaries of truffle plantations.

A NEW PERSPECTIVE IN CONTROLLING *TUBER* INFECTED PLANTS: THE USE OF ROC CURVES MAKE APPLICABLE A VISUAL ESTIMATION

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KEY WORDS: truffle cultivation, ROC curves, mycorrhizal plants, binomial response.

The production of seedlings infected with the inoculated *Tuber* spp. is the first important step in truffle cultivation. For this reason over the past several morphological methods for the evaluation of the degree of ectomycorrhizal infection of plants inoculated with Tuber spp. have been proposed. All methods developed (mainly in Italy and in Spain) are time consuming, requiring the direct count of a great number of tips for plant. Infact methods are based of quantitative scale (counts or percentages). Instead, the method adopted by the French, is less time consuming because it avoids the direct counting of mycorrhized root tips, by providing an estimate of the extent of mycorrhization directly using an ordinal scale. The scale is defined according to the quantity of mycorrhizal tips present, but this method is quite subjective because it is not supported by a statistical validation of the scale and of the inspector. In this work we applied ROC curves for the evaluation of the sensibility and specificity of inspectors who have to estimate a *Tuber* infected plants using a binomial response. A ROC curve was obtained by plotting sensitivity against the 1 – specificity for all possible cut-off points, i.e. the number of mycorrhizae. The area under the ROC curve is an assessment of the performance or diagnostic accuracy of the test. So, the ROC curve can be used to evaluate the degree between qualitative validator assessment and results of applying routine quantitative methods. We also applied k Cohen test that assesses the degree of agreement among the different estimates of many inspectors regarding the same sample. ROC curve application is particularly useful when a very large number of mycorrhized seedling must be assessed. Our method could also be used to develop a protocol to be used by in the assessment of mycorrhized seedlings. In this case, we must decide: 1) what the AUC values and the minimum sensitivity and specificity that derive from the assessments of an inspector must be in order to validate that inspector; 2) the minimum value of the *k* coefficient of Cohen and the number of seedlings necessary for the comparison among inspectors; 3) the minimum number of seedlings to be assessed.

KEEPING THE EARTH EGGS IN DIFFERENT BASKETS: THE FUTURE OF DESERT TRUFFLES IN QATAR

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KEY WORDS*:* desert truffles, *Tirmania*, *Terfezia*, cultivation, ecology, traditional ecological knowledge (TEK), natural resource management, desertification.

The State of Qatar has undergone rapid societal changes over the last 60 years due to the discovery of vast on- and off-shore oil reserves. The changes wrought by this new-found wealth have had significant effects on the nation's economy, health, and environment. The impacts of industrialization on Qatari traditional culture have not been thoroughly documented in a scholarly way – although one can imagine the changes resulting from the shift from an economy based on camel herding, pearl diving, and fishing to an economy based on oil and gas.

One Qatari cultural practice that has remained intact is hunting for desert truffles. These fungi, belonging to the genera *Terfezia* and *Tirmania*, are native to Qatar, and grow in association with the roots of the desert sunflower, *Helianthemum spp*. The desert truffle's geographic range extends across the Mediterranean to the Middle East. Desert truffles have been used as food and medicine since time immemorial. Their management and associated cultural knowledge constitutes a Traditional Ecological Knowledge (TEK) system and that has been managed for both food and medicine since time immemorial.

In its formulation of a National Biodiversity Action Plan, in signing the UN Convention on Biodiversity (CBD), and the UN Convention to Combat Desertification (UNCCD), the State of Qatar has committed to action in several priority areas: combating desertification, conserving biodiversity, documenting and protecting TEK, and mitigating/adapting to the effects of climate change. Desert truffles could be a suitable keystone *eco-cultural* species with which to model a responsive approach to the aforementioned goals set by the State of Qatar. In fact, as plant symbionts adapted to a hypogeous habit, as a food source for desert animals in a nutrient poor habitat, and most importantly their role as a fulcrum for the transmission of Qatari TEK, they are ideal candidates for the restoration of both the desert ecosystem and to reinforce the Qatari's threatened cultural relationship to this damaged and fragile landscape.

TUBER MACROSPORUM VITTAD. CULTIVATION

Vezzola Virgilio

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KEY WORDS: truffle production, harvest period.

In 1996, at Roè Volciano (Brescia, Italy), a truffle plantation was made using plants mycorrhized with *Tuber macrosporum* Vittad.; after five years, in 2001, some plants have started producing. Fruit-bodies were not collected until 2007, when 70% of the plants had become productive, and the plantation had achieved a total shading, a condition required by this species. The truffle harvest started in the middle of June and ended in December. In 2008, part of the plantation was tilled, to favour the formation of bigger fruit bodies as compared to those initially produced.

WIRELESS SENSORS NETWORK AND THEIR APPLICATION ON TRUFLLE CULTIVATION

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KEY WORDS: cultivation, wireless sensors network, ambient parameters, irrigation control.

A relevant aspect of the modern truffle cultivation is the opportunity to practice the irrigation in a controlled way. The evolution of climatic conditions plays a determinant role on the correct vegetative development of the symbionts with a direct influence on production in terms of quantity and quality. The possibility of locally monitoring, archiving, analysing and controlling some of the most important ambient parameters such as humidity and temperature both of the air and of the ground, atmospheric pressure and precipitation, wind speed and direction, etc. can be an important instrument to study the relations between them and the optimal conditions of development of the symbionts. The current technological state of the art permits the displacement of interconnected ambient sensors, even on huge areas. Periodically they acquire in situ data and transmit them through a wireless communication to a point devoted to gather, archive and area control. The possibility of visualization, analysis, interpretation and sharing of data and the remote control of the system can now benefit from the widespread and pervasive connection to Internet. Thanks to the relative simplicity of use and implementation the application adapts to a wider diffusion, not only in the academic and in the scientific community, thereby it has the possibility to access to large databases.

ROUND TABLE: EXPERIENCE OF AND PROBLEMS WITH TRUFFLE CULTIVATION 28TH NOVEMBER 2008

TRUFFLE-CULTIVATION: JOY AND PAIN

Bencivenga Mattia, Baciarelli-Falini Leonardo, Di Massimo Gabriella, Donnini Domizia

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KEY WORDS: *Tuber*, truffle cultivation, *Tuber* ecology.

Truffle-cultivation is a recent activity, widely increasing due to the necessity of finding farming activities alternative to the traditional ones, more productive and able to prevent ecological land degradation.
Truffle-cultivation has appreciably evolved over the last few decades, but new problems have arisen. The most evident progresses are:

– The large-scale production of mycorrhized plants;
– The presence of codified protocols for mycorrhized plant control and certification;
– A better understanding of suitable environments for truffle cultivation;
– A better knowledge of the cultivation techniques required by truffle-producing plants in an open field.

Nowadays, these results have enabled to plan rational truffle plantations, with very high probabilities of success and with satisfactory production levels: 50-100 kg per hectare in truffle plantations of *Tuber melanosporum* Vittad., *Tuber aestivum* Vittad. and *Tuber borchii* Vittad.; 1-5 kg per hectare in truffle plantations of *Tuber magnatum* Pico. Good productions have also been obtained in truffle plantations of *Tuber macrosporum* Vittad. and *Tuber brumale* Vittad.
However, joy is followed by pain:

– The scientific knowledge cannot yet guarantee the productive success of a plantation: a 10% possibility of failure still exists;
– Cultivation techniques in a truffle plantation cannot be generalized; useful cultivation modalities in a plantation can even be dangerous or useless when applied to another environment; this makes difficult the experimental result interpretation;
– A recent problem is the time-limited production of a truffle plantation: a truffle plantation starts producing, it produces for a few years and then it stops;
– The climatic changes have negative effects on truffle productions, expecially on those of black truffles: the severe drought and high summer temperatures, recorded over the last few years, have caused loss of production and have damaged the mycorrhizae, with negative effects on the years to come.

To conclude, we hope that the applied research in the area of truffle cultivation will increase, in order to limit the current problems and make the truffle cultivation a more reliable and productive farming activity.

POSTER SESSION
28TH NOVEMBER 2008

ECOLOGYCAL ASPECTS OF TRUFFLES POPULATIONS IN SALENTO (SOUTHERN ITALY)

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KEY WORDS: *Tuber borchii* Vittad., *Tuber aestivum* Vittad., *Quercus ilex* L., *Pinus halepensis* Miller, Salento, truffière.

In this work are reported preliminary data on the ecology and distribution of *Tuber borchii* Vittad. e *T. aestivum* Vittad., that form beneficial symbiosis (ectomycorrhizas) with some of the most representative local wood vegetation, providing also an economic values, thanks to the production of edible hypogeous fruit bodies. The results regard mainly, the eastern part of Salento, where the natural truffiere correspond to vegetal balanced ecosystems (climax): *Quercus ilex* L. woods, *Pinus halepensis* Miller woods, woods were *Q. ilex* and *P. halepensis* co-grow.

The average age of holm oak woods investigated was about 90 years and *Cyclamino hederifolii-Quercetum ilicis* represent the more diffuse association in the studied area. The average age of pine woods investigated, mostly constituted of Aleppo pine wood, was about 60 years. Pines represent structuring species that were introduced in Salento by the Italian "Corpo Forestale dello Stato" during multiple local vegetal reconstruction processes.

In this study, the individuation of the plants involved in the mycorrhizal symbiosis has been considered important for the introduction of mycorrhized plants in degraded areas, considering also the homogeneous climatic conditions and the ecological adaptability of *T. aestivum* and *T. borchii*. *Q. ilex and P. halepensis* represent the plants found in symbiotic association with the *Tuber* species collected.

Erbaceous and arboureus plant species of the natural truffière are important indicator of specifc pedo-climatic conditions; the evaluation of the more suitable truffière, their floristic-vegetational aspects as well as their position in relation to EU Community Interest Sites (pSIC) could provide useful information in the planning of reconstruction of degraded areas.

TRUFFLES POPULATIONS IN SALENTO (SOUTHERN ITALY): ASSOCIATION WITH *QUERCUS ILEX AND PINUS HALEPENSIS*

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KEY WORDS: Ectomycorrhizae, Salento, *Tuber borchii* Vittad., *Tuber aestivum* Vittad.

Morphological and molecular characters of *Tuber borchii* Vittad. and *Tuber aestivum* Vittad. populations have been investigated in the eastern and central Salento (Puglia, southern Italy) area throughout one year of sampling. From this preliminary study, they represent the most frequently encountered *Tuber* species in Salento and, on the basis of where they were collected, *Quercus ilex* L. and *Pinus halepensis* Miller have been identified as the symbiotic plants. In particular, *T. borchii* has been found in association with both kind of plants, while *T. aestivum* mainly with *P. halepensis*. Moreover, greenhouse tests to assess the capacity of both *Tuber* species investigated to perform an association with local flora (especially *Q. ilex*) are in progress, taking into account the main objectives of this research: (1) vegetal reconstruction processes and (2) increment of local truffle production.

TRUFFLED HOLMOAK (*Quercus ilex*) STANDS IN THE NORTH-WEST OF SORIA (SPAIN): PRESENT ECTOMYCORRHIZAE

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KEY WORDS: ectomycorrhizae, ecology, population biology.

Black truffle sporocarp production is determined, amongst many other factors, by an optimum mycorrhization degree in the roots of the host-tree. This process is triggered in balance with other fungal species that does not inhibit its sporocarp production. So, there is an ectomycorrhizal fungal community associated with the black truffle sporocarp productive host-trees that runs as any other living beings community, producing a certain fungal biodiversity and establishing connections in dynamic balance that will evolve. The absence of black truffle sporocarps production in some host-trees will be determined by its ectomycorrhiza absence, or because between the fungal community there are one or more species that inhibit this process or displace it.

With the aim of study those topics, the ectomycorrhizae present in 23 adult holmoak trees (*Quercus ilex* subsp. *ballota* (Desf.) Samp.) of seven *Tuber melanosporum* Vitt. productive areas in the North-West of Soria (inner Spain) are characterized and quantified. During the spring and the autumn of 1999 and 2000, black truffle productive and non-productive holmoaks were studied following the global method (Verlhac et al. 1990, La truffe guide pratique). Ectomycorrhizal types were characterized following the guidelines of Agerer (1999).

Tuber melanosporum, *T. aestivum* Vitt., *T. brumale* Vitt., *Cenococcum geophylum* Fr., *Pisolithus arrhizus* (Scop.) Rauscher, *Cantharellus tubaeformis* (Bull.) Fr., *Hebeloma* cf. *sinapizans* (Fr.) Sacc., *Tomentella galzinii* Bourdot, AD type, *Cortinarius* sp., *Hebeloma* sp. and *Scleroderma* sp. and many others Telephorales, Tuberales and Boletales ectomycorrhizal types were found.

Tuber melanosporum mycorrhizae are present both in productive and non-productive host-trees, as it happens for *T. aestivum*, while *T. brumale* ectomycorrhizae are only present in non-productive holmoaks. The rest of identified ectomycorrhizal types are present in productive and non-productive host-trees.

LIBYAN DESERT TRUFFLES

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KEY WORDS: ecology, desert truffle, *Terfezia, Tirmania*.

Desert truffles (hypogenous ascomycetes) are grown naturally in North Africa (Libya, Tunisia, Morocco) as well as in Kuwait, Bahrain and in Spain. In Spain, successful cultivation of *Terfezia* has been accomplished recently. Libyan desert truffles grow naturally in many parts of the country especially in Hamada al Hamra region where truffles are highly appreciated as a delicious food. Desert truffles are a kind of mycorrhizal fungus grown in association with the roots of the host plants such as *Helianthemum* spp., locally truffles are known as Terfasa and as A-Kamaa or Al-Faga in Qatar and Kuwait. Truffles usually appear in Libya after the rainy season in autumn between February and April. The price of the truffles increased dramatically in the local markets because the collectors exported their collected truffles to other countries such as Italy. The price of the desert truffles in international markets varied between 50 Euros to 250 Euros depending on the season and on the quality. Two genera of truffles are found in Hamada Hamra region *Terfezia* (known as red or black truffle) and *Tirmania (*known as white truffle) in this season (2007). The class of the soil habitat of the Libyan truffles was sandy loam with a pH over 8. Libyans consider truffles as a good source of protein and have used them as a substituent for meat in the old days. The chemical composition analysis showed that both truffles contained a good quantity of protein similar to meat. *Terfezia* contained 19% protein which was higher than *Tirmania* (16%).

TUBER MELANOSPORUM GROWTH-STIMULATING EFFECTS FROM VARIOUS CISTACEAE SPECIES

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KEY WORDS: *In vitro* cultivation, *Cistus*, *Tuber melanosporum*, transformed roots

ABSTRACT

In vitro culture of slow-growing ectomycorrhizal fungi can be fastened through dual (monoxenic) culture with its own natural hosts, as it has been already demonstrated by using *Agrobacterium rhizogenes*-transformed root organ cultures to stimulate growth of *Tuber melanosporum* (Wenkart *et al*., 2001; Coughlan *et al*., 2001) or *Terfezia boudieri* (Kagan-Zur *et al*., 2003) mycelia. Most studies are currently limited to a single host species: *Cistus incanus*. By transforming seedlings of *Cistus* and *Helianthemum* with *Agrobacterium rhizogenes* strain A4, we demonstrated they also stimulate *in vitro* mycelium growth of *Tuber melanosporum*. *Helianthemum* transformed roots served as a control, indicating the stimulation is due to taxa-specific compounds more likely to be present in common truffle-mycorrhizal species.

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TRUFFLE CULTIVATION AND PRODUCTION IN A 5-YEAR OLD *TUBER AESTIVUM* VITTAD. PLANTATION

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KEY WORDS: Cultivation, *Tuber aestivum*, production results

Up to the middle of the eighties, the truffle cultivation focused on plants mycorrhized with the most valuable truffle species, *Tuber magnatum* Pico and *Tuber melanosporum* Vittad.; no technical analyses of the land truffle production potentialities were undertaken. Over the last decades, both the planning and the cultivation of a truffle plantation have evolved, thanks to the scientific research results on the truffle ecology and on their cultivation.

The present work highlights a truffle cultivation of *Tuber aestivum* Vittad. located in Umbria (Terni) and made in 2003. Before carrying out the truffle plantation, the land owner has contacted a technical expert, who has analysed the area, to choose the right plant-truffle combination to cultivate and to identify the better cultivation techniques to be applied before and after bedding out the plantlets. The truffle plantation comprises 94 truffle-producing plants: *Quercus pubescens* Willd., *Quercus ilex* L., *Quercus cerris* L., *Ostrya carpinifolia* Scop. and *Corylus avellana* L., mycorrhized with *T. aestivum* and certified. Over the years, plants have been watered and pruned, while the soil around them has been weeded, covered on the surface, tilled, and inoculated with *T. aestivum* spores. The truffle plantation has started producing in 2007, and it has also produced in 2008, with a production of about 5 kg of truffle.

It is necessary that the new truffle plantations will be plant after a careful technical analysis, in order to favour the truffle production of the cultivated truffle species.

UNIVERSITY OF PERUGIA: 20 YEARS CHECKING AND CERTIFYING TRUFFLE PLANTS

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KEY WORDS: mycorrhization, *Tuber*, truffle cultivation.

Since the 1980's the Department of Applied Biology at the University of Perugia has dealt with checking and certifying single mycorrhized plants and large quantities of plants from several nurseries in Italy and France. In the beginning, the evaluation was carried out using the morphological method by various researchers on behalf of the Ministry of Agriculture and Forestry and the Forestry Corp in 1987. Then, in 1995, a group of researchers was requested by the local governments of 10 Italian regions to elaborate a new method, easier than the previous, to evaluate truffle plants. Since October 2007, the Department has checked and certified all plants, following the regional law n. 8 of 16.7.2007, using a morphological and molecular method of identification. The Department has drawn up agreements with many nurseries, checking approximately 80000 plants per year. Furthermore, the agreements with the nurseries also include the Department giving scientific and technical advice to improve the process of mycorrhization of truffle plants.
Other activities carried out by the Department are:

– checking and certifying root samples from truffle orchards to evaluate the situation, and/or carrying out expert analyses of these upon official request
– checking and certifying the fresh and preserved truffles and processed products, and again analyses upon official request.

Finally, the knowledge acquired in more than 30 years of studies and research has shaped the development of modern truffle cultivation.

THE POTENTIAL PRESENCE OF TRUFFLES IN VALLO DI DIANO AREA (SALERNO)

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KEY WORDS: Truffles, Vallo di Diano, *Tuber mesentericum,* GIS.

It's usually considered that the presence of truffles in Campania is reduced only to *Tuber mesentericum* Vittad., most of all produced and eaten in the area of Bagnoli Irpino. This theory is linked to the laking of scientific and popular works about truffles for this region.
However, the operators know that also the southern regions of our Country are great suppliers of large amounts of truffles for ages.
The presence of different species of truffles checked by collectors and mycologists in the "Vallo di Diano" area (Salerno), could represent an important chance of rural development for these territories.
In this area it was performed a cartographic survey with GIS software approach which had the aim to better understand the potential distribution of truffles wild production in forest environment and the localization of the most appropriate areas of cultivation of the different truffle species.
The thematic maps of Vallo di Diano have been studied, with reference to the geolitological map, the Corine Land Cover map (CLC 2000) and the Digital Elevation Model map (DEM).
The areas with appropriate ecological features for the different truffle species have been delimited in each map collected. Then these maps have been overlaid to estimate the areas where there was the concomitance of the appropriate environmental factors for each species.
The result of the research gave the chance to build a *potential truffles' presence map* for each truffles' species. In these maps is possible to notice that *T. aestivum* Vittad. is the most present and cultivable species followed by the *T. aestivum* Vittad. forma *uncinatum* (Chatin) and the *T. mesentericum* Vittad.. On the contrary *T. borchii* Vittad., *T. magnatum* Pico and *T. melanosporum* Vittad. are localized in smaller areas.
This research shows that "Vallo di Diano" area can be considered as a potential truffles' presence territory both for wild and cultivated truffle production.

FLAVOUR PROFILE OF THE *TUBER MELANOSPORUM VITTAD.*

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KEY WORDS: Sensorial Analysis, Truffle, *Tuber melanosporum Vittad.*

The Association Mondo Tartufo

The Mondo Tartufo Association was born in 2007 with the purpose to promote, plan, coordinate and develop study activities, research, documentation and realization of projects finalized to the spreading of the knowledge of truffle.

Among the various developed activities, Mondo Tartufo has created a Panel Test finalized to the recognition of the main describers that characterize every single typology of truffle, as the *Tuber melanosporum Vittad*. In order to make this, the Association has used the Sensorial Analysis.

SENSORIAL ANALYSIS

The Sensorial Analysis is a technique allowing an evaluation – through our sensory organs – of the products intrinsic value. The links created between the brain and the external receptors let us taste the different kinds of food, analysing them and describing their features: it is then possible to tell what the senses let us explore. The ability to detect smells and tastes belongs to the concentration of the element that defines such smells and tastes, but also to the experience: a product being analysed for a long time let us train our sensory memory, which keeps its knowledge.

Today many techniques are available, able to define to chemical level the presence of determined substances in the truffles, but only through training and passion for truffle, we become expert tasters, able to appreciate all the sensory interactions that no other professional instrument is able to give us.

Studies and searches have been effected in order to define the sensory profile of every typology of truffle. To this intention Mondo Tartufo has elaborated a card of evaluation that takes into consideration all the aspects involved in the evaluation of this appreciated tuber, describing some for every typology the sensory profile.

DESCRIPTION OF THE CARD

The evaluation cards take into consideration the three basic aspects for the sensorial analysis of the truffles: visual examination, tactile examination and olfactory examination. The evaluation of the gustatory examination is not considered in this phase, because it would be destructive and therefore the truffles, after such evaluation, could not be marketed anymore.

The most representative describers have been identified. They concur to determine the sensory profile of the *Tuber melanosporum Vittad*. and based on their presence and interaction with the other components the quality of the analysed truffle is determined.

Some of the parameters we considered are: the intensity of the aromas, the fineness or elegance, the complexity and the description of the single aromas that take part in the formation of the bouquet.

SOIL SUITABILITY TO TRUFFLE IN PIEDMONT REGION: METHODOLOGY AND RESULTS

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KEY WORDS*: Land evaluation; soil maps; cultivation*.

This work, financed by Piedmont Region, concerned *Tuber magnatum, T. melanosporum and T. aestivum*. Suitability maps to truffle were realised firstly at 1:250.000 scale for the overall region, secondly at 50:000 scale for a hilly area (500.000 hectares) where truffle harvest is practised.
The methodology used to produce suitability maps can be summarised as it follows.

– Bibliographical research.
– Interpretation of aerial photograph and remote sensing images on surveyed land.
– Land Units characterisation and drawing on topographic maps, according to main features (geomorphology, lithology, land use).
– Pedological survey by soil profiles (120) and cores (700); chemical-physical analyses on 400 samples; data were integrated by pre-existing data from the regional soil database.
– Definition of the main Soil Types and classification according to Soil Taxonomy (USDA-1999).
– Field verification of Land Units and Soil Types.
– Realisation of an evaluation table for the attribution of FAO Land Suitability Classes.
– Printing of the Land Units map.
– Printing of the Suitability Maps to the three species of truffle, according to the evaluation table of their specific environmental requirements, created by a synthetic work from bibliography and field experiences both.

In this evaluation table variability of dominant soils characteristics was classified according to the different pedo-environmental requirements, in order to attribute every Land Unit to FAO suitability classes (FAO Land Evaluation, 1976 and 1983).
Land was consequently divided into 4 suitability classes (high, moderate, low, very low), which were attributed to the three different truffle species, so that 3 specific maps were created, at 1:250.000 and 1:50.000 scale both.
The two maps show a complete frame of the truffle productive potential of the overall region, according to the resolution limitations of the reference scales.
It must be underlined that these maps show the truffle productive potential, and not the present use and management, which has a strong influence beside a land suitability evaluation.

TRUFFLES IN THE ENVIRONMENT: THE MOLECULAR ECOLOGY OF *TUBER*

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KEY WORDS: ecology, mycorrhizal community, *Tuber*

Truffles belonging to the genus *Tuber* are mycorrhizal fungi characterized by hypogeous fruitbodies. Molecular-based studies of mycorrhizal community composition in various habitats have documented *Tuber* mycorrhizae in many environments including floodplains, clearcut regeneration, tree nurseries, and orchards. However, it is often difficult or impossible to identify the particular species of interest because of a lack of (or biased) sampling of genetic diversity within this genus and taxonomic confusion residing in public databases. Work in our lab to resolve the phylogenetic relationships within *Tuber* has resulted in DNA sequencing from a large number of identified *Tuber* species representing a most of the known phylogenetic diversity of this genus. In this study we use a phylogenetic framework to place and identify a large number of "unidentified" *Tuber* sporocarps and mycorrhizae using ITS sequences from our own studies, Genbank, and colleagues. Our results show that the most commonly found mycorrhizae from *Tuber* species are from non-economic species in the Rufum, Puberulum, and Maculatum clades. Using molecular-based identification, we were able to taxonomically place 39 unrepresented phylotypes of *Tuber* including 9 known only from mycorrhiza.

EFFECTS OF SOIL MANAGEMENT ON THE EVOLUTION OF YOUNG PLANTS MYCORRHIZED WITH *TUBER MELANOSPORUM*

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KEY WORDS: truffle cultivation, mycorrhization, organic matters.

Soil management of young truffle plantations is a recurrent problem. In this trial, we study the effects of different crop management techniques on the evolution of *T. melanosporum* mycorrhization and on soil quality (organic matter and microbial biomass). The plot is located on the 'Causse de Martel' (Lot – Aquitaine region). The sub-soil is a hard cracked limestone (Jurassic period), with red clay pockets and a shallow stony surface (5 to 15 cm). The texture is silt loam-sandy-clayey with a C/N ratio of 8.9 and 5.1% organic matter. The plant material consists of controlled one-year-old oaks (*Quercus ilex* and *Quercus pubescens*) inoculated with *T. melanosporum.* The trial studied 4 treatments without repetitions (comparison test): mechanical tillage, chemical weed control (glyphosate), plastic mulch (black polypropylene weaving) and waterproof ensilage plastic much (plant density 1.5×10 m). The plantation was carried out in March 2002. Tree growth was measured each year. In 2007, organic and biological analyses of one soil sample per treatment were performed. A mycorrhization study was carried out, in 2008, on 15 trees (3 samples/tree) to compare the effect of soil management on the mycorrhizal development (morphological identification). After 6 years, the results show that the growth of *Quercus ilex* trees is greater than the growth of *Quercus pubescens,* whatever the soil management method used. For both oak species, the 2 treatments of plastic mulch give a greater trunk diameter than the chemical weed control. The least growth was observed under mechanical tillage. Compared to the grassed interrow, the results of soil analysis show that organic matter mineralisation is greater under the plastic mulch, particularly with the polypropylene material, under which the ratio fine organic matter/total organic matter is the lowest. Mechanical tillage has also an important impact on organic matter mineralisation. The mycorrhization analysis show that the *Q. pubescens* are well associated with *T. melanosporum,* while *T.brumale* is more frequent on *Q. ilex* root samples. Other mycorrhizal species developed on the root system: *Coenococcum* sp. and *Scleroderma sp*. on pubescence oak, and *Basiomycete* and "unidentified species" on holm oak. For *Quercus pubescens*, the mechanical tillage treatment provided the highest *T. melanosporum* frequence and the lower contamination level while the polypropylene mulch show a good *T. melanosporum* conservation but an important colonization with others ectomycorrhizal.
During the winter 2007/2008, the first truffles were harvested 5 years after plantation under evergreen oak with plastic mulch (4 trees produced *T. brumale* and *T. melanosporum*).

DEVELOPMENT OF BIOTECHNOLOGICAL TOOLS IN *TUBER B0RCHII*

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KEY WORDS: genomic and cell biology, vectors for transformation, EGFP, *Agrobacterium tumefaciens*.

During the last 10 years *Tuber* spp. have been subjected to a fairly extensive bio-molecular analysis. This included the cloning of several genes set up of in vitro culture conditions for mycelia and mycorrhizae, the development of a molecular phylogenetic framework plus species-specific identification tools, and a variety of gene expression studies. The latter led to the discovery of biotechnologically interesting genes as well as to the identification of regulatory traits that may be unique of the symbiotic lifestyle. Recently, initial steps toward a functional genomics analysis of these commercially and environmentally important fungi have been undertaken. Among these, the set up of an *Agrobacterium*-mediated transformation system for *Tuber* mycelia, based upon binary vectors bearing the *hygromycin resistance* and the *enhanced green fluorescent protein* genes as selection markers. Each of these genes has provided some piece of novel information and new hypotheses on truffle biology and metabolism. For example, the identification of a photoreceptor (TbWC-1) homologous to the well characterized *Neurospora crassa* NcWC-1 and the discovery of blue light controlled morphogenesis in an underground, conceivably "dark-growing" fungus has raised new scenarios on *Tuber* growth in general. A first step in this direction has been the recent development of a prototype genetic transformation system in *Tuber* (Grimaldi *et al.*, 2005 Curr Genet, 48:69-74). This relied upon *in vitro* cultured *Tuber* mycelia, a hypervirulent *Agrobacterium tumefaciens* strain (AGL-1) and on the binary plasmid pBGgHg, a pCAMBIA1300 vector bearing the hygromycin resistance (*hph*) and the enhanced green fluorescent protein (*EGFP*) genes under the control of the *Agaricus bisporus* GAPDH promoter and the CaMV 35S terminator. Following co-cultivation with *Agrobacterium* and hygromycin selection, ~20% of the treated mycelia became partially hygromycin resistant based on their appearance and on the results of fluorescence microscopy and PCR analyses, these outgrowing hyphae (~1% of which were EGFP fluorescence-positive) were interpreted as localized, yet heterogeneous regions of hygromycin resistance due to unequal penetration of the transgene and to the multinucleate nature of the starting mycelia. Low transformation efficiency and a highly heterogeneous population of untransformed and transformed hyphae are the main limitations of this procedure. Various ways to overcome such limitations, some of which are actively pursued will be presented.

PROGRESS TOWARD CULTIVATION OF *TUBER AESTIVUM* IN THE CENTRAL USA

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KEY WORDS: *Tuber aestivum* cultivation, seedling production, ecology and plantation population biology, *T. lyonii*, *T. rufum*, *T. maculatum*, and *T. whetstonense*.

Our research with the Burgundy truffle (*Tuber aestivum* syn. *T. uncinatum*) began in 2002, with an experiment that showed that the type of lime used to raise the pH of greenhouse potting mix can affect the growth rate of pedunculate oak (*Quercus robur*) root systems inoculated with certain selections of Burgundy truffle. Seedlings inoculated with one truffle selection produced larger root systems with more truffle mycorrhizae in substrate modified with crushed dolomitic limestone in comparison with high-Ca pelletized quick-release lime. Seedlings inoculated with another truffle selection experienced no inhibitory effects of high-Ca pelletized lime. In the same experiment, supplemental root dip inoculation at the time of planting into infested potting mix did not improve levels of colonization beyond those accomplished by ascospore infestation of the potting mix.
In a subsequent study, we evaluated the effects of three different potting media and two different container systems on the colonization of *Q. bicolor* x *Q. robur* seedlings by the Burgundy truffle fungus. Seedlings grown in book-style containers using a peat-based medium were smaller yet much better colonized than seedlings produced in RPM (Root Production Method) containers using a ground-bark-based RPM medium. In either container system, the peat-based medium supported development of more truffle mycorrhizae than did the RPM medium. Seedlings grown in book planters developed significantly more root tips l^{-1} potting mix than did seedlings grown in RPM containers. The optimum pH for truffle mycorrhiza development in the peat-based medium was between 6.7 and 7.5. We subsequently monitored the ectomycorrhizal community composition for two years after seedlings were transplanted into a field site that had been prepared by lime applications. Contaminant greenhouse ectomycorrhizal species declined to low levels after outplanting. After two years in the field, both *T. aestivum* colonization levels and indigenous ectomycorrhizal species richness and abundance increased, indicating that indigenous species, in the short term, did not displace *T. aestivum*. Among the indigenous ectomycorrhizal fungus species detected as ITS sequences from seedling mycorrhizae were four hypogeous fungi previously unknown from Missouri: *T. rufum*, *T. lyonii*, *T. maculatum*, and *T. whetstonense*. To date, only *T. lyonii* has been found fruiting at the plantation site, but a *T. rufum* ITS sequence identical to a plantation seedling-derived sequence has been recovered from mycorrhizae beneath a *Q. alba* tree in the adjacent forest. In addition, an apparent *Genea cazaresii* sequence was derived from an ectomycorhiza collected beneath a *Q. rubra* tree.

THE SOILS OF NATURAL TRUFFLE-GROUNDS IN ABRUZZO

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KEY WORDS: ecology and population biology, tuber, soil, soil evaluation.

In order to construct the Map of the Potential truffle producing earth in the Abruzzo Region to 1:250.000 scale [1], over 1000 sites of naturally occurring truffle-grounds were explored to study their ecological characteristics. Of these, 200 sites of *Tuber melanosporum* Vittad. and *Tuber magnatum* Pico were selected in which, beyond the environmental and vegetational characteristics, the types of soil were revealed. The study of soils was realized through the excavation of 190 mini pits and 10 profiles were chosen among the most representative sites.
In every mini pit and profile the soil was described and classified according to the methodologies adopted for the Map of Soils in Italy in 1:250.000 scale and the principal chemical-physical properties were determined. In the soil profiles a micromorfologic analysis of the functional horizons was done by means of interpretation of thin sections. The soils were subdivided and analyzed by type of truffle studied and the most important pedologic characteristics necessary for the development of the two species were verified.
From the study it was discovered that common soils exist and that these belong to a limited number of typical subunits of the soils present in the Abruzzo region.

[1] Research conducted as part of the "Map of the truffle vocation of the Abruzzo Region" project, cosponsored by the Abruzzo Region, within the triennial programs "Interventions of forestation and environmental development", 2001-2003 and 2004-2006 of the ARSSA through the Technical Assistance Programs of 2001-2002-2004-2005.

MICO PLANTS TRUFFLE CULTIVATION

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"Mico Plants" truffle cultivation is a family-run Garden Centre situated in S. Demetrio of Vestini AQ – Abruzzi – that offers its very high experience in the field of truffle cultivation. The company's main activity is the production of some mycorrized plants for truffle plantations.
The business philosophy is oriented towards the preservation of the Abbruzzi truffle germoplasm; this is the reason why mycorrhized plants are obtained from certified seeds and truffles both coming from the Abruzzi. Upon request, mycorrhized plants obtained from seeds and truffles of other Italian Regions can be produced.
Since the year 2000, the product certification of the Garden Centre is issued by the Department of Applied Biology of the University of Perugia, that certifies carpophores used for the inocula and truffle-producing plantlets. Certification is based on carpophore morphological analysis and on mycorrhizal morphological and bio-molecular studies.
Production features.
The Garden Centre assortment comprises:

– *Quercus pubescens – x T. melanosporum – x T. aestivum*
– *Quercus ilex – x T. melanosporum – x T. aestivum*
– *Corylus avellana – x T. melanosporum – x T. aestivum*
– *Ostrya carpinifolia – x T. melanosporum – x T. aestivum*

After the inoculum, plantlets are bred in cells characterized by a dimension of 7×7×17 – 9×9×13 – 11×11×17 cm, made of a substrate represented by corrected and sterilized natural soil. In this way plantlets not only have an elevated degree of mycorrhization, but also a strong and expanded root apparatus.
Truffle plantations obtained with our mycorrhized plants, on suitable sites, are already productive.

MAPS AND DATA BANK OF TRUFFLE HABITAT IN ABRUZZO (CENTRAL ITALY)

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KEY WORDS: ecology truffle habitat, *Tuber magnatum*, *Tuber melanosporum*, *Tuber aestivum*, *Tuber uncinatum*, *Tuber borchii*.

For a long time Abruzzo has been considered a place of interest concerning truffles by a few employed in this sector, in fact, only in the last years, the remarkable vocation of our land is largely perceived and recognized on extraregional field, too.
So it arose a necessity of protecting this important natural patrimony, from the immoderate and irrational exploitation, with a greater awareness of the zones potentially suitable for the production of truffles, evaluating the referring to each species investigated, and the location on the territory.
Joining all the experiences that professional searchers have about their area, we carried out a careful survey, individualizing, through 'GPS' system, over thousands of sites that are concerned with the four most important species: *Tuber magnatum, Tuber melanosporum, Tuber aestivum and/or uncinatum, Tuber borchii.*
The researches for the GIS elaboration were done putting on the information of the sites that were indicating the truffle-grounds, with other knowledge concerning soil science, geomorphology, climate and vegetation.
The project has been done in 5 years and a thematic map has been drown, in scale 1:250.000, for the 4 species investigated. In addiction, for the value that the two most esteemed species have, we have carried out the widening of the inquiry with more detailed cartographies, in scale 1:100.000.
The information obtained shows the remarkable extension and the location of the areas where the production of different species of truffle naturally occurs. This knoledge represents the start to defend and improve them. Moreover, the amount of the data-bank of recorded dates is an important reference point in the design of a cultivation plan.

Note: This is a research carried out in the field of the project "Carta della vocazionalità tartuficola della Regione Abruzzo" financed by Regione Abruzzo, in the sector of "Interventi di forestazione e valorizzazione ambientale" Triennal plans 2001-2003 and 2004-2006, and by ARSSA, through "I Programmi di Assistenza Tecnica" 2001-2002-2004-2005.

FDR (FREQUENCY DOMAIN REFLECTOMETRY) TECHNOLOGY FOR SOIL MOISTURE DETECTION IN PRODUCTIVE TRUFFLE ORCHARDS

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KEY WORDS: FDR, Diviner 2000, *Tuber*, soil moisture, dielectric constant.

The productivity of truffle cultivation of *Tuber melanosporum* Vittad. and *Tuber aestivum* Vittad. is contingent on the climate, especially rainfall and snowfall. Irrigation is an essential practice to stabilize production to the highest values (70-120 kg\ha). The water uptake positively affects the number and size of truffle fruit bodies, but may encourage the emergence of other mycorrhizal species more suited for moist and fresh micro-habitats.

In this research, we studied changes in soil moisture in a *T. melanosporum* and in a *T. aestivum* truffle orchards located close to Spoleto.

Data were collected weekly from July 2007 to September 2008, every 0.1 m and up to a depth of 0.8 m.

In the black truffle plantation, data were recorded in 2 productive and irrigated sites, and 2 productive non-irrigated sites.

In the summer truffle plantation, measures were carried out in 2 productive and hoed sites, and in 2 productive but not hoed sites.

The measurements were made using the *Diviner 2000* (Sentek Pty Ltd., South Australia) which detects the soil water content through frequency domain reflectometry (FDR) technology.

FDR systems are based on propagation speed difference of high-frequency signals (greater than 100 MHz) in the soil that consists of solid particles, air and water. These differences are due to dielectric constant (ε) fluctuations, determined by changes in soil water content (for water $\varepsilon=80$, for dry land $\varepsilon=4$ to 8 and for air $\varepsilon=1$ at 20 °C).

Every frequency measure recorded by the instrument is then converted in volumetric water content by applying an appropriate calibration equation. For this work, the equation proposed by the manufacturer (Default Sentek Calibration) has been applied, which gave very satisfactory results in terms of relatives changes of soil water content, although lacking in the respect of absolute values.

The results show that changes in soil moisture content are closely tied to the soil features of the site. The soils of *T. melanosporum* orchard show a steadier and more uniform moisture pattern than those of the *T. aestivum* plantation.

The summer truffle, as opposed to the black truffle, bears fruit bodies even in soils where strong soil water content excursions are present, confirming its adaptability to different environmental conditions.

The weeding generates large moisture fluctuations in the soil surface layer. This upper layer responds with rapid increases to rain events, but shows also quick stages of draining; the charging effect of a rainfall is exhausted more quickly than in deeper soil layers.

BOTANICAL AND ENVIRONMENTAL ASPECTS OF THE NATURAL TRUFFLE BEDS

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KEY WORDS: ecology, *Tuber magnatum*, *Tuber melanosporum*, flora, vegetation.

Each species of truffles presents a specific ecology. The environment where grow *Tuber magnatum* Pico and *T. melanosporum* Vittad. are quite different in terms of climate and botanical aspects. The natural truffle beds of *T. magnatum* are located in two most situations: woods slopes and riparian vegetation. In any case these truffle beds require an undisturbed environment where the structure of vegetation is complex, with all layers present and total coverage. On the other hand, the growth environment of the natural truffle beds of *T. melanosporum* is characterized by a dynamic vegetation: open, dry and sunny, with a low coverage. In a research carried out between 2006 and 2008 in the territory of Rieti Province (Central Italy), a lot of natural truffle beds of the most important species of truffles are analysed from different point of view. The aims of this research were to increase the knowledge about pedological, botanical and ectomycorrhizal aspects of natural truffle beds, to preserve and manage this important resource and moreover to improve the truffle cultivation. Here are reported the preliminary results about the natural truffle beds of *T. magnatum* and *T. melanosporum*. The phytosociological data indicate that the sites of *T. melanosporum* are included in the series of *Quercion pubescenti-petraeae*, *Lonicero-Quercion pubescentis* and *Laburno-Ostryon*. About *T. magnatum* the interested series are *Salicion albae* and *Alno-Ulmion* in the riparian environment, while *Aquifolio-Fagion*, *Tilio-Acerion*, *Lonicero-Quercion pubescentis*, *Ostryo-Carpion orientalis* and *Quercion pubescenti-petraeae* in the hilly woods. The floristic data show several endemic taxa: that means the ecosystems are characterized by a good level of conservation, without an important human impact. The biological spectrum shows an high percentage of Hemicryptophytes, followed by Therophytes in *T. melanosporum* and by Phanerophytes in *T. magnatum*. The chorological spectrum shows a prevalence of Mediterranean s.l. chorology for *T. melanosporum* and Paleotemperate for *T. magnatum*. These knowledge are important to support programs of conservation, management of natural truffle beds to increase the production level and suitability of the environment for productive truffle cultivation.

Research carried out within the "Le aree ed i suoli della Provincia di Rieti vocati alla coltivazione delle principali specie di tartufi eduli (TUBERI)" project, financied by Italian Ministero delle Politiche Agricole, Alimentari e Forestali, Coordinator Dr. M. Raglione, CRA – Istituto Sperimentale per lo Studio e la Difesa del Suolo di Rieti, Research Unit of Centro Appenninico "C. Jucci" del Terminillo – University of Perugia.

RHIZOSPHERE MICRO-ORGANISMS UNDER POPLAR AND OAK PLANTATIONS WITH DIFFERENT PRODUCTIVITY OF *TUBER MAGNATUM*

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KEY WORDS: ecology and population biology, white truffle.

Proves of specific relationships between soil micro-organisms and plant roots are so evident that a new word has been coined, 'rhizosphere', which indicates soil microbic flora (fungi and bacteria) placed near the root apexes. *Tuber magnatum* fructification is still a mysterious phenomenon, maybe influenced by micro-environmental factors among which the 'rhizosphera'. This research has the aim to prove, by a quali-quantitative comparison, hypothesised relationships between the 'micorhizospheres' of a poplar plantation and those of an oak plantation. In the Cuneo province two natural truffle-grounds were chosen as study-sites so far enough to be not reciprocally influenced (Priero oak plantation and Monchiero poplar plantation). Interesting, even if still preliminary, results were obtained by microbiological isolation, carried out using the 'subsequent dilutions method', more effective than the "rhizospheric soil direct incorporation technique". At Priero oak plantation the bacterial intensity was higher than at Monchiero poplar plantation, with greater values referred to the productive site. No relations were detected between the fungal intensity and *T. magnatum* productivity, even if at the oak site mean values were slightly higher. Qualitative differences of fungal flora were demonstrated to be dependent on study-area, host plant species and *T. magnatum* productivity.

At Priero site thirty-five fungal forms were isolated with prevalence from the unproductive area. Six of these forms seem to be dependent on productivity (*Gliocladium viride*, *Penicillium nigricans*, *Acremonium* sp., *Penicillium terreus* etc.), vice versa others were isolated only on the rhizospheric soil of the unproductive oak site (*Aspergillus terreus*, *Monilia* sp., *Dictyuchus* Leitg., *Gliocladium roseum*, ecc.). At Monchiero site thirty-one fungal forms were identified, among which eleven seem to be dependent on the poplar site productivity (*Aspergillus terreus*, *Gliocladium roseum*, *Penicillium nigricans*, *Aspergillus niger*, etc.), vice versa other thirteen were isolated from the 'rhizosphere' of the unproductive species (*Mucor* sp., *Penicillium albicans*, *Aspergillus ochraceus*, *Rhizopus stolonifer*, *Trichurus spiralis*, etc.). Comparison of qualitative data from all the samples is a difficult operation as some 'mycetes' (i.e. *Penicillium albicans*), are ubiquitous and not correlable to host plant, productivity and site location. Few fungal forms seem to be dependent on productivity as they were found out only under productive oak and poplar (rhizoctonic mycelium, *Penicillium nigricans*). Finally the presented preliminary results about 'rhizosphere' telluric micro-organisms are encouraging research continuation.

This work was realised by Piedmont Region financing.

FIELD RESPONSE OF *QUERCUS ILEX* SEEDLINGS INOCULATED WITH *TUBER MELANOSPORUM* PRODUCED BY THREE DIFFERENT COMMERCIAL NURSERIES

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KEY WORDS: Mycorrhizal colonization level, fine roots, truffle cultivation.

The objective of this work was to evaluate the field response of *Quercus ilex* (holm oak) seedlings with various levels of *Tuber melanosporum* mycorrhizae after 18 months in 3 different plantation sites. The seedlings, produced by 3 different commercial nurseries, were evaluated prior to outplanting to determine the numbers of total fine roots, non-mycorrhizal roots, *T. melanosporum* mycorrhizae, and non-*T. melanosporum* mycorrhizae. After 18 months in the field, plants from each treatment were carefully dug-up from each plantation and the roots were divided into 2 groups: old roots from the initial nursery plug and new roots, which had developed outside the plug. We measured root length of the taproot, root length of new roots, total fine roots, mycorrhizal levels, and plant height. Seedlings from Nursery A had significantly greater numbers of *T. melanosporum* mycorrhizae prior to ouplanting than seedlings from the other 2 nurseries. Eighteen months later we observed no differences in the number of truffle mycorrhizae in the original root plug based on nursery source. Seedlings from Nursery C had developed greater numbers of truffle mycorrhizae as well as greater numbers of fine roots in the field-developed new roots. Mycorrhizae colonization level is important for successful truffle plantations but not the only prognostic factor for successful truffle plantation establishment.

THE TRUFFLE TERRITORIAL SPECIALIZATION IN AN INNER AREA OF THE CENTRAL APENNINE USING GRAVITY MODEL APPROACH

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KEY WORDS: conservation, commerce and valorization.

The truffle cultivation has interested, for a long time, the areas located near the Italian Apennine, becoming an element of multifunctionality and farm diversification, able to reduce the marginalization of the rural areas.
This research has studied in an Italian inner highland area, as the province of Rieti, during a long period (1990-2006), the territorial specialization of truffle cultivation; the aim of this research was to appraise the existence of some indispensable conditions for the definition and the recognition of the rural district by the political administrations. In the first part of this research it used a simple model to describe the truffle territorial specialization, as the modified index of Balassa-Hoover; in this case the specialization index was modified with the aim to standardize the statistical data in a dimensionless variation range of ±1. In the communes of the area of study, close to the Apennine mountain ridge, some values of index of specialization have been significant, that have strengthen their position inside the rural district, even if, it has observed a substantial decrease of the authorized truffle pickers during the second period of observation.
The following phase of this research has been characterized by the application of a linear gravity model, used in physics, transformed with the least ordinary squares; in this case it has been possible to estimate a linear equation that connected, in all the communes of the study area, the farm multifunctionality, represented by the dependent variable truffle pickers, with a lot of independent variables linked to the rural and socioeconomic development of the territory, highlighted the existence of some significant relationships in the statistical model. The results have affirmed as the truffle can represent a variable able to increase the socioeconomic development of the rural areas, reducing the phenomenons of marginalization. The analysis has shown as in the next years it should intervene on the rural territory with some specific cultural paths, represented by the ways of taste, and by shared marketing actions, that are able to reduce the economical crisis of rural communities, to reduce the conditions of economic isolation of rural areas and to put the truffle from a market of niche to a wide market economically interesting, utilizing completely the advantages of the rural district, shortening the brokerage costs and the chain and exploiting the territory and the rural peculiarities of the multifunctionality.

NEW DATA ON IMPACT OF EARTHWORMS ACTIVITY ON BLACK TRUFFLE SOILS

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KEY WORDS: Truffle ecology, lime movement, earthworm casts, truffle culture.

Among soil invertebrates, the earthworms (Annelida, Oligochaeta) are unique in their ability to integrate physical, chemical and biological processes in the soil ecosystem (Edwards and Bohlen 1996; Shuster *et al.* 2002). Several authors have outstanding the research on earthworm feeding ecology and its implications for nutrient cycling and soil fertility. However Curry and Schmitd (2007) remarked that many aspects of the interactions between earthworms and soil mineral constituents are still poorly understood.

The amount of carbonates in soils depends on soil forming factors such as relief, biological factors, human and natural disturbances, time and climate (Jenny 1941; Breemen & Buurman 1998). Chan (2003) explained that soil carbonate mobility is related with the direct action of earthworms and other soil fauna, and demonstrated the earthworm ability to incorporate lime into subsoil in the calcareous amendments applying to ameliorated acid soils where the incorporation by tillage is not feasible.

On truffle research, many authors pointed that black truffle (*Tuber melanosporum* Vittad.) production is closely linked to calcium carbonate soil availability and mobility on overall soil profile. Callot (1999) empathises the currently study on the relationship of cause and effect among earthworms activity, pH of the soil and development of black truffle. Lulli *et al.* (1999) and Castrignano *et al.* (1999) also point out that black truffle require considerable soil porosity originated by biological activity, above all, by earthworms and ants.

The objective of the present study is to analyse the pH, carbonate fractions and abundance of total organic carbon (TOC) in a representative number of soil samples from *Tuber melanosporum* burns and earthworm cast samples, to establish statistical patterns on these soils parameters and to analyse the impact of earthworms activity on black truffle soils.

OPTIMAL METHOD FOR CULTIVATING *TUBER MELANOSPORUM*

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KEY WORDS: *Tuber melanosporum* Vittad., soil, mycorrhized trees, watering, pruning.

The results of cultivating *Tuber melanosporum* in France are recognized to be variable. A few plantations created and managed with exceptional care show that it is possible to specify an optimal method for cultivating *Tuber melanosporum.*

The reference plantation for this study is situated in the département of the Tarn. It consists of 57 ever green oaks mycorrhized with *Tuber melanosporum* and planted in 1995. It is compared with other plantations established in similar conditions but with different methods of management. The outstanding method includes progressive working of the land, mowing the grass, watering, pruning the trees and disseminating spores. Each operation is carried out with hand tools or light equipment, with a constant concern to avoid compacting the soil or disturbing the habitat.

The burnt areas appeared from the third year onwards, and production started in the fifth: production which started under 10% of the trees and can be seen under more than 90% in the twelfth year, varying between 15 and 45 kilos per hectare.

These results show that if the maintenance of truffle plantations (working the soil, watering for recovery and production, pruning the trees) is carried out diligently, constantly and regularly, production generally starts early and increases steadily. New generations of mycorrhized plantings with carefully managed cultivation, re-uniting traditional practices and recent progress, allow us to look to the future with confidence.

DIVERSITY OF THE GENUS *TUBER* FROM THE WEST BALKAN AREAS USING MOLECULAR CHARACTERISATION

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KEY WORDS: truffles, molecular diversity, Balkan area.

The area of SE Europe, particular the Balkan area, has only recently been more extensively explored for the presence and diversity of hypogeous fungi, compared to several other well characterized European areas, despite the suitable climate and vegetation conditions of the region. Several surveys have been performed in last decade generating substantial collection of hypogeous fungi, particular from the genus *Tuber*, in official herbaria. The latter material, mainly identified by regional specialists, was subjected to molecular diversity assessment and identification. We attempted to characterize selected collections at the molecular level and to explore them for potential new molecular variants as expected due to known high endemic characteristic and diverse ecosystems composition of the Balkan region.

Molecular approach supported morphological identification results and confirmed the presence of all commercially important species including *Tuber magnatum* Pico, *Tuber melanosporum* Vittad., and *Tuber aestivum* Vittad. We have also observed high diversity (polymorphism) within few other morphological species (*Tuber rufum* Pico*, Tuber brumale* Vittad.), which partially confirmed up to date scientific literature. The presentation aims to show point out selected results, new for science and present the high biodiversity aspect of hypogeous fungi in the Balkan region.

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FEW EXAMPLES OF TRUFFLE-PRODUCING *TUBER MAGNATUM* PLANTATIONS IN ITALY

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KEY WORDS: truffle plantations, carpophore production, *Tuber magnatum*.

The cultivation of the white truffle (*Tuber magnatum* Pico), highly developed in the eighties, due to the great value recognized to this species, has slowly decreased and eventually stopped. The lack of productivity of the majority of these truffle plantations and the following scepticism towards the white truffle cultivation is one of the main reasons of this halt. However, a few successful plantations do exist, and even if their productivity is modest if compared to that of other truffle species (see *Tuber melanosporum*, *Tuber aestivum*, among others), they prove that the white truffle can be cultivated, despite its complexity.
The authors show a few cases, in Italy, of productive white truffle plantations, and describe both their common features and the peculiar techniques applied to each one.
The aim is to publicize these positive results and to find a possible scientific elucidation of the carpophore production by *Tuber magnatum* Pico, to promote a renewed interest and research work on this species and its cultivation.

RECOVERY OF TRUFFLE PLANTATION BY *TUBER MELANOSPORUM* ABANDONED AND UNPRODUCTIVE

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KEY WORDS: truffle cultivation, *Tuber melanosporum*, ecology.

A truffe plantation, rather dense (3 m×3 m) of oak mycorrhized by *Tuber melanosporum*, made in 1980 in the Community Montana of Nerone and Catria that had never entered into production was subject to a number of cultural practices for groped productivity to recover after about twenty years from making omeless.
After first verifying, by taking radical, while the mycorrhized by *Tuber melanosporum* on the apparatus radical plant made homeless, it was the cleaning up of the entire surface area (about 6.000 m^2) by chopping machine. Subsequently, the first Pianella of *Tuber melanosporum* has made a slight "forconatura" them (and also gradually in subsequent years, as demonstrated such a will on other plants) while the areas under the plants and grass still remain productive of *Tuber brumale* were scattered hydrated lime. In a second time pruning were made to raise the canopy and better illuminate the ground below. In turn a few years, the plants treated in this way have started to produce truffles, the first in a sporadic and small amounts then resulting in discrete quantities collection also excellent size.
The authors point out the positive results achieved may be considering a way, all things fairly simple and economical, groped for the recovery of many truffle plantation executed and then, for a variety of causes, abandoned and then never come into production.

TUBER BRUMALE TESTING OF TRUFFIERES IN NEW ZEALAND

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KEY WORDS: Truffle cultivation, *Tuber brumale*, *T. melanosporum*, mycorrhizae, molecular biology.

Following the first discovery by the Edible Fungi Team of the New Zealand Institute for Crop & Food Research Limited (C&FR) of *Tuber brumale* mycorrhizae in C&FR nursery and in two truffières in 2006, a nationwide survey was conducted to determine how widespread *T. brumale* was in New Zealand truffières. A protocol for collecting roots was sent to growers who had planted oak and hazel trees between 1990 and 2005. One tree of each tree species present was randomly sampled. A combination of microscopic and DNA analyses was used to detect the presence of *T. brumale* mycorrhizae. We also recorded the presence of *T. melanosporum* mycorrhizae.

A total of 28 truffières (19 from the North Island and 9 from the South Island) provided 67 distinct root samples, collected from 30 hazels and 37 oaks. The presence of mycorrhizae of *T. brumale* was confirmed on trees from 10 of the 28 truffières (8 in the North Island, 2 in the South Island). *T. brumale* was detected on 11 trees (16.4% of the sampled trees). Out of these 11 trees, 7 also had *T. melanosporum* mycorrhizae. *Tuber melanosporum* mycorrhizae were identified in 23 of the 28 truffières (on 67% of the hazels and 54% of the oaks).

The present testing cannot assess the abundance of brumale in the plantations found to have brumale mycorrhizae. A negative result does not necessarily mean that brumale is absent from the entire tested tree, nor the truffière. To determine conclusively that brumale is absent from a truffière would involve more extensive and expensive testing. Results from this survey combined with preliminary work by C&FR and MAF (New Zealand Ministry of Agriculture and Forestry) confirm that brumale mycorrhizae have been detected in 12 of 30 truffières throughout New Zealand, i.e. 10 sites in the North Island, 2 sites in the South Island. Only trees produced by C&FR have been tested so far. To date there are no records of brumale truffles having ever been found fruiting in New Zealand. Due to the biosecurity regulations set by MAF, imports of truffle inoculum from overseas are subjected (since 2005) to morphological and molecular testing of every individual truffle. Provided that similar standards are put in place to inspect both domestic truffle inoculum and truffle trees produced in New Zealand, there is very little chance that brumale will expand in New Zealand through the establishment of new *T. melanosporum* truffières.

ESTABLISHMENT OF *TUBER AESTIVUM* VITT. ECTOMYCORRHIZAE ON *QUERCUS ROBUR* AND *CORYLUS AVELLANA* SEEDLINGS IN POLAND

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KEY WORDS: *T. aestivum, Quercus robur, Corylus avellana*, truffle cultivation, Poland.

1-year old seedlings of *Quercus robur* and *Corylus avellana* taken from bare root nurseries were inoculated with spores of *Tuber aestivum,* according to method of IPLA (Torino), which had been collected from fruiting bodies found in Poland in 2007. Ectomycorrhizal development was monitored for 6 months in the greenhouse and compared to the performance of the seedlings. Seedling survival in both host species was at similar level, only 2% of oak and 7% of hazel seedlings died, but mycorrhization showed major differences. The colonization rate was very high in case of *Q. robur* seedlings; 100% of investigated seedlings possessed ectomycorrhizae of *T. aestivum* whereas only 20% of *C. avellana* seedlings were colonized by the fungus. In both species, ectomycorrhiza of adventives fungi could be observed, probably *Thelephora* and *Scleroderma*.
The seedlings are to be outplanted in October 2008 to the site with natural soil of pH=7.8.
Preliminary results showed that successful colonization by *T. aestivum* under greenhouse conditions opens up the possibility of cultivating this truffle in Poland.

TRUFFICULTURE'S GREEN REVOLUTION STARTS IN TERUEL (SPAIN)

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KEY WORDS: Black truffle, cultivation.

The climate and the soils of the highlands of Teruel have never been capable of producing sufficient amounts of agricultural products to support a large population. Nowadays this is changing with truffle cultivation. There are more than 3,000 hectares of truffle orchards in this area and the yields are starting to support more people living in better conditions. The bases of this change were laid in the late 80's with the first successful plantations, and continued in the 90's with a strong program of subsidies to farmers. These efforts have created a strong truffle based sector of the economy that now is demanding technological support. The initial "plant and wait" approach was adequate to obtain the first truffle crops but a completely new body of knowledge is needed to bring trufficulture into the realm of modern agriculture, where yields are consistently high and predictable, and product quality meets global standards. With this objective in mind we have established a network of experimental plots in this area to provide answers to the questions that the growers identify as the most critical to their activity: watering strategies to achieve precocity and maximum sustained production, impact of cultivation decisions, and the influence of different soil textures in mycelia behaviour and fruitbody production. We are accompanying these experiments with monitoring of *T. melanosporum* mycelia development in a yearly cycle and during the early years of a plantation, as well as the evolution of the populations of other soil microorganisms as plantations age.

THE EXPERIMENTAL CENTRE OF THE MYCORRHIZATION AND PRODUCTION OF MYCHORRHIZED PLANTS IN PORTO VIRO (ROVIGO) AND THE VALORIZATION OF TRUFFLE PLANTATION IN THE VENETO REGION (ITALY)

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KEY WORDS: *Quercus robur, Corylus avellana, Ostrya carpinifolia, Tuber albidum, T. brumale, T. melanosporum, T. aestivum*, cultivation, valorization, mycorrhized plants, mycorrhiza, truffle, experimental truffle fields.

The protection and the restoration of the natural truffles heritage are important purposes to achieve in our country. The Region of Veneto promoted the L.R. 30/88 to increase the natural truffle productions. Within the limits of the law, the Forest Regional Service of Padua and Rovigo realized a new Experimental Centre of Mycorrhization and Production of young mycorrhized plants. The greenhouse and laboratory for the investigation of mycorrhizae are located in Porto Viro (Rovigo). The Centre produces, sells and uses the plants in reforestation areas to encourage a wider distribution of trees associated with every commercial truffle species into Veneto territory. An increase in plant number is very important both for ecological-environmental improvement and for economic profits deriving from the cultivation and the valorization of truffle plantations. In the nursery Centre from 2000 to 2007:

1) The production of mycorrhized plants associated with different species of *Tuber* increased of about 30% and is maintaining a constant trend till nowadays.
2) The rate of success in root mycorrhization process is improved of 5-10%, due to the use of spores, seeds and shoots of local ecotypes.

Growth and production data of plantations with *Tuber melanosporum* Vitt. planted out in four different experimental truffle fields in the district of Rovigo are presented in the poster.
These thick unproductive plants, 10-12 years old, began to produce truffles again after they were subjected to cuts and strong prunings by the Forest Service.

THE CHAIN OF TRUFFLE AND THE VALORIZATION OF THE PRODUCT IN TOSCANA E ABRUZZO

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KEY WORDS: Conservation, commerce and valorization.

Through this poster we want to present the multi-regional research project: "Initiatives of search and development related to the sector of the truffle", promoted by ARSIA for the regions: Abruzzo, Emilia Romagna, Molise, Toscana with Decree No. 321, 25-09-2007. The poster refers to the subproject B) and it is focalized on the "Study of the chain of truffle, with particular attention on the possibility of valorization of the product and on the analysis of investments for the construction of specialized woody plant in symbiosis with mycorrhizal truffle. Partners who won the public research project for the subproject B) are: DEART – Department of Agricultural and Land Economics – University of Florence (Coordinator); DSA – Department Environmental Science – University of Aquila; URATT – Unione Regionale Associazioni Tartufai Toscane; Associazione Tartufai delle Colline; Samminiatesi; Associazione Tartufai Senesi; Centro di Ricerca per la Selvicoltura di Arezzo. The project is made up of two objectives. Objective 1 regards the analysis of investments for construction of specialized plants (valuation of the potentialities and limits of the development) and it is articulated in two actions. The first action wants to reconstruct the universe of the subjects that plays a role in the truffle market through the partners involvement which are directly in contact with many of the existing operating units on the territory; the second action focalizes on the knowledge of the operators involved in the chain through the study of socio-economic aspects, the farm structure analysis, the choice model behavior of the gatherers or producers, the description of the environmental area of production, on the productive system and on the production and harvest costs. The aim of this first part of the analysis is the investment valuation (profit of the systems, different type of enterprises, actual criticalities). Objective 2 concerns a deep analysis of the truffle chain (in particular protection and valorization of the product). Also in this case are planned two different actions. The first one is referred to the data collection. The second one regards the implementation of a survey based on a collection of samples with the aim of underline the gatherer profile, the characteristics of the market and of the product. The results attended for this objective are those to underline a profile of the gatherers, to analyze the costs of carrying out this activity, to verify the consistence of the market structure and to value the volume of product that transits from the chain. The project, besides, is finalized to the evaluation of the economic relapses that can be foreseen for the territory in terms of increase of the production or in terms of cost of the resources directly or indirectly linked to the truffle activities, of increase of the defense of the environment and the activity of safeguard and environmental improvement, of activation of paths of valorization for the product and for the territory.

A *TUBER MAGNATUM* TRUFFLE-GROUND: AN APPROACH OF ENVIRONMENTAL GENOMICS

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KEY WORDS: *T. magnatum*, DGGE, soil, ecology and population biology, truffle productivity, microbial populations.

Truffle-grounds are special environments where the ectomycorrhizal truffles interact with their host-trees defining a nutrient-rich niche. While *Tuber* mycorrhizas are obtained in laboratory conditions, fruiting bodies are exclusively found in nature. As several biotic factors could influence truffle life and enhance or inhibit ascocarp formation, our first aim was to look for a connection between soil microbial populations and truffle productivity. We took advantage of an extensive study involving the survey of truffles and mycorrhizae (Mello *et al.* 2005; Murat *et al.* 2005) carried out for about eight years in an Italian *T. magnatum* truffle-ground where productive and non-productive areas were identified.
In this context, soil samples from both areas were compared in DGGE. In order to reach this goal, the metagenomic DNA extracted from these soils was amplified with both primers for 16S rDNA and 18S rDNA. The DGGE profiles demonstrated a positive relationship between microbe populations and the sampled, productive *versus* non-productive areas. As an example, *Moraxella osloensis* population appeared to be associated with productive sites. Fungal communities revealed several populations, yet showed no obvious patterns in relation to productivity, although *Mortierella* and *Fusarium oxysporum* appeared to be more abundant in the productive area. The results from the sequencing of some bands corresponding to the most represented populations offer a first glimpse into microbial communities thriving in truffle productive niches, and open the question whether microbe-mediated mechanisms may facilitate/inhibit truffle fruiting-body production or, viceversa, truffle sporocarps have an impact on the microbes living in the rhizosphere.

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UMBRAFLOR S.R.L. – ISO 9001 CERTIFIED GARDEN CENTRE TRUFFLE-PRODUCING PLANT PRODUCTION, USING LOCAL TRUFFLES AND NATIVE SEEDS, CERTIFIED ACCORDING TO EUROPEAN (DIR. 1999/105/CE), NATIONAL (D.LGS 386/2003) AND REGIONAL LAWS

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The nursery "Il Castellaccio", managed by UmbraFlor s.r.l., has been making truffle-producing plants for 30 years, relying on the careful experience of its workers and of 5 graduates in Agronomy and Forest Science, present in the Company.
UmbraFlor s.r.l. is also well known for other garden products, such as in particular cancer-resistant cypresses, Dutch Elm Disease-resistant elms and walnut trees grafted with fruiting cultivars.
Umbraflor s.r.l. has put much interest in the truffle-producing plant line production, due both to the local and national interest in this field, and to the direct effects on its turnover. All our greenhouses, recently renewed, have been designed to maintain strict sterile conditions, with air filtration systems and internal environmental parameters controlled by automated electronic stations.
The average annual production is of about 30.000 plants, belonging to various forest plants and truffle species more suitable to the Italian habitats, with the exclusion of *Tuber magnatum* Pico.
All the production line, from the beginning to the final plant delivery to the customer, is daily monitored according to the ISO 9001 certification, which the Garden Centre has acquired several years ago.
To obtain truffle-producing plants, exclusively native seeds are utilized, certified on the basis of present rules (Dir. 1999/105/CE – D.lgs. 386/2003 – Regional laws and regulations in force in specific areas). The origin of both seeds and carpophores reflects the different Italian regions where UmbraFlor s.r.l. has its main markets.
All the carpophores, prior to the *inoculum*, are sectioned one by one and microscopically analysed by the University of Perugia, who verifies and guarantees the identity of the truffle species required by the nursery. In October and November, all the mycorrhized plants are controlled by the Department of Applied Biology of the University of Perugia, who certifies the plant quality for truffle cultivation.
This certification is issued on the basis of mycorrhizal morphology characterization, according to the agreement made in 1995 between ten Regions and eight University and Research Institutes. Following the Umbria Region Reg. 8/2007, this certification is granted also on the basis of biomolecular analyses, extended to all the marketed truffle-producing plants.

TYPOLOGY OF ECOLOGICAL TRUFFLE SITES AND MAPPING OF THE PRODUCTION POTENTIAL

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KEY WORDS: *Tuber melanosporum*, suitable soil, production potential, mapping, ecological truffle site typology, cultivation, Mont Ventoux.

The black truffle, *Tuber melanosporum,* is an emblematic product from Mont Ventoux (Vaucluse, France), where it is hunted since early 19th century in natural field. However, as in many other production sites in France, production has substantially decreased in the past century.
Therefore it is all the more an important challenge for the rural and forest activity of this Mediterranean mountain to re-establish truffle production in the Mont Ventoux. However, prior to developing any schedule or management project a thorough assessment of the production potential of *Tuber melanosporum* was mandatory, and at forest scale. We elaborated an efficient tool: a specific map based on the typology of ecological truffle sites.
The method was applied to 13 000 ha of private forest. It is based on the crossing of geographic data (using GIS ArcGis 9.2):

– elevation and topography
– bioclimate (18 types)
– soils from pedologic maps at 1/20 000 (37 types)

We obtained 600 ecological units after crossing. 21 were significant on the study area (over 30 ha). The theoretical production potential of each ecological unit was characterized through an expertise based on the knowledge in truffle ecology and on the participation of Gabriel Callot (INRA former Research Director).
This characterization was validated on the study area. 100 data points were obtained/taken, depending of their representativity (in surface) on the study area. The variation factors of the production potential on each unit were determined using field validation.
The map obtained (at a 1:25 000 scale) classifies the production potential into 5 levels modulated by factors that could induce variation of the production level. These factors have to be checked on the field.

ESTIMATION OF CROP COEFFICIENT FOR IRRIGATION IN BLACK TRUFFLE ORCHARDS DURING ITS FIRST STAGES

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KEY WORDS: Holm oak, irrigation, evapotranspiration, ectomycorrhiza, *Tuber melanosporum*.

The symbiosis between *Tuber melanosporum* and *Quercus ilex* was analyzed from the point of view of the atmospheric evaporative demand on *T. melanosporum*-inoculated seedlings in young truffle orchards. Reference evapotranspiration (ETo), based on data from the climatic stations nearest to five truffle orchards studied, was determined using the Penman-Monteith method. The ETo was used to establish five doses of irrigation: 0, 25, 50, 75 and 100% of the ETo. Three treatment application periods were tested: from April through July, August and September, and from April through September. The experimental design consisted of 5 irrigation treatments and 3 periods in 5 blocks yielding 75 experimental units. Every unit consisted of a tree inoculated with *T. melanosporum*. The irrigation doses were chosen to cover a wide range of the crop coefficient (Kc). The highest numbers of *T. melanosporum* ectomycorhiza were observed on seedlings irrigated with the dosis of 50% of ETo during the April through July period. From the data on irrigation and rainfall we suggest that one of the characteristics of the Kc for young truffle orchards would be a decreasing function from April to August to increase again in September. Based on these observations, when the air temperature reaches the highest values of the year, the still non-productive young truffle orchards should be maintained in moderate water stress.

THE HYPOGEOUS FUNGI HARVESTED TOGETHER *TUBER AESTIVUM*

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KEY WORDS: taxonomy, hypogeous fungi, harvesters, truffles, identification.

The practical training stage of the Master in Micologia Agroalimentare (Agrifood mycology) organized by University of Bologna was carried out by Federico Paci in the Company Urbani Tartufi s.r.l. located in Sant'Anatolia di Narco (Perugia, Italy). The macroscopic and microscopic studies of truffles led to the identification of twenty-five species of hypogeous fungi belonging to Ascomycetes and Basidiomycetes. Some rare truffles such as *Tuber panniferum* Tul. and *Tuber malenconii* Donadini, Riousset, G. Riousset & G. Chev. were identified together *Fischerula macrospora* Mattir. and other species belonging to the genera *Genea, Melanogaster, Gautieria* and *Hymenogaster.* Some of the identified species, although interesting for rarity or for the large quantity of fruit bodies found, are not edible. These species were harvested by truffles collectors ("cavatori") in different parts of Italy and erroneously included in the lots of marketable *Tuber aestivum* Vittad. sold to the Company. They were put aside for us to study (but usually descarted) by the highly specialised personnel during the careful and thorough screening of the truffles before commercialization.
From this study we can make two important considerations: 1) there are many other different of hypogeous fungi in the same site where *T. aestivum* grows, probably sharing similar ecological requirements; 2) it is evident that some collectors are sometimes unable to recognize the hypogeous fungi harvested together with *T. aestivum.* Therefore the identification by the Company's personnel is very important before the commercialization in particular as concern truffles used as inoculum to produce truffle-infected plants, in order to avoid undesirable truffle contamination in the nurseries.

INTERNAL STRUCTURE AND QUALITY ASSESSMENT OF FRESH TRUFFLE BY MEANS OF MAGNETIC RESONANCE IMAGING SPECTROSCOPY

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KEY WORDS: MRI, *Tuber melanosporum*, freshness, fungicolous fungus, Conservation, commerce and valorization.

Magnetic Resonance Imaging spectroscopy (MRI), being a non destructive and non invasiveness analytical technique, offers a very powerful tool for the investigation of the internal morphology of foodstuff and of modifications induced by "stress agents", e.g. fungal invasion, diseases, contaminants, etc. In the present paper MRI was applied, to the best of our knowledge for the first time, to study the internal structure of fresh black truffle; decaying process and fungal invasion were observed and quality evaluation, which up to now has not yet yielded an accepted technical standard being based on smell and consistency on handling, was exploited by means of MRI.

HEAVY METAL CONTENT OF ABRUZZI TRUFFLES

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KEY WORDS: Truffle, Heavy metals, Bioaccumulation, AAS, ICP-OES, Conservation, commerce and valorization.

Many kinds of food have been analyzed for their content of heavy metals over the past few years. In some cases, it has turned out that foods of vegetal origin, including fungi, exhibit a strong tendency towards "bioaccumulation", defined as the capability to concentrate particular toxic substances inside somatic structures. Since truffles have been analyzed only in terms of the nutritionally relevant salt content, we deemed it necessary to undertake this kind of research on a representative sample of the diverse species and of the various soil types of the Abruzzi Region. The analyses have been conducted by using Atomic Absorption Spectrometry (AAS), to determine the presence of barium, cadmium, chromium, nickel, lead, copper, zinc and manganese, and by using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). In contrast to what has been found out about some fungi considered excellent edibles – and until now allowed to be traded by the food law in force – which, in point of fact, have been proved to be bio-accumulators of heavy metals, Hg and Cd, in particular, or of radioisotopes (Cs and Sr), at the moment, truffles appear to be a perfectly safe food from this point of view. In fact, the results obtained by two different analytic methods match up about the selectivity in ion attraction from the solutions present in the soil by truffles. It has repeatedly been emphasized that truffles strongly accumulate useful ions such as K and S, while very little attention has been devoted to harmful bioaccumulations, which, luckily however, do not seem to occur in truffles. The data gathered in this thesis is consistent with what emphasized by the only work on the subject which focused on Abruzzi truffles. It remains to be clarified, therefore, whether the absence of heavy metal bioaccumulation is a general feature of truffles or rather it is related with the features of the truffle-producing soils. Abruzzi soils favourable to the fruiting of truffles are, in fact, characterized by a low content of heavy metals and an alkaline pH which limits metal gain. Even though it is necessary to carry on further analyses in order to obtain statistically significant data, Abruzzi truffles, both from natural truffle-grounds and farming productions, have been proved to be safe and of top quality and the soils of production may be expected to be the same.

BLACK TRUFFLE (*TUBER MELANOSPORUM* VITTAD.) OBTENTION IN A MYCORRHIZED OAK ORCHARD OF A TRADITIONAL IRRIGATED LAND

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KEY WORDS: Truffle cultivation, agricultural techniques, alternative crops, mycorrhization.

The background, features and farming methodology to set up a *Tuber melanosporum* Vitt. mycorrhized oak orchard in order to harvest black truffle harvest in Zaragoza (Ebro Valley, Spain), are described in this paper.

Although truffle cropping is a common activity of diversification in mountain depressed areas the establishment on irrigated and calcareous soils could be considered in the same way as a fruit orchard.

A first black truffle was obtained in an experimental plot of Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA), located in Zaragoza. This new event in this field supports this assertion in contrast with the orthodoxy of the current trends in truffle farming.

VEGETATION ANALYSIS: A TOOL TO IDENTIFY TRUFFLE VOCATED AREAS IN MOLISE REGION

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KEY WORDS: *Tuber magnatum* Pico, Plant ecology, Phytosociology.

Owing to the climatic and soil characteristics in Molise region, the truffles are there widely diffused. Since this Region is one of the most important white truffles (*Tuber magnatum* Pico) area in Italy, the aim of the present study is to provide some preliminary information about the ecology of *T. magnatum* plantations in Molise region. The study, carried out during three years (2005-2007) in Busso (Molise region), investigated the relationships between the plant community and the truffle presence as well as its frequency in order to plan a flexible approach in preserving natural regional resources (Paura *et al*., 2008). Truffle presence, in fact, is closely related not only to symbiotic plants and pedosphaere environment but also to phytocoenosis characteristics (i.e. plant association, forest structure). In this connection, several data have been recorded in order to identify floristic, vegetation and structural features, in a natural white truffle plantation. The truffle plantation experimental area (100×100 m) was divided in 16 plots (25×25 m), to improve the accuracy of the data detected. In each plot were adopted the following methods:

a) phytosociological method to analyse the truffle plantation by means of the floristic and ecological characterization of plant communities;
b) analysis of the relationships between forest structure (i.e. diametric classes and canopy) and presence/frequency of white truffle;
c) correlation between the presence of floristic species and the white truffle presence/frequency.

A lot of white truffle occurrence in the plots were linked with the *Daphno laureolae-Quercetum cerridis* Taffetani & Biondi, 1995 association. Moreover the occurrence of truffle seems to be related to the landscape use history. For instance, the lack of *T. magnatum* seems to be associated to ancient pasture re-colonized by wood, the latter was attested by the presence of *Brachypodium rupestre*. In the some way a "buffer effect" was also recognized owing to the absence of the shrub in the truffle plantation peripheral areas that caused an higher surface invested by radiation energy and consequently allowing dryness environment. All the studied parameters let us to edit thematic maps, in according to the model elsewhere reported (Marchetti *et al.,* 2006). A deep in knowledge on the truffle plantations ecology will be useful to identify the most suitable areas for truffle cultivation and to improve the correct management of truffle production.

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INDICATOR-BASED APPROACH FOR MAPPING AND ASSESSING *TUBER MAGNATUM* PICO PRESENCE IN WESTERN LIGURIA (ITALY)

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KEY WORDS: ecology, mapmaking, white truffle.

The potential mapping of *T. magnatum* distribution is very important mainly to protect the areas where this species grows or might grow.
The work aims at presenting the procedure we followed in order to realise potential maps of white truffle for some Western Liguria areas.
Such maps were made according to a method which starts from data collected on the field (mycological and botanical sampling, pedological investigations, etc.). Analytical data (physic and chemical) are then processed and integrated with modern techniques for land surveys including GIS. The latter involves the processing of different layers, for instance wetness index, slope, aspect etc.
The preliminary map is being tested in the territory and the first results are encouraging. Such results make us suppose that this method can also be applied successfully in other regions.

TRUFFLE IN BHUTAN

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KEY WORDS: ecology, whitish truffles, Himalayas.

In 1991 thirty oaks inoculated with *Tuber melanosporum* Vittad., *Tuber magnatum* Pico and *Tuber aestivum* Vittad. obtained from the Research Centre in San Angelo in Vado (Italy) were sent to the National Mushroom Centre, under the Ministry of Agriculture in Bhutan through the then Director of the Institute. These plants were planted in the area around the Centre. Only later on starting from 1995 periodical management (removal of the lower branches to allow light) and adding of lime was carried out. In 2001 upon digging around one of the trees some truffles were discovered. They were very small 1-2 cm in diameters, soft and without any aroma. It has been concluded that they were not the main truffles inoculated on the plants but contaminations which were encouraged to take over because of the nature of the soil and pH. The pH of the soil is found to be below 6 also after liming.

There is only one report of natural truffles in Bhutan in 1998 while eight truffles of around 200 g each were discovered collecting pine saplings from a forest nursery near Thimphu, Western Bhutan. These truffles were similar to *Tuber borchii* Vittad. (bianchetto) for the smooth yellowish red peridium and the reticulated spores. The host plant was *Pinus wallichiana* (2 years old) and the soil pH 6.2. Molecular analysis will be necessary to identify these whitish truffles.

The spores of these truffle might have come along with the forest litter collected from the near by forest where lime stone and dolomite are found.

T. himalayensis and *T. indicum* has not been reported in Bhutan but has been suspected since they were found in the other parts of the Himalayas.

MOLECULAR IDENTIFICATION OF TRUFFLES

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KEY WORDS: Molecular biology, ITS, Restriction enzimes, *Tuber aestivum*, *T. brumale*, *T. indicum*, *T. melanosporum*, Ectomycorrhizal identification.

The organoleptic properties of some truffle species have notable importance in gastronomy and therefore significant economic value. Two of these species are *Tuber melanosporum* Vittad., and *T. aestivum* Vittad. the black truffle and the summer truffle, respectively. These hypogeus fungus were first collected in their natural forest habitat, but now are cultivated in high extension fields due to increasing market demand. The species identification for micorrization procedure, evaluation of propagation in seedlings, and monitoring of plantations is of critical importance for cultivation, avoiding undesired species as *T. brumale* Vittad. and *T. indicum* Cooke & Masse and assuring that the desired species is present at different stages of plant growth. Morphological characters do not allow in all cases the correct identification of truffles, especially at ectomycorrhizal stages. Therefore, this study has developed an inexpensive and simple DNA-based technique for the characterization of four *Tuber* species. Ribosomal DNA region (ITS) have been analysed using conserved and general primers (ITSl-ITS4), only positive PCR ITS products were digested with a combination of restriction enzymes. The result is distinctive band patterns allowing the identification of each species. This technique can be used for typing fruit bodies avoiding possible frauds in the case of morphological similar glebas as *T. melanosporum* and *T. indicum*, and for typing ectomycorrhizae to detect the presence of the desired species in few months, rather than wait 4-6 years until the formation of the fruit body. This is an initial step for the characterization of truffles, following steps include a restriction map of the most important species of the genus *Tuber*.

PLANTATION DENSITY OF HAZEL TREES (*CORYLUS AVELLANA*) INFLUENCES THE PRECOCITY AND KINETICS OF BURGUNDY TRUFFLE (*TUBER AESTIVUM*) PRODUCTION

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KEY WORDS: Cultivation; Burgundy truffle; *Tuber aestivum*; hazel orchard; *Corylus avellana*; plantation density; production.

Recommendations for truffle orchards establishment and further management are not always adapted to the ecology of each truffle species, in interaction with its host trees. As an example, the most common plantation density for Burgundy truffle orchards in the past 40 years was often adopted from Périgord black truffle.
In this context, a study was launched 23 years ago, with the objective to test the influence of tree density on the production of burgundy truffle and its evolution up-to-date.
A Burgundy truffle hazel orchard was planted in 1985, with four plantation densities in Commercy located in the North East part of France (48°45'42 N, 5°35'31 E), 250 km East of Paris. Four densities were tested: 2000, 1000, 666 and 333 trees per ha.
The first harvest was obtained at the highest densities (2000 and 1000 trees. ha^{-1}), 11 years after plantation. Then, high densities produced during four years and then ceased almost to produce 9 years after the first harvest (from the 20^{th} year after plantation). The lowest plantation density (333 trees. ha^{-1}) starts to produce significantly 15 years after the establishment of the orchard. Then, the production increased every year and the production peak is not reached yet.
Recommendations to the growers of Burgundy truffle orchard need to include advices (plantation density and tree pruning), not only at the establishment, but also during the life span of the truffière. This is crucial in order to optimize the economic yield of this crop.

PEDOLOGICAL ASPECTS OF NATURAL TRUFFLE BEDS OF *TUBER MAGNATUM* PICO IN THE RIETI PROVINCE (CENTRAL APENNINES, ITALY)

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KEY WORDS: soil, *Tuber magnatum* Pico, truffle ecology.

A study on natural truffles beds of *Tuber magnatum* Pico in the Rieti Province was carried out. From a macroclimatic point of view the truffles beds are located in two different elevation belt: from 100 m a.s.l. to 250 m in low Sabina and from 550 m a.s.l. to 900 m in mountainous inland Sabina (valley of the Salto river in the main). The analysis of climatic data, according to phytoclimatic map of the Latium region (Blasi, 1994), indicates that the truffles sites are all located inside the Temperate Region. In particular the sites of low Sabina belong to lower hilly thermotype, lower moist ombrotype of the mesaxeric Region, while the sites of the Salto valley are included in upper hilly thermotype, upper moist ombrotype of the mesaxeric Region. The truffles beds were discovered on terraced hilly slope, often of anthropic origin, or along the banks of little rivers, both affected by superficial landslide and solifluction, or above alluvial plains. The parent material of the soils is represented by sandy and marly sediments of plio-pleistocenic age in low Sabina, while the silty arenaceous rocks and marls of miocenic age are dominant in the valley of the Salto river. The results of chemical analysis show that the average values of pH measured in water and KCl, are respectively 8.09 and 7.35 and that the minimum value of pH in KCl not fall below 7.09. The content of total $CaCO_3$ presents a mean of 6.7% which is low if compared to the average of other environments suitable for *T. magnatum* growth, characterized by more marly parent material. The particle size analysis indicates that the means of sand, silt and clay are respectively 34%, 51% and 15% with the lowest CV (coefficient of variation) relative to the silt while the clay content shows a higher variation. The bulk density of the horizons suitable for the carpophore growth varies from 1.06 g/cm^3 to 1.37 g/cm^3. The particle size composition also indicate that the soils in the low Sabina differs from those of the Salto valley for a lower presence of sand and higher content of silt. The limited number of examined cases, in this initial phase of the study of the pedological aspects of natural truffle beds of *Tuber magnatum* Pico in the Rieti Province, would seem to indicate that the truffles sites are not associated with either a particular type of parent material or well defined "external" climate. It could be hypothesize that the factor which unite the different studied cases (low Sabina and Salto valley) is due to particular situations of geomorphological dynamics that activate not catastrophic processes of slope, associated to particular local microclimatic conditions in low Sabina.

HISTORIA MAGISTRA ARTIS *TUBERUM REPERIENDORUM* THE VALUE OF HISTORICAL DOCUMENTS FOR THE EXPLORATION OF UNKNOWN TRUFFLE-AREAS IN EUROPE

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KEY WORDS: Conservation, commerce & valorisation, Unexplored truffle-producing regions, Central & Eastern Europe, Historical documents (maps, travel reports, encyclopaedias).

One of the major problems that is posed when prospecting for new truffle areas in Central and Eastern Europe is the complete absence of a truffle hunting culture in these countries rather than a lack of truffle *terroirs*. This missing tradition, however, at the same time also offers a great opportunity to explore new truffle-producing areas, and to open up a valuable natural resource for some regions in Central and Eastern Europe.
With regard to the tracking of these unexplored truffle areas, historical documents – such as historical maps, travel reports or encyclopaedic articles – can be very useful instruments. The most instructive documents originate from the 18th century, especially the **Zedler** encyclopaedia, the first encyclopaedia of the German-speaking world (published from 1732 onwards). This work contains several articles on truffles, which extensively discuss not only their nature, but also the regions in which they actually grow. Furthermore, mycological maps, designed by scientists like Carolus Clusius (1526-1609) and the Bolognese count L. F. Marsigli (1658-1730), may also contain information on the provenance of truffles in Central and Eastern European countries. These maps not only show where which species of mushroom can be found, but frequently also refer to truffle-producing terrains. Finally, travel reports from the 18th century, which often mention the activity of truffle-hunting and of truffle commerce, may also prove to be a useful tool in the discovery of unexplored truffle areas.
In this paper, I would like to give some illustrations of the practical value of these historical documents in the exploration of unknown truffle-producing regions in Central and Eastern Europe.

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MANIFESTATIONS OF *TARTUFOMANIA* IN NORTHERN-EUROPE IN THE 18TH CENTURY

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KEY WORDS: Conservation, commerce & valorisation, Tartufomania, Northern Europe, 18th century

In the 18th century, the truffle conquered the northern parts of Europe. And if this cryptogam could have been cultivated like a champignon, the truffle would have definitely 'colonized' the whole of Europe, like its look-a-like tuber, the potato, which later was diffused all over the continent, and even in Great Britain and Ireland. Northern Europe, it seemed, was under the spell of nothing less than a tartufomania.

Drawing upon hitherto unknown historical documents from archives in Turin, Vienna, London and Berlin, I would like to describe some of these manifestations of tartufomania and try to explain this sudden and almost obsessive interest in the truffle against the background of the socio-cultural climate of that time.

With this research I intend to demonstrate that there is actually a long tradition of **pan**-european interest in the truffle, which implies that the economic-agricultural truffle policy can not be regarded as an issue of marginal importance, as it merely concerns – as is usually said – a *niche* in the economy of some Southern European countries.

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PRODUCTION OF HIGH QUALITY TRUFFLE PLANTS UNDER ISO 9001 QUALITY LABEL AND IN THE FRAME OF SPECIAL GROWING CONTRACTS

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KEY WORDS: Mycorrhizal plants, Truffle, Growing agreements, nursery.

The ROBIN tree nursery company is involved in controlled mycorrhization of forests plants since 1960 and in large scale production of mycorrhizal plants including edible mushrooms since 18 years. The ROBIN nursery is the first European nursery for the production of mycorrhizal plants under ISO 9001 quality label.The nursery has been involved in several European research programs adressing mycorrhizal symbiosis related to three main goals: reforestation purpose, selected mycorrhizal seedlings for phytoremediation of contaminated area, and establishment of EDIBLE MUSHROOM ORCHARD®. These participations allowed the nursery to get a considerable experience both at scientific and practical level on mycorrhizal symbiosis and high quality mycorrhizal plants production. A staff specially trained for mycorrhizal studies manages all steps of production of truffle plants from controls and selections of fruitbodies, to final control before delivery, according to schedule defined under ISO 9001 label aimed for high quality products. The truffle plants can be produced according to the special request of our customers in the frame of a special growing contract using selected batches of seeds and truffles supplied by the customers (ie: Angellozzi special growing contract for the production of truffle plants especially adapted to the Italian region of the Marche). Each batch of seeds and truflles we receive is well identified in order to keep track of these batches all along the steps of production. Each truffle we received is controlled under microscope with the aim to identify their spores. This control allows us to check the product supplied and to evaluate the quality of our inoculum. These truffle plants are produced in patented ROBIN ANTI SPIRALLING® containers. In specially designed greenhouses, the production of the truffle plants is daily monitored by trained staff in order to survey the watering and to avoid eventual trouble (diseases, insect damages). The development of the mycorrhizal association is regularly followed in order to maintain the permanence of this association. At the end of the growing season each batch of this production is sampled using statistical law. Each sample done is observed under stereoscopic microscope by trained staff in order to evaluate the mycorrhizal rate of the batch (in house control). After that, the final control (Certificate of mycorrhizal guarantee) is performed first by National French institute INRA in France and on the second hand by University of Perugia according to the regional rules for contracts in the Italian region of Marche. The control of the mycorrhizal rate is done laying particular stress on the good distribution of the mycorrhizas of the inoculated truffle along the root system and on the amount of these mycorrhizas and on the lack of other contaminant fungus.

THE GENUS *TUBER* IN SPAIN: NEW TECNICHES FOR YOUR INVESTIGATION

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KEY WORDS: *Tuber*, cryogenic scanning electron microscopy.

Several methodologies have been employed during decades for the identification and classification of Ascomycetous: the study of the ecosystem were they are isolated and their symbiont, light microscopic and magnifying glass observations for the study of their morphological characters, organoleptic recognition, fungi reaction front diverse reactives, etc. The continuous appearance of new equipment and their application to the fungi investigation are allowing us to a better knowledge of this fungus kingdom no described so far. The success of the application of cryogenic scanning electron microscopy (cryo-sem) to the study of microscopic fungi in previous investigations done in our laboratory allow us to discover new morphological characters no described by other researchers yet. This technique has not been applied to the study of these hipogeus fungi. Early studies say that genus *Tuber* in Spain it's composed by twenty species, six edibles nor the rest. The purpose of this investigation was the study of diverse autochthonous species of this genus by means of classical techniques and cryo-sem and confocal microscopical observations.

MONITORING THE DIVERSITY OF ECTOMYCHORRIZAL AND SOIL FUNGI UNDERLYING NATURAL AND MAN-MADE *TUBER MELANOSPORUM* PLANTATIONS

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KEY WORDS: fungal biodiversity, ectomycorrhizas, soil fungi, ITS, truffle plantations, ecology.

The production of truffles is the offspring of several intermingled factors that mediate the transition from the vegetative to the reproductive phase. Among these determinants, the fungal and microbial diversity of the soil likely stands out as a key player. Thus, monitoring microflora dynamics next to and on apical root-tips within truffle grounds is of paramount importance. Below ground, ectomycorrhizal communities are often species-rich. Yet, our knowledge on fungal biodiversity underpinning truffle plantations is still scant. Now, characterization of the different ectomycorrhizas (ECM) at the species level can be achieved by combining detailed morphological and anatomical descriptions with molecular approaches. Further to this, reliable procedures are available to isolate and analyse microbial and fungal DNA from soil samples. Lastly, the ongoing acquisition on public databases of sequence information relative to phylogenetically important genomic traits such as the small subunit (SSU) and the internal transcribed spacers (ITS) of the rDNA region for an increasing number of fungi has greatly improved the chances of giving species name to mycorrhizal structures and unculturable soil fungi. To investigate the fungal biodiversity underlying both productive and unproductive *T. melanosporum* natural and/or man made truffle plantations, we have sampled ECMs from truffle grounds located in central Italy (Latium and Umbria) and analysed them morphologically and molecularly via ITS rDNA region amplification. From these collection sites, by isolating and amplifying the fungal ITS region from soil samples, we generated a dataset of the soil fungal community. Both ectomycorrhizal and soil samplings were performed during the years 2007-2008. Until today, we were able to cluster ECMs into different morphotypes while approximately 50 ECM taxa on the basis of ITS analysis have been monitored. Interestingly, most of these ECMs have been also sampled into soil, likely as free living mycelia.
The ongoing analysis will help up tracking the fungal biodiversity on truffle fields over time and under different ecological situations. It will also enable us to test whether the presence or absence of specific ECM taxa is associated with truffle production.

CURRENT CHALLENGES IN TRUFFLE RESEARCH IN KOREA

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KEY WORDS: Edible ectomycorrhizal mushrooms, truffle, *Tuber* spp. isolation, *Tuber* infected plants production.

Growing the edible ectomycorrhizal mushrooms (EEMM) has proved to be a greater challenge than first anticipated due to the complex interdependencies in which fungi play a critical role. The challenge we face is to tilt the balance so that species of our choice can take up residence in such a complex natural setting-to design habitats in which it can grow. Many EEMM growers hope for huge profits when they try to grow European truffles, that sell at very high prices. Perigord truffle cultivation is also encouraged by governments in several countries as an alternative source of income to conventional agriculture. There are significant problems with the cultivation of EEMM on their host plants, and there are difficulties delivering quality products to the consumer. This paper will outline science and industry to this challenges in Korea, their successes in first steps of truffle fungi isolation and identification, production of *Tuber* sp. infected plants and some failures, the current state of our knowledge and will suggest a vision for the future.

CLIMATE CHANGE THREATENS THE TRUFFLE HARVEST

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KEY WORDS: Climate change and truffle.

History is full of poor or disastrous crops, as well as of environmental disaster.
Such arguments in themselves would not help to explain either the range or the quality of modern climate change. Rather, one might be led to consider it almost as a matter of routine.
However, worries and warnings are coming from the scientists about what is in store for us in a not so distant future. And it is just in this light that we should start considering poor crops, as they turn out to be directly connected with the radical changes which are taking place.
In the field of growing and harvesting all sorts of truffles, a quite remarkable decline in production has been recorded since last year.
It has all started from the drought in the second half of the year 2007, whose effects are still noticeable in spite of the late, heavy springs rains.
Having through the years gained a good deal of experience in this field, as far back as my memory goes I can say that an event of such prominence has never been recorded before.
Truffles are known to be reliable environmental indicators. Their presence is guarantee of the woods' healthy condition, and a drastic reduction in truffles must be seriously considered as the ringing of an alarm bell, as it goes beyond the specific field to show what is likely to happen in a more general context.
Anybody can perceive these worryng signs on going to the market or to the grengrocer's. There is less fruit on sale, and it is more expensive.
Being confronted with such an alarming prospect, we'd rather make a virtue of necessity – as the sayng goes.
This is why we now welcome the Truffle International Conference, which will be held in Italy in the next autumn.
It will be a good opportunity to investigate the changes, reflect upon the prospects in the field, and adopt the necessary measures which will protect the truffle's life and biodiversity, so that such a niche economy – which is so important not just in agriculture, but also in the marketing of quality products-may continue to exist.

CULTIVATION OF TUNISIAN *TERFEZIA B0UDIERI* CHATIN IN FIELDS CONDITION

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KEY WORDS: *Terfezia boudieri*, inoculation *Helianthemum sessiliflorum*, cultivation, Tunisia.

The transplantation of inoculated host plant of *Terfezia boudieri* Chatin (*Helianthemum sessiliflorum* Desf.) by ascosporous on an experimental field (containing two types of soils originated from the south of Tunisia, the first one is constituted by sand and the second by gypsum) and the inoculation of the same plants directly in the field permitted to produce a fruit body after two years of transplantation. For the sowed and inoculated plants, fruition takes place after the third year of cultivation.

TAXONOMIC STUDY OF DESERT TRUFFLES IN TUNISIA

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KEY WORDS: Taxonomic study, desert truffle, morphological criteria, *Terfezia*, *Tirmania*, *Picoa*.

Desert truffles are met in the arid and semi arid areas with warm climate around the Mediterranean basin, associated with roots of some perennial and annual species of *Helianthemum* and *Cistus* of the Cistaceae family. Their fruitbodies are very appreciated by local people for their organoleptic qualities and their therapeutic virtues.
Based on morphological criteria (form of spores and asci, spores ornamentation, structure of the peridium and the gleba), a mycological study has been done in the Range Ecology Laboratory of the Arid Lands Institute of Médenine, in order to identify desert truffles species which are present in southern Tunisia. Six species of truffles have been identified; *Terfezia boudieri* Chatin., *Terfezia claveryi* Chatin., *Tirmania nivea* Trappe., *Tirmania pinoyi* (Maire) Malençon., *Picoa carthusiana* Tul. & C. Tul. and *Picoa juniperi* Vittadini. The last two species have been recently found in Tunisia.

THE VIRULENCE OF *TUBER MELANOSPORUM*

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KEY WORDS: *Tuber melanosporum* Vittad., burnt area, foliage tree, equilibrium, tree pruning.

The production of *Tuber melanosporum* is generally that much better when the spread of the burnt area exceeds that of the host tree. This phenomenon, marked by a balance between the size of the tree and that of the burnt area has been called the 'virulence' of the truffle. It shows up on the root system of the tree which is deteriorated in the place where burning is the most active.
Studies of this phenomenon have allowed the definition of different characteristics relating to the state of the habitat and its cultivation. When the virulence is correct, the radius of the burnt aera (Rb) is usually greater than or equal to one and a half times the radius of the foliage (Rf); that is Rb ≥ 1.5 Rf. In function of the fertility of the soil or its water reserves, this equilibrium can be naturally encouraged (shallow soil) or restrained (deep soil) if the growth of the host trees is too rapid. The progression of the burnt area (Pb) is of the order of 10 to 15 cm per year under a good productive tree. This advance (Pb) can be faster when there are positive trophic points in the development zone. The plants beneficial to truffle grounds (dog roses, blackthorns or sloes and grapevines) are natural elements favouring the progress of the burnt area and the presence of fruiting bodies close to or in contact with their roots. The presence of animals on a truffle ground or plantation can be a factor which stimulates the virulence, with increased truffle production.
One experiment demonstrated that heavy pruning, unnecessary for the desired equilibrium, led to a zero progression of the burnt area (even a regression), with no yield at all in the harvesting season. Observing the virulence – or more precisely the Rb ≥ 1,5 Rf balance – has practical consequences for the management of the plantation. Tree-pruning is carried out taking care to limit the spread of the foliage. The potential development zone is reduced by pruning the trees, followed by thinning to regain space.
In some situations we have observed trees where the burnt area remains confined under the foliage and advances very slowly; with production at greater depths than usual. On the contrary, in other circumstances very marked virulence may be accompanied by lack of production despite a mycorrhizal profile dominated by *Tuber melanosporum*. The concept of virulence has been extended to include the productivity of truffle plantations, with characteristics common to truffle cultivation in France, Spain and Italy.

A FIRST STEP TO THE UNDERSTANDING OF MYCELIAL DATA IN *TUBER MELANOSPORUM-QUERCUS ILEX* ORCHARDS

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KEY WORDS: genomic and cell biology/ecology and population biology, *Tuber melanosporum*, soil mycelium, truffle cultivation, DNA, burn, rock cover.

Black truffle cultivation has become an important alternative in marginal agricultural lands, but understanding the biology and ecology of this fungus is essential to achieve successful production. We carried out observational studies in which, trees from *Tuber melanosporum* orchards in different stages of development were randomly selected to study the relationships between soil mycelial abundance, tree growth and surface rock cover, with the presence and extension of the *burn* and with truffle production. Mycelial distribution and abundance were estimated by molecular tools. We found that the allelopathic effect of *T. melanosporum* mycelium initially became evident on the surface around the trees after a certain biomass of *T. melanosporum* mycelium had formed below-ground, although its DNA could be detected before the appearance of the *burn.* The amount of black truffle mycelium was greater in newly productive trees than in nonproductive trees, but when trees were productive for many years, we observed a decrease in the soil mycelial biomass, perhaps because the investment of fungal resources are shifted from vegetative growth to ascoma-ta (truffle) formation. In 8-yr-old productive trees, *T. melanosporum* mycelium was mainly found in the first 35 cm of the soil profile although it could be detected as deep as 60 cm but in lower quan-tities, and not necessarily growing within the visual edge of the *burn.* Tree growth was positively related to the appearance of the *burn,* and surface rock coverage was dissimilarly related to the pro-ductivity of truffles, depending on the average rock cover of the orchard. Further and more exten-sive studies are needed to explain and to interpret these results in order to understand the black truffle “behaviour”.

RESEARCH ON HYPOGEOUS FUNGI AND FIRST HARVEST OF A CULTIVATED TRUFFLE IN AUSTRIA

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KEY WORDS: truffle cultivation, *Tuber*, mycophagy, spore dispersal.

1. In the past decade, a program for the inoculation of tree seedlings with one autochthonous (*Tuber aestivum* f. *uncinatum*) and with one introduced (*Tuber melanosporum*) truffle species was initiated. The first *Tuber aestivum* f. *uncinatum* was harvested under *Corylus colurna* on October 26th, 2008, on a 5 years old plantation established in a an alluvial plain with naturally calcareous soils, about 50 km south of Vienna. Thus far, no harvests of *Tuber melanosporum* were recorded.
2. Accompanying inventory research on truffles revealed the presence of about 13 naturally occurring taxa of the genus *Tuber*, depending on species concepts. *Tuber aestivum* f. *uncinatum*, *T. mesentericum*, *T. brumale* f. *brumale*, *T. brumale* f. *moscatum*, *T. rufum* and *T. excavatum* were most frequently found. The group of small white truffles was less represented, but this bias may be due to our targeted search for *T. aestivum* f. *uncinatum.* There are no Austrian records of the most precious edible truffle species, *T. magnatum*, *T. melanosporum* and *T. macrosporum*.
3. Molecular analyses of anamorphs present in the vicinity of *Tuber* ectomycorrhizae revealed the first known conidial states in the genus Tuber. The anamorphs were genetically linked to *T. borchii* and *T. oligospermum*.
4. An ongoing research project is dedicated to the investigation of the role of small mammal mycophagy in vectoring fungal spores, particularly those of hypogeous ectomycorrhizal fungi, in Austrian mountain forests dominated by *Piceae abies*, *Fagus sylvatica* and *Abies alba*. A multidisciplinary approach, including the microscopic and DNA based analysis of fungal spores in fecal pellets, the inoculation of tree seedlings and the inventory of the ECM fungal communities is being used. First results indicate that mycophagy is widespread but variable among different species of forest small mammals. Species of many different genera of hypogeous fungi, including *Elaphomyces, Tuber, Balsamia, Melanogaster, Hymenogaster, Hysterangium, Chamonixia, Octavianina* and others were detected. Some of the species which are frequently consumed by small mammals were deemed as rare thus, since there are only few herbarium records of these species. Only few of the species vectored by small mammals could be detected in the mountain forest ECM communities, including *Tuber puberulum* and *Elaphomyces* sp.

TRUFFLE CULTIVATION IN SICILY: PRELIMINARY RESULTS

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KEY WORDS: Truffles, Cultivation, Sicily.

In recent times truffles were found in several natural forest ecosystems and reaforestation of Sicily (Bencivenga & Venturella 2001; Pecorella & Venturella 2006; Venturella 1995; Venturella *et al*. 2004, 2006). On the basis of these findings a number of research projects were activated. In the framework of an agreement between the Laboratory of Micology of the Department of Botany (University of Palermo) and the Farm "Le Due Sicilie", located in the territory of the Madonie Natural Park, a first attempt to cultivate truffles of economic importance were carried out (Venturella *et al*. 2007). In particular the seedlings were provided and certified by "Raggi Vivai" (Cesena, Italy). In the spring of 2006 the experimental truffle-bed was developed on the basis of 100 seedlings of *Corylus avellana* L. inoculated with *Tuber aestivum* Vittad. (80%) and *T. melanosporum* Vittad. (20%). Besides 110 seedlings of *Quercus pubescens* Willd. s.l., inoculated with *T. melanosporum* (20%) and *T. aestivum* (80%) were planted. The techniques of cultivation and the scheme of planting of the truffle-bed together with the preliminary results of two years of observations are here pointed out.

TUBER MAGNATUM PICO SPORE GERMINATION

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KEY WORDS: biology, ascus, mycelium.

The aim of this work was to elucidate and to describe the processes that favour *Tuber magnautum* Pico spore germination. All the phases that follow spore release from the *ascus* up to the primary mycelium formation are described. Spore behaviour in the presence of the symbiotic plant and in its absence is also analysed.

TUBER MELANOSPORUM VITTAD. SPORE GERMINATION

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KEY WORDS: spore, mycelium, symbiotic plants.

The author describes the various phases occurring during *Tuber melanosporum* Vittad. spore germination, from the spore release from the *ascus* up the primary mycelium formation. Spore behaviour in the presence of the symbiotic plant and in its absence is also analysed.

THE *MAGNATUM* PROJECT (*M*ONITORAGGIO DELLE *A*TTIVITÀ DI *G*ESTIONE DELLE TARTUFAIE *NA*TURALI DI *TU*BER *M*AGNATUM)

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KEY WORDS: *Tuber magnatum*, conservation, ecology, management, soil mycelium, real time PCR.

MAGNATUM is the acronym for a four year inter-regional cooperative project co-funded by the Abruzzo, Emilia – Romagna, Molise and Tuscany Regions established through a public initiative and coordinated by ARSIA (Agenzia Regionale per lo Sviluppo e l'Innovazione nel settore Agricolo-forestale) of the Tuscany region. The aim of this project is to obtain scientific information that will be of use in safeguarding natural *T. magnatum* truffières. It will integrate well established methodology and molecular technology.

Four natural *T. magnatum* truffières, one in each of the collaborating regions will be chosen which will be closed to the public so that the collected scientific data will be more meaningful. The chosen truffières will also have pedological, vegetational and climatic characteristics considered ideal for each region.

In each truffière the effects of selective thinning and different methods of soil tillage will be observed for their effect on truffle production, the ectomycorrhizal fungal communities, and soil physical and chemical characteristics. Their effects on the presence and dynamics of *T. magnatum* mycelium in the soil will be also evaluated by real time PCR techniques.

The integrated use of different methods of analyses should provide insights into truffle biology giving information on the relationship between truffle production and the vegetative development of *T. magnatum* in the soil. The molecular quantification of *T. magnatum* mycelium in the soil of natural truffières, were the variability of fruiting body production often gives results difficult to evaluate, may also allow us to establish the effects of different management treatments.

ECOLOGY AND DISTRIBUTION OF HYPOGEAL FUNGI IN ARMENIA

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KEY WORDS: ecology, Armenia, hypogeal fungi, rDNA-ITS sequences.

Hypogeal fungi (HF) are mainly mycorrhiza forming mushrooms. They belong to phyla Zygomycota (*Endogonales*), Glomeromycota (*Glomerales*), Ascomycota (*Pezizales, Elaphomycetales*) and Basidiomycota (*Agaricales, Boletales, Phallales, Russulales)*. Regular rainfalls 700-1200 mm per year, sylvan brown and calcareous soils are necessary ecological factors for their growth.
Six species of HF (*Tuber aestivum*, *Elaphomyces granulatus, Octaviania stephensii*, *Rhizopogon roseolus, R. luteolus*, *Cremeogaster klikae*) were previously reported in Armenia. Our collections were identified by morphological and/or molecular methods using rDNA-ITS sequence data. Described 12 species belong to Glomeromycota (*Glomus macrocarpum*), Ascomycota (*Elaphomyces muricatus, E. granulatus, Hydnotrya tulasnei, Tuber rapaeodorum, T. rufum, T. scruposum, Tirmania pinoyi, Picoa juniperi*) and Basidiomycota (*Hymenogaster griseus, H. olivaceus, Octaviania stephensii*). Ten species (except *O. stephensii* and *E. granulatus*) were firstly reported in Armenia and South Caucasus region. Molecular analyses suggest that *T. scruposum* is a valid taxon which morphologically (peridium thickness and structure, characters of the cystidia and spores) differs from other whitish *Tuber* species (*T. puberulum, T. maculatum, T. foetidum, T. rapaeodorum, T. borchii).*
Distribution and growth territories of HF in Armenia were uncovering. Ecological characteristics, particularly type of soils (sandy-clayish, sylvan turf-carbonate, etc.) and host plants (*Cornus mas, Fagus orientalis*, *Pinus kotschiana,* etc.) of Armenian HF were revealed.

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