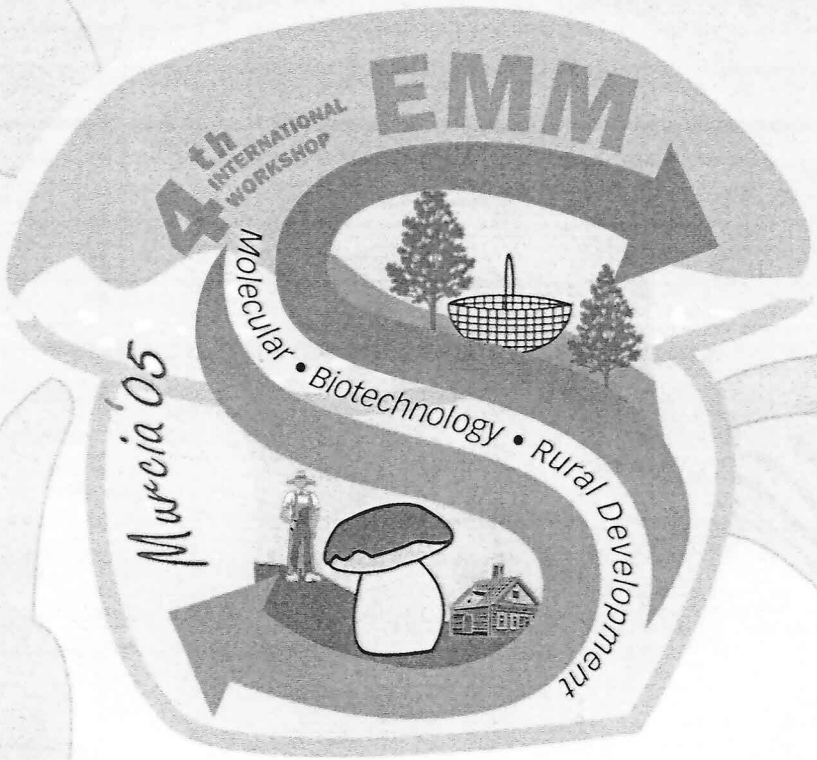

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IWEMM4 PROGRAMME

MONDAY 28th November

- 11.00 h. **ANTI JET-LAG SESSION** in Bullas.
  A visit to the Wine Museum of Bullas and a tasting of the famous Bullas' wine are included.

- 17.00 h. – 20.00 h. **Registration.**

- 19.00 h. – 20.00 h. **Put up Posters (Sessions A & B)**

- 21.00 h. **Welcome to the participants.**

TUESDAY 29th November

- 8.00 h. – 9.15 h. Late Registration.
  Put up Posters (Sessions A & B)

- 9.15 h. – 9.45 h. **Plenary Session:**
  EDIBLE ECTOMYCORRHIZAL MUSHROOMS: MANAGEMENT AND HARVESTING.
  Yun Wang. 123

- 9.45 h. – 11.00 h. **ORAL SESSION 1**

  Chairs: **Mario Honrubia and Alexis Guerin-Laguette**

  - 9.45 h. – 10.00 h.: FROM MOLECULAR TAXONOMY TO SPECIFIC MARKERS FOR *BOLETUS EDULIS* SENSU LATO GROUP.
    Antonietta Mello, Stefano Ghignone, Alfredo Vizzini, Clizia Sechi, Pino Ruiu and Paola Bonfante. 86

  - 10.00 h. – 10.15 h.: IDENTIFICATION OF *TUBER MAGNATUM* GENOTYPES FROM TRUFFLE GROUND SOIL SAMPLES.
    Claude Murat, Antonietta Mello, Matteo Cagnasso and Paola Bonfante. 91

  - 10.15 h. – 10.30 h.: THE PRODUCTION OF TRUFFLE INFECTED PLANTS USING MYCELIAL ISOLATES.
    Alessandra Zambonelli, Micro Iotti, Enrico Bonuso and Ian Hall. 124

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- 13.30 h. – 15.00 h. **LUNCH**

- 15.00 h. – 16.45 h. **ORAL SESSION 3**

Chairs: David Pilz and Fernando Martínez-Peña

16.45 h. – 17.00 h. **COFFEE**

17.00 h. – 19.30 h. **POSTER SESSION "A"**

Chairs: Yun Wang and Manuela Pérez-Gilabert

17.00 h. – 19.30 h. **POSTER SESSION "B"**

Chairs: Koji Iwase and Guillermo Pereira

- 19.45 h. **SPANISH AND FLAMENCO PROFESSIONAL DANCE: *BALLET ENTREDANZAS.***

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WEDNESDAY 30th November

- 8.30 h. EXCURSION TO:

  "MUSEO DE LA HUERTA" IN ALCANTARILLA,
  "MUSEO ARQUEOLÓGICO LOS BAÑOS" IN ALHAMA DE MURCIA,
  "SIERRA ESPUÑA REGIONAL PARK" AND
  THE PROTECTED LANDSCAPE OF ***"LOS BARRANCOS DE GEBAS".***

- 18.00 h. Arrive in Murcia.

- 18:30 h. **TAKE OFF POSTER SESSIONS A & B.**
  PUT UP POSTER SESSIONS C & D.

THURSDAY 1st December

- 9.00 h. – 11.00 h. **ORAL SESSION 4**

  Chairs: Koji Iwase and Fernando Martínez-Peña.

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- 13.30 h. – 15.00 h. **LUNCH**

- 15.00 h. – 17.00 h. **ORAL SESSION 6**

Chairs: Santiago Reyna and Alessandra Zambonelli

- 17.00 h. – 17.30 h. **COFFEE**

- 17.30 h. – 19.00 h. **ORAL SESSION 7**

Chairs: Yun Wang and Almudena Gutiérrez

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- 18.00 h. – 18.15 h.: THE WILD EDIBLE MUSHROOMS OF MEXICO: STATE OF KNOWLEDGE AND PERSPECTIVES.
Jesús Pérez-Moreno. 102

- 18.15 h. – 18.30 h.: ASSESSMENT OF SUSTAINABLE COMMERCIAL HARVESTING IN KHABAROVSK AND PRIMORSKY KRAIS, RUSSIA.
David Pilz. 104

- 18.30 h. – 18.45 h.: RADIOACTIVITY AND METAL BIOACCUMULATION BY *CLAVARIADELPHUS TRUNCATUS*.
María Isabel Gaso, J.L. López and Ángela Machuca. 62

- 18.45 h. – 19.00 h.: THE NUTRITIONAL AND MEDICINAL VALUE OF WILD FUNGI: BALANCING THEIR POSITIVE CONTRIBUTIONS TO LIVELIHOODS AND POTENTIAL SAFETY CONCERNS.
Miriam de Román and Eric Boa. 38

- 21.30 h. **IWEMM4 DINNER**

FRIDAY 2nd December

- 9.00 h. – 10.00 h. **ORAL SESSION 8**

Chairs: Asunción Morte and Alexis Guerin-Laguette

- 9.00 h. – 9.15 h.: RHIZOSPHERE MICRO-ORGANISMS IN NATURAL TRUFFLE-GROUNDS OF *TUBER MAGNATUM* WITH DIFFERENT PRODUCTIVITY – PRELIMINARY SURVEY.
Francesco Tagliaferro, Anna Maria Ferrara and E. Viotto. 118

- 9.15 h. – 9.30 h.: TRUFFLE-GROWING IN NAVARRA (NORTHERN SPAIN): FUNGAL BIODIVERSITY OF *TRUFFIÈRES*.
Ana de Miguel, Begoña González-Armada, Vanessa Clavería and Raimundo Sáez. 37

- 9.30 h. – 9.45 h.: MACROFUNGI ASSOCIATED WITH *CASTANEA SATIVA* IN NORTHEAST OF PORTUGAL. TEMPERATURE AND RAINFALL EFFECT ON FUNGAL FRUCTIFICATION.
Paula Baptista, Anabela Martins, Rui Tavares and Teresa Lino-Neto. 25

- 9.45 h. – 10.00 h.: REGULATION OF WILD MUSHROOM HARVESTS IN PINEWOODS OF ALMAZÁN FOREST RANGE (SORIA, SPAIN): SOCIO-ECONOMIC BALANCE.
Miguel López Estebaranz, Fernando Martínez Peña, Mercedes Molina, Ana Hernández Fernández and Juan Antonio Lucas. 80

- 10.00 h. – 10.30 h. **COFFEE**

- 10.30 h. – 13.00 h. **POSTER SESSION "C"**

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Chairs: **Santiago Reyna and Shannon Berch**

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- 10.30 h. – 13.00 h. **POSTER SESSION "D"**

Chairs: Alessandra Zambonelli and David Pilz

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- 13.00 h. – 13.30 h **CONCLUSIONS & CLOSSING SESSION**

LACTARIUS DELICIOSUS (L.: Fr.) S. F.Gray PERFORMANCE IN A PURE AND MIXED PINUS STAND OF *PINUS PINASTER* Ait. AND *QUERCUS PYRENAICA* Willd. IN THE PROVINCE OF SORIA (SPAIN).

Ágreda Cabo, T.[1], Fernández Toirán, M.[2], Martínez Peña, F.[3]

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Key words: Fruit body production, forest succession, mushroom hunting, *Pinus pinaster*, *Quercus pyrenaica*, *Lactarius deliciosus*, saffron milk-cap.

Mycological production has been studied in a pine forest of *Pinus pinaster* Ait. located in the south-east of the province of Soria in Central Spain. Altitude ranges from 1000 to 1200 m and annual rainfall from 500 to 700 mm, with a marked summer drought. Average annual temperature is 10.1ºC, with cool winters. Traditionally, resin was the main product of these forests, but changes in resin value have led to a gradual shift towards wood production. Mushroom harvesting is becoming a major activity, mainly focused on *Lactarius deliciosus* Fr. but also including *Hygrophorus* and *Tricholoma* species.

Since in wide areas of this stand the pine is mixed with *Quercus pyrenaica* Wild. the study that initially was reduced to the pure pinewood, was extended to all the stand. As the production is quantified depending on the age of the stand, the study examined three groups of age-classes: 0-40, 41-60 and more than 61. They were established thirty three 150 m^2 plots to perform a random stratified survey. These fixed plots were sampled every week over three falls from 2002 to 2004.

The objectives of this study are: 1) quantifying *L. deliciosus* production, 2) the description of fungal succession among forest age-classes, 3) the comparative study between the pure and mixed zone, and 4) the phenology of this production.

Sporocarp production presents a wide variability between different years consequence specially of the climatic conditions. In this way, the year 2004 *L. deliciosus* did not appear in the plots. Sporocarps yielded during the falls of 2002 and 2003 11.3 kg/ha in the pure zone and 23.0 kg/ha in the mixed zone. In early-age ecosystems the production is higher, with 11.8 and 46.8 kg/ha respectively. In November it has produced the 64 % of the *L. deliciosus* biomass in the pure zone and the 81% in the mixed zone.

Production where pine and oak coexist is twice higher than that of pure areas, which suggests that tree diversity has favoured *Lactarius deliciosus*. In both cases, the greatest productions take place in early-age stands and there is a remarkable upturn in older stands in the pure pine forest.

CHARACTERIZATION AND QUANTIFICATION OF THE ECTOMYCORRHIZAE OF THE TRUFFLE PLANTATION "LOS QUEJIGARES" (SORIA, SPAIN)

Águeda Hernández, B.[1], Fernández Toirán, M.[2] and de Miguel, A.M.[3]

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Key words: *Tuber nigrum* Bull., *Quercus ilex* L., ectomycorrhizae, truffle-growing, Arotz-Catesa.

The truffle-plantation "Los Quejigares" is situated in the province of Soria (Castilla y León, Spain), in the Southern slope of the Sierra de Cabrejas. It is a 600 ha plot situated at 1250 m a.s.l., on calcareous soil, the medium annual precipitation is 821,4 mm and the medium annual temperature is 8,6 °C. The holm-oaks (*Quercus ilex* L. subsp. *ballota* (Desf.) Samp.) mycorrhizated with *Tuber nigrum* Bull. were planted in 1979 by Arotz-Catesa. This plantation is one of the oldest truffle-plantations of Spain and the largest of the world and, nowadays, it is in fully production. Soil cultivation, calcareous stone crushing, irrigation and pruning have been done every year for plantation maintenance. Between 1999 and 2001, the ectomycorrhizae of this plot were studied with the aim of knowing *T. nigrum* mycorrhization percentages and the diversity and abundance of other ectomycorrhizal types presents on the fungal community in these truffle-productive trees.

The roots of 16 holm-oaks were sampled, 4 did not produce truffle sporocarps and 12 produced them. Two annual sampling were doing in spring and autumn, following the Global Method (Verlhac *et al.*, 1990).

It was found a high percentage of *T. nigrum* mycorrhizae, with a media about 70%. All the studied trees had this fungi in their roots, independently of their capacity to produce or not truffles. Numerous ectomycorhizal types were found, 39 of them are identificated: *T. nigrum*, *T. brumale* Vitt., *T. aestivum* Vitt., *Cenococcum geophylum* Fr., *Pisolithus tinctorius* (Pers.) Coker & Couch, Type *Hymenogaster*, Type *Scleroderma*, Type *Hebeloma*, Type *Cortinarius*, Type *Genea*, Type Boletal and 28 Types of Thelephorales.

In spite of the high number of morphotypes found, it seems that they do not replace *T. nigrum*, showing that there is a dynamic balance in the fungal community associated to the tree roots. These conclusions agree completely with other authors like Horton & Bruns (2001).

BASES FOR ECONOMIC VALUATION OF MUSHROOM PRODUCTIONS IN FOREST ECOSYSTEMS

Álvarez Nieto, Amparo[1] and Oria de Rueda Salgueiro, J. Andrés[2]

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Key words: Non-timber forest products, mushrooms, economic valuation.

Forest evaluation was initially connected to timber. Forests were mainly studied as wooden producers. As the society takes conscience of forest systems as biological multifunctional systems that offer a big diversity of goods and services apart from wood, forest evaluation has advanced and also it has developed new tools.

Valuation of wooden products is currently well documented. On the contrary, valuation of forest mushroom productions is much less developed. There are still very few papers on this subject. Moreover, a synthesis of theoretical foundations and main techniques is needed. This will be the main objective of this work.

To carry out this conceptual and methodological approximation, foundations of agrarian and forest valuation have been adapted to particularities of fungi production. As result, a theoretical development of skills that allow fungi valuation in different situations has been carried out. Characteristics of this resource to delimit the guidelines of investigation conducive to its properly valuation in different types of forests have been also analysed. Finally, implications in forest management are evaluated.

ECTOMYCORRHIZAL FUNGI IN WOODED BANKS: WHAT IS THE ECOLOGICAL AND ECONOMIC VALUE?

Jacqueline Baar

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Key words: Ectomycorrhizal fungal diversity, forest management practices

Wooded banks are characteristic for the agricultural landscape in The Netherlands and have a relatively high ecological value. However, the economic value is often considered as low and a considerable number of wooded banks have been removed over the last decades. This has resulted in reduced diversity of flora and fauna in agricultural areas.

From 2002-2006, the Dutch Ministry of Agriculture has funded a project to study the ecological and economic contribution of ectomycorrhizal fungi to wooded banks to prevent further removal of wooded banks. The economic contribution could be that edible mycorrhizal fungi can be sold by land owners as a delicacy for human consumption.

This study focused on monitoring the diversity of ectomycorrhizal fungi above- and belowground in two wooded banks of different age (3 and 60 years old) in an agricultural area in The Netherlands since 2002. Furthermore, this study investigated whether two different management practices could raise the number and diversity of ectomycorrhizal fruitbodies, including edible fungi. This management practices comprised removal of topsoil and addition of rain water. The dominant tree species in the old wooded bank was *Quercus robur* while the young wooded bank consisted of a mixture of tree species, including *Q. robur*.

The highest diversity of ectomycorrhizal fruitbodies was observed in the old wooded bank from 2002-2004. Molecular analysis with fungal specific primers revealed that the diversity of ectomycorrhizal fungi below ground in the old wooded bank was higher than in the young one. The old wooded banks contained numerous ectomycorrhizal species, including edible species as Boletus edulis and various *Russula* species. Fruitbodies of only one ectomycorrhizal species were observed in the young wooded bank. Addition of rain water raised the number of ectomycorrhizal fruitbodies in the old wooded bank, especially in dry periods of season. Removal of topsoil was not effective within the first three years after application, but longer term effects are expected.

MACROFUNGI ASSOCIATED WITH *CASTANEA SATIVA* IN NORTHEAST OF PORTUGAL. TEMPERATURE AND RAINFALL EFFECT ON FUNGAL FRUCTIFICATION

Paula Baptista[1], Anabela Martins[1], Rui Tavares[2] and Teresa Lino-Neto[2]

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Key words: macrofungi, abundance, diversity, *Castanea sativa*, ecology.

Macrofungal fructification is a seasonal event related with environmental factors, such as temperature and rainfall. A relationship with climatic factors and sporocarps production has been established but these correlations differs considerably from environment to environment. In this context, the present work aims were *i*) to known the macrofungal community associated with chestnut tree, *Castanea sativa* Mill. and the community changes over time in the Northeast of Portugal; and *ii*) to evaluate the possible effect of temperature and rainfall on macrofungal fructification during three consecutive years.

The study was carried out from 2002 to 2004, in a non-tilled *C. sativa* orchard located in Bragança (29T PG 80 9 36 UTM), Northeast of Portugal. In the orchard, five plots of 100 m^2 each were selected and all the sporocarps were collected weekly (during autumn and spring) or monthly (in the winter and summer periods). All the sporocarps were separated, counted and identified by genera or species. Meteorological data were collected in a climatic station located nearest to studied area. Total rainfall and mean, maximum and minimum temperatures were correlated with number of species, number of sporocarps, as well as total or different trophic groups. This correlation analysis was performed with weather data collected in different periods (5-30 days) before the sampling date. In the course of the work, 87 macrofungal species belonging to 23 genera were identified. About 85% of the collected species were mycorrhizal, from which the genus *Inocybe* spp. and *Russula* spp. contained the highest number of species. The environmental conditions, especially the rainfall, seem to have influence on macrofungal fructification mainly during the autumn period when the number of species and individuals were more abundant. The abundance and biodiversity as well the evolution of number of macrofungal species along the three years will be discussed. Correlation analysis between data weather and fungi variables will be also analysed in order to evaluate their effect on macrofungi production in Northeast of Portugal.

Acknowledgements: The authors are grateful to AGRO Project 689 for financial support of this work.

STUDY OF THE MACROFUNGI COMMUNITY ASSOCIATED WITH PINUS (*PINUS PINASTER*), CHESTNUT (*CASTANEA SATIVA*) AND OAK (*QUERCUS PYRENAICA*), IN THE NORTHEAST OF PORTUGAL

Baptista P.[1]; Rodrigues P.[1]; Sousa M.J.[1]; Rodrigues A.P.[2]; Borges A.[3] and Martins A.[1]

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Key words: Biodiversity, macrofungi, chestnut, oak, pinus, mycorrhizal, edible.

The work here presented concerns the preliminary results of Project AGRO 689 "Agronomic, economic and environmental interest of macrofungi associated with pinus (*Pinus pinaster*), chestnut (*Castanea sativa*) and oak (*Quercus pyrenaica*), in the Northeast of Portugal: Production of mycorrhizal pinus, chestnut and oak plants".

We intend to evaluate the biodiversity of macrofungi associated with each habitat and to get insight into the differences between mycorrhizal and nonmycorrhizal species as well as of edible and non edible ones. Edible mycorrhizal and nonedible mycorrhizal ratios will also be calculated in order to know the impact of their collection in either ecological and economical aspects.

During the two seasons of collection (Autumn 2004 and Spring 2005), oak habitat showed the highest number of species and of carpophores while pinus was the poorest habitat either in terms of number of species and of carpophores. We discuss the results obtained, comparing the three habitats in terms of biodiversity and potential economical interest of edible macrofungi collection and of ratio of the different trophic levels. We intend to contribute for the evaluation of risk assessment of overcollection of edible species and to a sustainable macrofungi management.

This work was financed by the Project AGRO 689.

PRODUCTIVITY OF *SUILLUS LUTEUS* IN PONDEROSA PINE PLANTATIONS FROM PATAGONIA (ARGENTINA)

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Key words: Field plantations, fungal sylviculture and ecology.

In Argentina, along the piedmont of the Patagonian Andes, there are vast grasslands located within the ecotone between native forests on the west and steppe on the east, which are suitable for afforestation with fast growing non-native conifers. The main objectives of this activity are to produce high quality timber, to foster social and economic development of the region, and also to ameliorate or even reverse erosion in areas that have been heavily overgrazed. Ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) is the most widely planted species in Patagonia; plantations were initiated around 50 years ago, and approximately 50,000 ha are currently forested, while 2,225,000 ha of suitable lands are still available. Previous works have reported ectomycorrhizal species present in ponderosa pine nurseries and plantations, and *Suillus luteus* (Fr.) S.F. Gray has been reported as widely distributed and abundant. This fact reveals not only good adaptability of the species in the region, indicating its efficiency as an EM fungus, but also the opportunity to harvest, process and commercialize its fruit bodies in order to obtain an alternative income to the forestation plan, that can help to finance plantation and management costs. Preliminary observations showed that sporocarp productivity varies between plantations, but the factors responsible for this variability are largely unknown. The aim of this study was to evaluate *Suillus luteus* productivity in ponderosa pine plantations under different sites conditions and management practices, and to develop recommendations regarding site selection and forest management options to maximize fructification.

Eleven plantations between 13-22 years old, with null to minimum slope located around Esquel City (Chubut-Argentina) were selected, considering a range of precipitations, soil types, stand densities and pruning situations. Each one was described by a circular 300 m^2 plot. Variables of soil and stand characteristics were measured, along with light, needle cover and depth, and under canopy vegetation. Four 100 m long x 2 m wide permanent transects were installed in each plantation. They were visited weekly from march to June (rain season), to measure sporocarp production, soil water content, air humidity, soil and air temperatures.

Preliminary results are presented from autumn 2005 (the survey will continue for at least two more seasons). Differences in sporocarp production were detected, with values between 0 to 14.03 Kg dry weight per ha per season. Plantations with higher productivity were associated with abundant herb cover and scarce needle depth, while plantations with lowest productivity were associated with sandy soils with little shrubs cover or with not pruned stands with abundant needle cover of 7-8 cm depth.

ESTABLISHING PERIGORD BLACK TRUFFLE PRODUCTION IN BRITISH COLUMBIA, CANADA

Shannon Berch, Sharmin Gamiet, Quentin Wyne, Wayne Haddow, Richard Winder, Bill Chapman and Dan Durall

Members of the Research Committee, Truffle Association of British Columbia, Duncan BC, Canada

Key words: field plantations and fungal silviculture and ecology, *Tuber melanosporum*

The Truffle Association of BC (TABC) is a registered non-profit organization established in 2004 and dedicated to education, research, and commercial development of culinary truffles, especially Perigord Black Truffles, in British Columbia. One of the goals of the Association is to secure research and development funds in support of new truffle orchards in the province. TABC currently has 15 members consisting of producers, small business owners, university faculty and private individuals. Truffle orchards have not previously been attempted or established in BC, although they have been successful using hazelnuts and oaks as host trees in New Zealand, Australia and parts of the US. Our analysis of the conditions in all of the areas where Perigord truffle is successfully grown indicates that parts of the southern interior and south-western BC should be suitable for truffle production.

Some TABC members have entered into agreements with a supplier to establish BC's first truffle orchards. Since the initial inputs are relatively high when establishing a truffle orchard, and the returns are delayed by up to 10 years, it is essential that *Tuber melanosporum* mycorrhizas remain dominant on the host. It is therefore critical that experienced and trained personnel examine mycorrhizas and the DNA of the fungus before inoculated seedlings are transplanted and while the mycorrhizas develop in the orchard. This will ensure that the fungus is correctly identified and that it dominates on root systems at time of establishment and throughout the orchard over time. It is also essential that soil characteristics are monitored for pH, organic matter, major and minor nutrients, especially calcium, nitrogen and phosphorus, key elements that are critical for mycorrhizal development.

To ensure that the investment of time and money is not lost, we must develop a best practice guide on tree seedling inoculation and monitoring, a framework for providing assistance, monitoring and publishing on the establishment of truffle plantations, certification processes for verification of truffle species at planting and for quality assurance reporting to industry and consumers, and plant health protocols.

DISTRIBUTION AND PRODUCTIVITY OF PACIFIC GOLDEN CHANTERELLES AND OTHER MUSHROOMS ON NORTHERN VANCOUVER ISLAND, BRITISH COLUMBIA, CANADA

Shannon M. Berch[1], Richard Winder[2], Tyson Ehlers[3] and Gary Hunt[4]

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Key words: field plantations and fungal silviculture and ecology, *Cantharellus formosus*, commercially harvested mushrooms

This report provides a summary of information that has been collected on commercial mushrooms on Northern Vancouver Island as part of the North Island Non-timber Forest Product Demonstration Project. The overall aims of this component of the project were to identify fungal species from a range of forest types, and gather information on the ecology, distribution and abundance of some important commercial mushrooms that is needed to develop management strategies to sustain or enhance their production. Forty commercial mushroom species were identified. Pacific golden chanterelle (*Cantharellus formosus* Corner) is the most widely harvested and economically important mushroom species on Northern Vancouver Island. Pacific golden chanterelle was found on 86 sites, 33 of which were considered highly (commercially) productive. Based on the information gathered from these sites, a habitat model was developed to map the distribution of Pacific golden chanterelle habitat across portions of Canadian Forest Products' and Western Forest Products' operating areas on Northern Vancouver Island. Some field-checking has verified predictions made by the habitat mapping model; additional data collected this year could be used to refine the model.

Three sites predicted to be similar in Pacific golden chanterelle productive capacity in the Nimpkish Valley were selected for further study to estimate production levels. Chanterelles were harvested from plots every 2 weeks throughout the fruiting season. Total biological chanterelle production across the 3 sites averaged 8.3 kg/ha/yr fresh weight. Chanterelle fruiting locations (patches) were mapped within the plots and habitat characteristics surrounding some of these patches were described in detail. We discuss the challenges we encountered in conducting this type of a study, and suggest ways to improve it in the future. This research provides a foundation for integrating production of commercial mushroom resources, particularly chanterelles, into forest management planning on Northern Vancouver Island and elsewhere in British Columbia.

PHYLOGENETIC RELATIONSHIPS OF NORTH AMERICAN TRUFFLES IN THE GENUS *TUBER*

Gregory Bonito[1] , Rytas Vilgalys[1], Omoanghe Isikhuemhen[2] and James Trappe[3]

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Key words: Tuber, phylogenetics, systematics / taxonomy, molecular biology, genetic diversity

Due to development of the hypogeous habit and dependence on mycophagy for spore dispersal, convergent evolution of hypogeous fungi present unique challenges for morphological systematics. However, the advancement of molecular methods has created new opportunities to examine genetic diversity among hypogeous taxa and to test morphological hypotheses of relationships in an independent ! manner.

In this study, we examine molecular-based diversity of the genus Tuber in North America. DNA was extracted from a broad collection of herbarium specimens originating from across the North American continent. A phylogenetic analysis based on ITS and RPB1 genetic loci was used to ascertain phylogenetic relationships of these North American truffle species and to explore cryptic diversity in species-complexes such as the Oregon white truffle, Tuber gibbosum. Molecular tools developed through this research will be used for: 1) sequence-based identification of mycorrhizal root tips; 2) competition and ecological studies; and 3) to explore patterns of Tuber molecular diversity on host trees across North American.

COMPARISON BETWEEN THE VARIABILITY OF THE ITS AND IGS REGIONS IN *TUBER BORCHII*.

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Keywords: molecular biology, *Tuber borchii*, genetic variability, ITS, IGS, rDNA

Tuber borchii Vittad. is an excellent truffle with a significant local market in the northeast of Italy. This truffle has a high ecological adaptability and a low host specificity, forming ectomycorrhizae with broad leaf trees and coniferous species in different natural habitats. Recently there has been great interest in *T. borchii* cultivation for the possibility of extending truffle cultivation also in areas not suitable for the most precious *T. melanosporum* and *T. magnatum*. Moreover *T. borchii* is easily cultivable in pure culture and *T. borchii* - *Tilia platyphyllos in vitro* infected plants have been extensively used as a model for understanding the molecular bases of mycorrhizal development and regulation. Despite the great importance of this truffle, the knowledge on its genetic variability is scarce. Our work analysed the genetic variability of the internal transcribed spacer (ITS) and of the intergenic spacer (IGS) of the nuclear ribosomal DNA. Several fruit bodies from different geographical origins and host plants were analysed in order to discover differences in size and/or in sequence of these regions. The first results obtained validated the absence of 5 S region in rDNA in all strains analysed and confirmed the size of 3,7 kb of *Tuber borchii* IGS region. We found a higher number of SNP in the IGS than in ITS region.

EFFECTS OF THE INITIAL LEVEL OF MYCORRHIZATION ON THE EVOLUTION OF YOUNG PLANTS INOCULATED WITH *TUBER MELANOSPORUM*

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Key words: Field plantation and ecology, mycorrhization, *T. melanosporum*, young plant quality

An experimental plot to monitor the evolution of the quality of mycorrhizal plants was designed in 2002 by the Ctifl, in collaboration with the Creysse experimental station, the agriculture chamber of Corrèze and the Martel truffle association. The aims of the work are two fold: to study the effect of the initial level of mycorrhization of young oaks inoculated with *T.melanosporum* on the development of mycelium after planting, and to determine the selection criteria used by the Ctifl for the control of nurseries that produce mycorrhized plants.

The plot is located on the 'Causse de Martel' (Lot- Aquitaine region- 300m altitude, mean annual precipitations 900mm, mean annual temperatures 12°C, annual insolation 2000h/year). 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 one-year-old oaks inoculated with *T.melanoposum*. The young plants were graded into 6 categories depending on the intensity and the homogeneity of the mycorrhization. The trial studied 6 treatments with 5 repetitions for each one. Every unit contained 24 trees (plant density 2x2m) totaling 824 trees.

The observations were carried out in 2002, 2003, 2004 and 2005. The trees were gently pulled up aided with a backhoe to avoid root damage. We measured the root system (hair root density from 1 to 3), the mycorrhization level, the presence of contaminant ectomycorrhizes (*T.aestivum, Coenococcum graniforme, Scleroderma sp..*) and the growth of the trees (trunk diameter and size). For statistical interpretation, the results on the mycorrhizal level were divided into 3 categories (low, moderate and high) and the mesurements of tree height and diameter also. The factorial correspondence analysis was applied to a database of 232 trees to study the link between the qualitative variables. The results shown here are based on the measurements obtained in 2005. We found that the level of mycorrhization of the 4-year-old trees depends on the initial level of mycorrhization of the plants after leaving the nursery. The higher category of mycorrhization correlates with the best initial mycorrhized trees and inversely. The high-mycorrhized oaks show the best growth in diameter and the highest hair root density. A good level of mycorrhization doesn't protect the trees from development of natural ectomycorrhizal mushrooms which affect all the trees whatever the final level of mycorrhization, particularly *Coenococcum graniforme*.

At this moment in time statistical analysis hasn't revealed a spatial effect on the evolution of the mycorrhization of the trees.

OPTIMIZATION OF FACTORS FOR THE PRODUCTION OF *PINUS HALEPENSIS* MYCORRHIZED WITH *LACTARIUS DELICIOSUS* UNDER NURSERY CONDITIONS

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Key words: *Lactarius deliciosus,Pinus halepensis,* mycorrhizal seedling cultivation, mycelial inoculum

Taking into account the practical applications on reafforestation of the use of seedlings mycorrhized with edible fungi, this study was aimed at optimizing the methods to improve the success of controlled mycorrhization in nursery.

Several methods of production of mycelial inoculum (mycelial slurries, mycelium entrapped in alginate beads, produced in bioreactors, in peat-vermiculite substrate) were evaluated and improved. Inoculation procedures in nursery were adapted to the *P. halepensis* seedling production. The effectivity of the different types and doses of inoculum were tested. On the other hand, several factors related with culture conditions such as watering, fertilization or substrate were considered. The percentage of mycorrhized seedlings and the rate of colonization were determined as a measure of the success of the established protocol. Data corresponding to plant growth and nutrient content were also determined as a measure of plant quality.

A protocol to obtain *P.halepensis* seedlings mycorrhizaed with *L. deliciosus* is proposed. The possibility of use of this technology to nursery scale is an application of both, mycological and forestry resources.

SUMMER TRUFFLES CULTIVATION AND OTHER EDIBLE MUSHROOM FROM TERUEL (SPAIN)

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Key words: summer truffles, *Tuber melanosporum*, *Tuber uncinatum*, *Tuber aestivum*, *Lactarius deliciosus*, edible mycorrhizal mushrooms.

The cultivation of winter cereals in the interior regions of the Iberian Peninsula (Teruel is a very good example) is kept thanks to the European Union aids. But these aids will disappear with the new Common Agricultural Policy. Thus, the truffles and other mushrooms represent a good opportunity to increase the profits in the agrarian sector in that region.

Forestry and Mycology Cultivations S.L. is a company situated in the rural environment of Teruel (Aragón, Spain), which, for fourteen years, has been working in the field of the applied mycology (growing of mycorrhizal plants) proposing plantations of black truffle (*Tuber melanosporum*) in order to make agricultural fields profitable in the rural environment. Right now the company is working in the plantation of summer truffle (*Tuber aestivum)*, as well as some species of pine tree with *Tuber uncinatum* due to the interest that these truffles can have in environments and lands where the black truffle does not grow up in good conditions. As far as other types of mushrooms are concerned (*Lactarius deliciosus*), at the moment that company is working in the plantation with these species of different pine trees (*Pinus* sp.). This plant has a forestry use, as well as to the creation of mycological environments for leisure. The company cultivates and grows mycelium from saprophytes mushrooms (*Pleurotus* sp., *Lentinus edodes, Pholiota aegerita, Marasmius oreades, Calocybe gambosa,* etc). Its main production is thistle mushroom (*Pleurotus eryngi*). This mushroom has very good qualities and an interesting demand.

The profit of the cultivation of those mycorrhizal plants is proved in many fields and plots in several regions as Teruel, Castellón, Soria, Navarra... The agrarian farms with mycorrhizal plantations are producing a plus-value for its owners.

The research on this sector is of crucial importance to develop new products demanded by the customers and to fix population in the rural areas diversifying the production

PIEDMONT WHITE TRUFFLE (*TUBER MAGNATUM* PICO) MYCORRHIZATION : NEW BASIS

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Key words: cultivation technology, truffle, *Tuber magnatum*, contaminating mycorrhizal fungi, mycorrhization checking.

Throughout more than 15 years, *Tuber magnatum* mycorrhizae have been mixed up with those from other « white truffles » from the *T. borchii* group. As a result the plants presumed mycorrhized with *T. magnatum* had been in fact mycorrhized with other truffle species. No wonder the results of cultivation have been disappointing until now.

The use of biochemical then molecular markers allows now to identify undoubtedly the mycorrhizae of *T. magnatum*, moreover to control the quality of mycorrhizal seedlings used when planting in the field.

The fact that inoculating plants with *T. magnatum* fruitbodies with proved methods has lead to mycorrhization with other species, sometimes in abundance, has remained a mystery for years. Mycorrhization experiments carried out under axenic conditions showed that *T. magnatum* fruitbodies carry, on the surface and inside the gleba, propagules of foreign fungi, mycorrhizal or not, that grew in the near or more distant area. Among the numerous saprophytes isolated, some have been clearly identifyed. Among the mycorrhizal ones, the most frequent ascomycetes are truffles: *T. borchii*, *T. maculatum*, *T. uncinatum*, *T. macrosporum*, even *T. melanosporum*. It is surprising to notice that in contact with *T. magnatum* fruitbodies, mycorrhizae from this species are rare, on the contrary, those from other mycorrhizal fungi (including *Tuber*) are very abundant. The existence of contaminating fungi on and inside *T. magnatum* fruitbodies is understandable because of their presence in the growing area of fruitbodies, and also because of their transport by many animals which attack truffles to feed themselves (slugs, worms, insects, small rodents...). They have an important impact on the spread of truffles by ingesting parts of fruitbodies and discharging spores within their faeces, sometimes far around. The germinative ability of *T. magnatum* spores being weak compared with the one of other fungi species involved by accident in the fruitbody, a few spores from the contaminant are enough to lead to a general mycorrhization of the seedling.

The obvious fact that *T. magnatum* fruitbodies do carry propagules from other mycorrhizal fungi must be taken into consideration in order to produce reliable mycorrhizal seedlings.

TRUFFLE CULTIVATION IN MOROCCO: THE FIRST RESULTS

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Key words: field plantation, truffle, *Tuber melanosporum*, mycorrhizal seedlings

The truffle cultivation method perfected in the early 70s by the INRA of Clermont-Ferrand (France) is based on the choice of a favourable environment (soil and climate), the seeding of truffle through mycorrhizal seedlings previously grown in greenhouses, and the maintaining of the environmental conditions in a favourable way for the mycorrhizal development then the fructification.

An area presumed suitable for the cultivation of Perigord truffle (*Tuber melanosporum*) has been chosen near Debdou (Eastern Marocco), on a calcareous plateau located at an altitude of 1,700 metres, in an environment of evergreen oaks. One thousand evergreen oaks and twenty hazels produced by the French company Agritruffe were planted in December 1998. Three hundred plants that had suffered from frost were replaced in December 2000. The plants edges are cultivated by hand with a hoe. On drought periods the plants are irrigated.

The plant growth has been medium (average height of the evergreen oaks: 140 cm, average height of the hazels: 70 cm). The first burnt areas (brûlés) were observed in 2003. Some mycorrhization checkings were done in April 2005 and show a high mycorrhization level with *T. melanosporum* in its pure state.

These results are very encouraging and allow to expect an imminent first production (winter 2005/2006?).

TRUFFLE-GROWING IN NAVARRA (NORTHERN SPAIN): FUNGAL BIODIVERSITY OF *TRUFFIÈRES*

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Key words: Black truffle, ectomycorrizae, hypogeous fungi, competitors, field plantations.

The aim of this work is to show the situation of the black truffle cultivation in Navarra (Northern Spain) from its beginning in the 1980s to the present. Nowadays, truffle cultivation in this region is a reality, since many farmers decided to introduce trees mycorrhized with *Tuber melanosporum* in their land, which are already producing truffles.

One of the most important aspects carried out for the knowledge and improvement of truffle production has been the monitoring of truffle plantations, which involves the confirmation of the presence of black truffle mycorrhizas on the roots of the inoculated trees as well as the study of some important competitors and other hypogeous fruit bodies occurring in truffières.

All these studies carried out over more than twelve years, show the great fungal diversity of field plantations.

THE NUTRITIONAL AND MEDICINAL VALUE OF WILD FUNGI: BALANCING THEIR POSITIVE CONTRIBUTIONS TO LIVELIHOODS AND POTENTIAL SAFETY CONCERNS

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Key words: Wild edible fungi, medicinal fungi, nutrition, health, safety, livelihoods, mushroom hunting, consumption

Wild fungi are a natural resource with a high nutritional and economic value. About 1200 species of fungi are used in 85 different countries as food and/or because of their medicinal properties. In this study, we review all global information on nutritional and medicinal value of fungi in relation to diet and health, addressing also safety issues and concerns.

In terms of nutrition, fungi are a good source of digestible proteins and fibre, are low in fat and energy, and make a useful contribution to vitamin and mineral intake. Dried mushrooms and concentrated extracts are used as medicines and dietary supplements. Some species have strong anti-tumour and antioxidant activity: they are commonly said to enhance immune system functions and lower cholesterol levels, for example.

Some of the potential safety concerns when eating wild edible fungi (WEF) are that edible species might be mistaken for poisonous ones; high heavy metal concentrations in WEF might be a source of chronic poisoning; and the consumption of WEF might contribute significantly to radioactive caesium intake of humans.

Exaggerated and unwarranted doubts about various species and sources of WEF often cloud rational judgements on what can and should not be eaten. Safety concerns need to be properly examined and then evaluated in the light of a more rigorous and balanced assessment of risk. Wild fungi are a valuable existing and promising alternative source of food in developing countries, and a suitable and nutritional alternative to the daily diet in the developed world.

MYCOCOENOSYS IN A TRUFFLE PLANTATION

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Key words: truffle, mushroom, truffle plantations.

In some truffle plantations the production of truffles is accompanied by fruit body formation of other mycorrhizal and saprophytic fungal species. In most cases, the ectomycorrhizal fungi present are considered competitor versus *Tuber* spp.

In order to asses the possible competitive phenomenon, a truffle plantation that produces high amounts of both *Tuber aestivum* and epigeous basidiomycetes fungi has been studied. This truffle plantation is located near Spoleto (Umbria – Italy), it has been planted in 1985 using 4 symbiontic species *(Quercus ilex* L., *Q. pubescens* Willd., *Corylus avellana* L., *Ostrya carpinifolia* Scop.) and it has an extension of 5 hectares. A pedologic analysis has been carried out, and macroscopic fungi have been collected between May and December 2004. The fungal species found have been identified, dried and kept at the *herbarium* of the Dipartimento di Biologia Vegetale e Biotecnologie Agroambientali e Zootecniche of the University of Perugia (Italy).

Twelve epigeous mycorrhizal fungal genera, and three hypogeous fungal species belonging to the *Tuber* genus have been identified. The fungal species distribution within the truffle plantation is influenced by the plant symbiont species and by soil characteristics, granulometry and pH in particular.

The observation that a copious fruit body formation of both *Tuber aestivum* and *Tricholoma argyraceum* (Bull.) Kumm. can be found at the same site and with the same plant symbiont is consistent with the hypothesis that mycorrhizal fungi present in productive truffle plantations are not competitor versus *Tuber* spp.

It is highly probable that in truffle plantations symbiotic fungi characterised by similar ecological needs can co-exist creating a relation similar to that observed in the higher plants forming phytocoenosys.

ASCOCARPS DENSITY IN A TRUFFLE PLANTATION

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Key words: truffle, truffle plantations, ascocarp.

Over the last years, truffle plantations have started recording high levels of production (70-100 kg per hectar). At present, the actual potential productivity of a plantation, ie the number of truffle primordia that are formed and the number of those that develop into ripe truffles, is unknown. An initial study has been undertaken on a highly productive truffle plantation of *Tuber aestivum* Vittad. (140 Kg per hectar). The truffle plantation has been planted in 1985, near Spoleto (Umbria – Italy), using hornbeam (*Ostrya carpinifolia* Scop.) as the plant symbiont. Over the first decade of May 2005, a 1 square meter area comprised between 2 productive plants has been delimited, and all the roots and the ascocarps present within it have been collected, at a depth of 0-5 cm. Plant roots have been analysed for mycorrhization, while fruit bodies have been observed in terms of macro- and micro-morphology.

52 unripe ascocarps have been collected, 0.6 to 2.2 cm wide. The morphological analysis of their peridium revealed the presence at the verrucae apex of hyphal whorls. The hyphae are simple, with an average length of 600 m and an average basal diameter of 3 m. Hyphal septa are rare, cell walls are slightly thickened and appear darker than the intracellular content, which shows a light amber colour. The hyphal apex can be pointed, or more commonly enlarged, and finely granular. The morphology of these hyphae resembles that of the cystidia observed in the mycorrhizae of *T. aestivum*. Plant roots and/or mycorrhizae have never been observed next to the numerous ascocarps of *T. aestivum* that have been found.

The absence of mycorrhizae and the presence of hyphal whorls on the peridium are consistent with the theory advanced by many authors of a saprophytic stage in the life cycle of the truffle.

The high number of ascocarps found per unit of surface suggests a potential productivity higher than that observed. This finding enhances the interest in identifying the possible agronomic measures able to promote the development and the ripening of the majority of the truffles that are formed in a plantation.

COMPARATIVE STRUCTURAL ANALYSIS OF THE MYCORRHIZA FORMED BY *LACTARIUS INDIGO*, *L. DELICIOSUS* AND SEVERAL NEOTROPICAL OR EUROASIATIC PINE SPECIES

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Key words: *Lactarius indigo*, mycorrhizae description, cultivation technology, mycorrhizal synthesis

The objective of this study was to establish the symbiotic ability of the edible fungi *L. indigo* or *L. deliciosus* to form ectomycorrhizae with a range of Neotropical (*Pinus ayacahuite* var. *ayacahuite*, *P. hartwegii*, *P. oocarpa* var. *oocarpa*, *P. pseudostrobus* and P. *rudis*) or Euroasiatic pine species (*P. halepensis*, *P. nigra*, *P. pinaster* and *P. sylvestris*). This study was also aimed at morphotyping and characterizing these *Lactarius* mycorrhizae. Special attention was focused on distinguishing features of the mycorrhizae among the pine species.

The synthesis was carried out in a controlled growth chamber using plastic containers with peat moss-vermiculite substrate and mycelial inoculum. A description of the morphology, appearance and structure of mantle and Hartig net is given for each combination.

L. indigo mycorrhiza were similar in all the pine. The main characteristics are: saffron to cinnamon greenish with age, with a net of saffron laticifers visible through outer mantle, cystidia-like emanating hyphae on the mantle surface, plectenchymatous mantle with interhyphal gelatinous material, being this gelatinous layer thicker in Euroasiatic pines than in Neotropical ones.

L. deliciosus was able to form ectomycorrhizae with Neotropical pines, what corroborated the lack of specificity in the ectomycorhizal relationship. Mycorrhizae were similar to previously described.

The ability to form mycorrhiza was independent of the origin (Guatemalan or Spanish) of the fungal strains and pine species

COMPARISON OF TRUFFLE AROMA FROM DIFFERENT CULTURES OF *TUBER MELANOSPORUM*

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Key words: biochemistry, aroma, HS-SPME, solid phase microextraction, truffle, *Tuber melanosporum*, *Quercus*, truffle culture

Tuber melanosporum Vittad. and other truffles belonging to the genus *Tuber* F.H. Wigg are subterranean fungi highly appreciated for its unique and characteristic aroma. Their culinary and commercial value is mainly due to their organoleptic properties, such as aroma, whose quality clearly provides the economic value of such edible fungi. To objectively describe the aroma of the truffles, headspace-solid phase microextraction (HS-SPME) combined to Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS) has been used. Previously, the optimal conditions to maximize the volatile compounds extraction have been obtained by Díaz *et al.* (2003; 2004; 2005).The aim of the present work has been to use this objective method of analysis by means of headspace-solid phase microextraction (HS-SPME) combined to GC and GC-MS, to study the influence of the different cultures on the aroma of *Tuber melanosporum*.

Truffle samples have been harvested from two cultures of Campillo de Paravientos (Cuenca) (*Quercus ilex* and *Q. cerris*). Then they were cut and analysed in fresh to avoid the changes of their organoleptic properties. HS-SPME has been used to extract the volatile compounds of truffle aroma. A fiber of medium polarity (so called for flavors) was used to avoid discrimination towards very non-polar and polar volatile compounds. Then, the extracted compounds have been analysed by GC with flame ionization detector and by GC-MS.

The obtained chromatograms of the truffle samples from the two different cultures have been compared and the compounds have been determined. A comparison among truffles from different *Quercus* species has been established in terms of qualitative and quantitative differences on volatile composition.

EFFECT OF THE ECTOMYCORRHIZAL FUNGI *LACCARIA LACCATA* AND *SUILLUS LUTEUS* ON DAMPING OFF BY *FUSARIUM MONILIFORME* AND *F. OXYSPORUM* ON SCOTS PINE SEEDLINGS

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Key words: Forest nursery, ectomycorrhiza, *Laccaria laccata*, *Suillus luteus*, damping off, biological control.

The role of the ectomycorrhizal (ECM) fungi *Laccaria laccata* and *Suillus luteus* as biological control agents against pre-emergence, post-emergence and late damping off caused by *Fusarium moniliforme* and *F. oxysporum* on Scots pine was studied in greenhouse experiments. Preinoculations with ECM fungi did not improve germination of seeds compared to treatments without the mycorrhizal fungus. However, reduction of germination caused by *F. oxysporum* became not significant when *L. laccata* was previously inoculated. Eighteen weeks after sowing, inoculation with both ECM fungi significantly reduced damage by *F. moniliforme* in post-emergence assays, but reduction of disease symptoms was only observed with *S. luteus* and *F. oxysporum*. Significant late damping-off damage on Scots pine seedlings was only caused by *F. oxysporum*. Inoculation with *L. laccata* reduced disease expression in *F. oxysporum* treatments to a value not significantly different than in controls. However, no significant differences in damage were observed between plants treated with *F. oxysporum* either inoculated or non-inoculated with both ECM fungi. Mycorrhizal formation was significantly lower in plants treated with *F. moniliforme* or *F. oxysporum*. It is shown that the protective effect by ECM fungi against damping-off by *Fusarium* spp. in Scots pine seedlings varied among damping off stages and ECM species.

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MYCORRHIZATION PROFILE IN A TRUFFLE PLANTATION OF *TUBER AESTIVUM* VITTAD.

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Key words: ectomycorrhiza, truffle, truffle plantations.

According to the present knowledge, mycorrhizae of *Tuber* are localised within the superficial soil layer, where truffles are often collected. This is particularly true in the case of *Tuber melanosporum* Vittad. and *Tuber aestivum* Vittad., that are frequently found in shallow soils. It is indeed common opinion not to remove soil from the surface of a truffle plantation and to till the soil at a depth of a few centimetres.

In order to evaluate the distribution of ectomycorrhizae on the basis of the soil profundity, a study has been undertaken on a truffle plantation of *T. aestivum*. The plantation was realised in Umbria (Central Italy) in 1985, using various plant symbionts; the plot investigated was planted with hazelnut trees (*Corylus avellana* L.) and hornbeams (*Ostrya carpinifolia* Scop.). The area is about 8000 square meter wide, and truffle production is localised only at the hornbeam site. The lack of production of hazelnut trees, next to the hornbeams, stimulated the investigation of the nature of both the soil and the mycorrhizae present.

During the Autumn of 2004, an excavation was made along the borderline of the productive and the unproductive truffle plantation parts. Along the excavation profile, which is 45 m-long and 1.5 m-deep, plant root samples have been collected at a distance of 1 m one from the other, and at 3 different depths: 0 to 20 cm, 21 to 40 cm, and 41 to 60 cm. In some cases further deep samples have been taken (61 to 80 cm).

The examination of the root samples collected revealed the presence of mycorrhizae of *Tuber aestivum* down to a depth of 60 cm, more rarely at a major depth (61 to 80 cm). The percentage of mycorrhized root apexes was also high (up to 80%). Samples collected from deeper soil layers showed the presence of mycorrhizae scarcely branched: the percentage of mycorrhized root apexes decreased at increased soil profundity, while the presence of mycorrhizae of another morpho-type designated *Forma 13* and also found in other truffle plantations, increased. Moving from the unproductive to the productive area of the plot, along the excavation site, a higher frequency of high precentage levels of mycorhization was observed. The lack of truffle production observed in the area where hazelnut trees were planted, despite the presence of mycorrhizae, may be due to different soil characteristics, which have started been studied and will be shown in a later work.

The results of the investigation relative to the distribution of *Tuber aestivum* mycorrhizae at different soil depths have important applications, because it establishes that less shallow soil tillages in a truffle plantation, if necessary, do not affect the mycorrhization process and the production of sporophores.

MUSHROOM PICKING DOES NOT IMPAIR FUTURE HARVESTS

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Key words: mushroom hunting, trampling.

Forest mushrooms not only have important functions within the forest ecosystem, but picking them is also a popular pastime activity, as well as a source of income in many developing and developed countries. Harvesting pressure has increased in many parts of the world and it is claimed that fungal species diversity has decreased over the past decades. This has led to widespread concern about overharvesting and possible damage to fungal resources. Several countries or regions have introduced legal measures to restrict harvesting of edible mushrooms in natural habitats because they fear that the removal of mushrooms from the forest, often before spore dispersal, might impair their reproduction. This has caused some controversies, since there is no scientific evidence regarding the effectiveness and the validity of such restrictions. In order to find out what the impact of harvesting mushrooms on subsequent fruiting really is, we started a research project in 1975. The study was carried out in two fungus reserves. The reserve "La Chanéaz" is located in a typical mixed forest of the Swiss Central Plateau, an area rich in edible species and frequently visited by people from nearby cities. The reserve "Moosboden" is located in a subalpine pure Norway spruce forest, another prevalent Swiss forest type and popular area for mushroom harvesting.

We investigated the effects of the two harvesting techniques picking and cutting, as well as the concomitant trampling of the forest floor on fruiting body appearance.

The analysis of the 29-year study reveal that harvesting has no significant impact on the fruiting body productivity or fungal species richness, irrespective of whether the harvesting technique was picking or cutting. The concomitant trampling of the forest floor, however, significantly reduces the number of fruiting bodies produced. But they reappear when the sites are left to recover. We therefore conclude that trampling does not damage the soil mycelia in the long run.

VALDEMECA (CUENCA, SPAIN): AN EXPERIENCE IN LOCAL REGULATION OF MYCOLOGICAL RESOURCES

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Key words: Valdemeca, mycological flora, mushroom hunting, local regulation, rural development.

The present work is an introduction to the regulation and sorting of mycological resources and represents an unique experience in Castilla-La Mancha (Spain). The municipality studied, Valdemeca, finds itself in the Serranía Alta of Cuenca, an extensive mountainous district of the Iberic System with a well-preserved forest cover, dominated by *Pinus sylvestris, P nigra ssp sallzmanii* and *P pinaster.* Many visitors keen on mycology are attracted each season by the rich and varied mycological flora or ecosystems of this area. Most of them come from the bordering provinces to pick up different mycorrhizal species, principally from the genera *Lactarius, Boletus, Tricholoma, Hygrophorus,* etc... Furthermore, the commercial gathering of the species of *Tuber* is also important.

The work focuses on the study of the flora itself, the species collected traditionally at the zone and the evolution and incorporation of new species collected, as well as their potential harvest. In addition, it has been studied and examined the experience of regulation at municipal level of mycological resources and its consent level for part of the local population.

The ethnomycological research has been carried out to improve the use of mycological resource like an element for the rural development in this area.

THE MORPHOLOGICAL AND MOLECULAR IDENTIFICATION OF THE MAIN TRUFFLE SPECIES IN CHINA

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Key words: truffle, morphology, molecular biology identification, PCR, ITS sequence

Truffle is one of the most precious mycorrhizal edible mushrooms in the world. It has a symbiosis life with some trees. An amount of truffles were exported to Europe annually from the main product regions of China, such as Yunnan and Sichuan. But few researches on the taxonomy and phylogeny are done in China, especially classical techniques of identifying the ascocarps or mycorrhizas of truffles do not allow certain species to be distinguished, for example *Tuber melanosporum* and *T. indicum*, *T. uncinatum*. The molecular technique was used in this study to identify the mains species of truffles in China.

Thirteen truffles used in this study were collected in Yunnan and Sichuan from where truffles were exported to Europe. Their characters of ascocarps, the structure of gleba and the shape of ascospores were described. And their rDNA ITS region were amplified and sequenced. Based on both the morphological and molecular characteristics, the 13 strains were divided into 6 groups. Among of them, two species, *T. chineseexcavatum* and *T. pseudoborchii*, may be new species. And *T. aestivum* and *T. uncinatum*, *T. sinense* and *T. himalaynese* may be same species.

ANTIOXIDATIVE PROPERTIES OF WILD EDIBLE MUSHROOMS: INDIVIDUAL CAP AND STIPE ACTIVITY

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Key words: wild edible mushrooms, *Lactarius deliciosus*, *Tricholoma portentosum*, scavenging effect, reducing power, total phenols

Reactive oxygen species are formed during normal cellular metabolism, but when present in high concentration they become toxic. Mammalian cells possess intracellular defences such as superoxide dismutase, catalase or glutathione peroxidase in order to protect the cells against excessive levels of free radicals. Also exogenous addition of compounds such as vitamins (A, E, -carotene), minerals (selenium, zinc), or proteins (transferring, ceruleoplasmin, albumin) can provide additional protection[1]. These natural antioxidants or other sources that can neutralize free radicals may be of central importance in the prevention of vascular diseases, some forms of cancer and oxidative stress responsible for DNA, protein and membrane damage.[2,3] The northeast of Portugal, due to their climatic conditions and flora diversity, is one of the European regions with higher wild edible mushrooms diversity, some of them with great gastronomic relevance.

We will report chemical assays on the antioxidant activity of two wild edible mushroom species, *Lactarius deliciosus* (L.) Gray and *Tricholoma portentosum* (Fr.) Quél., from northeast of Portugal. For the first time, the entire mushroom, the cap and the stipe individually were used in order to compare their antioxidant properties. Total phenolic content was determined using Folin and Ciocalteu's phenol reagent. The reducing power was evaluated measuring absorbance at 700 nm after mixing the samples with ferric compounds; higher absorbance indicates higher reducing power. The scavenging effects on DPPH (1,1-diphenyl-2-picrylhydrazyl) radicals were determined measuring the decay in absorbance at 517 nm due to the DPPH radical reduction, indicating the antioxidant activity of the compounds in a short time.

Overall, *L. deliciosus* revealed better antioxidant properties than *T. portentosum* (lower EC_{50} values), which is in agreement with the higher content of phenols found in the first species. The portion of the mushroom used has also influence in the results obtained, showing the cap methanolic extracts better results.

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GERMINATION OF BLACK TRUFFLE ASCOSPORES

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Key words: ascus, cultivation technology

Our understanding of the life cycle and ecology of the black truffle is essential to successful cultivation and also contributes to our understanding of fungal life strategies. Early methods in inoculation techniques for mycorrhizal colonization of host seedlings with *Tuber melanosporum* Vitt. emphasized the importance of breaking the protective wall of the ascus to encourage spore germination. Both physical and chemical methods have been proposed and it has been suggested that the spores cannot germinate in the absence of the host plant root. In this study we demonstrate that the spores of this *Tuber* species can in fact germinate within the ascus following a period of maturation and without the influence of an ectomycorrhizal host.

EDIBLE ECTOMYCORRHIZAL MUSHROOMS FROM BIRCH AND DOUGLAS-FIR CLEAR-CUT PLANTATIONS AND MATURE FORESTS IN THE SOUTHERN INTERIOR OF BRITISH COLUMBIA, CANADA

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Key words: Basidiomycete, clear-cut plantations, deciduous and conifer forests.

Five year old plantations and 75 - 125 year old birch (*Betula papyrifera*) and Douglas-fir (*Pseudotsuga menziesii*) and mixtures were sampled for 4 years for epigeous ectomycorrhizal fruiting bodies. Each forest type was replicated 3 times and a permanent transect at each site was used to sample fruiting bodies during the spring and autumn annually. Each transect was 20 X 10 metres and was divided into continuous 20, 10m X 10m plots. All taxa were recorded in each plot and was collected once for vouchering. Specimens were brought to the laboratory for analysis and were identified to the lowest rank. Cumulative percent frequency for each species for the 4 years was analyzed using the Analysis of Variance. Of the 189 total taxa collected, most were Basidiomycetes and 45 were edible. The majority of the edible taxa were found in conifer and mixed forests. *Leccinum scabrum* and *Laccaria laccata* were the only edible taxa found in both the plantation and forest stands. No edible taxa were collected in the plantations alone. There were 5 edible fungal taxa found only in the birch forests, but 4 of these are known to form ectomycorrhizae with conifers. *Leccinum insigne*, a deciduous mycorrhizal fungus, and *Cantharellus formosus*, *Boletus zelleri*, *Hygrophorus bakerensis* and *Russula aeruginosa*, known conifer mycorrhizal associates, were the only edible taxa found in the birch forests exclusively. Seven edible ectomycorrhizal taxa were found only in the predominantly Douglas-fir forests. Twenty seven taxa were found in the mixed forest stands. Results from this study show that mixed deciduous and conifer forests have the richest edible mushrooms when compared to each forest type alone. In addition, this study shows that young clear-cut plantations contain minimal edible ectomycorrhizal fungi.

INFLUENCE OF A LOW AND ECONOMICAL BLACK TRUFFLE MYCORRHIZATION ON *QUERCUS ILEX* SUBSP. *BALLOTA* GROWTH

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Key words: fungal sylviculture, *Quercus ilex*, mycorrhiza, truffle, *Tuber melanosporum*

Quercus L. spp. are associated with many mushrooms that increase their vigour in mediterranean areas. Owing to this fact the production of mycorrhized plants has been raised in recent years in Spain. The goal of the present study has been to analyse the influence of low *Tuber melanosporum* mycorrhization on *Quercus ilex* subsp. *ballota* growth, to produce extensive and economical reforestations with this black truffle.

A method of low intensity mycorrhization of *Tuber melanosporum* on 60 *Quercus ilex* subsp. *ballota* has been applied according to Manjón *et al.* (1991; 1994) and Bencivenga (1982). These plants and 24 control plants without mycorrhization were kept for 15 months under controlled environmental conditions in University of Alcalá. *Tuber melanosporum* mycorrhizae were analysed according to Bencivenga *et al.* (1987). 18 biometric measures of *Quercus ilex* have been carried out according to Rey-Benayas (1995; 1998).

Low correlations were found between the percentage of *Tuber* mycorrhization and root weight, total biomass and non photosynthetic biomass. These results show that *Quercus ilex* subsp *ballota* production used in reforestation with a low and economical mycorrhization of *Tuber melanosporum* does not increase the forest quality of this oak.

Results of low intensity mycorrhization on 60 *Q. ilex*

Mycorrhization mean	14%
Std. Desv.	8
Minimum	1%
Maximum	35%

Statistically correlations between the % of *Tuber* mycorrhization and Quercus growth

Pearson coeff.	Root weight	Biomass	Non photosynthetic biomass
% Tuber	0.272	0.242	0.238
p-value	0.035	0.061	0.066

STATISTICAL ANALYSIS OF *SPHAEROSPORELLA BRUNNEA* IMPACT ON *TUBER MELANOSPORUM* CULTURES

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Key words: cultivation technology, *Sphaerosporella*, Pezizales, mycorrhiza, *Tuber*

A casual finding of Sphaerosporella brunnea in some greenhouses from Spain has been studied. *S. brunnea* is a ectomycorrhicical discomycete (*Otideaceae*, *Pezizales*) which has produced some problems in Italian and French truffle culture. This fungus has contaminated many nurseries and competes with *Tuber* spp. under certain conditions (Chevalier & Dupré, 1990; Meotto *et al.*, 1992; Donnini & Bencivenga, 1994; Bencivenga *et al.*, 1995; Donnini & Bencivenga, 1995). García-Montero & Manjón (2001) propose that *Sphaerosporella brunnea* is not a dangerous contaminant in *Tuber melanosporum* nurseries. The goal of this study is to confirm this hypothesis.

In University of Alcalá there was an accidental contamination with *Sphaerosporella brunnea* affecting 580 small plants from 9 species that had been slightly mycorrhyzed with *Tuber melanosporum* (for using in extensive forest reforestations). The evolution of *Sphaerosporella brunnea* as a contaminant mycorrhizae has been studied for 3 years under controlled environmental conditions in those nurseries. These mycorrhizes have been analysed according to Bencivenga *et al.* (1987). The statistical treatment was performed with the Statistica Program v. 6 (StatSoft, Inc., Tulsa, OK 1999).

The 580 small contaminated plants show a low percentage of mycorrhizes of *Sphaerosporella brunnea* (mean: 11%; std. desv.: 10). The *chi-square* indicates that this fungus is associated in a selective way with the different host plants ($p<0.0001$) and it has been proven that *Quercus* spp. are more vulnerable. Spearman test also shows that the mycorrhization of *Tuber melanosporum* has not been significantly affected by *Sphaerosporella brunnea*. However, this statistical test indicates that other contaminant ectomycorrhicical fungi have significantly damaged to *Tuber melanosporum* mycorrhizae. These results show that the commercial nurseries devoted to the truffle culture are not threatened with *Sphaerosporella brunnea*.

Spearman Rank Order Correlations	Mycorrhizae interaction	R	t (N-2)	p-value
50 *Quercus* con *S. brunnea*	*S. brunnea* & *T. melanosp.*	0.157374	1.10408	0.275064
50 *Quercus* con *S. brunnea*	*S. brunnea* & other fungi	-0.300910	-2.18608	0.033718
50 *Quercus* con *S. brunnea*	*T. melanosp.* & other fungi	-0.698667	-6.76572	<0.0001
100 *Quercus* spp.	*T. melanosp.* & other fungi	-0.547942	-6.484454	<0.0001

INFLUENCE OF ACTIVE CARBONATE AND SOIL FEATURES ON *TUBER MELANOSPORUM* PRODUCTIVITY

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Key words: ecology, soil, active carbonate, truffle, production, *Tuber melanosporum*

Soil is one of the most studied factors in the biology of the *Tuber melanosporum*. This truffle is strictly calcicolous and grows in the surface horizons of moist, carbonated soils with C/N relationships close to 10 (biological active humus), in which the soil texture is balanced and tends to be simple and well-constructed. Souzart (2001) indicates that the soil parameters that *T. melanosporum* tolerates are highly variable. However there are very few soil studies related to truffle production. The aim of this study is to analyse the components and properties of surface horizons of 20 soils and to establish their relationships with *T. melanosporum* productivity.

Twenty soils were selected according to their production of carpophores of *Tuber melanosporum*: 8 samples were taken in high productivity burns, 6 samples in low productivity burns, and 6 were reference samples without production. In each soil, the first 30 cm of the profile were studied and the sampling was done according to FAO (1990). The following properties were analyzed: pH, total organic carbon, calcium carbonate equivalent, granulometric analysis, cationic exchangeable capacity, total sum of the bases and the base saturation percentage following the methods of ISRIC (1995); the textures were classified according to ISSS; the total nitrogen was analysed with a variant of the Bouat and Crouzet method (1965); the carbonate extractable with ammonium oxalate was determined according to AFNOR (1982). The determination of cation exchange of Ca^{2+} and Mg^{2+} was done by AAS (Philips UP9100x) and K^+ and Na^+ with a flame photometer (Sherwood 410).

Principal Components Analysis has shown that the productivity of *Tuber melanosporum* is influenced by the overall action of carbonate extractable in ammonium oxalate, stoniness, organic carbon, clay content and interchangeable cations present in the soil surface horizons. Nevertheless, their collective influence is low because PC2 only explains 27% of variance of the production of *T. melanosporum*. With respect to the study of the carbonates, a simple, positive and very significant correlation was found between the concentration of extractable carbonate (active carbonate) in the surface horizons and the production of *T. melanosporum*, explaining 42% of variance. Active carbonate is a finely divided fraction of the calcareous rock (smaller than 50 •'5fm in size), susceptible to rapid mobilization and very chemically active. The results obtained for active carbonate could have applications in truffle culture.

PRELIMINARY STUDY OF THE ATRACTION AND ECOLOGY OF *LEIODES CINNAMOMEA* (COLEOPTERA, LEIODIDAE) Y *SUILLIA FUSCICORNIS* (DIPTERA, HELEOMYZIDAE) IN SPANISH CENTRAL AREAS

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Key words: ecology, truffle, aroma, *Suillia*, *Leiodes*, *Tuber*

Different groups of insects parasitize truffles and this function is important to improve the soil areation, the fungi nutrition and the activation and dispersation of fungal spores. The beetle Leiodes spp. and the flies *Suillia* spp. are remarkable examples. In Spanish truffle areas, *Suillia gigantea* has been cited by Reyna (2000); *Suillia fuscicornis* and *Leiodes cinammomea* have been cited by Pérez-Andueza *et al.* (2005). Some habitats of *Suillia gigantea* have been studied by García-Montero *et al.* (2004). The aim of this work has been to report new studies about the ecological conditions of *Suillia fuscicornis* and *Leiodes cinammomea* in truffle areas from Alto Tajo Basin. Besides, first studies of insect attraction by truffle aroma have begun in this area.

In 1997 raw truffle samples and beetles have been collected in *Tuber melanosporum* burns from Alto Tajo Basin (Central Spain). These samples were kept under controlled environmental conditions using a culture store in University of Alcalá. Truffle taxonomy has been studied using a stereoscopic microscope (Leica WildMZ8) and an optic microscope (Leica LeitzDMRB). The macroscopic characteristics of the ascocarps and the biometry and morfology of spores have been studied according to Granetti *et al.* (1990), Montecchi and Lazzari (1993) and Pegler *et al.* (1993). By this method several adult fly specimens have been obtained. The insects have been determined in Catholic University of Ávila, by using a stereoscopic microscope (Nikon SMZ-2T) and taxonomy keys (Matile, 2000; McAlpine *et al.*, 1987; Papp and Darvas, 1998).

The truffle samples collected have been *Tuber melanosporum* and *T. mesentericum*. A first reference about the parasitism of *Suillia fuscicornis* in carpophores of *Tuber mesentericum* has been proposed. Habitats of *Suillia fuscicornis* and *Leiodes cinammomea* are studied in central Spain for first time: these areas have a high altitude (more than 1000 m) in a Supra-Mediterranean bio-climatic belt with a sub-humid, shadow climate; average annual precipitation is 800 mm, with low yearly average temperatures (10°C) and very cold winters. On the other hand, several authors indicate that the sulphur compounds are the most influential truffle compound in insect attraction regarding *Tuber melanosporum* and *T. magnatum*; so in this work it has been begun to study the influence of this type of compounds in flies and beetles from other truffle species.

STATISTICAL ANALYSIS OF PRODUCTION AND SIZE OF *TUBER MELANOSPORUM* BURNS IN DIFFERENT HABITATS IN CENTRAL SPAIN (ALTO TAJO BASIN)

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Key words: ecology, truffle hunting, *Tuber melanosporum*, Spain

There are still evident gaps in our knowledge of the black truffle's biological cycle, its adaptive responses to the physical environment, factors inducing fruiting, and its interactions with its host plants and other fungi. Ceruti (1990) argued the importance of correlating ecology and physiology studies. Scannerini (1992) states that efforts at incorporating biotechnology in truffle cultivation require more basic research into the biology of the mycorrhizae, mycelium and the fungus-plant relationship, as well as more research in the field over long periods. Finally, according to Chevalier (2001), the cultivation of *T. melanosporum* has barely advanced and current interests in truffle production are focusing more and more on ecological approaches.

Given this need to expand current knowledge on the ecology of *Tuber melanosporum*, the present investigation was designed to determine whether there is a correlation between burn size and truffle production in several habitats of this truffle in central Spain.

433 burns of *Tuber melanosporum* were monitored over 6 years in 8 types of forest habitats associated with *Quercus faginea, Q. ilex* subsp. *ballota. Corylus avellana, Cistus laurifolius* and *Tilia platyphyllos* inside a small area of Peralejos de las Truchas (Guadalajara, Spain). This region is located in a mountainous area at an altitude of more than 1000 m in a Supra-Mediterranean bioclimatic belt with a sub-humid, shadow climate. Average annual precipitation is 800 mm, with low yearly average temperatures (10°C) and very cold winters. The maximum production of this truffle in these natural un-managed burns was confirmed in interviews conducted on 14 truffle-gatherers. A statistical analysis has been made to know the best host plant in similar intermediate ecological conditions. The statistical treatment was performed with the Statistica Program v. 6 (StatSoft, Inc., Tulsa, OK 1999).

Tuber melanosporum has shown a great ecological adaptability in a landscape of high variability (8 different habitats in a small region). The truffle yield varies by up to 60% among these habitats. The size of the burns explains between 39 and 51% of the variance of production of *Tuber melanosporum*. However, the surface area of the burns is not significantly different among the 8 habitats. *Quercus faginea* is the best host plant in this region of central Spain.

SOIL IMPACT IN *CISTUS LAURIFOLIUS* L. POPULATIONS WITH LOW PRODUCTION OF *TUBER MELANOSPORUM*

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Key words: ecology, soil, *Cistus laurifolius*, truffle, *Tuber melanosporum*

There have been many studies of Cistus salviifolius L., *C. incanus* L. subsp. *incanus*, *C. laurifolius* L., *C. albidus* L., *C. crispus* L. and *C. monspeliensis* L. being mycorrhized with *Tuber melanosporum* (Chevalier *et al.*, 1975; Fontana & Giovannetti, 1978-79; Giovannetti & Fontana, 1981; 1982; Manjón *et al.*, 1994; García-Montero & Manjón, 2001). There are also studies of the mycorrhiza ultrastructure of *Tuber melanosporum* in *Cistus incanus* and their relation with bacterias (Fusconi, 1983; Bejerano *et al.*, 2001). However, there are limited studies involving truffle ascocarps production with *Cistus* spp. The objective of this study has been to analyse the soil conditions and natural production of *Tuber melanosporum* associated with *C. laurifolius* in a central region of the Iberian Peninsula (Alto Tajo Basin).

Twenty-six burns of *Tuber melanosporum* with *C. laurifolius* and *Quercus faginea* have been monitored during 3 years in Peralejos de las Truchas (Guadalajara, Spain). This region is situated in a mountainous area at an altitude of more than 1000 m in a Supra-Mediterranean bio-climatic belt with a sub-humid, shadow climate. Average annual precipitation is 800 mm, with low yearly average temperatures (10°C) and very cold winters. Vegetation consists of forests of *Quercus faginea* (*Cephalanthero longifoliae-Querceto fagineae S.*) with brushland of *Cistus laurifolius* (*Genisto scorpii – Cistetum laurifolii*). Soil sampling was done according to FAO (1990). The following properties were analysed: pH and calcium carbonate equivalent following the methods of the ISRIC (1995); the carbonate extractable with ammonium oxalate (active carbonate) has been measured according to AFNOR (1982). The statistical treatment was performed with the Statistica Program v. 6 (StatSoft, Inc., Tulsa, OK 1999).

The statistical results have shown a low production of *Tuber melanosporum* with *C. laurifolius* and *Quercus faginea* (annual maximum production mean: 216 g/burn). These shrubs develop small burns and their soils have 80 to 90% less of active carbonate than other burns from other host plants in this region. The statistical treatment shows that these low levels of active carbonate allow the *Cistus* to grow but damage *Tuber melanosporum* production. These 26 burns show much *Cistus* death and in old burns with minimum production, a new colonization of young *Cistus* it has been observed. These results indicate that *C. laurifolius* have difficulty producing *Tuber melanosporum* and they seem to be vulnerable to *T. melanosporum* action.

INFLUENCE OF ACTIVE CARBONATE OF THE SOIL ON THE PRESENCE OF BLACK TRUFFLE SPECIES: *TUBER MELANOSPORUM, T. AESTIVUM* AND *T. MESENTERICUM*

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Key words: ecology, soil, active carbonate, truffle, *Tuber melanosporum*, *Tuber aestivum*, *Tuber mesentericum*

Although soil is one of the most studied factors in the biology of the black truffles, however there are very few soil analyses that examine the habitat selection of these mushrooms. The aim of this study is to analyse the components and properties of the surface horizons of 12 soils and establish their relationship with habitat's selection of Tuber melanosporum, T. aestivum and *T. mesentericum.* Twelve soils were selected according to truffle species: 6 samples were taken in burns of *Tuber melanosporum*, 3 samples in burns of *T. aestivum* and 3 samples in burns of *T. mesentericum.* The sampling areas are situated in a mountainous region at an altitude of more than 1000 m in a Supra-Mediterranean bio-climatic belt with a sub-humid, shadow climate. Average annual precipitation is 800 mm, with low yearly average temperatures (10ºC) and very cold winters. Its vegetation consists of forests of *Quercus faginea* Lam. (*Cephalanthero longifoliae-Querceto fagineae S.*). Two soil samples were taken in these burns according to FAO (1990). The following properties were analysed: pH, total organic carbon, calcium carbonate equivalent, granulometric analysis, cationic exchangeable capacity, total sum of the bases and the base saturation percentage, following the methods of the ISRIC (1995); the textures were classified according to the ISSS; the total nitrogen was analysed with the variant of the Bouat and Crouzet method (1965); the carbonate extractable with ammonium oxalate was determined according to AFNOR (1982). The determination of the cation exchange of Ca^{2+} and Mg^{2+} was done by Atomic Absorption Spectroscopy (Philips UP9100x), and K^{+} and Na^{+} with a flame photometer (Sherwood 410). The statistical treatment was performed with the Statistica Program v. 6 (StatSoft, Inc., Tulsa, OK 1999).

Principal Component Analysis has shown that the presence of *Tuber melanosporum* versus *T. aestivum* and *T. mesentericum* is low influenced by their collective soils parameters. Nevertheless an Analysis of Variance (Anova) and a Logistic Regression indicate that the content of carbonate extractable in ammonium oxalate (active carbonate) is the variable that accounts for the habitat selection of *T. melanosporum.* Also, this type of carbonate is associated with other factors that affect the successful growth of *T. melanosporum.* The results obtained for active carbonate could have applications in truffle culture.

SOIL FEATURES AND CLIMATE CONDITIONS IN A *TUBER DRYOPHILUM* HABITAT IN CENTRAL SPAIN (ALTO TAJO BASIN)

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Key words: *Tuber dryophilum*, ecology, taxonomy, soil, climate, Spain

Tuber dryophilum is a white truffle whose ecology and distribution is still not well known. Montecchi and Lazzari (Ed.) (1993) observed that this species is located in Europe in high altitude regions. This truffle dwells in the ground, at notable depth. *T. dryophilum* was cited once in Spain, but there are doubts regarding its identification because it was not a ripe specimen (D. Calonge, pers. com., 2004). The goal of this study was to confirm *Tuber dryophilum's* presence in Spain and to analyze the soils and the climate that make up suitable habitat for this truffle in central Spain (Alto Tajo Basin).

One *Tuber dryophilum* habitat was located close to the village of Beteta (Cuenca, Spain). One soil sample was taken in this point according to FAO (1990). The following properties were analyzed: pH, total organic carbon, calcium carbonate equivalent, granulometric analysis, cationic exchangeable capacity, total sum of the bases and the base saturation percentage, following the methods of the ISRIC (1995). The textures were classified according to the I.S.S.S.; the total nitrogen was analyzed with the variant of the Bouat and Crouzet method (1965); the carbonate extractable with ammonium oxalate was determined according to AFNOR (1982). The determination of cation exchange of Ca^{2+} and Mg^{2+} was done by Atomic Absorption Spectroscopy (Philips UP9100x), and K^{+} and Na^{+} with a flame photometer (Sherwood 410). Pluviometric data were collected from 4 meteorological stations.

Tuber dryophilum's presence was confirmed in Central Spain (Alto Tajo Basin). Their habitat is a high altitude area (1215 m) and *T. dryophilum* dwells at notable depth in the ground (>50 cm). This region is situated in a mountainous area at an altitude of more than 1000 m in a Supra-Mediterranean bio-climatic belt with a sub-humid, shadow climate. Average annual precipitation is 800 mm, with low average yearly temperatures (10ºC) and very cold winters. Vegetation consists of forests of *Quercus faginea* (*Cephalanthero longifoliae-Querceto fagineae S.*) with brushland of *Cistus laurifolius* (*Genisto scorpii – Cistetum laurifolii*). This truffle is strictly calcicolous and grows in the deep horizons of carbonated soils with C/N relationships close to 10 (biological active humus), in which the soil texture is balanced and tends to be simple and well-constructed.

SOIL FEATURES AND *TUBER MELANOSPORUM* PRODUCTION IN *TILIA PLATYPHYLLOS* POPULATIONS IN CENTRAL SPAIN (ALTO TAJO BASIN)

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Key words: ecology, *Tuber melanosporum*, soil, truffle production, *Tilia*, Spain

Tilia spp. have been mycorrizhed with several truffles: *Tuber magnatum* with *Tilia cordata* (Tocci *et al.*,1985; Tocci & Lopiparo, 1990), *T. platyphyllos* (Granetti *et al.*, 1995) and *T.* spp. (Giovanetti, 1990; Bencivenga *et al.*, 1995; Gregori & Ciapp., 1990); *Tuber melanosporum* with *Tilia cordata* (Chevalier *et al.*, 1973; Tocci & Lop. (1990) and *T.* spp (Bencivenga *et al.*, 1995); *Tuber borchii* with *Tilia platyphyllos* (Granetti, 1995); Granetti *et al.*, 1995; Giomaro *et al.*, 1999); *Tuber uncinatum* with *Tilia cordata* (Chevalier *et al.*, 1973); and *Tuber brumale* with *Tilia* spp. (Fontana & Centrella, 1967). However, there are limited studies involving truffle production with *Tilia* spp. The goal of this study has been to analyse the soils and natural production of *Tuber melanosporum* associated with *Tilia platyphyllos* in central Spain.

Nine burns were selected close to the village of Peralejos de las Truchas (Guadalajara, Spain). This region is located in a mountainous area at an altitude of more than 1000 m in a Supra-Mediterranean bio-climatic belt with a sub-humid, shadow climate. Average annual precipitation is 800 mm, with low yearly average temperatures (10°C) and very cold winters. Vegetation consists of forests of *Quercus faginea* Lam. (*Cephalanthero longifoliae-Querceto fagineae S.*) with river forests with *Tilia platyphyllos* (*Astrantio-Coryleto avellanae S.*). Four soil samples were taken in these river forests according to FAO (1990) (2 samples from *Tuber melanosporum* burns). The following properties were analysed: pH, total organic carbon, calcium carbonate equivalent, granulometric analysis, cationic exchangeable capacity, total sum of the bases and the base saturation percentage, following the methods of the ISRIC (1995); the textures were classified according to the I.S.S.S.; the total nitrogen was analysed with the variant of the Bouat and Crouzet method (1965); the carbonate extractable with ammonium oxalate was determined according to AFNOR (1982). The determination of the cationexchange of Ca^{2+} and Mg^{2+} was done by Atomic Absorption Spectroscopy (Philips UP9100x), and K^{+} and Na^{+} with a flame photometer (Sherwood 410). The statistical treatment was performed with the Statistica Program v. 6 (StatSoft, Inc., Tulsa, OK 1999).

Tuber melanosporum with *Tilia platyphyllos* has been monitored for 3 years and the statistical results have shown a low production of truffles with these host plants. Soil analyses indicate that soil properties of *T. platyphyllos* burns are similar to other host plants burns of this region.

MEAN PRECIPITATION INFLUENCE ON THE NATURAL PRODUCTION OF *TUBER MELANOSPORUM* IN CENTRAL SPAIN

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Key words: ecology, *Tuber melanosporum*, truffle, production, precipitation, Spain

Climate and precipitation influence on *Tuber melanosporum* production is still not well known. A preliminary study of pluviometric data and truffle producing capacity of *T. melanosporum* was done in Peralejos de las Truchas (Guadalajara, Spain). This region is situated in a mountainous area at an altitude of more than 1000 m in a Supra-Mediterranean bio-climatic belt with a sub-humid, shadow climate. Average annual precipitation is 800 mm, with low yearly average temperatures (10ºC) and very cold winters. Vegetation consists of forests of *Quercus faginea* Lam. (*Cephalanthero longifoliae-Querceto fagineae S.*) with river forests with *Tilia platyphyllos* (*Astrantio-Coryleto avellanae S.*).

Maximum annual *Tuber melanosporum* production was estimated in Peralejos de las Truchas´ County between 1960 and 1990. Mean annual precipitation differences were also analyzed for the same period. Pluviometric data were collected from 4 meteorological stations. Besides, a weekly record of the grams of *T. melanosporum* collected by two harvesters during 10 years in natural habitats of Peralejos de las Truchas was done. Monthly mean precipitation and *T. melanosporum* production were compared for these 10 years. Statistical analyses were performed with the Statistica Program v. 6 (StatSoft, Inc., Tulsa, OK 1999).

For the 60´s, maximum annual production of *Tuber melanosporum* was calculated up to 700 Kg for the 6.900 ha of Peralejos de las Truchas´ County surface area. However, in the 90´s, maximum annual production was estimated between 400 and 500 Kg. Therefore, the maximum annual yield has been reduced up to 35%. This reduction can be partially explained by a decrease in mean annual precipitation of 30% for the same period, despite summer mean precipitation increased in 28%. Precipitation in winter, spring and autumn decreased in 66%, 19% and 10% respectively. On the other hand, the effect of weekly mean precipitation on *T. melanosporum* production was analyzed. This weekly production was estimated based in the weekly harvest of two harvesters during 10 years.

NEW ADVANCES IN ASIATIC TRUFFLE SPECIES COMMERCIALIZED IN SPAIN

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Key words: taxonomy, aroma, commercial aspects, Asiatic truffles, truffle market, Spain

Importation of a great quantity of exotic fungi could have a negative effect on the canning industry and truffle breeding. Truffle breeding is mentioned because of the possible substitution (for economical reasons) of cheaper fruitbody varieties and their influence on the production of mycorrhizal plants. Regarding this problem, a first step is to study the taxonomy of the fungi involved. In this study line, the aim of this work has been to report new researches of asiatic truffles commercialized in Spain: market, taxonomy and aroma.

Some optic and electronic microscope studies are supplied. A comparison with the type of material of *Tuber indicum* and *T. himalayense* is also provided. The SEM photographs have been taken with a Zeiss DSM-950 microscope. Spore samples were rehydrated with 100% ammonium hydroxide for 30 min, then dehydrated in aqueous ethanol solutions (70%) for 1-1.5 h, before fixation in formaldehyde dimetilacetal, (after immersion in acetone for at least 2 h). The spores were subsequently dried to a critical point, mounted onto an aluminium stub, and coated with gold-palladium in a Polaron E-5000 sputter coater for 120 sec at 1.4 kV and 18 mA (argon atmosphere) to create a metal coating, approximately 500 Å thick. Light microscopy (LM) photos were taken with a Nikon Labophot microscope equipped with an automatic photographic system. Headspace solid phase microextraction combined to Gas Chromatography and Gas Chromatography-Mass Spectrometry has been used to analyse the volatile compounds from Asiatic truffle aroma.

The first results on taxonomy of Asiatic truffles are reported in Manjón *et al.* (1995), García-Montero *et al.* (1997), Moreno *et al.* (1997), and Wang *et al.* (1998). In this work has been reported new data on Asiatic truffles commercialized in Spain: a new species of *Tuber* and fraudulent sales. The presence the Asiatic truffles in canned truffle have been verified in products called *T. aestivum* as well as in products called *T. melanosporum*. Besides, different volatile compounds have been found and their aroma is less intense than the *T. melanosporum* aroma.

RADIOACTIVITY AND METAL BIOACCUMULATION BY *CLAVARIADELPHUS TRUNCATUS*

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Key words: Radioactivity and metal accumulations, ecology, ectomycorrhizal fungus.

Radiocesium, originating from global fallout from nuclear weapon tests (long term), and from recent nuclear accidents (short term), is accumulated in the surface layers of forest soils. Species dependent variation in the accumulation of ^{137}Cs by fungi under similar deposition conditions can split among several orders of magnitude. In Mexico, the main source of radiocesium is the global fallout from nuclear weapon tests. Edible mycorrhizal mushrooms can accumulate significant amounts of radionuclides and can therefore contribute directly to the radiation internal dose to man. The edible ectomycorrhizal mushroom *Clavariadelphus truncatus* (Quel.) Donk, belong to Aphyllophoralles order and is widespread but not common in Asia, Europe, and North America. For this specie, the sampling was performed in Mexico, from 1993 to 1999 in a seminatural forest (19 05' 16" N; 99 22' 43" W) located in the middle part of the Mexican Volcanic Belt, at altitudes between 3000 and 3500 m. ^{137}Cs (740) and ^{40}K (1354) average value activity concentrations (Bq kg^{-1}), were determined in mushroom samples, with a gamma-ray spectrometer system HpGe (Hyper-pure germanium detector). The Al (75), Ca (3969), Cu (131), Fe (221), Mn (15), Na (521), Pb (17), Sr (9) and Zn (187) elemental content (mg kg^{-1}), were analyzed by flame atomic absorption spectrometry and the standard addition method, with a Varian SpectrAA 220 equipment. Trace elements (mg kg^{-1}) as: Cs (23), Rb (284), Cd (2.3), Se (1.4), Mo (0.54), Co (0.4) and As (0.9) were measured by inductively coupled plasma-mass spectrometry (ICP-MS) with a VG Elemental-PQ3 equipment. A higher content of D-manitol forming crystalline compounds were identified by X-ray diffraction (XRD) with a Siemens D5000 diffractometer. Siderophores (low molecular weight Fe^{3+} coordination compounds) were detected in the extracts obtained from fruit bodies of *C. truncatus*, by the universal colorimetric assay employing chrome azurol S (CAS).

For *C. truncatus*, the average ^{137}Cs concentration value was 24 times higher than the geometric mean (31 Bq kg^{-1}) for the whole Mexican mushroom samples analysed at the same site (N=138). This behavior makes *C. truncatus* useful as biological indicator of environmental radiocesium long term fallout. Relatively high concentrations of Fe, Zn and Cu were measured in this specie. Cs and Rb high concentrations were also obtained for *C. truncatus*, showing a positive correlation between these trace elements. The external secretory substances (siderophores) produced by fungi to sequester ferric iron and other transition metals (radioactive and stable) from the environment should be related with the radiocesium and other natural radionuclides uptakes.

NOTES TO THE ECOPHYSIOLOGY OF SOME IMPORTANT HYPOGEOUS FUNGI IN THE CARPATHO-PANNON REGION

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Key words: fungal ecology, *Tuber uncinatum*, *Tuber magnatum*, *Terfezia terfezioides*, Carpatho-Pannon region, habitat preference, phytoindication.

Numerous data on *Tuber uncinatum*, *Tuber magnatum* and *Terfezia terfezioides* have been reported so far from the Carpathian Basin. *Tuber uncinatum* is widespread here, except the highly acidic, light and sandy soil areas, while sporadic occurrence of *Tuber magnatum* were revealed only from the submediterranean regions of south western Hungary. Habitats of *Terfezia terfezioides* are restricted to the sandy soils deponated by river Danube.

Soil samples were collected in truffle beds and detailed analysis was carried out. Phytoindication method of Ellenberg (1974) adapted by Simon (2000) to Carpatho-Pannon region was used for habitat characterization. Based on the above mentioned analysis 5 regions of *Tuber uncinatum* with different climate effects (continental, submediterranean, atlantic) and 1-1 region of *Tuber magnatum* and *Terfezia terfezioides* were compared.

Tuber uncinatum soils were neutral or weekly basic, heavy or extremely heavy with high humus and variable $CaCO_3$-content. Phytoindication showed similarities in soil parameters to the above mentioned results and reflected the differences of climate effects amongst regions. The two most common host plants are *Carpinus betulus* and *Quercus cerris* in hilly and mountainous areas whereas semi natural forests of *Quercus robur* in the Great Plain produce *T. uncinatum* in exceptional quantity and quality. In contrast of French results shrub woodlands are not typical habitats of *T. uncinatum* in these regions while the most important ones are Carpineta and Querceta forests of closed valleys and North-oriented slopes.

T. magnatum habitats were mainly found in older gallery forests of *Quercus robur* on floodplains along rivers. Soils are heavy, weekly basic with high level of humus and variable $CaCO_3$-content. Phytoindication reflects Illyric broad-leaved forest conditions, the abundance of wetland herbs, high rate of Atlantic species and strong groundwater effect.

T. terfezioides soil analysis shows neutral-slightly basic soils with low $CaCO_3$, clay and humus content. Phytoindication revealed some submediterranean climate effect, semi-humid and intermediate moisture conditions and indicated slightly basic soils, disturbed, secondary and artificial *Robinietum cultum* habitats.

SOME DATA TO THE KNOWLEDGE OF CHINESE TRUFFLE'S (*TUBER INDICUM*) TAXONOMY AND HABITAT PREFERENCE IN YUNNAN PROVINCE

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Key words: *Tuber indicum, Tuber himalayense*, ITS region

Tuber spp. from China have an increasing importance in the truffle-market of the world since the early 1990s. Several new species have been described among these black Chinese truffles, but their identification and distinguishing from each other and from *Tuber melanosporum* is complicated. Besides, molecular taxonomical methods of investigation on habitat characteristics (soil, vegetation) may also provide a possibility to separate different species more effectively. Unfortunately the literature on the ecological demands of Chinese truffles seems to be very poor.

The objective of our study was to identify Chinese truffles found in Yunnan Province based on morphological and molecular methods and to characterize their habitats. Some ascocarps of Chinese truffles from the Hungarian market were also analyzed.

Truffle materials and soil samples were collected from 7 habitats of two districts (Dongchuan, Kunming) in 2003. After the morphological characterisation analysis of the sequences of ITS regions of the nuclear ribosomal DNA was carried out. Detailed analyses of soil samples were taken. Main woody and herbaceous species were also listed.

Comparing our ITS sequences to the results of Zhang et al. (2005) we can conclude that truffle samples from the two districts belong to *Tuber indicum* group. However *Tuber himalayense* was also detected as present in the Hungarian truffle market.

Habitats of collections originated from Dongchuan District can be characterized as a *Pinus armandii* Franch. woodland alike those three of Kunming, but two habitats proved to be mixed forests (*Keteleeria evelyniana* Mast., *Cyclobalanopsis glaucoides* Schottky, *Alnus nepalensis* D. Don., etc.).

Truffle soils can be characterized as acidic to subneutral (pH 5,2-6,5) with the absence of $CaCO_3$, except samples from Dongchuan district where higher, neutral pH (6,8-6,9) and some $CaCO_3$ (3,6-4,6%) have been detected. High humus content and variable macronutrient levels have been measured.

GIS ANALYSIS TO MANAGE *LACTARIUS DELICIOSUS* HARVESTS IN THE ALMAZAN FOREST RANGE (SORIA, SPAIN)

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Key words: Wild edible fungi, GIS spatial analyse, forest management, conservation and laws.

In the scope of the INIA RTA 03/046 research project, mycological variables have been analysed using GIS technologies in order to develop a sustainable management methodology involved in a Forest Resources Management Plan. These items are: sporocarps production of *Lactarius deliciosus* L., land accessibility and harvesting rates in the Almazán forest range (Soria, Spain).

The source information has been obtained from the 3rd Spanish Forest Map, a Digital Elevations Model, geological cartography, infrastructures and other spatial elements. These analyses were linked to production, social and economic estimates, as well as field surveys and inventories achieved between 1997 and 2004.

Working with the Manifold System 6 and ESRI ArcGis commercial software packages, we made a progressive data process, looking for final production spatial layers, as well as accessibility layers and harvesting rates.

We estimated a "*Lactarius deliciosus* production area" (LPA) of 24,846 hectares in this range, based on GIS analysis of *Pinus* stands, its canopy cover, its development grade and its dominancy versus other species.

Considering the harvesters' habits, we classified in zones the LPA in four accessibilities ranks, according to drive-time zones from population cores, roads and paths' influence areas and the terrain steep grade. LPA 61% showed a good harvesting accessibility.

Taking into account the LPA accessibility and the estimated number of *Lactarius deliciosus* harvesters´ visits from each population core, we zonified the estimate harvesting rate (number of visits per hectare) in order to map the higher harvesting impact areas.

THE *MÍSCARO* PROJECT: ECOLOGY AND MANAGEMENT OF THE COMMERCIALLY HARVESTED *TRICHOLOMA FLAVOVIRENS* IN MARITIME PINE FORESTS OF BEIRA LITORAL, PORTUGAL

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Key words: *Tricholoma flavovirens*, adaptive forest management, sustainable productivity, non timber forest products, fungal ecology.

Míscaro (*Tricholoma flavovirens*) is very common in maritime pine (*Pinus pinaster*) forests in Beira Litoral, Portugal, and it is the most commonly harvested mushroom in these forests. Timber production is the main management goal of these forests and *míscaro* harvesting used to be a traditional activity for local inhabitants. However, the harvest of this mushroom has increased much in the last years and there are reports of non-careful harvesting, disturbing the forest floor.

Although small in relation to edible mushroom world markets, the commercially harvesting of the *míscaro* can make a significant contribution to our regional economy and to the income of harvesters in a seasonal basis. The emergence of this new trend in *míscaro* harvesting comes at a crucial time for the region, in both economic benefits and changing attitudes towards forest management. Its development coincides with recent efforts and interest from local entities, namely INOVA-EM and CMC, to diversify the products one can obtain from pine forests.

With the onset of widespread commercial harvesting, sustainable *míscaro* production is to become an important issue for harvesters, consumers, forest managers and owners alike. Given the interdependence between *míscaro* and live trees appropriate forest management influences mushroom abundance as well as timber production and quality.

This project aims: i) to gather a better understanding of the reproductive mechanisms, ecology and population genetics of the *míscaro*; ii) to compare *míscaro* productivity across different pine forest management regimes and iii) to evaluate the harvesting impacts in the subsequent fruiting.

Our goal is to be able to provide forest owners, mushroom harvesters, managers and policy makers accurate information and guidelines for an appropriate management of maritime pine forests. If *míscaro* harvest and maritime pine forest habitats are managed properly, *míscaro* is likely to remain a sustainable forest product in Beira Litoral for many years to come.

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VASCULAR AND MYCORRHIZAL BIODIVERSITY OF TRUFFIÈRS IN NAVARRA (NORTHERN SPAIN).

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Key words: vascular flora, black truffle, ectomycorrhizae, burned areas, brûlé, field plantations.

Despite the numerous studies on the biology and the development of truffle, important aspects on its ecology and on the environment in which it is developed are still unknown. The ecosystem of truffières constitutes a special habitat in which the plants that grow inside the burnt area or "brûlé" are very influenced by the inhibiting substances produced by the mycelium of the fungi.

The periodical visits to different field plantations of black truffle in Navarra (Northern Spain), have allowed us to observe the change of the flora as the truffières evolve and mature. This encouraged us to start a study in depth, not only of the mycorrhizae both of black truffle and of other competitors, but also of the vascular flora, sampling and identifying the vascular species present in burned areas of two truffières of different ages in Navarra, as well as in nearby areas of natural production. The phytosociological inventories raised in these areas, as well as the characteristics of the flora, show up that the inhibiting substances produced by the mycelium of the fungi act as a powerful disturbing agent that explains the vascular flora of burned areas and its characteristics.

RAPID IDENTIFICATION BY MOLECULAR TOOLS OF THE MOST COMMON SPECIES OF THE *TERFEZIA GENUS* IN THE IBERIAN PENINSULA

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Key words: DNA, *Terfezia*, RFLP, taxonomy, sequencing

Desert truffles included in the genus *Terfezia* (Ascomycetes) constitute one of the main edible fungi in semiarid areas of the Mediterranean Region. *Terfezia boudieri, T. claveryi* and *T. olbiensis* grow typically in basic soils and *T. arenaria* and *T. leptoderma* in acid ones. *T. claveryi* and *T. olbiensis* are often confused because they are collected when spores are immature. Thus, a rapid method to identify these two species is required.

DNA was extracted both from ascocarp and mycelium from fungal isolation, of these five species. DNA extraction was done with E.Z.N.A. Fungal Kit (Omega). Fungal DNA was amplified using ITS1 and ITS4 primers. PCR was performed in 25 L using Ready To Go TM PCR Beads. Then, PCR products of the Internal Transcribed Spacer (ITS) region were directly sequenced. A search for restriction sites in the ITS region that differentiates both species was performed using Webcutter (*on line*). PCR products were digested with three restrictions enzymes, *Hinf* I, *Alu* I and *Msp* I.

The amplified ITS showed an interspecific size polymorphism. ITS size was approximately 580 bp for all species. The nucleotide sequence of the different species showed differences among them, and it was highly conserved within each species without intraspecific and intraindividual variation. Some of these sequences were deposited in GenBank.

Different restriction patterns can be observed after treating the PCR fragments with *Hinf* I, *Alu* I and *Msp* I. *T. claveryi* and *T. olbiensis* can be identify with the three restriction enzymes.

In conclusion, sequence analyses allowed us to design a rapid PCR-based method using ITS of the ribosomal DNA in order to identify these species.

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CULTIVATION OF TRUFFLES ON ACIDIC SOILS – THE CONSEQUENCES OF LIMING

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Key words: Field plantations and fungal sylviculture and ecology, liming, trace elements, acidic soils, *Tuber*

The Périgord black truffle (*Tuber melanosporum*), bianchetto truffle (*Tuber borchii*) and Burgundy truffle (*Tuber aestivum*) are primarily found associated with host plants in alkaline soils rich in calcium carbonate. However, in New Zealand such soils are restricted to relative small areas in North Otago, North Canterbury, Marlborough, Nelson, Hawkes Bay, Poverty Bay and Northland. While a few truffières have been established in these areas the majority are on acidic soils to which large quantities of lime have been applied. On average this has necessitated the application of about 2 tonnes of lime per hectare for each 10 cm depth of soil treated. More than 50 tonnes of lime has had to be applied per hectare in particularly acidic soils. The most acidic soil that has produced Périgord black truffles is a volcanic ash with a natural pH of only 5.3.

The enhanced pH appears to have disadvantaged contaminating fungi providing the soil pH was stabilised at > 7.5 before planting. Another beneficial effect of liming was an improvement in soil structure in some soils. However, there have also been detrimental effects from liming, in particular on the reduced availability of soil iron, manganese, copper and boron which resulted in sometimes severe deficiencies in host plants. The application of trace elements either as foliar sprays, in irrigation water or as dry powders mixed into the soil in the rooting zone, were generally successful in correcting the deficiency symptoms. The exception was the failure of foliar sprays containing iron EDTA to correct symptoms on already severely iron deficient plants.

SIX YEAR OF THE *TERFEZIA CLAVERYI* CULTIVATION IN MURCIA (SPAIN)

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Key words: desert truffle plantations, cultivation technology.

Desert truffles are of considerable interest for ecological reasons because the low water input required for cultivation makes them an alternative agricultural crop in arid and semi-arid areas. In addition, the price fetched in the open market by desert truffles gives them an obvious economic interest.

Six different plantations have been carried out in the Region of Murcia (southeast Spain), with several thousands of plants, from April 1999 until January 2003. *Helianthemum almeriense* Pau, a shrub species and member of the *Cistaceae* family, was used as host plant. *Terfezia claveryi* Chatin was selected as the symbiotic mycorrhizal fungus as it is the most frequent in calcareous soils on marls with gypsum from the semiarid western Mediterranean area. Mycorrhizal plants were obtained both in nursery and *in vitro* conditions. In general, soils used for plantations were characterized by a clay-loamy texture, basic pH (8.5) and low rates values of electrical conductivity (123 µs/cm), organic matter (0.9-3.9) and C/N rate (7-10).

The first truffles were obtained during the spring of 2001, 23 months after the planting date but, with the adequate agricultural management, this time has been reduced to 12 months in more recent plantations.

During the last five years, the carpophores have fructified yearly and this production has been increased because of an adequate irrigation when it was necessary. During the last fructification season, truffle production from one of the plantations reached 600 kg/ha.

The introduction of desert truffles into dry environments may be a useful way to rehabilitate lands which until now have been considered unproductive. The productive sectors of nursery and agriculture of unirrigated lands would be both favoured by the installation of an alternative cultivation in, up to now, unproductive lands. This crop would improve, not only the quality of the land, avoiding soil erosion, but also the standard of living of social and economically depressed areas, what will add worth to these territories.

In addition to its interest as an alternative ecological crop, truffle cultivation is considered as a model to study the life cycle of hypogeous fungi (truffles) associated with their partner green plants.

A PROJECT TO DEVELOP AN APPLIED FOREST MYCOLOGY IN CHILE

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Key words: forests, forest mycology, management.

Chile is a country that is characterized by its forest land. The native forests cover approximately 13,4 million hectares of surface, whereas the plantations with exotic species (mainly *Pinus radiata* and *Eucalyptus* sp) reach the 2.1 million hectares, representing a 20.6 % of the total surface of the national territory. The management of these resources, generally has been oriented to mono-functional sylviculture, where the wood production has been the main target. Nevertheless, the increasing demand of the society for goods and services of the forests makes think that they must be handled under the concept of multiple use, where gets up the rational use of all the material and non-material resources that provides the forests. Among the forest material resources are the Non-Wood Forest Products (NWFPs), that in many cases are ignored or considered worthless. In this context appear the edible mushrooms, and particularly the ectomycorrhizal fungi, as natural resources that can contribute to the development and sustainability of rural and farmers' communities of low income in Chile. Under this perspective, researchers of the Universidad de Murcia (Spain) and Universidad de Concepción (Chile) try to develop lines of work to implement an Applied Forest Mycology in Chile, to medium and long term, understood as strategic element for the diversification of the forest productivity. The idea is to define the forest, the forest mass (natural and/or artificial), not only like wood producer, but also like generating of other resources, particularly mushrooms and truffles. In this work, four areas would be the base of sustentation of this project, considering the know-how of the Spanish Group respect to an applied forest mycology : a) to study the fungal diversity, in terms of Mycological Resources, whose advantages are feasible in the Spanish and Chilean territories, b) to define action lines for the sustainability of the Mycological Resources and their ecosystems, c) to make possible the technological and methodological transference of work, from the Spanish Group to the Chilean Group, to implement a Forest Mycology in the Latin American countries, d) to delimit areas and possible strategies for the introduction of fungal species of special socioeconomic and commercial interest that extend the supply of advantages of the Mycological Resources in Chile.

This project is supported by the Agencia Española de Cooperación Internacional (AECI).

AREAS OF POTENTIAL PRODUCTION OF *TUBER NIGRUM* IN ALBACETE AND CUENCA PROVINCES (SPAIN).

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Key words: Truffle Potential Areas, Southern Spain.

The decrease in edible truffle production in their traditional collection areas in France, Italy and Spain, during the last 50 years, has promoted a growing interest in developing different culture techniques. In the last decade, truffle-culture has increased in calcareous zones of Spain but there is not an adequate knowledge of the current situation of the natural areas of truffle production. In this study, the location of the more productive zones of two provinces of Spain with predominant calcareous soils, is pointed out taking into consideration the following objectives:

1. Determination of the production potentiality of black truffle in Albacete and Cuenca provinces.

2. Location of the most productive zones, current ecological status and perspectives

3. Compilation of national and regional normative and legislation for exploitation of the natural truffle production areas

4. Elaboration of a "good practice manual for collecting truffle, truffle-culture and truffle-sylviculture to preserve the resource"

Potentiality of truffle areas was grouped in the next types:

- Type I. Areas where truffle presence has been observed in field sampling and/or traditionally collected

- Type II. Areas where truffle presence is probable (up to 60%), considering climatic, topographic and vegetation parameters.

- Type III. Areas where truffle presence is probable (up to 40%) considering climatic, topographic and vegetation parameters.

After carrying out the field sampling (2003) and data collection, correspondent cartography E:1:200.000 of the selected zone was made overlapping geographical information referenced throughout the ArcView software tool.

TRACKING EDIBLE *LACTARIUS* STRAINS IN DIFFERENT PHASES OF THE MANAGED MYCORRHIZAL SYMBIOSIS

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Key words: mycorrhizal inoculation, edible fungi, *Lactarius*, molecular characterization, field plantations, PCR, SSCP

Mycorrhizal inoculations of pine species with edible *Lactarius* is a promising alternative for sustainable production of these appreciated fungi. In the framework of a project aimed at optimizing the factors to produce and manage mycorrhizal plants to obtain fruiting bodies of *Lactarius deliciosus*, we have selected different strains with high colonization ability. Thus, it is necessary to develop methods for qualitative and quantitative tracing of these introduced strains both in the plant and in the plantation soil. Molecular methods offer the possibility of characterizing the strains where morphological methods are not sensitive enough. The objective of this study is to test different molecular methods for inter and intraspecific identification of edible *Lactarius* species in pure cultures, mycorrhizas and extramatrical mycelium in experimental plantations.

The methods tested include PCR with specific primers in the ITS-rDNA region, microsatellite-primed PCR and single strand conformation polymorphism analysis (SSCP) for qualitative analysis, and real-time PCR for DNA quantification. Eventual sequencing of the PCR products was carried out to interpret the results obtained. A total of 18 strains of *L. deliciosus* were tested, as well as five of *L. sanguifluus*, two of *L. semisanguifluus*, one of *L. rufus* and one of *L. tesquorum*. DNA extractions were performed from pure cultures of all the strains, from mycorrhizas of 10 *L. deliciosus* strains and from rhizospheric soil of outplanted mycorrhizal seedlings inoculated in the nursery with two *L. deliciosus* strains.

A specific reverse primer (LDITS2R), combined with the universal forward ITS1, allowed to specifically amplify DNA of *L. deliciosus* from pure-culture mycelium, mycorrhizas and rhizosphere samples. In spite of the low intraspecific variability in the ITS-rDNA region, 9 groups with different SSCP pattern were identified among the 18 strains of *L. deliciosus* tested. Samples from mycorrhizas and rhizospheric soil showed the same pattern as the corresponding mycelium. Microsatellite-primed PCR showed polymorphisms among the different *L. deliciosus* strains but plant DNA was also amplified in mycorrhizas samples. Real-time PCR using TaqMan probes and specific primers allowed quantifying the amount of extramatrical mycelium in the rhizospheric soil.

In conclusion, the molecular techniques tested are suitable for both inter- and intraspecific characterization of *Lactarius*. All of them are sensitive and rapid enough to monitor the survival and persistence of the introduced strains in managed mycorrhizal plants.

REHABILITATION OF SATOYAMA, THE COPPICE WOODLAND SUSTAINABLY UTILIZED IN RURAL AREA IN JAPAN, WITH ASSISTANCE OF FUNGI

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Key words: Field plantations, fungal sylviculture, Satoyama, coppice woodland

Satoyama is a traditionally used term for the area of coppice woodland around the cultivation field in rural area in Japan. It had been sustainably utilized with skillful management and brought many kinds of products such as bamboo sprout, firewood, charcoal, etc., which then gave money to the people until around 1960s. Furthermore, these managements (disturbance) resulted in relatively high biodiversity. However, the change of the way of life afterward turned Satoyama into wasteland. This is a trial project to revive productive Satoyama with advanced usage of fungi.

A rather small area (ca. 0.5 ha) belonging to local government (Ujitawara) was used as a model forest. All of the trees existed were surveyed for the positioning, and a bird's-eye view was taken from a radio-controlled helicopter. Several kinds of saprobic edible mushrooms such as *Grifola frondosa, Pleurotus ostreatus, Hypsizygus marmoreus,* etc. were cultivated with long or short logs, which were buried underground. On the other hand, edible mycorrhizal fungi such as *Tricholoma matsutake, Lyophyllum shimeji, Boletus reticulates,* etc. were inoculated to the seedlings or adult trees of *Pinus densiflora* or *Quercus serrata.* Most of the fungal strains and the seeds of all tree species used in this study were locally collected. Sawdust clolonized with fungi before or after mushroom harvesting was used to breed grubs.

This model forest consisted mainly of *Chamaecyparis obtusa* and *Pinus densiflora.* The former timber tree should be felled and replaced by *Pinus densiflora* and *Quercus serrata* (and *Quercus acutissima*) to make them into productive Satoyama. Mushrooms of wood decomposers fruited even in the first year, but those of mycorrhizal fungi are expected to occur several years later. The total management system in this Satoyama project will be introduced and discussed.

TERFESS COMMON TO MOROCCO AND TUNISIA

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Key words: truffles, *Tuber*, *Terfezia*, *Tirmania*, *Picoa*, geography, climate, soil, vegetation.

Within the framework of a programme of study of truffles of the north of Africa (case of Tunisia and Morocco), tours of prospecting have been done in different regions of Morocco and Tunisia in order to harvest truffles and to note the ecological and pedological features of these truffle sites.

The authors noted that there are high similarities between *Terfezia* species grown in the two countries (Morocco and Tunisia). Six common species have been identified: *Terfezia arenaria*, *T. boudieri*, *T. claveryi*, *Tirmania nivea*, *T. pinoyi*, and *Picoa juniperi*.

Terfezia leptoderma, *T. olbiensis*, and two species of genus *Tuber* (*Tuber asa*, *T. oligospermum*) found in Morocco, probably do not exist in Tunisia. One species probably *Terfezia terfezioides* grow in Tunisia but has never been found in Morocco.

The influence of climate, soil and vegetation between Tunisia and Morocco are discussed.

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PRODUCTION OF MYCORRHIZAL PINE SEEDLING WITH "SHIRO" OF MATSUTAKE IN A LARGE CULTURE BOTTLE

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Keywords: Matsutake, cultivation technology, mycorrhizal seedlings.

Matsutake (*Tricholoma matsutake*) is one of the most famous edible mushrooms and is associated with various conifer plants. Recent studies established basic in vitro techniques for ectomycorrhization of matsutake with Japanese red pine. Toward the success of matsutake production, scale-up of the mycorrhizal system to be the "shiro" has been strongly desired. Shiro of *T. matsutake* is the large-scale visible whitish soil area consisting of mycorrhizas and extraradical mycelium that develop solely in the mineral layer. Here we show a new technique for mycorrhization of matsutake with shiro structure. The mineral layer soil was collected from the matsutake sampling site in a Japanese red pine forest. Dried soil was moistened with distilled water into 10 % (W/W), packed in a 1 L wide mouth polycarbonate bottle, and autoclaved for one hour. Five pieces of cultured matsutake mycelium (1-2 g wet weight) were inoculated in the packed soil and incubated for 3 months at 20C. A sterile juvenile seedling was transplanted in the bottle. Another void bottle was directly connected upward of the culture bottle for the seedling growth, which was aerated with four holes (6mm in diam) sealed with fulorocarbon membrane filter. These fungus-plant growing bottles were incubated in a growth chamber at 20C under continuous illumination of 20000 lx. A year after transplantation of seedlings, the shoot height reached ca. 10 cm, and the root systems developed amounts of mycorrhizas with shiro structure in the diameter of 3-5 cm. This is the first report on "shiro" formation *in vitro*. These mycorrhizal seedlings may be spawns for the matsutake production.

VEGETATION STRUCTURE AND FUNCTION IN *TRICHOLOMA MATSUTAKE* PRODUCTION PINE STANDS

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Key words: Vegetation structure and function, *Tricholoma matsutake*, below-ground ectomycorrhizal colony, ergosterol, field plantations and fungal silviculture and ecology

Pine stand and soil water is critically important for *Tricholoma matsutake*(*Tm*) production because they are the unique source of carbohydrate or water for the growth of the ectomycorrhizal fungus. The objectives of this study were to understand the vegetation structure and function of *Tm* production pine stands, water relations and characteristics of below-ground *Tm* colony.

The investigation was carried out at *Tm* fruiting pine stands in Sogni Mt National Park, South Korea, where the *Tm* fairy-ring colonies have been recorded for several years. Ecological properties of the pine stands i.e. vertical vegetation structure, leaf area index, water relations of the vegetation and soil water status within the *Tm* soil colony were measured during September 2004 to July 2005. Dominant woody species were *Pinus densiflora* and *Quercus mongolica*, and major under-shrubs were *Rhododendron mucronulatum*, *R. schlippenbachii* and *Fraxinus sieboldiana*. In the *Quercus* dominant stand pine trees die back with much less crown. Leaf area index (LAI) of the pine stand varied 1.4 to 3.7 depending on the seasons and the LAI change were greater in a *Quercus* dominant stand than in the pine one. Soil water content changed 8.4 to 15.4%. Leaf water potential changed 1.02 to 2.15 Mpa in pine, and 0.33 to 12.8 MPa in the other woody species. Leaf water potential values correlated to the soil water contents within the same season. Whereas it was 12.8% at non-colony, soil water content within *Tm* colony was 6.2 - 8.0% in the width of 0 - 40 cm from the colony front edge, and 12.1% at 80cm from the edge. Soil water content in the colony was lower by 1.0 - 4.0% than that in the non-colonized soil. In contrast, ergosterol content was 0.4 g g^{-1} fresh soil at the uncolonized soil, while 1.1 - 4.9 g g^{-1} fresh soil where the hyphae actively grow, and 0.4 g g^{-1} in the dead colony area. The water content changes were reversed to the ergosterol content changes in the *Tm* fungal colony.

We conclude that *Quercus* is the most competitive species to pine trees and that *Tm* colony consumes more soil water than non-colony does.

IDENTIFICATION OF THE NATURAL HOST PLANTS OF *MATTIROLOMYCES TERFEZIOIDES*

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Keywords: molecular identification, species specific PCR, ecology, Mattirolomyces, host plants, anatomy, TEM.

Most of the records of *Mattirolomyces terfezioides* (Matt.) Fischer (*Terfezia terfezioides* (Matt.) Trappe) are from the Carpathian basin, where the ascocarps of the fungus have been generally collected in mixed black locust forests on sandy soil. Although *M. terfezioides* has not got such gastronomic importance like some *Terfezia* species in the Mediterranean region, the interest in the fungus has been increased in Hungary due to the popularity of truffle collecting. The connection of *M. terfezioides* with different plant species were hypothesized and presented previously, however the fungus did not show clear mycorrhizal characteristics in *in vitro* experiments. The main purposes of our work presented here were to identify unambiguously the host plants of *M. terfezioides* in its natural habitat and to infer to the function of these connections by detailed anatomical characterization. A species specific PCR was developed for identifying the fungus in the collected roots. The primers were designed to amplify an approximately 400 bp long part of ITS region of the nrRNA gene and so a sensitive nested PCR could be carried out, at first using the fungal specific ITS1F-ITS4 primers. The typical intracellular structures of *M. terfezioides* were checked both by light and electron microscopy.

Several host plant species of *M. terfezioides* have been identified; in the studied area the fungus colonized both herbaceous and woody plants. For example *Viola cyanea* Celak. and *Salvia glutinosa* L. were identified as hosts, both herbaceous species are common in the forests where *M. terfezioides* have been found. Woody hosts were the shrub *Ligustrum vulgare* L. and *Celtis occidentalis* L. a very common invasive tree frequently found in mixed *Robinia* forests. In the case of *C. occidentalis* not only the hyphae of *M. terfezioides* could be detected, but also small, premature ascocarps of the fungus were found on the roots of the tree.

The results presented here and the planned future experiments with the identified hosts could serve important data about the ecology of *M. terfezioides*, which could be particularly interesting in the recently started attempts of the cultivation of the fungus.

This work was supported by the Hungarian Research Fund (OTKA, D048333) and János Bolyai Research Fellowships awarded to GMK.

COMPARISON OF THE MYCORRHIZATION IN THREE YOUNG TRUFFLE ORCHARDS OF DIFFERENT AGES IN EL TORO (CASTELLÓ, EASTERN SPAIN)

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Key words: *Tuber melanosporum*, *Quercus ilex*, ectomycorrhiza, field plantations, contamination

Around 1970, the first Spanish truffle orchards were planted to fight against the decline in spontaneous production. Since then, truffle cultivators and researchers have shown that the most important factors in the success of a truffle plantation are the initial plant mycorrhization, the ecological conditions of the plot and its previous land use. Adapting the cultivation to the ecological requirements of the black truffle and diminishing the ectomycorrhizal competition it suffers are the principles guiding the cultural treatments. Nevertheless, the fact that the first carpophores do not appear until several years after plantation causes many truffle cultivators to have misgivings with respect to the correct criteria for the cultural interventions. As an indicator of orchard evolution during the establishment phase, an assessment of the mycorrhization was carried out in three truffle orchards of different ages (four, six and eight years of age), with homogeneous environmental conditions and seedlings from the same nursery. The black truffle was found to dominate the ectomycorrhizal community although many plants suffered contamination by other fungi. The usefulness of mycorrhiza analysis for predicting carpophore production and evaluating previous management applications is discussed, as well as the ecological significance of the mycorrhizal contamination found in the orchards.

REGULATION OF WILD MUSHROOM HARVESTS IN PINEWOODS OF ALMAZÁN FOREST RANGE (SORIA, SPAIN): SOCIO-ECONOMIC BALANCE.

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Key words: Wild edible fungi, regulation, rural development.

It is presented a socio-economic balance of the first year of a pilot experience of regulation of wild mushroom harvests using harvest permits carried out in the autumn of 2003 in public pinewoods in the Almazán forest range (Soria, Spain).

The regulated area comprises 30030 hectares, mainly public pinewoods of *Pinus pinaster* Ait., pure or mixed with *Quercus pyrenaica* Willd., where it is harvested an average of 252t/year of *Lactarius deliciosus* Fr. among other wild edible mushrooms. Forest management is done by Castilla y León regional government.

Data about the system of permits established are analysed together with those from 478 phone surveys carried out with three different population groups in the province of Soria.
An amount of 4479 permits were issued (1259 to foreign "mycotourists") that brought about an income of 20350 euros in an autumn when the production of *Lactarius deliciosus* was 16.3 kg/ha, 1.8 times superior to that of the average year. 67% of population accepted the regulation model which was established. It manages all the aspects involved in the process: environmental, legal, socio-economic and functional at a cost of 46553 euros (1,5 /ha).

This regulation model does not generate important direct income for the public forest property but can become a viable socio-economic form of sustainable management of a resource that generates positive externalities in the forest range, in the form of harvesting income, "mycotourism" and wild fungi commercialization companies.

This pilot experience was carried out within the framework of the LIFE00 ENV/E/544 MYAS "Mycology and sustainability" project.

NATURAL RESOURCES AND LOCAL DEVELOPMENT: THE MYAS MODEL.

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Key words: Wild edible fungi, regulation, rural development.

The natural resources constitute at the present time a key element for the necessary socio-economic transformation of numerous rural areas of Spain characterised by their scarce economic dynamism and excessive dependence on regional and sectorial policies. The new concepts of development which are becoming more and more rooted in society, based on the principles of sustainability, together with the concept of local development and the profound changes in demand - both those related to the desire for different, safe and high quality food, as well as the changes in leisure activities and free time - are some of the factors which are contributing to this socio-economic transformation. The mycological resources fit perfectly into this hypothesis and may contribute to an effective rural development in Spain.

In recent years a progressively increasing demand for fungi has grown up. The need to modify the local supply in order to adapt it to the new demands, with the idea of increasing the profits of the producing areas and the need to consider fungi as a genuine productive resource, persuaded us to present the MYAS project (Mycology and sustainable use) to the EU "Life Environment" programme with the aim of developing an authentic management model for these resources which may positively effect rural development in the region of Pinares Llanos Centrales (Soria, Spain). The MYAS aims to be a philosophy, a method and a model capable of being introduced in other regions. The importance and impact of its results determined the presentation of a new project which is in operation at the moment - in this case of Inter-territorial co-operation - in which 15 LEADER and PRODER groups from Castille and Leon participate. Its objective is to improve the model of sustainable management of the mycological resources, defined in the previous project, and now incorporating new initiatives. It brings together research, territorial planning, vocational training, environmental education, commercial management and the integration of the mycological resources with up-market tourism. The objective is that this mycological resource provides a unifying force for the territory, that it creates a distinct identity and that it brings about a social change by means of a participatory methodology which makes the local society the driving force for change.

The project is structured in 6 general action programmes: the sustainable management of the resources: planning and regulation quality, safety and differentiation: commercial management mycological tourism vocational training environmental education: marketing and diffusion. The MYAS is more than just a project and our aim, with all its limitations and risks, is for it to become a model for the sustainable management of the mycological resources, orientated towards rural development with a real future.

CONTRIBUTION TO UNDERSTAND *XOLANTHA GUTTATA* MYCORRHIZAL ASSOCIATION WITH DESERT TRUFFLES IN MIDLAND OF PORTUGAL

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Key words: *Terfezia* spp., Mycorrhiza, *Xolantha guttata*, desert truffles, field Plantations, fungal sylviculture, ecology

In Portugal, desert truffles include fungus within genera such as *Choiromyces* Vitt., *Pachyphloeus* Tull., *Stephensia* Tull., *Terfezia* Tull. and *Tuber* Mich., which is known to live nearby shrubs such as *Cistaceas*. Few studies have been done in Portugal on this subject and the references are confused. Thereby, this study contributes to identify the species occurring in selected areas in midland of Portugal.

During this work, mycorrhizal association with an annual shrub *Xolantha guttata* (L.) Raf. (syn. *Tuberaria gutatta* (L.) Four.) was studied in three different areas with well known productive and non productive spots, in order to understand the characteristics which determine the best natural productive areas and to create basis for further establishment of new productive areas. Macroscopic and microscopic characterization of *Xolantha guttata* roots showed mycorrhizal association in both good productive and non productive spots, however different types of mycorrhiza were revealed. Fertility, pH and active calcium from soil samples were analysed to understand the correlation between different morphological types of mycorrhiza observed in natural field condition.

To prove the ability to establish mycorrhizal association with *Terfezia* spp., new experimental plots were created in non productive areas, using *Xolantha guttata* seeds spread with dried *Terfezia* spp. ascocarps and not inoculated seeds as a control area.

This work was supported by programme AGRO 8.1, project 449 "Establishment of mushroom and toadstool new areas in midland of Portugal".

COLLECTION OF CARPOPHORES OF EDIBLE ECTOMYCORRHIZAL FUNGI IN MINE SITES.

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Key words: wild edible fungi, heavily polluted sites, ectomycorrhizal fungi, fungal ecology.

Collection and/or consumption of wild-growing mushrooms is a common practice in many places of the world, in where the rural and agricultural communities use this forest resource as an alternative that helps to increase their incomes. The recognition in field of those fungal species which are not poisonous for the human health is information that in many countries is transmitted from generation to generation. However, the importance of the place where these edible wild fungi grow is often ignored or underestimated. It is too evident that soil pollution in our planet is increasing each day as a result of several human activities, such as the use of chemicals in agriculture and forestry, the manufacturing of pulpwood and paper, mine waste disposal, etc. This fact contributes to that the soils in the proximities of industrial zones accumulate high amounts of heavy metals, which can be captured and stored in the carpophores (fruit bodies) of some edible fungi that grow there. This work is part of an investigation project (DIUC 204.415.005-1.0) to monitor heavy metals concentrations in several edible wild fungi and their soil substrates to know the level and features of metal accumulation and possible risk to the health of local consumers in Chile. The purpose of the present study is to evaluate the relationship between the concentration of heavy metals in the soils and the amount that the carpophores of certain species of ectomycorrhizal fungi can accumulate when grown in sites with high or low environmental pollution. For this the carpophores of *Suillus bellinii* (Inz.) Kuntze were collected from adult plantations of *Pinus radiata* D. Don growing in metalliferous areas with high concentrations of heavy metals, mainly Cu (1000 ppm). On the other hand, the capability to accumulate metals also was studied in *S. bellinii* carpophores growing in sites considered unpolluted. The physical and chemical analyses of carpophores and soil substrates even are developing, but previous results demonstrate to the existence of a correlation between the concentration of some metals in the soil and its accumulation in the carpophores of the edible ectomycorrhizal fungi. It would demonstrate the enormous risk for the human health that the collection and/or consumption of edible wild fungi in sites next to zones of industrial activity or highly contaminated as metal smelters, represents.

POST-FIRE FOREST REGENERATION WITH CASH CROP: TRUFFLES

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Keywords: fire, *Tuber melanosporum*, resprouting vegetation.

Fire is a serious threat to Mediterranean forests and the corresponding rural populations. The current increase in forested areas and fuel loads increase the probability of large forest fires. After a devastating fire, these lands lose profitability and frequently are no longer managed, resulting in fire-prone shrub communities that inhibit forest regeneration. Reforestation immediately following fire poses a high investment cost with poor financial returns given the trends in wood markets. Reforestation with seedlings inoculated with black truffle (*Tuber. melanosporum* Vitt.) that potentially produce a marketable commodity could provide a viable alternative for these sites. A major biological concern for this solution is the ectomycorrhizal inoculum resident in the soil, which may compete or displace the truffle mycorrhizae in the inoculated out planted seedlings. We address this concern with the vision of establishing multi-use forest cover as opposed to establishing more labour-intensive truffle orchards.

Following the 1998 forest fire, which burned 26,000 h of conifer forests with occasional oaks in the understory in north-eastern Spain, we established experimental plots with holm oak (*Quercus ilex* L.) nursery seedlings inoculated with *T. melanosporum* where climate and soil properties were adequate for black truffle habitat. Four plot conditions were evaluated: post-fire with resprouting vegetation (T1), post-fire without resprouting vegetation (T2), post-fire cleared (T3), and cereal fields (T4). After 3 years we removed whole plants to evaluate the influence of the plot history on seedling growth and the persistence of black truffle mycorrhizae, and the overall mycorrhizal diversity present in the roots. We found that seedlings in T3 had significantly higher total shoot and root biomass, and lateral root expansion. Plot history influenced the diversity of mycorrhizal morphotypes, but the proportion of *T. melanosporum* colonization remained constant, suggesting that *T. melanosporum* can thrive in post-fire conditions within the ectomycorhizal fungal communities just as well as in a cereal field lacking inoculum of ectomycorrhizal fungi. Follow-up work is necessary to observe truffle productivity.

RELATIVE QUANTIFICATION OF DNA FROM *TUBER MELANOSPORUM* MYCELIA IN SOIL AROUND PRODUCTIVE AND NON-PRODUCTIVE TREES IN A TRUFFLE ORCHARD

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Key words: molecular biology, field plantations, fungal ecology, black truffle, ITS-PCR, soil hyphae.

Due to dramatic declines of *Tuber melanosporum* Vitt (black truffle) production in Mediterranean regions, several truffle orchards have been established for intensive truffle culture. Different ascomata productions are usually registered between trees in the same truffle orchard. Variations in the amount of *T. melanosporum* mycelia in soil could explain those differences. Our objective was to check if the amount of *T. melanosporum* mycelia in soil could be different around productive and non-productive inoculated *Quercus ilex* L. trees.

In spring of 2005, we collected in an 11-year-old truffle orchard in Teruel (Spain), soil samples around 12 productive trees (>1 kg of truffles produced the previous season) and 12 non-productive trees (no truffles produced). All of them presented a burn. Direct isolation of total DNA from soil was assessed with a modified CTAB-based protocol. PCR was carried out with fungal specific and *T. melanosporum* specific primers. The resulting amplicons were compared with a known DNA standard to measure the relative DNA-band intensities.

Preliminary results showed that in soil around productive trees, we detected significantly lower amounts of DNA from black truffle mycelia. The stone cover of the burns around these trees was significantly larger. Ecological and physiological explanations to these findings are needed. At this moment parallel studies of tree growth variables and soil features are carried out to design management strategies to improve the fructification of this fungus.

FROM MOLECULAR TAXONOMY TO SPECIFIC MARKERS FOR *BOLETUS EDULIS* SENSU LATO GROUP

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Key words: molecular biology, ITS, specific markers, *Boletus edulis*.

Boletus species belonging to the section *Boletus* are the most frequently eaten fungi among those harvested in natural conditions in Europe. This section groups a whole of 10 taxa which are hardly distinguishable on the basis of their morphology. Some of them have shown to induce allergic IgE-mediated symptoms either through inhalation, ingestion or contact.
Since questions related to the presence of allergens in any of the most appreciated species (*B. edulis, B. aereus, B. pinophilus, B. aestivalis*, all together called *B. edulis* s.l.) remain opened, together with the absence of tools which distinguish the species, we faced this problem by the development of specific primers.

Thirty-five fruitbodies were investigated by morphological and molecular analyses. Twenty-eight of them originated from Italy, three from Sweden, one from Croatia and three from China. They were classified according to the classical mycological features and DNA was extracted for ITS analysis of the rDNA.

ITS was amplified from all fruitbodies with the primers ITS1F and ITS4. In order to design specific primers for the *B. edulis* s.l., sequences were obtained from 28 samples. These sequences were first used to generate a phylogenetic tree to verify whether all the samples we classified as belonging to *B. aereus*, *B. pinophilus*, *B. edulis* and *B. aestivum* grouped in each clade. Once we distinguished in the tree the four taxa of the *B. edulis* s.l., we designed 4 pairs of primers intended to be specific. The efficiency and specificity of these primers were tested on DNA from all the available fruitbodies and mycelia. These amplifications gave a band of the expected size for each species (*B. aereus*, *B. pinophilus*, *B. edulis* and *B. aestivum*, Fig. 3) and no band from all the other samples.

The possibility to identify the four taxa inside fungal mixtures usually sold in the market could be a prerequisite for medical studies on toxic and allergic reactions following ingestion of these mushrooms.

Since the discrimination of the 4 taxa, by their morphological features only, is not easy and as they are sold in Europe without distinction, the availability of specific DNA probes is a crucial point.

MORCHELLA FRUITING PATTERNS RELATIVE TO CLIMATE AND ASSOCIATED VEGETATION

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Key words: fungal silviculture and ecology, *Morchella*, precipitation, soil temperature, spatial pattern, vegetation associations.

Although the fruit bodies of the facultative mycorrhizal fungi in the genus *Morchella* are highly prized and avidly collected, there are many aspects of the life cycle which are still poorly understood. Thus, attempts to cultivate morels or enhance natural fruiting have had mixed results. In order to more precisely characterize morel fruiting biology, we have made mapped collections of morel fruiting bodies over five years (2001-2005) at the University of Missouri Horticulture and Agroforestry Research Center, New Franklin, Missouri, USA. Air and soil temperature, as well as precipitation, have been recorded daily from the site. Preliminary analyses suggest that cumulative soil temperature degree days > 2 C is a good predictor of the initiation of morel fruiting. The intensity of morel fruiting is well predicted by the number of rain events of at least 10 mm. During the 2005 field season, we observed enhanced morel fruiting where precipitation was enhanced by irrigation of other mushroom cultivation experiments, leading us to speculate that natural fruiting can be enhanced through appropriately timed irrigation.

In 2003, all woody stems of at least 1 cm diameter were mapped in the site from which mapped fruit body collections have been made. Diameter, species, and crown condition were noted for each stem. We are using nearest neighbor and other spatial statistics to characterize the association of morel fruiting with specific woody plant species. The results of these analyses should help in the evaluation of anecdotal claims regarding the floristic associates of morel fruiting. These results will guide our efforts to optimally locate sites for enhancement of natural fruiting.

Production of sclerotia by *Morchella* spp. is easily accomplished in the laboratory. Over several years, we have placed laboratory-produced sclerotia in the field to investigate the conditions under which fruiting might be stimulated. We have found that sclerotia placed in the field in the autumn remain viable the following spring, germinating both on agar and in response to apple seedling root systems. We are investigating the utility of incorporating germination stimulants within sclerotia which might enhance germination and ultimately fruiting.

The dependence of the morel life cycle on outcrossing is still an open question. We have obtained pure cultures from the majority of the mapped field collections. We are interested in determining whether or not these collections represent clonal populations. Neither classic somatic compatibility tests nor AFLP analysis have proven satisfactory thus far.

OPEN FIELD INOCULATION OF ADULT HAZELNUT GROVES WITH *TUBER MELANOSPORUM* VITT.

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Key words: *Tuber melanosporum*, cultivation technology, hazel, field inoculation.

The hazelnut tree has a great social and economic value in the NE of Spain. Nowadays sunk in an economic crisis, most of these fields lays on potential truffle producing areas. *T. melanosporum* and *T. brumale* appear in some of them naturally.

Our aim is to coordinate the truffle and hazelnut cultivation, trying to get an added value to the traditional harvest.

In this work inoculations have been carried out in mature hazel trees (from 11 to 30 years old) with *Tuber melanosporum's* sporal inocula.

They were carried out two inoculations in all the treatments (spring-fall). Four years later, the mycorrhization qualitative analysis reflects the presence of *T. melanosporum* among all the inoculated fields.

Four hazels from two different experimental plots began to produce black truffle one year after the second inoculation. Three years later, eight hazels produced 460g of the inoculated truffle.

PLAN OF CONSERVATION AND SUSTAINABLE USE OF MUSHROOMS AND TRUFFLES OF ANDALUSIA. PLAN CUSSTA.

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Key words: Others

Andalusia is a rich region in fungi, possibly one of the most bio-diverse regions in the world when referring to the truffle species and paradoxicalally, of little micologica tradition.
In the last few years a social phenomenon without precedent, has taken place. Society has experimented a tremendous flood of interest in mushrooms and truffles, as much as for its recreational purposes as in for its economic and gastronomical interest. This unusual and unexpected interest has made a great impact upon the people of Andalusia, where today, it is the natural element that arrouses most interest.

The lack of micological tradition, to which we alluded earlier, is now indeed an ally in the ordered control of this resource. We stared from zero, without too many previous interests that could cloud a good management directed at the sustainability of this resource and of the conservation of the species that consequently, or for other reasons, could be threatened. All of which becomes of much greater importance as a result of the latest technical and scientific advances that bring to light several new aspects:

1) the paper of the fungi as essential components of the ecosystems,
2) the general decline of the mushrooms and truffes population,
3) flattering economic evaluations on these micological resources.

As a result, at the end of the year 2001, the Council of Natural Resources of the Board of Andalusia started the Plan CUSSTA, which outlined the following guidelines:

1) Social Participation and Environmental Education.
2) Investigation.
3) Conservation.
4) Sustainable Use.
5) Regulation of uses.

INVERTASES IN THE ECTENDOMYCORRHIZAL ASSOCIATION *HELIANTHEMUM ALMERIENSE* X *TERFEZIA CLAVERYI*

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Key words: fungal biochemistry, desert truffle, invertase

The aim of this study was to characterize in *Helianthemum almeriense* roots the modifications of activity of invertases, following colonization by the desert truffle *Terfezia claveryi*. After three months from the *in vitro* inoculation, a positive growth response could be observed due to the mycorrhizal colonization, which was significant for roots but not for shoots. As a consequence the plant root/shoot ratio was higher in non-mycorrhizal roots (1.26) than in the mycorrhizal ones (0.91). However, the root: shoot ratio does not take into account development of extraradical mycelium, root exudation and changes in root and fungus respiration. These factors increase the carbon request of mycorrhizal vs nonmycorrhizal roots grown in conditions of limiting P, even if the root : shoot ratio does not increase.

The extraction buffer HEPES-KOH 0.2 M, pH 8.5 gave the best results in comparison with sodium-tetraborate 0.1 M, pH 8.8. Invertase activities were detected in the soluble fraction at pH 4.5 and 8, and at pH 4.5 in the cell wall-bound fraction. Great significant differences in soluble acid invertase activity were observed between mycorrhizal (14.8 nkat/mg protein) and non-mycorrhizal roots (0.6 nkat/mg protein). However, no acid cell wall-bound invertase and alkaline invertases (soluble and cell wall-bound) were detected in either mycorrhizal or non-mycorrhizal roots. The stimulation of the activity of vacuolar invertase within root cells may provide hexoses for uptake by either intercellular or intracellular hyphae, although this would first require the transport of the hexoses to the apoplast. Alternatively, this enzyme may produce hexoses to provide energy to drive the uptake of nutrients, such us P, from the intracellular hyphae.

This work was supported by Project REN2003-08241/GLO from M.E.C. MPG holds a contract from "Programa Ramón y Cajal", MEC, Spain and FEDER.

IDENTIFICATION OF *TUBER MAGNATUM* GENOTYPES FROM TRUFFLE GROUND SOIL SAMPLES

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Keywords: *T. magnatum*, soil DNA extraction, genetic variability, Molecular Biology and Biochemistry

Many studies have investigated the molecular structure of ectomycorrhizal fungal populations, but only a few of them have faced the genetic variability of truffle populations. In a previous work, we analysed ascomata of a *T. magnatum* population collected for as long as 5 years, and we identified two genotypes (Mello et al., 2005, Environ. Microbiol., 7(1), 55-65). As a second step, in order to collect information about the distribution of *T. magnatum* mycorrhizas, root tips were sampled, revealing only a few *T. magnatum* ectomycorrhizas. Therefore, this investigation did not unravel the below ground distribution of *T. magnatum* (Murat et al., 2005, FEMS Microbiol. Lett., 245 (2): 307-313).

The aim of the present work was to elucidate the molecular structure of the *T. magnatum* mycelium directly from truffle ground soil samples through: i) an assessment of the presence/absence of T. magnatum and ii) the identification of *T. magnatum* genotypes.

We collected 26 soil samples in a natural truffle ground of *T. magnatum* (Montemagno, Asti, Italy). From each sample three DNA extractions were performed and pooled. DNA was then used in nested PCR reactions with primers amplifying a specific genomic region of *T. magnatum.* The bands obtained were then sequenced.

DNA was extracted successfully from all the soil samples and the good quality of the extractions was confirmed by using universal primers in PCR reactions. Eight out of 26 soil samples gave positive result with specific markers of *T. magnatum.* The sequencing of PCR products allowed us to identify 2 genotypes, which correspond to the genotypes already identified through the previous ascomata analysis.

The present work represents the first report concerning the analysis of soil DNA extracted from a truffle ground. The technique we used proved to be a good tool to identify *T. magnatum* in soil samples. Moreover, *T. magnatum* was identified in a non productive area of the truffle ground, suggesting that biotic or abiotic factors could inhibit the ascomata production.

CHARACTERIZATION OF PHOSPHATASE ACTIVITY IN *TERFEZIA CLAVERYI* CHATIN ASCOCARPS

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Key word: fungal biochemistry, phosphatase, *Terfezia claveryi*, ascocarps.

Terfezia claveryi Chatin is an edible hypogeous mycorrhizal ascomycete called desert truffle. In natural ecosystems, it establishes mycorrhizal symbiosis with several species of genus *Helianthemun*. Phosphatases are a wide group of enzymes which catalyze the hydrolysis of different phosphate esters. They differ in their pH optimum, substrate specificity, reaction mechanism etc. Phosphatases located on the surface of mycorrhizal fungi permit phosphate mobilization, making it accessible to the plant. In some cases, this process may be responsible for the differences in growth between mycorrhized and no mycorrhized plant. To date, data on the effect of phosphatase on ectendomycorrhizical fungi are very limited.

This is the first report on the phosphatase activity from *T. claveryi* ascocarps. Both acid (ACP) and alkaline (ALP) have been detected using *p*-nitrophenylphosphate (*p*-NPP) as substrate. ALP activity was partially purified using phase partioning with PEG_{8000}. With this method an important reduction in the lipid content of the extract was obtained. This phosphatase was kinetically characterized using *p*-NPP as substrate. Its pH optimum was pH 10.0 and the K_m 7.5 mM. The effect of different inhibitors on phosphatase activity was also studied.

In addition, ALP was histochemically localized within *T. claveryi* ascocarps using the bromo-cloro-indolphosphate and nitro blue tetrazolium reaction.

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MEASURING SPOROCARPS PRODUCTION OF EDIBLE ECTOMYCORRHIZAL FUNGI IN SIX MEDITERRANEAN FOREST ECOSYSTEMS IN THE PROVINCE OF PALENCIA (SPAIN).

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Key words: mushrooms productivity, xeric locations, forest management.

In some locations of mediterranean area where summer rains are almost nonexistent, edible ectomycorrhizal fungi production in autumn may be very high and explosive. In Spain, many forest ecosystems that are being abandoned because of their low economic value, are associated with the most important and appreciated edible fungi of the world. The study was located in the province of Palencia (northern Spain), in six different forest ecosystems with particular xeric conditions. *Pinus sylvestris*, *P. pinaster*, *P. halepensis*, *Quercus ilex ballota*, *Q. faginea* and *Q. pyrenaica* stands were studied.

Autumn mushrooms were collected for two years to examine the fungal diversity and sporocarps production. Three plots of 100 square meters (2*50 m) were established randomly in each studied habitat. The plots were visited at one week interval from October to December of 2003 and 2004, and were evaluated collecting complete sporocarps productions. Then they were identified in the laboratory and fresh weight was measured. In some samples the identification was only to Genus as in some cases of *Cortinarius* and *Russula*. Sporocarps were dried and weighed.

More than 10.000 sporocarps were collected during the study. Average productivity of edible ectomycorrhizal marketed mushrooms of *Pinus sylvestris* was 218.87 kg/ha, *P. pinaster* 160.51 kg/ha, *P. halepensis* 16.99 kg/ha, *Quercus ilex ballota* 41.44 kg/ha, *Q. faginea* 6.36 kg/ha and *Q. pyrenaica* 53.53 kg/ha. The high production of *B. aereus* (11.07 kg/ha) and the presence of *Cantharellus cibarius* (2.12 kg/ha) and *Cratherellus cornucopioides* (1.96 kg/ha) in the *Quercus ilex ballota* stand under an annual precipitation lower than 400 mm, were particularly important. In *Q. pyrenaica* forest *Amanita caesarea* production is surprising (14.04 kg/ha in 2003) but differed strongly among years (0.01 kg/ha in 2004).

This study is currently in course with the purpose of obtaining the realistic data of mushrooms production that can be used in forest management, increasing economic interest of these habitats by non-wood forest production.

ECOPHYSIOLOGICAL AND PEDOLOGICAL FACTORS AND BLACK TRUFFLE (*TUBER MELANOSPORUM* VITT) PRODUCTION IN AN EVERGREEN HOLM OAK (*QUERCUS ILEX* L.) TRUFFLE ORCHARD

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Key words: field plantations, ecophysiology, *Quercus ilex*, soil, stoniness, *Tuber melanosporum*

Truffle production varies considerably among trees within truffle orchards. Ecophysiological and pedological factors could explain this variability. Our aim was to determine the relationships between truffle production and variables such as tree diameter, spring shoot growth, leaf water potential, soil type and stoniness. The study was carried out on spring 2004 near Roullens (France) in a productive truffle orchard of 101 trees from 3 different nurseries. Truffle production, i.e. number and weight of collected truffles around each tree, had been registered every year since the orchard began to produce. A detailled soil study showed that five soil classes could be identified within the same orchard. Differences of truffle production were observed between classes ($p=0.06$). Major production was registered on a calcosol lithic area. Soil stoniness appeared to be associated with higher truffle productions, but stoniness was also associated with higher productive soil classes. Leaf water potential and other ecophysiological parameters were independent of production. In view of our results, soil heterogenety can be found within the same orchard, and this heterogeneity could be responsible for the differences in production.

FUNGAL SYLVICULTURE. FOREST MANAGEMENT TO IMPROVE PRODUCTION OF WILD EDIBLE MUSHROOMS

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Key words: Fungal sylviculture, edible mushroom, production

Technologies tending to preserve and improve wild edible mushrooms production are known as a whole as Fungal Sylviculture. (MARTÍNEZ DE AZAGRA & ORIA DE RUEDA, 1996). This science is based on preserving and promoting fungi richness in forests using technical management for necessary interventions in these aspects. Inside Fungal Sylviculture there are included a varied set of aspects and technologies such as different patterns of timber harvesting which could be suitable for promoting mushroom production. Other topics like selection of producing species, rotations, use of fire and mycorrhization are also studied. In general, there are included the most interesting aspects applied to the natural forests and forest plantations. Furthermore, current skills as GIS (Geographic Information Systems) are applied to the management of fungi producing forests. In this paper several examples of this use are presented.

In numerous natural ecosystems and forest areas, production of edible fungi can overcome other classical productions as wood, resin, cork or hunt. For instance, in oak forests, holm oak forests , mountain groves and chestnut groves which produce *Boletus* gr. *edulis* fungi, production from 10 to 200 kg/ha in one year (sometimes 400 kg/ha) can be reached. This produces high annual revenues. Utilization of valued fungi capable of growing in dry forests is very important. For example, forests of *Quercus ilex ballota* produce high crops of *Amanita caesarea, Boletus aereus, Cantharellus cibarius* and *Craterellus cornucopioides* in acid soils and crops of *Tuber nigrum* and *Tuber aestivum* in basic soils.

Fungal Sylviculture has developed related to great demand on forest edible fungi and its entry in international market circuits. Currently, apply Fungal Sylviculture is essential to avoid the depletion of this richness. In fact, production is seriously decreasing in some very industrialized countries.

BOLETUS EDULIS PRODUCTION IN XEROPHILIC AND PIROPHITIC SCHRUBS OF *CISTUS LADANIFER* AND *HALIMIUM LASIANTHUM* IN WESTERN SPAIN.

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Key words: *Boletus edulis*, *Cistaceae*, production, scrubs, rural development.

Fungal communities in xerophilic and pyrophitic scrubs composed by *Cistaceae* plants can reach high economic importance. These shrubs are able to grow in very poor soils and are associated with some of the most important edible ectomycorrhizal species as *Boletus edulis*, *B. aereus*, *Cantharellus cibarius*, *Russula cyanoxantha*, etc. Some species of the *Cistus* and *Halimium* genus can produce large harvests of these apreciate ectomycorrhizal fungi since they are 3-4 years old. *Cistus ladanifer* and *Halimium lasianthum* shrubs cover wide areas in western Iberian Peninsula, both in Spain and in Portugal.

In this work the presence of *Boletus edulis* and *B. aereus* in ecosystems covered with *Cistus ladanifer* and *Halimium lasianthum* was studied along west Spain. Also, an experiment was set up to evaluate edible fungi production of these scrubs in the area of Aliste (Zamora) during the autumn of 2003 and 2004. Fungal production was collected weekly in permanent plots (2*50 m). Both fresh and dry weights were measured.

We observed the presence of *Boletus edulis* and *B. aereus* associated with *Cistus ladanifer* in the provinces of León, Zamora, Salamanca, Ávila and Toledo. Appearance of these fungi associated with *Halimium lasianthum* have been also recorded in dry and poor soils of Zamora, León and Palencia. In the study of Aliste (Zamora), an average production of 36 kg/ha of *Boletus edulis* in *Cistus ladanifer* plots has been observed in 2003.

The ability to produce very valuable fungi by these scrubs since they are 3 or 4 years old, very much before than other ecosystems like *Pinus* or *Quercus* stands, whose production of *Boletus* was delayed until they are 40. For this reason *Cistus* scrubs are very interesting according to forest valuation and economics in rural areas, especially for marginal lands and poor countries.

MANAGEMENT OF EDIBLE MYCORRHIZAL MUSHROOMS AS A TOOL FOR WILDFIRE PREVENTION IN MEDITERRANEAN ECOSYSTEMS

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Key words: edible mushrooms, fire, diversity, production, Mediterranean ecosystems

Mushrooms play a fundamental role in the ecosystem functioning and are also a high valuable forest resource. An adequate management would improve conservation and production of mushrooms and this would also contribute to wildfire prevention. We studied an area where wildfire is frequent and mycological production is economically important.

The plots were placed in the area of Aliste (Zamora) in two different ecosystems, covered by *Pinus pinaster* Ait. and *Cistus ladanifer* L. respectively as main species and wildfire effects on edible mushrooms communities were analyzed during the autumn of 2003 and 2004.

The studied *Pinus* and *Cistus* dominated ecosystems provide a high production of edible mushrooms and several of these species are marketed in local industries. For *P. pinaster* stand, fresh weight production for edible taxa represented 82% of the total in unburned plots (272.2 kg/ha). Fresh weight for edible mushrooms in *Pinus* and *Cistus* plots decreased significantly following wildfire and the trend was similar for the most important marketed fungal species, *Hygrophorus gliocyclus, H. agasthomus, Lactarius deliciosus* (in *P.* pinaster plots) and *B. edulis* (in *C. ladanifer* plots). However, fire significantly increased the abundance of the pyrophytic mycorrhizal species such a *Leccinum corsicum* associated to *C. ladanifer.* In addition, some mycorrhizal fungi associated to *C. ladanifer* are common to *Pinus* forests, so these pyrophytic shrubs can play an important role in the regeneration of *P. pinaster* stands after wildfire.

In conclusion, developing methods for conservation of a sustainable productivity of edible mushrooms in natural forests can be also useful as a tool for wildfire prevention.

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COMPARATIVE ANALYSIS OF THREE METHODS TO ESTIMATE THE SPOROCARP PRODUCTION OF EDIBLE WILD MUSHROOMS IN PINEWOODS OF SORIA FOREST RANGE (SPAIN).

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Key words: Sporocarp production, sampling method, ecology, forest management.

Within the framework of the INIA RTA 03/046 research project, it is compared the efficiency and cost of 3 sampling methods used to estimate sporocarp production of edible wild mushrooms in managed forest stands in the pinewood range of Soria-Burgos, Spain.

The comparison is made with 3 types of sampling plots randomly established in mature stands of *Pinus sylvestris* L. homogeneous in age, physiography and density. In method 1, six 35x5 m^2 fenced permanent plots were used, where all the sporocarps were collected weekly in the autumn from 1995 to 2004. In method 2, three 330 m long permanent transects were used where all the sporocarps were collected weekly in the autumn from 1997 to 2003. Method 3 consisted in three 330 m long permanent transects where all the observed sporocarps were registered (not collected) to study their weekly evolution during the autumn of 2004.

Method 3 allows to estimate weekly sporocarp production in the forest (P), differentiating between: the potential production (p), the production collected by harvesters (R), the production consumed by domestic or wild forest animals (G) and the production wasted by putrefaction, trampling etc., (M), in such a way that P=p+R+G+M. Method 2 merely estimates (p), while method 1 calculates (P) but does not allow to know the values of (R), (G), or (M) in the forest.

In estimating the production of sporocarps of *Lactarius deliciosus* L. in the autumn of 2004, method 3 obtained a decrease of a 30% in relative error with respect to method 1. Compared 2 years of similar production, the estimate of (p) of *Lactarius deliciosus* with method 3 reduced relative error a 43% with respect to method 2. Maintenance costs of method 1 are superior to those of 2 and 3. And sampling cost of method 3 is a 30% superior to that of methods 1 and 2.

Consequently, method 3, though more expensive to sample, is more efficient and reveals important data for sustainable management of edible wild fungi such as: harvesting rate, production loses due to wild animal consumption or trampling.

OPTIMIZATION OF THE MYCORRHIZATION PROCESS OF *PINUS HALEPENSIS* IN CONTROLLED CONDITIONS

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Keywords: *Pinus halepensis*, ectomycorrhizae, mycelium inoculum, cultivation technology, growth and mineral nutrition.

The study of the growth of the autochtonous ectomycorrhizal mushrooms of the Haouz region notably *Suillus collinitus* (Fr.) O. Kuntze, *Suillus bellini* (Inz.) Watl, *Hebeloma mesophaeum* (Pers.) Quélet, *Tricholoma terreum* (Sch.: Fr.) Kummer and two strains of *Suillus mediterraneensis* (Jaquet. & Blum) Redeuilh, the strain 3530 and the strain 3532, showed the capacity of all these species to develop on MNM and PDA media. The optimum of the mycelial growth was observed in a pH of 5.5 to 6. The effect of the type of inoculum (obtained on peat-vermiculite or produced on liquid or agar medium) of these symbiotic species on the mycorrhization of pine seedlings has been experimented in controlled conditions.

The use of mycelium grown on peat-vermiculite allows obtaining degrees of mycorrhization superior to those registered by using mycelium of the same mushroom under the other types. The mycorrhizal percentages of the inoculated seedlings by *S. collinitus*, *H. mesophaeum*, *T. terreum* and the strain 3530 of *S. mediterraneensis* were superior to those of the seedlings inoculated with the other fungal species. The inoculation of pine seedlings by the mycelium of various symbiotic species cultivated on solid substrate showed a very significant improvement of the plant growth and the mineral nutrition. *S. collinitus*, *H. mesophaeum* and the strain 3530 of *S. mediterraneensis* showed a very important efficiency and a positive correlation between the colonization and the stimulation of the growth and the mineral nutrition.

COMPARATIVE ASSAY OF MYCORRHIZATION WITH DIFFERENT DOSES OF INOCULUM FROM DIFFERENT YEARS. PRECOCITY OF SYMBIOSIS ESTABLISHMENT.

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Keywords: Black truffle, cultivation technology, mycorrhization

The aim of this essay was to relate precocity and efficiency in *Quercus ilex* L. mycorrhization by *Tuber melanosporum* Vitt., with the dose and age of the fungus inoculum used.

Plants were obtained from the acorns of a thousand-year-old evergreen oak, and inoculated in their naked roots by sprinkling dried truffle powder from 2002, 2003 and 2004. The dose applied was one, two and three grams per plant.

According to the results, the inoculum source lot presented a decisive importance independently from the year. The first micorrhiza were obtained 75 days after inoculating one gram per plant of a two-year-old inoculum. Only in the case of old inocula, the final percentages of mycorrhization were significantly different according to the dose. A three-gram dose per plant was necessary to mycorrhize 42 % of the plant root system.

CHARACTERIZATION OF AN ESTERASE FROM ASCOCARPS OF *TERFEZIA CLAVERYI* CHATIN.

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Key words: fungal biochemistry, desert truffle, esterase, *Terfezia claveryi*, TX-114

A non-specific esterase from ascocarps of the desert truffle *Terfezia claveryi* Chatin is described for the first time. The increase in the levels of this enzyme has been taken as an indication of the enhanced metabolic activity produced during the establishment of arbuscular mycorrhizal associations. In addition, the esterase isozyme pattern has been used as a tool for the characterization of arbuscular mycorrhizal fungi and in taxonomic studies of edible mushrooms.
The enzyme was partially purified using phase partitioning in the non-ionic detergent Triton X-114. The enzyme showed maximum activity toward short-chain *p*-nitrophenyl esters, and no interfacial activation was observed, indicating that the enzyme responsible for this activity is an esterase and not a lipase. The enzyme presennted its maximum activity at pH 7.4 and 60ºC. The values obtained for K_m at pH 7.4 were 0.3 mM for *p*-nitrophenyl butyrate and 0.6 mM for *p*-nitrophenyl acetate. *T. claveryi* esterase was inhibited by phenylboric acid, indicating that serine residues were involved in the enzyme activity. This enzyme was localized only in the hypothecium and was absent from the peridium and gleba.

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THE WILD EDIBLE MUSHROOMS OF MEXICO: STATE OF KNOWLEDGE AND PERSPECTIVES

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Keywords: ecology, rural development, biotechnology.

Mexico has a great biological and cultural diversity, which includes around 25 000 plant species, 40% of which are endemic to the country, 45 vegetation types and more than 50 ethnic groups. As a result of this mixture, more than 270 wild mushrooms are eaten in the country. They have been widely consumed, for centuries, since the time of Pre-Columbian cultures. More than one thousand traditional names, in different local languages, have been recorded for them, in Mexico. Most of the species (around 80%) are distributed in coniferous and *Quercus* forests, and the rest are in cloudy mountains and tropical forests. Annually, thousands of tons, of more than 100 species, are gathered and sold in popular markets mainly in Central and Southern Mexico.

Apart from the great ecological importance in the structure and functioning of local natural ecosystems, mainly through mycelial networks which nurture and connect trees in nature, ectomycorrhizal (ECM) fungi play also an important social role in Mexico. More than 150 ECM species, in 38 genera are consumed in the country, including for example species of *Amanita, Boletus, Cantharellus, Hebeloma, Helvella, Laccaria, Lactarius, Leccinum, Ramaria, Russula, Suillus, Tricholoma* and *Xerocomus*. Commercialization of these ECM fungal species follows, at least, four levels: i) collection for self-consumption; ii) direct sell to consumers, in small communities; iii) sell in local or regional markets, in some cases, through intermediaries; and iv) exportation to international markets, which includes mainly species of *Amanita, Boletus, Cantharellus,* and *Tricholoma*. Despite the richness of native ethnomycological knowledge in Mexico, along with dramatic deforestation rates, cultural erosion is being carried out at a rapid pace. Integral approaches in order to improve forest conservation, including enhanced knowledge of non-timber products, like wild edible mushrooms, is an urgent need in the country. Currently a pilot project in Central Mexico, in the Izta-Popo Zoquiapan National Park is been carried out, with financial support from Mexican Government (through the Project SEMARNAT-CONACyT 2004-01-45). This project includes ecological, social and biotechnological studies of the wild edible mushrooms of the region. Currently more than 45 species have been identified in the area, of which 85% are sold in regional markets. Additional to the importance to the local diet of communities, commercialization of these mushrooms is an important source of income, accounting in some families for example, more than 80% of their total incomes, during the mushroom season. ECM inoculum has been produced with more than 20 local species, and successful inoculation of pines has been carried out using 10^7 to 10^9 spores per plant.

EFFECTS OF THINNING YOUNG FORESTS ON CHANTERELLE MUSHROOM PRODUCTION

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Key words: Fungal silviculture, chanterelles, forest thinning, productivity

The Pacific golden chanterelle *(Cantharellus formosus)* and the white chanterelle (*C. subalbidus*) are the two most commonly collected chanterelle species in the Pacific Northwest. Both fruit in young dense Douglas-fir and western hemlock stands that are common throughout the region as a result of logging native forests during the latter half of the 20th century. Many of these young stands are now slated for thinning to produce wood fiber, enhance the growth and health of residual trees, reduce fire danger, and meet other management goals. Foresters are grappling with how to fit forest products such as chanterelles into their management plans because so little is known about how various management practices and silvicultural regimes affect the persistence and productivity of ectomycorrhizal mushrooms.

Chanterelle productivity responses were investigated in a landscape-scale thinning experiment in 50-year-old Douglas-fir stands in the Cascade Range of Oregon. Three thinning treatments (control, light and heavy thins) were replicated (in stands of 20-40 ha) in four areas (logging sales spaced 5-50 km apart). All stands began at around 625 trees per ha, lightly thinned stands had about 275 residual trees per ha, and heavily thinned stands about 125 remaining trees per ha. Elongated rectangular strip plots were used to sample systematically across all micro-environmental conditions in each stand (including such features as logging skid trails and creeks) in order to obtain representative stand-level estimates of chanterelle productivity. Chanterelle numbers and weights were sampled repeatedly during the fruiting season to obtain total seasonal productivity for each stand. Stands were sampled one year prior to thinning (logging) and four out of six years afterwards.

Chanterelle numbers and weight were significantly decreased by thinning the first year after logging, more so in heavily thinned stands than lightly thinned stands for chanterelle numbers. Nearly all evidence of differences in chanterelle productivity among thinning treatment means disappeared within 6 years. The effect of thinning on chanterelle productivity appears proportional to the percentage of host trees removed. Silvicultural tradeoffs exist however. For instance, frequent light thinning might conserve mushroom production levels, but frequent thinning entries are costly, and can result in more soil compaction and residual tree damage if not conducted carefully.

ASSESSMENT OF SUSTAINABLE COMMERCIAL CHAGA HARVESTING IN KHABAROVSK AND PRIMORSKY KRAIS, RUSSIA.

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Key words: Chaga, *Inonotus obliquus*, medicinal fungi, conservation and sustainable harvest, commercial nontimber forest products, Russia

Chaga is a medicinal fungus harvested from birch trees by inhabitants of the boreal forests in the Northern Hemisphere since time immemorial. It is a sterile conk-like growth that is produced by the canker wood-rot fungus *Inonotus obliquus* as it interacts with the birch tree's physiological defense mechanisms. It was traditionally used as a tea for stomach ailments and as a skin-cleansing solution. Currently, its pharmaceutical properties are being investigated for immunological-enhancing and anti-cancer properties; consequently world markets are expanding. Russians have a long tradition of using chaga, and are now beginning to export it internationally. The increased demand for chaga products raised concerns about over-harvesting. Although Russia has a long history of regulating and promoting commerce in nontimber forest products, little is known about potential harvest levels for this fungus product.

A sustainability analysis for chaga harvesting was conducted using printed sources and local interviews in Khaborovsk and Vladivostok, in the Russian Far East. Using information on chaga biology, birch forest silviculture, and exports by the business sector; data were combined into Excel worksheets that calculated sustainable harvest levels using various assumptions. Results showed that even using pessimistic values, the biological resource is exceedingly abundant and at no risk of over-harvesting, even with dramatically increased harvest levels. Nevertheless, it is possible that chaga might be harvested in areas near rural communities or along roads at such a rate that buyers would eventually obtain it more inexpensively elsewhere in Russia or the world. Thus the economic sustainability of the resource near rural communities in Khabarovsk and Primorsky Krais could be of eventual concern because road networks are relatively underdeveloped.

Recommendations to enhance local access to the resource and to locally and regionally retain more profits from the processing and sale of chaga products are presented. These recommendations describe ways that the development projects, the NTFP business community, scientists, foresters, and local administrators could cooperate to enhance economic development while ensuring sustainable chaga supplies.

Chaga harvesting shares many of the same issues of sustainability as commercial harvesting of edible ectomycorrhizal mushrooms, but also differs in significant ways. These comparisons and contrasts will be discussed.

SUCCESS OF BURGUNDY TRUFFLE OAK HOST GROWN IN SEVERAL POTTING MIXES

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Key words: *Tuber aestivum*, potting media, *Quercus robur, Quercus bicolor*, Cultivation Technology

The European Burgundy truffle (*Tuber aestivum* syn. *Tuber uncinatum*) is a valuable food commodity and may be a profitable commercial crop in the south central USA. Truffle cultivation involves germinating host trees such as white oaks and hazels in the presence of truffle spores. The spores produce hyphae that surround the root tips and form ectomycorrhizae. High levels of *Tuber* root colonization early in the tree life cycle may reduce competition from other fungi and improve future truffle production. The purpose of this study was to develop a host growth medium that satisfied the environmental requirements of the truffle fungus and the host while reducing colonization by other mycorrhizal fungi. This study evaluated the performance of the *Quercus robur* x *Q. bicolor* host in seven potting mixes. The mixes consisted of different proportions of rice hulls, ground bark, sand, vermiculite, lime and fertilizer and were based on the RPM (patent pending) potting process developed by Forrest Keeling Nursery in Elsberry MO.

CURRENT STATE AND PERSPECTIVES OF TRUFFLE CULTIVATION IN CHILE

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Key words: truffle, *Tuber melanosporum*, cultivation, Chile.

Chile presents a clear potential for truffle cultivation, because favourable areas exist to establish truffle (*Tuber melanosporum*) plantations mainly between 35° and 40° S latitude and in the colder locations between 33° and 35° S., where climate predominant is a Mediterranean Temperate. Also, due to the ecological conditions, ectomycorrhizal fungi that can compete with new introduced species are barely represented in some areas of the country, mainly in soils used by the traditional agriculture and the cattle raising.

The establishment of truffle cultivation in Chile began in the year 2002, through a Research Project developed by the Catholic University of the Maule in association with CEAM Foundation of Spain and supported by the Chilean Ministry of Agriculture. Currently, Starting from this project nine hectares of experimental truffle orchards have been out planted, distributed between the Metropolitan Region (María Pinto) and XI Region (Coyhaique). The results of these plantations are promising and a very good initial adaptation of *Tuber melanosporum* has been observed from the field cultivation conditions.

In August 2004 the company Agrobiotruf S.A. has been formed, being the first one in Chile in developing a nursery specialized in the commercial production of truffle mycorrhized plants and consultantship services with the objective of achieving a critical mass of plantations that allows to enlarge the cultivation bases and this way to be able to establish an industry.

After the first year of operation, the company has been able to produce 4.400 mycorrhized plants with *Tuber melanosporum*, which will be established between October 2005 and May 2005 by means of a coordinated approach with different producers in different areas of the country. These areas were selected based on rigorous analysis of climate and soil conditions.

Currently, the company Agrobiotruf is developing a certification program with a independent organism to validate the quality of the produced plants and it has also begun a production of 10.000 truffle plants that will be available for out planting in September 2006. With this work, it is hoped to establish an approximate surface of 35 hectares of truffle orchards in 2006.

The present work aims to show the current situation and perspectives for trufficulture in Chile, also to give to know the technical advances in the cultivation development.

EVALUATION OF THE MYCORRHIZATION PROCESS ON PLANTS OF THE SPECIES *CASTANEA SATIVA* (FAGACEAE) WITH MYCORRIZHAL *TUBER AESTIVUM* AND *MORCHELLA CONICA* (ASCOMYCETES).

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Keyword: *Morchella conica, Tuber aestivum* and Chestnut.

Castanea sativa (Chestnut tree) is an emergent species in Chile included in the program of forest diversification of Chilean Government. It constitutes a valid option in the future plans of forestation, this species is distributed from the VIII and X regions and like other forests species do not present a positive display net income during the rotation of their culture. In order to revert this situation it has been begun to use edible mycorrizhal fungi that generate an additional entrance of source, diminishing the costs of replant and to a large extent of the period of the rotation, which can make the investment in forestation more attractive.

For this work we inoculated plants of Chestnut tree with *Tuber aestivum* (white truffle) and *Morchella conica*. These species are considered quite attractive anywhere in the world by their high value in the commercial markets.

For this work, we took 18 plants of the breeding ground of INFOR company for random analysis, which represent 3 treatments with: 6 plants witnesses without inoculation, 6 plants inoculated with *Morchella conica* (inoculate spore) and 6 plants inoculated with *Tuber aestivum*. First, we began with the documentation of the homogeneity and guarantee of the plants, was come to eliminate the substratum by the roots of the plants, one by one, and the mycorrizhal analysis was begun until to have observed and counting 300 apexes according to methodology described by Fischer and Colinas (1996). The data were incorporated in a table for the determination of the proportion of the mycorrizhal effective (PME) and pollution (PMP).

The mycorrhizal plants with *Morchella conica* had the tendency to present micelium of cream-brown color forming a mantle around the primary, secondary and tertiary roots. For the plants inoculated with the species *Tuber aestivum* or white truffle, we obtained good results with a mycorrhizal average of 49.4 %. The presence of mycorrhizal micelium ventures a good beginning in the future plans of mycorrhization of the forest plantations, finally it is to evaluate the behavior of these land species.

EFFECT OF TRUFFLE SYLVICULTURE TREATMENTS ON THE MYCORRHIZATION AND TREE REGENERATION OF A MIXED FOREST WITH SPONTANEOUS PÉRIGORD BLACK TRUFFLES

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Key words: *Tuber melanosporum*, *Quercus ilex*, ectomycorrhiza, recovering of spontaneous truffières, fungal sylviculture and ecology

In recent decades, spontaneous black truffle production has suffered a generalized decline due largely to forest densification. From 1998 to 2001, a truffle sylviculture pilot project was carried out in El Toro (Valencian region, eastern Spain) to improve the production of spontaneous truffières by adapting the ecological conditions to the requirements of the black truffle. In 2001, the monitoring of truffle production, mycorrhization and tree regeneration was begun in some of the treated truffières. Four years later, both truffle production and populations of black truffle ectomycorrhizas are recovering, and the holm oak has become the dominant species in the regenerating forest. The present threats to truffle production in this forest, the most probable evolution of the truffières and the needs for the coming years are discussed.

SPANISH TRUFFICULTURE

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Key words: *Tuber melanosporum*, Spain, truffle production, field plantations and fungal sylviculture and ecology, research.

Although inspired by French and Italian cultivation, Spanish trufficulture has developed its own personality and is beginning to produce good results. In this work, we describe the most outstanding aspects of Spanish trufficulture in three basic fields:

1) Forest harvesting of spontaneous truffle production. This began in an intense way around 1950 and continues today; it still accounts for most of the production.

2) Truffle plantations, which were initiated around 1970. Especially notable is the 600 ha Arotz plantation in Soria, which produces 2500 kg of truffles a year. In recent years, many truffle orchards have started producing, thus increasing the importance of plantations as opposed to harvesting of spontaneous truffières. In Mora (Teruel), one of the most important Spanish truffle markets, it is estimated that more than 20% of the truffles sold already come from plantations. In many cases, the different public administrations provide economic support for the plantation of truffle orchards; as a result, more than 500 ha of truffle orchards are planted each year in Spain. Another incentive is the collective of truffle nurseries that produce more than 100.000 inoculated seedlings every year.

3) Extensive research and technical activities carried out in research centres and universities. They include areas as diverse as nursery mycorrhization, certification of inoculated seedlings, black truffle ecology, recovery of spontaneous truffières, cultivation and monitoring of truffle orchards, chemical taxonomy, truffle species control, reforestation and truffle sylviculture. 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 work includes information on production, prices, truffle distribution, markets and trade, plantations, research activities and publications. Lastly, a diagnosis is made of future perspectives for the truffle sector in Spain.

MICROBIOLOGICAL CHARACTERIZATION, DECONTAMINATION AND PRESERVATION OF *TUBER AESTIVUM*

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Key words: decontamination, controlled atmospheric, *Tuber aestivum*

Among the edible mushrooms, truffles (*Tuber* spp.) are the most coveted species both for their gastronomic and economic value. To satisfy actual demand it is necessary to increase production and to optimize storage methods to increase shelf life and preserve freshness. To this end, we applied decontamination and barrier techniques to establish conditions that would increase the shelf life of fresh summer truffles (*Tuber aestivum*).

The summer truffles (*Tuber aestivum*) used in these studies were obtained from the Aragón region. Upon arrival to the laboratory, the soil was removed from the truffles by brushing them with a wet soft brush and rinsing with tap water. The samples were submitted to chemical and physical decontamination procedures by washing them with either 70% ethanol or commercial bleach diluted to 500 ml l^{-1} and sonicated. The truffles were then stored with a low permeability plastic film and under control atmospheres. The controlled atmospheres utilized consisted of gas mixtures of 50% CO_2+30% N_2+20%O_2 or 10%CO_2+10%O_2. Truffles were stored at 4°C for 28 days. The efficacy of the decontamination procedures was established based on the total microbial recovery of meshophilic aerobic bacteria, mold and yeast as wells as *Pseudomonas* spp. and Enterobacteriacea family. To establish the ideal controlled atmospheric treatment the truffles were analyzed every 7 days based on pulp and surface color, water activity, humidity, texture, respiration rate and microbial growth.

Preliminary results found that treatment with 70% ethanol and sonication were the most efficient method of decontamination by reducing the initial microbial burden by 3 logs. None of the storage conditions had a negative impact on external appearance or on the typical odor of fresh truffles during the 28 days of storage. We only observed changes in the texture at the end of the storage period more likely caused by the loss of humidity due to the truffle's own metabolic activity.

Based on these results we can conclude that decontamination with 70% ethanol combined with sonication and storage under modified atmospheres preserve a low microbial load and ensure the maintenance of organoleptic characteristics during at least 4 weeks

BIOLOGY, ECOLOGY AND MANAGEMENT OF EASTERN CANADIAN MUSHROOMS

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Key words: field plantations, fungal sylviculture, forest mushrooms ecology, ECM ecophysiology, boreal forest, commercial harvest, forest management,

Trees of Boreal forest rely on symbiotic ectomycorrhizal fungi for their survival. Many of these fungi produce edible sporocarps, also known as wild or forest mushrooms. Annually, the boreal forest of Eastern Canada produces thousands of tons of edible forest mushrooms. There is a potential to develop this relatively new industry in Eastern Canada, but very few studies have considered the mycological flora of this forest. In 2005, the Natural Sciences and Engineering Research Council of Canada founded our three years project, focussing entirely on edible mushrooms in the boreal forest of Eastern Canada. This project aims to 1) Establish, at both the macro or forest scale, and at the micro or fungal colony scale, the relationships between stand types, environmental factors and distribution of the five potentially most valuable fungal species occurring on the study sites: two species of chanterelle (*Cantharellus cibarius, Craterellus tubaeformis*), the matsutake (*Tricholoma magnivelare*), the lobster mushroom *(Hypomyces lactifluorum)* and the turtle mushroom (*Sarcodon squamosum*); 2) Elucidate the mechanisms underlying the spatial and temporal development of individual colonies, and sporocarp formation of these five species and; 3) Establish the correct taxonomic identification of the five selected species.

During the first year of the project, 25 to 50 colonies for each of the five species under study will be localized on a natural stand of Jack pine. Stand composition and associated vegetation will be noted, together with stand structure, age, soil characteristics, and meteorological data. Those data should permit the establishment of a correlation between the presence and abundance of the five selected species and a limited number of ecological characteristics. In addition, they will permit us to establish how these parameters interact with geographical and environmental factors, as well as with natural and human perturbations. During the second and the third years of the project we will identify which part of the soil profile the fungus occupies, when and how expansion of the colony occurs during the growing season, and when and where primordia develop under the soil surface. Furthermore, the colonies or groups of colonies for each of the five species will be verified for genetic homogeneity using clonal specific DNA probes.

INITIAL DEVELOPMENT OF A *TRUFFIÈRE* IN CENTRAL CHILE

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Key words: field plantations and fungal sylviculture and ecology, *Tuber melanosporum*, Chile, plantation.

The agro-ecological conditions of Central Chile (35° - 40° S latitude) are very similar to the best truffle producing regions in Europe. The main differences are related to soil acidity and summer rainfall. In the natural distributions of black truffle in Europe, soils are calcareous with high pH (7.5 - 8.5) and summer rain is 150 - 250 mm. In Chile, the best truffle producing areas have soils with pH of 5.5 - 7 and minimal summer rain. These conditions can be modified in Chile with lime application and artificial irrigation. An experimental *truffi re* was established to investigate *in situ* production of *Tuber melanosporum* Vitt. in Chile. The site selected in the central valley (35° 32' S and 71° 36' W) is characterized by soils with a pH of 5.8. Five months before planting, soils were prepared by plowing, lime application (18 ton per hectare), and disc harrowing. The inoculated plants used during establishment were *Quercus ilex*, *Q. robur* and *Corylus avellana*. The average number of mycorrhizal root tips per plant was 6,325, 4,300, and 6,025, respectively. After nine months, a root assay was conducted and results indicate a mycorrhizal index of 43% for *Q. ilex*, 47% for *Q. robur* and 68% for *C. avellana*. No other ectomycorrhizal fungi were found.

EFFECT OF IRRIGATION ON FRUITBODY PRODUCTION OF MUSHROOMS IN A FINNISH SCOTS PINE FOREST

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Key words: Fungal ecology, mushroom yield, irrigation

Picking up mushrooms and using them as food have a long tradition in Finland. Finnish forest mushrooms are known about their purity and high quality. That is why they are highly appreciated for exportation to other European countries. However, variation in mushroom yield between the years complicates commercialising. It is known that several climatic factors such as precipitation and temperature during the growing season affect fruitbody production. However, no consistent pattern of the effects of climatic factors has been elaborated suggesting a complicated regulation mechanism. Our aim was to monitor the growth of fruitbodies in a Scots pine forest during three growing seasons and to study the effects of irrigation on the fungal yield.

Eight experimental plots (20x20m) were established in a Scots pine forest in western Finland. Four of them were non-irrigated controls and the rest four were irrigated from the beginning of June to the end of August in 2002, 2003 and 2004. Irrigation was repeated twice a week. The irrigated plots received twice the amount of water, which had fallen on the control plots through natural rainfall during the summer. All fruitbodies of the fungi were collected once or twice per week from the plots until the end of the season. Number and dry mass of the fruitbodies were determined. Fungal biomass from the humus layer was measured as ergosterol with HPLC. In the early summer 2004 Nylon mesh bags (mesh size 50 •'5fm) filled with quartz sand were buried in soil and picked up in September in order to measure the growth and species of mycorrhizal fungal mycelium in the soil. Dissolved nitrogen (NH_4^+, NO_3^-, dissolved organic N) was determined with flow injection analyser. Fungal community structure in the mesh bags will be studied with PCR-DGGE method.

The effect of irrigation on fruitbody production was species specific. Irrigation increased the total number of fruitbodies of *Cortinarius* sp. and *Lactarius* sp., whereas no effect was found for example in the total number of *Suillus variegates*, *Leccinum* sp. or *Rozites caperata*. Irrigation did not affect significantly the total number of the fruitbodies but it advanced timing of the yield. Precipitation in the growing season 2004 was higher than in 2003, which may explain the smaller effect of the irrigation treatment in 2004. As a conclusion, irrigation affected significantly the fruitbody production of some fungal species, but did not affect significantly the total number of the fruitbodies or the fungal biomass in the soil.

ENVIRONMENTAL, ECOLOGICAL AND VEGETATIVE PROPAGATION STUDIES OF LIBYAN TRUFFLES

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Key words: Field plantations, truffle, ecology, truffle habitat soil, physical and chemical properties

Truffle is a common name for the subterranean fungi belonging to the class ascomycetes. Several genera are recognized around the world. These include *Terfezia, Tirmania* and *Tuber*. In Libya, the most common truffles are *Tirmania* (the white truffle) and *Terfezia* (the black or red truffle). The present study was initiated to study the environmental and ecological conditions which promote natural growth of Libyan truffles. An attempt was made to examine the vegetative propagation of truffles in several synthetic media. The physical properties of the soil sample which was obtained from the truffle habitat showed that Libyan truffles grow mostly in the sandy loam region with high value of porosity and considerably low value of field capacity. The true and bulk density of the soil studied was found to be 2.7 and 1.4 gm/cm3 respectively.

The chemical analysis of the truffle habitat soil showed that the pH value is in the low side of alkalinity with low value of electrical conductivity. The mineral element analysis showed that the calcium concentration was the highest followed by sodium, magnesium, potassium, nitrogen and phosphorus. The microbial analysis of the soil sample from truffle habitat showed that spore forming bacteria, such as *Bacillus macerans*, *B. polymyxa* and *B. coagulans* are the most common organisms. *Mucor*, *Rhizopous* and *Pencillium* constitute part of the microflors.

Plants that are expected to have influence on the growth of the Libyan truffles were collected and identified. The most common among the plants collected was *Helianthemum* which was found wherever there were truffles. For the vegetative propagation of fresh truffles, three different synthetic media were tried. Heavy cottony growth of mycelium was observed in the flasks containing mixed media.

APPROACH OF THE FRENCH TRUFFLE CULTIVATION

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Key words: cultivation technology, *Tuber melanosporum* Vitt., *Tuber brumale* Vitt., truffle trees cultivation, lawn ecosystem, tilling.

The truffle cultivation techniques applied in France are not homogeneous from one region to another and within each region. There can have together different types in one area.

From the technical point of view, the study undertaken since 10 years shown positive and negative points of three types. 1) Traditional truffle cultivation still persists in traditional truffle areas of South-West and South-East. It used seedling without any mycorrhization controls which are planted on tilled soils with modern mechanical machines. The results are rather uncertain. 2) Truffle trees cultivation has been developed when the first mycorrhizated seedlings has appeared on the market, in 1974. Today, this dominant type in many French regions consist to grow the host trees with the method used in orchard : tilling, water managing with irrigation, pruning, fertilization and soil improvement, weeds and pest control. The results are variable : in some plantations, host trees can give high yield ; in other one, they can get too much undesirable truffle species such as *Tuber brumale* Vitt. 3) Truffle cultivation in lawn ecosystem correspond to a truffle areas managing which preserved equilibrium observed on natural truffieres growing notably on fallow lands. It suppose three stages in plantation technical itinerary : a) host trees with as good as possible settlement during the two first years ; b) maintenance of the plantation to facilitate lawn ecosystem setting and maintain it until the beginning of the production ; c) the cultivation during truffle production stage with tilling and irrigation. This new truffle cultivation has the advantage not to be penalized by contamination.

From the sociologic and professional point of view, people which are no farmers are first truffle growers, excepted in areas where truffle cultivation is a new activity, in particular where cereal farmer are trying new economic ways for their farms. Professional organization, under the aegis of Féderation française des trufficulteurs (French truffle growers organization or FFT), stays dynamical in their will to find means and grants or to get measures or laws to develop truffle cultivation. French research and experimentation try to resolve the uncertain feature of truffle growing but has limited people and modest means from Europe, Oniflhor for French State, and regional collectivities.

From the economic point of view, production which should have increase has difficulties to do it, in spite of one thousand hectares planted each year since for at less 15 years. We observe that the decline has been stopped and the French potential is waiting for a good summer without any heatwave and drought to show production recovery.

PRESSURE OF CONTAMINATION MYCORRHIZAL FUNGI AGAINST BLACK TRUFFLE IN SPONTANEOUS AND CULTIVATED TRUFFIERES IN FRANCE

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Key words: Ecology, *Tuber melanosporum* Vitt., *Tuber brumale* Vitt., *Tuber aestivum* Vitt., contamination pressure, black truffle aggressiveness.

We note on different areas that trees initially mycorrhizated by *Tuber melanosporum* Vitt. produce *Tuber brumale*. This results frequently observed in France penalize truffle cultivation development. The main factors at the origin of the phenomenon are *Tuber brumale* Vitt.'s affinity with host tree like *Corylus avellana* L.; technical itinerary, notably when the soil is frequently tilled to limit weeds expansion which compete with growth tree, irrigation installed and used the first years; fungi contamination pressure from wooded environment. Now, in those landscape with many *Quecus pubescens* Willd woods with oaks, on fallow land or *Juniperus communis* L. moors where human activity is rare; we have observed development of spontaneous truffieres which produce *Tuber melanosporum* Vitt. In the South of France, spontaneous truffieres study, by analysis of roots mycorrhizae and truffles collects with a dog, has shown that among this contaminants fungi, two *Tuber* are mainly setting: *Tuber aestivum* Vitt. and *Tuber brumale*, but sleeping or never fruiting. Moreover, the three main species of *Tuber* are distributed in the roots system area in order to get a fungi train. Similar observations has been done in very good cultivated truffieres. More recent data show that contamination pressure of *Tuber brumale* and *Tuber aestivum* are without any negative effect on *Tuber melanosporum* production if this specie is aggressive against grass and bush with brules which increase of 20 to 30 cm per year in average. Observations done in different areas seam to indicate that faster the brule increase, the best is the black truffle production. We can't say if it is the aggressiveness of *Tuber melanosporum* or its resistance to fungi pressure contamination are a natural quality of the environment (or soil). On the opposite, black truffle could have a poor resistance at the fungi pressure contamination, either by default of good conditions that cause fruiting (and aggressiveness), or because of the presence of pernicious factors missing in certain areas or regions. We think that the linear tilling, by reducing biodiversity and disorganizing fungi train, contribute to increase fungi contamination pressure. Some experiences on acid soils with limestone improvements have been installed to know if pernicious factors exist in natural chalky soils.

"VERCHAMP", MYCORRHIZAL EDIBLE FUNGI CULTIVATION AS A SUSTAINABLE USE IN MARGINAL AREAS

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Key words: field plantation, mycorrhizal plants, edible fungi, certification methodologies, spreading.

In the framework of Interreg IIIA ALCOTRA Italy - France program, a new project, named "VERCHAMP" (Development of a new cultivation: arboreal plantations for excellent edible fungi production), has been approved in Autumn 2004. HAD - Hautes Alpes Développement, as coordinator, has presented this project together with the Chambre d' Agriculture des Hautes Alpes and the Regione Piemonte – Settore Gestione Attività Strumentali per l'Economia Montana e le Foreste, as Italian partner.

The main aim of this triennial project is to spread the cultivation of edible mycorrhizal valuable fungi, like truffles, *Lactarius* and *Suillus*, particularly interesting to recover marginal lands, with low fertility.

HAD charged with a public bid the Robin nursery to realise a strain collection of different clones of these fungi, to supply the mycorrhizal plants and to set up at least 12 experimental orchards for each country, in selected areas chosen by specialist technical teams; the action is supported by training of land owners and by spreading to all the stakeholders.

Actually 24 experimental plots have been already set up in France and 15 in Italy; they well be surveyed for the next fifteen years to improve the knowledge on the host-fungi cultivation and to get practical informations for spreading the interest for such new cultivation to all the stake holders Regione Piemonte has equipped its research Institute, IPLA s.p.a. (Forestry and Environment Institute) with a Laboratory able to carry out experimentations for mycorhization techniques improvement and to control mycorhization quality, according to microscopic and bio-molecular techniques; a proposal to conform check and certification methodologies of mycorrhizal plants in Europe will be drawn up too.

In natural environments in France and in Italy two hundred plant – fungus couples from different symbiotic species and on different ecologic sites will be identified, aiming to have vegetable materials fitting for different field peculiarities.

Finally, the project provides the realisation of truffle suitability pilot maps, classifying Piemonte pedotypes according to their different aptitude to truffle production of the considered species.

RHIZOSPHERE MICRO-ORGANISMS IN NATURAL TRUFFLE-GROUNDS OF TUBER MAGNATUM WITH DIFFERENT PRODUCTIVITY – PRELIMINARY SURVEY

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Fey words: fangal ecology, white truffle, rhyzosphere.

For a large variety of micro-organisms, soil is a natural habitat suitable to live in. The presence of a microbiotic *facies* derives from different microscopic site conditions. The micro-organisms influence can vary from place to place, even if the sites characteristics appear to be the same. In spite of difficulties, the study of different *facies* can help to understand the reason of this variability. Bibliographical surveys confirm the existence of specific relationships between soil micro-organisms and plant roots. For this reason 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 and can be influenced by micro-environmental factors among which the rhizosphera.

The aim of this work has been the quantitative and qualitative evaluation of rhizospheric micro-organisms and the study of their influence on truffle production.

During November, a month which is very favourable to *T. magnatum* fructification, microbiological isolations have been carried out from soil samples taken from oak and poplar natural truffle-ground in Viarigi (Alessandria). In each natural truffle-ground, two different sampling areas have been located: the first site is productive and the second site is unproductive. The microbiological isolation has been carried out using the "subsequent dilutions technique".

The results seem to be particularly interesting even if they are preliminary. A faster mycelium growth and a higher fungal mass have been observed in the productive soil, with poplar and oak both. As for bacterial presence, no substantial differences have been found among the analyzed samples. The results of qualitative analysis on fungi have been very interesting: it has been possible to identify some more ubiquitous fungal forms, not related to *T. magnatum* production, as well as other forms depending to productivity and to different species of the host plant. In the natural truffle-ground with poplar, *Cephalosporium* sp. and one species of *Aspergillus*, with orange conidia, seen to be related with *T. magnatum* production. Under the productive truffle-ground with oak, a certain amount of fungal forms, belonging to *Penicilium, Trichoderma, Rhizopus, Acremonium* and *Aspergillus* genera, has been isolated.

These first results seem to point out a correlation between amount of fungal colonies and site productive capacity. This evidence must be confirmed by statistical analysis on a greater number of samples.

FUNGAL DIVERSITY AND BIOPROSPECTING IN SICILY (SOUTHERN ITALY)

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Key words: mushroom hunting, bioprospecting, Sicily.

Sicily is considered a hot-spot region for plant and fungal diversity. A high number of fungi, i.e. approximately 2000, arise from investigations on Sicilian territory carried out in the last ten years. Information on ecology and distribution of taxa recorded are also available for some provinces of the region. Besides bioprospecting actions are in progress for specific taxonomic groups, i.e. *Pleurotus* growing on Umbelliferous (*Apiaceae*) plants and *Tuber* species. Ethnomycological data and scientific papers on the nutritional value and organoleptic characters of edible mushrooms of economic importance are very scarce. But a number of edible mushrooms growing in forest ecosystems, meadows and pastures of Sicily are well known for their good organoleptic characters by fungi gatherers. Besides the economic importance of biodiversity is one of main aspect for the safeguard and valorization of natural genetic resources. Sixty taxa could be considered as a potential source of food in Sicily but only two of them, *Agaricus bisporus* and *Pleurotus ostreatus*, are cultivated at industrial level. New cultivation techniques were experimented with *Pleurotus* growing on Umbelliferous plants but there is a need to improve the methodology with the main object of reducing the influence of weather conditions and pathogens and also to improve marketing actions. For the reasons stated above the inclusion of *Pleurotus* species in indutrial processes is still in progress. The recent findings of *Tuber* species (*Tuber aestivum*, *T. borchii*, *T. puberulum*, *T. mesentericum*, *T. brumale*) gave new opportunities to local populations for the exploitation of natural truffle-beds and the establishment of experimental fields for truffles cultivation. Other mushrooms growing in the wild, such as agaricus, amanitas, boletes, chanterelles, funnel cup fungi, inky fungi, lactarius, russulas, lepiotas, etc., are widely used as food by local populations. The mushrooms are usually collected in the wild for personal use or buy in greengrocers or costermonger along the country roads during the autumnal season. This type of use slipped from any control and rules on sanitary measures with evident risks of poisoning for the population. A huge number of wild edible mushrooms (33 taxa) are used in the preparation of courses in restaurants but, again, the picking is entrusted to local fungi gatherers without any control by the health-officers. Considering that a number of wild mushrooms are potentially utilizable as high-quality food or could be inserted in production cycle, the studies on the nutritional value, economic importance, traditional uses and recipes are in progress. The main objective of such studies is to suggest to local Administration the inclusion of some edible mushrooms of high or good organloeptic qualities within the basket of the typical food productions of Sicily.

GROWTH OF THREE DIFFERENT *LACTARIUS* SPECIES UNDER DIFFERENT *IN VITRO* CONDITIONS.

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Key words: ectomycorrhiza, edible fungi, *Lactarius*, growth *in vitro*, cultivation technology.

Lactarius deliciosus (Fr) S. F. Gray is probably the most appreciated edible mycorrhizal fungus in Spain. According to Hall (2001), more than 1000 tons of saffron milk cap are collected world-wide, representing considerable income for many families. In several regions of Spain there is a great interest in making plantation of pine mycorrhizal with *Lactarius* species. The first step for this procedure is to optimize the inoculum production. We have studied the growth parameters of fungi *in vitro*: pH, temperature and culture media.

In the present work, the effect of 4 different growing media (BAF, MMN, 5X and WB) adjusted to 5 different pH values (5, 5.5, 6, 6.5, 7) and at two growth temperatures (18 and 23 °C) was studied on fungal biomass formation of nine strains of *Lactarius* (seven *L. deliciosus*, one *L. sanguifluus* (Paulet) Fries and one *L. semisanguifluus* Heim and Leclair)

Results showed that, in general, *L. deliciosus* presented a greater growth than the other two species (*L. sanguifluus* and *L. semisanguifluus*). The growth culture medium, WB produced the highest biomass formation for almost all fungi of the study, followed by 5X and BAF. The effect of the temperature was especially important and 23 °C encouraged the greater fungal development for all the species. Finally the effect of the pH was not relevant and little difference was found among the species.

Further research is needed to study carbon assimilation and storage in fungi, as well as the effect on development of feeding with different carbon sources.

EFFECT OF DIFFERENT CARBON SOURCES ON GROWTH OF THREE DIFFERENT *LACTARIUS* SPECIES.

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Key words: ectomycorrhiza, edible fungi, *Lactarius*, carbohydrates, sugars cultivation technology.

Carbohydrates are needed for ectomycorrhizal development. In nature fungi find this carbon source by establishing symbioses with the root of trees, forming mycorrhizas. Previous data have focused on ectomycorrhizal development, using glucose, but no other sugars have been studied in depth in this respect.

In the present work the effect on the biomass formation of 3 different *Lactarius* species (*L. deliciosus* (Linnaeus ex Fries) S. F. Gray, *L. sanguifluus* (Paulet) Fries and *L. semisanguifluus* Heim and Leclair) was compared after feeding with different sugars (fungus-specific mannitol and trehalose, plant-specific saccharose and non-specific sugars glucose, arabinose, and fructose). The culture medium was WB. The effect of pH was studied at three different levels (5, 5.5, and 6). Mycelium was dryed in an oven (70 ° C, 48 hours) and weigh.

In general, biomass formation was higher in media containing glucose for all fungi and pH levels. Arabinose produced the worst growth at all pH levels. The fungus *L. semisanguifluus* showed lower values of biomass formation compared with the other fungi. The lower pH value (5) produced better results in biomass production for almost all treatments.

Further research is needed to decode the relation between feeding sugar and storage sugar in fungi, in order to optimise growth conditions of mycorrhizal fungi in pure culture.

TUBER SPECIES FOUND IN NEW ZEALAND

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Key words: truffle, *Tuber maculatum*, taxonomy, ectomycorrhizal

The first black truffle (*Tuber melanosporum*) was harvested in 1993 from a black truffle plantation in Gisborne, New Zealand. Since then more than 40 specimens of *Tuber* species, either collected or received, from all over New Zealand as well as a further 16 specimens from Herbarium of the Plant Diseases Division, Landcare Research, Auckland, New Zealand, have been analysed. Morphological and molecular examinations of these 60 specimens confirmed that *T. maculatum* and *T. rufum* were present amongst the collection. Another three species were unresolved. The *Tuber* spp. were growing in association with the tree genera *Quercus, Salix, Populus, Corylus, Tilia, Pinus* and *Pseudotsuga.*

All of these truffle species have been introduced from the Northern Hemisphere. No native *Tuber* species have been found in New Zealand. The most common species present in New Zealand is *Tuber maculatum,* which has been collected and sold to local Italian restaurants. *T. maculatum* has been found in a few black truffle plantations, arousing concern among New Zealand black truffle growers that it may have displaced *T. melanosporum* in their truffières. The results of taxonomic and ecological studies of these species are discussed in this paper.

EDIBLE ECTOMYCORRHIZAL MUSHROOMS: MANAGEMENT AND HARVESTING

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Key words: edible, ectomycorrhizal, mushrooms, harvest, management

There are more than 2500 species of edible mushrooms with the most valuable and highly sought after belonging to the mycorrhizal group, including *Tuber melanosporum* Vitt., *Tuber magnatum* Pico & Vitt., *Tricholoma matsutake* (Ito & Imai) Sing., *Tuber uncinatum* Chatin (*T. aevistivum* Vitt.), *Boletus edulis* Bull: Fr. sensu lato, *Cantharellus cibarius* Fr., *Amanita caesarea* (Scop.: Fr.) Pers: Schw., *Lyophyllum shimeji* (Kawam.) Hongo and *Lactarius deliciosus* (L.) Fr.

In many countries it is traditional to collect edible mushrooms from forests for recreation, personal consumption and trading. Commercial harvest of edible ectomycorrhizal mushrooms, such as truffles in Europe and matsutake in Asia, has been an industry for centuries. These activities have made a substantial financial contribution to local economies and employed millions of people. In some regions and communities commercial harvesting is the major source of income. The new concept of non-timber forest products has recently emerged and gained popularity with forest owners and managers. The economic and ecological value of ectomycorrhizal edible mushrooms makes them an attractive non-timber forest product with significant potential.

Ectomycorrhizal edible mushrooms are mainly obtained from natural forests. Unfortunately, the production of many important mycorrhizal mushrooms has declined dramatically in the last century. Cultivation, managing the natural forests, and discovering new resources are major ways to increase their production. However, so far *Tuber melanosporum* and very few other species of truffle have been commercially cultivated. The cultivation of species such as *Lactarius deliciosus*, *Cantharellus cibarius* and *Lyophyllum shimeji* looks promising while that of the most expensive mycorrhizal mushrooms, including *Tuber magnatum* and *Tricholoma matsutake,* has proved challenging. Managing forests within which ectomycorrhizal edible mushrooms flourish to improve their production is a challenge for researchers and practitioners. The health of natural forests, particularly in developing countries, has declined making protection of ectomycorrhizal edible mushrooms difficult. Recently discovered resources of ectomycorrhizal edible mushrooms in China and other developing countries is encouraging. However, international research effort is required to maintain the habitats of ectomycorrhizal edible mushrooms in many developing counties.

This presentation discusses the importance, ecology, and management of edible ectomycorrhizal mushrooms.

THE PRODUCTION OF TRUFFLE INFECTED PLANTS USING MYCELIAL ISOLATES

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Key words: cultivation technology, truffles, mycelial inoculation

The first steps in truffle cultivation are to produce *Tuber* infected plants in greenhouses, use these to establish plantations (truffières) in suitable areas and then maintain the *Tuber* mycorrhizae to maximise productivity. Unfortunately, nurserymen not infrequently fail to closely follow the spore inoculation techniques perfected during the 1970s and 1980s for producing infected plants. As a consequence commercially produced plants are often only poorly infected with the inoculated species of *Tuber*, contaminated with less valuable species of truffle or ectomycorrhizal fungi that may not even produce fruiting bodies. While similar inoculation techniques are also used in the forestry industry infected plants are also produced using mycelial inocula. This has the advantage that the nurseryman is not reliant on fruiting bodies and by employing pure cultures the risk of contamination is minimised. This method involves isolating the mycelium, the preparation of stable cultures, and then using bulked up inocula grown on a suitable medium to inoculate clean seedlings.

We have recently prepared pure cultures of *T. borchii, T. melanosporum, T. macrosporum, T. maculatum, T. rufum, T. brumale* and *T. aestivum* which were then characterized using both morphological and molecular tools. In greenhouse inoculation trials using pure cultures of various isolates of *T. rufum, T. borchii, T. aestivum* and *T. melanosporum* we were able to obtain mycorrhizas of *T. rufum* and *T. aestivum* on *Ostrya carpinifolia*, mycorrhizas of *T. melanosporum* on *Corylus avellana* and *Quercus pubescens* and of *Tuber borchii* on *Quercus robur* and *C. avellana* five months after inoculation in a sterile calcareous soil. The isolates exhibited differing abilities to infect. The most effective strains were *T. melanosporum* Tme4 and *T. borchii* 10RA that were able to infect 70 % and 90 % of root tips respectively. The identity of the mycorrhizas was confirmed from the morphological characteristics of the mycorrhizas and by sequencing the ITS region and comparing the sequence with those in the GenBank database.

AN *IN VITRO* FLOWERING HOST PLANT FOR MYCORRHIZAL DESERT TRUFFLE PLANT PRODUCTION

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Key words: desert truffle, *Terfezia*, *Helianthemum violaceum*, micropropagation

Terfezia claveryi establishes mycorrhizal symbiosis with the genus *Helianthemum*. The species *H. almeriense* has been already micropropagated and *in vitro* inoculated with desert truffles mycelia with a great success for the mycorrhizal plant production by our Group. Since truffle formation is related to the plant flowering, the perennial species *H. violaceum* was selected as a new host plant for its high spontaneous *in vitro* flowering. Moreover, this species colonizes different territories than *H. almeriense* does and both could be used in desert truffle plantation programs in Spain.

The *in vitro* micropropagation protocol has been carried out for *H. violaceum* from shoot tips and nodal segments of *in vitro* germinated seeds. Kinetin was more effective than BA (benzylaminopurine) at the multiplication phase. The best results were obtained with low kinetin concentrations (0.05-0.2 mg/l) with a multiplication rate of 2.5 shoots per initial explant. Due to its good elongation and spontaneous rooting during the multiplication stage, the *in vitro* protocol was quite short.

The rooted explants were *in vitro* inoculated with *T. claveryi* mycelium. After two months, an average of 47% of mycorrhization was obtained. Phenolic compounds were observed into the root cells which limited the mycorrhizal colonization probably due to the long time in *in vitro* conditions. Significant differences on biomass were observed between mycorrhizal and non-mycorrhizal plants.

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