

# **The Third Symposium on Hypogeous Fungi of the Mediterranean Basin HYPOGES3**

**Under the theme “Truffles and Desert Truffles in the  
Face of Climate Change”**

**The Faculty of Sciences, 8-12 April, 2025**

## **BOOK OF ABSTRACTS**



*The 3<sup>rd</sup> Symposium on Hypogeous Fungi of the Mediterranean Basin  
Truffles and Desert Truffles in the Face of Climate Change -HYPOGES 3, 2025*

## 3<sup>rd</sup> Symposium on Hypogeous Fungi of The mediterranean Basin



### Foreword

#### Dear participants,

Welcome to the third Symposium on Hypogeous Fungi of the Mediterranean Basin (HYPOGES3).

Held every ten years, HYPOGES is a unique platform for researchers, scientists, and enthusiasts to explore the fascinating world of hypogeous fungi, including truffles and desert truffles (*Terfezia* and *Tirmania*). This symposium celebrates the ecological, culinary, and socio-economic significance of these fungi, fostering collaboration and knowledge exchange among experts from diverse fields.

Since its inception in 2004, HYPOGES has grown to become a major event in the study, culture and management of hypogeous fungi. The previous editions highlighted groundbreaking research on their biology, taxonomy, ecology and conservation while emphasizing their role in rural development and environmental preservation. Post-conference excursions showcased the rich fungal biodiversity of Morocco, underscoring its importance as a host country for this prestigious event.

HYPOGES3 continues this tradition under the theme “Truffles and Desert Truffles in the Face of Climate Change”. Over the next few days, participants will engage in discussions on sustainable development, mycorrhizal associations, culinary applications, ecotourism potential, and the fight against desertification. This symposium also aims to address challenges such as declining production, over-exploitation, and gaps in knowledge regarding cultivation and conservation.

We are thrilled to welcome you to Rabat, a city where history meets innovation. Together, let us advance scientific progress, strengthen international collaboration, and celebrate the remarkable diversity of hypogeous fungi. May HYPOGES3 inspire new ideas and partnerships that will shape the future of this vital field.

Enjoy the symposium!!

**The HYPOGES3 organizing committee**

## **3<sup>rd</sup> Symposium on Hypogeous Fungi of The mediterranean Basin**



### **ORGANIZING COMMITTEE**

#### **Presidents**



**Pr. Lahsen KHABAR**  
**UM5, FSR**  
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# **HYPOGES 3 Program**

**Tuesday April 8<sup>th</sup>, 2025**

**17 :00 – 20 :00 Registration.**

**Wednesday April 9<sup>th</sup>, 2025**

**08: 30 – 10: 00 Opening ceremony**

- **Pr. Mohamed RHACHI**, President of Mohammed V University in Rabat.
- **Pr. Mohammed REGRAGUI**, Dean of the Faculty of Sciences.
- **Pr. Lahsen KHABAR/ Pr. Sarah BOUZROUD**, HYPOGES 3 chairman.

**•Coffee Break•**

**•Group Picture•**

**—Session 1 — Truffle production and Climate resilience**

**Chairs: José Antonio BONET & Jalila BENJELLOUN**

- |                          |   |
|--------------------------|---|
| <b>10 : 45 – 11 : 15</b> | <b>La trufficulture dans le monde après 50 ans d'utilisation intensive du plant mycorrhizé.</b><br>Gérard CHEVALIER, INRA, France.  |
| <b>11 : 15 – 11 : 45</b> | <b>Truffle polycrisis in the Carpathian Basin</b><br>Istvan BAGI, Talajtérkép Kft., Hungary.  |
| <b>11 : 45 – 12 : 00</b> | <b>Oral presentations :</b> <ul style="list-style-type: none"><li>• <b>Croatian case study: development of methods for mycorrhization of forest seedlings, promotion of green economy and increasing the stability of forest ecosystems.</b><br/>Ivana ZEGNAL, Croatian Forest Research Institute, Croatia.</li></ul> |
| <b>12: 00 – 12 : 00</b>  | <b>Discussion</b>   |

**•Lunch Break•**



## 3<sup>rd</sup> Symposium on Hypogeous Fungi of The mediterranean Basin



### —Session 2 — Truffle cultivation and Agroforestry practices in the Mediterranean regions

#### Chairs: Zoltan BRATEK & Laila SBABOU

- 14 : 00 – 14 : 30**     **Current situation and perspectives of the truffle sector in the Mediterranean area.**  
Daniel OLIACH, Forest Science and Technology Center of Catalonia, Spain.
- 14 : 30– 15 : 00**     **Oral presentations :**
- **Truffle sector in Croatia, from wild to plantations.**  
Anton BRENGO, Croatian Forest Research Institute, Croatia.
  - **Effect of mycorrhization on growth, and phenols accumulation in *Argania spinosa* seedlings.**  
Awatef SLAMA, University of Carthage, Tunisia.
- 15: 00 – 15 : 30**     **Soil conservation and improvement through agroecological management of the cultivation of desert truffles (TURMASOIL project)**  
Asuncion MORTE, University of Murcia, Spain.
- 15 : 30 – 16 : 00**     **Discussion**

#### •Coffee Break•

#### — Poster session (16 : 00 – 16 : 30) —

### —Session 2 — Truffle cultivation and Agroforestry practices in the Mediterranean region

#### Chairs: Istvan BAGI & Kaoutar TAHA

- 16 : 30 – 17 : 00**     **The Truffle and Truffle-farming in Morocco: Current status of knowledge and perspective.**  
Lahsen KHABAR, Mohammed V University, Morocco.
- 17 : 00– 17 : 30**     **Oral presentations :**
- **Production de plants de deux espèces arborescentes mycorrhizées par des truffes du desert: perspectives d'application dans le reboisement des regions semi-arides et sahariennes en Algérie.**  
Zohra FORTAS, University of Oran, Algeria.
  - **Stimulating the effect of climate change on mycorrhization of millet (*Pennisetum glaucum* L.) cultivated in contrasting soils (beneath and outside shrub) in Senegal.**  
Hassna FOUNOUNE, Senegalese Institute of Agricultural Research, Senegal.
- 17: 30 –18 : 00**     **Discussion**

## 3<sup>rd</sup> Symposium on Hypogeous Fungi of The mediterranean Basin



Thursday April 10th, 2025

- Excursion to Maamoura Forest -

Friday April 11<sup>th</sup>, 2025

### —Session 3 —Truffle Ecology, Biodiversity and Conservation

#### Chairs: Francisco ARENAS & Loubna ELFELS

- 08 : 30 – 09 : 00      Interaction of the white truffle *Tuber magnatum* with bacteria and plants.  
Alessandra ZAMBONELLI, University of Bologna, Italy.
- 09 : 00 – 10 : 00      Oral presentations :
- Diversity of Diptera feeding on truffle species.  
Marco LEONARDI, University of L'Aquila, Italy.
  - Genome diversity of *Tuber borchii* in Sardinia.  
Martina DE MATTHEIS, University of L'Aquila, Italy.
  - Study of mycology of Morocco: Truffles and desert truffles.  
Marwa OIKRIM, Faculty of Sciences of Rabat, Morocco.
  - Moroccan *Tuber* spp. Identification and distribution.  
Chaimae MEYAD, Faculty of Sciences of Rabat, Morocco.
- 10 : 00 – 10 : 30      Discussion

•Coffee Break•

— Poster session (10 : 30 – 11 : 00) —

### —Session 1 —Truffle Ecology, Biodiversity and Conservation

#### Chairs: Daniel OLIACH & Chouhra TALBI

- 11 : 00– 11 : 30      Deciphering biodiversity and soil-plant feedbacks in the truffle environment for fitting best management practices to optimize the production of truffle systems.  
José Antonio BONET, University of Lleida, Agrotecnio-CERCA center, Spain.
- 11 : 30– 12 : 00      Oral presentations :
- Influence of habitat (plantation vs wild sites) structuring soil fungal communities associated in truffle sites (*Tuber melanosporum* and *Tuber aestivum* sites).  
Yasmin PINUELA, ID-Forest, Agrotecnio-CERCA center, Spain.

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- Lavender intercropped truffle cultivation in Türkiye.  
Ismail SEN, Forest Science and Technology Centre of Catalonia,  
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12: 00 – 13 : 30

Discussion

•Lunch Break•

—Session 4 — Sustainability and Environmental Aspects

Chairs: Marco LEONARDI & Houda TAIMOURYA

15 : 00 – 15 : 30

Hungarian and European Protection of Truffles and Traditions.  
Zoltan DR BRATEK, Hungarian Truffling Federation, Hungary.

15 : 30 – 16 : 00

Gastro-tourism using Moroccan Truffles as an economic driver  
for rural development.

Helena WILLIAMS, Mohammed VI Polytechnic University,  
Morocco.

16 : 00 – 16 : 30

Oral presentations :

- Exploring truffle tourism: an overview of experiences across  
Europe.  
Marta ROVIRA, Forest Science and Technology Centre of  
Catalonia, Lleida, Spain.

—Coffee Break —

17 : 00 – 17 : 30

La première truffière de *Tuber melanosporum* au Maroc: histoire  
de succès et défis

Dr. Abdelaziz LAQBAQBI, Morocco.

17: 30 – 18 : 00

Discussion

Attendance certificates' distribution

—GALA DINER (Optional) – 20 : 00 – 23 : 00 —

**Saturday April 12<sup>th</sup> , 2025**

—Middle Atlas Trip (Optional) -



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# ORAL COMMUNICATIONS



### Truffle Polycrisis in the Carpathian Basin

BAGI István

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Commercial truffle harvesting in the Carpathian Basin was revived in the late 1990s. Since the mid-2000s, we have been monitoring the yield and the harvestable species at a national level. In the first 15-20 years, the production fluctuated depending on the weather. The annual yield of harvested truffles was mostly dependent on the amount of rainfall, with no order of magnitude difference between the best and worst years.

However, in the last 6-8 years, we have observed a drastic decrease in the national and regional yields of harvested truffles. Based on available data for forest areas and regions, the quantity of all commercially harvested Carpathian Basin underground fungi species (*Tuber aestivum*, *Tuber brumale*, *Tuber magnatum*, *Tuber macrosporum* *Mattirolomyces terfezioides* *Choiromyces maeandriiformis*) has decreased significant to varying degrees. The annual quantity of Hungary's most abundant truffle species (*Tuber aestivum*) has decreased by approximately 70 percent in the past decade. In the most significant producing region (Jászság), the decrease reaches 80-90 percent. The emblematic underground mushroom of the Carpathian Basin, the Hungarian honey truffle (*Mattirolomyces terfezioides*), practically fails to produce during the increasingly frequent dry summers. This decline is caused by several factors that reinforce each other. The main causes are linked to climate change, such as changes in precipitation, rising temperatures, and an increase in sunshine hours. These changes also affect groundwater levels and the health of forests. In addition, human activities, like truffle harvesting and forest management practices, are likely contributing to the decline. We attribute a significant negative impact on truffle production to introduced oak pests, most notably the oak lace bug (*Corythucha arcuata*), which reduces assimilation.

**Keywords :** Climate change, forest health, Hungary, *Mattirolomyces terfezioides* production data, *Tuber aestivum*, *Tuber magnatum*.



## Hungarian and European Protection of Truffles and Traditions

BRATEK Zoltán<sup>1</sup>, BRANT Sára<sup>1</sup>, HELLER Ádám<sup>2</sup>, BALAZS DOMONKOS Péter<sup>2</sup>,  
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Legal protection of intellectual property accumulated in the collection of Hungarian traditional and regional foods as the TTR (=HÍR in HU) and collection and promoting their economic utilisation. Hungarian truffles awarded by Traditions-Tastes-Regions (TTR) trade mark system are the following species:

- Jászsági nyári szarvasgomba 2016 (Summer Truffle of Jászság, *Tuber aestivum*) Registered PGI by the EU since 2021
- Homoki édes szarvasgomba 2021 (Hungarian Sweet or Honey Truffle, *Mattirolomyces terfezioides*)
- Fehér szarvasgomba, White truffle 2024 (*Choiromyces meandriformis*)

Main objectives of the Geographical indication Program based on:

- Increasing the number of protected GIs
- Making better use of the potential of protected GIs

The first truffle of whole Europe awarded by PGI (protected geographical indication): Jászsági nyári szarvasgomba (Summer Truffle of Jászság, *Tuber aestivum*) Registered PGI by the EU since 2021. Case of Jászsági nyári szarvasgomba PGI Summer truffle of Jászság: Chernozem soil, oaks, plant associations typically rich in humus in Jászság: „Jászság” Characteristic of freshly mowed grass (coumarins). Optimum maturation period: July to mid-August: “Summer” truffle

Local knowledge: Fame “Gold of Jászság”/Diamond of Jászság

The trademarks and/or geographical indications are offered to the producer groups:

- They provide quality guarantee to the consumers
- Legal protection of the protected register name
- Increase consumers’ confidence.

The production of GI products match to the criteria of sustainability, based on local sources than local materials, local labour force and traditional knowledge. The trademarks and/or geographical indications have distinctive ability, increase the added value, marketing value of these products and can be sold on higher price. (According to the EU statistics, at least more than twice than price of standard products).

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**Keywords :** Geographical indication (GI), protected geographical indications (PGI), trademarks, Traditions-Tastes-Region (TTR) trademark, truffles.

## **Truffle sector in Croatia: from wild production to plantations**

**BRENKO Anton <sup>1\*</sup>, OLIACH Daniel<sup>2</sup>, DASHEVSKAYA Svetlana <sup>3,4</sup>, ALDAY Josu G<sup>3,4</sup>,  
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The truffle trade in Croatia is mainly dependent of wild production from forests and of import from neighboring countries. According to Regulation on collection of native wild species (Official Gazette 114/2017), the species that can be collected for commercial and private purposes are *Tuber magnatum* Picco, *Tuber borchii* Vittad., *Tuber melanosporum* Vittad., *Tuber aestivum* (Wulfen) Spreng., *Tuber aestivum* var. *uncinatum* (Chatin) I.R. Hall, P.K. Buchanan, Wang{?} & Cole, *Tuber brumale* Vittad., *Tuber mesentericum* Vittad. and *Tuber macrosporum* Vittad., which proves the fungal diversity of the forests ecosystem and the potential for development of new agroforestry practices in form of establishing truffle orchards. Also, the existence of *T. magnatum*, *T. melanosporum* and *T. aestivum* has a significant positive impact on local cuisine, gastronomy and tourism.

Although in nature exists a great variety of truffles that can be collected for commercial purposes, the orchard production in Croatia does not exist or it exists as small scale trials of individuals. The orchard production and seedling inoculation in Spain, France and Italy exists for several decades. In order to give the seedling inoculation in Croatia a 'head start', a new research program aiming to investigate the ectomycorrhizosphere conditions of *T. aestivum* natural production sites in pilot area of Istrian peninsula, Croatia has recently started. The specific objectives of the program are (i) to identify the appropriate ecosystem conditions and soil factors (chemical and physical) that influence the presence of *T. aestivum*, (ii) to identify the mycorrhiza helper bacteria (MHB) in natural areas, (iii) to investigate the appropriate fertilizer for best seedling and mycorrhiza development and to (iv) implement those research results into the newly established production of inoculated seedlings of indigenous tree species in Croatia. Such seedlings can later be used, except for orchard establishment and new agroforestry practice development, for reforestation, prevention of soil erosion and degradation and for recuperation of areas burnt in forest fires.

**Keywords :** Inoculation, mycorrhiza helper bacteria, orchard establishment, soil protection, reforestation.

### La trufficulture dans le monde après 50 ans d'utilisation intensive du plant mycorhizé

CHEVALIER Gérard

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Clermont-Ferrand, France.

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L'utilisation intensive du plant mycorhizé par la truffe à partir de 1973 a constitué le plus grand progrès en trufficulture. Malheureusement les méthodes de trufficulture n'ont pas permis de profiter de cet acquis essentiel. Elles n'ont pas évolué et la truffe est toujours un produit rare donc cher. Les trufficulteurs se sont contentés de reproduire les techniques de leurs prédécesseurs sans comprendre ce qu'ils faisaient. La recherche n'a pas progressé non plus. Au lieu d'aller sur le terrain, les chercheurs ont tout misé sur de nouvelles techniques, comme la biologie moléculaire. Les résultats obtenus au laboratoire ont été décevants et même ont conduit à des inepties, par exemple l'incapacité des plants mycorhizés possédant un seul sexe à produire des truffes. L'avenir de la trufficulture suppose un changement radical des méthodes de trufficulture actuelle et l'adoption de méthodes modernes, comme la perma-trufficulture et la trufficulture syntropique.

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**Mot-clés:** Biologie moléculaire, perma-trufficulture, plant mycorhizé, trufficulture, trufficulture syntropique.

### Genome diversity of *Tuber borchii* in Sardinia

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*Tuber borchii* plays a key ecological role in Mediterranean ecosystems as ectomycorrhizal fungus. It is distributed from Iberic Peninsula to Iran and from Mediterranean islands to Finland. Among Mediterranean islands, Sardinia is recognized as a biodiversity hotspot and offers a unique opportunity to explore the genetic diversity of *T. borchii*. It can be found from south to north and from coastal areas to the central highlands of the island. In this study, we analyzed the genome diversity of 24 *T. borchii* specimens collected across Sardinia. Preliminary barcoding analyses showed that all ascomata belonged to the haplotype 1 after Bonuso et al. (2010), the most widespread lineage in Italy. haplotype 2 of *T. borchii* was not detected among collected ascomata although it was previously found in some localities of Sardinia. The whole genome sequencing was performed using the Illumina MiSeq platform with an average coverage of 30×, generating approximately 4 Gb per sample. The reference genome used for reads mapping was the *T. borchii* strain Tbo3840 available at MycoCosm portal of JGI (Murat et al., 2018), which has a genome size of 98 million base pairs. Phylogenetic relationships were inferred through clustering analysis. This research contributes to expanding the knowledge of *T. borchii* genomic diversity in the mediterranean areas, with new insights on the ecology of this truffle species and implications for its conservation, valorization and cultivation.

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**Keywords :** Fungal biodiversity, Illumina sequencing, mediterranean mycobiota, truffle ecology.

### The Truffles and truffle farming in Morocco : Current Status of knowledge and perspective

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The climate, soil, and vegetation conditions have an impact on the distribution and abundance of the different truffle species. The cultivation of the promising species requires the establishing of these features. Here, we discuss the ecological traits of Moroccan truffles and desert truffles as well as their relationships with host plants. We also analyze the climate and soil characteristics to better understand the geographic distribution and fructification of truffles and desert truffles in Morocco. Desert truffles are found in semi-arid and arid regions of Morocco, as opposed to truffles, which are found in sub-humid settings. *Helianthemum* species are frequently associated with the spread of desert truffles in Mamora forest and eastern regions of Morocco, although *Quercus ilex* and *Q. faginea* are necessary for the survival of truffles (*Tuber* spp.) in the Middle Atlas. The fructification of truffles and desert truffles is primarily dependent on the frequency of precipitation. The two main desert truffles of Mamora forests, *Terfezia arenaria* and *Tuber oligospermum*, need an average of 435 mm of rain annually and a slightly acidic soil. While *Terfezia boudieri*, *T. claveryi*, and *Tirmania* spp., which are found in the oriental and Highland deserts, require a high CaCO<sub>3</sub> content and an average annual precipitation of 123 to 267 mm. As an alternative, there is *Tuber aestivum*, which is only found in humid areas with rainfall rates of more than 650 mm. It grows in calcareous soil that is rich in organic matter and is surrounded by possible host plants like oaks, cedars, and pines. Our findings suggest that by comprehending the biological requirements of desert truffles in Morocco, it may be possible to successfully cultivate truffles and create a market for them.

**Keywords :** Climate, desert truffles, geographic distribution, Morocco, soil properties, truffles.



## Simulating the effect of climate change on mycorrhization of millet (*Pennisetum glaucum* L.) cultivated in contrasting soils (beneath and outside shrub) in Senegal.

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Projected climate change in the Sahel, characterized by increasing temperatures and erratic rainfall, threatens soil fertility and crop production. Promoting indigenous shrubs like *Guiera senegalensis* into agroforestry systems may enhance soil health and plant resilience through mycorrhizal interactions. This study combines field observations and controlled climate chamber experiments to assess the effects of water and heat stress on millet (*Pennisetum glaucum* L.) mycorrhization, soil microbial activity, in agroforestry soils. Field experiments conducted in Sanghaïe (Niakhar, Senegal) revealed that mycorrhization intensity of millet was higher outside the shrub canopy, suggesting spatial variability in root colonization and microbial interactions. To further explore these dynamics under climate stress, a climate chamber experiment simulated water and heat stress (37°C, 25% Water Holding Capacity (WHC) vs. control: 32°C, 75% WHC) on soil microbial diversity, enzymatic activities (dehydrogenase,  $\beta$ -glucosidase, and urease), microbial biomass (measured as soil organic carbon and total nitrogen), and plant mycorrhization rate. Water and heat stress significantly impacted soil microbial community and millet growth. Enzymatic activities, microbial biomass and mycorrhization intensity declined under stress conditions. Despite initial positive effects of AMF inoculation, prolonged stress reduced its efficiency in improving millet biomass. These findings highlight the complex interactions between mycorrhizal fungi soil microbial communities, and climate stressors in shaping plant responses to environmental challenges. This study emphasizes the importance of integrating *G. senegalensis* into climate-resilient agroecosystems and considering root mycorrhization, enzyme activities, and microbial biomass when evaluating the effects of water and heat stress on soil microbial communities and millet growth.

**Keywords :** Agroforestry, arbuscular mycorrhizal fungi, millet, water and heat stress.

### Production de plants de deux espèces arborescentes mycorhizées par des truffes du désert : perspectives d'application dans le reboisement des régions semi-arides et sahariennes en Algérie

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Cette étude a pour principal objectif d'évaluer et de caractériser d'une part le potentiel mycorhizien de deux espèces de truffes du désert *Terfezia leptoderma* Tulasne. sur le développement du chêne vert (*Quercus ilex* L.) et *Tirmania nivea* Maire Malençon sur celui de l'arganier (*Argania spinosa* L. Skeels) cultivés en serre et d'autre part produire des plants mycorhizés vigoureux afin de les introduire dans les programmes de plantation du chêne vert dans des zones semi-arides et de l'arganier dans les régions littorales et sahariennes initiés par notre gouvernement, Le but de ces programmes est de rechercher des moyens de lutte contre la régression surtout la densité de l'arganeraie et celle du chêne vert.

Des synthèses mycorhiziennes ont été réalisées dans des conditions de serre sur un substrat naturel en inoculant, *in vivo*, les plantules de chêne vert avec *Terfezia leptoderma* et celles de l'arganier *T. nivea*. Les résultats révèlent que la symbiose mycorhizienne a un effet bénéfique sur la croissance et les paramètres physiologiques des deux plantes. La symbiose mycorhizienne a conduit à la formation des ectomycorhizes chez le chêne vert et d'endomycorhizes chez l'arganier.

L'aptitude des truffes du désert à s'associer avec ces espèces ligneuses et la production en pépinière de plants mycorhizés plus vigoureux et résistants ouvrent une voie prometteuse pour renforcer l'échec des reboisements de *Quercus ilex* et de l'arganier dans des zones semi-arides littorales, restaurer l'arganeraie dans les régions sahariennes sud-ouest et également la préserver ces espèces de truffes du désert.

**Keywords :** *Argania spinosa* L. Skeels reboisement, ectomycorhizes, endomycorhizes, plants mycorhizés, *Quercus ilex* L., régions semi-arides et sahariennes, *Terfezia*, *Tirmania*, truffes du désert.

### La première truffière de *Tuber melanosporum* au Maroc : histoire de succès et défis

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La trufficulture représente une pratique agricole prometteuse, non seulement pour sa valeur économique et gastronomique, mais également pour son impact environnemental positif, par sa contribution au développement durable des zones rurales et la préservation de la biodiversité et des essences forestières. Bien que cette pratique agricole soit implémentée en Europe, en particulier en France et en Italie, au Maroc, la trufficulture reste encore en développement, offrant néanmoins un potentiel économique et écologique significatif grâce à ses conditions géographiques favorables. La première expérience de trufficulture a débuté en 2006 avec la production réussie de truffes noires de Périgord, *Tuber melanosporum*, à Debdou. Cette initiative, menée par Dr. Abdelaziz Laqbaqi, a marqué le Maroc comme l'un des rares pays producteurs de cette espèce, aux côtés de la France, l'Italie, l'Espagne, la Nouvelle-Zélande, l'Australie et les États-Unis. Les plantations mises en place à Debdou et à Imouzzar Kandar, ont montré une adaptation exceptionnelle du champignon au terroir local, avec des productions dès la troisième année, démontrant ainsi le potentiel significatif de la trufficulture pour une production durable des truffes tout en préservant les forêts de chêne vert, essentielles pour le maintien de la biodiversité et la lutte contre la désertification. Cependant, plusieurs défis subsistent, en particulier la vulnérabilité des truffières aux fluctuations climatiques, notamment aux sécheresses récurrentes, qui affectent la productivité et la régularité des récoltes. À ce défi s'ajoute la surexploitation des essences forestières de chêne vert et l'absence de cadres législatifs clairs pour la collecte et la commercialisation des truffes ce qui peut limiter sa rentabilité à long terme.

**Mot-clés :** Biodiversité, développement durable, Maroc, Trufficulture, *Tuber melanosporum*.

## Diversity of Diptera feeding on truffle species

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True truffles, the hypogeous fruiting bodies of fungi belonging to the genus *Tuber*, are well known for their ecological and economic significance. While extensive research has focused on their symbiotic relationships with plants and interactions with mammals, the role of insects—particularly Diptera—in truffle ecology remains underexplored. This study investigates the diversity of Diptera species associated with truffles, focusing on their feeding behavior and potential ecological roles. Field surveys were carried out in several truffle-producing regions of Italy, where mature truffles of different species were collected. Diptera larvae were extracted from the truffles, and the adults that successfully developed in the laboratory were identified using both morphological and molecular techniques. A total of 20 Diptera species from 8 families were recorded, with 15 species newly associated with truffles. The most abundant families included *Heleomyzidae*, *Mycetophilidae*, and *Phoridae*, suggesting a specialized adaptation to feeding on fungal tissues. Feeding surveys and gut content analyses revealed that Diptera larvae primarily consume truffle glebae, while the role of adults in spore dispersal remains speculative and requires further investigation. The presence of truffle-specific volatiles, such as dimethylsulphide and 2,4- dithiapentane, seems to play a key role in attracting Diptera to truffles. Furthermore, the temporal and spatial distribution of Diptera species varied significantly among truffle species and habitats, highlighting the complexity of these interactions. This study provides a comprehensive assessment of the diversity of Diptera associated with truffles and sheds light on their ecological roles as consumers of truffle tissues. These findings contribute to a better understanding of truffle ecology, conservation, and cultivation, as Diptera may influence truffle biology in ways that are not yet fully understood. Future research should explore the potential for Diptera to act as bioindicators of truffle health and examine the impact of environmental factors on these interactions.

**Keywords :** Biodiversity, Diptera, fungal-insect interactions, *Tuber*, true truffles.

### Moroccan *Tuber* spp. identification and distribution

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Morocco displays a strategic location at the northwest corner of Africa, yielding a unique blend of bioclimatic conditions, ranging from humid to arid climates. This diversity supports a wide range of ecosystems, influencing the distribution and abundance of truffle species through climate, soil and vegetation. Ecological and edaphic studies have highlights Morocco's potential for truffle diversity, particularly in the Middle Atlas and Maamora Forest. These regions offer contrasting yet favorable conditions for various *Tuber* species. The Middle Atlas is the most conducive area for black truffle development due to its humid to subhumid bioclimatic stage, moderate temperatures, and adequate rainfall. The region's calcareous soils, which are basic and rich in organic matter, provide ideal conditions for truffles to thrive. Dominated by *Quercus* species (e.g. *Quercus ilex*), these forests foster ectomycorrhizal relationships essential for truffle growth. The most predominant *Tuber* species found in this area are *Tuber melanosporum*, *Tuber rufum*, *Tuber uncinatum*, and *Tuber excavatum*. In contrast, the Maamora Forest in northwestern Morocco supports different *Tuber* species due to its temperate climate and acidic sandy soils and low organic matter content. Despite these differences, it remains a significant habitat for species such as : *Tuber oligospermum*, *Tuber asa*, and *Tuber gennadii*.

**Keywords :** Distribution, climate, Maamora forest, Middle Atlas, Morocco, *Tuber* spp..



## **Soil conservation and improvement through agroecological management of the cultivation of desert truffles (TURMASOIL project)**

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*Terfezia claveryi* Chatin, a hypogeous edible fungus, is a valuable natural resource in the Mediterranean basin regions. It establishes mycorrhizal symbiosis with Cistaceae shrubs and is the only desert truffle species cultivated in Spain. Desert truffle farming, also known as Turmiculture, is highly adapted to drought conditions in semi-arid regions, requires minimal management, does not depend on pesticides and thrives without the use of fertilisers. We hypothesize that the agroecological practices associated with *T. claveryi* farming improve the quality of the soil and attenuates its degradation, so it is an ideal candidate for ecological farming systems, promoting sustainable agriculture and environmental conservation. The TURMASOIL project aims to study the evolution of physico-chemical and biological parameters in different types of soils in the Region of Murcia (Spain), dedicated to *T. claveryi* cultivation, under different climatic conditions, to verify whether this crop improves the fertility of the soil and reduces its degradation. The results revealed clear improvements in physical, chemical and biological soil characteristics in the plots cultivated with *T. claveryi*. Physically, cultivated soils exhibited higher porosity and structural stability compared to controls, with notable variability among soil types and plantation ages. Chemically, organic carbon and total nitrogen levels were higher in cultivated soils, although phosphorus levels remained slightly elevated in controls. Biologically, soil enzyme activity (phosphatase,  $\beta$ -glucosidase and dehydrogenase) and soil microbial respiration were significantly higher in cultivated soils than in uncultivated control soils. Microbial diversity analysis revealed significant bacterial enrichment in cultivated soils, while fungal communities displayed distinct compositional differences between cultivation and control areas. These results confirm that the cultivation of *T. claveryi* improves soil quality and fertility, supporting the development of this crop in the sustainable management of degraded soils in semi-arid regions.

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**Keywords :** Desert truffle cultivation, metagenomics, pedology, *Terfezia claveryi*.

### Study of the mycology of Morocco: truffles and desert truffles

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Truffles are the edible hypogaeal Ascomycota (Pezizaceae) fruiting bodies produced by the genera *Terfezia*, *Delastreopsis*, *Balsamia*, *Delastria*, *Leucangium*, *Mattirolomyces*, *Phaeangium Picoa*, *Tirmania*, and *Tuber*. These are fungi that spend most of their life cycle in symbiotic association with the roots of certain higher plants, such as Pines, Quercus, Olea... The classification of truffle species has traditionally been based on both the morphological characteristics of ascocarps and ascorporal ornamentation. *Terfezia* is different from most *Tuber* in that each ascus contains eight ascospores, whereas most tubers have between one and eight ascospores. Desert truffles are mycorrhizal fungi native to the Mediterranean region's dry and semi-arid environments. The Maâmora woodland in Morocco's northwestern region, the plains of the highlands in the east, and the Sahara in the south and southeast are very favorable environments for desert truffles. On the other hand, truffle species (genus *Tuber*) were harvested in the Middle Atlas between 1600 and 2000 meters above sea level, in a sub-humid or humid climate, and on limestone soils. These edible species are the subject of a very active and important local seasonal trade. Most are spring bearing, while some (*Delastria rosea*, *Terfezia leptoderma* and *Tuber oligospermum*) appear from November or December. These diverse climatic conditions and host plant associations contribute to the development and expansion of the diversity of terfess species in Morocco. Morocco now possesses a dozen "Terfess" species (genera *Delastria*, *Picoa*, *Terfezia*, and *Tirmania*) and six truffle species (genus *Tuber*). The purpose of this study is to present the different *Terfezia* and *Tuber* species collected in Morocco as well as their geographical distribution and to evaluate the results of a comparative study of plant mycorrhizae by desert truffles.

**Key words:** Geographical distribution, Maâmora, Morocco, mycorrhizal fungi, Terfess, Tuber.

### Current situation and perspectives of the truffle sector in the Mediterranean area

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Truffles are a highly valued non-wood forest product. Over a hundred and sixty species of the *Tuber* genus have been reported globally, but only a minority have a commercial interest. Still, the majority of the truffles are collected from the wild, but an arising economy based on truffle cultivation has emerged in the last two decades in different part of the globes, where Mediterranean climate occurs. Among the cropped species, *Tuber melanosporum* Vittad., *Tuber aestivum* Vittad. and *Tuber borchii* Vittad. are successfully cultivated, while only recently the cultivation of *Tuber magnatum* Picco. has taken place in the field. However, the progress of truffle cultivation has been uneven across the Mediterranean region. This communication, examines the current landscape of the truffle sector, highlighting challenges and outlining priority actions essential for its development in key producing countries such as Spain, France, Italy, and Croatia. Key areas of focus include enhancing the legal framework and bolstering consumer awareness to stimulate truffle consumption.

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**Keywords :** Non-wood forest products, supply-chain analysis, Truffle tourism, *Tuber*.

**Influence of habitat (plantation vs wild sites) structuring soil  
fungal communities associated in truffle sites (*Tuber  
melanosporum* and *Tuber aestivum* sites)**

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Truffles are edible hypogeous fungi highly appreciated in culinary due to its genuine aroma. Among all, the black truffle (*Tuber melanosporum*) and the summer truffle (*T. aestivum*) are the most traded ones. Its high commercial value has encouraged the establishment of plantations all over the world and, particularly, in the Mediterranean climate regions. However, there is a lack of information about the accompanying fungal species and community structure present at natural (wild) and artificial truffle habitats (plantations) when both truffle species cohabitate. The main objective of the present study was to assess the effects of climate region (Mediterranean versus temperate) and habitat type (wild versus plantation) on the soil fungal community structure of these truffle systems. We investigated overall and ectomycorrhizal soil fungal composition, fungal diversity, and fungal functional guilds to identify differences between habitats and regions in three sampling seasons: Winter 2018 (December 2018), Spring 2019 (April 2019) and Summer 2019 (July 2019), and we assessed the effect of several soil properties, (pH, organic matter, and C:N ratio) on the structure of the fungal communities present. We showed that soil fungal community composition and ectomycorrhizal species composition are filtered by habitat type rather than climate regions. Also, we observed the influence of soil pH, organic matter content and C:N ratio structuring total and ectomycorrhizal fungal assemblages. Greater soil fungal diversity was found in temperate compared to Mediterranean sites when considering all fungal guilds. Ectomycorrhizal diversity did not differ between climate regions but was significantly higher in wild sites compared to plantations. Our results reflect the possible potential dominance of management practices, soil properties, and ecosystem age in shaping soil fungal assemblages compared to other ecological factors, leading to encourage the proliferation of pioneer species, like *Tuber* spp.

**Keywords :** Fungal diversity, soil fungal community, truffle ecology, truffle plantations.

### Deciphering biodiversity and soil-plant feedbacks in the truffle environment for fitting best management practices to optimize the productions of truffle systems: The TUBERLINKS project

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Truffle culture is coming of age as we celebrate half a century of modern truffle cultivation. A lot of excellent work has been done along these five decades to reliably get truffle-inoculated plants and produce truffles all over the world. Yet, most of this research has focused on generating very pragmatic information for direct use of farmers, developing a very much-needed emergency mycotechnology that was able to provide growers with basic tools to manage plantations. Yet, at this point, this pragmatic research is unable to carry us any forward on its own.

With this in mind, we have devised the project TUBERLINKS that will focus on the ecological interactions of truffle forests and plantations, both above- and belowground, and with the host-tree physiology. TUBERLINKS will help us get our hands into the “black box” and gain the much-needed understanding of how the entire soil-truffle-plant system “works”.

The overarching hypothesis is that understanding the truffle life cycle and the ecology (biodiversity, functioning) of the whole soil-truffle host system, and of its interactions with the environment (climate, soil, water/nutrient availability), will enable the optimal management of black truffle agro-forestry systems.

TUBERLINKS proposes three General Objectives:

- To study the impact of truffle mycelium on the soil environment, its temporal dynamics, and the functional consequences.
- To evaluate the physiological performance of truffle-colonized trees and the impact of water and nutrient limitation on the host tree-soil-system.
- To refine the truffle cultural techniques based on the physiology and abiotic/biotic soil dynamics, considering the whole tree-truffle-soil system.



TUBERLINKS will generate new knowledge for a wide range of end-users on: (i) the truffle ecology and biodiversity interactions, (ii) the functioning of the soil truffle-environments, (iii) the host-tree ecophysiological cues, (iv) the susceptibility of truffle systems to management and environmental limitations, and (v) the sustainable truffle production management.

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**Keywords :** Ecology, physiology, *Tuber melanosporum*, water relations.

### Exploring Truffle Tourism: An Overview of Experiences Across Europe

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Truffle tourism in Europe has emerged as a captivating niche within the travel industry, offering unique experiences for tourists seeking to explore the world of truffle culinary delights and cultural discovery. Throughout Europe, regions such as Provence in France, Piedmont in Italy, and Teruel in Spain, have gained renown for their rich truffle heritage attracting visitors from around the globe. These destinations offer a wide range of activities designed to showcase the attractiveness of truffles, including guided truffle hunts in orchards and forests, interactive showcooking classes, and tastings with truffle delicacies.

A truffle orchard tour offers an immersive experience into the world of truffle cultivation where participants can learn about various aspects of truffle farming, observe truffle-hunting dogs in action, and have the chance to taste fresh truffles, allowing to appreciate its unique aroma. While truffle farm visits offer firsthand insight into the cultivation and harvest process, other tourism avenues exist for learning about truffles, such as truffle festivals, with lively markets, competitions, and culinary demonstrations.

Despite being an increasingly popular activity, truffle tourism lacks scientific studies characterizing it or analyzing factors influencing its development. Through 12 in-depth semi-structured interviews with European truffle tourism providers, particularly in Spain, our aim is to address these questions. Thus far, it is observable that truffle tourism primarily emerges as a complementary activity to truffle harvesting, rather than originating from the tourism sector. The main motivations are educational, aiming to showcase the cultivation process and gastronomic value of truffles, while also raising awareness about their authentic taste compared to artificial flavors. In addition, activities are carried out pairing truffles with other local products, such as wines, cheeses, etc., thus promoting awareness not only on truffles but also on the territory and its products.

**Keywords :** Ecotourism, truffle experiences, truffle tour offer.

### Lavender intercropped truffle cultivation in Türkiye

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Truffles have great culinary value due to their unique aromas, and the demand for truffles continues to increase in international markets. This has incentivized the truffle cultivation as a valuable agricultural alternative, particularly in regions with a Mediterranean climate. Türkiye is one of the Mediterranean countries where truffles grow naturally, with at least 13 *Tuber* species reported in the literature. Because the ecological conditions in Türkiye are suitable for truffle cultivation, it has gained popularity in recent years. Several entrepreneurs have started to establish orchards for *T. aestivum* Vittad., and a lesser extent, *T. melanosporum* Vittad. Particularly in *T. aestivum* orchards, the owners prefer intercropped lavender between the rows with the aim to cultivate multiple products. This intercropping model also allows for lavender honey production. However, this approach requires a deep understanding of the growing needs of both truffles and lavender to be successfully implemented. The best practices for *T. aestivum* orchard intercropped with lavender are examined in Seydikemer, located in the Muğla province of Türkiye. Additionally, the future perspectives for truffle cultivation in Türkiye is discussed.

**Keywords :** *Lavendula angustifolia*, non-wood forest products, *Tuber*.

### Effect of mycorrhization on growth, and phenols accumulation in *Argania spinosa* seedlings

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The objective of this study is to assess the mycorrhizal association between the edible desert truffle *Terfezia boudieri* and the argan tree (*Argania spinosa*). Mycorrhization was established by inoculating *A. spinosa* seeds with *T. boudieri* ascospores under greenhouse conditions. Plant length and leaf number of *A. spinosa* (25 days old) were evaluated every ten days for mycorrhizal (M) and non-mycorrhizal (NM) seedlings. Leaf phenol and flavonoid contents were measured at 115 days. Mycorrhization significantly promoted plant growth and secondary metabolite synthesis. Plant length increased, reaching 1.5 times more than NM seedlings. Flavonoid leaf content was  $1.899 \pm 0.06$  mg RE/ml for NM and  $2.478 \pm 0.05$  mg RE/ml for M seedlings, while total phenol content was  $2.648 \pm 0.05$  mg EAG/g for NM and  $3.814 \pm 0.06$  mg EAG/g for M seedlings. Root colonization by *T. boudieri* showed an endomycorrhizal type, with no mantle structure observed. This finding demonstrated that *A. spinosa* grows more vigorously when associated with *T. boudieri*. This mycorrhization process could promote ecological solutions and support local development in forest regions by enhancing truffle and argan fruit production.

**Keywords :** Argan tree, inoculation, mediterranean region, *Terfezia boudieri*.

## Gastro-tourism using Moroccan Truffles as an Economic Driver for Rural Development

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Gastro-tourism as part of a distinctive destination brand can **attract tourists across every economic category who spend more, travel more often, stay longer and create more word-of-mouth marketing**. Gastro-tourists are valuable destination influencers. The pursuit of truffles is an excellent motivator for gastro-tourists, both as an experience (truffle hunt) and for consumption at rural local businesses.

As a powerful economic driver, gastro-tourism is a destination development multiplier; especially attractive for rural development. Over 50% of all travelers admit to being a gastro-tourist, or “foodie”; over 54% of Millennials choose food experiences over music experiences; 65% of Gen Z’s ranked travel and seeing the world as the most important way to spend their money; over 94% plan the food components of their vacations or holidays before even leaving their home. On average, their food and beverage expenditures have been reported to be 24% or more above the average, and are statistically significantly above the median or mode (in Williams et al., 2022).

Gastro-tourism is defined as the intentional pursuit of appealing, authentic, memorable culinary experiences of all kinds, while travelling internationally, regionally or even locally...a Gastro-experience is an authentic, memorable, food- or beverage-related activity (Williams, 2014). Our studies reveal that gastro-tourists are eager to travel to a destination and stay overnight if there is a minimum of six memorable gastronomic experiences within a 2-hour travel radius. Our 6+ Gastro-cluster Process Model lays out a roadmap for gastro-tourism success, which can be used to establish co-branded 6+ Gastro-clusters/networks by grassroots stakeholders interested in sharing their local/regional food & beverage customs with international/domestic, and deliberate/incidental travelers. More importantly, linkages between 6+ micro-establishments expand the reach of each individual business.

The truffle industry in Morocco, including both experiential “truffle hunting” as well as the consumption of truffles, can be a unique and valued part of a 6+ gastro-tourism cluster.

**Keywords :** Destination branding, economic development, gastro-tourism, influencers, rural development, truffles.

## Interactions of the white truffle *Tuber magnatum* with bacteria and plants

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*Tuber magnatum* Picco (the Italian white truffle) is the most valuable and widely appreciated truffle. Despite recent successful cultivation attempts, it remains more challenging to grow than other edible *Tuber* species, with many aspects of its biology and ecology still unexplored. For example, *T. magnatum* mycelium is extremely difficult to cultivate under *in vitro* conditions. Recently, it has become possible to obtain some strains of *T. magnatum* in association with specific *Bradyrhizobia*. These bacteria appear to play a role in the nitrogen nutrition of *T. magnatum* mycelium, establishing a symbiotic relationship between them. Phylogenetic analyses using the 16S rRNA, *glnII*, *recA*, and *nifH* genes revealed that these bacterial strains form a monophyletic group divided into five subgroups. These strains belong to a previously undescribed lineage within the *Bradyrhizobium jicamiae* supergroup. Phylogenomic analyses conducted with over 400 single-copy orthologous genes conserved in bacteria (BUSCO) confirmed their placement within the *B. jicamiae* supergroup.

Despite *T. magnatum* mycorrhizas can be obtained under greenhouse conditions, their mycorrhizae are rarely observed in the field. This suggests that *T. magnatum* may employ alternative strategies, such as endophytism within the roots of non-ectomycorrhizal plants. Recent studies have demonstrated the endophytic abilities of *T. magnatum* in the herbaceous plant *Carex pendula* Huds, using molecular methods and fluorescence *in situ* hybridization (FISH) technique with, newly designed, highly specific *T. magnatum* probes. These newly developed FISH probes will facilitate further investigations of the endophytic tendencies of *T. magnatum* and will help understanding their influence on the life cycle and biology of this fungus.

**Keywords :** Bradyrhizobia, FISH, *Tuber magnatum*.



### **Croatian case study: Development of methods for mycorrhization of forest seedlings, promotion of green economy and increasing the stability of forest ecosystems**

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Although the production of inoculated seedlings with *Tuber* spp. is widely known in some European countries for several decades, as is their use in truffle orchard establishment, these practices are fairly unknown in Croatia. Croatian Forest Research Institute (CFRI) has enrolled a project financed by the Environmental Protection and Energy Efficiency Fund (EPEEF) of Republic of Croatia with the purpose of fostering the use of inoculated seedlings as a tool for achieving stability of forest ecosystems and green and rural economy promotion. The value of the project is reflected in the innovations that the project results provide: evaluating the use of inoculated seedlings in forestry as a departure from traditional management methods, encouraging the development of the rural economy through a new agricultural product and developing a laboratory and greenhouse inoculation protocol as a basis for educating interested stakeholders. The general objective of the project is to increase the carbon sink in natural ecosystems and encourage "green" economy, which will be achieved through two specific objectives : Development of a methodology for inoculation of commercial tree species as a basis for the development of new knowledge and skills, and Development of a new product and encouraging the development of the rural economy. During the project duration of 30 months, CFRI has successfully inoculated nine commercially important forestry species with *Tuber aestivum* and *Tuber melanosporum*. An overview of project activities and inoculation results will be given.

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**Keywords :** Agroforestry, black truffles, green economy, inoculation, mycorrhiza, rural development.

# POSTERS



### Diversity of Hypogeic and Semi-Hypogeic Fungi Associated with *Quercus Rubra* L. In Hungary: First Insight

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Northern red oak (*Quercus rubra* L.), a species native to North America, is one of the most important exotic commercial timber species planted in Hungary. Although it prefers loose and neutral to acidic soils, because the high tolerance of abiotic stress and resistance against powdery oak mildew (*Erysiphe alphitoides*) and oak lance bug (*Corythucha arcuata*), it was planted in wide range of environmental conditions and habitats (from parks, gardens to semi-natural forests). As pests and especially climate change reduces the survival of native *Quercus* species, the importance of northern red oak will increase, so additional plantations are likely to be established. The goal of the research is to examine the ECM community of northern red oak and compare to native ECM-forming trees. We collected soil samples from each sampling sites. The roots were gently cleaned of soil particles, then we snipped each root tips that shown signs of ECM colonization. DNA was extracted from the collected root tips and sequenced using new generation Illumina sequencing. Our results suggest that hypogeic and semi-hypogeic fungi are important, and in some cases dominant species of the ECM community of northern red oak in Hungary. The most common genera is *Scleroderma*. Although we get sequences belonging to an undescribed *Pachyplodes* species is new for science in one of the sampling sites, we were not able to find the sporocarps belonging to that species yet. Combined with our fungarium's data, we recorded 12 species of hypogeic fungi associated with northern red oak. We will continue the research in the coming years, so the species list will presumably be extended and new species description is expected.

**Keywords :** Climate change, ectomycorrhiza, fungal community, hypogeic fungi, *Quercus rubra*.

### Diversity of Ectomycorrhizal Fungi in the oldest *Castanea sativa* Mill. (European Chestnut) Orchard in Nagymaros, Hungary

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*Castanea sativa* is an important multipurpose tree species known for its various socio-economic and ecosystem services. The symbiotic association between plant roots and ectomycorrhizal fungi is a common natural phenomenon that plays a crucial role in tree health and forest ecosystem stability. However, little information is available on the diversity of ectomycorrhizal fungi in *C. sativa* orchards in Hungary. This study was conducted to identify the diversity of ectomycorrhizal fungi in the oldest sweet chestnut orchard of Hungary. Soil samples were collected from four plots within the orchard, and DNA sequencing was used for species identification. The data were analysed using SPSS version 20. A total of 169 ectomycorrhizal species belonging to 45 genera and 27 families were identified. The dominant families included Thelephoraceae with 32 species, followed by Inocybaceae with 25 species, Russulaceae and Sebacinaceae with 19 species each, and Cortinariaceae with 18 species. The most species-rich genera were *Tomentella* (28 species), *Inocybe* (21 species), *Cortinarius* and *Sebacina* (17 species each), and *Russula* (15 species). Hyphogaeic fungi comprises a minor but diverse part of the ECM community, from which *Elaphomyces holstii* is the most abundant species. The Shannon diversity index indicated that the ectomycorrhizal fungal community exhibits moderate to high diversity, suggesting a stable and resilient ectomycorrhiza fungi community with a well-distributed species composition. The evenness value indicates that ectomycorrhizal species are moderately evenly distributed, though some species are more dominant than others. Together with our previous study based on sporocarp collection, 22 species of hypogaeic fungi present in this orchard. In conclusion, the *C. sativa* orchard harbors a diverse ectomycorrhiza and hypogaeic fungal community. Further, physiological and molecular studies are needed to explore the functional roles of these fungi in tree growth and ecosystem dynamics.

**Keywords :** *Castanea sativa*, ectomycorrhiza, fungal community, symbiotic association, species diversity.

### Research in Hungarian sand truffle habitats

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The sand truffle (*Mattirolomyces terfezioides* (Mattir.) E. Fisch.), or Hungarian sweet truffle has a special culinary value, because unlike other truffles, it has a very sweet taste and can be used for sweets. Our group has carried out several different researches on the Hungarian sand truffle habitats in recent years. The only known large-scale occurrence in the world is in the Carpathian Basin in Central Europe, where it is mainly found in *Robinia pseudoacacia* forests planted in sandy soil areas along the Danube river. Soil surveys have been carried out on many habitats. These results show that the species prefers neutral, slightly basic, sandy soils with low calcium and high humus content. Field surveys have revealed mycelial mats in the known truffle growing sites. The examination under a stereo microscope revealed that they produced conidia. Sequencing of the samples showed us that the conidia most likely belong to the sandy truffle, this species' asexual form has not been described before. Truffle hunters have also reported the presence of a previously unknown hypogeous fungus in several sand truffle habitats in Hungary. During other field investigations, we found this species in other sand truffle habitats, and after morphological and molecular genetic analyses we identified it as a subterranean *Geastrum* species. Interestingly, in all cases, honey locust (*Gleditsia triachanthos*) trees were present around the mushroom's growing site. The species, based on genetic data, eventually identified as *Geastrum nadalii*, described from Spain in 2024. However, this new species of earthstar has only been described in the Mediterranean yet, this is the first occurrence in continental region. These examples demonstrate that the sand truffle habitats in Hungary continue to provide us with many new scientific results and they are worthy for further research.

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**Keywords :** Habitat preference, mitospore, new species, sand truffle.



### Desert truffle diversity and potential mycorrhizal connections in Moroccan ecosystems

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In Morocco, there are many different types of desert truffles, including *Terfezia*, *Tirmania*, *Delastria*, *Picoa*. The Maamora Forest, Doukkala-Abda Sahel, northeast of Morocco, and the Moroccan Sahara are the four truffle regions where the geographical distribution of these ascomycetes has been noted most frequently. In addition, to being a great source of protein, amino acids, carbon hydrates, and fiber, desert truffles are also regarded as a valuable source of antibiotic alternatives for pathogenic bacteria that are resistant to antibiotics. Due to the widespread appreciation of truffles, we could use these resources to develop local populations, especially in truffle-producing regions. A remarkable feature of truffles is the biodiversity of desert truffles in Morocco and other Maghreb countries. The discussion covers ecology, biology, geographical distribution and concluding remarks, including the mycorrhizal association of truffles, as well as biochemistry and physiology.

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**Keywords :** Biodiversity, biochemistry, desert truffles, mycorrhizal association, taxonomy.

### Micropropagation of *Tuber* host plant: *Quercus* spp.

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The *Quercus* genus plays a vital role in improving ecosystems in both urban and forest settings. However, the extensive exploitation of *Quercus* species without regulation leads to deforestation in many areas. Reforestation of the affected regions with *Quercus* plantations obtained through *in vitro* propagation is the optimal solution. *Quercus* micropropagation enables the cultivation of various plant parts in different medium compositions. Buds, meristems, and shoot tips can produce genetically stable clones, while meristems and embryos may be utilized to minimize contamination. Alternatively, the highest multiplication rate of *Quercus* is achieved through somatic embryogenesis. During *in vitro* multiplication, several issues can arise, including basal calluses, vitrification, tissue necrosis, and phenol accumulation. Additionally, incorporating supplements into the basal medium, such as polyvinylpyrrolidone, ascorbic acid, and casein hydrolysate, enhances the *Quercus in vitro* culture by controlling phenolics and tissue necrosis, respectively. Increasing agar and calcium concentrations while removing chloride from the medium helps reduce vitrification. The Woody Plant Medium is ideal for *Quercus in vitro* multiplication. Moreover, acclimatization is ensured by transplanting plantlets into sterile soil and maintaining a high relative humidity level along with regular irrigation to prevent plant dehydration.

**Keywords :** Acclimatization, *Quercus* propagation, Sterilization, Shoot multiplication, Rooting.

## Micropropagation of host plants for desert truffles: *Helianthemum guttatum* and *Helianthemum ledifolium* grown in Morocco's arid and semi-arid regions.

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*Helianthemum guttatum* Mill and *Helianthemum ledifolium* are two herbaceous plants belonging to the Cistaceae family. They exhibited a great drought tolerance potential due to their mycorrhizal association with desert truffles. Plant propagation through seeds encounters different difficulties due to the tegument imposed morphological dormancy. To overcome this, micropropagation can be used to produce many plants in reduced time. The current study focuses on the development of a productive in vitro plant regeneration method for *Helianthemum guttatum* Mill and *Helianthemum ledifolium* plants by establishing the optimal conditions for in vitro proliferation of *Helianthemum* axillary and apical buds using Murashige and Skoog (MS) basal medium supplemented with different concentrations of 6-benzylaminopurine (BAP) (0, 0.5, 1 and 2 mg. L<sup>-1</sup>). The greatest results of shoot proliferation were obtained with BAP-free MS medium and MS culture medium supplemented with 0.5 mg/l BAP the shoot regeneration percentage from the axillary buds and apex varied from 100% to 95%, respectively, after five weeks of the culture of *Helianthemum ledifolium* and *Helianthemum guttatum* Mill. Moreover, the rooting percentage in the medium containing MS supplement 0.5 mg. L<sup>-1</sup> BAP was observed to be lower than in the medium lacking BAP, which reaches 100%. As the concentration of BAP rises to 1 mg. L<sup>-1</sup> and 2 mg. L<sup>-1</sup>, the proportion of callus induction also increases. The outcome results demonstrated the efficiency of the developed protocol for the production of vigorous vitroplants, with a longevity rate of 90% *Helianthemum ledifolium* and 70% *Helianthemum guttatum* Mill after being transferred to greenhouse conditions.

**Key words :** *Helianthemum guttatum* Mill, *Helianthemum ledifolium*, BAP, shoot regeneration, rooting.

### ***Terfezia Boudieri* performance within mycorrhizal association on *Quercus* sp. species**

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The development of mycorrhizae can improve the resilience of forest ecosystems to climate change besides several mycorrhizal fungi are known to have a significant effect in improving plant performance and adaptation to stressful environmental conditions. Accordingly, this research aims to investigate how the mycorrhizal fungus *Terfezia boudieri* affects the growth (primary root length, aerial plant weight) and physiological behavior (net/intercellular photosynthesis [CO<sub>2</sub>] and photosynthesis/photosynthetically active radiation intensity) of *Quercus* sp. (*Q. coccifera* and *Q. suber*). Inoculated and non-inoculated seedlings of both *Quercus* species were grown in pots (1 L) in a greenhouse (with temperature between 25 and 30°C, natural lighting and irrigation applied twice a week with surface water). The data revealed that primary root length and above-ground biomass increased with mycorrhization. In addition, mycorrhization promoted net photosynthesis (at 400 ppm and saturation point), water use efficiency and photosynthetic pigment content. The effectiveness of *T. boudieri* inoculation on *Quercus* sp. performance revealed by this research explains the positive effects generated by the mycorrhization process on the plant partner and establishes the potential of mycorrhization to improve the resilience of forest management to environmental change.

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**Keywords :** Biomass, inoculation, mediterranean region, *Quercus* sp., *Terfezia boudieri*.

## Desert Truffles and Their Mycorrhizal Symbiosis

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Desert truffles, known as terfess, belong to the Terfeziaceae family and play a crucial role in the arid and semi-arid ecosystems of Morocco. Their development relies on a symbiotic relationship with the roots of specific host plants, mainly from the Cistaceae (*Helianthemum* genus). In Morocco, different truffle species associate with various host plants depending on the region. *Terfezia arenaria* colonizes *Helianthemum guttatum* in Maamora, while *Terfezia boudieri* forms a symbiosis with *Helianthemum lipii* var. *sessiliflorum* and *Helianthemum ledifolium* in Doukkala-Abda and the Oriental region. *Terfezia clavaryi* is associated with *Helianthemum lipii* and *Helianthemum apertum* in the Moroccan Sahara and the Oriental region, whereas *Terfezia leptoderma*, also associated with *Helianthemum guttatum*, is found in Maamora and the Oriental region. *Terfezia olbiensis* establishes a symbiosis with *Pinus halepensis*, while *Tirmania nivea* colonizes *Helianthemum hirtum* and *Helianthemum lipii*, notably in Doukkala-Abda and the Moroccan Sahara. Other species, such as *Delastria rosea*, are associated with *Pinus pinaster* var. *atlantica* in Maamora, while *Picoa juniperi* is associated with *Helianthemum lipii* in the Moroccan Sahara. The development of these truffles is strongly influenced by arid or semi-arid climates and environmental factors, including calcareous or sandy soils and water availability, which play a key role in the fructification process. Given the challenges posed by climate change and soil degradation, further research on controlled cultivation of desert truffles is essential. Studying mycorrhizal symbiosis could optimize production and improve understanding of the complex interactions between these fungi and their environment. Enhancing resource management could promote a sustainable desert truffle production sector in Morocco while preserving their ecological and economic role.

**Keywords :** Desert truffles, *Helianthemum*, mycorrhizal symbiosis.



## 3<sup>rd</sup> Symposium on Hypogeous Fungi of The mediterranean Basin

