1ST International Congress of

TRUFFICULTURE

I Congreso Internacional de

TRUFICULTURA

2013

TERUEL

5.6.7.8 | 3 | 2013 Spain

ABSTRACT BOOK

LIBRO DE RESÚMENES

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ABSTRACT BOOK

LIBRO DE RESÚMENES

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5th March 2013

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6th March 2013

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Chair *Dr. Carlos Colinas.* University of Lleida & Forest Sciences Center of Catalonia (Spain).

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13:00 **Closing Speech.** Challenges after sequencing the black truffle genome. *Dr. Claude Murat, Claire Fourrey, Annegret Kohler, Emmanuelle Morin, Francis Martin.* French National Institute for Agricultural Research (France). 79

13:45 **Closing Ceremony and Awards Ceremony.** *José Vicente Lacasa Azlor,* president of the 1st International Congress of Truficulture 2013 and authorities. Coordinated by *Dr. Juan J. Barriuso.* Agrifood Research and Technology Centre of Aragon (Spain).

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15:00 **SESIÓN 4: Biología y genética de poblaciones aplicadas a la gestión.**
Modera *Dra. Alessandra Zambonelli*. Universidad de Bologna (Italia).

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9:00 **Visita de campo.**

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9:00 **Conferencia invitada.** Sexualidad en el ciclo biológico de las trufas: la importancia y las repercusiones de ser compatible. *Andrea Rubini, Claudia Riccioni, Beatrice Belfiori, Dr. Francesco Paolocci.* CNR de Perugia (Italia). 67

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Las trufas del desierto en Túnez: diversidad e intentos de cultivo. *Abdelatif Boudabbous, Imed Sbissi, Mohamed Neffati, Maher Gtari.* 68

Polonia como lugar para trufas. *Maria Lawrynowicz, Dominika Slusarczyk, Malgorzata Polatynska.* 69

La trufa de verano en Suiza: fenología y requerimientos ecológicos. *Simon Egli, U. Stobbe, W. Tegel, L. Sproll, Martina Peter, R. Elburg, D. Nievergelt, U. Büntgen.* 70

Gran diversidad y amplia ocurrencia de esporas mitóticas en los Pezizales ectomicorrícicos. *R.A. Healy, Matthew Smith, Gregory M. Bonito, D.H. Pfister, Z.W. Ge, G. Guevara, G. Williams, K. Stafford, L. Kumar, T. Lee, C. Hobart, Jim M. Trappe, Ritas Vilgalys, D.J. McLaughlin.* 71

Desarrollo de ascocarpos de *Tuber mesentericum. Marie-Anne French.* 72

Alta diversidad filogenética en el grupo *Tuber excavatum. Alexander Urban, Benjamin Stielow, Ákos Kund I Orczán, Torda Varga, Gabriella Tamaskó, Zoltán Bratek.* 73

Análisis filogenético de *Tuber brumale* aggr. *Zsolt Merényi, T. Varga, J. Geml, Gerard Chevalier, Zoltan Bratek.* 74

11:30 **SESIÓN 6: Estrategias para la conservación en fresco de las trufas y comercialización.** Modera *Dr. Ian R. Hall.* Truffles & Mushrooms, Ltd (Nueva Zelanda).

11:30 **Conferencia invitada.** Estrategias para en la post-cosecha y la conservación de *Tuber melanosporum* en fresco. *Dr. Domingo Blanco, M. Eugenia Venturini, Pedro Marco, Carmen S. Rivera.* Universidad de Zaragoza (España). 75

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Cómo detectar *Tuber magnatum* y *Tuber melanosporum* en productos procesados. *R. Rizzello, Elisa Zampieri, Andrea Vizzini, A. Autino, M. Cresti, Paola Bonfante, Antonietta Mello.* 77

Efecto de cubiertas comestibles y de los envases de atmósfera modificada en la población microbiana y en las características de calidad de *Tuber aestivum* (trufa de verano). *Pedro Marco, Carmen S. Rivera, Domingo Blanco, M. Eugenia Venturini.* 78

13:00 **Conferencia de clausura.** Retos tras la secuenciación del genoma de la trufa negra. *Dr. Claude Murat, Claire Fourrey, Annegret Kohler, Emmanuelle Morin, Francis Martin.* Instituto Nacional de Investigación Agronómica de Francia. 79

13:45 **Acto de clausura y entrega de Premios.** *José Vicente Lacasa Azlor,* presidente del I Congreso Internacional de Truficultura 2013, y autoridades. Modera *Dr. Juan J. Barriuso.* Centro de Investigación y Tecnología Agroalimentaria de Aragón (España).

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Trufficulture as tool for private forests management

Medrano Ceña, P.A.[1]

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Abstract

In the province of Soria, and, by extension, in other many areas of Castilla y León region and continental and mediterranean Spain, the most important forest areas are prevailed over holmoaks (*Quercus ilex*). Traditional use of those stands had been based on the integration of its different potential productions: pastures, firewood, charcoal, hives, edible wild mushrooms, etc.

However, the change in the way of life, primarily in rural areas, has caused the leave of those traditional forest management practices, causing the decline of those woods. To the loss of capacity of production in the rural cores, it should be added the environmental damage that means the lack of management in those forests: loss of biodiversity, forest fires risk, pests, etc. Its recovery, management and value enhancement strategy, entail all sort profits: environmental, economics, socials, etc., and also an important strengthening and means of support effect in the rural areas.

Value enhancement actions in those zones, that are in most of the cases the result of the ancient integration between the men and its environment, should be approach as a whole, using tools that try to copy the traditional interaction between the different agents and resources. In the definition of this new environmental order, black truffle could and should have a central role, by its economic value and also by the fact that its optimal management practices are the same to the multiple-use forestry practices in those stands.

The most of the stands that produce black truffle are of private property, so it is also very important get the interest and implication of its holders, generally dispersed along all the national territory as a result of the migratory flows. In the majority of the cases, due to the fact that those forest are lands tenures *pro indiviso*, purchased during the seizures of XIXth Century end, to the problem to find groups formed by several hundreds of people, it has to be added the complexity to resume the line of succession in four and five generations, in order to confirm its ownership in a proper way.

Different work proposals developed from the Soria´s forestry association are outlined in this speak, the ones related with local experiences point to reach multiple-use forestry practices around the black truffle (*Tuber melanosporum*), and also the ones related with the finding and implication of the owners in order to build collective management models for the territory in the Montes de Socios (forests of fellows) project.

KEYWORDS

Black truffle, multiple-use forestry, private property stands, collective management, forest of fellows.

Practical overview on the relationship between climate and truffle harvest

Olivier J.M.[1],; Doublet, J.M.[1]; Sourzat, P.[1]; Tabouret, P.[1]; Tourrette, J.F.[1]

[1]**French Network for Experimentation in Truffle Production**
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Abstract

The link between truffle harvests and climate during spring and summer seems to be evident since more than 120 years, but without real demonstrations based on robust data allowing practical advices to the farmers. One problem is induced by the long delay between climatic events and the harvest, other factors being involved in the development of the fruitbodies. Another requirement is to take into account the real events occurring during many years in well definite locations where observations and harvests take really place together; the correlation between macro-data (analysis of the weather on too large geographical scales, harvests from too big markets...) cannot be useful for identification of biological behaviours and practical propositions, only to propose tendencies. It is the reason why we tried to build a network including truffle fields under various climates, including constraints on facilities (like wheather stations to get informations from air and soil, irrigation), collection of fruitbodies all over the picking season…

In this presentation we provide data about the rainfalls during different years considered as “good or not” for truffle production. We discuss the real problems encountered to propose conclusions and practical advices, mainly in management of water supplies. We also provide not rare observations linked to the production of truffles even after a long dryness period during the formation of the ascocarps (over 40 or 50 days). That induces a discussion on the resistance of the young truffles to the lack of water. But that also requires progresses in the knowledge of the fungal physiology (i.e. effect of temperatures, gaz exchanges in soil, dates for fruiting induction..) to avoid mistakes as previously done with some excesses in water supplies.

Desert truffle potential zones: A GIS approach with multiple applications

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Abstract

A Geographic Information System (GIS) is a technological tool for understanding geography and making intelligent decisions. In order to establish a distribution model of the desert truffle potential areas within the Region of Murcia (southeast Spain), a methodology based on a GIS computer tool with geographic information was used. To obtain the thematic cartography it was necessary to select the main characteristics that define the desert truffle mycorrhizal ecosystem: host plant, soil characteristics, bioclimatic zone and a specific precipitation regime.

The mapping was carried out by overlapping different vector and raster layers that codified the selected aspects. Nine different thematic maps have been designed: six maps for each *Helianthemum* species and a map with all the *Helianthemum* species, a bioclimatic zone map and an annual precipitation map.

The obtained cartography provides the optimal bioclimatic zones and precipitation regime for each mycorrhizal host plant. These maps offer specific information about potential desert truffle locations within the Region of Murcia. This cartography will enable users:

- To make systematic surveys in the potential zones that will permit the construction of data bases that reflect actual fungal presence to compare with the potential maps.
- To select the specific mycorrhizal host plant for future desert truffle orchards.
- To edit locally distribution maps.
- To calculate new maps of density and diversity.
- To search for areas to protect and manage.
- To create routes, interactive presentation programs (in museums, interpretation centers, natural parks), which is of special interest to enterprises and environmental institutions.

Acknowledgments: This work was funded by project CGL2011-29816 (Spanish Ministry of Science and Innovation, Spain).

KEYWORDS

Desert truffle, *Helianthemum*, GIS, habitat, orchard.

A potential model for black truffle cultivation in the province of Huesca (Spain)

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Abstract

Black truffle is a demanding organism regarding the conditions for its development. The land where it will be grown must accomplish some soil and climate requirements, mycorrhizal plant quality must be guaranteed and subsequent management of truffle grower must be adequate. Still, uncertainties remain about the moment when field begins to yield and about phases of the biology of the fungus. If the need of a large initial investment is added to this, it seems particularly important and necessary to determine the optimal location of crop establishment and future actions for the sake of profitability.

This paper uses Geographic Information Systems (GIS) to generate a spatial potential model or aptitude for planning and make technical decisions with a rigorous scientific knowledge, allowing a more orderly trufficulture in the province of Huesca.

To do this work it has been used the Climate Atlas of Aragon, Digital Terrain Model, the Forest Map of Aragon, the map of potential vegetation and land use maps of Corine Land Cover, among others, joined to own mapping data generated from historic data belonging to actual plantations spread by 15,626 km^2 in the province of Huesca. It has been finally determined the most appropriate regions for growing truffles, their spatial distribution and the actions to be performed on those established plantations.

Results of this work show a great truffle potential in the province of Huesca, a fact that can be useful when the territory is managed toward a profitable agriculture in an area where traditional crops have very low profitability due to poor soils and the hardness of the climate.

KEYWORDS

Map, GIS, cartography, Aragón, *Tuber melanosporum.*

Mapping and characterization of the mean black truffle producing area in Europe

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Abstract

The Drôme and Vaucluse basin appeared to take in a large truffle production sector associated with a presence of all the actors of the industrial network, through the decline of the European production. But no specific item allowed declining its importance, provides the features and is able to offer development opportunities tailored to the territory and the economic context.

At the request of the elected representatives of the territory and the labour union (syndicate) of Truffle growers responsible for this region, a work of exhaustive mapping of the main part of the concerned sector was confided to the “Regional Center of the Forest Property”

It consisted in visiting 120 municipalities in their entirety, to identify and to describe all truffle stemming from plantation, after a work of observation on air photos, a visit of ground, a transfer on map and an IT processing by means of software SIG. Thanks to an access to the cadastral data, not only the characterization of the production tool was possible but also the profile of the truffle grower was able to be obtained.

It appears that on the main zone of production, covering 522 km2 and three cantons, it is more than 3600 ha of truffle fields that feed mainly the market of Richerenches. The farmers are not the main owners of these plantations with a possess of only 45 %. The intercalary cultures essentially with lavandin, accompany (45 % also) the young plantations. The mixture Quercus pubescens, Quercus ilex and Quercus robur was held retained as basis of production by a majority of planters (75 % of the surface). 51 % of truffle growers are more than 60 years old.

A complementary work with the various actors approvals of the network allowed finally to highlight the weak points and the key points, to propose 3 possible axes of development to maintain or strengthen the future of this production:

1) Constitute a powerful, representative interprofession of the various segments of the network able to be a strength of political proposal.
2) Assure the continuation of the effort of plantation and of renewal of the production-tool.
3) Develop the experiment to guarantee a more regular fruiting, less dependent on climatic chances and better adapted to the various local ecological contexts.

These main points must be implemented in the next years in the interest of all.

KEYWORDS

Mapping, characterization, network, interprofession, renewal, experiment.

Field performance of truffle plantations in firebreaks

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Abstract

Firebreaks are a common measure to fight against wildfires in the Mediterranean. The vegetation is reduced in wide strips, and the maintenance of this open structure is very expensive. *Tuber melanosporum* grows in open forests and its phytotoxic effect inhibits the growth of some plants. Thus, black truffle could be cultivated in firebreaks to reduce maintenance costs and to improve firebreak sustainability.
The success of these plantations depends on the ability of *T. melanosporum* to persist in firebreak soils. Truffles plantations are usually established on plots previously cultivated for non-ectomycorrhizal plants (cereals, almond trees, etc). But in firebreaks it is common to find isolated ectomycorrhizal trees and shrubs (*Quercus*, *Pinus*, etc), which support a native ectomycorrhizal community. In addition, the soil depth is usually lower than in agricultural lands, resulting in a lower soil quality that conditions seedling survival and growth.
In 2008 *Quercus ilex* seedlings inoculated with *T. melanosporum* were planted in three areas with a potential value as firebreaks. The plantation holes were treated with the aim of reducing the ectomycorrhizal infectivity of the soil before planting. Five treatments were tested: a heat treatment, overliming, addition of plant compost, addition of sodium hypochlorite, and control. Two different stocklots of seedlings were planted: the first presented a commercial mycorrhization (mean: 3390 mycorrhizas of *T. melanosporum*, colonising 45% of the root tips), and the second presented a lower mycorrhization (mean: 1711 mycorrhizas of *T. melanosporum*, colonising 24% of the root tips).
Two years after plantation, 30 seedlings were extracted to assess the short-term field performance of the seedlings in the firebreaks, as well as the influence of plantation site, soil treatment and initial mycorrhizal state.
The seedlings with a higher initial mycorrhization showed a higher size in the field, and the seedling size was also influenced by the plantation site. The number of *T. melanosporum* mycorrhizas per plant was very variable within treatments and did not show any significant difference. The percentage of roots colonised by *T. melanosporum* and the richness of native ectomycorrhizal fungi colonising the seedlings were significantly influenced by the interactions among initial mycorrhizal state, the plantation site and the soil treatment.
T. melanosporum has shown able to persist on firebreak soils in the short term, although the plantation site influences the persistence of the introduced fungus. The results support the role of nursery-seedlings quality on the early performance of truffle plantations: the influence of site and soil treatment was much lower in the seedlings with a high initial mycorrhization than in the seedlings with a low mycorrhization.

KEYWORDS

Tuber melanosporum, firebreak, truffle cultivation.

Effects of soil tillage on *Tuber magnatum* development in natural truffières

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Abstract

Tuber magnatum Pico, the Italian white truffle, commands the highest prices for any truffle. Despite its commercial value it is the only edible European truffle which was not yet been successfully cultivated. In the absence of methods for the cultivation of *T. magnatum*, it is appropriate to identify cultural practices to maximise production in natural truffières. Soil tillage is extensively used in black truffle cultivation to increase ascoma formation, however its effects are not known on the development of *T. magnatum.*

Recently a specific real-time PCR assay using TaqMan chemistry has been developed to detect and quantify *T. magnatum* in soil. We used this technique to observe the effects of superficial soil tillage (10-15 cm depth) in two different natural *T. magnatum* truffières in Tuscany and Emilia Romagna. Ascoma production was also followed in the three years the research was conducted. A randomized block design was used and included tilled and untilled plots in both the truffières. The effects of soil tillage on ectomycorrhizal fungal communities was also assessed. Statistics were carried out using before-after control-impact (BACI) analysis.

Tilling significantly increased the quantity of *T. magnatum* soil mycelia which seemed to be related to an increase in soil porosity by up to 34%.

The effect of soil tillage on ascoma production was inconclusive owing to the variable climatic conditions in the three years of the experiment with summer rain only sufficient to guarantee ascoma production in the first year.

However, the results confirm that real time PCR is an effective tool for quantifying soil mycelium in field experiments such as ours. Because of the ascoma production is scattered and inconsistent and *T. magnatum* mycorrhizas are extremely rare, mycelium is the only reliable target to monitor this precious fungus in soil.

KEYWORDS

Tuber magnatum, soil tillage, mycelium, real time PCR, ectomycorrhizal community.

Promoting rural marginal areas with the truffle production: an analysis of the convenience of investments in Italy

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Abstract

The multifunctional role of agriculture and forestry activities is well known and the production of truffles represents a valid way of combining the private interest with the public one. As a matter of fact, to enhance the truffle is necessary to protect the area through good forest management practices as the production level of the truffle is directly proportional to the state of maintenance of the territory. Defending socio-environmental interests in this case means also to increase the income of the entrepreneurs. Especially in marginal rural areas, this production can be a viable alternative to give a new impetus to revitalize economically territorial contexts for which, otherwise, the risk of abandonment would increase. The truffle activity can be developed in two different directions, one relating to the improvement and management of existing forests and the other connected to the creation of new areas for the cultivation of black truffle. In both cases, however, it is necessary to provide entrepreneurs with an economic-financial framework, as clear and objective as possible, so that they can decide on the most appropriate investment strategies. In this context, public decision-maker may be also involved in, as it might be interested to assess the convenience of public investment to support a multifunctional activities like the truffle production.

The objective of this work is to analyze the economic/financial convenience of the investments in the sector of truffle production, in order to provide useful information to those who want to invest in this area or who intends to optimize its management from an economic point of view.

This issue was addressed through cost-benefit analysis approach. The methodological procedure refers to an analysis of a sample of twenty-one areas for the truffle production in Tuscany. This analysis made possible the definition of commonly used cultural practices and the evaluation of the costs of production of truffles. Indeed, in literature there are no data for a coded cultivation and production technique. Our research was also useful for the identification of a standard technique for the production of black truffle. Direct surveys was specifically conducted to estimate the gross salable production. These estimates showed a high variability in truffle production between plant and plant and a significant influence of microclimatic conditions and environmental factors on productivity. This implies that the total productivity is not directly proportional to the area under cultivation. We have, therefore, suggested three possible scenarios (pessimistic, optimistic and realistic) linked to the productive potential of a truffle cultivation. Finally, the convenience of the investment was evaluated through the analysis of three indicators: Net Present Value (NPV), Internal Return Rate (IRR) and cost benefit ratio.

The results show a high variability of indices of convenience of the investments in relation to the scenario. In particular, the optimistic hypothesis generates very satisfactory indicators, in which all investments have positive NPV and high SIR. The pessimistic scenario, on the contrary, determines a negative trend of convenience indicators. The realistic assumption is intermediate to the two scenarios. Finally the proposed study, thanks to the identification in detail the structure of the production costs, has enabled the identification of ideal economic conditions and silvicultural practices for the optimization of the management of truffle cultivation.

KEYWORDS
Economic analysis, Truffle production, Investment Evaluation

Truffle cultivation: a global reality

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Abstract

Truffles have been appreciated from ancient times, as revealed by the fact that its name is similar not only in Latin languages (trufa, truffe, trifola, tartufo) but also in some Arabic countries (terfes, terfez). The harvesting of wild truffles is documented since 2,500 BC in Egypt; however its cultivation was attained very late compared to other agricultural products also mentioned in the Bible as cereals or legumes.

In recent decades the cultivation of black truffle (*Tuber melanosporum*) and other species of truffles has expanded around the world to all the regions with Mediterranean climate, and to other regions with similar climates. Some people has tried to cultivate it in regions too rainy, dry or warm outside the European standard for the species, with varying degrees of success. In all truffle-growing areas there is an element of random "luck" still very important: unfortunately or fortunately, depending on where you look, neither art nor science have solved this limitation.

In parallel, Asian and American truffles have entered into the market. Due to their different qualities and lower prices these truffles have served as lower-prize substitutes for the greater value, gourmet truffles. This expansion implies important ecological and economic risks such as nonnative species becoming invasive and competing with European valuable truffles for ecological niches.

This conference analyzes the state of the art of truffle cultivation around the world: France, Italy, Spain, Australia, New Zealand, Chile, South Africa, Argentina, Israel, etc., elsewhere it is being cultivated. Its role as a transitional element between agricultural and forestry activity makes truffle cultivation one of the most important paradigms of sustainable development in rural areas. The truffle is very attractive for tourism with a great sense of curiosity and high gastronomic interest, offering a very positive added value in regions which are mostly undeveloped and depopulated.

KEYWORDS

Tuber melanosporum, truffle cultivation, Europe, Australia, New Zealand, Chile, USA.

Teruel, a global model for truffle cultivation: origins, present and future

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Abstract

Through the last 10 years, Teruel has become a global example of the black truffle (scientific name *Tuber melanosporum*) cultivation. It is a geographic area with 4,000 hectares of truffle plantations at present, producing 80-90% of the total black truffle production in Spain.

In this paper we show the climate and soil conditions found in this area, as well as the reality of the limited farming viable and the factors that favoured the first plantations during the 90s decade that evolved to the present situation.

We have also analysed the factors that, as we understand it, have caused the *Tuber melanosporum* production decline. This truffle grows wild in our mountains but it has practically disappeared and is now in danger of extinction.

The paper shows as well the beginning and organization of a sector thanks to the creation of the truffle farmer association, with over 500 members, and the most important monographic fair in Spain as for number of assistants.

We want to highlight the support our community politicians have given to the truffle farming in terms of financial contributions to the establishment of plantations. This sector is expected to be consolidated with the funding of a social watering plan by means of which around 1.200 hectares of truffle plantations will be irrigated. It is also remarkable the economic impact the truffle cultivation is having on the economy of the Community: creation of garden centres and watering and fence companies; summing up: creation of work and wealth. Besides, it is imperative to note the positive impact it is having on the tourist area, resulting in new business activities like gastronomic tourism, guide visits to truffle plantations which have helped to create new organisations to cover these new tourist activities. The truffle farming is a real revitalizing engine that contributes to attract population in a poor and depressed area.

KEYWORDS

Teruel, black truffle (*Tuber melanosporum),* global model, 4,000 hectares, truffle farmer, cultivation, social watering, irrigation, rural tourism.

The technical framework of truffle cultivation

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Abstract

Since the introduction of mycorrhiza-inoculated plants, the development of truffle production in France has been based on two main methods of cultivation which have replaced the traditional model. The 'Pallier' method was inspired by arboriculture, with working of the soil, pruning the trees and irrigation. The 'Tanguy' method was based on habitat changes during the formation of natural truffle grounds. Recently other models have challenged them. Technical guidelines can be defined from the results obtained with different types of husbandry to best adapt to different environmental conditions.

This framework – or precautionary technical plan – includes five phases: the moment of planting, maintenance before fruiting, cultivation during the harvest, renovation or regeneration, and grubbing-up.

At the beginning of the 1st phase an assessment is made of the soil's potential, the availability of water resources for irrigation and the presence of woods around the plantation. These factors will govern the improvements made to the soil (sub-soiling, crushing, lime fertilisers), the choice of tree species, planting density, orientation of the rows of trees and the clearance between the outer rows and the surrounding woods.

Phase 2 is the most debated (because it is the most influential) and is very variable with regard to soil maintenance. Grassing over creates biodiversity conditions at ground level close to those of natural truffle grounds. On the other hand it can hamper the growth of host trees and in some cases limit the spread of the truffle mycorrhiza-inoculated root system in the surface layer.

Phase 3 doesn't seem to vary much between the different methods of truffle cultivation tested in France and abroad. It consists of keeping the soil aerated, controlling the truffles' water requirements and limiting the closing-up of the plantations by pruning or lopping the trees.

The goal of the 4th phase is to recover space for the truffles to invade by removing trees, thereby getting closer to the pioneering system preferred by *Tuber melanosporum*.

Phase 5 ends the production cycle by uprooting the plants.

With nearly 40 years of experience since the introduction of tested mycorrhiza-inoculated plants (in 1974), it is desirable to develop and impose one's own method of truffle cultivation at a local level. The identification and control of interacting factors is the key to achieving ongoing truffle production.

KEYWORDS

Tuber melanosporum, "Pallier" method, "Tanguy" method, soil aeration, irrigation, pruning.

Impact of irrigation period and dose on the growth of *Tuber melanosporum* in young truffle orchards

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Abstract

In Mediterranean climate, the young truffle-oak plantations are subjected to drought episodes that can compromise the development of *Tuber melanosporum*. Irrigation is used to mitigate these negative effects although the water needs to optimize fungal growth remain uncertain. We investigated the responses of *T. melanosporum* to water supply in three periods: May to July, August to October and May to October. In each period, five water doses were established: 0, 25, 50, 75 and 100% of the reference evapotranspiration (Eto). Five orchards were planted with Holm oaks inoculated with *T. melanosporum*, and in each orchard, we arranged a two-factorial design with irrigation period and irrigation dose as main factors to test the interaction between them on the growth of *T. melanosporum* and Holm oaks after three years in the field. The seedlings that were evaluated before planting for the quality of ectomycorrhizal colonization did not had any ectomycorrhizae other than *T. melanosporum*. Irrigation period significantly interacted with irrigation doses for the absolute presence per seedling of *T. melanosporum* mycorrhizae. Irrigation in May-July increased significantly *T. melanosporum* colonization in seedlings irrigated with 50% Eto dose compared to the 0% Eto dose. Similar pattern with fewer differences in means was observed in August-October period, but the irrigation doses did not change *T. melanosporum* colonization in May-October period. We found ectomycorrhizae different from *T. melanosporum* in 51% of the seedlings studied, but their presence was not significant. A total of 52% root tips were mycorrhizal and in 80% of those, the fungal partner was *T. melanosporum*. Only higher irrigation doses significantly affected the height of seedlings and the total root tips per seedling, but the total fine root length was unaffected. Our results suggest that a moderate irrigation dose may promote the seedlings growth and number of fine root tips per unit of fine root length, which may be potentially colonized by *T. melanosporum*. This results with the fact that the seedlings evaluation before planting did not yield other ectomycorrhizae than *T. melanosporum* suggests that irrigation had not effects on the potential competitors of *T. melanosporum*. Further studies are needed to improve the irrigation programs of truffle orchards, which should pay attention to the growth periods of the symbionts.

KEYWORDS

Ectomycorrhizae, holm oak, black truffle, drought episodes, preproduction

The role of irrigation in the rot of truffles in Western Australia

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Abstract

The success of truffle production in Australia has been hampered by the occurrence of disease and pest problems. Many growers from across the country have experienced heavy losses, and estimates from 2010 put these losses at ca. 900kg or 35% of the national crop. Losses are strongly associated with surface forming truffles, that are susceptible to insect predation, desiccation, high temperatures, and disease. This study demonstrates that the rate and frequency of irrigation, affects the depth of truffle formation and the proportion of healthy truffles at harvest.

The experiment was conducted over two truffle seasons between 2010 and 2012, on a 14 year-old commercially managed *T.melanosporum-Corylus avellana* plantation in Manjimup, Western Australia. A split-plot trial was established, testing two (weekly or monthly) irrigation intervals at four rates of irrigation calculated by the replacement of evapotranspiration (ETc) (100, 50, 9 and 0% ETc). In the second season, the 9 and 0% treatments were replaced with 37 and 50% ETc, respectively.

During the truffle harvesting season (late May until August), the trial area was searched on a weekly basis by a team of trained dogs that located both mature and rotten truffles. The nearest tree, truffle depth, condition and size were recorded for each of the 7,863 and 11,717 truffles harvested in 2011 and 2012, respectively.
Log-linear analysis showed that weekly irrigation and the 100% ETc rate both resulted in a higher proportion of truffles becoming exposed at the soil surface in 2011 and 2012. Data from 2012 showed that these treatments also resulted in a significantly greater proportion of truffles being affected by rot. The relationship between irrigation interval and truffle rot was conditionally dependent on truffle depth. When water was applied weekly, there was no difference in the proportion of truffles becoming rotten at the soil surface in comparison to below. This suggests that there is a unique cause of rot affecting truffles below the surface when irrigation is applied weekly. This is possibly related to anoxia.

While adequete moisture is essential for truffle production, irrigation requires careful managment to ensure the health of the developing truffle through to maturation.

KEYWORDS

Rot, disease, irrigation, water, Australia.

Use of mobile devices for truffle fields optimization

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Abstract

The rise of trufficulture, especially of plantations, in recent times, suggests us the need of tools that allow sector development and technological advancement. Regarding the importance and the choices that new technologies offer us, we are therefore obliged to use them to achieve progress in this sector, so old and so little developed.
The main aim of this work is to provide a management tool to truffle managers which allow them to take specific, clear, organized, easy access and easy to use data of their truffières.
To achieve this goal we have relied on the possibilities that mobile devices (tablet and Smartphone) give us, considering that actual society is used to them, they are very easy to operate and its price is affordable.
The proposed solution includes two parts:

- Devices for Smartphone
- Desktop devices for data processing and analysis.

Smartphone App

Its development is based on the ability to collect and send data through mobile devices, thanks to 3G, WIFI and GPS. All the information got in the field by truffle growers can be sent and processed at real time, giving information such as:

- Geolocation of each of the plants in the field.
- Production of each plant in the field, by date, quantity and ripening.
- Phytosanitary status of each of the plants.
- Task performed on the field, weeding, watering, pruning...
- Pictures through the mobile device camera.

The app for mobile devices can be implemented for the main operating systems in which mobile devices work (Android, IOS).

Desktop App

The desktop app is a tool for PC, where is collected all the information took by the mobile device; it is an easy use tool whose main advantages are:

- Information is showed organized and available.
- Ability to perform statistical analysis of the information.
- Reports creation.
- Export data to others formats (interoperability between different systems).

KEYWORDS

Mobile application, Management, Statistics, Production control.

Developing novel tools and processes for sustainable cultivation of *Tuber borchii*

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Abstract

Tuber borchii, also called biachetto, are ectomycorrhizal (ECM) Ascomycetes producing excellent truffles with inherent commercial value as highly prized food product with an increasing demand worldwide. Differently from the premium *Tuber* species (*T. magnatum* and *T. melanosporum*) that are patchily distributed in limited areas in southern Europe, *T. borchii* has a broad range throughout Europe, from southern Finland to Sicily, from Ireland to Hungary, from Poland to Portugal. The wide host range and ample variety of habitats enclosing multiple soils and climates, ranging from the Mediterranean basin, subtropical and temperate forests to boreal forests allow the cultivation of this species also in non-endemic areas, where other species cannot grow. Truffle fruit body production usually occurs after 3-5 years of seedling establishment in the field. However, the influence of site factors and management practices in the development of truffières, their successful establishment, maintenance, and productivity, as well as the mycorrhizosphere signaling pathways, are not thoroughly understood. The knowledge about suitable *Tuber* traits for cultivation, either by increasing production or preventing undesirable characteristics, is still insufficient. We attempt to assess direct and indirect impacts of *T. borchii* cultivation practices in soil properties and processes, underlying genetic and physiological mechanisms that potentially promote/compromise the symbiosis, and interpreter the *T. borchii* cultivation connected to soil functions. Two field trials were established in Alentejo region (spring 2010, 2011) for *T. borchii* cultivation. The field trials include: i) introduction of plantlets of *Quercus suber* (Qs) and *Pinus pinea* (Pp) inoculated with *T. borchii*; and ii) inoculation of adult trees of Qs and Pp with pellets of *T. borchii*. In this method inoculations are performed producing root traps in the drip zone of trees, which are then inoculated with native Mediterranean *T. borchii*. The cultivation practices included mobilization to clean the soil and balancing the pH to favour the *T. borchii* establishment. We focused our study on the estimation of colonising progress of the *T. borchii* and its competitive ability over the ECM fungal community by combining biochemical and molecular tools. We also collected environmental and ecological data, which include vegetation and ECM fungal community assessments, and soil physical-chemical parameters. *T. borchii* traits, environmental and ecological data, land-use variables were analysed using different types of GIS functions/extensions. Results are discussed in terms of the cost-effective practices for sustainable cultivation of *T. borchii* and the impact of land use in shaping those interactions.

KEYWORDS
Tuber borchii, sustainable cultivation of truffles, truffle traits, communities processes above/below-ground, Mediterranean forests.

Out of the Wild : toward cultivating the Burgundy Black Truffle in the Central USA – A holistic undergraduate research team effort

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Abstract

Students' introductions to research often focus narrowly on aspects of much larger systems that they never fully appreciate. This lack of broad understanding narrows the intellectual growth of undergraduate students and limits their eventual contributions as members of research teams. The objective of our "Mizzou Advantage" program Undergraduate Research Team effort was to give a small, highly interdisciplinary and highly motivated team of undergraduate students and faculty the opportunity to explore all facets of the Burgundy truffle life cycle, with a view to its eventual commercial cultivation in the central USA and the associated transformational growth of regional food- and hospitality-based industries. Our group included mentors and students from Mycology, Agroforestry and Horticulture, Biochemistry and Bacteriology, Food Science, Soil Science and Microbiology, Rural Sociology, Agriculture Education, and Sustainable Agriculture (individuals whose diverse interests were piqued by the project résumé, and who might otherwise never have discovered one another). The initial learning curve was steep, as the entire team studied the nature of mycorrhizal relationships in general, and then what is known of truffle biology specifically. Information gaps were identified, and sub-teams took on more focused challenges while remaining connected to the larger group. A Burgundy truffle "banquet" introduced the team to the potential rewards of truffle cultivation in Missouri. Visits to a nascent truffière and to the highly innovative Forrest Keeling Nursery lent a sense of imminent implementation to our efforts. One sub-team focused on identification of bacteria which may enhance Burgundy truffle survival and fruiting. Another sub-team evaluated soil amendments (including biochar) that might prove useful to improve soil structure and nutrition while delivering truffle and bacterial inocula. A third sub-team worked on a guide for potential "trufficulteurs", delineating proposed inputs and activities required to establish a productive truffière. Although the program lasted only 9 months, substantial information was generated, enduring relationships were formed, and a novel approach to undergraduate education was validated.

KEYWORDS
Burgundy truffle, *Tuber aestivum,* cultivation, biology, agroforestry, sustainable agriculture, interdisciplinary undergraduate research.

Empirical or rational truffle cultivation? It is time to choose

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Abstract

Up to now truffle cultivation methods were empirical. They were based on processes applied in France by our ancestors during XIX th Century and up to the first world war, as well as noting the conditions under which the fungus developed spontaneously and its fruiting. This has given irregular results and hampered the realisation of the full value of the truffle mycorrhizal young plants, available now for 40 years.

On the other hand, the rational methods are developed from scientific knowledge from the biology of the fungus and its host. There are two basic principles to take into consideration: first, the dynamic system between the roots and their truffle mycorrhizae, second, the way the fungus feeds itself and in particular its saprophytic capacity to use complex components such as tannins. Respecting these two principles means that there must be different soil maintenance techniques; it must ensure cohabitation of areas where the mycelium is present and active, and areas where it is absent.

In the areas where the truffle mycelium is present, the soil must be worked to aerate it and loosen it; the truffle must be 'helped to form its brûlé', particularly when its environment is contrary (superficial soils, heavy soils). When the fructification has ended, the aim is to cut the roots to allow a regeneration of the root system to also encourage new local growth of mycorrhizae, but also to help break down existing roots to encourage their decomposition and the release of nutrients and tannin components sought by the truffle mycelium and the truffle. Root pruning indirectly provokes the slowing down of the development of the canopy forcing the tree to use its resources to regenerate the root system at the detriment of its foliage.

Contrary to what is advised in all referenced guides for truffle cultivation, the work must be carried out sufficiently deep from the beginning, and not superficially. It must be carried out early in the season, manually or with agricultural adapted equipment (discs, not hooks), aside of the tractor.

The trees must be hard pruned, not only in winter but in summer also. It must be carried out early and followed up regularly. They are several objectives: indirectly renew the root system, control the foliage water evaporation in summer, limit the tree growth and foliage development and indirectly the root system to reduce a development assessed too great, to thin the outer foliage of the tree to allow the penetration of the sunrays, to provide a mulch with the trimmed branches to cover and protect the 'brûlés' in the summer and during the heat waves.
Only the careful application of this rational method in truffle cultivation could lead to an intensive truffle cultivation: first truffles harvested four years (even three) after plantation, whatever the species of the tree used, high proportion of productive trees at four years (more than 70%), possibility to plant at high density (more than 500 trees per hectares for *Tuber melanosporum*), sustainable production (more than thirty years).

The Europeans who do not have access to vast areas to cultivate truffles, like others on the Southern hemisphere, such as Australia, will only remain competitive by utilizing methods to obtain early, heavy and sustainable harvests.

KEYWORDS Truffle cultivation, root system, mycorrhization dynamics, root regeneration, saprophytic capacity, pruning, soil work, adapted tools.

Towards an European regulation on the quality of truffle mycorrhized seedlings

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Abstract

The successful production of truffle by cultivated orchards has improved markedly in recent years as a result of the inspection and certification of mycorrhizal plants, mandatory in some European countries, and encouraged by many commercial nurseries and truffle growers. Methods of truffle-seedlings certification vary from country to country and even within the same country, making the comparison at least impossible. For example in Italy, although an official method is present, providing the counting of ectomycorrhizae in several portions of the root system, most of the controllers adopt a personal method faster and quicker than the official one, essentially based on visual estimation of the mycorrhization level in the entire root system. Moreover, all the current methods, does not agree on the amount of ectomycorrhizae which a mycorrhized-plant must possess to be certifiable and then regarded as suitable for the purposes of truffle cultivation.
For several years it is increasingly urgent the need to identify an official method that can be used throughout Europe and finally making it possible to standardize and make comparable the quality of the truffle-seedlings produced and commercialized in different countries.
Following several years of investigations on mycorrhized-seedlings produced by different Italian nurseries and subsequent findings in the field, we propose a new method for the analysis and certification of *Tuber*-infected seedlings.
The proposed method consists of a first part which provides the evaluation of a mycorrhized-seedling by the visual evaluation of the percentage of truffle ectomycorrhizae in the whole root system. In cases of contention or when is not possible to evaluate, with absolute certainty, whether or not a plant exceeds the quality control, then you switch to the count of vital ectomycorrhizae present in certain portions of the root system. Furthermore is being established, the minimum percentage of ectomycorrhizal tips that a plant must have to be certified as well as the limit of any other mycorrhizal contaminant. In the second part of the method has been defined the criteria for considering a homogeneous batch of mycorrhized-seedlings, the minimum percentage of plants to be analyzed, the sampling procedure and criteria for considering a batch of truffle-seedlings finally suitable for truffle cultivation. In the last part it is also determined the maximum period of validity of a certificate issued for a batch of truffle-seedlings.
Finally, it is hoped that a committee of experts would be instituted and work on the proposed method, make appropriate changes, translate it in all European languages and propose it as the European official method of analysis of mycorrhized truffle-plants.

KEYWORDS

Mycorrhized-seedling – mycorrhization control – certification.

Comparative analysis of different methods for evaluating evergreen oaks mycorrhized with black truffle

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Abstract

Controlled production of mycorrhized plants with black truffle (*Tuber melanosporum* Vittad.) is nowadays one of the most important factors for truffle cultivation. In Spain there is, however, a lack of legal framework that regulates the production, and certifies the quality and the purity of mycorrhized plants. Any type of certification should include at least a series of broad controls that guarantee good practices within nurseries dedicated to mycorrhized plant production.

There are five commonly used and documented methodologies for assessing and certifying mycorrhized plants. Three of them are used in different Spanish regions: University of Lleida (Fischer and Colinas, 1996), CEAM-Valencia (Reyna, 1997), and CITA-Aragón (Palazón et al., 1999). The other two methods are commonly used in France, INRA-ANVAR (Chevalier, 1972), and in Italy, University of Peruggia (Bencivenga, 1987). All five methods were applied to mycorrhized plants coming from two different nurseries, evaluating 6 batches with 12 plants per batch. We also measured different parameters related to forestry quality, as well as the time needed to carry out each method, its level of difficulty and its efficacy.

A high correlation between the percentage of mycorrhization given by each method and the capacity of the method to evaluate the different batches was found. The advantages and inconveniences of each method are discussed. Including criteria that guarantee the absence of invasive mycorrhizal fungi found in our ecological niche is another important factor to include in future unifying regulations.

Globally, results obtained herein give valuable information toward a much-needed unification of evaluation criteria.

KEYWORDS

Quercus ilex, Tuber melanosporum, certification, nurseries, mycorrhization.

Production of quality seedlings of *Quercus ilex* and mycorrhization with *Tuber aestivum*: a comparative of substrates with an high fertilization

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Abstract

Low fertilization regimes is widely considered to increase seedling mycorrhization rates. One aspect of forest nursery management is to produce quality seedlings with high survival capacity and high growth rates in dry and oligotrophic plantation sites Also, much evidence indicates that increase in size and tissue nutrient concentration improves seedling survival and growth rates in Mediterranean forest plantations -in the case of holm oak is recommended fertilize with no less of 75 mg N/seedling-. An experiment has been carried in order to study the effect of four substrates commonly used in forest nursery production with an high nitrogen fertilization regime on the mycorrhization of *Quercus ilex* seedlings inoculated with spores of *Tuber aestivum* (a production of quality seedlings). Substrates were: sterilized calcareous soil mix with perlite (1:1), unsterilized fertilizated peat moss, unsterilized fertilizated peat moss mix with vermiculite and finally, unsterilized unfertilized peat moss. With the purpose to obtain a substrate pH of 7.5 to 8.0, fine-grained calcium carbonate ($CaCO3$) was added to peat moss. Acorns pregerminated have been inoculated at 3-month-old in 0,4 L pot. Ascospore inoculum was produced by dried and pulverized fruiting bodies of *T. aestivum*, suspending the fragmented fertile in tap water. 1,0 g of fresh truffle was inoculated on each seedling pot. The seedlings were housed in a greenhouse with an average daily temperature of 15-30°C, were well irrigated and were fertilised with 120 mg of N/year for seedling at the end of experiment. For each treatment, the percentage of mycorrhization, morphological and physiological attributes was measured. All treatments have presented a good mycorrhization level. Morphologically, in soil with perlite has produced the worse seedlings. Physiologically, the use of peat moss mixed with vermiculite is no recommended. Stock quality mycorrhizal seedlings is possible with common forest nursery management.

KEYWORDS

Quercus ilex, Tuber aestivum, nursery fertilization, quality seedling.

Proposed visual assessment-based method for inspecting mycorrhized plants

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Abstract

While the importance of a high level of mycorrhization of the root system of these plants has long been recognised, the problem of their widescale inspection has not yet been fully resolved. Generally speaking a good method of inspecting mycorrhized plants produced in a nursery must meet several basic criteria: it must be precise, reproducible, but above all must be fast and easy-to-use. Hence practicality is assessed in terms of time taken to assess correctly the degree of mycorrhization and intrinsic ease of calculation in order to extrapolate this result to the [plant] batch. Previous methods involving counting root apexes falling within random areas or counting root apexes sampled, from the proximal to the distal portion, are difficult to use on a large scale due to the amount of time spent gathering samples and carrying out the subsequent count. The method proposed by us employs an ordinal scale to assess the mycorrhization of plants. The operator assesses the degree of mycorrhization of each plant sampled, judging whether or not this is valid using the visual assessment method that does away with the need to make a physical count of mycorrhizae present. It is therefore necessary to standardize the capacity of operators to discriminate in this way in order to harmonize their decisions, quantifying their sensitivity and specificity. Sensitivity is the rater's ability to identify correctly as "well-mycorrhized" any plants judged valid through the application of a quantitative method. It is an index that varies between 0 (the operator doesn't recognize any well mycorrhized plant) and 1 (the operator recognizes all well mycorrhized plants. However, on examining this matter further, we reach the conclusion that this ability alone is not enough. Indeed, something more is required; specificity is in fact the ability to identify correctly plants that are not "well mycorrhized". This also is an index varying between 0 and 1. An operator's specificity and sensitivity may be represented on a graph using ROC curves. Appropriate statistical tools exist for this purpose, such as ROC curves for example (which give a graphical representation of the discriminatory characteristics of raters) and Cohen's Kappa. Therefore it is possible to deduce the quality of the original batch from the rater's decisions; in any case the common criteria applied by experts in the sector need to be defined. Furthermore, it is advisable to continue to use a method based on an actual count of mycorrhizal apexes in the event of disputes. It should be noted that in Italy, visual assessment based on the above methods has already been approved as a method for certifying mycorrhized plants in the Emilia Romagna and Marche Regions.

KEYWORDS

Ectomycorrhiza, estimation, ROC curve, truffle infection.

Critical points in the production of the mycorrhized seedlings of *Quercus ilex* subsp. *ballota* x *Tuber melanosporum*

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Abstract

Among the activities carried out by CEDER-Serranía de Cuenca and the Asociación de Desarrollo Rural Molina-Alto Tajo, in the Project MICOTRUFA, we evaluated more than two hundreds seedlings of *Quercus ilex* subsp. *ballota*, 1 to 2 years old, mycorrhized with *Tuber melanosporum*. In total, 16 lots of seedlings, from 7 nurseries (from four Spanish autonomous communities: Aragón, Comunidad Valenciana, Castilla León and Castilla La Mancha) were analysed. We studied whether the lots complied with the parameters recommended for the sale and distribution of forest materials for reproduction purposes, as described in Royal Decree 289/2003, 7 March, paying particular attention to age and size criteria and quantitative norms (height of the aerial part and root neck diameter) and criteria related with rootball adequacy and qualitative norms (damage or wounding of plants, root deformations, multiple stems). To quantify the degree of mycorrhization the method described by Fischer and Colinas (1997) was followed. The results indicated that the lots in general did not conform to the criteria defining forest quality materials. As regards the mycorrhizal quality of the plants (taken as the degree of colonisation), it was very heterogeneous and clearly influenced by the inoculation method and time at which it was evaluated. In light of the results, we define a set of critical points for the production of plants mycorrhized with *Tuber melanosporum*, based on which modifications that improve nursery inoculation methods and cultivation are recommended.
This work was supported by MICOTRUFA-project

KEYWORDS

Quercus ilex subsp. *ballota, Tuber melanosporum*, micorrhization, quality, nursery.

First results of truffle plantations in Austria – is ectomycorrhiza development a good predictor of truffle harvests?

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Abstract

We report results of controls of ectomyorrhiza (ECM) development in Burgondy truffle (*Tuber aestivum* s.l., incl. *T. uncinatum*) plantations established between 2003 and 2007 in Austria, and first truffle harvests. Analysis of mycorrhizal samples was done as a service on request by plantation owners, or as part of the monitoring of cooperative experimental plantations. Mycorrhizal samples were taken between 2 and 9 years after plantation from the upper soil layer (0-30 cm). Mycorrhizal samples were analysed under a dissection miroscope, assisted by microsopy of mantle preparations. Occasionally, DNA tests were used to control the results of microscopic species identification.
In all 8 plantations controlled, *T. aestivum* ECM were still present, the intensity of mycorrhization with *T. aestivum* and with other fungi was variable. All most frequently used host trees (*Carpinus betulus, Corylus colurna, Corylus avellana, Quercus cerris, Q. pubescens, Pinus nigra*) presented a high level of mycorrhization with the target truffle species in at least some cases, while in others competing ectomycorrhizal fungi had developed and sometimes taken over.
The first Burgondy truffle was harvested in 2008, under *Corylus colurna*, in a plantation with about 150 mycorrhized trees (*C. colurna*). In 2012, three *Carpinus betulus* trees (7 years after out-planting) and one *Corylus colurna* tree (8 years after out-planting) produced first Burgondy truffles in the oldest part of our cooperative experimental plantation in Lower Austria. In this part of the plantation, *T. aestivum* ECM are less abundant than in other, mostly younger plantations, despite seemingly favourable edaphic and climatic conditions. In most samples Burgondy truffle had lost dominance and was in part replaced by other species, most frequently by *Tuber rufum*. Moreover, *Tuber rufum* ascocarps had been observed already two years before the first harvest of *T. aestivum,* along with *Hymenogaster* sp. and *Hebeloma* sp. The three producing *Carpinus betulus* trees are no exception to this tendency of replacement of *T. aestivum* ECM – on the contrary, in in the investigated root samples *T. aestivum* ECM were hardly detectable, if at all. In samples from the producing *Corylus colurna* tree, *T. aestivum* accounted for about one half of the ECM tips. A more detailed analysis of the ECM community in this plantation is in progress.
The very low quantity of *T. aestivum* ECM in samples from producing *Carpinus betulus* trees challenges the predictive power of the analysis of ECM communities. The relationship between ectomycorrhizal abundance, community structure and productivity merits further study. Future observations will show 1) whether the productivity of *T. aestivum* can persist if the species is far from being dominant, or if the replacement by other ECM fungal species will continue and 2) whether it is possible to influence the outcome of interspecific interactions by plantation management.
In January 2013 we found first ascocarps of Périgord truffle (*Tuber melanosporum*) and winter truffle (*Tuber brumale*) in a private truffle orchard planted in 2005 in a garden about 60 km south of Vienna. Ascocarps were submature and damaged by cycles of frost and thawing. This is the first report of a harvest of these species from a plantation in Austria. According to available data, the natural range of *T. melanosporum* does not reach Austria.

KEYWORDS
Truffle cultivation, competition, experimental ecology.

Persistence and detection of black truffle ectomycorrhizae in plantations

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Abstract

The presence or absence of black truffle mycorrhizae and mycelium in a plantation cause continous doubts to its owners. Until the appearance of burnt areas or the start of the sporocarps production, at least 4-7 years after the seedlings plantation, farmer is not able to know if the handling that he is carrying out is correct or not.

In order to study the presence and abundance of *Tuber melanosporum* ectomycorrhizae among plantations located in Teruel province (Northern Spain), we analysed 48 truffled evergreen oaks of three different age groups, half of them already producing black truffles and the other half non-producing. On each tree between five and 10 samples of roots were performed, depending on its age. Two different sampling methods were also applied: direct sampling of root tips and soil cores.

The presence (in 46 out of 48 trees) and dominance (65% of root tips colonised) of *T. melanosporum* ectomycorrhizas were widespread throughout the studied plots. Its abundance is significantly higher in productive trees and also in young trees. No differences were found when comparing the capacity of both sampling methods to detect this species, although it was found in a higher percentage of soil cores than of samples obtained by direct sampling.

In order to know how many samples should be taken to detect the presence of *T. melanosporum* in a given plantation with more than 95% of probability, we found that it would be necessary to analyze no more than three samples, taking a single sample per one random tree in the plantation. Although the presence of the mycorrhizae is not a sufficient indicator of the future production of black truffles in a plantation, its absence itself could determine the decision-making on its future.

KEYWORDS

Black truffle, *Quercus ilex,* ectomycorrhiza sampling methods.

Monitoring the birth and development of truffles in *Tuber melanosporum* orchads

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Abstract

The presence of mycorrhizal symbiotic plants, soil suitability, temperature and humidity are by general consensus considered decisive factors in truffle production. However, there is no other system, apart from the harvesting of the final product, to experimentally define the environmental conditions which 1) stimulate the formation of primordia and 2) favour their growth to maturity.

In order to fill the above informational gap, in 2007, it was installed a unit to record a continuous,large number of atmospheric and soil parameters within a *Tuber melanosporum* orchard. By analyzing this data, some trends of moisture and soil temperature, correlated with the variations of the production of CO_2 which occur during the summer, have been identified as being a cause of mycelial stress. After periods of eight-ten days since the start of the trends, it was possible to collect primordia of *Tuber melanosporum* using the technique of wet-sieving developed by Pacioni (1985). These events take place several times during the summer and early autumn to coincide with the production of primordia, many of which die or develop too early and consequently rot or are eaten by insect larvae. Those which "sprout" in late summer, show a steady growth in size and reach the mature state. It was possible to identify individual truffles after they had reached a size of at least 6 mm in diameter, and follow their growth (increase in volume and diameter) over time. For this purpose, it was used a particular GPR (ground penetrating radar) able to scan the soil to the depth of one metre, set up so as to discriminate truffles, roots, animals and different types of stones.

The statistical analysis of the parameters observed will be presented to focus attention on the ones which have a key role in the development of *Tuber melanosporum*. The step-wise regression technique, based on meteorological and soil parameters, will be used to describe the development of *T. melanosporum* by a simple regression model.

We have been trying to further improve the monitoring technique also by considering the quantitative changes of the mycelium in the soil and therefore make the method more reliable with a view to employing it to the management of truffle orchads.

KEYWORDS

Cultivation, production, instrumental monitoring, management model, *Tuber melanosporum.*

Counter-season production of truffles - from humble beginnings to an industry

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Abstract

From 1800 to the early 1970s truffle mycorrhized trees were produced by growing seedlings in the root zone of mature trees, a method which has become known as Talon's technique. A shortcoming of this technique is that the seedlings can become infected not only with the desired truffle but a range of other mycorrhizal fungi, and a whole suite of rhizosphere organisms including pathogens and pests. Importation of these plants into New Zealand and Australia was therefore prohibited. However, with the development of sporal inoculation techniques in France and Italy in the early 1970s meant that truffle growing in the Southern Hemisphere could begin with the aim of producing truffles counter-season to the Northern Hemisphere. Practical research eventually began in New Zealand in 1985, the first experimental truffières were established in 1987, and the first truffles were found in 1993. From the very start the research was unashamedly applied and received adequate funding. The proviso was that the research had to be confidential and results unpublished. But all was to change in 1992 when the Crown Research Institutes were established and the measure of the success of research largely shifted from the establishment of new industries for the good of New Zealand to the number and quality of scientific papers.
In Australia in the early 1990s there was no need to repeat the research that had been conducted in New Zealand, so some very large truffières were established right from the outset. These were aided by handsome tax write offs and government grants, unlike New Zealand which removed virtually all agricultural subsidies in the 1980s and discontinued state funding for research on truffles in 1996. As a consequence Australia now produces almost 100 times more truffle than New Zealand. The potential returns from the cultivation of truffles in New Zealand and Australia has not escaped the attention of people in other Southern Hemisphere countries so that Chile has already produced its first truffles, and truffières in Argentina, South Africa and Uruguay are likely to produce within the next 5 to 10 years.
Southern Hemisphere truffières can be viewed as open air laboratories where the development of the inoculant fungus can be followed unshackled by traditional ideas and competing mycorrhizal fungi. Some significant issues have surfaced. For example, in Australia even if both mating types of *Tuber melanosporum* are present, fruiting may still not occur suggesting that other parts of the genetic makeup of inocula, soil microbial diversity, or management practices may be responsible. The introduction of *Tuber brumale* and other contaminating ectomycorrhizal fungi either by accident or negligence has also created many problems. The resolution of these issues are directly applicable to truffle cultivation in the Northern Hemisphere. In our paper we will briefly outline the history of truffle cultivation in the Southern Hemisphere, problems that inoculation methodologies may have engendered, where there are deficiencies in our knowledge, and how this information is relevant to truffle cultivation in the Northern Hemisphere in both traditional and non-traditional countries.

KEYWORDS

Cultivation, truffles, problems, management, inoculation, methodology, Southern Hemisphere, New Zealand, Australia, Chile, Argentina, Uruguay, South Africa.

Lessons learned in twenty years of monitoring mycorrhizal status in cultivated truffle grounds

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Abstract

In 1989 the first orchards of trees inoculated by the black truffle were panted in Navarre. Farmers, over the complete ignorance of the process of their fields, sought technical and scientific advice in the ITGA. In 1992, with the clear objective of contributing to the progress of truffle growing in Navarra, begins a tripartite relationship, Truffle farmers-ITGA-University of Navarra, which has lasted until today,
One of the issues raised in concrete a long these years has been the study of mycorrhizal status of truffle grounds.

At first, it was important to know if the trees were mycorrhizated by the black truffle. If not, see if they were mycorrhizated by others truffle species and what, or by other fungi and identify them to know their role in the process of evolution of trees and in the production time.

Soon, competing mycorrhizae were detected (species of *Scleroderma, Pisolithus, Hebeloma* and other well-known as AD type and SB type but in those years still unidentified, currently already identified as *Trichophaea woolhopeia* and *Tomentella galzinii*).

Twenty years monitoring mycorrhizal status in cultivated truffle grounds, not only of Navarre, also of Aragon, Castilla-Leon and other communities, have allowed us to have at this time an extensive and exhaustive catalog of competing mycorrhizae present in truffle grounds, that collects the evolution in them, from the pioneer species composition in the first moments of planting to the mature production.

Not all recognized mycorrhizal species have been identified yet, but the results achieved by the morphological identification, complemented by molecular techniques allow us to affirm that truffle plantations have a high diversity of mycorrhizal species, among which the genus *Tuber* is represented by many species like *T.aestivum, T.borchi, T.brumale T.excavatum, T.mesentericum, T.panniferum* or *T.rufum*, fruiting also in the fields, some of which, for its abundance, cause disappointment in farmers

Among the other fungi, Thelephoroid species constitute an interesting group with a high number of species that grows in plantations, accompanying productive and unproductive trees. In this case, exploration strategies, such as abundant emanating hyphae and/or presence of rhizomorphs make this group one of the best represented in this environment.

In summary, truffle stands are ecosystem in natural evolution, with a diverse and rich ectomycorrhizal flora. So, truffle production is reached in the conjunction of many factors together with a mycorrhizic fungal cortege.

KEYWORDS

Mycorrhization monitoring, *Tuber melanosporum* stands, ectomycorrhizae, *Tuber,* Thelephoroid.

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Is truffle *brulis* a case of perturbational niche construction?

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Abstract

Niche construction is the process whereby organisms, through their metabolism and activities modify their own and/or each other's niches. It is perturbational when organisms physically change one or more components of their external environments (Odding-Smee *et al.*, 2003). According to these definitions, *brulis* could be a case of perturbational niche construction. It satisfy at least two requirements for nice construction: a working organism and an activity governed by information from prior natural selection which are the *Tuber* species and a biochemical process determining the disappearance of grasses, respectively.
The present investigation was aimed at testing a third requirement - if the disappearance of grasses in the *brulis* induces a non-arbitrary perturbation in the soil physical environment, aggregation of soil particle in particlar – and understanding if these changes satisfy the other requirements for niche construction. The investigation was done comparing a *brulis* and the neighbouring grass-covered area in terms of size of soil aggregates, and testing if changes in aggregation were attributable to freeze/thaw cycles. Since the effect of freezing on soil structure depends on the size of pores filled with water – i.e. from soil water tension, the test was carried out by comparing samples wetted at different water tensions.
The investigation was done in a natural truffle bed using as response variables the 0.25-2.00mm and 2.00-5.66mm aggregate size classes and the mean weight diameter (*MWD*), a measure of the degree of soil aggregation. They were determined in 36 soil samples collected in a *brulis* and its neighbouring grass-covered area according to a systematic sampling design. Data were analysed with a geostatistical approach to compare the spatial pattern of aggregate size parameters with the shape of the *brulis*. A large soil sample was then collected in the grass-covered area and partitioned in 15 sub-samples, three of which were immediately analysed whereas the remaining ones were wetted at four different water tensions, submitted to a number of freeze/thaw cycles equal to those on average recorded in the area and analysed for aggregate size distribution. In the end, data comparison was done with univariate ANOVA.
Results of the geostatistical analysis showed about a 30% of variation in the aggregate size of samples, and a spatial distribution of variables that overlapped the pattern of the *brulis*. The laboratory experiment then showed that freeze-thaw cycles of a single winter season may produce a significant decrease in aggregate size (- 30% at field capacity) compared to the grass-covered area. Both results indicate that the disappearance of grasses in the *brulis* fulfils the requirement of modifications in the physical state of some factors, in this case the physical organization of soil particles and related physical characteristics.
Two further short-term requirements should be met for niche construction: i) energetic profitability, and ii) enhancement of fitness inside the *brulis*. At a first glance both requirements may be fulfilled in the *brulis*, but their quantitative analysis is out of my area of expertise and I can only suggest them to researchers interested soil microbial ecology in relation to truffle species.

Odding-Smee F.J., Laland K.N., Feldman M.W. 2003. Niche construction. The neglected process in evolution. Princeton University Press, New Jersey.

KEYWORDS

Aggregates, *brulis*, niche construction, soil structure.

Physical properties of soils also drive black truffle fructification in plantations

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Abstract

The role of pH and carbonates content in the soils where black truffles (*Tuber melanosporum*) are cultivated has been reported by numerous authors. Nonetheless, little is known about the influence of soil physical properties. This study focuses on the physical soil characteristics that drive the success in the production of black truffles in orchards.

Seventy-eight *Quercus ilex* ssp. *ballota* plantations older than ten years were studied in the province of Teruel (eastern Spain). Soil samples were extracted to analyze the edaphic characteristics and to identify *T. melanosporum* ectomycorrhizae. The influence of cultivation practices, climatic features and soil properties on sporocarps production was assessed.

Low contents of fine earth and silt appear to be promoting fructification, while high levels of bulk density, clay content and water-holding capacity seem to be positive for truffle production. Watering is also highly positive for truffle fructification, as reported by other authors in previous works. A simple logistic model is provided to predict the probability of fructification.
The balance between water availability and aeration play a crucial role in achieving success in black truffle plantations.

KEYWORDS

Bulk density, texture, water availability, aeration, factors triggering truffle fructification, ecology.

Alkalinity, structure and organic matter of soils determine the truffle production in Pyrenean Regions

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Abstract

The program "Typology of truffle stations in Pyrenean Regions" was based on the survey of 213 wild and cultivated truffle beds in Navarra, Catalonia, Midi-Pyrénées and Languedoc-Roussillon. The data collected in the field consisted of photographs and samples of soil, roots and mycorrhizae. The success of this program was based on the close partnership with regional unions of truffle hunters and growers that have agreed to open their truffle beds and share their knowledge with scientists. Statistical analysis has highlighted several key findings:

1. Mycelium that colonizes roots, survives but does not necessarily bear ascocarps: the biological analyses show that the roots of planted truffle trees, which have not yet borne, or which had already borne but bear no longer, ascocarps are often heavily colonized by *Tuber melanosporum*. Planting mycorrhizal plants has led to the establishment of the mycelium, which can persist for many years on the roots of trees, but the mycelium does not necessarily bear ascocarps. It is therefore essential to distinguish the vegetative phase (root mycorrhization and development of truffle mycelium) of the fruiting phase (sexualization and production of ascocarps).

2. Light soils, which remain light even when they are dry: the soils most favorable to the fruiting of *Tuber melanosporum* are always light, as can be the soil of a garden. They can be dug by hand without effort. This property is based on a low cohesion of the material, which may result from several factors: a granulometry from sandy to sandy-loam, a high content of iron oxides or organic matter well decomposed that improves the soil structure, or frequent tillage of the soil surface.

3. Alkaline, neutral, dolomitic or moderately calcareous soils: *Tuber melanosporum* is known to thrive in limestone soils. But our results lead us to specify this statement: many wild truffle beds are developed on dolomitic sandy soils which are not calcareous, and some wild truffle beds can develop on slightly acidic soils locally re-carbonated by debris or colluvium. Moreover, our results show that wild truffle beds are sparse on very calcareous soils, which therefore are not the best for truffe production.

KEYWORDS

Mycorrhizae, Production, Soil properties, *Tuber aestivum, Tuber brumale, Tuber melanosporum.*

Calcium, key to truffle development and truffle cultivation

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Abstract

The natural geographic area where the Perigord truffle (*Tuber melanosporum* Vitt.) in Southern Europe (France, Italy, Spain) is not due to factors of the soils, but the climatic requirements of that species which is thermophilic. On the other hand, the Burgundy truffle (*T. uncinatum* Chat. syn. *T. aestivum* Vitt.), able to withstand more the cold temperatures, is found all over Europe, from Spain to Sweden, and Ireland to Belorussia and Ukraine. It is even found in Armenia and Azerbaijan.

The truffle geographical distribution is due to the existence of exchangeable calcium in the soil. Both species can grow in soils without calcium carbonate, but they must at least be calcic. In France, in Eastern Pyrenees, *T. melanosporum* is harvested in soils derived from the decomposition of calcic granites. The minimal rate of exchangeable calcium is around 3 per thousand for the formation of both species. The more the soil is light, sandy and non clayey, the lowest the rate can be.

Truffle cultivation is therefore subject to how rich in calcium the soil is; the rate of which in the soil will have to be checked regularly. The management of the calcium by a calculated addition of chalk , lime and/or calcareous gravel, either by raising its quantity in soils too low in this element, or to introduce it in soils where it is lacking completely, is one of the key solutions in rational truffle cultivation. Truffle cultivation is therefore possible in soils without calcium, but recarbonated. The examples are seen frequently in the Northern hemisphere (U.S.A., France, Spain), and in the southern hemisphere (New Zealand, Australia).

The dependence of the two species on calcium (even more pronounced for *T. melanosporum*) conditions their competitiveness amongst other mycorrhized fungi which are calcifugous. As a result, the absence in the soil of calcium before planting could hold a certain advantage for truffles, by the absence of propagules from calcicolous mycorrhizal fungi which could be damaging to the truffle.

KEYWORDS

Tuber melanosporum, T. aestivum, T. uncinatum, truffle cultivation, liming, exchangeable calcium.

First identification of the pathogen causing tumor malformations of evergreen oaks in Spain

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Abstract

In recent years it has been detected in Spain an increase in pests and diseases associated to truffle plantations. The appearance of tumor malformations in trunks and branches of *Quercus ilex* L. highlights. These bumps, occasionally described in the eastern mountains of the Iberian Peninsula, have expanded dramatically from the increase in the number and density of truffle plantations. This pathology is placed not only in plantations, but also in forests, and in all age trees. In fact, damages have been also detected in nurseries.

Disease manifests itself as an irregular thickening of wood from branches of any age and from main trunk until the woody tissue is opened, forming wounds. The affected branches usually undergo necrosis and in case of affect the main trunk, the affected tree dies.

After an extensive literature review and failed attempts of isolating fungal and bacterial species of these tumors and wounds, it was found that the possible disease-causing organism corresponds to a *Candidatus Phytoplasma*. Using two types of PCR: Real-Time PCR and PCR-nest, carried out with primers that amplify 16S ribosomal gene sequences, common to all known phytoplasmas, positive results have been obtained. Thus one phytoplasma could be implicated in the development of this disease. Furthermore, It has been confirmed the presence of this organism in phloem tissue from wood by DAPI stain (4 ', 6-diamidino-2-phenylindole) for epifluorescence microscopy.

In order to start controlling this disease we are currently identifying the possible methods of transmission of the organism both mechanically and by means of seeds or vectors, as well as the actual distribution of the disease in the Iberian Peninsula.

KEYWORDS

Candidatus phytoplasma, real time PCR, DAPI, *Quercus ilex.,* black truffle.

Preliminary study of the mites community structure in different black truffle producing soils

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Abstract

Soil biodiversity is important to agricultural productivity and ecosystem health. The relationship between soil fauna and biology of *Tuber melanosporum* Vittad. has been among the less studied aspects of its ecology. Only Callot (1990) wrote about the importance of the soil fauna. One of the least studied the mites (Acari), which help regulate relationships between microbes and fungi and contribute to spore dispersal. The importance of mites to fungus biology and ecology led us to begin identification of the mites associated with the black truffle in productive soils both in wild areas and orchards.

The study was carried out in two black truffle production zones in Navarra (northern Spain) with four samples from four different orchards and five from the corresponding natural production areas. The fauna was extracted with Berlese-Tullgren funnels. Animals were categorized by taxonomic groups, and mites were classified to species.

The purpose of the study is to analyze the composition and community structure of the different biotopes, by use of parameters such as abundance, species richness, real diversity (H') and equitability (J'). In addition, a dendogram was constructed to observe affinities between communities and species.

In all, 305 mites were identified, belonging to 63 species from the four major taxonomic groups (Oribatida, Prostigmata, Mesostigmata and Astigmata) that compose the edaphic community. The results show a possible trend that natural production soils have greater diversity and species richness than orchard soils. Furthermore, community analysis shows differences in species composition in different study areas. Three families, nine genera and five species were recorded for the first time for the Iberian Peninsula.

Fesults from the preliminary study provided the basis for a more comprehensive, current study designed to more accurately determine oribatid mite community structure throughout the year in different areas of production.

Callot, G. 1999. La truffe, la terre, la vie. INRA Editions. 210 pp.

KEYWORDS

Acari, *Tuber melanosporum,* mite communities, oribatid, truffle orchards.

The truffle beetle *Leiodes cinnamomeus* (Coleoptera, Leiodidae), key pest of black truffle (*Tuber melanosporum*) in Central Spain: bioecological contributions and mass trapping control

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Abstract

The truffle beetle *Leiodes cinnamomeus* (Panzer) is one of the most important mycophagous insects producing economic losses to black truffle (*Tuber melanosporum* Vittadini) in Europe, becoming the primary pest of this fungus in Central Spain. Other mycophagous insects, such as truffle flies *Suillia* spp. (Diptera, Heleomyzidae), produce less damages than the truffle beetle, despite of its frequency in the field, so they can be considered secondary pests. In this producing area, at Soria province in the region of Castilla y León, the black truffle is a valuable economic resource, but *Leiodes* is a limiting factor due to the galleries its larvae burrow in the young carpophores of truffle. Besides, during its trophic activity the larvae produce toxic saliva that rots the carpophores and do it accessible to truffle flies and pathogenic fungus.

During 6 years (2005-2011), a research project has been carried out in one of the biggest plantations of black truffle in the world, property of Arotz Foods SA and placed in Abejar (Soria). In the first two years the bioecology of the insect was studied, establishing life cycle, damage levels and testing different trapping systems and smells. In the third and fourth years, high scale mass trapping trials were carried out with different trap density. Finally, during the fifth and sixth years the trapping system was improved and registered, implementing the mass trapping strategy in the entire plantation for controlling the pest.

The life cycle of *Leiodes cinnamomeus* has been confirmed in our conditions, both in field and laboratory trials, resulting in five stages (egg, larva 1, larva 2, larva 3, pupa and adult) closely related with truffle cycle. The ecology of the pest has been studied, particularly its phenology and population estimates, with three activity peaks in November, January and March. Three levels of damage have been established in order to determine the threshold of economic losses. The trapping system has been improved (receptacle, attractant, trap density), patented and tested in true conditions, with positive results in controlling population.

This work has been possible thanks to the supporting of Arotz Foods SA and, partially, through a grant funded by CDTI (Ministerio de Industria) and ADE (Junta de Castilla y León).

KEYWORDS

Truffle beetle, Leiodes cinnamomeus, black truffle, *Tuber melanosporum,* Central Spain, insect pest, bioecology, mass trapping.

Quantification of extraradical fungal mycelium of *Tuber melanosporum* by molecular techniques

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Abstract

Molecular techniques based on Real-Time PCR have been recently used for quantification of extraradical fungal mycelium in the soil. This technique has represented a step forward to undertake studies on spatial-temporal distribution of the extraradical fungal mycelium, usually neglected because of the limited detection techniques but considered as the most metabolically active phase of the symbiosis. The Real-Time PCR technology has been adapted for the quantification of soil fungal biomass of key edible species as *Amanita caesarea*, *A. ponderosa*, *Boletus edulis*, *Lactarius deliciosus*, *Rhizopogon roseolus*, *Terfezia olbiensis* and *Tuber melanosporum*, both in plantations and natural forests. Taqman® probes, considered highly specific, were designed for the different target fungi using the appropriate software. Standard curves were made with DNA extracted from known amounts of tissue belonging to the different fungal species (either mycelium cultures or young sporocarps) mixed with the target soils to take into account the presence of possible inhibititors of the PCR. Fluorescence values corresponding to soil DNA extractions from each site were interpolated in the corresponding standard curve. The limit of mycelium detection was, approximately, 2 µg mycelium per g of soil. Soil samples taken from the burnt areas of seven plantations and a natural truffle ground producing *T. melanosporum* were sampled to track the persistence of the inoculated fungi and the dynamics of the symbiosis in a non-destructive way. The extraradical mycelium biomass detected in the soil from the natural truffle ground was significantly greater (up to ten times higher) than the mycelium biomass detected in any of the orchards. Soil from productive, nonirrigated orchards contained significantly more extraradical mycelium than the rest of orchards irrigated, productive of *T. brumale*, or nonproductive. The obtained quantitative results are of special interest to evaluate the fungal response to cultural treatments and to monitor the seasonal dynamics of the extraradical mycelium of *T. melanosporum* in the soil. In conclusion, Real-Time PCR is a promising technique for non-destructive assessment of fungal persistence since soil mycelium may be a good indicator of root colonization. However, the accuracy of the technique will ultimately depend on the development of appropriate soil sampling methods because of the high variability observed.

KEYWORDS

Tuber melanosporum, Truffle orchards, Extraradical mycelium, Real-time PCR.

Integrated approaches to evaluate the productivity of *Tuber melanosporum* in a model plantation

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Abstract

Natural production of *Tuber melanosporum* is almost exclusively in France, Spain and Italy, which are the main distribution areas for this species. Cultural practices have been developed in many countries throughout the world. Nevertheless cultivation requires long-term investments: production usually begins 6–10 years after seedling inoculation. The fruiting body is usually collected in the burned area, an area devoid of vegetation near or around the host trees, where the truffle competes with other ectomycorrhizal fungi (Napoli et al., 2010). Nevertheless, potential truffle yield remains highly unpredictable. The genome sequencing of the ectomycorrhizal fungus *Tuber melanosporum* has revealed that the fruiting body production depends on the availability in the soil of two mycelia with opposite mating types (Martin et al., 2010; Rubini et al., 2011). This finding has suggested that seedlings produced for truffle-culture programs have to be inoculated with both the mating types. In order to investigate if the productivity is also correlated to a certain amount of *T. melanospoum* in the soil, in addition to the co-presence of mating type genes, we set up a protocol in a model plantation presenting productive and unproductive trees. The quantity of *T. melanosporum* in soil samples was assessed by quantitative PCR (qPCR) on ITS region and its mating types were searched. Results showed that mating type genes were detected in the stand under productive trees and when more than 0.3 ng of *T. melanosporum* DNA was present (Zampieri et al., 2012). Up to now the establishement of a *T. melanosporum* plantation has been exclusively based on soil features. In future, the proposed analyses can help truffle operators in the management of their plantation by attesting the occurrence of *T. melanosporum*, after seedling inoculation and before the harvest of the fruiting bodies.

References

Martin et al., (2010). The black truffle genome uncovers evolutionary origins and mechanisms of simbiosis Nature, 464, pp. 1033–1038.

Napoli A, Mello A, Borra A, Vizzini A, Sourzat P, Bonfante P. (2010). *Tuber melanosporum*, when dominant, affects fungal dynamics in truffle-grounds New Phytology, 185, pp. 237–247.

Rubini A, Belfiori B, Riccioni C, Tisserant E, Arcioni S, Martin F, Paolocci F. (2011). Isolation and characterization of MAT genes in the symbiotic ascomycete *Tuber melanosporum*. New Phytology, 189, pp. 710–722.

Zampieri E, Rizzello R, Bonfante P, Mello A. (2012). The detection of mating type genes of *Tuber melanosporum* in productive and non productive soils. Applied Soil Ecology, 57, pp. 9–15.

KEYWORDS

Tuber melanosporum, mating type genes, truffle-ground soil, qPCR.

The evolving soil fungi community associated with the establishment of black truffle plantations reflects an increasing dominance of *Tuber melanosporum* with the concurrent decrease in soil saprotrophic and pathogenic fungi

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Abstract

The decline in natural black truffle (*Tuber melanosporum*) production in its native Mediterranean habitat has led to the development of cultivation methods that have resulted in the establishment of extensive black truffle plantations, with variable measures of successful ascocarp (truffle) production-an important part of the fungal life cycle. One of the factors of interest in understanding the life cycle of this fungus is the diversity of the soil community in which this fungus interacts. Studying the diversity of soil fungi communities is challenging, in part, because it is impossible to observe their vegetative and reproductive structures belowground. In this study we apply molecular techniques to examine the evolving soil fungi community found beneath *Quercus ilex* trees inoculated with the black truffle in established plantations using a chronosequence design with host trees of six ages (3, 5, 7, 10, 14 and 20 years old) in Teruel (eastern Spain). Soil samples were collected at 30 cm in depth and at a distance of 40 cm from the trunk of host trees. Soil DNA was extracted and amplified using ITS1F and ITS4 fungal primers and then cloned and sequenced to compare soil fungi composition among all ages. We obtained 480 sequences that include 89 unique operational taxonomic units (OTUs), with 27 contigs and 62 singlets. Of these OTUs, 59 belong to the phylum Ascomycota and 13 belong to the phylum Basidiomycota. Among all OTUs, 68% could be identified to species level, 92% could be identified to genus level, and 99% could be identified to phylum level. Out of the 228 successfully sequenced colonies, the most abundant and fully identified species were *T. melanosporum* and *Fusarium oxysporum*, which represented 34% and 13% of all OTUs. *T. melanosporum* was the only *Tuber* species recovered in this study and was present in all six age classes. As tree age increased we observed a remarkable increase in the abundance of *T. melanosporum* and the subsequent decrease in *Fusarium* and *Phoma*. In the 10 year-old plantation, we no longer detected *Phoma* sp, and *Fusarium* was represented by only 1 of 31 OTUs in the 20 year-old tree, where *T. melanosporum* comprised 71% of sequences. This inverse relationship between the frequency of soil saprotrophic and pathogenic fungi and that of *T.melanosporum* provides a new insight into the functional community that develops with the dominance of *T. melanosporum*.

KEYWORDS

Tuber melanosporum, soil fungi diversity, *Fusarium*.

Survival of *Tuber melanosporum* mycorrhiza in sub-optimal climatic conditions

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Abstract

Climatic variables are known to be very important in the successful cultivation of *Tuber melanosporum*. Monthly temperatures, sunshine hours and precipitation levels can all have an impact on the success of a plantation.

In Europe, the truffle species *Tuber melanosporum*, has a very clear ecological niche. The northern boundary of this species within Europe is thought to be dictated by climatic parameters. Cultivation is rarely attempted outside of areas within these climatic boundaries.

The survival and fruiting ability of *Tuber melanosporum* outside of its natural climatic range has not been widely tested. From 2007, trial plots of *Tuber melanosporum* inoculated trees were established across England (UK). The latitudes of these plots are far beyond the natural extent of *Tuber melanosporum* in Europe. The growth of the out-planted inoculated trees was monitored and the survival of the *Tuber* mycorrhiza was annually logged. Data was collated from 5 geographic regions with distinct climatic variation. Ranges include precipitation from below 500mm to over 1,000mm per annum and recorded sunshine hours across all sites were significantly below those found in areas where *Tuber melanosporum* is successfully cultivated and naturally exists.

In these sub-optimal geographic zones, *Tuber melanosporum* mycorrhiza was sustained and correlation of climatic variables to mycorrhiza survival rates produced several interesting results. The most important climatic variables for sustaining mycrorrhiza in these sub-optimal geographic regions are discussed.

Although, *Tuber melanosporum* has shown an ability to grow and develop well in sub-optimal climatic conditions, the ability to produce fruiting bodies has not yet been recorded. Sampling for fruiting bodies has not yet been attempted on the trial sites within this study.

The ability of the mycorrhiza to grow and thrive in these sub optimal conditions is intriguing and creates questions as to the limiting climatic factors for cultivation of this species. Although fruiting bodies may not be produced in the current climatic conditions of the sites within this study, correlations of mycorrhiza rates with variables such as sunshine hours provide some interesting insights. Relevance for fruiting triggers and cultivation methods is discussed.

KEYWORDS

Climate, UK, mycorrhiza, *Tuber melanosporum,* temperature, precipitation.

Tuber melanosporum rapidly colonizes the soil surrounding its host tree in *Quercus ilex* plantations

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Abstract

Black truffle (*Tuber melanosporum*) is one of the most highly prized edible fungi from Europe. Successful cultivation of the truffle requires a long-term investment in the establishment of young trees inoculated with this ectomycorrhizal (ECM) fungus and the maintenance of the symbiosis throughout its preproductive and productive years. Monitoring the symbiosis is challenging, as it requires methods that can detect the belowground proliferation of the fungus associated with its host tree. In this study we applied a chronosequence design to observe the pattern of expansion of extramatrical mycelium (EMM) of this fungus as the host tree matures. We hypothesized that the dynamics of this expansion can be observed by monitoring the quantity of *T. melanosporum* DNA from soil beneath colonized host trees. Our study was carried-out in established truffle plantations of Sarrión, in Teruel Spain, where we collected soil from beneath trees of different ages (3, 5, 7, 10, 14 and 20 years old) and at 3 distances from the trunk of the trees (40, 100 and 200 cm. We also studied the presence *T. brumale* and *T. indicum*, potentially problematic truffle species in these plantations. Multiplex species-specific primers were used to amplify DNA of *T. melanosporum* and quantification was based on comparison of band intensities from agarose gels. We detected DNA of *T. melanosporum* in 50 of the 54 soils surrounding host trees from the 18 truffle plantations. The main factors, age and distance and their interaction had significant influences on mycelium quantity. We observed greater quantities of *T. melanosporum* mycelium at 40 cm from the tree trunk than at 100 or 200 cm in younger plantations. There were no quantitative differences between 40 and 200 cm from the tree trunk in the older plantation age classes of 7, 10, 14 and 20 years. No DNA from *T. brumale* or *T. indicum* was detected in these plantations. Our results suggest that *T. melanosporum* colonizes the soil surrounding the seedlings in just three years. Subsequently it expands outwards, but its quantity does not increase beyound an apparent maximum.

KEYWORDS

Tuber melanosporum, soil DNA, chronosequence.

Persistence of *Tuber melanosporum* mycorrhizas and extraradical soil mycelium in a *de novo* plantation

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Abstract

When establishing a *de novo* truffle plantation with mycorrhizal plants, one crucial factor is the persistence of the fungus in tree roots and its expansion trough the soil. Using molecular techniques, as real-time PCR, we can detect and quantify the *Tuber melanosporum* extraradical soil mycelium.

In the present study extraradical mycelium and mycorrhization of plants have been monitored in a *de novo Quercus ilex* x *T. melanosporum* plantation, since its establishment (in spring 2010) until 18 months after (autumn 2011). The orchard is located in the oriental Pre-Pyrenees (Fígols, Catalonia) and occupies 0,25Ha. It was planted with 229 nursery inoculated *Q. ilex* plants mycorrhizal with *T. melanosporum*. Interspersed among these trees 14 non-inoculated plants (controls) were randomly planted. Mycorrhizal and control plants and soil samples were taken 6, 12 and 18 months after their establishment (4 soil samples at 5 cm from the trunk of each sampled plant). The number of *T. melanosporum* mycorrhizas of each plant was counted. DNA was extracted from soil samples, and *T. melanosporum* mycelium was quantified by real-time PCR using specific primers and TaqMan® probe. Weather data, available from an automatic weather stations located near the plantation, were used to establish any relation with the analyzed variables (mycorrhization and soil mycelium concentration).

Tuber melanosporum extramatrical soil mycelium was detected from the first sampling (6 months), and although there was an increase of mycelium along time, the amounts were not statistically different. Positive correlation between the abundance of mycorrhizas and the concentration of extraradical soil mycelium ($r=0,7814$; $p=0,0129$) was detected in spring (after 12 months of the plantation establishment), when the number of mycorrhizas per plant was the highest. After one year, mycorrhization (%) increased significantly ($p=0,0236$).

No correlations between soil mycelium biomass and weather conditions were detected. The number of *T. melanosporum* mycorrhizas was positively correlated with mean and maximum temperatures, the monthly average of the daily global solar radiation, the mean evapotranspiration rate, and with the precipitation of the previous month.

According to our results, in this plantation the fungus persisted in the radical system of all nursery inoculated plants and soil mycelium of *T. melanosporum* was increasing with time. Mycorrhization seems to be more affected by climate variations than soil mycelium. TaqMan ® real-time PCR technique is a good method for the traceability of *T. melanosporum* without disturbing host trees, allowing us to detect the persistence and expansion of the fungus through a truffle orchard from its establishment.

KEYWORDS

Tuber melanosporum, Real-time PCR, mycorrhiza, extraradical mycelium.

Genetic diversity and structure of the Iberian populations of *Tuber melanosporum* Vittad.

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Abstract

The black truffle is the fruiting body of the Ascomycete fungus *Tuber melanosporum* Vittad., which is distinguished by its intense taste and odor, making this fungus one of the most appreciated and quoted within gastronomy. Several studies have been focused in breeding, production and determining the principles of the organoleptic properties, but fewer in the population genetics of the species. Nevertheless, some studies have been conducted over the French and Italian distribution ranges. This is the first time that the genetic diversity and genetic structure of the Spanish natural populations have been studied. To achieve this goal, four to fourteen fruiting bodies from different host trees of each population were collected across 23 populations covering almost the entire geographical distribution range of the species in the Iberian Peninsula.

The neutral-dominant molecular marker Inter Simple Sequence Repeats (ISSR) were used. Four of the sixteen assayed ISSR primers were selected for the genetic analyses. This technique demonstrated their reliability and capability to detect high levels of polymorphic bands in the species. Spanish populations of *T. melanosporum* showed high level of genetic diversity (hN = 0.393, hS = 0.678, Hs = 0.418). This indicates a good genetic conservational status of the Iberian populations, and corroborates the heterothallic reproduction of this species, which was suggested recently by other authors. This high level of genetic diversity may be a consequence of the wide range of distribution of the species, of its dispersion by zoocory and by its evolutionary history. Molecular Variance Analysis showed a high degree of genetic structure among populations (47.89%), demonstrating a different evolutionary history of the Iberian populations with respect to the French and Italian geographical ranges. Bayesian analysis of genetic structure differentiated two main Spanish groups separated by the Iberian mountain system, and showed the genetic singularity of some populations (Jaén, Murcia, Navarra, Soria, Guadalajara and Lérida). Our results suggest the survival of some of these populations during the last glaciation, not strictly applied to the southern ones as in the French or Italian distribution ranges, but in specific areas which may have acted as refugia for the later dispersion to other calcareous areas in the Iberian Peninsula and France.

KEYWORDS

Bayesian population analysis, biogeography, genetic diversity and structure, Iberian refugia, ISSR, interglacial expansion.

Large scale identification and mapping of single nucleotide polymorphisms in black truffle (*Tuber melanosporum*) by genome resequencing

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Abstract

Tuber melanosporum is naturally distributed in Italy, France and Spain and it is found in contrasting climates such as mediterranean warm climate in south Spain and continental colder climate in Alsace (France). During the last century the production of *T. melanosporum* decreased dramatically due to rural depopulation, landscape modifications and climate changes. The potentiality of *T. melanosporu*m to adapt to these changes is directly linked to its genetic diversity and reproduction mode. During many years, *T. melanosporum* was considered as a selfing species with a low level of genetic diversity using RAPD and microsatellites markers. Recent studies demonstrated that *T. melanosporum* is a heterothallic species. Moreover, thanks to the genome sequencing of *T. melanosporum* and the progress of next generation sequencing it is now possible to assess the real intraspecific genetic diversity comparing the genome of different isolates.

The aims of this study were 1) to quantify the *T. melanosporum* intraspecific genome diversity by isolate resequencing; 2) to identify and map single nucleotides polymorphisms (SNPs) in the whole-genome of this species and 3) to identify putative genes and genomic regions under selection. To reach these aims six genomes of *T. melanosporum* were sequenced using illumina technology. These isolates were chosen in different populations covering the northern and southern limit of distribution of this species in order to improve the chance to find polymorphisms.

For each genome the ~34 to ~39 millions reads generated were aligned to the reference genome using BWA. The coverage for each resequenced genome was ~20 X and the mapping covered more than 97% of the reference genome. The majority of the reads mapped uniquely confirming that the mapping criteria used were sufficiently stringent to avoid multi-mapping even considering the ~60 % repeated sequences in the *T. melanosporum* genome. From 2,658,743 (2.12%) to 3,218,206 (2.57%) nucleotides present in the reference genome had no equivalent in the resequenced genomes. The *T. melanosporum* core genome, i.e. genes present in all resequenced genomes, was estimated to have 7446 genes. A total of 526,792 SNPs were identified corresponding to one SNPs each 237 bp. SNPs were not distributed equally along the genome since 77 % of them were present in transposable elements. 3,143 genes (42 % of all gene models) presented SNPs in their sequences (intron and exons) and 2,047 (27 % of all gene models) in their coding regions. Statistical analyses using different indexes (e.g. dN/dS and Tajima's D) allowed to identify gene models as well as genomic regions putatively under selection. Moreover, several genes categories seems more rich in mutations although other presented less mutations.

To our knowledge this study present the first large-scale identification of SNPs for a mycorrhizal species by genome resequencing. These results demonstrated that population genomic allows to investigate how truffles could adapt to climate changes.

KEYWORDS

Tuber melanosporum, genome resequencing, adaptation, SNPs, population genomic.

Sexuality in the life cycle of truffles: importance and repercussions of being compatible

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Abstract

The spontaneous production of the most valuable truffles has been facing with a severe decline all over the distributional range of these species. Further to this, cultivation of these fungi is possible only for some species and, in many cases, with controversial results.

About two decades ago, we started studies with the aim to assist truffle growers and nurseries providing them with innovative tools to boost the production of these fungi. The initial goal was to prevent economic frauds and ecological damages linked to the paucity of reliable morphological traits to identify with certainty the truffle species present on the roots of host plants and/or their ascocarps. Several molecular markers (i.e. simple sequence repeat, SSR loci) and methods have then been developed so that it is now possible to type not only the species but also the strains that give rise to fruit bodies and mycorrhizas of the most valuable *Tuber* species.

As a follow up of these studies, we demonstrated that it is possible to differentiate by molecular markers natural populations of both *Tuber melanosporum* and *Tuber magnatum* according to their different geographic provenance (Rubini et al., AEM 2005; Riccioni et al., New Phytol 2008). Even more intriguingly, we found that, within any given truffle population, strains not only share but also exchange alleles with each other. This last finding has propelled basic studies to obtain an advancement in our understanding of life cycle and sexual reproduction of these symbiotic species (Paolocci et al., AEM 2006; Rubini et al., New Phytol 2007). The hypothesis to be tested was whether truffles were strictly selfing species, at that time the most accredited thesis (Bertault et al., Nature, 1998), or, instead, they could outcross as hinted at by our preliminary data.
The sequencing of *T. melanosporum* genome has enabled us to look at the structure and organization of the *MAT* locus that is the genomic region controlling the sexual reproduction strategy of fungi (Martin et al., Nature 2010). As the two alternative genes, known as mating type genes, at this locus are harbored by different strains it has been concluded that *T. melanosporum* is a heterothallic, thus an obligatory out-crossing fungus (Rubini et al., New Phytol 2011a). In turn, this finding has promoted efforts to monitor the spatial and temporal distributional patterns of strains of different mating type in the soil and on host plants, either from natural or man-made truffle plantations (Rubini et al., New Phytol 2011b). Further to this, the mining of the *MAT* genes from *T. melanosporum* genome has set the ground for deciphering the genetic control of the sexual reproduction in other *Tuber* spp.

Here we report on our recent advances on the life cycle and propagation patterns of *Tuber* spp. These findings call for a profound reconsideration of the strategies to inoculate and grow host plants and manage productive truffle plantations. Additionally, approaches to unveil the interplay between environmental and genetic determinants controlling the switch from vegetative to the sexual phase in these fungi are discussed. Understanding the dynamics that govern this transition is crucial to promote truffle fructification.

KEYWORDS

SSR, mating type, MAT genes, mycorrhiza, heterothallism, strain distribution.

Desert truffles in Tunisia: Diversity and cultivation attempts

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Abstract

Desert truffles (called terfess) are widespread in Tunisia. They are found also in North Africa countries, Middle East, Turkey, southern France, Italy and Spain. The most important desert truffles are those included in *Terfezia* and *Tirmania* genus because of their appreciated edible and commercial value. We have assessed the diversity of desert truffles in Tunisia by means of morphological and molecular tools of collected fruit bodies. Three species of the genus *Terfezia* (*T. boudierie*, *T. claveryi* and *Terfezia arenaria*), two other of *Tirmania* (*T. nivea* and *T. pinoyi*) and two of the genus *Picoa* (*P. juniperi* and *P. lefebvrei*) were described. *Helianthemum sessiliflorum* is the most common host plant while, *T. claveryi* was found in association with H*. kahirikum* and *H. almeriens* and *T. arenaria* with *H. guttatum*. Also, we have described specimens of an unusual truffle collected from Center East of Tunisia in mixed stands dominated by *Acacia cyanophylla* Lindl. Morphological features of the new specimens are similar to *Reddellomyces* species Analysis of large subunit and intergenic spaces (ITS) of rDNA showed that this species is closely related to two species of *Reddellomyces* (*R. westraliensis* and *R. donkii*), but differs by the size of the spores and paraphyse. Nevertheless, the new specimen shows similar morphological features to those of *R. parvulosporus*, but similarity corroboration of these two species remains difficult in the absence of DNA sequences. The new species was likely brought from Australia together with their host plant, and they seem to have facilitated the establishment of *Acacia* plantations.

In parallel, Experimental truffle field (20 m x 40 m) was established between 2006-2007 in Arid Lands Institute of Medenine. Seedlings of *Helianthemum sessiliflorum* host plant were inoculated by spores of *T. boudieri* (the most appreciated by the local population) from Tunisian southeasterly regions. The first cultivated desert truffles were obtained after two years of the planting date (four fruit bodies). Plantations have lost their productivity in the following years due to the rainfall limitation. For that we decided to install an irrigation system to adjust and achieve the ideal level of precipitation.

KEYWORDS

Desert Truffles, Tunisia, *Terfezia, Tirmania, Picoa, Reddellomyces*, truffle cultivation.

Poland as an environment for truffles

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Abstract

Research on hypogeous fungi in Poland was successively growing starting from the sixties of 19th century. Before only scant information was available in the literature. In 1981 *Tuber mesentericum* was discovered in the south of Poland and an attention was extended to other edible truffles. Yet, only in the last decade, after joining the researchers by amateurs with dogs, several new sites of black truffles have been indicated in the south and central part of the country.

On the basis of soil, climate, chorological and coenological analysis it appears that Poland offers a suitable environment for truffles of the *T. aestivum/uncinatum* complex. Truffle occurrence is restricted to single places in the areas with proper ecological conditions. Results of monitoring indicate that only in some years the climatic conditions enable formation of fruit bodies. Yet, the most of them do not ripe in temperatures which appear to be too low.

However in the last years one observes an increasing of fruit bodies having ripe spores production, indicating improving climatic conditions for truffles in the area of northern Europe. Field observations and research results from Poland support the opinion that trufficul-ture is possible in the most of Europe.

The Burgundy Truffle in Switzerland: its phenology and ecological requirements

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Abstract

In the sixties, mycologist Charles Schwärzel claimed that the Burgundy truffle (T. aestivum syn. uncinatum) was the most abundant edible fungus species in Switzerland, based on his observations in the Basel region (Schwärzel 1967). Over the past few years, this truffle species has gained more and more interest in Switzerland. As evidenced by the increasing sales of trained truffle dogs, truffle hunting has become an upscale leisure activity. New truffle plantations are being created as an alternative to traditional agricultural practices, and the gastronomic scene shows growing interest in locally harvested Burgundy truffles.

Compared with other truffle species, the Burgundy truffle displays a large ecological amplitude and a wide distribution throughout Europe. Although classified as 'Very Rare' on the German Red Lists, the species seems to be more abundant than generally supposed on calcareous soils (Stobbe et al 2012). Considering that the Burgundy truffle seems to benefit from a slightly warmer and drier climate north of the Alpine arc (Büntgen et al. 2012), it can be hypothesized that the calcareous regions north of the Alps may transform into more suitable Burgundy truffle habitats in the future.

In an ongoing monitoring project, selected truffle sites will be monitored in three-week intervals with trained truffle dogs. The harvests will be weighted and sampled to determine the degree of maturation and to conduct genomic studies to evaluate truffle individuals and mating types within populations. On each site, the tree growth of associated host trees will be assessed using high-resolution dendrometers. Soil temperature and moisture levels will be continuously measured to link micrometeorological parameters with tree growth and truffle harvest. Additionally, in situ excavation of truffle sites, using geo-archaeological methods, data will be provided about the ecological components of each site in relation to fruit body production and maturation.

This project aims to gain more knowledge about the ecological requirements of the Burgundy truffle in Switzerland; specifically, to understand truffle phenology and how climate affects growth, either directly, or indirectly, via their symbiotic host plants. This new knowledge will foster a better understanding of the growth requirements of the Burgundy truffle in order to provide a solid basis for sustainable use of this valuable forest resource and to evaluate an eventual potential for cultivation.

References:

Büntgen et al. 2012. Drought-induced decline in Mediterranean truffle harvest. Nature Climate Change 2: 827-829.
Stobbe U et al. 2012. Spatial distribution and ecological variation of re-discovered German truffle habitats. Fungal Ecology 5: 591-599.
Schwärzel, C., 1967. Beitrag zur Hypogäenflora des Kantons Basel-Stadt und seiner näheren Umgebung. Schweizerische Zeitschrift für Pilzkunde 9: 129-141.

***KEYWORDS** Tuber aestivum, Tuber uncinatum,* phenology, mycorrhiza, climate change, tree growth.

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High diversity and widespread occurrence of mitotic spore mats in ectomycorrhizal Pezizales

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Abstract

Fungal mitospores may function as dispersal units (e.g. asexual spores, anamorphs, or conidia) and/or spermatia and thus play a key role in distribution and/or mating of species that produce them. Mitospore production in ectomycorrhizal (EcM) Pezizales is rarely documented but has been was previously reported for two European *Tuber* species. However, the ecological importance of mitospores in the life cycles of ECM Pezizales, including truffles, is not yet clear. Here we document mitospore production by a high diversity of EcM Pezizales on three continents, in both the Northern and Southern hemispheres. We sequenced the internal transcribed spacer (ITS) and partial large subunit (LSU) nuclear rDNA from 292 spore mats (visible mitospore clumps) collected in Argentina, Chile, China, Mexico and the USA between 2009 and 2012. We collated spore mat ITS sequences with 105 fruit body and 47 EcM root sequences to generate operational taxonomic units (OTUs). Phylogenetic inferences were made through analyses of both molecular data sets. A total of 48 OTUs from spore mats represented six independent EcM Pezizales lineages and included truffles and cup fungi from multiple genera. Three clades of seven OTUs have no known meiospore stage. Mitospores were present over many months and were usually found both prior to and during the truffle fruiting season. Mitospores failed to germinate on sterile media, or form ectomycorrhizas on *Quercus*, *Pinus* and *Populus* seedlings, consistent with a hypothesized role of spermatia. The broad geographic range, high frequency and phylogenetic diversity of spore mats produced by EcM Pezizales suggests that a mitospore stage is important for many species in this group in terms of mating, reproduction, and/or dispersal. The widespread occurrence of mitospore production across the Pezizales phylogeny (and in the sister lineage Orbiliales) suggests that mitospore production may be present but undocumented in many or most Pezizales. Understanding the biology of mitospore production in ECM Pezizales may be a key step in fully understanding the biology of truffles and maximizing truffle production.

KEYWORDS

Cryptic diversity, ectomycorrhizal Pezizales, environmental sequencing, mitospore, truffle.

Tuber mesentericum ascocarp development

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Abstract

For the sustainability of the truffle industry, just like for other end-consumer products such as asparagus, soft fruit, which are highly seasonal perishable foods, growers may have to satisfy the markets most of the year using the different species seasonality and marketing them on their specific qualities by species in 'gourmet' recipes. The vulgarisation of a luxury item may be one way of increasing the sustainability of this industry. One key element for this to happen is the accurate identification of ascocarps, ripe or unripe, by all handlers of truffles in the food chain from pickers to consumer. Few truffle species have had their full development explained. *Tuber mesentericum* was analysed from the attached roots to fully grown ripe specimen during wet and long draught spells. The specific kidney shape with a cavity and the pattern of the sterile veins is not always found. The small specimen do not have a perfume that the human nose may be able to note either. *Tuber mesentericum* however, retains its cavity, but this may change shape. It is not a basal cavity as the birth root is found as part of the truffle peridium, opposite the cavity in its early life. The roots found in the cavity will generate several lines of sterile veins in eccentric form, but are only found in a proportion of ascocarps. In period of draught, the ascocarp remains firmly attached to the root, and may even have up to 3 attach points. The birth roots have apex of warts which will give the peridium of the fully ripe specimen still a unique rough feel. In draught condition certain ascocarps develop almost flat, where the cavity becomes a dip between a stack of peridium warts with minimal gleba. The changes in the weather as the season progresses may develop the kidney shape into a tall U shape, or curling inward the cavity. *Tuber mesentericum* does not retain its birth root, so it attracts other roots which may feed it and help shape the ascocarp and its cavity. It may also take the form of a rounded Y, but in all cases the peridium retains the hooked peaks in the warts as they detached initially from the birth root(s), giving it this rough feel.

KEYWORDS

Ascocarp development, draught adaptation, *Tuber mesentericum,* birth roots.

High phylogenetic diversity in the *Tuber excavatum* group

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Abstract

The *Tuber excavatum* species complex is poorly investigated, probably due to a lack of economic interest. However, *T. excavatum* s.l. is frequently co-ocurring with valueable gourmet truffle species such as *T. melanosporum* or *T. aestivum* f. *uncinatum*, and according to field observations it might be part of fungal communities replacing these gourmet truffle species, when environmental conditions promote their senescence and succession of ectomycorrhizal communities.

The classic monographers of the genus *Tuber* recognize only one or two species, i.e. *T. excavatum* and *T. fulgens,* and a few varieties of *T. excavatum*, but available DNA date suggest that this group might be more species rich. We generated nuclear ribosomal internal transcribed spacer (nrITS - the region selected as barcode for fungi) sequence data of *T. excavatum* samples from temperate and mediteranean environments in several European countries (Austria, France, Germany, Italy, Hungary). Phylogenetic analyses revealed the presence of at least seven groups with mutual sequence differences larger than 3 % (ITS1 and ITS2 combined), the conventional cutoff used for distinguishing operational taxonomic units. Currently used species concepts fail to describe the diversity of this group adequately. We investigate potential correlations of DNA sequence data and morphological characters, to prepare a revision of species concepts in this group.

KEYWORDS

Cryptic speciation, species recognition, barcoding, biodiversity.

The phylogenetic analysis of *Tuber brumale* aggr.

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Abstract

The *Tuber brumale* aggr. is a black truffle which can be found just about everywhere in Europe and because of its spiny spores it is usually classified into the *T. melanosporum* species group (Jeandroz et al. 2008; Riousset et al. 2001). Its fruiting body is of a smaller size, so it is less marketable than the *T. melanosporum*, but due to its exquisite smell the trading significance of *Tuber brumale* is not negligible. The macromorphological and organoleptic properties of its fruiting body are characterized by a remarkable versatility. The debate about the rate of taxonomic differences between the more marketable, smaller, more sweet-scented and the bigger, less redolent (moschatum) varieties which appear on *T. melanosporum* plantations and causing a significant decrease of production, has been going on for a long time.

Due to the clarification of this question the molecular taxonomic study of the species with the widest coverage of its distribution has became more important. The haplotype analysis is based on ITS, while the phylogenetic analysis happens with the supplement of LSU and PKC loci. The samples based on the three loci can be divided into two clades, which show distinctions considering their species. One of them is only present in the Carpatho-Pannon Region and in the Balkans. On the basis of the ITS region three main varieties can be differentiated, from which only one can be found in Western Europe where the variability of the region is lesser than in other regions. In order to differentiate the variations we worked out an ITS-RFLP method. The molecular results can not be unambiguously supported by morphological studies, so far we have not been able to find a single marco- or micromorphological feature which unequivocally separates the two molecular species. The ecological needs of this two species do not show significant differences in the Carpatho-Pannon region.

In conclusion, it seems that so far the *Tuber brumale*, which was mostly regarded as belonging to one, can be separated into two molecularly clearly distinguishable species, which have not been supported by morphological properties, and which is not compatible with the traditional brumale/ moschatum division.

KEYWORDS

Tuber brumale, Tuber brumale f. moschatum, phylogeny, biogeography.

Strategies for the post-harvest handling and conservation of fresh *Tuber melanosporum*

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Abstract

Tuber melanosporum is the most appreciated truffle species collected in Spain. Its organoleptic properties, especially the aroma, resulting from a blend of hundreds of volatile compounds, makes *T. melanosporum* the queen of truffles, and also one of the most prized foods worldwide. Consumers prefer fresh to processed truffles (canned, frozen or sub-products), the fresh produce having the highest culinary and economic value.

Nevertheless, fresh black truffles are highly perishable and have a very limited shelf life (10 days) due to several factors such as being harvested at full maturity, their high microbial load (10^7 to 10^8 microorganism/g), their physico-chemical characteristics (a high presence of macro- and micronutrients, a water activity near to 0.97, a neutral pH, a positive redox potential and the absence of antimicrobial compounds), and their high respiratory activity (truffles are a live feed). The common presence of larvae and the poor application of post-harvest technologies are also factors that accelerate the deterioration of these products.

It has been observed that the most important problems in the post-harvest conservation of fresh black truffles are the bacterial growth, especially of fluorescent pseudomonads, and to the development of a superficial mycelium.

Taking into account these factors and our previous research, a successful strategy to prolong the shelf-life of these fruiting bodies, ensuring their commercial and sanitary qualities, includes the following steps:

1ª Selection of the truffles according to the current legislation (Real Decreto 30/2009). We have to remove completely the cover soil and use optical magnifiers to identified the parasitized carpophores.

2ª Quick and constant cooling of truffles (0ºC-3ºC) immediately after harvest to decrease the microbial growth rate, the negative action of larvae and the respiratory activity.

3ª A sanitizing treatment has to be applied to reduce the high initial microbial load and the risk of presence of pathogens. Among the several methods investigated, chemical and physical, we selected the combination of ultrasounds (35 Hz) with 70% ethanol for 10 minutes. However, the presence of parasitized carpophores requires the use of other technologies. The application of ionizing radiation (1,5 kGy) has demonstrated to be an attractive methodology because its efficacy is not only limited to the surface but it can penetrate the product and eliminates the microorganisms present in crevices and creases as well as internal larvae of insects.

4ª Stored under modified atmosphere conditions (~10% O_2/10% CO_2). This technology besides protecting the product of the environment reduces the microbial growth and the respiratory activity and facilitates the handling and marketing.

Therefore, if we want to extend the shelf-life of these fresh condiments beyond 30 days, we have to applied the postharvest strategies commented below or similar ones.

KEYWORDS

Truffles, *Tuber melanosporum*, decontamination, irradiation, modified atmosphere packaging.

Picoa melospora a dark truffle sometimes canned with other black truffles - the first smooth-spored species in the genus *Tuber*

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Abstract

The genus *Tuber* P. Micheli ex F.H. Wigg is one of the best-known lineages of hypogeous ascomycetes. It is thought to comprise at least 9–11 clades and contains more than 180 species (Bonito et al. 2010, Kinoshita et al. 2011). However, many taxonomic problems still remain.
ITS molecular analysis showed that *Picoa melospora* G. Moreno, Díez & J.L. Manjón is closely related to the genus *Tuber* Rufum clade and hence, should be combined as *Tuber melosporum*. Its morphological features are in accordance with molecular data, since this species has a black-brownish ascoma covered with flat warts, dark whitish to brownish gleba and stipitate subglobose to ellipsoidal asci. In light of the present results, *T. melosporum* presents smooth spore ornamentation unique in the genus *Tuber*, which was the cause of its proposition as a new species in *Picoa*. Spore ornamentation, although a valuable feature to characterize subgenera of the genus *Tuber*, should be regarded cautiously since some species could present spore types that are different from that of their lineage.

Tuber melosporum (G. Moreno, Díez & J.L. Manjón) P. Alvarado, G. Moreno, J.L. Manjón & Díez, *Bol. Soc. Micol. Madrid* 36: 26 (2012).
≡ *Picoa melospora* G. Moreno, Díez & J.L. Manjón, *Bull Féd. Assoc. Mycol. Méditerranéennes* 18: 88 (2000).

References

Bonito G, Gryganskyi AP, Trappe JM, Vilgalys R. 2010. A global meta analysis of *Tuber* ITS rDNA sequences: species diversity, host associations and long-distance dispersal. Mol Ecol 19:4994–5008, doi:10.1111/j.1365-294X.2010.04855.x

Kinoshita A, Sasaki H, Kazuhide Nara K 2010. Phylogeny and diversity of Japanese truffles (*Tuber* spp.) inferred from sequences of four nuclear loci. Mycologia 103(4):779–794, doi: 10.3852/10-138.

Authentication of *Tuber magnatum* and *Tuber melanosporum* in processed products

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Abstract

Tuber magnatum and *T. melanosporum*, are greatly appreciated throughout the world, both as fresh fruiting bodies and as ingredients in processed products. Truffle species are usually identified on the basis of their spores, asci, peridium and gleba. These features are readily recognizable in fresh truffles by specialists, but are difficult to see in processed products. To solve these problems and to limit frauds due to the use of species of truffles different from those declared on the label of truffle-processed products, a protocol was set up to authenticate *T. magnatum* and *T. melanosporum* in products. Microscopic techniques have been used in order to support molecular identification. Good quality DNA was quickly obtained in a few hours using a kit generally employed for DNA extraction from soil. A new primer pair specific for *T. magnatum* was developed inside the internal transcribed spacer-2 (ITS2) and employed in a quantitative PCR assay (qPCR). A qPCR assay has also been developed to authenticate *T. melanosporum* in processed foods using primers developed by Bonito (2009) and previously applied by Zampieri et al (2012) in *T. melanosporum* soils. The optimized assays for *T. magnatum* and *T. melanosporum*, showing good values of efficiency, are specific, sensitive and applicable to products that have undergone intensive transformation processes. They have been successfully tested on commercial samples such as cream and butter (Rizzello et al, 2012). In conclusion, the designed methods will help in detecting fraudulent practices in order to assess food quality and to protect the consumer.

References

Bonito, G. (2009). Fast DNA-based identification of the black truffle *Tuber melanosporum* with direct PCR and species-specific primers. **FEMS Microbiology Letters**, 301: 171–175.

Rizzello, R., Zampieri, E., Vizzini, A., Autino, A., Cresti, M., Bonfante, P., Mello, A, (2012). Authentication of prized white and black truffles in processed products using quantitative real-time PCR. **Food Research International,** 48: 792–797.

Zampieri, E., Rizzello, R., Bonfante, P., & Mello, A. (2012). The detection of mating type genes of *Tuber melanosporum* in productive and non productive soils. **Applied Soil Ecology,** 57: 9–15.

KEYWORDS

Tuber magnatum, Tuber melanosporum, processed products, qPCR, food quality.

Effect of edible coatings and modified atmosphere packaging on the microbial population and quality characteristics of *Tuber aestivum* (Summer truffles)

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Abstract

Fresh summer truffles (*Tuber aestivum*) have a very short postharvest shelf life due to several factors such as the bacterial growth, the development of a superficial mycelium, the dehydration, and the process of senescence. A combination of edible coatings and modified atmosphere packaging would extend the shelf life increasing the possibility for a foreign market.

Thus, the objective of this research was to determine the effects on the microbial populations and the organoleptic quality of the application of an edible coating based on chitosan and formulated with different antimicrobials (potassium sorbate, nisin and nisin + EDTA). The truffles were cleaned with a wet soft brush, rinsed with tap water and sonicated for 10 min. The truffles were then divided in five batches, three of them are coated with chitosan, one with a mix of chitosan and potassium sorbate (1,25 g / 100 ml) and the last one with a mix of chitosan and nisin (0,40 g / 100 ml) and EDTA (50 mM). Finally, truffles were packaged with a low permeability plastic film and stored at 4 ºC for 42 days. The antimicrobial efficacy of the treatments was established based on the effects on the total mesophilic aerobic bacteria, the *Pseudomonas* genus, the *Enterobacteriaceae* family and the mold and yeast. A sensorial analysis was also carried out to determine the influence of the coatings on the organoleptic quality. The results showed that the postharvest shelf-life of control truffles stored in air only reached 14 days, the truffles packaged in modified atmospheres achieved 28 days whereas the coated batches maintained their quality during 6 weeks. The batches coated with chitosan + potassium sorbate and with chitosan + nisin + EDTA showed the lowest microbial counts with differences of 2 logarithmic units. The sensorial analysis did not showed differences between coated and uncoated samples.

KEYWORDS

Truffle, *Tuber aestivum,* edible coatings, chitosan, modified atmosphere packaging.

Challenges after sequencing the black truffle genome

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Abstract

Thanks to the genoscope and the *Tuber* Genome Consortium, the genome of *Tuber melanosporum* was sequenced and published in 2010 (Martin et al., 2010, Nature, 464: 1033-1038). The main characteristics of this genome are: 1) its large size (125 Mbp) with a high proportion of repeated elements (60 %); 2) the low number of genes (7500 genes); 3) the limited gene set for degrading plant polysaccharide and 4) the genes coding for sulfur metabolism pathway that are upregulated in fruiting body suggesting a role in the volatile synthesis. Using these genomic resources we were able to unravel the sexual reproduction mode by identifying mating type genes and to characterize new polymorphic markers allowing population genetic analyses. In the recent years the next generation sequencing technologies and the reference genome of *T. melanosporum* allowed to start a population genomic project with resequencing of isolates. This opens the way for studying the adaptation mechanisms of *T. melanosporum* to environmental stresses.

The ectomycorrhizal (ECM) symbiosis is a key phase of the *Tuber melanosporum* life cycle. More generally, the ability to establish ECM is a widespread characteristic of various soil ascomycetes and basidiomycetes. The genetic mechanisms underlying this symbiosis, that contribute to the delicate ecological balance in healthy forests, also provide insights into plant health that may enable more efficient carbon sequestration and enhanced phytoremediation. Sequencing mycorrhizal species genomes allow investigation of ecological adaptation and functional variation within and between the various lineages. The two first mycorrhizal genome sequenced (*Laccaria bicolor* and *T. melanosporum*) suggested that the ECM symbiosis has evolved very differently in the two major fungal phylum, the Ascomycotina and Basidiomycotina. Within the framework of the JGI Mycorrhizal Genomics Initiative and the Genoscope TUBEREVOL project, we are sequencing a phylogenetically and ecologically diverse suite of mycorrhizal fungi (Basidiomycota and Ascomycota), which include the major clades of symbiotic species associating with trees and woody shrubs. To date the genome of 17 mycorrhizal species are sequenced including three *Tuber* spp (*T. aestivum, T. melanosporum* and *T. magnatum*). The comparison of the three *Tuber* spp. genomes allowed investigating synteny as well as identifying the *Tuber* core genome. Comparative genomic analyses including mycorrhizal, saprotrophic and pathogenic fungi allowed the investigation of mycorrhizal life style highlighting a genome expansion for fungal species interacting with plants and the importance of small-secreted proteins.

To conclude, the sequencing of the black truffle genome was only the starting point to investigate its evolution, adaptation, life cycle and their ecology. With the continuous progress of technologies and the reduction of sequencing cost the genome of many new truffles species will be available in the next years.

KEYWORDS

Genome sequencing, evolution, adaptation, ectomycorrhizal.

Typology of truffle grower in France: implications in sustainable truffle cultivation for the future

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Abstract

Truffle production in France is a factor in long-term development despite its inherent limitations due to different practices and environmental conditions. A simplified classification allows us to define three types of truffle grower according to cultivation techniques, financial investment and environment types.

The 'gardener' truffle producers develop a part of their family property by exploiting old inherited plantations or planting modest-sized plots.
The 'farmer' truffle producers are few in the traditional areas and remain prudent with regard to their specific investments. On the large cereal and wine-producing plains (Poitou, Touraine, Berry, the Rhône valley and the Valensole plateau), the farmers invest in bigger areas.
The 'investor' truffle producers, from varied social backgrounds, generally group together into companies to set up large-scale cultivation with important technical and financial means.

The 'gardener' truffle producers employ techniques integrated into their environments, which are mostly derived from local traditions or a great curiosity about Nature. The 'gardeners' slow down the closing-up of the environment by planting plots which 'eat into' the wooded areas that result from abandoning agriculture. They thereby create barriers which prevent fires from spreading in ever more overgrown landscapes. The practices are meticulous and the hours of work often very long.

The 'farmers' use truffles as a way of diversifying their cultures. Mechanisation is often associated with plots of larger areas - sometimes with an excessive number of operations.

The 'investors' use methods derived from fruit production without any fixed ideas.

When truffle cultivation is carried out by farmers or wine producers in new truffle growing areas, watering is anticipated right from the start of the plantations and in their husbandry. The same principle guides the investors in France.

Truffle cultivation contributes globally to the sustainable development of the land. Nevertheless, in the traditional regions it is handicapped by environmental effects and the lack of water resources for irrigation - coupled with a certain inertia in developing this practice. The absence of fungal contamination in the regions of the great plains allows better results, further improved by the practice of watering.

KEYWORDS

Gardener, farmer, investor truffle producers, practices.

Amycoforest: an international project (ALCOTRA) on the interaction between macrofungi production and forestry

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Abstract

Amycoforest is an European project AlCOTRA (*Alpi Latine COoperazione TRAnsfrontaliera Italia-Francia*), involving a group of Italian and French public institutions (Liguria and Piemonte Regions, Province of Imperia, University of Genoa for Italy; *Centre Régional de la Propriété Forestière* – CRPF, for France). It is a demonstrative and training project based on technical and scientific experiences: the main aim is to develop a sylviculture that harmonizes wood and macrofungal production (including truffles), according to a concept of multiple-use forestry. The 2-years project is structured in a series of activities: I) the mapping of suited areas; II) the realization of thematic cartography to propose a management model for the selected areas; III) the preparation of demonstrative stands in order to present the forest management treatments useful for a fruitful fungi production; and IV) the promotion of research, education, sustainability, marketing, rural development associated with forest mushrooms. The dissemination task is devoted to inform forest owners and keepers about possible economical benefits that can derive by a proper and specific management actions addressed to increase the fungal production. As concerns truffles, hypogeum fungi, the selected areas are located in Italy, in Piedmont and Liguria. The state of truffle cultivation in wild stands in these regions is significantly different: Piedmont boats very ancient traditions regarding the truffle harvesting and also its cultivation; on the other hand, Liguria only recently started cultivating truffles. From this perspective, the demonstrative stands in Piedmont are aimed at confirming and disseminating the most effective practices for cultivating truffles. In Liguria, the selected stands are located in some marginal areas, which have been progressively abandoned in the last decades. Nowadays, these areas were found to be particularly suitable for spontaneous truffle growth. Here, the purpose is to restore the marginal lands by means of practices for enhancing truffle production (*Tuber melanosporum* Vittad., *T.aestivum* Vittad., *T.brumale* Vittad.). Until now a total number of 10 (6 in Piedmont and 4 in Liguria) demonstrative stands have been implemented. The works, started on 2012, are still in progress. The adopted practices regard the sylvicultural treatments, the introduction of mycorrhized plants, and the techniques for in-situ mycorrhization. For edible mushrooms, different species are taken into account, like *Boletus edulis* group, *Cantahrellus cibarius* Fr., and others, in different ecological conditions and forest stands. All plants and fungi used are autochthones.

KEYWORDS Sylviculture management, *hypogeous fungi,* marginal land restoration.

Mapping the potentialities of truffle-producing trees implantation in the Haute-Garonne département

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Abstract

The French département of Haute-Garonne is not known to be a traditional area of truffle's production. However, there are currently some producers in this département, and some associations are now trying to promote this activity, to exploit the great potential of this region of France.

The association of producers of Haute-Garonne, "La truffe sur son 31", is by now trying to map the potentialities of implanting truffle-producing trees on the soils of Haute-Garonne. This map will help them support their actions before the local authorities to find some subsidies and finance their projects.

By crossing information we have found in the literature, we defined the factors that could influence on the growth and production of truffles. These factors are of different types: climatic, pedological, botanical, topological, etc. To combine all the factors, we created a key that made the links between all these factors, to allow us to estimate the potentials. Then we worked on the software ArcGis to compile all the data regarding each factor.

The result of our work is a map of the département on wich you can see different zones classified from potential zero to four (potential zero : none of the factors is favorable to the implantation of truffle-producing trees ; potential four : the combination of all the factors of the zone gives the best conditions for the implantation of truffle-producing trees)

The work already done has to be improved, but it is the beginning of something that could be very useful in the future to help the association decide where and how implant truffle-trees. The methodology is the good one, but it has to be given better tools to become more accurate : more precise data, ratifications by experts in truffle, soils, climate, etc.

KEYWORDS

Mapping, soil potential, climate, botanical indicators, topography, criteria combination.

Spanish trufficulture

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Abstract

Trufficulture is one of the few agro-forestry profitable activities that grows in the Mediterranean area.

In this work, the more noteworthy aspects of the Spanish trufficulture: the wild-truffle hunting, that starts strongly in the 50 decade of the past century and persist nowadays; and the development of the black truffle plantations, which started in the 1970s and between them stands out Arotz´s plantation in Soria with its 600 ha and producing more than 2,500 kg per year. Currently, production in the forest is going down and the majority of the plantations begin its production being it more than the one in the wild. In the Mora de Rubielos market (Teruel), more than the 40% of black truffles come from plantations, increasing in years with low rain. Many times, plantations have the economic support of the public administration, so every year more than 500 ha are installed. At the same time, there is a group of more than 15 nurseries that produce annually more than 200,000 mycorrhized seedlings.

There is also a very wide scientific and technique activity, with many research projects and experimental areas developed by Spanish Research Institutes and Universities. Between them, it highlight the research project for the integral development of the trufficulture in Teruel, promoted by the INIA (Spanish Government) and that had seven subprojects. From the scientific-technique area, in collaboration with the truffle-famers associations, there is supported the consultancy and broadcasting activities. Nursery and cultivation techniques are also being exported to Chile and Argentina.

Information about black truffle production, prices, harvest areas, markets, plantations, research activity and publications is included. Last, a diagnosis of the future perspectives of the sector is done.

KEYWORDS

Rural development, agro-forestry profitable activities, research activities, trufficulture development.

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Evolution of black truffle culture in the province of Huesca (Spain)

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Abstract

Of all the species of the genus *Tuber* naturally appearing in Spain, black truffle (*Tuber melanosporum* Vittad.) is the most valued not only culinary but also economically, ecologically and socially. It represents a sustainable alternative in rural areas economically and demographically disadvantaged. In this work we have collected the evolution of its culture in Huesca (Northern Spain), one of the provinces with the highest weight in the truffle sector in Spain, in relation to the market and support it has received from public administration.

In Huesca, its wild presence is evidenced since the sixties, what allowed the establishment of a relevant market statewide in Graus. Around 1990 appeared the first plantations devoted to its cultivation in the regions of Sobrarbe and Ribagorza. Since then, the number of parcels dedicated to trufficulture in Huesca has been increasing. They are installed at an average rate of about 86 new hectares each year, dedicating to this activity almost a thousand hectares and generating a significant socio-economic framework (collectors associations, specialized nurseries, processing industry and product processing, tourism associated, markets, etc.).

This increase is mainly due to the high prices that this product reaches in the market, its ability to be installed in poor soils, unsuitable for other crops, the achievements in research and subsidies granted by public administration. The support of Gobierno de Aragón and Diputación Provincial de Huesca has been decisive from the economic and scientific standpoint. The actions of this last organization have been essential for the province of Huesca allowing on one hand, fostering the truffle by grants which led to increase the number of truffle plantations and on the other hand their early production starts because of the installation of irrigation systems.

On the other hand, the fungus is disclosed in different areas without truffle tradition, and both truffle growers and other professionals (chefs, transformers, etc.) receive technical information. It gives them knowledge for a better plantations management and a better use of available resources. Finally, all this effort is consolidated through the creation of the only truffle specialized center in Spain, the Centro de Investigación y Experimentación en Truficultura (CIET), placed in Graus, which works jointly with the Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA). It provides several technical services essential for the truffle sector, such as the identification of different truffle species, the mycorrhization degree by *T. melanosporum* in nursery plants and tracking truffle plantations through roots analysis.

Thereby, present and future lines of action are aimed to set the truffle production together with deep in the knowledge of *T. melanosporum* and its management in plantations.

KEYWORDS

Rural developement, government grants, *Tuber melanosporum,* black truffle, Aragón.

The Situation of Truffles in Germany

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Abstract

Jean-Marie Dumaine, president of the Ahrtrueffel Society, will give a brief lecture on the history of truffles in Germany and the Ahrtrueffel Society's initiatives to further the rediscovery of truffles in Germany. He will provide an account of the creation of the Society's Truffière which is the first of its kind in Germany after 100 years. He will also explain the special circumstances of truffle collection in Germany which is highly limited due to a total ban on collection and ansportation of truffles. The ban was effectuated due to the fact that, in Germany, truffles are on the "Red List" of highly endangered species. He will elaborate which species of truffles have been found and identified around Sinzig. Furthermore, he will try to provide some insight into what the future of truffle cultivation in Germany might be. As there are many chalky grounds in Germany, truffle cultivation has a lot of agricultural potential. The truffle can be considered as a very innovative product. There already are some new Truffières and companies who offer trees infected with truffle spores. The yearly Truffle Symposium which is organised by the Ahrtrueffel Society has found international recognition.

KEYWORDS

Trufficulture, truffiere, cultivation

Truffle orchards realized in Romania

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Abstract

Romania has a total area of 237 500 km^2 and a forest fund of 6,5 million ha., from which approximately 4 million ha are represented by common beech and Quercus, species, favourable for truffles.
Although we do not have a precise situation concerning the harvesting of truffles in our country, after the investigations and discussions realized with truffle pickers and merchandisers, it is appreciated that approximately 10 tons of truffles are harvested in Romania, per year, from which 90% are represented by black truffles (*Tuber aestivum* Vittad.) and 10% by white truffles (*Tuber magnatum* Pico).
It can be observed that in our country the interest concerning truffles is very recent (after the year 1990) and the plantations with inoculated seedlings are scarcely: 5 with *Tuber aestivum* and 2 with *Tuber melanosporum*. Only in the year 2012 almost 60 truffle plantation projects were drawn in order to obtain European funds, but none was approved. Two from the realized plantations were accomplished by specialists from the Forest Research and Management Institute:
1. The *Tuber aestivum* Plantation from Prejmer from the year 2011. There were 57 seedlings brought from Hungary and planted at a distance of 2x2 meters: 2 year old sessile oak (*Quercus petraea*), Turkey oak (*Quercus cerris*), hazelnut (*Corylus avelana*), Turkey hazelnut (*Corylus colurna*) and 3 year old oak (*Quercus robur*). The soil has a pH of 7.9, a very high quantity of humus (7.2% in the first 10 cm and 7.5% between 10 and 30 cm) and many carbonates even at the surface ($CaCO_3$ = 21.3%). After the first year of vegetation, the seedling's conditions are very good (growths of 20 cm). Only 2 seedlings were lost due to beetles and moles, even though the year 2012 has recorded the most severe drought from the last 50 years.
2. The *Tuber melanosporum* Plantation from Moldova Noua from 2012, where 40 Turkey oak seedlings from Italy were planted. The most favourable climate area from Romania was chosen, more precisely, an area with temperate-continental with Mediterranean influence climate. This type of climate is characterized by a warmer climate in comparison with other hill regions (moderately-continental), with relatively scarcely precipitations, even abundant sometimes (an average of over 700 mm/year) with two maxims, at the beginning of summer and during autumn and average annual temperatures of 8-10°C.
The plantation scheme was of 5 m x 5 m, the seedlings being planted in 5 rows, 8 on each row. Because the soil had an unfavourable pH (5.5), lime amendments were realized before plantation. The lime (calcium hydroxide) was brought from retail totalizing 400 kg (16 bags of 25 kg each) on a total area of 0.14 ha. After these works, the pH of soil has become 6.8. The seedlings were watered twice a week together with the measures for protecting the cultures against rabbits. The success of the culture is of 100%.
In order to diminish the effects of frosting (the restrictive factor concerning the spreading toward east of the Burgundy truffle) we will take measures concerning the soil's mulching around the seedlings by applying mulch from the grassy vegetation from between the planted rows.
Even though we know that the Burgundy truffle does not have a natural spreading that can exceed the East of France, Switzerland and the West part of Italy, if this plantation can be accomplished a big leap (of approximately 1500 km) can be realized concerning the repartition of this kind of truffle.

KEYWORDS

Truffles, plantations, *Tuber aestivum, Tuber melanosporum*.

Ten years of quality control of seedlings inoculated with *Tuber melanosporum* in Viveros Alto Palancia

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Abstract

The successful cultivation of *Tuber melanosporum* is based on the plantation of mycorrhized seedlings on suitable soils. The attributes of the inoculated seedlings, particularly their mycorrhizal state, influence the performance of the plant and the fungus in the field. However, in Spain there is no official regulation on the certification of mycorrhized seedlings.
In 2003, the CEAM (Centro de Estudios Ambientales del Mediterráneo) signed an agreement with the nursery Viveros AltoPalancia to check and certify seedlings inoculated with *T. melanosporum*. From then, the stocklots of mycorrhized seedlings have been checked every year following the methodology developed by Reyna *et al.* (2001). The quality control involves: (a) checking all the sporocarps used as inoculum, (b) assessing the mycorrhizal state of the commercial stocklots on the basis of the number and the percentage of root tips colonised by the fungus and the occurrence of other ectomycorrhizal fungi, and (c) assessing the risk of contamination by other ectomycorrhizal fungi during the nursery period through bait seedlings.
The annual control has allowed to evaluate the progress in the nursery quality, to identify limitants and potential improvements of culture practices, and serves as a guarantee to both the client and the nursery. The number and percentage of root tips colonised by *T. melanosporum* have improved over the decade, specially in recent years. During the first years the efforts concentrated in controlling the occurrence of the ectomycorrhizal contaminations, particularly *Sphaerosporella brunnea*, whereas in recent years the focus was on increasing the size of seedlings and the number of *T. melanosporum* mycorrhizas.
The quality control has turned into a continuous quality improvement scheme. The main challenges for the coming years are the homogeneity of the seedlings at the time of inoculating, the use of alternative substrates, and the introduction of molecular methods in the certification process. As truffle growers are more informed about the influence of seedling quality, nurseries are more interested on an official seedling certification.

KEYWORDS

Tuber melanosporum, nursery, cultivation, mycorrhization, quality control.

Tuber borchii mycorhization depending on host tree and stage of inoculation

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Abstract

Tuber borchii is an excellent edible truffle, also known as “bianchetto” or “marzuolo” in Italy and “blanquilla” in Spain. Widely spread all over Europe, from UK to Italy, Spain and Portugal. In the markets can be usually found mixed with other whitish truffles from the *Puberulum* section.

Due to its broad range of ecology and habitats, in this project several infection techniques to inoculate different host trees to this truffle were studued, in order to cultivate it in all these potencial areas.

Seedlings of *Pinus pinea, Pinus sylvestris, Pinus radiata, Quercus ilex, Quercus suber*, *Quercus striatula* (a native oak from México) and *Coryllus avellana* formed mycorrhizas with *Tuber borchii* at levels from 29% to 54%, seven months after the inoculation. All trees were outplanted to follow their performance in the field.

Tuber borchii has claimed to be an agressive truffle able to fast infect trees. On the other hand hazelnut industry is growing fast in Chile, and hundreds of hazel orchards are being planted in the regions also suitable for growing truffles. Most hazel seedlings come from layering in clump strangulation, with other mycorhizas in the root system, mainly *Scleroderma*. An experiment with hazels was done in oder to evaluate three different inoculation methods: a) nursery inoculation on seedlings coming from seeds; b) rootdip inoculation of commercial seedlings at the outplanting time; c) direct inoculation in mature hazels in the field. Results are a) all nursery inoculated hazels got mycorhizas with the inoculated truffle over 30% of infection, b) 83% of hazels got *T. borchii* mycorhizas and c) 66% of hazels got *T. borchii* mycorhizas. Although the levels of mycorhization on b) and c) were always lower than 10%.

At the same time different inoculations in mature forests *(Pinus pinea, Quercus suber and Quercus rugosa*) have been carried out with this white truffle in Spain, Portugal and México.

KEYWORDS

Tuber borchii cultivation, infected truffle trees production, Mycoforest Technology®.

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Fluctuations in the development of the yield of a truffle plantation

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Abstract

Truffle production varies within plantations as a result of tree ageing, culture methods and climatic vagaries. A study of the development of one plantation has been carried out since its creation in 1995 and 1996.

The first harvest started after 6 years. 10 years later we can see clearly how it evolved with time and evaluate its possible future development as well as the effects of the difficulties encountered.

The plantation consists of 2000 trees on 4.5 hectares, including 28% of evergreen oaks, 58% of pubescents and 14% of pedunculates. The number of productive trees has constantly and regularly increased each year, with 2 exceptional years - 2008-2009 and 2011-2012 - due to extremely favourable climatic conditions. A chart with the producing trees in red displays their geographical distribution on the plot, where groups and non-productive zones show up, as well as the negative effect of surrounding woods on their development and the start of their production. The producers can be classified in 4 categories: regular, which yield every year; alternating, which stop producing from time to time; those which have only yielded 1 year out of 10; and those which don't yet yield or never will. The quantity of truffles harvested has constantly increased, reflecting the increase in the number of productive trees.

The climate plays a decisive role. The 2008-2009 season was very good, but 2011-2012 which promised to be exceptional (judging by the large number of surface-growing truffles and that of producing trees) didn't live up to expectations because of climatic surprises which caused the surface truffles to rot and froze all those of the month of February. Rainfall charts show wide variations in distribution from one year to another. All the same, lack of rain isn't a major problem if there are enough water resources for irrigation. However this isn't the case for excessive rainfall, which can be harmful and impossible to regulate. Too much lasting frost or heat waves have catastrophic effects.

Predation of the truffles by millipedes, click beetles and Liodes mites is a factor which limits production. These predators appear successively over time as the truffle plantation ages. it is essential to regulate them if you want to harvest ascocarps with a fine attractive appearance. The increase in the number of these creatures is probably linked to conserving calcicole grassland, in view of their habitat and the nutritional value that truffles represent for these insects.

This plantation is particularly used for experiments carried out in the context of 'SYSTRUF'. As truffle producers we are very interested in improvements which could be made through scientific work and technical experiments in the field, to allow us to advance with our practices.

KEYWORDS

Tuber melanosporum, chart producing trees, climatic variations, truffle predators.

Comparative ecology of wild truffle grounds and truffle plantations

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Abstract

In France a small part of *Tuber melanosporum* truffle production comes from natural truffle grounds and the majority from plantations of mycorrhiza-inoculated trees. Comparing the ecology of natural truffle grounds and plantations throws light on the decline of the former and the difficulties of cultivation.

In uncultivated environments, natural truffle grounds decline because of:

1. Rapid development to the limit of moorland with shrubby plants (junipers, sloes, brambles, broom), in the absence of grazing resulting from changes in human activity.
2. Natural afforestation which extends further and further
3. Ever drier summers.

In plantations poor results are noted in two different types of situations:

1. In traditional regions characterised by shallow calcareous soils and a strong decline of agriculture, forests of pubescent oaks in the South-West or evergreen oaks associated with Aleppo pines in the South-East give rise to the pressure of fungal contamination. Mycorrhizal fungi of a forest ecosystem originating from these woods contaminate the inoculated plants in truffle plantations. Drier and drier summers also constitute an unfavourable factor.
2. In regions of cereal or wine-growing plains where truffle cultivation is becoming more and more established, the climate is considered as being the principal disadvantaging factor. Delayed triggering of fructification or a reduced life span of truffle production raise questions when the cultivation practices (working the soil, watering and tree-pruning) have been carried out correctly. Experiments with bringing in spores of *Tuber melanosporum* seem to encourage the start of truffle production when contaminating Basidiomycetes are sometimes detected.

The difficulties encountered in truffle production in plantations suggest that we haven't yet reached the optimal functioning of the truffle ecosystem as it existed during the truffle's period of abundance. Truffle culture experiments and scientific work towards a deeper knowledge of the biology of the fungus are necessary to avoid the pitfalls encountered.

KEYWORDS

Tuber melanosporum, climate evolution, traditional areas, cereal and wine-growing plains.

The current state of somatic embryogenesis in holm oak and its potential for yielding clonal material

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Abstract

The holm oak (*Quercus ilex* L.) is one of the most important mycorrhizal host species in the cultivation of truffles. The development of propagation procedures to obtain oak clonal material from selected trees is of great interest to the truffle industry, both scientifically to better understand host-guest relations and economically to likely attain improved productions. However trees of the genus *Quercus* are very difficult to propagate vegetatively. Although rooting of cuttings taken from very young plants is possible, this organogenic ability is rapidly lost a few months after germination. Therefore cloning trees selected by reliable and high ascocarp production is no longer possible.

An alternative to rooting cuttings is the complete plant regeneration by somatic embryogenesis, which has shown positive results in the cloning of several oak species. Despite the progress made in recent years, regeneration of viable plants from somatic embryos still remains costly on most forest species. Furthermore, somatic embryos are usually obtained from immature zygotic embryos and thus the regenerated plants are of unproven genetic and phenotypic value. However in cork oak we obtained somatic embryos from tissues of adult trees, and virtually any genotype can be cloned with our protocol. Using this protocol experimental plots for field evaluation of clonal material have been established by TRAGSA in a cooperation project.

In the holm oak protocols for inducing somatic embryogenesis from zygotic embryos and regenerating plants were described. We recently developed induction methods that allow cloning of adult holm oak trees using floral organ tissues. Besides our experience with cork oak embryogenic cultures has allowed us to progress quickly in the phases of recurrent proliferation, differentiation and germination. Cultures of embryogenic lines with high proliferative capacity in liquid medium to facilitate handling have been initiated. Currently we are working to get *ex vitro* acclimatization of selected holm oak clonal material. A description of the methods employed in each phase of plant regeneration will be presented.

This work is funded by the Spanish National R&D program, project AGL2010-22292-CO3-01.

KEYWORDS

Cloning, Forest biotechnology, Micropropagation, *Quercus ilex,* Tissue culture, Vegetative propagation.

Experiences of quality valuation in truffle seedlings by the University of Perugia

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Abstract

Truffles are hypogeous ectomycorrhizal fungi that belong to the genus *Tuber* (Pezizales). Some *Tuber* spp. are successfully cultivated by the establishment of orchards using mycorrhized truffle-plants, previously infected with truffle inocula by commercial nurseries. The selection of the plantation sites as well as the quality of mycorrhized seedlings are considered fundamental prerequisites to allow these fungi complete their life cycle and produce truffles. In this scenario, some regional Italian governments required a specific certification of mycorrhization level and plant quality for marketing, out-planting and realization of truffle orchards.

Since the late 80s, the Department of Applied Biology at the University of Perugia carries out analysis and certification of truffle-plants produced by commercial nurseries. The inspection activity is performed following an official method arranged by several Italian researchers in 1995. This method is based on morphological analysis subsequently supplemented by a biomolecular control. Moreover, specific changes on the basis of acquired experiences after several years of truffle-plant certification were included. In this work we have compared data on truffle-plants produced in seven Italian nurseries in the last 12 years, for a total of over 10000 samples analyzed. We have measured differences in mycorrhization level concerning truffle-plants of: i) different plant species; ii) different plant age; iii) inoculated with different *Tuber* sp. A similar comparison was performed regarding the presence of concurrent fungi and their mycorrhization percentage.

We showed that different mycorrhization performance often occurs in some nurseries between different plant species and ages, with cases of significant increase of plant quality in the second year. The processing of this large amount of data, gathered through years of experience of inspection and certification, allowed us to identify the parameters defining the quality of homogeneous batches of truffle-plants, especially in terms of minimum percentage of *Tuber* ectomycorrhizae and presence of other mycorrhizal contaminants. This experience is proposed to stimulate discussion on the potential establishment of an European official method for truffle-plant certification, that as argued by technicians and professionals since many years, it represents an approach to protect truffles and truffle-farmers.

KEYWORDS

Truffle cultivation, truffle-plants, mycorrhization level, *Tuber*, commercial nursery.

The INRA Mycorrhized Truffle Tree

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Abstract

The large-scale production of mycorrhized Truffle Tree began in 1973. The Company Agri-Truffe, based on the work performed by the laboratory Centro di Studio per la del Terreno micologia CNR and INPL (Istituto Nazionale per da Legno e Piante the Ambient) of Turin. The first plants were planted in autumn 1973. The first truffles (*Tuber melanosporum*) were collected in December 1977 in Burgundy, under hazel.

Since 1973, the truffle tree has been the subject of continuous improvement. Three generations of plants have succeeded.
Those of the first generation were produced according to the method of Mannozzi-Torini, enhanced by INRA (Institut National de la Recherche Agronomique) in collaboration with the INPL.
Plants of the second generation were produced in "ball rolled Melfert" using an inert substrate based on peat and composted softwood bark, fertilized, modified to fit the mycorrhization by the nose.
For third-generation plants, the substrate has been modified and the envelope "nonwoven" of the Melfert bag was replaced by a plastic container.

The development of the forth generation obtained by vegetative propagation of individuals particularly successful has been delayed by technical difficulties vegetative propagation of trees, pubescent oak (*Quercus pubescens*) and holm oak (*Q. ilex*).

However, it is clear from the analysis of parameters of mycorrhization (host, geographical origin truffles, environment) on trees obtained by vegetative propagation or not, every tree could be infected by the truffle. The geographical origin of truffles in the production is greater than anticipated in the limited genetic diversity within the species *Tuber melanosporum*.

The species most suitable for the production of truffles plantations, *T. melanosporum*, pubescent oak and holm oak, *T. uncinatum* (syn. aestivum), oak (*Quercus* spp.), the common hazel (*Corylus avellana*), hornbeam (*Carpinus betulus* and *Ostrya carpinifolia*), pine Austrian black pine (*Pinus nigra* austriaca) and cedar of Atlas (*Cedrus atlantica*).

The mycorrhizal plant now produces the majority of truffles harvested in France, the production of natural truffle declining more and more.

It has allowed the introduction of the Truffle in other countries of the northern hemisphere: USA (Agri-Truffle company, a subsidiary of Agri-Truffe), Morocco, Sweden, but also in the southern hemisphere: Australia, New Zealand.

KEYWORDS

Truffle, mycorrhizal seedling, host plant, plant selection.

Weed control using adequate mulch can improve *Tuber melanosporum* mycelial expansion regarding the traditional mechanical strategies in new established Black truffle orchards

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Abstract

Conventional methods of weed control in truffle orchards rely primarily on tillage in the first few years after planting. The use of synthetic mulches in truffle orchards is an alternative to these methods with unclear results due to the wide range of materials available and the lack of experiences. In a young orchard of Holm oak (*Quercus ilex*) seedlings inoculated with Black truffle in north-eastern Spain, the traditional mechanical weed control methods were compared with the use of synthetic mulches and stone cover during three years. Herbaceous cover, seedling growth and abundance of *Tuber melanosporum* mycelium were evaluated to determine the effectiveness of these treatments in controlling weeds and supporting the growth of the host tree and the expansion of the desired fungus. The untreated and white single layer polypropylene-mulched plots had the highest percentage of herbaceous cover with significant differences in seedling stem diameter increases. Herbaceous cover was significantly lower in units with double-layer white and single and single and double-layer black polypropylene mulches while mechanical soil tilling and stone much provided minimal weed control after 3 years. The quantity of *T. melanosporum* mycelium detected in the soil at 30 cm distance from the host plant was greater in double-layer white much than all the other treatments, although at 15 cm distance there was very little difference among treatments, based on PCR analysis using *T. melanosporum* specific primers. The effect of weed control on reflection of light, and soil temperature and moisture were monitored to help explain observed differences and are discussed further, confirming the positive results obtained with the mulch of double-layer.

KEYWORDS

Soil mycelium, polypropylene mulch, holm oak, preproduction stage, mechanical tilling.

A 10-year review of plant quality and mycorrhizal status observed in the certification program for seedlings inoculated with *Tuber melanosporum* for the establishment of black truffle plantations

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Abstract

To provide technical support to the developing Spanish truffle industry during the past 10 years, the Center for Forest Technology of Catalonia and the Government of Castilla and Leon have offered similar services to evaluate the mycorrhizal status of seedlings inoculated with *T. melanosporum* for truffle growers and forest nurseries that specialize in this product. The evaluation method combines both qualitative and quantitative observations. Qualitative parameters are based on the Spanish legal requirements published in the Royal Declaration 289/2003 Annex VII, which governs commercial forestry material intended for reforestation. Quantitative evaluations are based on estimates of total fine root tips, the number and percentages of *T. melanosporum* mycorrhizae, of non-mycorrhizal root tips and of non-*T. melanosporum* mycorrhizae. Written reports as well as technical recommendations based on plant data are provided to potential truffle growers.

During this period we have evaluated more than 3000 seedlings from more than 15 nurseries, primarily in Spain. The preference for *Quercus ilex* as the desirable host plant in Spain has increased over this time period, but we have evaluated other host plants including *Corylus avellana* and *Q. faginea* and *Q. coccifera*. The most frequently observed non-*Tuber* mycorrhizae belong to the Humariaceae family. Seedling quality problems have been quite variable and include root structural problems as well as root necrosis. We report the frequency of other *Tuber* species, encountered in our evaluations, particularly *T. brumale.*

During the past 10 years it has been necessary to update the original method based on our observations of plant material from the nurseries and field performance. A minimum number of fine roots has been established because we have observed the loss of up to 50% of fine roots in *Q. ilex* seedlings one year after outplanting. We have also incorporated a routine molecular analysis to screen for the presence of *T. brumale* and *T. indicum* in order to reduce the probability of cultivating less desirable *Tuber* species. We discuss the value of testing for mating types in the seedling evaluation process and further interventions that could increase the success of truffle cultivation.

KEYWORDS

Tuber melanosporum, seedling quality, mycorrhizal status.

Organic substrates "with potential value added" in establishing *Tuber melanosporum* on *Quercus ilex* subsp. *ballota*

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Abstract

In Spain, there is a gradual implementation of the truffle. This culture is at the crossroad between the forest sector and the agriculture, and mainly focuses on the sustainable management of forests and forest areas, using the minimum possible inputs, according to the principles of organic farming, and thus excluding fertilizers and pesticides chemicals.

In the context of sustainability exposed, the Truffle needs to replace substrates currently used peat-based, for others who are not contributing to the exploitation of peatlands, nonrenewable resources. This is the case of the use of substrates obtained from composting organic waste in agriculture and the food industry.

In this paper we have tried to contribute to the study of new means to promote the colonization of roots of *Quercus ilex* by the fungus and mycorrhizal development, using composted agricultural byproducts, with characteristics similar to traditional substrates. For this purpose, we have studied the feasibility as a means for growing *Quercus ilex* subsp. *ballota* inoculated with *Tuber melanosporum* Vitt., two organic substrates obtained from composting organic waste in agriculture and the food industry, compared to a commercial preparation obtained from peat. There have been a total of seven treatments, where in some of them *Trichoderma harzianum* (Th-78) has been inoculated. During the experiment, it has been observed that some composts can be a viable alternative to commercial substrate, and it has also been demonstrated that some of them allow further development of the specific root system of *Q. ilex* that it is susceptible to be colonized by *Tuber melanosporum*.

KEYWORDS

Quercus ilex, ectomycorrhizae, Tuber melanosporum, black truffle, Truffle, compost, soilless.

Effectiveness of preservation of dry inoculum of *Tuber melanosporum*

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Abstract

The production of ascocarps of *Tuber melanosporum* is subjected in most occasions to fluctuations. This means that the price per kilo may increase from one season to another with the inconvenience that this might mean for the buyer. For nurseries, purchases are made from the first months of year to the end of harvest season, and the variation in production can affect the acquisition of material in good condition for inoculation. In order to assess the conservation status of spore inoculum of the years 2010, 2011 and 2012 was raised this paper. Truffles were laminated and dried at 40° C in a hot air oven, and subsequently be stored at 5° C until use. Acorns of *Quercus ilex* subsp. *ballota* and *Quercus faginea* subsp. *faginea* pregerminated with 1 cm root was seeding in 0,3 L container filled with unsterilized fertilized peat, which is corrected by amending the pH of 8,5 with calcium carbonate. Triturated truffles were hydrated for 48 h before inoculation that was performed by injecting 20 ml of distilled water which had been suspended 1 g of fresh truffle per pot. The seedlings were kept in a greenhouse with a daily average temperature of 5-30° C, watered to saturation 2-3 times a week, and fertilized with 120 mg of N/seedling at the end of the experiment. For each treatment the percentage of mycorrhiza was evaluated, and morpho-physiological attributes to characterize forest quality was analyzed. The spores were stained with fluorescein diacetate and tetrazolium salts to estimate viability.

KEYWORDS

Quercus ilex subsp. *ballota*, *Quercus faginea* subsp *faginea*, *Tuber melanosporum*, viability, spores.

The ecology and cultivation of Oregon truffles

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Abstract

Four indigenous truffle species with recognized culinary and commercial value, known collectively as Oregon truffles, are harvested in the Pacific Northwest region of North America, primarily in Oregon and Washington west of the Cascades Mountains. They include *Tuber oregonense* Trappe, Bonito & Rawlinson, *T. gibbosum* Harkn., *Leucangium carthusianum* (Tul. & C. Tul.) Paol., and *Kalapuya brunnea* Trappe, Trappe & Bonito. Until recently their reputation suffered from indiscriminate harvest of immature truffles, but education of chefs and consumers, as well as promotion and education regarding the use of truffle dogs, have contributed to both improved quality and prices that exceed some better-known European truffles.

Oregon truffles are observed fruiting naturally in a wide variety of common soil types, and are widespread in natural and managed forests throughout the region. Despite considerable breadth in habitat characteristics, they reach their greatest abundance in young afforested *Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco plantations, established on former agricultural land. In suitable soils, a high proportion of these plantations eventually produce one or more, and occasionally all four, Oregon truffle species. Their early successional behavior and adaptability to anthropogenic environments suggest that the Oregon truffles may lend themselves to commercial cultivation. Because their principal habitat is ephemeral, abundance of these species is largely a function of trends in human demographics, agricultural economics, and land use taxation taking place over periods of decades. Sustainable long-term supply of these species is likely to depend on development of agricultural systems.

Cualitative characterization of two truffle-plantations, five and six-years-old in the Maule region (Chile)

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Abstract

The Maule region, located in central Chile, presents a series of climatic, topographical and soil conditions that allow the development of various agricultural and forest crops.

In these cultures, the black Périgord truffle has positioned itself as a real alternative investment for domestic producers, especially producers who Maule region through the implementation of integrated crop management to their plantations allowed entry production in the fifth and sixth years of the first black truffles in Chile.

After various research projects, supported by the government of Chile, have allowed qualitatively characterize potential areas for development of the truffle Chile.

In this context, this study presents the characterization and agronomic edaphoclimatic two of the black truffle producing areas Chilena, located in the Maule region, making it possible to validate the crop in Chile.

KEYWORDS

Tuber melanosporum, Chile, edaphoclimatic, black truffle.

Effect of irrigation treatments on soil water potential and truffle productivity in a *Quercus ilex* – *Tuber melanosporum* orchard

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Abstract

Irrigation is a key treatment in Truffle orchard management, yet there is lack of experimental studies on the amount of water needed to optimize truffle yields in mature plantations. In order to provide plantation managers with watering guidelines, in the spring of 2011, we instrumentalized a mature *Quercus ilex* plantation with water potential meters and we took readings every week from mid spring to mid autumn to measure the effects of the watering treatments which consisted of watering at a rate of 32 l/m2 every 2, 3, and 5 weeks, with no irrigation as control. In the winter, truffles were collected by tree and weighed in the lab. Preliminary results indicate that watering has a significant effect on truffle production that is highest when watering frequency is two weeks.

KEYWORDS

Tuber melanosporum, *Quercus ilex*, irrigation, water potential.

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Production of high quality truffle plants under ISO 9001 quality label and their performances in truffle orchard

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Abstract

The ROBIN tree nursery company is involved in controlled mycorrhization of forests plants since 1960 and in large scale production of mycorrhizal plants including edible mushrooms since 18 years. The ROBIN nursery is the first European nursery for the production of mycorrhizal plants under ISO 9001 quality label. The nursery has been involved in several European research programs adressing mycorrhizal symbiosis related to three main goals : reforestation purpose, selected mycorrhizal seedlings for phytoremediation of contaminated area, and establishment of EDIBLE MUSHROOM ORCHARD® .These participations allowed the nursery to get a considerable experience both at scientific and practical level on mycorrhizal symbiosis and high quality mycorrhizal plants production.A staff specially trained for mycorrhizal studies manage all steps of production of truffle plants from controls and selections of fruitbodies to final control before delivery according to schedule defined under ISO 9001 label aimed for high quality products.
The truffle plants are produced in patented ROBIN ANTI SPIRALLING® containers of various volumes and different ages. A large range of host trees are associated with all the main truffles : *Tuber melanosporum*, *T.uncinatum*, *T.aestivum* and with *Tuber magnatum* in close collaboration with INRA.
Custom made plants can be produced according to the special request of our customers in the frame of a special growing contract using selected batches of seeds and truffles supplied by the customers(ie : from different regions of France or from different foreign european countries). Each batch of seeds and truflles we received is well identified in order to keep track of these batches all along the steps of production. Each truffle we received is controlled under microscope with the aim to identify their spores. This control allows us to check the product supplied and to evaluate the quality of our inoculum.
These truffle plants are set up in specially designed green houses and the development of the mycorrhizal association is regularly followed in order to maintain the permanence of this association. At the end of the growing season each batch of the production is sampled using statistical law. Each sample done is observed under stereoscopic microscope by trained staff in order to evaluate the mycorrhizal rate of this batch (in house control) .After that the final control is performed by National French institute INRA. The control of the mycorrhizal rate is done laying particular stress on the good distribution of the mycorrhizas of the inoculated truffle along the root system and on the amount of these mycorrhizas and on the lack of other contaminant fungus. Within the framework of European VERCHAMP® based on principle of sustainability and the concept of local development, 68 EDIBLE MUSHROOM ORCHARD® have been set up during the years 2004-2007: in total 15 000 plants in various ecological conditions using truffles plants and also plants mycorrhized with *Lactarius* and *Suillus luteus* edible species.
The first plants mycorrhizal with *Tuber magnatum* and controlled by molecular tools, have been planted out since 2006 in 55 differents orchards located in France, Italy and Switzerland. The analysis of the data obtained from an experimental network set up in 2000 devoted to the trees CHAMPION®, concept defined in 1995 by ROBIN nursery, gave very interesting results. In MUSHROOM ORCHARD® regular and sustained production can be expected earlier (4-7 years) than in the past due to improved quality seedlings. Controlled truffle plants production under custom request is the best way to extend the preservation and the extension of the harvest of the truffles in their wide natural habitat.

KEYWORDS

Mycorrhizal plants, Truffle, EDIBLE MUSHROOM ORCHARD®, nursery, tree CHAMPION® concept.

In vivo and *in vitro* experimentation with the singular ectomycorrhizal fungus *Sphaerosporella brunnea* (Alb. & Schwein.) Svrček & Kubička

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Abstract
Sphaerosporella brunnea (anamorph: *Dichobotrys brunnea*) is a pioneer and opportunist ectomycorrhizal species presenting a facultatively saprophytic behaviour, and it is the most common competitor fungus in nurseries producing plants mycorrhized with *Tuber* species. Our objective in this work was to learn more about its lifestyle as the first step to manage its presence in greenhouses.

Monosporal isolates obtained from ascospores of *S. brunnea* were employed to develop a series of *in vitro* and *in vivo* assays. First we studied their vegetative growth and asexual reproduction in different culture media and conditions. Second, two inoculation trials with *Quercus ilex* were performed in climate chambers, monitoring ectomycorrhiza formation and ascocarp production. In the first trial plants were inoculated with different combinations of two monosporal isolates or with just one of them. In the second trial different watering frequencies were applied to plants previously inoculated with conidia obtained from a single monosporal isolate.

S. brunnea grew rapidly on most standard media and produced conidiophores and arthrospores under appropriate conditions. The composition of the culture medium did not only influence the growth of the *S. brunnea* mycelium (better growth rates in PDA –Potato Dextrose Agar- and OMA – Oat Meal Agar- media) but also in the formation of asexual spores (just in CMA -Corn Meal Agar- and MEA –Malt Extract Agar- media). The effect of aeration was decisive for conidiogenesis. Both mycelia and conidiospores were found to be effective sources of inoculum for mycorrhization. *In vivo S. brunnea* was able to form ectomycorrhizae and ascocarps rapidly, in less than two months and in three months from the inoculation date respectively. Ascocarp production was dependent on watering frequency but independent from the presence of potential mating partners.

Concerning sexual reproduction, homotallism of this species seems to be proven as apothecia have been obtained after inoculations with material, mycelium or conidia, from single monosporal isolates. The life cycle traits of *S. brunnea* as assessed in this study, i.e. fast growth, homothallism and rapid production of mitospores and meiospores are characteristic of a very effective colonizer and may help to understand its virulence under greenhouse conditions. The arthrospore-like structures observed in pure cultures may function as resistant resting spores. Substrate moisture seems to be a determinant factor for the development of this fungus. Therefore, its management can be very useful to control its presence in greenhouses devoted to the production of mycorrhized plants.

Propagation by mitospores and homothallism are poorly known in ECM fungi, therefore these results may be of fundamental interest beyond the initial question of greenhouse management.

KEYWORDS Dichobotrys, homotallism, conidiogenesis, *Tuber melanosporum* nurseries, fungal vegetative growth, fungal reproduction, pest management.

Phytosanitary problems that has been detected in truffle plantations in the province of Teruel (Spain)

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Abstract
The cultivation of the mycorrhized plant with *Tuber melanosporum* (above all, oaks, *Quercus ilex*) has had a spectacular growth in the last 20 years, at the end of this year (2012) we can find nearly 10.000 hectares as a regular plantation.

The dispersion of the cultivation in places without natural *ilex*, the different seedlings' origin, the lack of knowledge about the cultivation techniques that some of the farmers have about the theme and the improvement in the conditions of the plant's development (the fertilisation, the work in the fields, the watering, etc…). All of these matters have as a consequence the emergence of pests and diseases that in a natural environment they go unnoticed.

In this study, it has been done an exposition about the parasites that have been detected in the truffle plantations along the last 20 years in our province.

First of all, it's exposed the parasites that affect to the nursery plants and the damages to the crob (root and the over ground part) and at the end, those parasites that affect to the truffle.

Some damages produced by eriophyid *Cecidophyes tristernalis* N. and *Aceria ilicis* C.), curculionidae (*Otiorphynchus sulcatus* F.) and rodents (*Microtus* and *Apodemus*) have been observed in nursery.

In the plantation and in the first years of growth, we find worms that damage the roots (*Otiorhynchus sulcatus* F., *Melolontha*, *Vesperus* sp.) and rodents (*Microtus*).

On top of that, it has been noted that in the over ground part we can find some damages in the leaves produced by crixomélidae (*Lachanaia hirta* F.), eriofidae (*Aceria ilicis* C.), curcuoliónidae (*Polydrosus setifrons* J. du Val) and cinípidae (*Plagiothrochus quercusilicis* F.). The branches and twigs have been attacked by homopterous of genre *Kermes* (*K. vermilio* P. and *K ilicis* L.) and *Targionia* (*T vitis* S.).

The attack from fungus (*Taphrina kruchii* B. and fumaginas) and bacteria (*Erwinia quercina*) have lower incidence over the plantations.

In the truffles, damages caused by *Leiodes'* larvae are particularly serious.

In the same way, frosts and hailstones are found as occasional damages.

Finally, it is mentioned several damages whose origins are unknown, and they consist in some irregular swags that appear in the branches, as time passes these bulgings increase their size and they may cause the drying of the branch, and it is also possible the drying of all the plant's over ground part. The plant's answer is that it gushes, and this action keeps this root zone alive.

KEYWORDS
Tuber melanosporum, Quercus, parasites, damages.

Truffle and truffle cultivation in Italy: main aspects and news

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Abstract

The author reviews the main aspects regarding truffles and truffle cultivation in Italy. First of all, the various species of truffles now present in Italy and the quantity of truffles gathered and sold in Italy every year are described; the economic, commercial, environmental and social importance of truffles and truffle cultivation on upland and underdeveloped areas in various Italian regions is also explained. Second, the various procedures of production, the quantity of truffle trees planted in Italy every year and the hectares of truffle ground currently under cultivation are briefly mentioned; then, the technical procedures used in the cultivation of truffles are explained, focusing on the main "cultivation models" with particular reference to the new approach now in use in Italy (integrated model or Brothers Angellozzi's model). Finally, the results of the average production per hectare of each cultivated truffle species are provided, together with the review of the business brought about by truffles and truffle cultivation in Italy (more than 100 million Euros!).

The first production results of *Tuber melanosporum* in Sweden

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Abstract

With this report of the first production of *Tuber melanosporum* in Sweden, the northern cultivation border of this truffle species has been greatly expanded. The possibility of growing the black truffle *T. melanosporum* in truffières planted with inoculated tree seedlings, together with the dramatic decline in natural production during the last century, has lead to vast establishment of truffle orchards. These antrophogenic activities have widely expanded the geographical distribution of the species. Today, successful truffières are harvested in *e.g.* the USA, New Zealand and Australia. However, with the main harvesting season between December and March, the Northern European expansion for *T. melanosporum* has had its climatic limits.

Until recently, the northernmost producing *T. melanosporum* truffière was situated in France on the 50th latitude. However, on the 14th of November 2011 the first truffles were found in a planted *T. melanosporum* truffière at the 58th latitude on the Swedish island of Gotland. One of these fruitbodies was close to full maturation, while the second was immature, still with a whitish interior. Both specimens have been confirmed as *T. melanosporum* by DNA sequencing and phylogenetic analysis of the ITS sequence, as well as microscopical examination of the typical spiny spores, macro-morphology, and olfactory testing.

The now producing Swedish *T. melanosporum* truffière was established with *T. melanosporum* inoculated hazel (*Corylus avellana*) seedlings on Northern Gotland in year 2002. The island of Gotland has during the last decade nurtured a growing reputation for its naturally occuring Burgundy truffles, *Tuber aestivum* syn. *T. uncinatum*, which has created a new Nordic market for this delicacy. With Gotland, being the northern outpost even for the more cold-resistant *T. uncinatum* truffle, planting *T. melanosporum* inoculated seedlings was a far shot, but one that turned out successful, thus introducing a new and valuable cash-crop on rural Gotland. The distribution of soil and climate parameters potentially suitable for *T. melanosporum* production in this region are further investigated.

The authors are most thankful to Konsul Faxes Foundation for supporting the research on Swedish truffle cultivation, and to the truffle orchard owner Mrs Maria Karlsson at Bräntings farm in Rute parish on Gotland, Sweden.

KEYWORDS

Tuber melanosporum, truffle production, Sweden.

Long-term alteration of the soil properties and ectomycorrhizal community of former charcoal kilns in a truffle-producing region

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Abstract

Spanish truffle hunters point out that many historic charcoal kilns have produced *Tuber melanosporum* sporocarps at some time after abandonment in the first half of the 20th century. The traditional production of charcoal involved piling the wood, covering it with soil, and carbonising it through a slow combustion. The kiln reached up to 400-500ºC and the process took three to ten days.
The main factors that have been claimed to contribute to the occurrence of *T. melanosporum* in charcoal kilns are: (a) the open vegetation that is required to construct these kilns persists some years after the abandonment of the kilns, and this environment is propitious to *T. melanosporum*; (b) heating damage on ectomycorrhizal propagules decreases the ectomycorrhizal infectivity of the soil; (c) heating and incorporation of charcoal particles modify the soil properties; (d) wood distillates incorporating into the soil could inhibit fungal activity; and (e) wild boars (which act as vectors for dispersal of truffle spores) are attracted to abandoned kilns for wallowing.
The current characteristics of the soil and the ectomycorrhizal community of former charcoal kilns have been assessed with the aim of improving the understanding of the ecological relation between former charcoal kilns and the spontaneous formation of *T. melanosporum truffières*. The physical and biological soil properties of 18 former charcoal kilns in three different sites were compared with those of the surrounding forest. Two bioassays were performed in soils of the three sites to assess the infectivity of soils and the competitiveness of *T. melanosporum* in this environment.
Although the kilns have not been used for decades, the soil showed lower resistance to penetration, lower water infiltration, a different colour and a higher daily temperature fluctuation. The soil respiration was also lower, although not significantly. The occurrence of herbaceous and subshrubs is lower, whereas the roots of *Quercus* are more abundant.
In the soil of the charcoal kilns neither the ectomycorrhizal infectivity nor the richness of native ectomycorrhizal fungi colonising the roots of seedlings was significantly different from those in the surrounding forest. However the composition of the community colinising the seedlings was different in the charcoal kilns and the surrounding forest. When the soil was heated the infectivity was reduced in the control soil but not in the charcoal kiln. The proliferation and competitiveness of *T. melanosporum* mycorrhizas was similar in charcoal kilns and control soils.
The recovery of the ectomycorrhizal community in charcoal kilns and the changes in composition suggests that these soils are not currently better for the development of the symbiotic phase of *T. melanosporum*. On the other side reduced penetration resistance, the increased daily temperature fluctuation, the reduced soil respiration and the reduced abundance of herbaceous are characteristics of spontaneous *truffières*, suggesting a better environment for *T. melanosporum* reproduction.

KEYWORDS
Tuber melanosporum, charcoal kiln, ecology.

Characterisation of Pseudomonadaceae populations in *Tuber melanosporum* wild truffieres

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Abstract

In the last decade several surveys have revealed an important role of *Pseudomonas* genus as a Mycorrhizal Helper Bacteria (MHB). Moreover, the bioaugmentation of environmental isolated *Pseudomonas* strains to the substrate at inoculation time in different plant species throughout mycorrhization assays have been related on the occurrence of a greater number of mycorrhiza of *Tuber* genus.

In this work, a selective culture medium was designed to isolate bacteria belonging to *Pseudomonaceae* family from soil samples taken from two different wild *truffières*. The amount of culturable *Pseudomonadaceae* populations was monitored using the selective medium throughout one year in different wild *truffières*. A total of eight samples per monitored *truffières* were taken every 40 days for the two monitored *truffières*. In the same area, non-truffle producing evergreen oaks were used as negative controls being sampled simultaneously to the monitored *truffières*. The results revealed both a low diversity of colonial morphology and a similar abundance of culturable *Pseudomonadaceae* strains on the selective-designed agar medium throughout the one-year survey in both monitored *truffières* ($6.44 \times 10^4 \pm 3.54 \times 10^4$ CFU · $g^{-1}$and $5.71 \times 10^4 \pm 3.25 \times 10^4$ CFU · g^{-1} respectively (± standard deviation of n=8). Non-producing trees showed similar colonial morphologies to those found from the *truffières*, but less abundant ($2.54 \times 10^4 \pm 2.54 \times 10^4$ CFU · $g^{-1}$of soil (± standard deviation of n=8)).

Finally, thirty samples from both *truffières* and controls were characterized by means of 16SrRNA gene-based PCR-DGGE (Polymerase Chain Reaction (PCR) with a subsequent Denaturing Gradient Gel Electrophoresis (DGGE). We obtained a low diversity (1-4 DGGE bands) in all the studied samples (both *truffières* and controls).

It is noteworthy that most of detected bands migrated mainly in two defined positions from the DGGE gel, corresponding to a two different ribotypes belonging to *Pseudomonas chlororaphis* and *Stenotrophomonas spp*. respectively.

KEYWORDS

Pseudomonas, MHB, *Tuber melanosporum,* wild truffieres.

Recent advances of research on diversity, conservation and cultivation of truffles in China

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Abstract

Since 1985 to 2008 around 20 truffle species were recorded. *Tuber indicum* and *Tuber aestivum* have been commercialized. Last five years more than 15 new truffle species were discovered, all of which are white truffles. One of them, *Tuber panzhihuanense* has been published, which has big ascomata, peasant aroma and commercialized. *T. bominense* from Tibet and other 13 new species will been published soon. Recent discovery of Chinese truffle new species further reveals biodiversity of truffles is much richer than we expected. Increasing demand for Chinese edible truffle products led to large-scale commercial harvesting of these species, which caused severe damages of their forest environments. Production has declined by 1/2 to 1/3 in the last few years, even to the extent that truffle resources may face extinction in some regions if destructive harvesting methods are not stopped. Management and legislation on the commercial harvesting and conservation of truffles in China are now drawn attention. Proposals of protection the truffle resources are launched in a few regions. A few truffle reserves have been established. More than 10 new truffle orchards have been established recently. Ascocarps of *Tuber indicum* and *Tuber melanosporum* have been produced from two orchards. Post harvesting processing has also been advanced.

KEYWORDS

Advance, Chinese truffles, biodiversity, protection, cultivation.

Habitat characteristics and phylogeny of *Tuber aestivum* and accompanying truffles in Poland

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Abstract

Research on truffles revealed that at least ten species of *Tuber* genera is present in Poland. The identification was done basing on morphology, anatomy and genetic features of examined species.
So far, only a few conducive to *Tuber aestivum* sites have been characterised taking into account both biotic and abiotic ecological conditions.

The sites are mainly located in Nidzianska Basin, in southern part of Poland. The soils in the area are characterized by bedrock that belongs to marlstone, marly limestone and gypsum. The type of soil is rendzina. Despite its high latitude 50°32'N and longitude 20°32'E, the region which was the richest in fruiting bodies of *T. aestivum* belongs to one of the warmest Polish zones. The annual mean precipitation between 1997 and 2012 was 600 mm and the annual mean temperature for the same period was 8.0°C. Localities are situated at an elevation of 230 – 300 meters.

For the localities physical and chemical soil parameters such as: texture, pH, Ca, K, Mg, N, P, organic matter were analysed. Diversity of host species and forest floor plants were summarised. Last year two new truffles' species were found and the genetic analysis confirmed presence of *T. maculatum* and *T. macrosporum*. Presently, only one locality for the latter species is known. Yet, we have been focusing on key factors that might determine its occurrence. Since the number of truffle fruit bodies is often limited we have studied also mycorrhizal structure in order to widen our knowledge of ecological factors in truffles' environment. Assessment of mycorrhizae was done for such tree species as: *Quercus robur*, *Corylus avellana* and *Carpinus betulus*. On the investigated host species mycorrhizae of *Tuber aestivum, T. macrosporum, T. maculatum* and *T. excavatum* were present.

Second point of our study is to find a possible route of *T. aestivum* migration. Comparison of the species basing on DNA sequences (deposited in Genbank NCBI) for different geographical regions in Europe showed that the Italian refugee is most probable. The affinity between truffle species found in Poland is also discussed.

KEYWORDS

Truffle habitat, soil conditions, mycorrhiza, phylogeny.

Does *Pseudomonas fluorescens* help the *Tuber melanosporum* colonization on *Pinus* sp. seedlings?

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Abstract

Pinus nigra and *Pinus halepensis* are forest species currently used to reforestation programs in calcareous soils of mediterranean regions, and their roots are able to form a mycorrhizal symbiosis with the black truffle. The inoculation of forest seedlings with ectomycorrhizal fungi can benefit the reforestation of Mediterranean areas and the reintroduction of inocula of mycorrhizal fungi into these areas. Also, some rhizobacteria can improve the establishment and functioning of this ectomycorrhizal symbiosis (Mycorrhizal Helper Bacteria) between the fungus and the host plant. In two similar studies conducted in 2009 (Dominguez et al., 2012) [2] and 2011, *P. halepensis* and *P. nigra* seedlings were inoculated with the *Tuber melanosporum* mycorrhizal fungus and the *Pseudomonas fluorescens* CECT 844 rhizobacteria, under non-limiting greenhouse conditions. While *P. halepensis* seedlings were cultivated without added calcium carbonate to a growing peat substrate, *P. nigra* seedlings were grown on the same substrate with added calcium carbonates, correcting its initial pH 6.

Five months after inoculation, we analysed the mycorrhizal colonization in seedlings roots. The addition of *P. fluorescens* CECT 844 did double the rate of the mycorrhization of *T. melanosporum* on *P. halepensis* seedlings growing in substrata without carbonates added; by contrast, this rhizobacterial inoculation did not significantly improve the colonization of *T. melanosporum* on *P. nigra* seedlings growing in substrata corrected.

From the results of these two trials, we hypothesized that the *P. fluorescens* CECT 844 strain may cause MHB (Mycorrhizal Helper Bacteria) effects under unsuitable pH soil conditions to the black truffle.

[2] Dominguez, J.A.; Martin, A. Anriquez, A & Albanesi, A, 2012. The combined effects of *Pseudomonas fluorescens* and *Tuber melanosporum* on the quality of *Pinus halepensis* seedlings. Mycorrhiza, 22 (6) 429-436.

The combined effects of *Pseudomonas fluorescens* rhizobacteria and *Tuber melanosporum* on the growth of *Pinus nigra* seedlings

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Abstract

The inoculation of forest seedlings with mycorrhizal fungi and rhizobacteria can improve the morphology and physiology of the seedlings and benefit the reforestation of Mediterranean areas and the reintroduction of inocula of mycorrhizal fungi into these areas. The ecological, economic and social values of the ectomycorrhizal fungi of the black truffle found in the rural Mediterranean are well known.

Pinus nigra is a specie currently used to reforestation in mediterranean regions, and their roots are able to form a mycorrhizal symbiosis with the fungus *Tuber melanosporum;* previously we howed that inoculation of *P. halepensis* seedlings with *Pseudomonas fluorescens* CECT 844 rhizobacteria improved plant growth and N absorption (Dominguez et al., 2012)[2]. At present work, *P. nigra* seedlings were produced in the nursery under well-watered conditions, growing on the peat substrate with added calcium carbonates, correcting its initial pH 6; we studied the physiological response of these seedlings to a combined treatment of *Tuber melanosporum* inoculation and/or a rhizobacteria *Pseudomonas fluorescens* CECT 844 inoculation. Five months after inoculations, growth parameters (seedling height, basal diameter, shoot and root dry weight), were measured ; the N, P, K total content and the concentration on seedlings roots and shoots were also measured. Subsequently, tests were performed to estimate the root growth potentials.

Few significant improvements on growth parameters were observed; only the combined inoculations improved the shoot weigh of the seedlings. Moreover, between two inoculations apparently opposing effects were observed regarding the P absorption by the seedlings.

[2] Dominguez, J.A.; Martin, A. Anriquez, A & Albanesi, A, 2012. The combined effects of *Pseudomonas fluorescens* and *Tuber melanosporum* on the quality of *Pinus halepensis* seedlings. Mycorrhiza, 22 (6) 429-436.

Results of long-term mycorrhization trials and spatial distribution of mycorrhiza of *Tuber macrosporum* Vittad.

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Abstract

Besides well-known and popular truffle species of wide market (*Tuber magnatum*, *Tuber melanosporum* and *Tuber aestivum/uncinatum*) some other truffle species also represent high organoleptic value. *Tuber macrosporum* is one of these species, however, due to its unbalanced presence, depending mostly on natural truffle sites, only small market is based on the species and fruit bodies are mainly sold mixed with *Tuber aestivum/uncinatum*. Cultivation of *T. macrosporum* mycorrhized plants could solve the problem and can open new perspectives for the species.

In the trial the most common plant partners of the truffle were selected for inoculation: *Quercus robur*, *Quercus cerris*, *Corylus avellana* and *Tilia cordata* seeds were surface sterilized and germinated in sterile substrate. Mature and unhurt carpophores of *T. macrosporum* were collected in natural Hungarian truffle habitats, cleaned, surface sterilized and stored at -20 C° until use. Spore slurry were made blending carpophores with water. 2 g/plant of spore slurry was added and plants were transplanted at 2-leaf stage into plastic containers of 1000 cm^3 filled with a special peat-perlite mix. Plants were nursed for 30 months under unheated greenhouse conditions. Seedlings were watered regarding the demand of seedling and keep in mind that on natural habitats *T. macrosporum* is mainly present on fresh, wet soils.

Fifteen seedlings of the total 30 inoculated were selected to evaluate the mycorrhization level in each plant species. Seedling roots were soaked in cold water for 4 hours and gently washed under tap water. Fine roots were clean used brush and pincers. Mycorrhiza description was carried out using stereo and light microscope. Root colonization level were determined on the following method: seedling root system were divided into distal and proximal sides; four parts were selected and 50 root tips in each root part were counted, total of 400 root tips helped to estimate mycorrhiza infection level.

Preliminary results showed variable *T.macrosporum* mycorrhiza presence on the root tips of different host species, and in some cases concurrent mycorrhizal species also appeared. The methodology used for mycorrhiza analysis made possible to draw a picture of mycorrhizal root tip spatial distribution, evidently limited by the container but still informative to carry out the right cultivation method.

KEYWORDS

Tuber macrosporum, mycorrhization, *Quercus spp., Corylus avellana, Tilia cordata.*

Vineyard and truffle : association between the roots of vine and the mycelium of truffle in a productive "truffière"

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Abstract

During the last century, truffle trees were often planted in vineyards or after them, particularly during the phylloxera crisis. Truffle growers think that this association was beneficial for truffle production. In this trial, we study the effects of the association between vine plants and truffle trees on the evolution of *T.melanosporum* production and the ecological advantage of vine introduction into the truffle zone.

The plot is located on the 'Causse de Martel' (Lot, Midi-Pyrénées region). The sub-soil is a hard cracked limestone (Jurassic period), with red clay pockets and a shallow stony surface (5 to 15 cm). The texture is silt loam-sandy-clayey. The plant material consists of controlled oaks (*Quercus ilex* and *Quercus pubescens*) inoculated with *T.melanosporum*. The trial studied 2 treatments with 4 repetitions: with and without vineyard. Every unit contained 5 trees (plant density 5x5 m) totalling 80 trees. The plantation was carried out in 1999. Two vine plants were introduced in 2000 between each Quercus tree at 1.6m from the trunk. Tree growth and truffle production were measured each year. In 2010, biomolecular analyses of soil samples around 2 productive *Q.ilex* and 2 non productive *Q.pubscens* (but with a "brûlé") and of vine roots were performed to detect *Tuber* species with specific markers (*T.melanosporum*, *T.brumale* and *T.aestivum*). In 2011, biomolecular analysis of soil samples around 14 non productive *Q.pubescens* and on vine roots were carried out, and a mycorrhization study of the trees by morphological identifications completed this work.

The results show that the growth of the trees is the same between the two treatments (with and without vine plants). *Q.ilex* have a greater growth than *Q.pubescens* and began to produce first (winter 2005/2006). Even though the number of *Q.ilex* truffle trees is greater in the treatment with vine during the first six years, a statistical analysis of the two last years of fructification show that there isn't any difference in the mean number of truffles per tree and in the mean weight of truffles per tree between the area with vine and the area without vine.

The mycelium of *T.melanosporum* is detected by PCR on the roots of the vines up 3.2m from the trunk of the oaks with productive as well as with non productive associated trees (66% of the vine tested with non productive *Q.pubescens*). Myceliums of *T.aestivum* and *T.brumale* are sometime present. Soil samples show the presence of *T.melanosporum* mycelium at each side of the trunk into the "brulé" but the mycelium is not detected farther. The mycorrhization of the trees (morphological identification) seems to be in relation with the detection with mycelium in the soil.

Eventhough the roots of vine don't contract mycorrhizes, the detection of *T.melanosporum* associated with this plant is an interesting new result. We suppose that vine could have a functionnal role on the ecology of the "truffière" which is to be determined (dissemination, reproduction ?).

KEYWORDS *T.melanosporum,* vineyard, truffle ecology, *mycelium* detection.

A method to evaluate ectomycorrhizal fungus richness in black truffled plantations

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Abstract

Tree´s ectomycorrhizal community is one of the factors that determines the success of truffles production. Many works have been done in the world with different sampling methods in orther to know the mycorrhizal status of truffled trees in plantations but specific sampling effort that must be done to study it remains uncertain until today.

A sampling in depth of nine evergreen oaks (*Quercus ilex*) from different plantations was carried out, with the aim of developing a sampling method using soil cores that assess reliably the species richness of each truffled tree. Three different categories of age (less than six, between six and nine and more than nine years) and two of black truffle production (productive or not productive) were chosen. In every tree 12-24 soil cores were taken, in order to cover the whole root influence area. Ectomycorrhizal morphotypes found in each soil core were differentiated by its anato-morphological characters, sampling sequence was randomized and species richness was estimated by Clench model.

Observed number of species per tree was from one to 15, with a strong dependency on the productive character and fewer on the age of the host tree. Species accumulation curves were stabilized quickly in asymptotes in three trees, or approached them in five ones, even when the observed number of species was high. Samples taken were not enough for just one of them. Gradual statistical analysis of the samples is recommended in order to reduce sampling effort.

KEYWORDS

Species accumulation curves, *Tuber melanosporum,* ectomycorrhizal fungal community, *Quercus ilex,* soil cores.

Diversity of ectomycorrhizae in black truffle plantations in Teruel

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Abstract

Trufficulture is an original agro-forestry activity in which a fungus is cultivated in the living roots of a tree. It needs more tools that allows its development evaluation than in any other case. Ectomycorrhizae root analysis must be done in order to verify the presence and evolution of the inoculated fungus along the years and the species that have colonized the roots and that may compromise the success of the plantations. This work focus on the identity of those fungi, their ecology and the ability to manage their presence on truffled trees.

48 truffled evergreen oaks of three different age groups (less than six, between six and nine and more than nine years), located in the province of Teruel (Spain), were studied. Half of them already producing black truffles and half non-producing. On each tree, five periodic ectomycorrhizae samplings were performed during two years, using two different sampling methods: direct extraction of roots and soil cores.

A total of 98 different ectomycorrhizal morphotypes were found, 11 of them were identified using anato-morphological techniques to species level and 18 of them to genus level. The most frequent found types were: *Tuber melanosporum*, *Trichophaea woolhopeia*, *Tuber brumale*, *Pisolithus arhizus* and *Quercirhiza squamosa*.

The most dominant ectomycorrhizal types belong to short distance exploration type and Thelephorales order was the most represented. We found neither any morphotype with the ability of displacing black truffle ectomycorrhizae from the roots, nor possible indicators of land suitability for truffle growing.

One to three different ectomycorrhizal morphotypes were found in 58% of the trees and the maximum number in a single one was 21. It was shown that both, species richness and distribution of their abundance, are higher in non-productive trees and in those older than nine years. Those trees also exhibited a higher proportion of more evolved exploration types.

This work increases the knowledge of black truffle fungal competitors, and could be the basis of future studies that contribute to managing its impact on truffle cultivation.

KEYWORDS
Black truffle, *Trichophaea woolhopeia, Quercirhiza quadratum*, AD morphotype, *Tuber brumale, Pisolithus arhizus, Quercirrhiza squamosal.*

Ectomycorrhiza community from natural and plantation *Tuber aestivum* sites in Mediterranean climate

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Abstract

Tuber aestivum Vittad. is an important commercial truffle species in Europe, frequently grown in truffle plantations and commonly collected from various stands and areas. *T. aestivum* is ectomycorrhizal with oaks, hornbeams, hazel and several other tree or shrub species. Several studies indicated the shift in ectomycorrhiza community after the plantation of inoculated seedlings in reducing the initial species and increasing of the “contaminant” species. Roles of the latter in truffle production are not clear.

We aimed to study and compare the ectomycorrhizal community in a representative natural and plantation *T. aestivum* sites with identical or differential plant partners of the same age (20-25 years after planting) and comparable climate and soil conditions. The populations of ectomycorrhiza were analysed using standardized sampling, morphologic and molecular identification approaches and basic diversity assessment. Communities were compared among the sites and plant partners.

The study represents the insight into the ectomycorrhizal community related to the natural *T. aestivum* ground in comparison to previously analysed plantations by Benucci et al. (2011). None of the *T. aestivum* sites showed only the presence of this truffle ectomycorrhiza. In plantations sites there were several other *Tuber* species, otherwise not observed in natural sites. The number of the “contaminant” species was comparable among sites and differed among host plants both, in natural forests and plantations with higher number in hazel and hornbeam and lower in oaks. The observed differences indicate the influence of the pioneer level of the host plant. The community diversity was comparable to other ectomycorrhizal sites and indicates the shift of the plantations toward natural conditions. In particular the presence of several ectomycorrhizal species in common indicates the parallel shift in communities.

Benucci G.M.N., Raggi L., Albertini E., Grebenc T., Bencivenga M., Falcinelli M., Di Massimo G. 2011. Ectomycorrhizal communities in a productive *Tuber aestivum* orchard: composition, host influence and species replacement. FEMS Microbiology Ecology, 76: 170-184.
Pinuela Samaniego Y. 2012. Ectomycorrhiza diversity in natural *Tuber aestivum* Vittad. ground. B. Sc. Thesis, Academic study Programmes, Department of forestry and Renewable Forest Resources, University of Ljubljana, Ljubljana, 65p.

KEYWORDS

Tuber aestivum, ectomycorrhiza community, diversity, natural ground, plantation.

Tree-shelters may modify the symbiotic relationship between the black truffle and its host depending on the host species

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Abstract

In Mediterranean Truffle Orchards, *Quercus ilex* and *Q. faginea* are common hosts for *Tuber melanosporum*, and after planting the seedlings are protected by tree-shelters during the early years. Studies about host species for *T. melanosporum* protected by tree-shelters are scarce especially in the case of tree-shelters built by translucent and opaque material. By combining *Q. ilex* and *Q. faginea* species with 3 tree-shelters (opaque with lateral holes, translucent and flexible black mesh) plus a control in five orchards, we establish a two-way factorial design to study the effects of the interaction between species and tree-shelters on survival of host seedlings and their growth and the growth of *T. melanosporum* and ectomycorrhizal competitors. Because, in the same nursery conditions, *Q. faginea* grows faster than *Q. ilex*, we also study the dynamic of *T. melanosporum* and ectomycorrhizal competitors in no sheltered seedlings before planting and after three years in field. The survival rates were independent of the tree-shelter and species. On average, seedling survival was 90%, which was considered that seedlings had a proper adaptation to the ecological conditions of orchards. The interaction between tree-shelters and species was significant for the absolute abundance of *T. melanosporum* (total ectomycorrhizae of *T. melanosporum* per seedling) and the absolute abundance of ectomycorrhizae (*T. melanosporum* plus competitors per seedling). The absolute abundance of *T. melanosporum* was lower in opaque tree-shelters compared with control in *Q. ilex*, but no significant differences were observed in *Q. faginea*. However, in the case of absolute abundance of ectomycorrhizae, we only found significant differences in *Q. faginea* where the values were up to twice higher on translucent tree-shelters than in control. Tree-shelters had no effects on the relative abundance of *T. melanosporum* (*T. melanosporum* per total ectomycorrhizae) and the relative abundance of ectomycorrhizae (total ectomycorrhizae root tips per total root tips). Basal diameter before planting was 1.3 fold larger in *Q. faginea* than in *Q. ilex*, but after three years in field this difference increased to 3.5 and was significant. The absolute abundance of ectomycorrhizae increased after three years in field compared with the values before planting although the differences between species remained constant. However, the absolute abundance of *T. melanosporum* did not change in the field for any of the two species, and thus the initial differences between species remained unchanged. The relative abundance of ectomycorrhizae was similar in both host species before planting and did not change in field. However, the relative abundance of *T. melanosporum* decreased in field for both host species from 48% to 32%. The results of this study suggest that more opaque tree-shelters may delay *T. melanosporum* growth in host species characterized by slower growth rate as *Q. ilex*. Translucent tree-shelters could promote the abundance of ectomycorrhizae in faster growth rate species as *Q. faginea*, with unclear effect about *T. melanosporum*. Further studies are needed about the dynamic between *T. melanosporum* and ectomycorrhizal competitors in different host species, and the real needs of tree-shelters in orchards protected by fences.

KEYWORDS

Tuber melanosporum, Quercus ilex, Q. faginea.

A molecular approach for molecular indexing of *Tuber melanosporum* Vitt. from micorrhized trees

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Abstract

Truffle (*Tuber melanosporum* Vitt.) culture is an agroforestry sector in Chile of increasing interest due to the high prices that truffles fetch in the international market and the recent evidence that its commercial production is possible in the Chilean climatic and soil conditions. Farmers have identified the molecular indexing of the black truffle along the production system as critical, considering that they have to invest a huge amount of money for establishing their commercial plantations. Also, quality control of the harvested truffle using molecular identification can give more security for a product coming from a non-traditional supplier, as Chile is.

For indexing the black truffle using molecular markers along the productive chain it is necessary to sort several technical barriers: 1) It is necessary to have a standard protocol for extraction of high quality DNA from the rizosphere of inoculated plants, both from the nursery or from the plantations.; 2) A reliable and accurate method for molecular identification using specific primers for *T. melanosporum*.

For DNA high quality DNA extraction directly from the rizosphere of inoculated nursery plants or trees from the field, three methods were evaluated. High quality DNA was obtained from 5g of soil and roots of *Quercus ilex* L. mycorrhized with *T. melanosporum* Vitt. The influence of Nitrogen during sampling processing was not critical and the addition of BSA to the sample increased the detection efficiency of *T. melanosporum* by Polimerase Chain Reaction.

Detection of *T. melanosporum* was performed by the technique of cleaved amplified polymorphic sequence (CAPS) from amplicons generated with the primers ADL1 (5´-GTAACGATAAAGGCCATCTATAGG-3´) and ADL3 (5´-CGTTTTTCCTGAACTCTTCATCAC-3`), where a restriction fragment of 160 bp specific for *T. melanosporum* was generated, which allows the discrimination of this species from the rest of the species belonging to the *Tuber* sp. genus. Direct detection of *T. melanosporum* in one step was also obtained by polymerase chain reaction (PCR) from total DNA isolated from mycorrhized roots and with the primers ITSML (5´-TGGCCATGTGTCAGATTTAGTA-3´) and ITSLNG (5´-TGATATGCTTAAGTTCAGCGGG-3´), generating a single amplicon of 440 bp. The molecular detection of *T. melanosporum* by the methods presented here will allow the rapid and accurate detection of mycorrhization of trees, both under nursery and field conditions. This technology will also provide more security to farmers by controlling the quality of the mycorrhized trees they will plant and also by following the mycorrhization status of established orchards. Also, this technology could be a complementary approach for a certification program in Chile and South America, standardized with some European practices of certification.

KEYWORDS

Tuber melanosporum indexing, High quality DNA extraction.

Historical biogeography and diversification of truffles in the Tuberaceae and their newly identified Southern hemisphere sister lineage

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Abstract

Truffles have evolved from epigeous (aboveground) ancestors in nearly every major lineage of fleshy fungi. Because accelerated rates of morphological evolution accompany the transition to the truffle form, closely related epigeous ancestors remain unknown for most truffle lineages. This is the case for the quintessential truffle genus *Tuber*, which includes species with socio-economic importance and esteemed culinary attributes. Ecologically, *Tuber* species form obligate ectomycorrhizal symbioses with a diverse species of plant hosts including pines, oaks, poplars, orchids, and commercially important nut trees such as hazelnut, chestnut, and pecan. Unfortunately, relatively limited geographic sampling and inconclusive phylogenetic relationships have obscured our understanding of their origin, biogeography, and diversification. To address this problem, we analyzed a global sampling of Tuberaceae based on DNA sequence data from four loci (ITS, LSU, EF1-alpha, and RPB2) for phylogenetic inference and molecular dating. Our well-resolved Tuberaceae phylogeny shows high levels of regional and continental endemism, with several lineages restricted to only one continent (e.g. the /gibbosum lineage in North America, the /japonicum lineage in Asia and the /gennadii lineage in Europe). We also identify a previously unknown epigeous member of the Tuberaceae – the South American cup-fungus *Nothojafnea thaxteri* (Cash) Gamundí. Phylogenetic resolution was further improved through the inclusion of a previously unrecognized Southern hemisphere sister group of the Tuberaceae. This morphologically diverse assemblage of fungi includes truffle forms endemic to Australia and South America (e.g. *Gymnohydnotrya* species) as well as epigeous, *Helvella*-like species classified in the genus *Underwoodia* sensu lato. The Southern hemisphere taxa appear to have diverged more recently than the Northern hemisphere lineages. Our analysis of the Tuberaceae suggests that *Tuber* evolved from an epigeous ancestor. Molecular dating estimates that the Tuberaceae diverged in the late Jurassic (~156 million years ago), with subsequent radiations in the Cretaceous and Paleogene. Intracontinental diversification, limited long-distance dispersal, and ecological adaptations have driven truffle evolution and Tuberaceae biodiversity.

KEYWORDS fungal evolution, phylogenetics, divergence time estimation, *Tuber, ectomycorrhizal lineages, hypogeous fungi,* biogeography.

Exploring the potential of co-cropping the Pecan Truffle (*Tuber lyonii*) with Pecan (*Carya illinoinensis*) in the Southeastern USA

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Abstract

Truffles are part of traditional European cuisine and the most highly prized and economically important truffles are of European origin, including *Tuber melanosporum*, *T. magnatum*, *T. aestivum* and others. Despite the fact that truffle consumption has been primarily a European phenomenon, particularly of France and Italy, interest and enthusiasm for truffles has grown throughout other parts of the world. Truffle cultivators have made great strides in producing truffles (such as *Tuber melanosporum)* in cultivated orchard settings. However, attempts to grow European truffles in the United States have produced mixed results. In the Western USA, truffle connoisseurs have turned to local species, such as *Tuber gibbosum* and *Tuber oregonense* (the Oregon white truffles). The result has been a surge in the interest and economy surrounding these truffle species. Although "new" truffle species such as *T. gibbosum* and *T. oregonense* may not be tempting to traditional European truffle-consumers, these unique delicacies are appealing to a growing number of consumers interested in distinctive local foods and cuisine. In the southeastern USA there is an abundant and delicious local truffle species, *Tuber lyonii* that is commonly referred to as the Pecan Truffle because it is often collected in commercial orchards with pecan trees (*Carya illinoinensis*, Juglandaceae). This truffle species is a spiney-spored member of the *Tuber rufum* lineage. The Pecan Truffle has not been widely collected or consumed in the past but it shows great promise as a local truffle crop for the eastern and southeastern USA, where it has a growing and regional niche market. In the past several years, our research team has begun studying the biology of *Tuber lyonii* across the southern USA to learn more about this species and to determine how it might be successfully cultivated with pecan trees. Here we provide an overview of our recent research on *Tuber lyonii* and clarify what is known about the distribution, fruiting phenology, and host associations of this truffle species. Preliminary molecular data based on ITS rDNA indicates that *Tuber lyonii* is a species complex distributed across a wide geographic range spanning much of eastern, midwestern, and southern North America. No clear geographic patterns are yet evident and further work is needed to delimit the species within this group. Our recent studies using trained truffle dogs in Georgia and Texas in combination with analysis of ectomycorrhizas from pecan root samples indicate that members of this species complex are widespread across many soil types and can be abundant in the belowground ectomycorrhizal community of some pecan orchards. We expect that minor manipulations to the management of pecan orchards in the USA could enhance the distribution and production of *Tuber lyonii* with pecans in the near future.

KEYWORDS

Co-cropping, ectomycorrhizal communities, Pecan truffles, truffle systematics, *Tuber lyonii.*

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Functional characterization of the *T. melanosporum* pheromone-receptor system by yeast complementation assays

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Abstract

The release of *T. melanosporum* genome has recently allowed us to identify the structure of the mating type locus (*MAT*) and in turn to find out that the two *MAT* genes at this locus are present in different strains (Martin et al., Nature 2010; Rubini et al., New Phytol 2011). This organization of the *MAT* locus typifies heterothallic ascomycetes. In these fungi mating occurs only between strains of opposite mating type, which attract each other by secreting pheromones. Each mating type produces its own specific pheromone These can be divided into two groups, α- and a-factor pheromones. Each pheromone interacts with its cognate receptor synthesized by a strain of opposite mating type: the α- and a-factor pheromones are bound by STE2 and STE3 receptors, respectively. This binding triggers the activation of a mitogen-activated protein kinase (MAPK) signaling pathway ultimately targeting a homeodomain transcription factor (STE12) that coordinates the mating program of the cell (Debuchy et al., ASM press 2010).

Most of the key genes of the pheromone pathway have been *in silico* identified in the *T. melanosporum* genome, among them, the putative genes for the α *-factor (ppg1)* and *a-factor* (*ppg2)* pheromones, similar to those of *Saccharomyces cerevisiae,* along with genes coding for their receptors (Martin et al., Nature 2010).

Besides *in silico* identification, however, no functional evidences for the *T. melanosporum* pheromone receptor system have been provided yet. To achieve this goal, here we employed the yeast *S. cerevisiae* as a heterologous model system. Thus, the *T. melanosporum* coding sequences for the pheromone receptors STE2 and STE3 and for the putative pheromone precursors *ppg1* and *ppg2* have been isolated by RT-PCR from *in vitro* grown mycelia of opposite mating type and each one cloned in a yeast expression vector (pPGK, Kang et al., Cell Biol 1990). The pPGK vectors harboring the above mentioned truffle cDNAs were then transformed into suitable yeast strains lacking the endogenous pheromone and receptor genes. Agar diffusion bioassay (Halo assay) was used to evaluate the interaction between yeast strains carrying the heterologous pheromone and cognate receptor genes. Further to this, a synthetic α-factor peptide, designed on the basis of the *in silico* analysis of the putative gene for α pheromone precursor, was tested for its capacity in triggering morphological changes in a yeast strain expressing the respective truffle receptor gene (STE2).

These experiments documented a positive interaction between the protein pair coded by the *T. melanosporum ppg1* and *STE2* genes. Thus, a functional evidence for a truffle pheromone–receptor system has been provided for the first time. Efforts are in progress to provide functional evidence for the a-factor pheromone and STE3 receptor system.
The identification of pheromone and receptor genes in *Tuber* spp. is fundamental to deep our insight into the genetic mechanisms and environmental stimuli that control the fertilization process, and thus the fructification, of these fungi.

KEYWORDS

Pheromone, G protein coupled receptors, halo assay, *Saccaromyces cerevisiae*, mating type, MAT genes, heterothallism.

Between gleba and ascus

Lüer, K.[1]

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Abstract

Karin Lüer of the Institute for Genetics of the Johannes-Gutenberg University in Mainz will give a brief lecture on the above topic which will provide an insight into the secretive micro world of truffle spores. Mrs Lüer will show microscopic images of the last life cycle of a truffle fruit body. She made the images herself.

She will also discuss some questions about the development of asco spores which were raised when examining light and fluorescence microscopic images. She will present her own thought and theories for discussion.

These questions, which thus far have not been fully answered, relate to the invasion of spore material into the finished ascus and the influence of nutritional factors on the number of spores that will be developed. Furthermore, one might want to discuss the meaning of the fluorescence and why the spores in different states of ripeness give off different fluorescences.

The microscopic images are of an exceptionally high quality. She will also show a number of colouring attempts designed to reveal the DNA or Membranes. Also, Mrs Lüer will present some REM and laser microscopic images.

KEYWORDS

Microscopic imaging, spores, fluorescence, life cycle.

Analysis of European Union imports of Italian truffles using panel data methodology

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Abstract

Truffles are found in many regions of Italy where their harvest and sale creates considerable income. A considerable proportion of the crop is exported to other European countries where they can command very high prices.

The truffle is not a typical agrarian commodity but a higher and luxury goods hence its trade and consumption is more sensitive to change caused by financial and economic downturn. In the same time European consumers has become very demanding towards ecological issues and organic food with some effects on the consumption and importation of truffle.

The aim of my research was to investigate over the past 12 years which variables have acted on the importation of truffle from Italy to other European countries as a consequence of a change in the economic context and in the level of income in different states of the European Union.

To analyse the main interrelationships able to implement the export of Italian truffle inside the European domestic market, I used a quantitative approach using statistical dataset published by Eurostat through the multiple regression model based on a fixed approach on panel data. The R^2 and adjusted R^2 have pointed out as the model fits well with the aim of the research and it has been able to explain more than 50% of variance. The parameters estimated by fixed effect panel data have pinpointed an indirect correlation among the dependent variable Italian truffle importations and the independent variables such as index of poverty, growth rate of Gross Domestic Product, skills and competence of customers over the time and the ecological behaviour. A direct correlation was found between the dependent variable importation of Italian truffle and the independent variable Gross Domestic Products in terms of purchase power parity value; thus, the model has underlined as a drop in Gross Domestic Product has been tightly linked to a fall in importation of Italian truffle towards other European Nations.

The analysis has pointed out as economic aspects in terms of wealth and Gross Domestic Product are pivotal to get better the importation of Italian products. If the goal of Italian farms is to improve the exportation power it is fundamental to take into account two variables such as the level of education in the European domestic market and also the ecological impact of packaging during the export phase, which has to be as low as possible.

KEYWORDS

Panel data, Italian truffle exportation, Italian truffle importation, poverty, Gross Domestic Product.

Assement of the aromatic profile of commercial truffle oils by headspace solid-phase microextraction and gas cromatogrophy-mass spectroscopy (HS-SPME/GC-MS)

Cullere, L. [1]*; **Chevret, B.** [1]; **Rivera, C.S.** [2]**; **Marco, P.** [2]**; **Blanco, D.** [2]**; **M.E. Venturini** [2]**

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Abstract

Because of its appreciated and intense flavor, different species of truffles, are used as seasoning in many food matrices: cheeses, sausages, pates, liqueurs, vinegars, honey, chocolates, oils, etc. Perhaps one of the foods that best retains the potent aroma of truffles is the oil. However, for a long time, the marketing of truffle oil is subjected to many controversies about the possible use of artificial or synthetic fragrances.
Thus, the objective of this work was to evaluate and compare the aromatic profile of commercial *Tuber melanosporum* truffle oils with those made in our laboratory mixing pieces of truffle with oils (natural truffle oils) and macerated them.
The aromatic profile of truffle oils was evaluated and identified by headspace solid-phase microextraction and gas cromatogrophy-mass spectroscopy (HS-SPME/GC-MS). The methodology used, with some modifications, was described in Cullere et al. (2012).
Significant differences were detected in 15 of the 70 aromatic compounds (belonging to 12 families of odorants) detected in the two groups of truffle oils. Of these 15, 13 had much higher levels in commercial truffle oils, especially sulfur odorants. Only toluene, octanol and 3-carene were higher in the natural truffle oils. 2,4-dithiapentane o bismethylthiapentane, the impact compound in the *Tuber magnatum* aroma, dimethylsulfide, a relevant aromatic compound in *T. melanosporum* aroma, and 1-octen-3-ol and 1-octen-3-one, very important compounds in the *Agaricus bisporus* aroma, were higher in the commercial truffle oils.
In conclussion, we have detected high concentrations of the impact aromatic compounds of *T. magnatum* and *T. melanosporum* aromas in the commercial truffle oils. This could indicate an artificial enrichment in these compounds which could be unpleasent to the consumers.

Culleré, L., Ferreira, V., Venturini, M.E., Marco, P. and Blanco, D. 2012. Evaluation of gamma and electron-beam irradiation on the aromatic profile of black truffle (*Tuber melanosporum*) and summer truffle (*Tuber aestivum*). Innov. Food Sci. Em. Technol. 13, 151-157.

KEYWORDS

Commercial truffle oils, *Tuber melanosporum;* 2,4-dithiapentane; dimethylsulfide.

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