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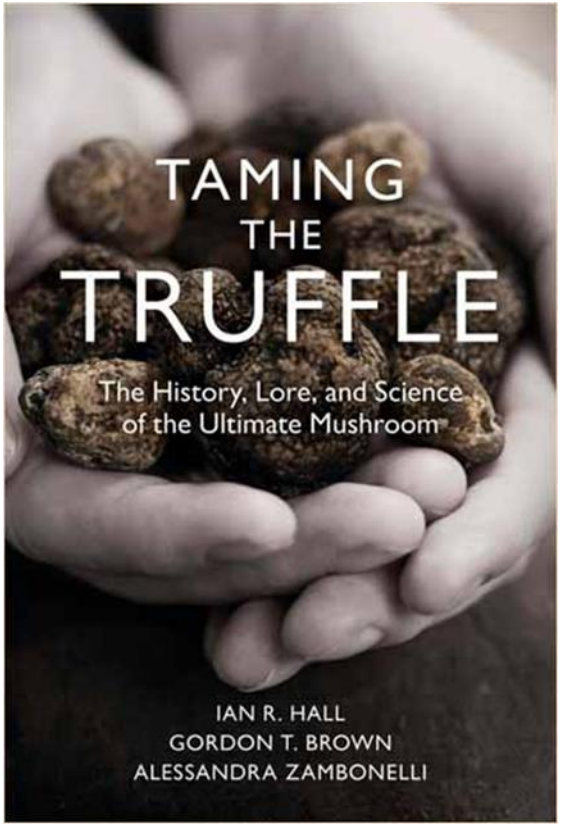
State of the Truffle Industry and Perspectives from Italy

Alessandra Zambonelli

Distal, Università di bologna



Truffles have a long tradition in Italy. They were already used in ancient Roman times.

However, these were not true truffles. They were desert truffles, from the *Terfezia genus*.

Information on true truffles starts in the Renaissance.

In the 18th century, Piedmont truffles *Tuber magnatum* began were highly valued.

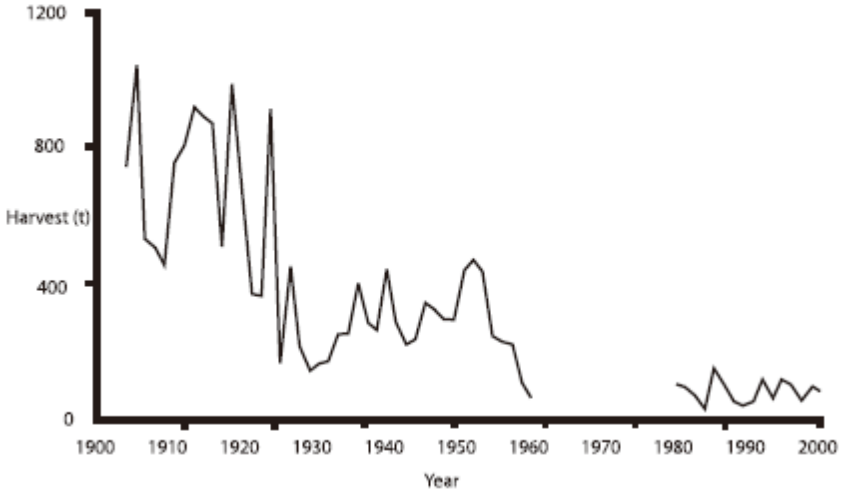
From this time on, interest in truffles increased in Italy.
So people started to think about cultivation.

At that time, people did not know that truffles are mycorrhizal fungi. So they tried to grow them like button mushrooms. Obviously , these attempts were not successful.

Only at the end of the 19th century did truffle cultivation really begin In France .
Joseph Talon planted acorns collected under truffle-producing oak trees.
However, his approach was still very empirical.
When this method was later applied in Italy, in the early 1900s, it did not succeed.

Modern truffle cultivation started only in the 1960s and 1970s

Tuber melanosporum production



Even today, most truffles in Italy are found in natural forests.
In the past, pigs were often used, especially in France.
Today, however, only trained dogs are used.

"Truffle hunting and extraction in Italy" was officially inscribed on the UNESCO Representative List of the Intangible Cultural Heritage of Humanity in 2021.

https://ich.unesco.org/en/video/57854

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Why are wild truffles declining?

1. **Less natural forest.**
2. **Forest abandonment**
3. **Climate change.**
4. **Too many collectors.**

3 - Climate change.

Climate change has direct effects on truffles.

- **Hotter summers and less summer rainfall**
- **Extreme events.**

Biology and Fertility of Soils
https://doi.org/10.1007/s00374-018-1296-3

ORIGINAL PAPER

Effect of summer soil moisture and temperature on the vertical distribution of *Tuber magnatum* mycelium in soil

Mirco Iotti [1] · Pamela Leonardi [2] · Giuliano Vitali [2] · Alessandra Zambonelli [2]

Received: 7 February 2018 / Revised: 5 June 2018 / Accepted: 12 June 2018

4- Too many collectors.

Intensive truffle harvesting can contribute to a decline in production. Studies have shown that repeated digging may damage Tuber ectomycorrhizae and the soil mycelial network, and may also reduce the chances of successful fertilization.

Spores seem to have a fundamental role in truffle fertilization and fruit body formation, providing further support for the need to protect natural truffle grounds.

Number of autorized truffle harvesters in italy

REGION	Population in2014	Number of Truffle hunters	%
Abruzzo	1333939	7277*	0.546%
Basilicata	578391	1652	0.286%
Calabria	1014316	68	0.007%
Campania	5869965	1249*	0.021%
Emilia Romagna	4446354	14945*	0.336%
Friuli Venezia Giulia	1229363	436	0.035%
Lazio	5870451	3284*	0.056%
Liguria	1591939	225	0.014%
Lombardia	9973397	3548	0.036%
Marche	1553138	12093	0.778%
Molise	314725	4601	1.462%
Piemonte	4436798	6350	0.143%
Puglia	4090266	309	0.008%
Sicilia	5094937	2**	0.000%
Toscana	3750511	7198*	0.192%
Trentino Alto Adige	1051951	416	0.040%
Umbria	896742	7422	0.828%
Valle D'Aosta	128591	1**	0.001%
Veneto	4926818	2521*	0.051%

Truffle spores are very important for truffle diffusion
It is well known that animals like mammals or marsupials in Australia are attracted by truffle aromas as well insects.

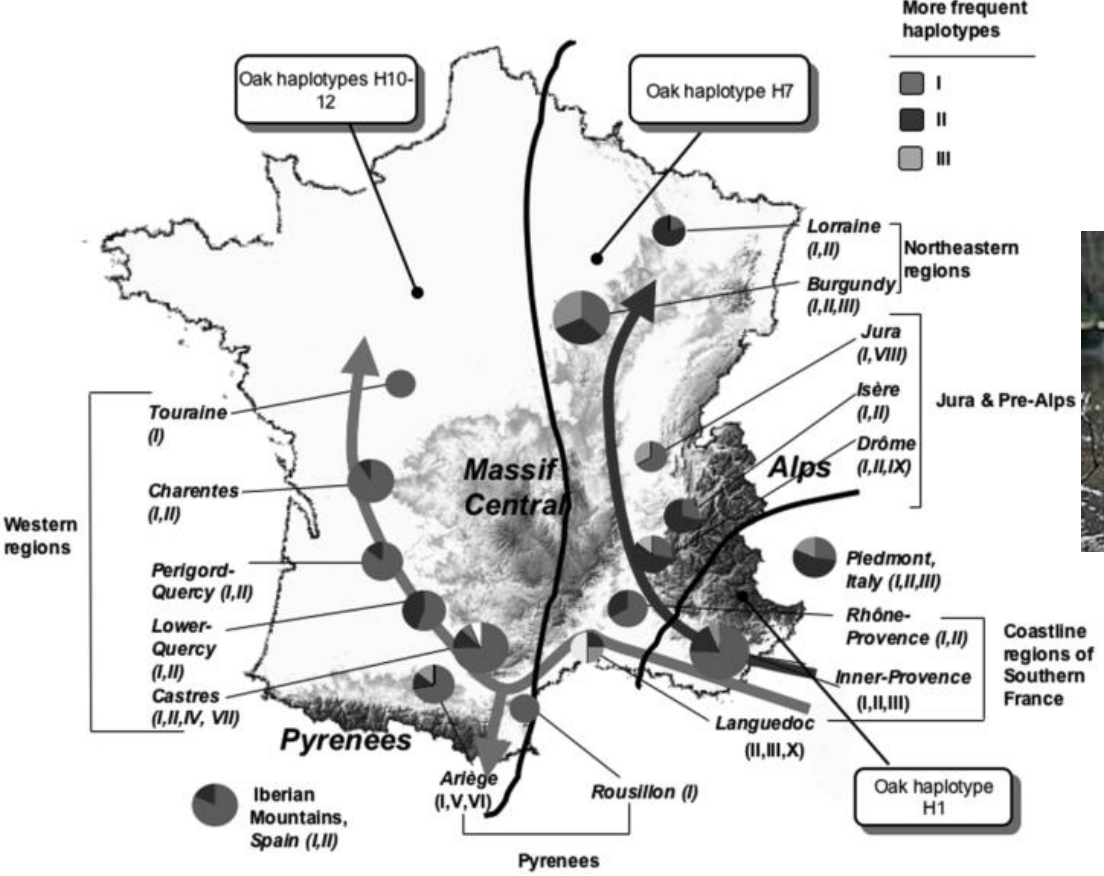
Pacioni G, Bologna MA & Laurenzi M (1991) Insect attraction by*Tuber*: a chemical explanation. Mycological Research 95: 1359–1363.

Olfactory orientation of the truffle beetle, *Leiodes cinnamomea*

Michael E. Hochberg[1,*], Guillaume Bertault[1], Karine Poitrineau[1] & Arne Janssen[2]
[1]*ISEM, Université Montpellier II, Place E. Bataillon, CC065, 34095 Montpellier, France;* [2]*IBED, Section Population Biology, University of Amsterdam, PO Box 94084, 1090 GB Amsterdam, Netherlands*

Accepted: 21 August 2003

Key words: attraction, truffle, odour, fungus, behaviour, Coleoptera, Staphylinoidea, Leiodidae



Their role in truffle long distance dispersal may be extremely important.

Recolonization after the last and the coldest glacial period (10 000–16 000 yr ago)

New Phytologist

Research

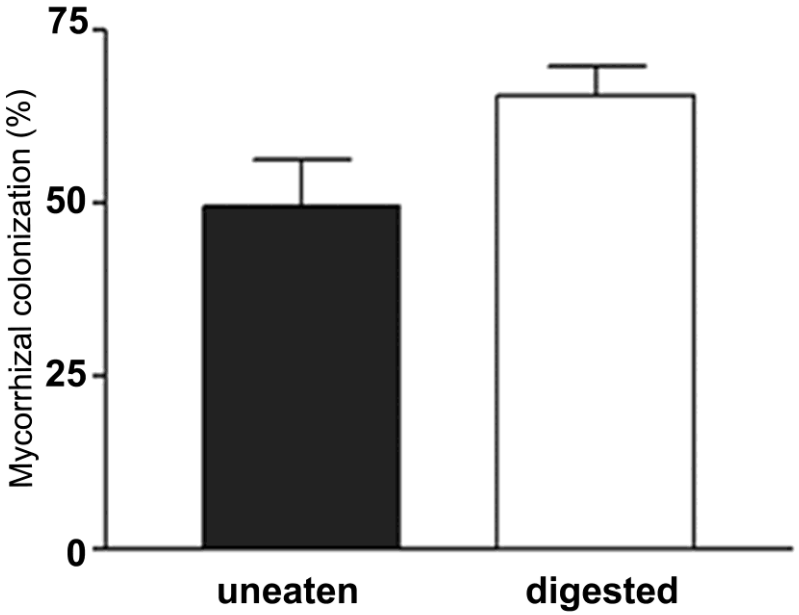
Polymorphism at the ribosomal DNA ITS and its relation to postglacial re-colonization routes of the Perigord truffle *Tuber melanosporum*

Claude Murat[1,2,5], Jesús Díez[1,3,5], Patricia Luis[1,6], Christine Delaruelle[1], Chantal Dupré[4], Gérard Chevalier[4], Paola Bonfante[2] and Francis Martin[1]

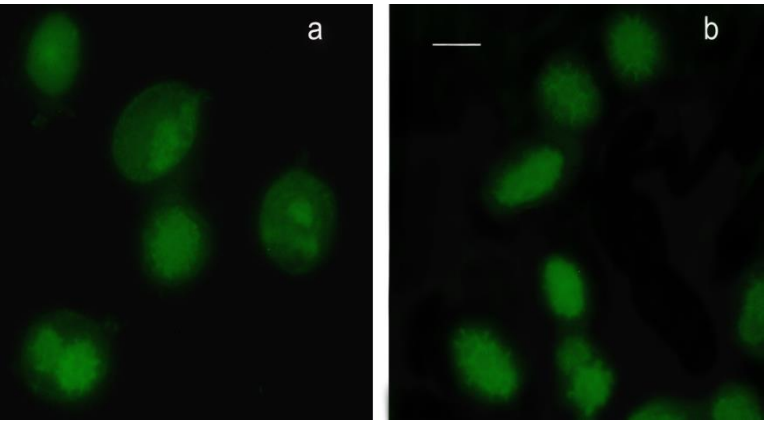
[1]UMR INRA/UHP 1136 'Interactions Arbres/Micro-Organismes', Centre INRA de Nancy, F-54280 Champenoux, France; [2]Dipartimento di Biologia Vegetale dell'Università di Torino, Viale Mattioli I-10125 Torino, Italy; [3]Departamento de Biología Vegetal, Universidad de Alcalá, E-28871 Alcalá de Henares, Spain; [4]UMR 1095 INRA-UBP 'Amélioration et Santé des Plantes', Site de Crouelle, 234 avenue du Brézet, F-63039 Clermont-Ferrand Cedex 2, France; [5]These authors contributed equally to this work; [6]Present address: Institut für Botanik – Terrestrische Ökologie, Universität Leipzig – Johannisallee 21–23 D-04103 Leipzig, Germany

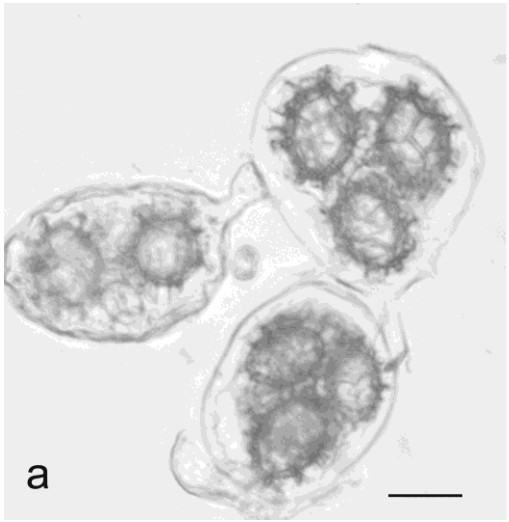
In order to support this theory we wanted to verify the vitality and infectivity of truffle spores after the passage through pig the digestive tract, are not negatively affected.

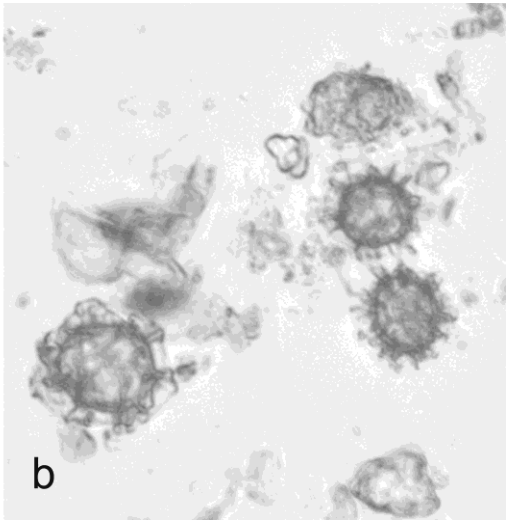
So I fed my pig with *T. aestivum* and inoculated oak seedlings with the spores digested by my big and with fresh spores coming from the same fruiting bodies.



The digested spores were vital but also had the ascus wall dissolved, and the spore wall seemed to be partially corroded when viewed using an atomic force microscope which may have favored their germination .







available at www.sciencedirect.com
ScienceDirect
journal homepage: www.elsevier.com/locate/funeco
ELSEVIER

Viability and morphology of *Tuber aestivum* spores after passage through the gut of *Sus scrofa*

Federica PIATTONI[a], *Antonella AMICUCCI*[b], *Mirco IOTTI*[a], *Francesca ORI*[a], *Vilberto STOCCHI*[b], *Alessandra ZAMBONELLI*[a,*]

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[b]Dipartimento di Scienze Biomolecolari, University of Urbino "Carlo Bo", Via Saffi 2, 61029 Urbino, Italy

British Mycological Society promoting fungal science

Fungal Biology

journal homepage: www.elsevier.com/locate/funbio

Effect of slug mycophagy on *Tuber aestivum* spores

Francesca Ori [a], Michele Menotta [b], Marco Leonardi [a, *], Antonella Amicucci [b], Alessandra Zambonelli [c], Hervé Covès [d, e], Marc-André Selosse [d, f], Laure Schneider-Maunoury [d], Giovanni Pacioni [a], Mirco Iotti [a]

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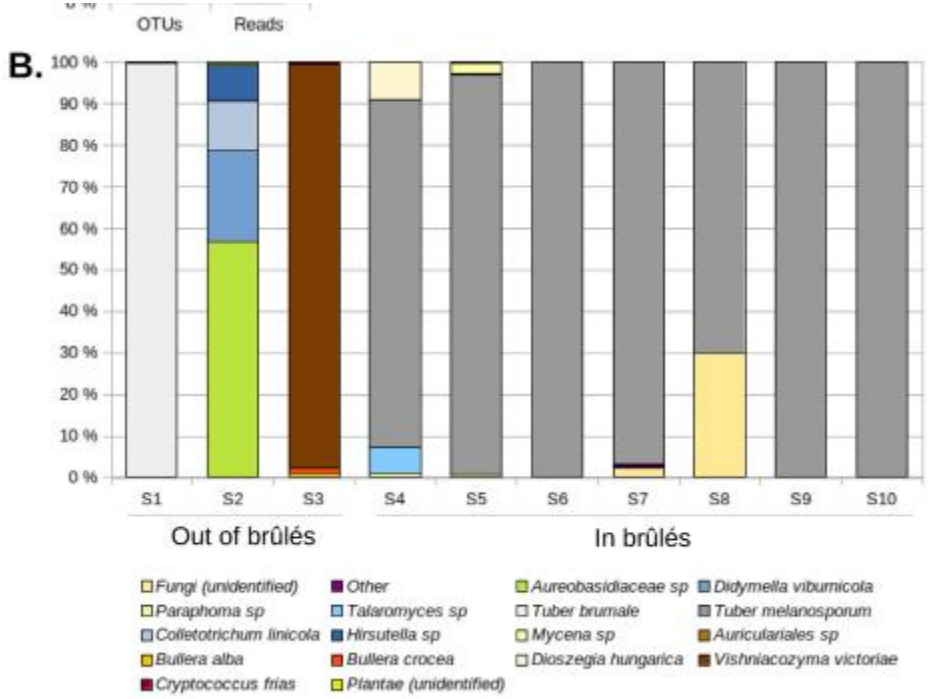
[c] Department of Agricultural and Food Sciences, University of Bologna, Viale G. Fanin 44, 40127, Bologna, Italy
[d] Institut de Systématique, Évolution, Biodiversité (UMR 7205 – CNRS, MNHN, UPMC, EPHE), Muséum national d'Histoire naturelle, Sorbonne Universités, 57 rue Cuvier, 75005, Paris, France
[e] Arbre et Paysage 32, 93 Route de Pessan, 32000, Auch, France
[f] Department of Plant Taxonomy and Nature Conservation, University of Gdansk, Wita Stwosza 59, 80-308, Gdansk, Poland



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Collecting all the truffles in the soil may reduce their reproduction.
It can also reduce the production of fruiting bodies.

Paolocci et al. (2006) and Belfiori et al. (2008) suggest that truffle are hetherothallic. This means they need two compatible partners to reproduce.

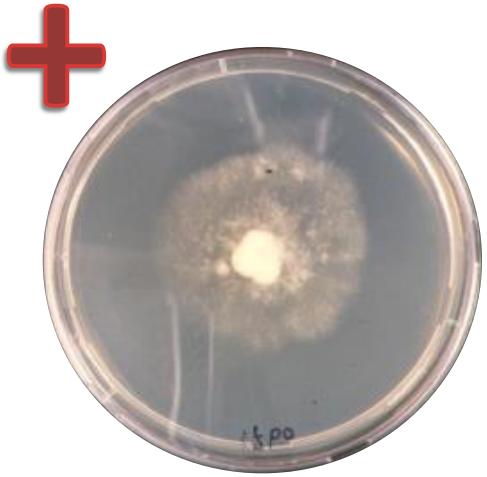
In 2010 black Truffle genome sequencing confirmed it

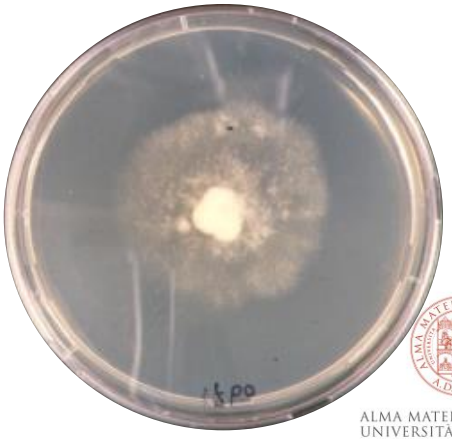
nature

LETTERS

Périgord black truffle genome uncovers evolutionary origins and mechanisms of symbiosis

Francis Martin[1], Annegret Kohler[1], Claude Murat[1], Raffaella Balestrini[2], Pedro M. Coutinho[3], Olivier Jaillon[4–6], Barbara Montanini[7], Emmanuelle Morin[1], Benjamin Noel[4–6], Riccardo Percudani[7], Bettina Porcel[4–6], Andrea Rubini[8], Antonella Amicucci[9], Joelle Amselem[10], Véronique Anthouard[4–6], Sergio Arcioni[8], François Artiguenave[4–6], Jean-Marc Aury[4–6], Paola Ballario[11], Angelo Bolchi[7], Andrea Brenna[11], Annick Brun[1], Marc Buée[1], Brandi Cantarel[3], Gérard Chevalier[12], Arnaud Couloux[4–6], Corinne Da Silva[4–6], France Denoeud[4–6], Sébastien Duplessis[1], Stefano Ghignone[2], Benoît Hilselberger[1,10], Mirco Iotti[13], Benoît Marçais[1], Antonietta Mello[2], Michele Miranda[14], Giovanni Pacioni[15], Hadi Quesneville[10], Claudia Riccioni[8], Roberta Ruotolo[7], Richard Splivallo[16], Vilberto Stocchi[9], Emilie Tisserant[1], Arturo Roberto Viscomi[7], Alessandra Zambonelli[13], Elisa Zampieri[2], Bernard Henrissat[3], Marc-Henri Lebrun[17], Francesco Paolocci[8], Paola Bonfante[2], Simone Ottonello[7]





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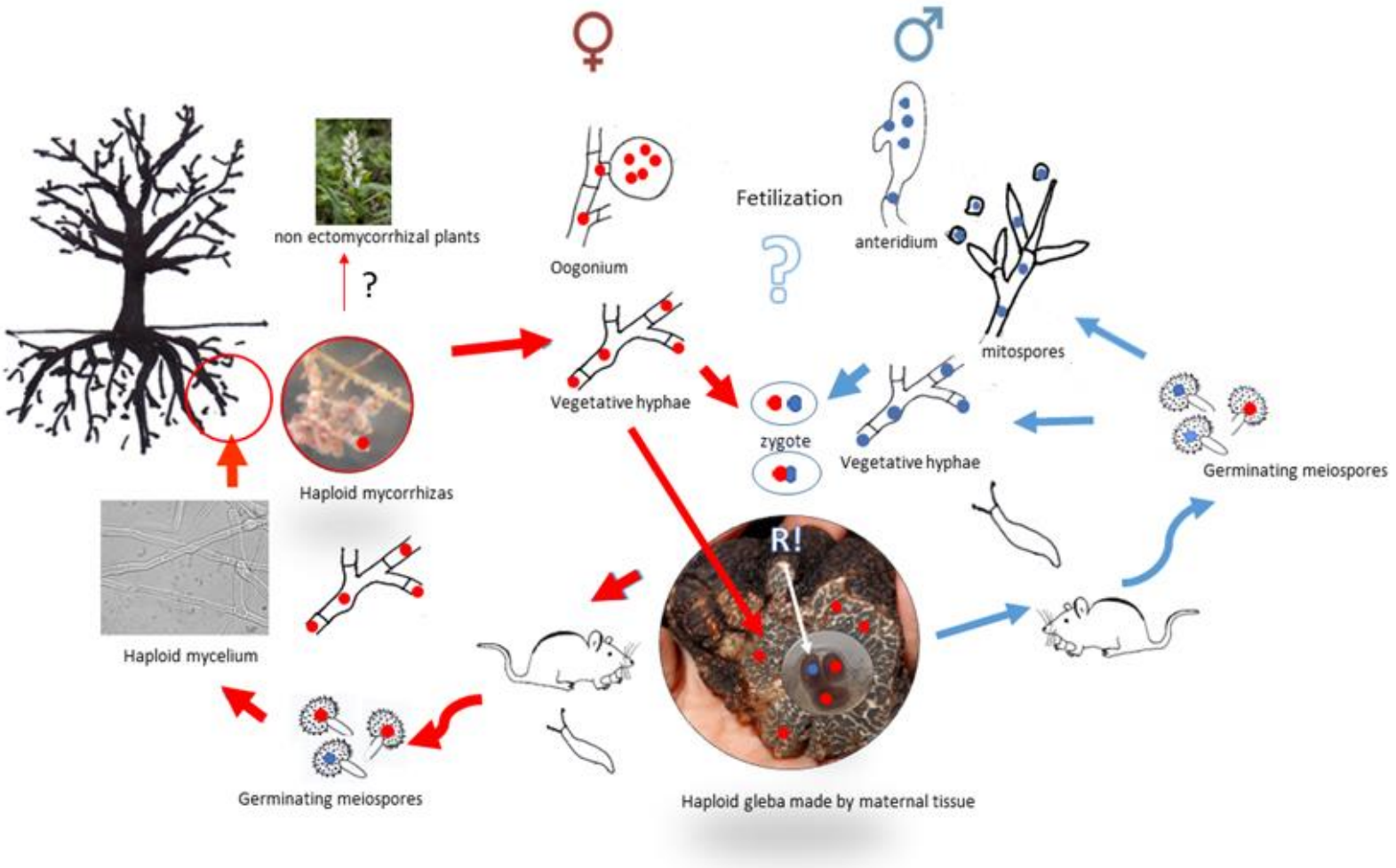


This finding emphasizes the importance of leaving at least some mature truffles in the soil. Their spores not only contribute to dispersal but may also provide the paternal partners necessary for successful fertilization and the production of new truffles.

Truffle traps or truffle nests

C. Murat, L. Bonneau , H. De la Varga , J.M. Olivier, F. Sandrine, F. Le Tacon
Italian Journal of Mycology vol. 45 (2016) ISSN 2531-734
DOI: 10.6092/issn.2531-7342/6346

Trapping truffle production in holes: a promising technique for improving production and unravelling truffle life cycle

Murat Claude[1*], Bonneau Lucien [2], De la Varga Herminia[1], Olivier Jean-Marc[3], Sandrine Fizzala[4], Le Tacon François[1]

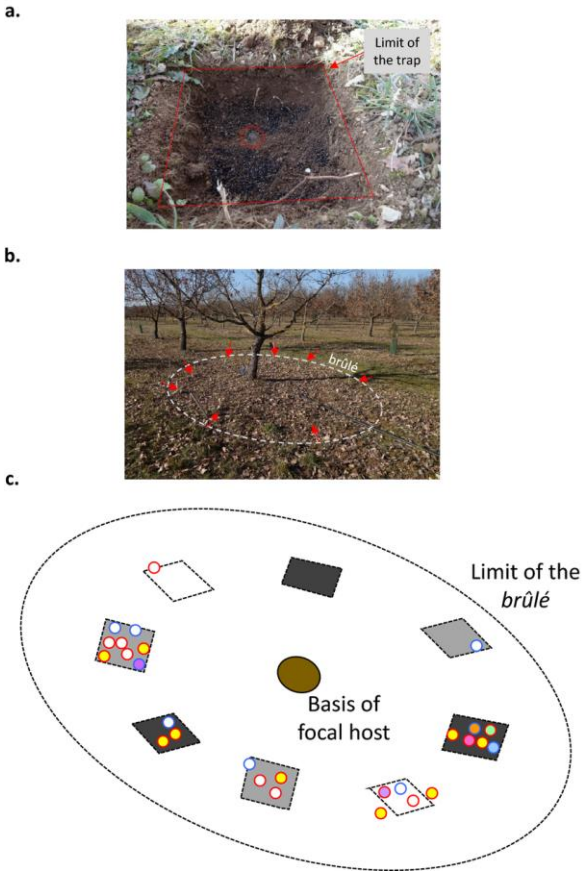
Mycorrhiza (2025) 35:2
https://doi.org/10.1007/s00572-024-01177-1

REVIEW

Advances in molecular genetics have increased knowledge of *Tuber* species' life cycle and population genetic structure, indicating ways to improve yield

Mahesh C. A. Galappaththi[1,2] · William A. Dunstan[2] · Giles E. St. J. Hardy[2,3] · Jen McComb[2] · Mark P. McHenry[2] · Alessandra Zambonelli[4] · Treena I. Burgess[2]

Received: 28 March 2024 / Accepted: 17 October 2024



scientific reports

OPEN

Efficiency of the traditional practice of traps to stimulate black truffle production, and its ecological mechanisms

E. Taschen[1], G. Callot[1,2], P. Savary[3,4], M. Sauve[4], Y. Penuelas-samaniego[4], F. Rousset[5], X. Parlade[6], M.-A. Selosse[7,8] & F. Richard[4]

The black truffle *Tuber melanosporum* was disseminated all over the world, propelled by the development of a wide variety of empirical practices. A widespread practice, called 'truffle trap', consists of placing pieces of truffles into excavations dug under host trees, and of collecting truffle in these traps in the next years. This research aims at (1) evaluating the effect of this practice on fruitbody production based on the analysis of 9924 truffle traps installed in 11 orchards across *T. melanosporum* native area in France and (2) exploring the mechanisms involved in fruitbody emergence using traps where the genotypes of introduced truffles were compared with those of fruitbodies collected in the same traps. We confirmed that truffle traps provide a major and highly variable part of truffle ground production, representing up to 89% of the collected fruitbodies. We evidenced a genetic link between introduced spores and collected fruitbodies, and then demonstrated that truffle growers provide paternal partners for mating with local maternal mycelia. We also highlighted that soil disturbance stimulate the vegetative development of established maternal mycelia. This research supports that a widely used traditional practice enhances fruitbody production by shaping favorable conditions and providing sexual partners required for fruiting.

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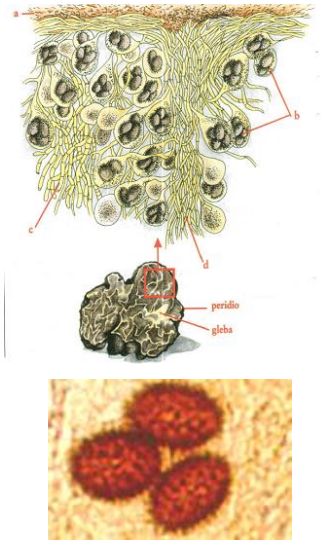
The decrease in production is also linked to cultivation.
Truffle cultivation has not always been successful.
A key point is the quality of the plants.

Spore inoculation

The spore inoculation technique perfected during the 1970s and 1980s

Spore suspension



inoculation

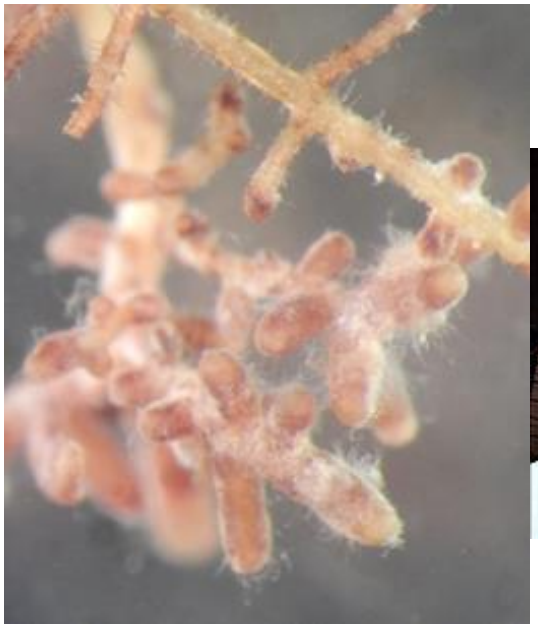
The spore method of inoculation is still the most used method of inoculation of truffles.

Spore inoculum is obtained from fresh or preserved ascocarps used to inoculate sterile seedlings or cuttings of forestal plants.

The market has two sides

On one side, there are good nurseries. They produce high-quality plants

On the other side, there are unreliable nurseries. Their plants are poorly mycorrhizal or not mycorrhizal at all with the declared truffle

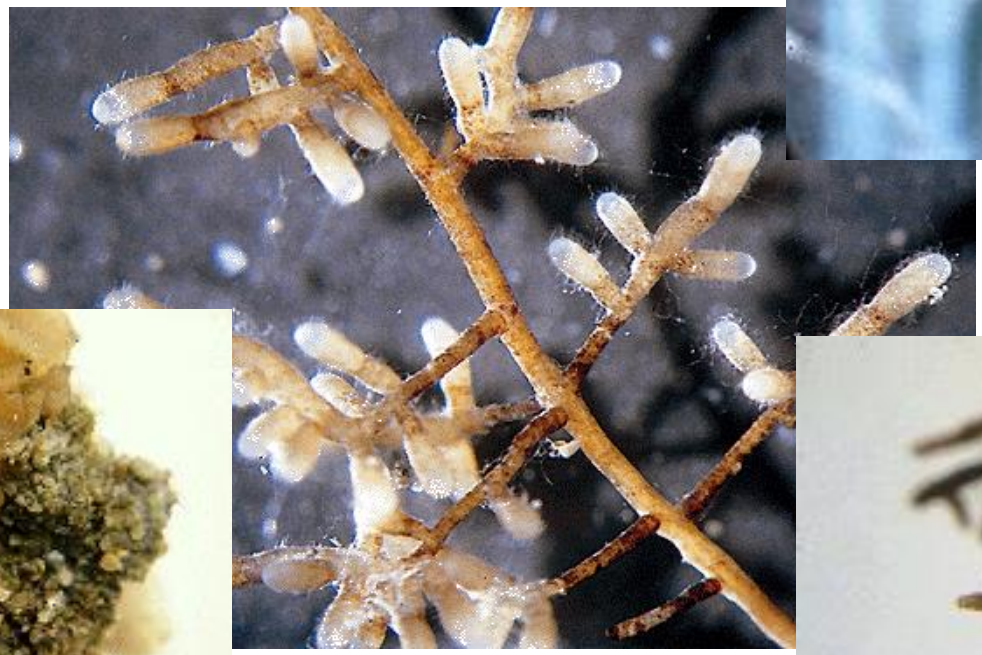


Inoculum control second condition

When large quantities of fruit bodies are used to prepare inocula operators may accidentally incorporate less valuable species of truffles which can then become established on the host plants. These less valuable truffles are often more infective and consequently can contaminate entire batches of plants.

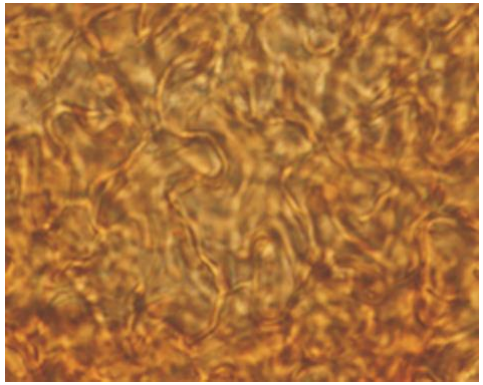
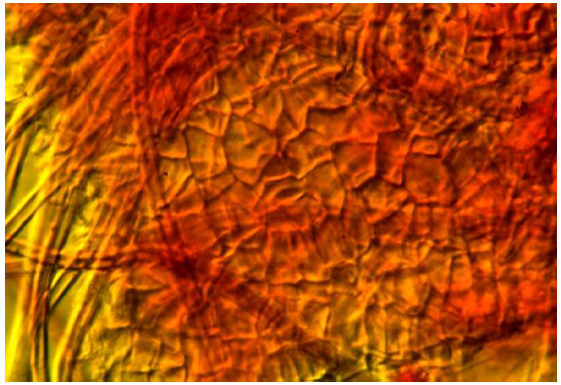
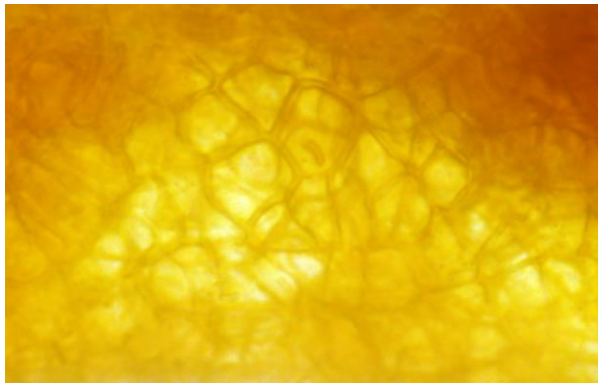
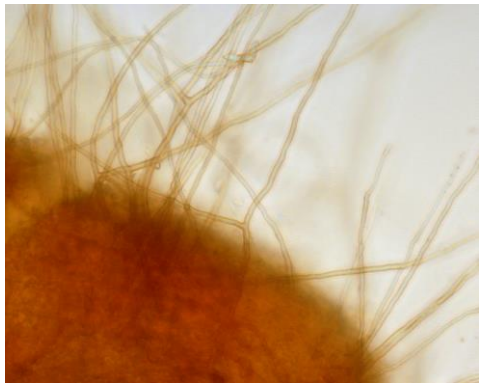
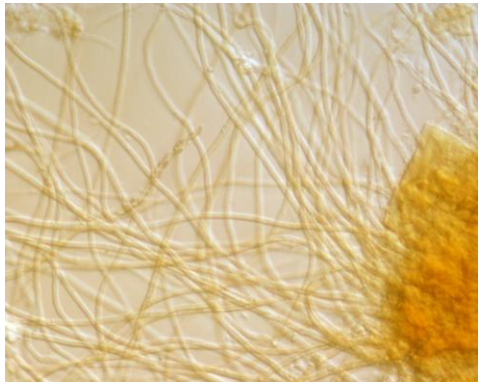
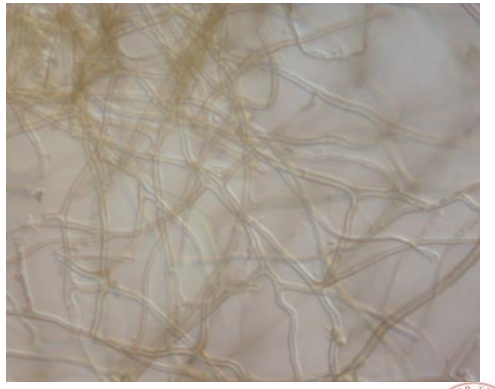
- *T. melanosporum* truffles can be beconfused with those of *T. indicum, T. brumale* and *T. aestivum*

Control of the produced plants



T. melanosporum *T.aestivum* *AD*

Trichophaea woolhopeia

Molecular methods such as PCR with specific primers and multiplex PCR, sequencing, have the potential of checking the inoculum and later identifying contaminating *Tuber* mycorrhizae on plants prior to outplanting

ectomycorrhizae

DNA extraction

PCR → ITS region amplification

MYCOLOGICAL RESEARCH 110 (2006) 60–65

available at www.sciencedirect.com

SCIENCE DIRECT

journal homepage: www.elsevier.com/locate/mycres

A quick and precise technique for identifying ectomycorrhizas by PCR

*Mirco IOTTI, Alessandra ZAMBONELLI**

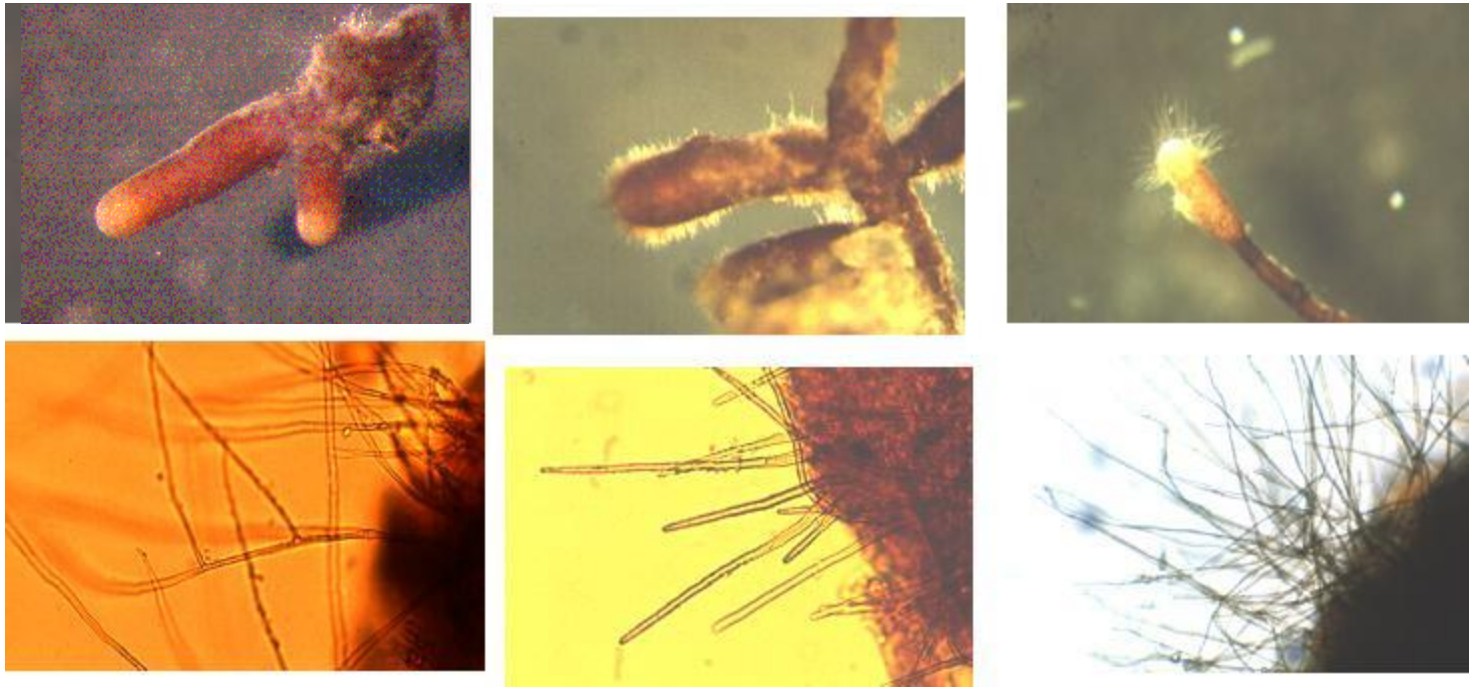
Dipartimento di Protezione e Valorizzazione Agroalimentare, Università degli Studi di Bologna, via Fanin 46, I-40127 Bologna, Italy

- Lenth variability
- RFLP analysis
- Specific PCR
- PCR multiplex
- Sequencing

The mycorrhizae of black truffle species are often very difficult to distinguish using morphological characters alone.

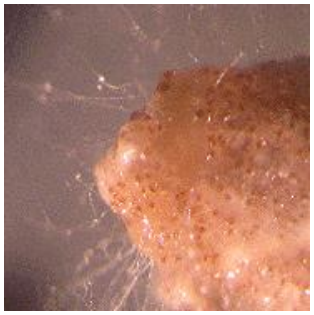
For this reason, accurate identification using molecular techniques is essential, especially when dealing with black truffle species whose mycorrhizae are morphologically very similar

Tuber melanosporum *Tuber brumale* *Tuber indicum*



Mycelial inoculation

Mycelial pure culture



Vegetative growth of the mycelium

Inoculation

In our lab we are perfecting the mycelial method of inoculation The advantage of this inoculation method is that fruiting bodies do not need to be purchased and there is no risk of introducing contaminants with the inoculum.

Experimental truffière of Cadriano (BO)

Establisled in 2007

5 different strains: Tb98, 2352, 2292, 1Bo, 2364 inoculated alone and in mixture

2016

100 truffles - 722 g in two months

The truffles were found above all in the plants inoculated with a single strain

Mycorrhiza
DOI 10.1007/s00572-016-0703-6

SHORT NOTE

First evidence for truffle production from plants inoculated with mycelial pure cultures

Mirco Iotti[1] · Federica Piattoni[2] · Pamela Leonardi[2] · Ian R. Hall[3] · Alessandra Zambonelli[2]

Ascoma genotyping and mating type analysis of the mycorrhizas and of the surronding soil mycelium

PCR multiplex

environmental microbiology

Environmental Microbiology (2019) 00(00), 00–00 doi:10.1111/1462-2920.14777

Ascoma genotyping and mating type analyses of mycorrhizas and soil mycelia of *Tuber borchii* in a truffle orchard established by mycelial inoculated plants

Pamela Leonardi,[1] Claude Murat,[2] Federico Puliga,[1] Mirco Iotti[3] and Alessandra Zambonelli[1]*

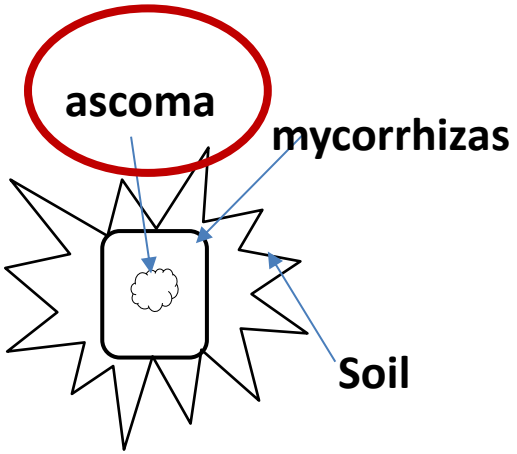
[1]*Department of Agricultural and Food Sciences, University of Bologna, viale Fanin 44, 40127, Bologna, Italy.*

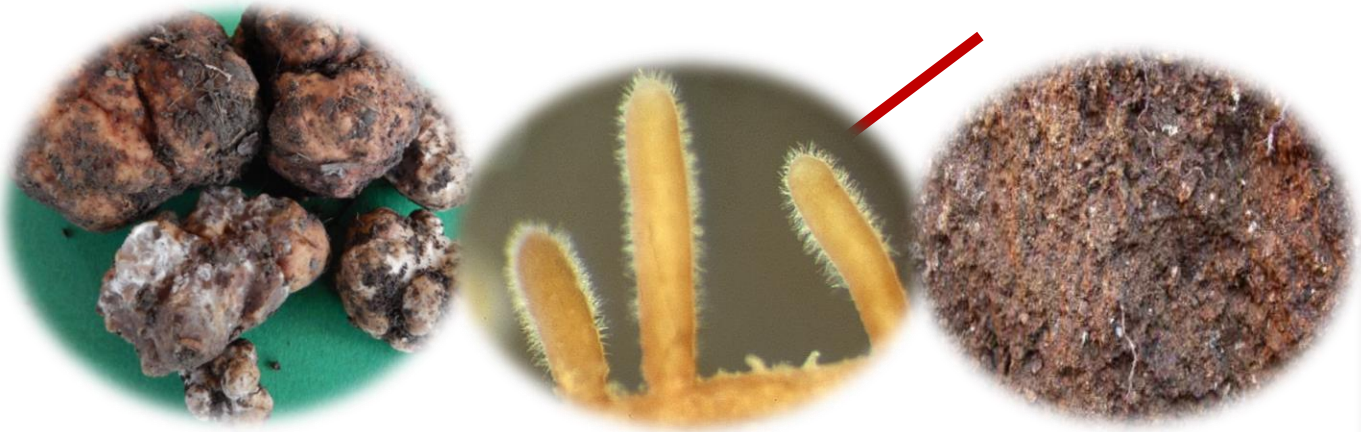
[2]*Université de Lorraine, INRA, UMR IAM, 54000, Nancy, France.*

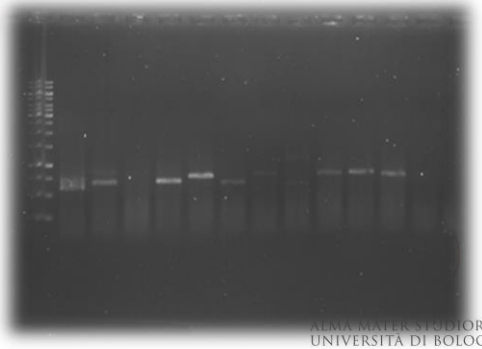
[3]*Department of Life, Health and Environmental Science, University of L'Aquila, via Vetoio, 67100, Coppito, L'Aquila, Italy.*

Introduction

Ectomycorrhizal fungi assist plants in their growth, therefore, playing key roles in forest ecosystem functioning. In addition, some of them produce edible fructifications representing income opportunities for farmers and foresters. True truffles are hypogeous fungi belonging to the genus *Tuber*, which live in ectomycorrhizal association with a wide range of shrubs and trees (Zambonelli *et al.*, 2016). The genus *Tuber* comprises around 200 species but only a few of them have a considerable value, such as the European



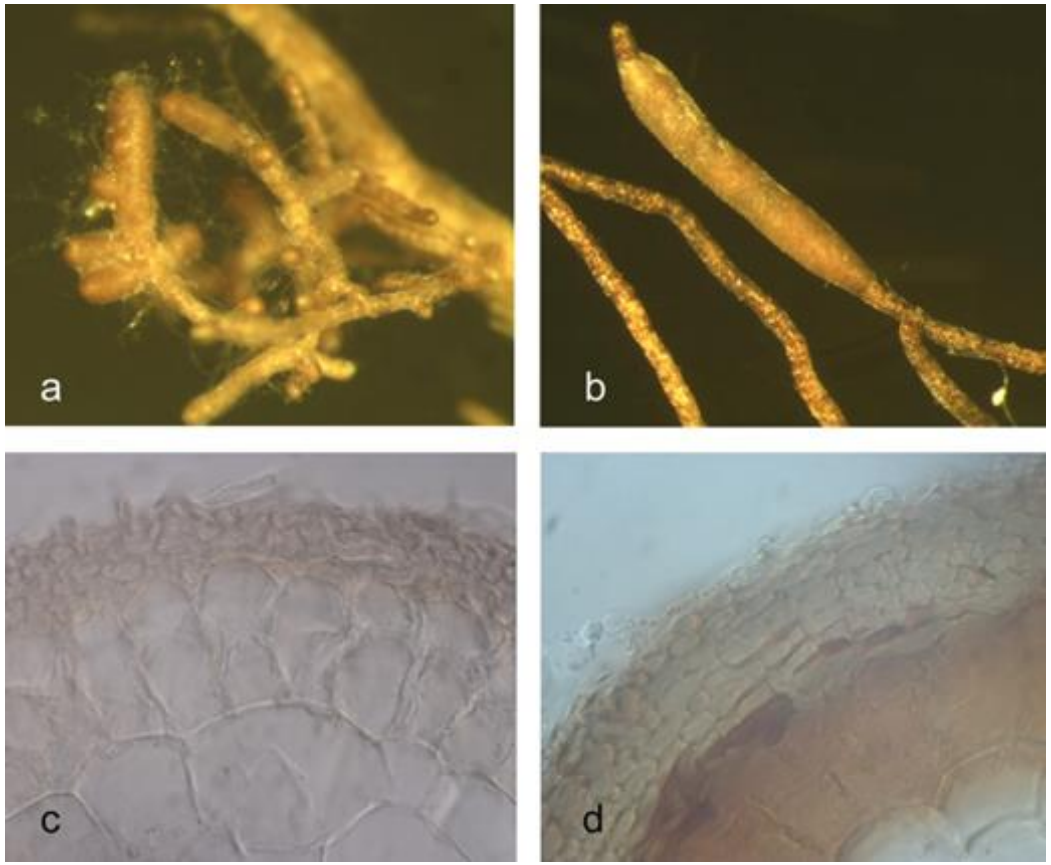




- This is the first demonstration of truffle production from plants inoculated with pure cultures and opens up the possibility of producing truffle-colonized plants using cultures rather than spores.

In a recent research we demonstrated that different *T. borchii* strains have a different tolerance at high temperatures.

28°C



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Morphological and functional changes in mycelium and mycorrhizas of *Tuber borchii* due to heat stress

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Tolerant strain

Sensible strain

We developed and patented a new technique for producing truffle-infected plants using pure culture mycelium.

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The truffle cultivation revolution

Truffle production is complex and expensive. Traditional methods involve high production costs, a significant risk of contamination, and genetic limitations. Moreover, natural truffle yields are declining due to environmental factors, and the market lacks adequate regulation.

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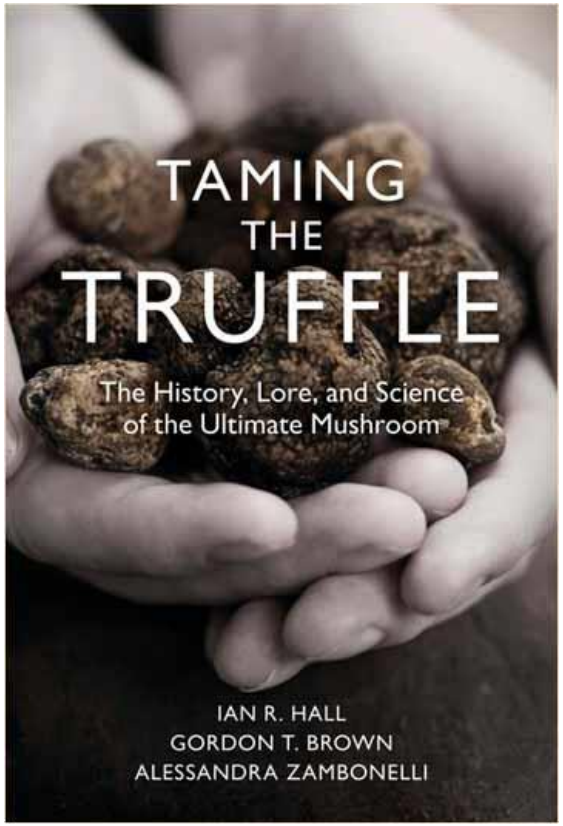

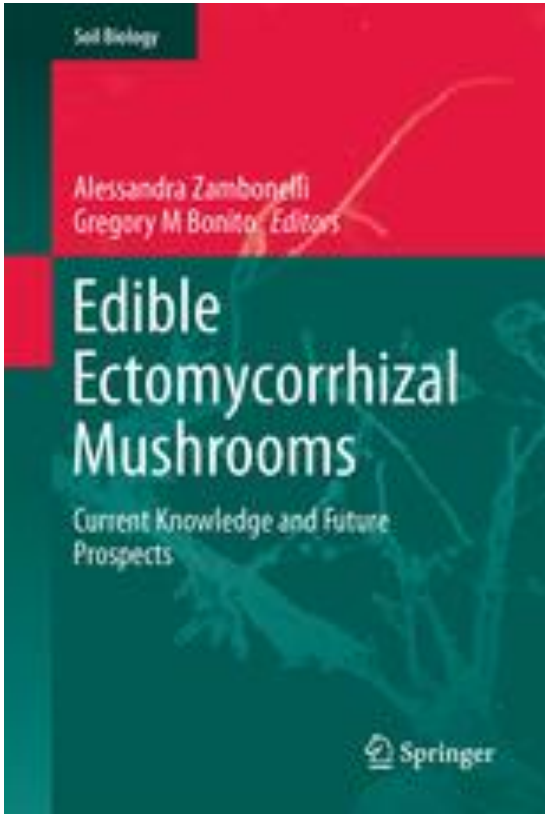

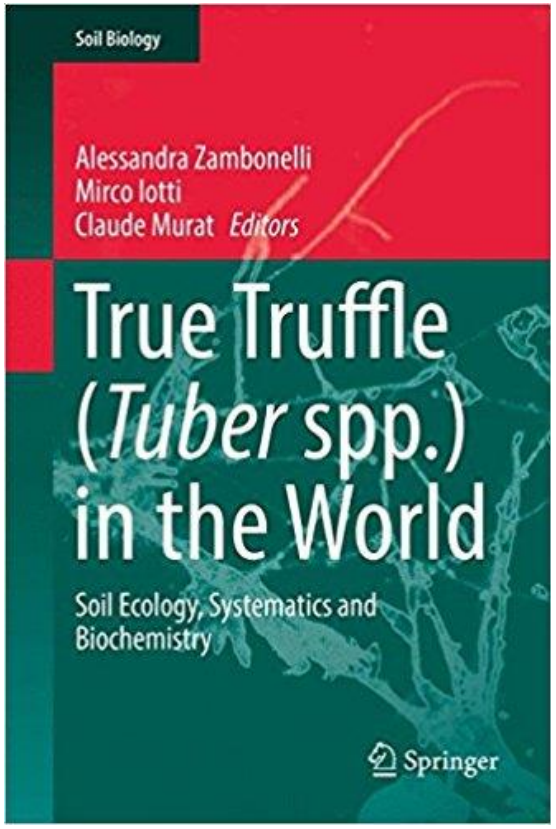

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Thank you very much for your attention