University of Belgrade

Institute For Multidisciplinary Research

Book of abstracts

Truffle Research Union of Europe Conference

Belgrade 27-30th September 2023

Editor
Prof. Dr Žaklina Marjanović

Truffle Research Union of Europe Conference
-Book of abstracts-

Publisher
University of Belgrade
Institute for Multidisciplinary Research
Kneza Višeslava 1, Belgrade

Editor
Dr Žaklina Marjanović

Technical editor
Dr Slobodan Stefanović

Print
Byzart d.o.o., Belgrade

Print run
65

ISBN
978-86-80109-25-1

Belgrade, 2023

CIP - Каталогизација у публикацији Народна библиотека Србије, Београд
635.85(048)

TRUFFLE Research Union of Europe. Conference (2023 ; Beograd)
Book of abstracts / Truffle Research Union of Europe Conference, Belgrade, 27 -30th September 2023 ; editor Žaklina Marjanović. - Belgrade : University, Institute for Multidisciplinary Research, 2023 (Belgrade : Byzart). - 40 str. : ilustr. ; 21 cm
Tiraž 65. - Registar.

ISBN 978-86-80109-25-1

a) Тартуфи -- Апстракти

COBISS.SR-ID 125505801

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50 years of development of seedling production and truffle cultivation

Chevalier, G.[1]

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Using the truffle mycorrhizal seedling has been the most important event in the long story of the truffle cultivation. The first conception was made by the researchers of the INPL of Turin in the late 1960ties. The production on a large scale was carried out in the early 1970 ties by INRA of Clermont-Ferrand in collaboration with Society France-Walnut in Saint- Maixant (Gironde). The first mycorrhizal seedlings (9000) were marketed in autumn 1973. The first truffles (*T. melanosporum*) were harvested 4,5 years later under hazel tree in Burgundy. The mycorrhizal seedlings plantation has allowed, on one hand, to decrease the delay of production, and on the other to be able to produce truffles in the whole world. Unfortunately, plantation of the mycorrhizal seedlings has not given always the desired results, sometimes because of the quality of the plants but more often because of the mistakes of management of the plantations. The nurserymen have been unfairly accused. In fact, at the very beginning, truffle cultivation has been carried out like the one of potatoes. In particular, the work of the soil has been carried out badly and the truffle growers have used tools too heavy that were compacting the soil. In the middle of the 1990s researchers came to the idea of saving the truffle orchards by using molecular biology methods. However, this did not bring the desired results, because instead of working in the field, the work has been mainly done in the lab. They have lost contact with the reality and have put forward erroneous conclusions - for example that it is dangerous to prune the trees because it reduces the photosynthesis, or that some mycorrhizal seedlings are not productive because they do not have the "good gender"! Is not late to correct these mistakes. Since app. ten years ago promising methods have arisen: e.g., agroforest cultivation, permacultivation, syntonic cultivation, in order to replace the old traditional cultivation practices that are completely outdated. We have lost lots of time, but it is still possible to make up for this loss, even though it is still a long way to victory. To modify the mentality, on the one hand of the researchers, and on the other hand of the truffle growers of the whole world is an attempt to the impossible.

Biodiversity of the genus *Tuber* from Europe: an update

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Most of the *Tuber* species were described in the 19th century, a few in the 20th, then in the 21st we have once again witnessed a new proliferation of proposals for new species, the first of which described only on a morphological basis and then with the support of genetic analyses. This phenomenon has mainly affected the non-European countries, but also some previously neglected European areas such as the Iberian Peninsula and the Balkans. In a very reductive way, it can be estimated that there are around fifty species of *Tuber* definitely identified from Europe so far, fourteen of which were described after 2017 (*T. alcaracense*, *T. buendiae*, *T. conchae*, *T. cryptobrumale*, *T. davidlopezii*, *T. lucentum*, *T. lusitanicum*, *T. magentipunctatum*, *T. mohedanoi*, *T. pulchrosporum*, *T. pustulatum*, *T. suave*, *T. suavolens*, *T. zambonelliae*). To these must be added some species originally described in other continents and whose presence has been ascertained in Europe both with the collection of ascomas (*T. anniae* and *T. wenchuanense*), or the analyses of the ECMs (*T. huidongense*). Their presence was also hypothesized through the use of soil metagenomics and the re-evaluation of some species considered synonyms of others, such as *T. bituminatum*, and status changes as a result of molecular analyses such as the new combinations *T. melasporum*, *T. monosporum* or *T. oligospermum*.

A strongly underestimated number of species is also due to the confusion that exists on the real taxonomy of the species of the distant past. Very complex clades such as Excavatum, Puberulum and Rufum lack genetic types and the large amount of sequences deposited in public genetic banks are subject to interpretations that only rarely correspond to reality. The GenBank problem was reported 15 years ago on the pages of Science but nothing has changed: GenBank is not taxonomically reliable if the sequences of the types are not deposited. After the presentation of some cases of incorrect use of the sequences deposited in GenBank, the invitation is made to young researchers who deal with *Tuber* and to all taxonomists: first of all designate the type or types otherwise the chaos of the system is increased.

Black truffle harvesting in Spanish forests: history, agro-climatic zoning and climatic variability of production

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In Spain, the black truffle (*Tuber melanosporum* Vittad.) reaches its southernmost, warmest and driest natural locations. The identification of the climatic factors driving its distribution and fruiting is necessary to optimize cultivation practices. Firstly, we defined the first agro-climatic zoning of the natural distribution areas of black truffle in Spain. Three different agro-climatic zones were identified, with dry environments that presented mean rainfall lower than the optimum for the fungus being dominant. Then, we analyzed the records of black truffle harvesting in Spain to investigate the relative role of climate, host tree growth and other environmental and human factors on the variability and trend of ascocarp production. Climatic factors largely explained the variability in annual production, but the major temporal trend was apparently linked to human factors. Among climatic factors, rainfall and temperature during the ascocarp development showed the highest relationship with truffle production. This allowed us to explore the use of tree ring, water deficit indices and satellite remote sensing as indicators of the drought impact on black truffle production.

Supported by: INTACT RISE-MSCA Project "INnovation in Truffle cultivation, preservAtion, proCessing and wild truffle resources managemenT" Grant Agreement n.: 101007623

Quantitative Magnetic Resonance Imaging (QMRI): a promising tool to evaluate truffle quality

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Water represents the main freshness factor for truffles and mushrooms and affects all biotic and biochemical activities during maturation of fruiting bodies. Quantitative Magnetic Resonance Imaging (QMRI) is a non-destructive technology that can be applied for evaluating both water content and its behavior. This technique has been applied to a few mushroom species such as *Agaricus bisporus*, *Lentinula edodes*, and *Volvariella volvacea* but it is underutilized for truffles. It was first applied by Pacioni and colleagues to assess the internal structure and physical changes of *Tuber melanosporum* ascomata over 1-month post-harvest storage.

Recently, our research group demonstrated that QMRI (1T scanner) can be successfully applied to evaluate freshness and residual mass of *Tuber aestivum* ascomata without affecting their integrity. Specifically, we analyzed uninjured and injured ascomata stored at 4 °C with two different cooling systems for a 17-day period. QMRI analysis showed that mass and volume losses were significantly faster in injured ascomata stored in the fan forced fridge. Free water content decreased linearly (about 1.7% per day) as well as the longitudinal (T1) and transverse (T2) relaxation times (12–14 ms and 1.2–1.3 ms per day, respectively) and the Apparent Diffusion Coefficient (ADC) which were reduced by half during the monitoring time. The estimated residual (dry) mass was nearly constant over the period of investigation. The same protocol was then used to test the effectiveness of a hypogeal display case equipped with an ultrasound humidity emanation apparatus (https://www.afood.it/ENG/Hypogeal-display-case) in improving the shelf-life of *Tuber borchii* and *Tuber melanosporum* ascomata. Research is underway to evaluate the potential of QMRI in identifying internal alterations of the gleba caused by microbes.

Mass spectrometry imaging applied to *Tuber aestivum* fruiting bodies

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This study explores and evaluates how mass spectrometry imaging (MSI) with Desorption Electrospray Ionization (DESI) can be used as a tool in natural product research. Since DESI MSI was first introduced in 2004, the technique has numerous times successfully been applied, mapping chemical compounds located on plant surfaces, animal tissue, or bacterial and fungal cultures. DESI was applied on *Tuber aestivum* and through MSI we were, for the first time, able to locate the compounds within the fruiting bodies of *T. aestivum*, mapping the fungus´ chemistry. Previous chemical studies have mainly focused on the volatile compounds of the aroma. In this study, untargeted profiling with ultra-performance liquid chromatography (UPLC) and a DESI coupled to a quadrupole time-of-flight mass spectrometry (QToF) was used to analyze different compounds in fruiting bodies of *T. aestivum*. By analyzing extracts and locating compounds inside the fruiting body the results presented clear differences between the gleba and peridium of the fruiting body. The study led to the findings of several new compounds, including yet unidentified peptides. In conclusion, the present study demonstrates how DESI MSI can provide information about natural products in truffles to better understand their chemistry and its role in nature.

First evidence of symbiotic relations between *Bradyrhizobium* spp. and *Tuber magnatum* mycelium

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Tuber magnatum Picco is considered the most valuable truffle species and until now his mycelium has never been grown reliably *in vitro* conditions. *Tuber magnatum* ascoma hosts a specific bacterial community generally dominated by Alphaproteobacteria with *Bradyrhizobium* spp. the most abundant taxa. Several studies hypothesized that these bacteria may have a specific role in the formation, nutrition, and maturation of ascomata. However, no information is reported on their possible role in *T. magnatum* mycelium development.

The main goal of this work was to study the effects of *Bradyrhizobium* spp. on the isolation and development of *T. magnatum* mycelium. Three *T. magnatum* strains were co-isolated with bacteria on mWPM (Iotti et al., 2005) from the gleba of immature ascomata. Our tests showed that both the isolated bacteria community and *T. magnatum* mycelia were unable to grow alone on mWPM. Moreover, when co-cultivated with the mycelia of *T. borchii* and *T. melanosporum*, the isolated bacteria did not positively enhance their growth. Phylogenetic analyses inferred by 16S and *glnII* genes showed that the isolated bacteria belong to the *Bradyrhizobium* genus and that they are grouped in a clade close to *Bradyrhizobium elkanii*. Furthermore, *nifH* genes were detected in all the bacterial isolates. The results obtained in this study suggest a specific obligate symbiosis between these *Bradyrhizobia* and *T. magnatum* mycelium. Although further studies are necessary to better understand the mechanisms of interaction between *T. magnatum* and *Bradyrhizobium* spp., these results could have important biotechnological applications in truffle cultivation.

The pF Tracer One ™ an innovation for managing irrigation in truffle orchards from the national experimentation project *CulturTruf*

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Truffles are ectomycorrhizal fungi growing in symbiotic association with many trees and shrubs. *Tuber melanosporum* is one of the truffle species most appreciated by consumers. Nowadays, production of this species worldwide is based on plantations of young plants inoculated by professional nurserymen. It is therefore vital to identify recognised and reliable technical paths to ensure good production. In particular, it is now accepted that summer water shortages and the resulting drought are a major obstacle to good truffle production. But how can this constraint be overcome and watering managed in a sensible way? In the national experimentation project *CulturTruf*, funded by *FranceAgriMer* between 2016 and 2022, one of the objectives was to determine the optimum water regime in truffle orchards using tensiometers placed in the soil. The hypothesis of monitoring the soil's water potential (abbreviated as 'pF') was tested, in order to triggering and applying watering adapted to the truffle's needs. The results obtained showed that the irrigation threshold set at pF = 4 ensures good production of *T. melanosporum* in drought conditions. The final conclusion is that it is essential to use a probe/sensor system to monitor and adapt the water regime. However, until now, there has been no pF reading tool on the market in the correct reading range sought (pF 2 to pF 4.5) and at an affordable cost for truffle growers. The aim of our project was to develop a reader and recorder adapted to low-cost sensors. To do this, we decided to work with gypsum sensors, enabling the pF to be read between 2.5 and 4.5. After calibration in the laboratory and field tests, the resulting technological innovation obtained rise to a declaration of invention, and a licence to exploit the secret know-how, co-signed by INRAE and the newly-created start-up WETRUF. WETRUF developed and named the first marketable version of this innovation, the **pF Tracer One ™**. Marketed since May 2020, it has sold over 300 units of reader and 1,800 sensors to date, both in France and internationally. Today, WETRUF continues to innovate through its Research & Development activity with the objective to develop a range of tools for managing watering in truffle fields, with different functionalities.

This subject is a good example of the rapid transfer of an innovation resulting from an experimental project (CulturTruf) to the socio-economic sector, through the licensing of know-how and the creation of a start-up (WETRUF).

Tuber thracicum a new european species of melanosporum clade

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Truffles in the genus *Tuber* are hypogeus fungi which live in symbiosis with the roots of many trees and shrubs. Within the genus *Tuber*, the Melanosporum clade includes some commercial species of great economic interest that are cultivated worldwide. At least four species of the clade have been described in Europe: *Tuber melanosporum*, *T. brumale*, *T. crytpobrumale* and *T. petrophilum*. Recent phylogenetic studies have shown the existence of cryptic species in this clade. While analyzing ascomata of *T. brumale* from Bulgaria, we found distinctive macro and micro-morphological features in some specimens. The ascomata of these samples were subglobose or depressed to slightly irregular, sometimes excavated at the base, brownish reddish brown to almost black, with greyish tones. The peridium was formed by two very thin layers, the outer irregular with slightly pronounced polygonal warts. The gleba was compact, marbled, at maturity with violet-brown tones and cream-colored sterile veins. Ascospores were spiny up to 32 μm long, ellipsoid or rarely spheroidal, dark brown, with black-dark brownish spines. Phylogenetic analysis conducted on the internal transcribed spacer (ITS) and the large subunit (LSU) rDNA sequences showed that these specimens form a monophyletic, well-supported taxon within the Melanosporum clade, closely related to *T. brumale* complex and *T. pethrophilum*. Morphological and molecular analyses supported the proposal of the new species *T. thracicum*.

Could *Tuber magnatum* and *Tuber melanosporum* co-occur in the same plantation?

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Tuber melanosporum is harvested mainly in plantation realized with inoculated seedlings. On the contrary, *Tuber magnatum* is harvested in woody environment. Only recently, in 2019, *T. magnatum* ascocarps were harvested in a French orchard realized with *Quercus pubescens* inoculated in nursery using INRAE/ROBIN know-how (Bach et al., 2021, https://doi.org/10.1007/s00572-020-01013-2). The plantation was realized in 2015 in an agricultural context. First truffles were harvested in 2019 as well as the successive years. In 2021, *T. melanosporum* ascocarps were also harvested in this *T. magnatum* orchard. Indeed, it has been implanted at only 12 m from an orchard realized with *T. melanosporum* inoculated seedlings. Some trees currently produced both truffle species. The aim of this study was to investigate the competition between both species addressing following questions: Which is the mycelium contamination evolution of *T. magnatum* and *T. melanosporum*? Is there a competition between both species mycelium in the soil? Could *T. magnatum* also colonize the *T. melanosporum* orchard? Using droplet digital PCR we quantified the mycelium of both species in soils of the plantation harvested in 2018, 2020 and 2023. We found that both species colonization increased since 2018. A negative correlation was found between the mycelium quantity, indicating a competition between both species. Interestingly, *T. magnatum* also colonized the *T. melanosporum* orchard. This site is an unique opportunity to investigate competition between both species as well as to investigate how we could favor one or another species using different management techniques (e.g. watering, shadowing, spore supply…).

Supported by: INTACT RISE-MSCA Project "INnovation in Truffle cultivation, preservAtion, proCessing and wild truffle resources managemenT" Grant Agreement n.: 101007623

First production of Italian white truffle (*Tuber magnatum* Pico) in an orchard in France

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The first attempt to cultivate *Tuber magnatum* started in the 1970s whithout sucess. This failure was due to the use of wrong mycorrhizal plants. The wrong quality of these plants was due to wrong identification and characterization of the mycorrhizae of *Tuber magnatum*. After twenty years of research, implementations and improvements, Robin nursery in collaboration with french national agronomic institute (INRAE) have succeed in production of well mycorrhized plants with *Tuber magnatum* (Process INRAE/ROBIN). Plants were commercialized since 2010 in the frame of know-how license with INRAE. Robin staff realizes first control on each plants produced in order to find and identify *Tuber magnatum* mycorrhizas wich are marked by a coloured pink. After INRAE realizes two types of control: 1) non destructive morphological checking of each seedlings to identify the magnatum mycorrhizas 2) the analysis by DNA of one or more mycorrhizas for each seedling confirming that each plant harboured *Tuber magnatum*. In 2018 INRAE researchers have sampled five orchards set up in different regions of France by customers of the Robin nursery, with plants mycorrhized by *Tuber magnatum* and confirmed by soil DNA analysis that *Tuber magnatum* survived in the soil 3 to 8 years after plantation. *Tuber magnatum* is not naturally known close to these plantations. In 2019, in one of these orchards set up in the NA region by the Robin nursery employee and under the recommandation of the management of this orchard by the Robin nursery, the first *Tuber magnatum* ascocarps were harvested 4,5 years after plantation under 4 differents trees. Until 2019 the production of *Tuber magnatum* ascocarps has continued and each year new trees began to produce, while the first ones continued production. In 2023 12 trees of the 52 planted have produced *Tuber magnatum* ascocarps. Since 2020, each year new customers who have set up orchards using our plants mycorrhized with *Tuber magnatum* reported us the production of *Tuber magnatum* ascocarps in their orchards in the NA region and in other regions of France. These results demonstrate the feasability of *Tuber magnatum* cultivation world wide by planting mycorrhizal plants.

Time-targeted production of black truffle (*Tuber melanosporum*) using different types of truffle nests and irrigation doses

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Climate change has been described as the main threat for the cultivation and growth of *T. melanosporum* in Mediterranean conditions, mainly due to decreasing annual rainfall and rising temperatures affecting the truffle's life cycle. Therefore, black truffle cultivation techniques need to be adapted to different conditions minimizing the effects of climate change. In order to predict the management of truffle cultivation under these conditions, we established a study orchard in an area whose climate is closer to what we expect in 50 years in the current optimal truffle growing areas of NE. We tested the productivity of *T. melanosporum* by implementing three different water irrigation regimes (irrigation when the soil water potential reaches -250, -500 and -750 kPa) to two different types of truffle nests (1: not mixing the peat with the original soil; 2: mixing the peat with the original soil). We evaluated the production and quality of black truffles. As an outcome solely derived from the first year of study the impact of irrigation and truffle nests was already evident. The 90% of total truffles harvested were found inside the truffle nests and 89% located in the north side (shady side) of the tree. A significant positive effect of irrigation on truffle production was observed, as well as the differential influence of the type of nest on harvesting time during the season. The findings in the following years will contribute to understanding the impact of water availability and truffle nest design on truffle cultivation, thereby supporting the sustainable development of truffle farming in Mediterranean regions.

Supported by: INTACT RISE-MSCA Project "INnovation in Truffle cultivation, preservAtion, proCessing and wild truffle resources managemenT" Grant Agreement n.: 101007623

Complexity of water requirements for truffle production in Burgundy

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Truffle production is dependent on water and may largely decrease as a consequence of climate change in the future. However, excess of water is not good either. Innovative methods are therefore required for water optimization in truffle plantations, through irrigation or careful plantation design.

Two plantations have been followed for several years in Burgundy (North Côte d’Or). The first one in Leuglay has been planted in 2013 in an area which is temporarily flooded every year. Nonetheless, the first truffles were collected in 2021. Temporary flooding has therefore no negative impact on truffle production.

The second plantation in Terrefondrée has been created in 2006 and contains trees inoculated with *Tuber melanosporum* or *Tuber aestivum* var. *uncinatum*. The first truffles were harvested after ten years and producing trees have increased regularly along the years. Irrigation experiments have shown a positive impact on truffle production. In this plantation, it has also been observed that the spatial distribution of producing trees is not regular. An analysis of soil electric conductivity has been carried out during winter, when the soil was saturated in water. This showed that 85% of truffle producing trees were present in areas with low or medium low conductivity. This means that high moisture is not good for truffle production. Soil profiles are being studied in these areas in order to better identify the soil properties linked to truffle production. Such data may enable a new design in truffle plantations for choosing most appropriate tree and truffle species and planting areas.

Use of selected bacterial inocula to improve the quality of mycorrhizal seedlings in greenhouses

Ranocchi, B.[1], Giorgi, V.[2], Landi, L.[2], Castelli, I.[2], Romanazzi, G.[2], Peroni, C.[3], Neri, D.[2], Zambonelli, A.[4], Amicucci, A.[1]

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Truffle cultivation started in the 19th century, meanwhile the production of wild truffles began to decrease due to increased anthropization and climate change. Starting from the 1960s, improvements in agricultural techniques were studied and applied (Callot 1999). Nowadays, over 50% of the truffles harvested worldwide (Hall et al. 2003) are produced in orchards. Research is hence focused in improving truffle cultivation by understanding the interaction between plant and fungus and evaluating agricultural techniques able to maximize production. In this scenario, the quality of the seedlings and the quality of their inoculation is a strong discriminant for the success of planting for truffle production. Several studies investigated growth conditions promoting *T. melanosporum* proliferation in the field (Bonet 2006, Garcia-Montero 2014, Buntgen 2015); others have focused on the standardization of the procedures for the evaluation of seedling quality in defense of customers (Alvarado 2013, Murat 2015), others focused on management practices in the greenhouse stage (Pruett 2009, Bruhn 2013).

In the present study, we report the experimentation carried out on *Quercus ilex* seedlings mycorrhized with *T. melanosporum* and inoculated with selected bacterial species, in order to evaluate the effects of bacteria to stimulate *Tuber melanosporum* root colonization and growth of young seedlings of *Quercus ilex* L..

T. melanosporum colonization was assessed using morphological and molecular methods. Shoot growth and root parameters were analyzed. Our investigation showed in the early stages of ectomycorrhizal symbiosis on roots, a higher ECM colonization in the proximal (closer to the collar) section of roots apparatus than in the distal one. Moreover, a major effect was observed on roots co-inoculated with *Pseudomonas* spp., increasing the truffle inoculation rates of root tips by more than 5% compared with seedlings that received non-bacterial inoculation treatments.

The results obtained so far are of great interest, as they lay the foundations for developing new bio-sustainable strategies, effective to improve the health and mycorrhization of truffle-producing seedlings. Further studies will concern experiments on other species of *Tuber* and different bacterial strains. It will also be our goal to deepen the studies on the biochemical and molecular interactions underlying this complex plant-fungus-bacteria relationship, using in vitro models.

Why analyzing and certifying truffle plants?

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Truffle cultivation has become an agricultural activity of great interest for productive results and farm income. For certain truffle species, it represents a way of conserving and protecting natural truffle grounds while at the same time supplying the truffle market. In any case, to achieve a successful cultivation it is essential to respect the cultivation requirements of the different truffle species and to carry out the necessary cultivation care. Moreover, the factor we can best control is the quality of truffle plants. In this context, it is strongly recommended to plant the best mycorrhized truffle plants to improve the whole sector. At the same time, the production of truffle plants is highly differentiated in various countries, with respect to the species of plants and truffles that are produced and marketed. Moreover, given the considerable variability of rules in the various EU and non-EU countries, and sometimes their complete lack, it is difficult to propose a single method of analysis and certification. To achieve this goal, within the INTACT project, a specific task deals with this topic. An internal questionnaire submitted to the project participants showed that there was total agreement on the common starting point: to make the certification of truffle plants produced in each country mandatory.

Supported by: INTACT RISE-MSCA Project “INnovation in Truffle cultivation, preservAtion, proCessing and wild truffle resources managemenT” Grant Agreement n.: 101007623

Evaluation of the colonization of five *Quercus* taxa native to Greece by *Tuber aestivum* (Ascomycota, Pezizales)

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The genus Tuber P. Micheli ex F.H. Wigg. includes ectomycorrhizal fungi forming hypogeous ascomata (truffles) famous for their unique organoleptic properties. *Tuber aestivum* (Wulfen) Spreng. presents a relatively wide range of distribution and plant symbionts as well as good adaptation to hot and dry environments. Five Quercus taxa native to Greece (i.e., *Q. coccifera*, *Q. ilex*, *Q. ithaburensis* subsp. *macrolepis*, *Q. pubescens*, *Q. trojana* subsp. *trojana*) were inoculated with spore suspensions obtained from a single *T. aestivum* ascoma. Seedlings were planted in a sterilized mixture of calcareous soil, peat and vermiculite, and inoculated by approx. 1.5*106 ascospores (per individual). Evaluation of fungal colonization of roots was conducted at 3, 7 and 12 months after inoculation by examining five individuals per species, and estimating the percentage of the colonized root tips. Results showed fairly good colonization rates three months after inoculation for three species (i.e., *Q. pubescens* 17%; *Q. macrolepis* 29%; *Q. trojana* 41%), rather high colonization (58 – 80%) at seven months for four oak species, and high colonization (54 – 87%) at 12 months for all oak species. In addition, positive correlations were assessed between the number of colonized root tips and the total root tips number, while no significant differences were detected between the inoculated plants and the respective control for parameters related to the above ground plant growth. Moreover, the morphoanatomic features of ectomycorrhizae formed by *T. aestivum* and *Q. macrolepis* (and *Q. trojana*) were examined and reported for the first time.

Acknowledgement: The research project was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the “2nd Call for H.F.R.I. Research Projects to support Post-Doctoral Researchers” (Project Number: 1057).

Where do we stand on the knowledge of *T. magnatum* environments? Progress and new research hypotheses

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Knowledge about the environments of *T. magnatum* has been consolidated in the 2000s, confirming the importance of microclimate and that of specific soil types that, in fact, restrict the areas of natural spread of the fungus. In recent years, investigations have focused on two distinct themes: on the one hand, mycorrhization techniques have been refined, leading to the commercial production of mycorrhized plants and the establishment of the first experimental truffle orchards; on the other hand, the hydrological aspects of natural truffle areas, previously left in the background, have begun to be studied. The first topic has yielded interesting results with the start of production in areas never reported as productive and promises to expand the territories in which to "cultivate" *T. magnatum*. The second line of investigation is providing insights into the need for specific water management of truffles - natural and cultivated - focused on soil water conservation. For the future, it will be necessary to work in depth on this aspect. The natural environments of *T. magnatum* are characterized by a great need for water, which we hypothesize can be managed at the scale of micro-hydrographic basin, since the exclusive use of irrigation practices adopted, for example, for *T. melanosporum* is not economically viable.

Tuber balkanicum – new taxon defined by molecular, ecological and biogeographical methods

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Truffle diversity of Balkan Peninsula does not stop to surprise the scientific community with three new species from the melanosporum clade described in last year's (Mereny et al 2017, Milenković at al 2015, Slavova et al 2023). Out of large set of ITS sequences, Mereny at al. (2017) described *T. cryptobrumale* as connected to Pannonian region, but two other genetically quite distinctive populations were not distinguished as species. In this contribution, we have analyzed larger set of sequences and their habitats and detected strong connection between specimens with specific ITS/LSU region variants to ecologically specific habitats of Balkan peninsula. Soils and vegetation where these specimens have been detected rather resemble descriptions of *T. melanosporum* sites in Italy, while habitats of *T. cryptobrumale* resemble those of *T. magnatum*. Additionally, we have detected numerous populations of *T. melanosporum* in the coastal regions of Croatia, while this species has never been officially detected eastern from Dinaric alps in Balkan peninsula. We hypothesize that *T. melanosporum* could never overcome low temperatures of high and long Dinaric massive and is therefore localized in the western Mediterranean regions. This could be the reason why the melanosporum clade has been highly diversifying in Balkan regions. Moreover, the genetic variant widely distributed all over Europe and described as "*T. brumale*" has never been detected in the Balkans. Therefore, we propose that ecologically and genetically specific taxon that is widespread only in Balkan peninsula (and to some extent northwards) should be recognized as a different species - *Tuber balkanicum*.

Supported by: INTACT RISE-MSCA Project "INnovation in Truffle cultivation, preservAtion, proCessing and wild truffle resources managemenT" Grant Agreement n.: 101007623

Impact of plastic pollution on soil biological activity in white truffle habitats in the large river plains in Serbia

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The impact of plastic pollution on forest soil biota is an emerging research area. There are knowledge gaps on a forest microbiome composition and activity as effected by wild waste deposition in forest ecosystems located on white truffle (*Tuber magnatum* Pico) habitat areas. Particles of plastic materials decompose into micro-plastic particles and are responsible for many changes in the soil physicochemical characteristics, including porosity, enzymatic activities, microbial activities, plant growth. One of the first investigations of plastic and microplastic (MP) in the soil on the territory of Serbia is currently underway and is being carried out within the project "Evaluation of the Microplastic in the Soils of Serbia - EMIPLAST - SoS". The research sites are located on the alluvial plains of the largest rivers in Serbia, which are the habitats of the white truffle.

Forest sites visibly polluted and non-polluted by plastic materials were sampled and analysed for soil main chemical, physical and biological properties. Potentially mineralizable soil organic carbon was measured in a long-term incubation of soil samples with sequential measurement of the emitted CO2 flax by alkali trap method.

Preliminary results showed that some soil properties such as pH, electrical conductivity, total C and N, CEC and mechanical composition are significantly affected by the presence of plastic materials. Generally, polluted sites showed elevated microbial respiration and the rates of C mineralization. For the Danube and Morava River basin the differences were significant, while for the Sava river basin the difference wasn't significant. In order to establish the level of the negative impact of microplastics on soil properties and microbial activity in the longer term, the study is ongoing.

Acknowledgment: This research was supported by the Science Fund of the Republic of Serbia, #GRANT No 7742318, „Evaluation of the Microplastics in the Soils of Serbia" - EMIPLAST S.o.S.

Detailed morphological analyses and distribution of truffles in the puberulum clade in North Macedonia

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The aim of this work was detailed morphological examination on the collections within clade puberulum of genus *Tuber*, deposited in the Macedonian Collection of Fungi. The collections with intermediate characteristics were separated for molecular analyses, while for the collections possessing determining features the morphological analyses are presented and their distribution is shown according to the defined biogeographic regions (BR) of the country. *T. borchii* is considered as the most common white truffle, widely distributed in 19 BR and it is present in broadleaf and coniferous forests. *T. anniae* is a species with only one finding in Europe, confirmed only in Finland. It is found in North Macedonia in *Picea abies* forest at 1800 m.a.s.l. on the mountain Shar Planina. The mixed forest of *Quercus pubescens* and *Carpinus orientalis* are widely distributed in the country and well researched for truffles, however *T. oligospermum* is rarely encountered species which was found only on the mountain Jakupica at 600 m.a.s.l. *T. dryophilum* is distributed in four BR in mixed forest of *Q. pubescens* and *C. orientalis*, mixed forest of *Fagus sylvatica* and *Abies borisii-regis*, as well as in *Pinus nigra* plantations at 350–1300 m.a.s.l. *T. puberulum* was recorded in nine BR in association with *Q. coccifera*, *Q. frainetto*, *Q. pubescens*, *C. orientalis*, as well as in mixed planted forests of *Tilia cordata*, *P. nigra*, *Q. pubescens* and natural mixed forests of *F. sylvatica* and *A. borisii-regis*¸ at 100–1500 m.a.s.l. The ongoing study on molecular identification and phylogeny of white truffles in the country is expected to yield higher diversity of species within this group.

Long-term study of the establishment of *Tuber melanosporum* in an experimental reforestation plot with black truffle: highlights and future developments

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There is currently a growing interest in establishing truffle plantations on non-economically productive land, such as abandoned pastures or old small agricultural fields in forested areas. Encouraging reforestations by establishing truffle plantations could be a strategy to recover the Mediterranean landscape mosaics, reducing the risk of forest fires, while providing economic income. In order to evaluate the feasibility of the introduction of these plantations within forest settings, a plantation with 250 *Quercus ilex* seedlings inoculated with *T. melanosporum* was established in May of 2010 on an abandoned pasture surrounded by an extensive *Q. ilex* forest. The only interventions was a mulching fabric treatment to prevent herbaceous competition. During the initial five years of cultivation, we observed a higher relative abundance of non-*T. melanosporum* ectomycorrhizal fungi in the trees located nearer to the forest. Moreover, trees with larger root collar diameters exhibited higher biomass of *T. melanosporum* mycelium, and their fungal community demonstrated less susceptibility to the proximity to the forest. Furthermore, we observed that removing mulch material after 5, 8, and 11 years of plantation establishment does not seem to be a negative effect on the amount of truffle mycelium in soil. In addition, we found that the root collar diameter of the seedlings is a good indicator of mycelial expansion in the plantation. These results confirm the feasibility of restoring and reclaiming wild areas that adequate characteristics for black truffle cultivation and thus increase their value with truffle production.

Supported by: INTACT RISE-MSCA Project “INnovation in Truffle cultivation, preservAtion, proCessing and wild truffle resources managemenT” Grant Agreement n.: 101007623

Growing truffles to save the world? Potentials, limitations and uncertainties of greenhouse gas capture by truffle cultivation

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Nursing, planting and raising truffle-mycorrhized trees is the foundation of truffle cultivation. Carbon (C) capture by establishing truffle plantations might be comparable to reforestation/afforestation projects, as long as the truffle plantations persist. Currently, claims about C capture by planting truffle-trees are hardly based on empirical numbers. We will present data on tree growth in productive burgondy truffle plantations, to calibrate models of tree biomass.

The impact of truffle mycelia on soil C is less straightforward to assess and model, given soil spatial heterogeneity and clustering of co-occuring ECM fungi. Land use history and pre-existing soil carbon stocks modulate the effect of truffle plantations, which are further complicated by liming, if applied.

Agricultural regulations need to account for the potential of truffle cultivation and should incentivise practices which improve long term C sequestration by truffle plantations, e.g. extending the rotation period of truffle plantations and converting the harvested timber into durable products at the end of the plantation cyclus.

Seasonal dynamics of Tuber melanosporum mating-type mycelium is influenced by climate

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The Perigord black truffle (*Tuber melanosporum*) is one of the most famous truffle species worldwide. Since the early 70s of the last century, the cultivation of this fungus is possible. In the frame of the CulturTruf project financed in France from 2016 to 2023 we investigated the water management in orchards as well as the influence of climate on the truffle life cycle. The aim of this study was to investigate the monthly variability of both *T. melanosporum* mating-type mycelium to determine the climatic parameter influencing the mycelium growth in the soil as well as to unravel its sexual reproduction dynamics. During four years each month soils were sampled under 18 trees from four orchards (two in southeast and two in south west of France). For each plantation probes recording soil temperature and hydric potential were installed. The mycelium of *T. melanosporum* MAT1-1 and MAT1-2 was quantified using droplet digital PCR. The quantity of *T. melanosporum* varied during the years with different pic of mycelium in the different seasons. According our data, we couldn’t exclude that several sexual reproduction initiations occurs during the season. Using an innovative statistical approach (Bliss method), we were able to demonstrate that the climate (soil temperature or soil water potential) has an effect on the quantity of mycelium two weeks later. This effect differs depending on the season, the site and whether or not trees are watered. This project provided us new information to guide orchard management by promoting soil mycelium growth.

Supported by: INTACT RISE-MSCA Project “INnovation in Truffle cultivation, preservAtion, proCessing and wild truffle resources managemenT” Grant Agreement n.: 101007623

Distribution of truffle-inhabiting insects and the range of their hosts using fungus beetles (*Leiodes* spp.) and truffle flies (*Cheilosia soror* and *Suillia* spp.) as examples

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In Poland there are several species of truffles (*Tuber* spp.), including the culinary prized Burgundy truffle (*Tuber aestivum*). Their fruiting bodies are inhabited by many insect species, including mycetophagous beetles and flies of the genera *Suillia* R.-D. 1830 and *Cheilosia soror* (Zetterstedt, 1843). The relationships between insects and their hosts are still poorly understood, and research requires interdisciplinary collaboration among specialists. Undoubtedly, the range of insect species included in the so-called monophages or oligophages is mainly determined by the presence of their host. The food selectivity of insects at certain stages of development and the way they colonise the fruiting bodies significantly influence the distribution of "truffle flies" and beetles. The objective of this study was to improve our understanding of the biology of insects associated with truffles. The study of the coleopterofauna of truffle fruit bodies, particularly representatives of the family Leiodidae, allowed us to obtain information on the biology of little-known beetle species that are sporadic in other microenvironments. So far, only *Leiodes cinnamomea* has been described as a species closely related to the truffle, while *L. oblonga* has been found in large numbers during the course of the research. Among the widespread mycetophagous Heleomyzidae flies (including *S. affinis*, *S. pallida*), a rare species closely related to the truffle (species group *Suillia gigantea-flagripes*) was discovered for the first time in Poland. Earlier conjectures about larval development of *Cheilosia soror* in the fruiting bodies of truffles were also confirmed.

Mycophagy of truffles by invertebrates

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Species within the *Tuber* genus (truffles) require mycophagy for ascospore dispersal. Previous evidence has largely been focused on the role of mammals, but data is emerging about the importance of invertebrates in the lifecycle of truffle species. Here we present results of a feeding experiment using Isopods and *T. melanosporum* as a food source. Fruitbodies were consumed at a rate of 4.0 mg per isopod, over 24 h. Most of the recovered faecal pellets contained ascospores after 12 h. Gut-transit inflicted little mechanical damage to ascospores, and the majority were still contained within an ascus. Secondly, invertebrates were collected from grounds naturally producing *T. aestivum* and the gut contents were observed. The results are framed in terms *Tuber* spp. lifecycles, including mating type distribution and the likely importance of such interactions in truffle ecology.

Wild truffles and desert truffles in Morocco: Geographical distribution, soil characteristics and ecology

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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 $CaCO_3$ 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.

Supported by: INTACT RISE-MSCA Project “INnovation in Truffle cultivation, preservAtion, proCessing and wild truffle resources managemenT” Grant Agreement n.: 101007623

First cultivated summer (Burgundy) truffles found in truffle orchards in Latvia

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Truffle orchards have been planted in Latvia since 2008. Most of orchards were planted around 2010-2011, the oldest one being from 2008. The first *Tuber aestivum* fruitbodies have been found in November 2020 (reportedly, pers. comm.) and August 2021 (author`s find). In the process of checking of planted truffle orchards some other fungi species are often found, both hypogeous and epigeous.

The truffle orchards have been visited and checked with a help of the truffle dogs in Latvia since 2011.

The first find of *Tuber aestivum* by the author (with the help of trained Welsh Springer Spaniel Hafren) was made in August 2021 in the oldest truffle orchard in Latvia (planted in 2008). Fruitbody was found around 15 cm deep under pedunculated oak *Quercus robur* (inoculated by from Robin Pepinieres, planted in 2008). Fruitbody was mature, weighted 90 grams.

Most likely, truffle fruitbodies could have been found earlier and more often, if the orchard owners had trained their own truffle dogs and checked their orchards on a regular basis. Until now, only 4 trained dogs have been working more or less regularly.

Truffle nests in Spanish *Tuber melanosporum* orchards: empirical development, pros and cons, and potential for research

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Truffle growers devote great efforts to improve black truffle orchards productivity, developing new agronomic practices such as “truffle nests” (peat amendments that are supplemented with truffle spore inoculum), which has widely spread among Spanish growers during the last decade. This technique is an adaptation of an old practice used by wild truffle harvesters. One of the innovative aspects of nests is the use of peat-based substrates, which clearly differ from mineral soils. Here, we describe how nests are used in Spain and how they affect the truffle ascocarp quality and related characteristics: depth in soil, weight, shape, spore maturity, profile of volatile organic compounds and probability of infestation by the pest insect *Leiodes cinnamomeus*. Besides, it has been previously hypothesized that the fruiting associated with nests is linked to the growth of the native truffle mycelium or to changes generated in the soil fungal community. For this, we assessed the abundance of black truffle mycelium and the composition of the fungal community in nests in comparison to the surrounding soil.

Supported by: INTACT RISE-MSCA Project “INnovation in Truffle cultivation, preservAtion, proCessing and wild truffle resources managemenT” Grant Agreement n.: 101007623

Mapping of areas suitable for black Truffle farming in Patagonia, Argentina

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Black truffle production is considered an intensive crop, introduced (nonnative) in South America that included afforestation with inoculated with truffles (mainly *Tuber melanosporum* Vittad.). Truffle farming presents sites requirements regarding climatic and edaphic features. Temperatures must range between 8.6 °C to 14.8 °C but tolerating 42 °C (warmer months) to -25 °C (colder months); optimal rainfall have to range between 425 to 900 mm, although resisting up to 1500 mm. Edaphic conditions include pH between 7.5 and 8.5, presence of carbonates, permeable and aerated soils, non-floodable and sandy loam - silt loam textures. On the other hand, irrigation is one of the management actions necessary for success, mainly in areas with low precipitation but with other suitable conditions, and in those with very scarce summer precipitation as Patagonia, with seasonal (autumn-winter) rains. In this sense, Patagonia Argentina is a huge piece of land (1,060,631 km^2) with temperate to cold climate, with temperatures decreasing markedly as the southern latitude increases. Western Patagonia includes the southern section of the Andes Mountain range, has a cold humid climate and native, subantarctic forests, with ecotonal areas to the east, with semi-arid climate. Eastern Patagonia is a plateau that reaches the Atlantic coast, with a desert climate and steppes or scrubland. These huge climate gradients and the abundant presence of microclimate spots, determines a wide range of soil types, that suppose the presence of wide areas suitable for black truffle requirements. In this context, the promotion of trufficulture as a profitable, against season crop for marginal and idle land in this region, requires accurate information about suitable areas on a regional scale that allows to quantify and identify surfaces, plan the use of the land, and develop management tools for the activity. For this reason, two truffle aptitude maps considering edaphic, climatic and irrigation requirements were developed for Patagonia Argentina, a regional map at a scale of 1: 10,000,000, and another for Chubut province, at a scale of 1: 40,000. The potential truffle growing area with appropriate soil and climatic conditions in Patagonia totals 854,000 ha.

Considering the possibility of using irrigation, the regional surface totals 2,680,000 ha, while only in Chubut province totals 489,877 ha. These maps are pioneering for Argentina and Patagonia, constitute a powerful decision-making tool to diversify the productive matrix in Patagonia incorporating this novel a profitable crop in vast areas.

Supported by: INTACT RISE-MSCA Project "INnovation in Truffle cultivation, preservAtion, proCessing and wild truffle resources managemenT" Grant Agreement n.: 101007623

New data on the diversity of *Balsamia* in Europe

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The genus *Balsamia* consists of at least 25 species of hypogeous fungi occurring in the Northern Hemisphere. The *Balsamia* species are ectomycorrhizal symbionts of both coniferous and broadleaved trees, and are often found accompanying some *Tuber* species, e.g. from the Rufum group, with which may initially be confused. Phylogenetic investigations recognized several lineages within the genus with some of them occurring in more than one continent. In Europe and adjacent areas at least 6 species are known and some of them have recently been described from the Mediterranean region. Field research carried out in the Carpathian beach forests of Central Europe led to the discovery of a new species of *Balsamia*, which is characterized by the very peculiar sequences of the ITS barcoding region. The ascocarps of this species are characterized macroscopically by almost smooth peridium and microscopically by the smallest spores among all the known *Balsamia* species. Phylogenetic analysis suggests, that the new species may occupy a basal position within the genus.

Exploring new biodiversity hotspots in *Tuber aestivum* natural distribution

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Tuber aestivum is associated with a wide range of tree species and is the most widespread edible truffle. Its capability of colonizing many different areas along with a long reaping period, increased in the last decades scientific studies and commercial interest.

A number of large- and fine-scale population genetics studies have been conducted to explore its genetic diversity and genetic structure.

Recent investigations based on ITS sequence analyses revealed a strong phylogenetic pattern and a higher level of genetic diversity in Turkish and southern European populations than in northern European populations, suggesting that Turkey and southern Europe may have acted as glacial refugia for this species.

Despite these findings, the southernmost distributional range of the species is not yet fully explored. With the aim of evaluating other potential diversity hotspots in this study we expand previous investigation, extending the sampling to other southernmost natural *T. aestivum* areas, such as Moroccan mountainous regions (Middle Atlas), Serbia and several Spanish regions.

The ongoing analyses based on haplotypes alignment of the ITS region of the rDNA, reveal as preliminary result a pronounced genetic variability among Moroccan *T. aestivum* populations, suggesting a possible phylogeographic differentiation also in these new areas of investigation. Additional analyses are underway to better trace natural populations of *T. aestivum* according to their geographic origin.

These aims could be critical both for truffle industry interest in selecting well-adapted inoculum for the production of mycorrhizal seedlings or traceability of truffles provenance, and for the establishment of programs devoted to biodiversity conservation.

Supported by: INTACT RISE-MSCA Project "INnovation in Truffle cultivation, preservAtion, proCessing and wild truffle resources managemenT" Grant Agreement n.: 101007623

Monitoring moisture and temperature in Swedish truffle orchards

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In a project financed by Leader Gute, the Gotland Truffle Association has placed 20 soil sensors (Watermark) in 5 different truffle producing areas, natural or orchards on the island Gotland in the Baltic Sea. The aim is to learn more about truffle mycorrhiza's demands on water and temperature to produce truffles (*Tuber aestivum* syn. *uncinatum*), in order to optimize irrigation regimes. The project started in the summer of 2022 and will run until January 2025. Two spots were chosen in each truffle producing area. Each spot has two sensors, one on 10 cm depth and one on 25 cm depth. The truffle produced within a 10-meter radius around the sensors are registered. The soil differs in the studied areas, but generally there are moraine soils with a low amount of clay and pH 7.0 - 8.5. One of the areas has pure sand, which has been treated with lime to raise the pH to pH 7. The sensors were put in the soil in June 2022. Results are registered on-line through the Sensefarm system, which can be followed by all participating truffle growers. Results are continuously analyzed. Soil humidity at 10 and 25 cm depth as well as temperature over time will be presented in diagrams and maps.

Anatomical study and morphological diversity of mycorrhizae formed by Moroccan desert truffles

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Numerous studies have shown that desert truffles form several types of mycorrhizae, endo, ecto or ectoendomycorrhizae, under different growing conditions. The aim of the present work is to study the anatomy and morphological diversity of mycorrhizae formed by Moroccan desert truffles under axenic and gnotoxenic conditions, and to evaluate the effect of mycorrhization on the growth and development of *Tuberaria guttata*. The rate of mycorrhizal infection and the growth and development of the plants were taken into consideration in order to assess the effect of the mycorrhizal association on the host plant. Anatomical studies were carried out to determine the morphological diversity of the mycorrhizae formed. Under axenic and gnotoxenic conditions, mycorrhized host plants grew better than non-mycorrhized plants (Control). Fertilization had a positive effect on plant development. The desert truffles studied, *Terfezia arenaria*, *Terfezia leptoderma*, *Terfezia claveryi*, *Tirmania pinoyi* and *Tuber gennadii* all produced a well-developed Hartig network without a mantle, but the mycorrhizae formed showed great morphological diversity between desert truffle species.

Supported by: INTACT RISE-MSCA Project “INnovation in Truffle cultivation, preservAtion, proCessing and wild truffle resources managemenT” Grant Agreement n.: 101007623

Characterization of *Tuber macrosporum* / *Populus alba* mycorrhizas from cultivated seedlings: case report

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The oldest data on hypogeous fungi from the territory of Serbia date back to the year of 1777, but first study on truffles and truffle-like fungi in Serbia started in 1992. Collected material, fruit bodies of *Tuber* species had been founded and identified and extensively studied during the last decade of the twentieth century.

Despite the current interest in truffles in Serbia, there is not much information about artificially established truffle plantations. The register orchards of truffles in Serbia have not been created yet.

Thus, the objective of this study was the morphological and anatomical characterization of an unknown ectomycorrhizas of host species *Populus alba* from cultivated seedlings from part of a ten-year-old truffles plantation.

In autumn 2016, we were invited to visit and determine what kind of mycorrhizae is situated in the plantation near the town of Požarevac (Eastern Serbia). The truffle-ground (about 3,000 m2) is placed in Kličevac where a plantation was established during the autumn 2007. According to the owner, he bought commercially produced seedlings of *Populus alba* inoculated with the black truffle.

Black truffle aroma transfer to food matrices and truffled products perception by consumers

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Diversity and quantity of truffled products in markets are increasing day by day. Meanwhile, there are gaps of knowledge on i) how and which truffle Volatile Organic Compounds (VOCs) are effectively transferred to the food matrices of these products or ii) how heating treatments, that have to be applied in the food industry, affect these transferred VOCs. We conducted two experiments, one working with different fat-based food matrices vs different times of exposure to the truffle, and another using home-made products vs different levels of heat treatment and different truffle concentrations. Products were analyzed by gas chromatography and olfactometry or tasting panel, and a list of VOCs showing best-aromatization power was made along with a determination of which VOCs are most affected by heat treatments.

On the other hand, most truffled products found in markets contain added flavors, mainly bis(methyltio)methane (BMTM), confusing most non-experienced consumers on how real truffle aroma smells like. The veracity of these products and the perception of consumers strongly affect truffle growers´ incomes. We investigated labelling and make-up of 51 different products and a tasting panel evaluated eight of them. Consumers were divided into two groups according to their previous knowledge about truffles. We found that confusing or incorrect labeling were frequent and that non experienced consumers tended to rate more positively BMTM-containing products than the Truffle-containing ones, showing that a training effort must be done to improve population knowledge about truffles.

Supported by: INTACT RISE-MSCA Project "INnovation in Truffle cultivation, preservAtion, proCessing and wild truffle resources managemenT" Grant Agreement n.: 101007623

Legal regulation of truffle sector in the Republic of Serbia

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General Laws on nature protection of Serbia (competent Ministry for Nature Protection) passively regulate fungi by regulating protection of the environment, biodiversity, diversity of ecosystems or landscapes in the country, in a way that should meet the requirements of the EU legislation system. The direct regulation of issues related to truffles is found in Rulebook on the declaration and protection of strictly protected and protected wild species of plants, animals and mushrooms and Decision on the determination of goods for which import, export, or transit requires the obtainment of certain documents. The truffle species included in these two documents are *Tuber magnatum*, *Tuber aestivum* and *Tuber macrosporum*. The Law on Forests, issued by the Ministry of Agriculture and Forestry directly treats mushrooms in two articles, one forbids collection of any secondary forest products, unless stated differently by that same Law, while other allows collection of secondary forest products only upon approval of the forest manager. It is not stated how these two articles were correlated with Rulebook and Decision of Law on nature Protection that refer to wild mushrooms including truffles. Apart from legal regulation in the commercial sense, there is absolutely no in purpose legal protection of the areas and ecosystems where truffles naturally occur. Without adequate protection of forest ecosystems that contain truffle species, there is a risk of losing these commercially important species. Establishment of truffle plantations is not regulated by any legal act, why the amendment of existing legislation is necessery in order to enable the developement of the entire truffles sector in the country.

Supported by: INTACT RISE-MSCA Project “INnovation in Truffle cultivation, preservAtion, proCessing and wild truffle resources managemenT” Grant Agreement n.: 101007623

Which is the mating type gene distribution in single spores inside Tuber melanosporum ascus?

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Truffles belong to ascomycetes for which sexual reproduction is guided by mating type genes called MAT1-1 and MAT1-2. According genomic and genetic analyses it has been proposed that truffles are heterothallic. Spores are produced in ascus, for *Tuber melanosporum* the number of spores is generally comprise between one to five. To date it is unknown if all the spores present in a single ascus are genetically identical and harbor the same MAT gene or if they are different. The aim of our study was to investigate the distribution of MAT gene in single spores inside ascus to better understand the mechanism of spore formation. From three *T. melanosporum* ascocarps, ten ascus for each class of spores number from one to four spores have been collected. Using a binocular magnifying glass, we broke off the asci and separated the spores for individual analysis. From each single spore MAT genes were determined using droplet digital PCR (dPCR). We first confirmed dPCR is suitable to analyze low quantity of DNA since 87 % of the spores analyzed gave results. The first results suggested that a single spore can have different nucleus since both MAT were detected for 87.7 % of the spores. Interestingly most of the spore with both MAT have been found in ascus with low number of spores. Since haploid mycelium forming coming from spore germination harbor only one mating type gene, these preliminary results open new questions on truffle sexual reproduction and mode of spore germination.

Supported by: INTACT RISE-MSCA Project “INnovation in Truffle cultivation, preservAtion, proCessing and wild truffle resources managemenT” Grant Agreement n.: 101007623

ISBN 978-86-80109-25-1
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