1st International Conference on TRUFFLE RESEARCH '14

Vic - Barcelona, 9 -12 March 2014

www.truffleresearch.eu

UVIC
UNIVERSITAT DE VIC
UNIVERSITAT CENTRAL
DE CATALUNYA

BOOK OF ABSTRACTS

1st International Conference on TRUFFLE RESEARCH '14

www.truffleresearch.eu

Published on the occasion
1st International Conference on Tuffle research'14

Vic, March 2014

Curators:
Consol Blanch, Alba Crespi GRBETA. Grup de recerca en biodiversitat, ecologia, tecnologia alimentària i Gestió Ambiental de la UVic-UCC

Authors are responsible of the contents of each abstract

Edition partially suported by the Micosylva Project +(SOE3/P2/E533) financed by FEDER "Mycosilviculture and valuation of wild mushrooms for forest sustainability and multifunctionality"

Design. www.eumodc.cat

ORGANISATION

ORGANISING COMMITTEE

Local permanent commission

- Consol Blanch-Colat (**Chair**), Polytechnic School, UVic-UCC, Vic, Spain
- Núria Barniol-Noguer, Polytechnic School, UVic-UCC, Vic, Spain
- Albert Hueso-Morell, Polytechnic School, UVic-UCC, Vic, Spain
- Jordi Villà-Freixa, Polytechnic School, UVic-UCC, Vic, Spain

Organising committee

- Consol Blanch-Colat (Chairwoman), Polytechnic School, UVic-UCC, Vic, Spain
- Núria Barniol-Noguer, Polytechnic School, UVic-UCC, Vic, Spain
- Mireia Bartrons, Polytechnic School, UVic-UCC, Vic, Spain
- Joan Bertran-Comulada, Polytechnic School, UVic-UCC, Vic, Spain
- Carme Casas-Arcarons, Polytechnic School, UVic-UCC, Vic, Spain
- Laura Culleré-Varea, University of Zaragoza, Unizar , Zaragoza, Spain
- Anna Fenosa-Bernardo, Polytechnic School, UVic-UCC, Vic, Spain
- Albert Hueso-Morell, Polytechnic School, UVic-UCC, Vic, Spain
- Elena Ibañez-Ezequiel, CSIC, Madrid, Spain
- Nandu Jubany, Can Jubany, Calldetenes, Spain
- Sarah U. Khan, Polytechnic School, UVic-UCC, Vic, Spain
- Daniel Oliach, Forest Sciences Centre of Catalonia, Solsona, Spain
- Xavier Parladé, IRTA Research & Technology Food & Agriculture, Cabrils, Spain
- Míriam Torres-Moreno, Faculty of Health Sciences and Welfare, UVic-UCC , Vic, Spain
- Josep Turet-Capellas, Polytechnic School, UVic-UCC , Vic, Spain
- Jordi Villà-Freixa, Polytechnic School, UVic-UCC, Vic, Spain

Technical Secretariat

- Maria José Gallego, Manners - Traduccions i Congressos, Barcelona, Spain

Scientific Secretariat

- Alba Crespi-Boixader, Polytechnic School, UVic-UCC, Vic, Spain

SCIENTIFIC COMMITTEE

Chairs

- Antonietta Mello, (**Honorary Chair**), Plant Protection Institute-CNR, Turin, Italy
- Consol Blanch-Colat (**co-Chair**), Polytechnic School, UVIC-UCC, Spain

Members of scientific committee

- Amadeo Alpi, University of Pisa, Italy
- Mireia Bartrons, Polytechnic School, UVic-UCC, Vic, Spain
- Joan Bertran-Comulada, Polytechnic School, UVic-UCC, Spain
- Domingo Blanco-Parmo, University of Zaragoza, Spain
- José Antonio Bonet, University of Lleida, Spain
- Gregory Bonito, University Autònoma de Madrid, USA
- Paola Bonfante, University of Turin – CNR, Italy
- Carme Casas-Arcarons, Polytechnic School, UVIC-UCC, Spain
- Alejandro Cifuentes-Gallego, CSIC-CIAL, Madrid, Spain
- Carlos Colinas, University of Lleida, Spain
- Laura Culleré-Varea, University of Zaragoza. Spain
- Vicente Ferreira-González, University of Zaragoza, Spain
- Francisco García Carmona, University of Murcia, Spain
- Buenaventura Guamis-López, Universitat Autònoma de Barcelona, Spain
- Andrea Gogan, Szent István University, Gödöllö, Hungary
- Ian R. Hall "Trufle" Ltd. New Zeland
- Elena Ibañez-Ezequiel, CSIC-CIAL, Madrid, Spain
- Korány Kornél, Corvinus University of Budapest, Hungary
- Jaume Llistosella, Faculty of Biology, University of Barcelona. Spain
- Michele Miranda, University of l'Aquila, Italy
- Claude Murat, INRA-Nancy/Université de Lorraine, France
- Giorgio Pennazza, Univ. Campus Bio-medico di Roma, Italy
- Guillermo Pérez-Andueza, Catholic University of Avila, Spain
- Guillermo Reglero, , Spain
- Richard Splivallo, Georg-August Universitaet Goettingen, Germany
- Míriam Torres-Moreno, Faculty of Health and Welfare, UVIC-UCC, Spain
- Alessandra Zambonelli, University of Bologna, Italy

Organizet by:

With the collaboration:

UVIC
UNIVERSITAT DE VIC
UNIVERSITAT CENTRAL
DE CATALUNYA

Escola Politècnica Superior

Vicerectorat de Recerca i Transferència del Coneixement

SACU. Servei d'Atenció a la Comunitat Universitària

FECYT
FUNDACIÓN ESPAÑOLA PARA LA CIENCIA Y LA TECNOLOGÍA

UCC+i
RED DE UNIDADES DE CULTURA CIENTÍFICA Y DE LA INNOVACIÓN

MAJOR SPONSORS

Ajuntament de Vic
Fires i Mercats

Ajuntament de Centelles

Centre Tecnològic Forestal de Catalunya

Generalitat de Catalunya
Departament d'Agricultura, Ramaderia, Pesca, Alimentació i Medi Natural

PLA ANUAL 2014
DE_TRANSFERÈNCIA_TECNOLÒGICA

micosylva+

SUDOE
Interreg IV B

UE / EU - FEDER / ERDF

Springer

alícia

trufapasión

Jubany

Laumont
www.laumont.net

COLL
tòfones i bolets

COLLABORATORS

gremi
hostaleria d'Osona

Fussimanya

LLONGANISSA DE VIC
INDICACIÓ GEOGRÀFICA PROTEGIDA

INDICACIÓN GEOGRÁFICA PROTEGIDA

Juvé & Camps

celler d'Osona
VINS CAVES LICORS

FORMATGES DE CIURET

EUROPASTRY

TABLE CONTENTS

CONTENTS

Preface of the Organising Committee

First of all, on behalf of the Organising Committee I would like to welcome you all to the first International Conference on Truffle Research (ICTR). I would like to thank all who have decided to attend the Conference and who have therefore shown their interest in our proposal.

The 1st ICTR has been promoted by the Environment and Food Industries Department of the Polytechnic School at the University of Vic – Central University of Catalonia (UVIC-UCC). The original aim of the Conference was to bring together the latest developments in truffle research from all over the world in the production, nutritional and gastronomic fields, encompassing areas such as trufficulture, truffle genomics, molecular biology, quality control, flavour characterization, olfactometric and sensorial analysis, conservation of truffles and their derivatives and, also, truffle marketing and consumption.

Now, less than one year since we made our first announcement about the Conference, this book of abstracts confirms that the aim has almost been achieved. Now, all that remains is to hold the Conference itself. What we have achieved so far and what we hope to achieve in the Conference is due to the help of many people, including invited speakers, authors and participants in general, technical and scientific "secretariat" and the organising and scientific committees. To all of you, truly, our warmest thanks.

We have received the confirmation of the participation of eighty truffle experts, coming from all the five "continents" of the Earth. The participating countries are: Austria, Portugal, Spain, France, Italy, Israel, United Kingdom, Germany, Poland, Hungary, Slovenia, Czech Republic, Estonia, Morocco, Egypt, South Africa, Saudi Arabia, Qatar, Australia, New Zealand, USA and Chile. Thanks to their contributions, we are able to offer you an event with: 14 invited lectures, four of them in a round table; 17 oral presentations and 12 poster communications. It is our pleasure to announce that a special *Food Analytical Methods* issue entitled "**Recent Food Analysis and Truffle Research**" will be devoted to bring together the most important contributions presented at this international meeting.

Several social activities have been arranged in order to introduce everybody to our culture and way of life, and we hope that they will prove to be of interest to everyone and you enjoy your stay in Vic

We hope that the Conference will prove to be useful to all participants and that it will be the first of many transversal events creating links between the worlds of academia, basic and applied research, trufficulture, cuisine and society in general. This first Conference is taking place in Vic, Catalonia, but we hope that future events will be held in other countries around the world, giving all of us the opportunity to see each other once again in pastures new.

Consol Blanch

Chair of the Organising Committee of the ICTR'14

Vic, 2th February 2014.

Preface of the Scientific Committee

As a member of the active community dealing with truffle research, it is a great pleasure to summarize in a few words the advancement of this field that will be illustrated in the conference from valuable Experts coming from all-over the world.

Although truffles are known since ancient Greeks and Romans, the life cycle of truffles remained obscure till recent times, on the one hand for the absence of an experimental system based on spore germination and therefore the classical breeding of the resulting mycelia, and on the other hand for the missing knowledge of mating-type genes unlike for other filamentous Ascomycetes.Inthelastyears,technologicaladvancessuchashigh-throughput DNAsequencing, supercomputing and the emergence of genomics have lead to the sequencing of the *Tuber melanosporum* genome and provided new insights on the formation of the truffle fruiting body, its interaction with the environment and the promotion of mycorrhization strategies. Beside to these recent discovers and post-genomics activities (focused on specific gene categories, e.g., cell-wall related genes), that represent a breakthrough in the truffle research, the following topics are here presented in invited lectures: advances in truffle soil ecology; evaluation of food quality and bioactivity for the identification of metabolite differences that could be used as food quality markers(Foodomics); modern green processes for obtaining bioactive compounds; co-evolution of truffles with their tree hosts and animal spore dispersers over millions of years. Latest advances on artificial sensorial systems and their application in food, the chemical ecology of truffle volatiles together with the impact on truffle aroma of the microbiome inhabiting truffle fruiting bodies, and responses of the pest truffle beetle *Leiodescinnamomeus* to volatiles are also discussed in invited lectures, from both an ecological point of view, as well as a food science perspective. Lastly, conservation strategies to improve the fresh truffle shelf-life and the quality of commercial truffle flavoured oils are also presented.

In light of the achievements ruled out from these contributions, local and National Agencies should become more sensitive to research and help the scientists to translate their results to industries in order to benefit truffle producers. Conferences as this are not only an exciting meeting for scientists, they facilitate the dissemination of results and link researchers to farms and industries. Wishing a successful conference and a stimulating debate I thank Dr. Consol Blanch, Chair of the Organizing Committee, for sharing with me a few words of a warm welcome.

Antonietta Mello

Honorary chair of the Scientific Committee of the ICTR'14

Turin, 2th February 2014.

SCIENTIFIC PROGRAMME

TOPICS OF THE CONFERENCE:

1. TrufficuLture and ecoLogy
2. Truffle genomics and moLecuLar bioLogy
3. Foodomics: quaLity controL, flavour characterization, oLfactometric and sensoriaL anaLysis
4. Truffle and derivative conservation
5. Nutrition and gastronomy

PRESENTATION CODE

PL	Plenary lecture
IL	Invited lecture
RT	Round table contribution
OP	Oral presentation
OPS	Oral presentation - Sponsor collaboration
P	Poster communication

Each code has: **capital letters**, according to the kind of presentation and **two figures**, the first one means **topic number** and the second one is the **order number** in each series.

PROGRAMME

Sunday 9th March

Time	Sessions	Location
18:30 – 20:00	Registration	El Sucre
20:00	Welcome cocktail	El Sucre

Monday 10th March

Time	Sessions	Location
08:30-09:00	Late registration	A-AM
09:00-09:30	Opening ceremony	A-AM
	Topic 1 & 2. Trufficulture and ecology & Truffle genomics and molecular biology. Chair: Antonietta Mello, **Plant Protection Institute-CNR, Turin, Italy**	
09:30-10:30	**PL1.1** *Advances in truffle soil ecology, the key to improve its cultivation.* **Alessandra Zambonelli**, University of Bologna, Italy	A-AM
10:30-11:00	Coffee break	Room T017
11:00-11:30	**IL2.1** *New insight in the truffle life cycle.* **Claude Murat**, UMR INRA-UHP, France	A-AM
11:30-12:00	**IL2.2** *Truffle research in the post-genomic era* **Raffaella Balestrini**, UOS Torino, CNR, Italy	A-AM
	Topic 5 (I). Nutrition and Gastronomy. Chair: Josep Sucarrats, director of "Cuina" and "Descobrir" Gatronomic Journals, Barcelona, Spain	
12:00-12:30	**IL5.1** *Three classical Truffle recipes at "El celler de Can Roca" a gastronomic point of view.* ***Chef Joan Roca,*** *El Celler de Can Roca, Girona, Spain*	A-AM
12:30-13:00	**IL5.2** *The Cooking of the Truffles. Manipulation and Experiences.* A-A ***Chef Nandu Jubany,*** *Can Jubany, Calldetenes-Barcelona, Spain*	M
13:00-13:15	Gatronomic Exhibition by Nandu Jubany	A-AM
13:15-14:15	Lunch	Room T017
14:15-15:00	Poster session	ER

LOCATION CODE

A- AM: Auditorium -Aula Magana of EPS-UVIC-UCC
ER: Exhibition Ramp, beside Auditorium
AE: Alícia Espace on Món Sant Benet

	Oral Presentations Topics 1 & 2. Chair: José Antonio Bonet, UdL, CFTC, Spain	A-AM
15.00-15.15	**OP1.1** *Soil factors and vegetation determining occurrence of tuber aestivum vitt. in natural stands.* Alessandra Rosa-Gruszecka, Forest Research Institute, Poland	A-AM
15.15-15.30	**OP1.2** *Relationship between production, extraradial soil mycelium and ectomycorrhizal diversity in a black truffle plantation: first results.* Mikel Queralt, University of Navarra, Spain	A-AM
15.30-15.45	**OP1.3** *The hypothetical "saprotrofic phase" of the tuber melanosporum mycelium should be permanently buried.* Michele Miranda, University of L'Aquila, Italy	A-AM
15.45-16.00	**OP1.4** *Integrated management and sustainable cultivation of the valuable truffle species Tuber borchii vittad.* Marcos Morcillo, Micologia Forestal & Aplicada, Spain	A-AM
16.00-16.15	**OP1.5** *Low summer soil temperature and moisture favours root tip colonization of Quercus ilex by Tuber melanosporum.* Antoni Olivera, University of Lleida-Agrotecnio Center, Spain	A-AM
16.15-16.30	**OP2.1** *How many filamentous fungi do a truffle?* Giovanni Pacioni, University of L'Aquila, Italy	A-AM
16:30-16.45	Coffee-break	Room T017
	Topic 3. Foodomics (I): Quality Control, Flavour Characterization, Olfactometric and Sensorial Analysis. Chair: Consol Blanch, University of Vic, Spain	
16:45-17:45	**PL3.1** *The Chemical ecology of truffle volatiles* **Richard Splivallo**, University of Frankfurt, Germany	A-AM
17:45-18:15	**IL3.1** *Chemical aspects of the truffle aroma* **Laura Culleré**, University of Zaragoza, Spain	A-AM
20:00	EPS-UVIC-UCC- Transfer to the restaurant	EPS Front door
20:30	Conference Dinner. Chef **Nandu Jubany**	El Serrat de Figaró

Tuesday 11th March

Time	Sessions	Location
	Topic 3 & 4. Foodomics (II) and Truffles and derivatives conservation. Chair: Elena Ibáñez, CSIC-CIAL, Madrid, Spain	
09:00-09:30	**IL3.2** *Foodomics: opening the door to a global evaluation of food quality and bioactivity.* **Alejandro Cifuentes**, CSIC- CIAL-Madrid, Spain	A-AM
09:30-10:00	**IL4.1** *New technologies that would be applicable to truffle conservation and in the maintenance and protection of its biocatives components.* **Buenaventura Guamis**, UAB Research Parc, Autonomous University of Barcelona, Spain	A-AM
10:00-10:30	Coffee-Break and taste of truffle products, presented by "Conserves Coll" (Castellterçol, Catalonia, Spain)	Room 1017
	Oral Presentations Topics 3 & 4. Chair: Alessandra Zambonelli, University of Bologna, Italy	
10:30-10:45	**OP3.1** *The quality of commercial truffle flavored oils assessed by means of GC–MS and electronic nose.* Giovanni Pacioni, University of L'Aquila, Italy	A-AM
10:45-11:00	**OP3.2** *Quality aspects of the Australian black truffle (T. Melanosporum).* Garry Lee, University of Western Australia, Australia	A-AM
11:00-11:15	**OP4.1** *A patented edible film to improve the fresh truffle shelf-life.* Giovanni Pacioni, University of L'Aquila, Italy	A-AM
11:15-11:30	**OPS4.1** *Company Laumont: Establishment, Development and Leadership in truffle business either fresh or preserved.* Jordi Serentill, Deputy CEO Laumont S.L, Tàrrega, LLeida, Spain	A-AM
11:30-12:00	Poster Session	ER
12:00-12:30	Visit to truffle stalls at the weekly market in Vic Main Square	Plaça Major- Vic
12:45-13:30	Transfer to Món Sant Benet	Coach Station
13:30-15:30	Lunch	La Fonda – Món Sant Benet
15:30-16:00	Guided visit to Alícia – Food and Science Foundation	AE
	Topic 5 (II). Nutrition and Gastronomy. Chair: Toni Massanés, Alicia Foundation, Spain	
16:30-17:30	**OP 5.1** *Heritage projects in Fundació Alícia. Why do we like eating truffle?* Núria May, Alícia Foundation, St. Fruitós del Baiges, Spain	AE
17:30-18:15	Transfer to Vic	
19:00-20:00	Guided Visit to Vic	Plaça Major-Vic
20:00-21:00	Reception and Welcome Drink at the Vic Town Hall Traditional regional products and wine by "El Celler d'Osona"	Vic Town Hall

Wednesday 12th March

Time	Sessions	Location
09:00-10:00	**Topic 3. Round table: New trends in authentification, preservation, flavour characterization and bioactive compounds extraction in truffles. Chair:** Alejandro Cifuentes, CSIC-CIAL, Madrid, Spain • **RT3.1** *Electrophisycal (EAG) responses of Leoides Cinnamomeus to volatiles isolated from tuber melanosporum.* **Antonio Ortiz,** Universidad de Jaén, Spain • **RT3.2** *Modern green processes for obtaining bioactive compounds.* **Elena Ibañez**, CSIC- CIAL-Madrid, Spain • **RT3.3** *Latest advances on artificial sensorial systems and their application in food.* **Giorgio Pennazza**, Univ. Campus Bio-medico di Roma, Italy • **RT3.4** *Foodomics: truffles quality control strategies.* **Giovanni Pacioni**, University of L'Aquila, Italy.	A-AM
	Oral presentations Topics 1 & 4. **Chair:** Xavier Parladé, IRTA-Sustainable Plant Protection. Cabrils, Barcelona, Spain.	
10:00-10:15	**OP1.6** *Biodiversity and conservation of desert truffles in Saudi Arabia.* Mohammed Amin Uddin Mridha, King Saud University, Saudi Arabia.	A-AM
10:15-10:30	**OPS4.2** *The future of the truffles, a projection of the present* **Ismael Ferrer, Mario Cequier, Trufapasion SC,** Estadilla, Huesca, Spain	A-AM
10:30-10:45	**Coffee-break**	Room T017
10:45-11.15	**Poster session discussion.** Chairs: Richard Splivallo, University of Frankfurt, Germany and Michele Miranda, University of l'Aquila, Italy	A-AM
	Oral Presentations. **Chair:** Alessandra Zambonelli, University of Bologna, Italy	
11:15-11:30	**OP1.7** *Truffle cultivation in Germany – current situation and prospects.* Ulrich Stobbe, Albert-Ludwigs-University Freiburg, Germany.	A-AM
11:30-11:45	**OP1.8** *The cultivation of truffles in the southern hemisphere – the problems and the potential.* Ian Hall, Truffles and Mushrooms (Research) Limited, New Zealand & Alessandra Zambonelli, University of Bologna, Italy	A-AM
11:45-12:00	**OP2.2** *Hyphal growth and life cycle in truffle: new research perspectives and applications in the field.* Antonella Amicucci, University of Urbino, Italy	A-AM
12:00-12:15	**OP2.3** *Heterothallism in T. melanosporum and T. indicum: warning and lessons from genomics to preserve identity and promote fructification of the Perigord truffle.* Francesco Paolocci, CNR Institute of Biosciences and Bioresources (IBBR) Division of Perugia, Italy	A-AM
12:15-13:00	**PL1.2** *Why Earth needs Truffles.* ***James Trappe***, Oregon State University, US. **Chair:** Christine Fischer, CTFC, Solsona, Spain	A-AM
13:00-13:30	Closing ceremony	A-AM
13:30-14:30	Farewell Cocktail, taste of truffle products by "Trufapasion", wine by "Celler d'Osona" and cava by "Juvé y Camps". Catalan Human Tower Exhibition "Castells", by "Emboirats" UVIC group.	EPS Courtyard

PLENARY LECTURES

PL 1.1

ADVANCES IN TRUFFLE SOIL ECOLOGY - THE KEY TO IMPROVING TRUFFLE CULTIVATION

Alessandra Zambonelli [(1)] *, **Ian Hall** [(2)], **Antonella Amicucci**[(3)], **Elena Barbieri**[(3)], **Mirco Iotti** [(1)]

(1) *Dipartimento di Scienze Agrarie, University of Bologna, via Fanin 46, 40127 Bologna, Italy*

(2) *Truffles and Mushrooms (Research) Limited and Edible Forest Fungi New Zealand Limited, P.O. Box 268, Dunedin 9054, New Zealand*

(3) *Department of Biomolecular Science, University of Urbin Carlo Bo, Via Saffi 2, 61029 Urbin, Italy*

*alessandr.zambonelli@unibo.it, Telelphone: +39 051 2096579

Keywords: environmental conditions, microorganisms, soil, truffle cultivation

Truffle cultivation is an economically important agro-forestry industry in Europe and is also becoming established in many other non-European countries. The well known important steps for successful truffle cultivation are: 1) to plant suitable plants fully colonized with the appropriate *Tuber* species, 2) to choose a site with climatic conditions suitable for the selected plant and truffle, 3) to select a soil suited (naturally or by liming) to the truffle [1]. However, even when all these prerequisites are satisfied yields are often lower than expected. The lack of both mating types, which need to cross for initiate fructification, was considered to have been the cause of most failures. However a recent study clearly shows that there are more factors involved in ascoma production than just the presence of both mating types on host trees [2]. The whole truffle life cycle is carried out underground where it not only establishes nutritional relationships with the roots of a suitable host plant, but it associates with other soil organisms. It is well known that inside the *Tuber* ascomata there are numerous bacteria and fungi which seem to be directly involved in ascoma nutrition and development [3]. At the same time Mycorrhizal Helper Bacteria are involved in establishing the ectomycorrhizal association. In soil truffle niches, *Tuber* ectomycorrhizas and its mycelium interact by molecular signals with other ectomycorrhizal fungi, saprobic and pathogenic fungal species, as well as bacteria, plants and animals. The presence of *T. melanosporum* in the soil drastically reduces the fungal and plant diversity [4]. In contrast, some microorganisms are likely to produce molecules capable of inhibiting the growth of *Tuber* in the soil. For example, it has been shown that volatile organic compounds produced by *Staphylococcus pasteurii*, an ubiquitous bacterium, are capable of completely inhibiting the growth of *Tuber borchii* [5].

Despite the importance of the underground environmental conditions and organisms associated with truffles remain mostly unknown and need to be thoroughly researched before we will begin to understand the ecological factors that favour fructificating. To this aim advances in molecular tools and new technologies show great promise.

[1] I. R. Hall, G. Brown, A. Zambonelli, A. Timber Press, Portland (2007) 304 p.

[2] C. C. Linde, H. Selmes, Applied and Environmental Microbiology **78** (2012) 6534-6539.

[3] E. Barbieri, P. Ceccaroli, R. Saltarelli, C. Guidi, L. Potenza, M. Basaglia, F. Fontana, E. Baldan, S. Casella, O. Ryahi, A. Zambonelli , V. Stocchi. Fungal Biology, **114** (2010) 936-942.

[4] C. Napoli, A. Mello,A. Borra, A. Vizzini, P. Sourzat, P. Bonfante, New Phytologist, 185 (2007) 237–247.

[5] E. Barbieri, AM Gioacchini, A. Zambonelli, L. Bertini, V. Stocchi, Rapid Communication of Mass Spectromectry 19 (2005) 3411-3415.

PL 1.2

WHY EARTH NEEDS TRUFFLES

James Trappe

Department of Forest Science, Oregon State University, EU

trappej@ucs.orst.edu, Telephone: +001541-737-8593

Keywords: ecosystem, mycorrhiza, truffle, mutations, evolution

Earth's ecosystems operate through interactions evolved mostly by genetic mutations selected in response to environmental selection pressures. Truffles elegantly represent adaptation and co-evolution of mycorrhizal fungi with their tree hosts and animal spore dispersers over millions of years. Most truffles evolved from mushroom ancestors ill equipped to produce spores under climatic stresses such as heat, drought and frost. The series of mutations that enabled mushroom-forming mycorrhizal fungi to fruit in the protected, moisture-conserving below ground habitat enable them to reproduce even when above ground climates and weather prevent spore production. Co-evolution with animal mycophagists was simultaneously required for spore dispersal. Truffle species thus appear particularly well adapted to maintain populations of mycorrhizal fungi during global warming.

PL 3.1

THE CHEMMICAL ECOLOGY OF TRUFFLE VOLATILES

Richard Splivallo(1),*

(1) *Goethe University Frankfurt, Institute for Molecular Bio Science, Max-von-Laue-Str. 9, 60438 Frankfurt am Main, Germany*

*richard.splivallo@a3.epfl.ch, Telelphone: +49 176 64 93 14 02

Keywords: truffle, volatiles, aroma, interactions, plants, bacteria, ecology

Truffle aromas have been studied for decades by sensory scientists in order to decipher the components which make those aromas so appealing to food connoisseurs. Truffles, however, do not produce their smell for the mere pleasure of humans but do so with a real ecological purpose. It is well accepted that volatiles produced by truffle fruiting bodies serve as attractants to mammals which feed on truffles and hence disperse their spores. Truffle aroma might also be involved in communications with plant roots as well as with soil microbes and insects.

Here we will review the current knowledge about the interaction of truffles with its environment and specifically the involvement of volatile organic compounds in these interactions. How truffles might manipulate plant roots through volatiles and how this might affect plant fitness will be discussed [1], [2]. Current knowledge on the biosynthesis of major truffle volatiles will also be addressed considering the recent insights brought in by the sequencing of the Périgord truffle genome in 2010 [3], [4]. Last the impact on truffle aroma of the microbiome inhabiting truffle fruiting bodies will also be discussed from both an ecological point of view as well as a food science perspective.

[1] R. Splivallo, M. Novero, C. Bertea, S. Bossi, and P. Bonfante, New Phytol, 175 (2007), 417–424

[2] R. Splivallo, U. Fischer, C. Gobel, I. Feussner, and P. Karlovsky, Plant Physiol., 150 (2009), 2018–2029

[3] R. Splivallo, S. Ottonello, A. Mello, and P. Karlovsky, New Phytologist, 189 (2011), 688–699

[4] F. Martin, A. Kohler, C. Murat, R. Balestrini, P. M. Coutinho et al. Nature, 464 (2010), 1033–1038

INVITED LECTURES

IL 2.1

NEW INSIGHTS IN THE TRUFFLE LIFE CYCLE

Claude Murat

INRA, UMR 1136 INRA Université de Lorraine « Interactions Arbres-Microorganismes », Labex ARBRE, IFR EFABA, 54280 Champenoux, France

claude.murat@nancy.inra.fr; +33 (0)3 83 39 41 27

Truffles are ectomycorrhizal fungi living in symbiosis with many trees and shrubs. The interest in truffle cultivation and commercialisation has greatly increased in the world. The truffle industry is dynamic due to efforts by truffle cultivators and plantations focused on increased production, currently at the rate of about 1,000 ha per year in France. But clear management guidelines geared 1) to enhance production; 2) to face climate changes and 3) to make truffle production less unpredictable are still needed. To draw these guidelines it is necessary to better understand the truffle life cycle.

In this presentation I will present the most recent new insights in the truffle life cycle obtained thanks to the black truffle (*Tuber melanosporum*) genome-sequencing project [1] and to the SYSTRUF project (Bases for a sustainable ecological monitoring of ecosystems producing truffles *Tuber melanosporum*) financed by the French National Research Agency.

The genomic resources allowed us to unravel the sexual reproduction mode of the black truffle by identifying mating type genes and to characterize new polymorphic markers allowing population genetic analyses. The genets distribution in truffle orchards have been investigated showing a non random distribution of ectomycorrhiza formed by both mating types as well as an annual turnover of the genets [2].

In the SYSTRUF project we demonstrated that the ascocarp carbon came from the tree by labelling experiments and that a link between the ascocarp and the tree existed late in season [3]. Finally we identified the bacterial community associated with the different phases of the black truffle life cycle [4] as well as a particular fungal community in productive versus non-productive soils.

Acknowledgments: I would like to thank all *Tuber* genome consortium and the SYSTRUF colleagues that contributed to the data I will present.

[1] F. Martin et al., Nature, 464 (2010) 1033-1038

[2] C. Murat et al., New Phytologist, 199 (2013), 176-187

[3] F. Le Tacon et al., Plos One, PloS one 8 (2013), e64626

[4] S. Antony-Babu et al., Environmental Microbiology, (2014), doi:10.1111/1462-2920.12294

IL 2.2
TRUFFLES IN THE POST-GENOMIC ERA

Raffaella Balestrini[(1)]*, Fabiano Sillo[(2)], Elisa Zampieri[(2)], Antonietta Mello[(1)], Francis Martin[(3)], Paola Bonfante[(2)]

[(1)] *Istituto per la Protezione delle Piante, UOS Torino – CNR, Viale Mattioli 25 – 10125 Torino*

[(2)] *DBIOS – UniTO, Viale Mattioli 25 – 10125 Torino*

[(3)] UMR INRA-UHP 1136 Interactions Arbres/Micro-organismes, Centre INRA de Nancy, 54280 Champenoux, France

*r.balestrini@ipp.cnr.it, Telephone: +390116502927

Keywords: genome and transcript sequencing, symbiosis, cell wall, RT-qPCR

Truffles, like other ectomycorrhizal fungi, have a dual lifestyle, living both in the soil as facultative transitory saprotrophs and within the plant roots as symbionts. Thanks to the symbiosis they establish with the roots of woody and shrubby plants, truffles produce prized fruiting bodies. During its life cycle, the mycelium undergoes morphogenetic changes, reflecting the expression of specific genes,which can be influenced by environmental factors.In 2010,the *T.melanosporum* genome sequencing project launched by a French-Italian consortium, was accomplished and the results led to the first information concerning an Ascomycete-symbiotic fungus [1]. The working hypothesis of the further steps was that identification of processes that condition and trigger fruit body and symbiosis formation, ultimately leading to a more efficient production, would be facilitated by a thorough analysis of truffle genomic traits. Starting from the genome data set, several post-genomics activities were developed, in order to focus on specific gene categories (*e.g.,* cell-wall related genes, environmental response genes, etc.) and, among them specific gene families [2, 3, 4]. These results have allowed us to obtain new knowledge on the fungal biology of the black truffle. By contrast, in the absence of the whole genome sequence of the white truffle *T. magnatum*, we have performed a gene expression analysis on specific genes that could be involved in changes during truffle post-harvest storage. In parallel, a metabolite profile has been obtained on fruiting bodies conserved in the same conditions.

Taken together, results coming from both black and white truffles have allowed us to highlight some of the molecular events that take place during truffle development in nature and their conservation for the market.

[1] F. Martin, et al., Nature, 464 (2010) 1033-1038.

[2] E. Zampieri, R. Balestrini, A. Kohler, S. Abbà, F. Martin, P. Bonfante, Fungal Genet Biol, 48 (2011) 585-91.

[3] R. Balestrini, F. Sillo, A. Kohler, G. Schneider, A. Faccio, E. Tisserant, F. Martin, P. Bonfante, Curr Genet, 58 (2012) 165-77.

[4] F. Sillo, C. Gissi, D. Chignoli, E. Ragni, L. Popolo, R. Balestrini, Fungal Genet Biol, 53 (2013) 10-21.

IL 3.1

CHEMICAL ASPECTS OF THE TRUFFLE AROMA

Laura Culleré Varea

Dpto. of Analytical Chemistry, Science Faculty, University of Zaragoza, c/ Pedro Cerbuna nº 12, 50009 Zaragoza

*email: lcullere@unizar.es, Telelphone: +34 876 553330

Keywords: *T. melanosporum*, *T. indicum*, *T. aestivum*, olfactometry, aromatic compounds

Truffles are highly appreciated due to their characteristic sulphur aroma and are used mainly uncooked in French and Italian cuisine, particularly black truffle (*Tuber melanosporum*) commonly known as the "black diamond of cuisine". Black Périgord truffle is considered the most aromatic, while white truffle (*Tuber magnatum*) from Italy is considered the finest because of its complex aroma, and it is also the rarest and the most expensive. Summer truffle (*Tuber aestivum*) is less aromatic than black and white truffles, but is moderately priced and has a good aroma quality. In China, there are 25 species of the genus Tuber. One of them is the species *Tuber indicum*, which looks very similar (dark gleba and black peridium) to *T. melanosporum* and it is difficult to tell them apart by traditional morphological observations [1, 2].

Inrecentyears,somestudieshaveanalyzedthevolatilecompoundsofdifferentspeciesoftruffles using mainly headspace solid-phase microextraction (HS-SPME) technique [3-5] and direct headspace analysis [6]. This information obtained by gas chromatography-mass spectrometry (GC–MS) analysis is interesting but incomplete given that it is necessary to evaluate which of the compounds are in fact important odorants. In order to rank these odorants attending to their potential importance in the aroma of truffle, a gas chromatographic–olfactometric study (GC–O) is necessary. Very few papers have undertaken olfactometric studies of truffle samples. Therefore, one of the main aims of our research was focused on the aromatic characterization of different species of truffles using this olfactometric strategy [7, 8].

On the other hand, it is known that truffles have their highest organoleptic value when fresh. However, like many other vegetable commodities, they are highly perishable mainly due to bacterial and mould growth and dehydration which contribute to the rapid loss of organoleptic properties such as texture, aroma, and taste [9]. Moreover, truffles are seasonal so long term storage methods are used to ensure their availability throughout the year. Freezing is one of the most important methods for retaining food quality during long-term storage. The goal of the present work was to study the effect of freezing and long-term frozen storage on the aroma composition of *T. melanosporum*, using sensory analyses and analytical techniques (HS-SPME coupled with GC–MS). The truffles were frozen at temperatures of -20ºC to -80 Cº during different times (1, 20, 40 and 60 days). Descriptive and discriminative sensory and chemical analyses, based on headspace solid phase microextraction followed by gas chromatography–mass spectrometry analysis (HS-SPME–GC–MS), were carried out after these periods of time. Fifteen compounds with high aromatic potential in truffles were determined. The volatile composition data revealed that *T. melanosporum* aromatic profile is deeply modified as a consequence of a freezing process [10].

As conclusion, it is important to highlight that we have developed different chromatographic strategies, based on HS-SPME-GC-MS and DHS-GC-O, through which it is possible to characterize from an aromatic point of view different species of truffles, allowing for example distinguish between *T. melanosporum* and *T. indicum*, (avoiding possible fraud practices). Another

useful application of the developed methodologies consisted on evaluating the effect of different storage processes as freezing on the truffle aroma.

[1] J.P. Douet, M. Castroviejo, D. Mambru, G. Chevalier, C. Dupré, F. Bergougnoux, J.M. Ricard, B. Médina, *Analytical Bionalatyical Chemistry* 379 (2004) 668-673.

[2] L. Riousset, G. Riousset, G. Chevalier, M.C. Bardet, eds. *Truffles d'Europe et de Chine*. ed. I.N.d.R.A.I.I. Éditions. 2001: Paris.

[3]F. Bellesia, A. Pinetti, A. Bianchi, B. Tirilini, *Flavour and Fragrance Journal* 13(1), (1998): 56-58.

[4]P. Buzzini, C. Gasparetti, B. Turchetti, M.R. Cramarossa, A. Vaughan-Martini, A. Martini, U.M. Pagnoni, L. Forti. *Archives Of Microbiology* 184(3), (2005) 187-193.

[5] P. Díaz, E. Ibañez, F.J. Señorans, G. Reglero, *Journal of Chromatography A* 1017(1-2) (2003). 207.

[6] R. E. March, D.S. Richards, R.W. Ryan, *International Journal of Mass Spectrometry* 249 (2004) 60-67.

[7] L. Culleré, V. Ferreira, B. Chevret, M.E. Venturini, A.C. Sánchez-Gimeno, D. Blanco, *Food Chemistry* 122 (2010) 300-306.

[8] L. Culleré, V. Ferreira, M.E. Venturini, P. Marco, D. Blanco, *Food Chemistry* 141(1) (2013) 105-110.

[9] C. S. Rivera, D. Blanco, M.L. Salvador, M.E. Venturini, *Journal of Food Science* 75(4) (2010) E225-E233.

[10] L. Culleré, V. Ferreira, M.E. Venturini, P. Marco, D. Blanco, *Food Chemistry* 136(2) (2013) 518-525.

IL 3.2

FOODOMICS: OPENING THE DOOR TO A GLOBAL EVALUATION OF FOOD QUALITY AND BIOACTIVITY

Alejandro Cifuentes

Institute of Food Science Research (CIAL), National Research Council of Spain (CSIC), *Nicolas Cabrera 9, Campus de Cantoblanco, 28049 Madrid, Spain.*

a.cifuentes@csic.es, Telephone: +34910017955

Keywords: Foodomics, metabolomics, transcriptomics, bioactivity, colon cancer, alzheimer, transgenic food

Nowadays, the use in food science and nutrition of advanced "omics" tools such as transcriptomics, proteomics or metabolomics allows investigating topics that were considered unapproachable few years ago; this trend has generated a new discipline defined for the first time by our group as "Foodomics" [1-3]. In Foodomics, food safety, quality and bioactivity are investigated through the application of advanced omics technologies in order to improve consumers' well-being, health and confidence.

Although no applications of Foodomics to truffle research have been found so far in literature, the possibilities of Foodomics in this area can be huge. To demonstrate this point, we introduce in this work the new discipline of Foodomics, describing its fundamentals, discussing the role of Foodomics to solve some current and future challenges in food science and nutrition, and showing several Foodomics applications carried out in our laboratory related to food quality and food bioactivity. Namely, these Foodomics works were done: i) to evaluate the quality of (transgenic vs. non-transgenic) foods, ii) to investigate the possibilities of Foodomics in Alzheimer's disease studies and, iii) to determine the anti-proliferative effect of food ingredients against different human cancer cell lines. Whole-transcriptome microarray, proteomics and MS-based non-targeted whole-metabolome approaches were employed to carry out the mentioned studies. These Foodomics strategies enabled: i) the identification of metabolite differences that could be used as food quality markers, ii) the identification of biomarkers for early detection of Alzheimer's disease which should allow to investigate the effect of diet on this illness, and iii) the identification of several differentially expressed genes alone and/or linked to changed metabolic pathways that were modulated by food ingredients in cancer cells, providing new evidences at molecular level on the antiproliferative effect of food compounds.

REFERENCES

[1] M. Herrero, C. Simó, V. García-Cañas, E. Ibáñez, A. Cifuentes, Mass Spec. Rev. 31 (2012) 49–69.

[2] A. Cifuentes, J. Chromatogr. A 1216, 7109-7110 (2009).

[3] V. García-Cañas, C. Simó, M. Herrero, E. Ibáñez, A. Cifuentes, Anal. Chem. 84 (2012) 10150–10159

IL 4.1

STRATEGIES FOR THE POST-HARVEST HANDLING AND CONSERVATION OF FRESH TRUFFLES

Buenaventura Guamis

Parc de Recerca UAB (PRUAB), Bellaterra, Spain

buenaventura.guamis@uab.es, Telephone: +34 93 586 88 91

The quest for healthier, nutritious, more natural foods with increased safety level and obtained with processes respectful of the environment has increased during the last 15 years. Sensory quality of marketed foods has also faced more exigent demands from consumers worldwide

The conservation of truffles and its derived products is one of the biggest current challenges for the food industry.

The truffle is a highly-appreciated seasonal product which has a very high market value. It is used in culinary preparations of a very high standard.

The technologies which can be applied to conserve the fresh product, whole or cut into slices or cubes, must assure that the organoleptic properties such as flavour, colour and texture are not altered.

Any alteration reduces the quality of the product and leads to it losing its market value.

Currently, there are 3 types of technology which can be used to maintain the product as fresh as possible and give it longer life.

The first of them is cryogenic freezing. This is an already traditional technology which is only applied to high-value products. In the case of the truffle, instant freezing of the fungus is achieved using liquid nitrogen or carbonic snow in cupboards or tunnels. In all cases adequate time and temperatures most be applied according to the size and water content of the fungus.

The second technology called hydrostatic high pressure (HHP) was developed in the EU in the 1990s. It consists of the application of high pressures of around 600 MPa for a few minutes at a controlled temperature. This technology needs the product to be packaged in a flexible pack and submerged in a solution which allows the transition of the pressure to the product.

The product must be conserved in refrigeration for several weeks.

The third technology is ultra-high pressure homogenization (UHPH) which appeared at the end of the 1990s and which was perfected in the first decade of the 21st century. The use of Ultra High Pressure Homogenization (UHPH) for food processing is based upon the application of a continuous system that combines shear, cavitation, high pressure and impact forces to insert small molecules into larger molecules to protect and also destroy microorganisms included spores

This technology continually processes liquid foods and it can also be used to produce emulsions and sauces. It has the advantage of sterilizing and stabilizing foods which once aseptically packaged can be preserved for months at room temperature

IL5.1

THREE CLASSICAL TRUFFLE RECIPES AT "EL CELLER DE CAN ROCA" A GASTRONOMIC POINT OF VIEW.

Joan Roca

El Celler de Can Roca, Girona, Spain

joan@cellercanroca.com Telelphone: +34 972 222 157

Keywords: truffle, food proximity culture, classical truffle recipes, seasonal products, Roca

Joan Roca will explain how the truffle is processed at El Celler de Can Roca from a gastronomic point of view, from a proximity culture and the technical applications for different treatments, on three classic recipes from the restaurant still in force: The brioche, the salty bonbon, and a truffle soufflé. The recipes will also be illustrated with video recipes, in a tribute to one of the few seasonal products that we still have, and that makes us wish from the kitchen the arrival of winter in order to receive all the truffle character.

IL5.2

THE COOKING OF THE TRUFFLES. MANIPULATION AND EXPERIENCES.

Nandu Jubany

Can Jubany Crta. Sant Hilari, s/n. 08506 Calldetenes (Barcelona)

*nandu@canjubany.com Telelphone: +34 93 889 10 23

Keywords: truffle, cooking, manipulation, experience, Jubany

The chef Nandu Jubany will explain the cooking of the Truffles and its manipulation. Also, he will speak about the characteristics of truffles from many points of view, as agricultural, technology, physical, nutritional and sensory characteristics, cuisine or entrepreneurship. Jubany will introduce the truffle season menu and a fun, original and stimulating way for the palate of the star desserts truffle season in his restaurant Can Jubany.

IL 5.3

HERITATGE PROJECTS I N FUNDACIÓ ALÍCIA. WHY DO WE LIKE EATING TRUFFLE?

Fundació Alícia

Camí de Sant Benet, s.n. 08072. Sant Fruitós del Bages, Catalonia, Spain.

may@alicia.cat, Telephone: +0034 875 94 02

Keywords: heritage, truffle, history, alimentation.

Fundació Alícia is a nonprofit culinary research center with a clear aim, working to make people eat better. This is achieved by a multidisciplinary group of professionals who develop projects in three different labor branches: healthy eating habits, product technology and sustainability and heritage. A visit to the installations of Fundació Alícia will be developed before the theoretical session.

One of the principal purposes of the heritage working branch is the revalorization of local, traditional or singular products, being themselves tangibles or not. The truffle is a product with a long history of recollection and consuming and in our country and the use is related to traditions and gastronomic and culinary customs.

The presentation will also try to give answer to the question proposed in the title of the session with the help of the experts present in the talk. As far as we know, humans eat ingredients, which are then referenced as food, that are available in the environment where they live. But this first definition is not the only condition necessary for an accurate supply of food, as American anthropologist Marvin Harris reflects in his theory books, the first problem to be solved for any human population is how best to use the resources it has available, so that it not only can maintain itself but also reproduce itself and sustain the next generation. Truffle will be analyzed by this point of view aiming assistants to provide their point of view on the subject.

Truffle is a product now rated as a gourmet ingredient but this condition has sometimes changed since ancient times. There is written evidence that truffles were once valued as precious ingredients in classical times, but as it is said it's use was partly left apart in the last centuries of medieval times as it is seen by the absence of written recipes in monastic books including it as a main ingredient. It was not until renaissance times when truffle was once again described as "the diamond of the kitchen" like Brillant Savarin notes in his Physiology of the gout published in 1825. The French culinary revolution gave recognition and gourmet use to the product, being desired by bourgeoisie all over the world, influenced by the innovative techniques and structures of the new French cuisine. Different recipes along history will be commented and described during the presentation.

ROUND TABLE

RT 3.1

ELECTROPHYSIOLOGICAL (EAG) RESPONSES OF *LEIODES CINNAMOMEUS* TO VOLATILES ISOLATED FROM *TUBER MELANOSPORUM*

Antonio Ortiz(1), **Guillermo Perez-Andueza**(2)*, **César Saucedo**(2) and **Fernando Herrero** (2)

(1) *Inorganic and Organic Chemistry Department. EPS Linares. Universidad de Jaén. C/ Alfonso X el Sabio nº 28. 23700 Linares (Spain)*

(2) *Agroforestry and Environmental Department. Universidad Católica de Ávila. C/Canteros s/n. 05005 Ávila (Spain)*

*guillermo.perez@ucavila.es, Telephone: +920251020 (Ext. 159)

Keywords: black truffle, *Tuber melanosporum*, truffle beetle, *Leiodes cinnamomeus*, volatiles, semiochemicals, electroantennography (EAG).

Tuber melanosporum Vittadini (black truffle) is a subterranean fungi highly appreciated for its culinary and market values. The truffle beetle *Leiodes cinnamomeus* (Panzer, 1793) (Coleoptera, Staphylinoidea, Leiodidae) is an important pest of black truffle in Central Spain. Both the adults and the larvae of *Leiodes* contribute to economic losses by the destruction of truffles, due to their feeding and oviposition habits, but little is known about the chemical communication (semiochemicals) between beetles and their host fungi [1] [2] [3].

Volatile compounds released by unripe and ripe truffles, were collected by dynamic headspace (DHS) on Tenax-TA, as well as by solid phase microextraction (SPME) adsorption. Gas chromatography-mass spectrometry (GC-MS) analysis of released volatiles by unripe and ripe truffles, showed 13 compounds in noticeable amounts. From a quantitative point of view, unripe truffles emit higher quantities of sulfur components (DMS and DMDS), anisole (methoxybenzene) and benzene 1,2-dimethyl, than ripe truffles. Detection by coupled gas chromatography-electroantennography (GC-EAG) revealed that four of the identified compounds elicited reproducible antennal signals in tested adult insects.

The overall EAG response profiles of male and female beetles to the tested fungus odors were similar. Among the compounds tested, dimethyl sulfide, 1-octen-3-ol and 4-methyl-3-octanol elicited consistently high EAG responses.

[1] G. Pacioni, M. A. Bologna, M. Laurenzi, *Mycological Research* **95** (1991) 1359-1363.

[2] M. E. Hochberg, G. Bertault, K. Poitrineau, A. Janssen, *Entomologia Experimentalis et Applicata* **109** (2003) 147–153.

[3] R. Splivallo, S. Ottonello, A. Mello, P. Karlovsky, *New Phytologist* 189 (2011) 688–699.

RT 3.2

MODERN GREEN PROCESSES FOR OBTAINING BIOACTIVE COMPOUNDS

Elena Ibáñez*

Institute of Food Science Research CIAL (CSIC-UAM). Nicolas Cabrera 9, Campus de Cantoblanco, 28049 Madrid, Spain.

*elena@ifi.csic.es, Telephone: +34910017956

At present, researchers are facing new challenges in the development of new extraction techniques/processes that can be used to both, analyze and obtain valuable compounds from natural sources. Up to now, traditional extraction methods (mainly solid-liquid extraction) have been used to extract bioactives; these methods have several drawbacks including time consuming, laborious, low selectivity and/or low extraction yields. New challenges involve the development of processes that are fast, selective, efficient, sustainable, green (without using toxic organic solvents), with high yields and low cost. The techniques able to meet these requirements are, among others, those based on the use of compressed fluids such as supercritical fluid extraction (SFE), pressurized liquid extraction (PLE) and subcritical water extraction (SWE), which are among the more promising processes [1, 2]. Depending on the polarity of the green compressed fluid, different "green" or environmentally clean technologies can be used, as can be seen in Figure 1.

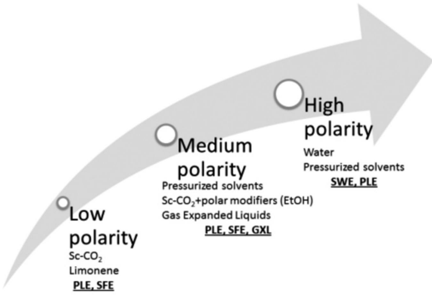

Figure 1. Green solvents and environmentally friendly technologies used to extract high added-value products from natural sources

The use of such technologies in different fields related to truffle research and applications will be presented and proposed to the audience; for instance, compressed fluids could be used: 1) as sample preparation technique for authenticity control of the raw materials and samples; 2) as extraction process to recover valuable compounds from low quality truffles or by-products; 3) as a new tool to develop new flavors and textures in molecular gastronomy.

Acknowledgments

This work was financed thanks to AGL2011-29857-C03-01 (Ministerio de Economía y Competitividad) and ALIBIRD, S2009/AGR-1469 (Comunidad de Madrid) projects.

[1] King, M.B. and Bott, T.R. *Extraction of natural products using near-critical solvents*. 1993, Glasgow: Blackie Academic & Professional.

[2] Mendiola, J.A., Herrero, M., Cifuentes, A., Ibáñez, E. *Use of compressed fluids for sample preparation: Food applications.* Journal of Chromatography A, 2007. **1152**(1-2): p. 234-246.

RT 3.3

LATEST ADVANCES ON ARTIFICIAL SENSORIAL SYSTEMS AND THEIR APPLICATION IN FOOD

Giorgio Pennazza*, **Marco Santonico, Arnaldo D'Amico**

Faculty of Engineering, University 'Campus Bio-Medico di Roma', Italy

*g.pennazza@unicampus.it, Telephone:

Keywords: gas sensor array, electronic nose, electronic tongue, chemical sensors

Looking at food analysis by a technological point of view, five ingredients make the recipe of an optimal technique: non-destructivity, consumer-oriented evaluation, reliability, effectiveness, easy and fast execution [1]. Sensorial systems aim at covering all these aspects but, in spite of twenty and more years of application in the field, they are still far from the goal. Three key-points must be considered to do the job: standardization, data fusion, specialized protocols and tools. Thus, the starting point is the identification of the target-food and of the specific problem to be faced. In this case, truffle is the object of the research and its authentication and preservation are the problem. The promising results so far obtained suggest the ability of an artificial sensorial system in monitoring shelf-life and in evaluating different preservation conditions [2]. Current and future developments are going towards a multi-sensorial system (volatiles, liquid and optical properties) [3] and a specific arrangement of the measuring system. Besides, correlation with other instruments pertaining analytical chemistry must be studied in order to support the scientific results. Other key-applications of this group are relative to the monitoring of ham curing processes, mushrooms shelf-life observation and fish freshness checking.

[1] Baldwin A, Bai J, Plotto A, Dea S, Electronic noses and tongues: Applications for the food and pharmaceutical industries, Sensors 11 (5) (2011) 4744-4766.

[2] Pennazza G, Fanali C., Santonico M., Dugo L., Cucchiarini L., Dachà M., D'Amico A., Costa R., Dugo P., Mondello L. Electronic nose and GC-MS analysis of volatile compounds in Tuber magnatum Pico: Evaluation of different storage conditions. Food Chemistry 2013,136 (2), 668-674.

[3] Santonico M, Pennazza G, Grasso S, D'Amico A, Bizzarri M. Design and Test of a Biosensor-Based Multisensorial System: A Proof of Concept Study, Sensors 2013, 13, 16625-16640.

RT 3.4

FOODOMICS: TRUFFLES QUALITY CONTROL STRATEGIES

Giovanni Pacioni*

Dept. of Life, Health and Environmental Sciences, University of L'Aquila, I-67100 L'Aquila, Italy

*giovanni.pacioni@univaq.it, Telephone: +390862433209

Keywords: instrumental control, adulteration, nutraceutical

The applications of technological and analytical tools to the study of truffles for quality control and their chemical composition are examined.

They show a strong interest in the analysis of aromas that have been the subject of investigations and patents for over a century.

Regarding taste, a great result can be achieved with the introduction of the electronic nose, and in the future also of the electronic palate, properly trained by a panel of experts.

However truffles are not spices but food, even if their cost is such that they are mainly used as flavoring.

As food they should be investigated for what concerns their nutritional value and adulteration / contamination processes; however, from this point of view, investigations are lacking.

Therefore, some cases of episodic analysis on fresh and differently preserved truffles are reported and discussed. They concern the presence of heavy metals and radio-nuclides, pesticides and herbicides, dyes, chemical preservatives and hydrocarbons; species and quantity of truffles present in processed products (RT-PCR); new non-invasive methods for investigations of internal conditions (NMR-I) and qualitative and quantitative composition of truffle flavors (DHS-GC-MS coupled with ES). The last two methods are now also useful for evaluating new systems to increase the shelf-life of fresh truffles.

A revisitation of the classical analyses of foods (proteins, amino acids, lipids…) carried out on truffles revealing the presence of steroid and non-steroid hormones, glutamic acid, neurotransmitter lipids, L-DOPA etc, emphasizes the need to prize "truffle" as food from the nutraceutical / functional point of view, which clears the way for a reconsideration of ancient ideas on truffles.

ORAL PRESENTATIONS

OP 1.1

SOIL FACTORS AND VEGETATION DETERMINING OCCURRENCE OF TUBER AESTIVUM VITT. IN NATURAL STANDS

Aleksandra Rosa-Gruszecka[(1),*], Dorota Hilszczańska[(2)], Hanna Szmidla[(1)], Katarzyna Sikora[(1)]

[(1)] *Forest Research Institute, Department of Forest Protection, Braci Lesnej 3, Sekocin Stary, 05-090 Raszyn, Poland, fax. +48 227150557*

[(2)] *Forest Research Institute, Department of Forest Ecology, Braci Lesnej 3, Sekocin Stary, 05-090 Raszyn, Poland, fax. +48 227150504*

*A.Rosa@ibles.waw.pl, Telephone: +48 227150353

Keywords: *Tuber aestivum*, soil factors, host-plants, phylogeny

The aim of this study was to check the relationship between the carpophore of *Tuber aestivum* presence and the value of soil variables. The survey done in the southern part of Poland, at six chosen sites, revealed that among ten host-plants of *Tuber aestivum* three species of orchids were present.

Statistical analyses shared positive correlation between *T. aestivum* productivity and concentration of Ca and $CaCO_3$. In all investigated sites at least one of orchids species was present: *Cephalanthera damasonium*, *Epipactis helleborine* and *Cypripedium calceolus*. Although no positive correlation between given orchid species and summer truffle was found, the aspect needs to be checked in the future, establishing higher number of investigated plots.

The identity of *Tuber aestivum* has been confirmed, basing on morphology, anatomy and genetic features. Morphologically determined ascocarps of *T. aestivum*, found in different localities in Poland, were used as source material for DNA extraction. Genetic analyses were performed on a variable part of rRNA gene, containing both ITS1 and ITS2 regions. Sequences were aligned using the Clustal algorithm of BioEdit program (version 7.1.3.0). Maximum-likelihood tree (bootstrap with 500 replicates) showed the presence of a tritomy, with the following groups: a) *T. aestivum* cluster from localities A and B, b) *T. aestivum* from locality C, c) *T. aestivum* from localities B, D and E. All nodes were supported with high bootstrap values.

Research on truffles in Poland is still very much in a pioneering phase and it is hoped that our results will help promote the establishment of a truffle culture and industry in our country.

OP 1.2

RELATIONSHIPS BETWEEN PRODUCTION, EXTRARADICAL SOIL MYCELIUM AND ECTOMYCORRHIZAL DIVERSITY IN A BLACK TRUFFLE PLANTATION: PRELIMINARY RESULTS.

Mikel Queralt[(1),*], Xavier Parladé[(2)], Ana De Miguel[(1)]

[(1)] *Deparment of Environmental Biology, University of Navarra.*
C/Irunlarrea s/n. 31008, Pamplona, Navarra, Spain

[(2)] *Institut de Recerca I Tecnologia Agroalimentària (IRTA). Centre de Cabrils.*
Sustainable Plant Protection. Ctra. Cabrils Km.2 E-08348 Cabrils, Barcelona, Spain.

*mqueralt@alumni.unav.es, Telelphone: +34 948 42 56 00

Keywords: *Tuber melanosporum,* mycorrhizas, real-time PCR, extraradical mycelium.

Black truffle (*Tuber melanosporum*) is one of the most highly prized edible fungi of the world. In the 90s, many holm-oak plants inoculated with black truffles were established in Spain. However, many of these plantations have not obtained the expected production, despite showing a burned area around the trees and truffle mycorrhizae in the roots. Although many scientific papers have reported the persistence of *Tuber melanosporum* mycorrhiza or the quantification of the extraradical mycelium in plantations, there is no work relating the production of truffles, the dominance of their mycorrhizae and the amount of mycelium.

In order to understand the relationship between these parameters we designed a study in a plantation located in Eraul (Navarra, Spain) and established in 2000. We analysed 20 mycorrhized holm-oaks with a recorded production of sporocarps in the 2013-2014 season. For the assessment of mycorrhizal dominance, four soil cores were taken around each tree from the upper soil layer (0-30cm). The mycorrhizae were separated by morphotypes and then identified using DNA sequencing. Quantification of extraradical mycelium was carried out from the same soil samples using molecular techniques based on Real-Time PCR. In October 2013 the first samples were obtained. Sampling will be repeated in January, April and July of 2014, with the purpose of knowing the variation of mycelium and mycorrhiza dominance over a year. The relationships between these parameters will be further analysed.

OP 1.3

THE HYPOTHETICAL "SAPROTROFIC PHASE" OF THE TUBER MELANOSPORUM MYCELIUM SHOULD BE PERMANENTLY BURIED

Osvaldo Zarivi, Antonella Bonfigli, Sabrina Colafarina , Pierpaolo Aimola,Anna Maria Ragnelli, Giovanni Pacioni, Michele Miranda*

Dept. of Life, Health and Environmental Sciences, University of L'Aquila, 67100 L'Aquila, Italy

* michele.miranda@univaq.it, +390862433264

Keywords: truffle nutrition, phenolic compounds, mycorrhiza

Since the Renaissance it has been hypothesized that truffles can grow on plant material rich in organic matter. Idea revived several times in the course of time, with the Boulanger's proposal to cultivate the "blanche de la truffe" such as *Agaricus bisporus* and in time much more recent with the theory of a saprotrophic phase of the *Tuber melanosporum* mycelium that would feed at the expense of polyphenols, such as tannins and lignin. Hence the suggestion to fertilize truffle orchards with plant material rich in phenols and prepare mycorrhizal seedlings on phenolic substrates.

The study of the genome of *Tuber melanosporum* (2010) has cast doubt on the possibility that its mycelium was able to implement this type of metabolism for lack of a suitable package of degradative enzymes of phenols. By using a 13C Pulse-Labeling Technique, Le Tacon et al. (2013)were able to demonstrate that the only way of carbon transfer is that from the host plant to Tuber melanosporum mycorrhizas and ascocarps.

Zarivi et al. (2013) investigating the transcriptional, biochemical and histochemical of laccases expression during T. melanosporum development have added a further element against the hypothesis of saprotrophic phase. Laccases are the main enzymes for polyphenol digestion. The cDNAs of Tuber melanosporum laccases (Tmellcc1 and Tmellcc2) have been cloned. From the cloned cDNAs probes were prepared to investigate the expression levels of the Tmellcc1 and Tmellcc2 genes in the free living mycelium (FLM), ectomycorrhizas (ECM) and different developmental stages of fruit body (FB) by quantitative PCR (qPCR). The mRNA expression levels agree with the changes of laccase activities. The histochemical data agree with the qPCR and biochemical results. The highest laccase expression occurs in the ECM, when the host plant roots are invaded by the fungal mycelium, but not reaching the levels of expression such as to justify a nutritional degradation of soil polyphenols by its free living mycelium.

OP 1.4

INTEGRATED MANAGEMENT AND SUSTAINABLE CULTIVATION OF THE VALUABLE TRUFFLE SPECIES TUBER BORCHII VITTAD.

João Trovão[1]; Marcos Morcillo*[2]; Maria Bagulho[3]; Helena Freitas[1]; Anabela Marisa Azul[1]**

[(1)]Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, 3001-401 Coimbra, Portugal

[(2)]Micologia Forestal & Aplicada, Rbla. Arnau 6 local D, Vilanova i la Geltrú, 08800 Barcelona, Spain

[(3)]Micoflora, SA Centro de Empresas, Edifício Clube Náutico, 7580-509 Alcácer do Sal, Portugal

*marcosmorcillo@micofora.com, Telelphone: +34 938155455

**amjrazul@ci.uc.pt, Telelphone: +351239 855 210

Keywords: *Tuber borchii*, sustainable cultivation of truffles, communities processes above/below-ground, Mediterranean forests.

Fruitbodies produced by *Tuber borchii* Vittad., also known as bianchetto truffles, has a broad distribution [1, 2] and its farming in non-endemic areas has being expanding worldwide.

We combined detailed experiments regarding truffle cultivation practices with multiple below and aboveground surveys to investigate the influence of integrated management strategies on *T. borchii* establishment, plant host establishment and performance, and on local plant distribution and soil processes through time. The study was conducted in the first two field trials of *T. borchii* in Portugal (Alentejo region), established in spring 2010 and 2011, in areas dominated by *Quercus suber* L. (cork oak; Qs) and *Pinus pinea* L. (stone pine; Pp). We used morphotyping and molecular tools to assess colonization progress of *T. borchii* and ECM fungal community below-ground [3].

Seasonal surveys attest that *T. borchii* have ample spectrum of host age and habitats. We observed that *T. borchii* exposed ability to disperse both from inoculated plantlets of Pp introduced in the field, and from adult trees of Qs and Pp inoculated of with pellets of the fungus. Cultivation practices involving the pH correction did not substantiate putative benefits in *T. borchii* establishment through time. The suppression of autochthonous vegetation did contribute to decrease the competition of *T. borchii* in relation to ECM fungal community, but may contributed to the establishment of *Thelephoraceae* and the species *Scleroderma meridionale* Demoulin & Malençon on roots. In contrast to the majority of ECM fungal species below-ground, the vitality of *T. borchii* ECM was more pronounced in winter but also during summer.

We conclude that traits of *T. borchii* may result in putative advantage to occupy different habitats and co-habit with ECM fungal community in Mediterranean forests. Integrated management and sustainable cultivation of *T. borchii*, underlying direct and indirect impacts of cultivation practices in soil functions and sustainability of forests are discussed.

[1] Urban A et al, *acte du colloque, brive la gaillarde*. Fevrier**2**(2008):19–34.

[2] Azul AM et al, *Mycorrhiza* **20**(2010):73-88.

[3] Suz LM et al, Soil Biology, Springer-Verlag, Berlin-Heidelberg, Vol 15 (2008) 437-474.

OP 1.5

LOW SUMMER SOIL TEMPERATURE AND MOISTURE FAVOURS ROOT TIP COLONIZATION OF QUERCUS ILEX BY TUBER MELANOSPORUM

Antoni Olivera[(1)]*, **José A. Bonet**[(1,2)], **Daniel Oliach**[(2)], **Carlos Colinas**[(1,2)]

[(1)] *University of Lleida-Agrotecnio Center (UdL-Agrotecnio)*

[(2)] *Forest Technology Centre of Catalonia (CTFC)*

*antoni.olivera@pvcf.udl.cat, Telephone: +34 679238208

Keywords: ectomycorrhizae, holm oak, black truffle, soil temperature, soil moisture

The development of the ectomycorrhizal symbiosis between young *Quercus ilex* seedlings and *Tuber melanosporum* often depends on the environmental conditions under which it occurs. Our purpose was to determine whether the formation of mycorrhizas between *T. melanosporum* and *Q. ilex* depended on soil temperature and moisture regimes during the warm season. We used a two-factorial combination of soil temperatures and moistures with five replications. *Q. ilex* seedlings inoculated with *T. melanosporum* were planted in 18.2-liter containers filled with soil extracted from a wild truffle bed mixed with perlite. After four months, the seedlings were extracted to determine the amounts of root tips colonized by *T. melanosporum,* uncolonized, or colonized with other competing ectomycorrhizal fungi. No interaction was observed between soil temperature and moisture on the amount of *T. melanosporum* ectomycorrhizas per seedling. Containers with cooler temperatures were the most favourable to the formation of *T. melanosporum* mycorrhizae, as well as those with medium-low soil moisture. High soil moisture increased the capacity of competitor fungi to form mycorrhizas, regardless of soil temperature. According to our results, strategies to limit substrate temperatures should be implemented in nurseries or when establishing truffle orchards in particularly warm sites. Irrigation should avoid keeping soil moisture at or above field capacity for extended periods.

OP 1.6

BIODIVERSITY AND CONSERVATION OF DESERT TRUFFLES IN SAUDI ARABIA

Mohammed Amin Uddin Mridha [(1), *], **Abdul Aziz Al-Qarawi** [(1)] **and Fahad Nasser Ibrahim Al-Barakah** [(2)]

[(1)] *Plant Production Department, and* [(2)] *Soil Science Department, Faculty of Food and Agricultural Sciences, King Saud University, P. O. Box. 2460 Riyadh 11451, Saudi Arabia.*

*mridha52@gmail.com ; Telephone: +966532057761

Keywords: Biodiversity, Conservation, Desert Truffles, Saudi Arabia

Desert truffles are available in certain areas of Saudi Arabia and they are being used in the Arabia Gulf countries for both food and medicine for centuries. These delicious wild mushrooms are growing under certain weather conditions. Although these naturally occurring mushroom are very valuable in relation to the restoration of their biodiversity and their conservation, but very little research activities are conducted in Saudi Arabia. Systematic field survey to identify truffle growing areas throughout the country and to have indigenous knowledge regarding the occurrence of truffles and their distribution, habitat, host range etc are required for baseline information to developed sound research program on desert truffles in the country is needed. The physical, chemical and biological properties of the soil to understand the population structure of the truffles is also needed to support the biodiversity and conservation of the fungi. Conventional taxonomic identification and the molecular identification of the truffles are very important to catalogue the diversity of desert truffles in Saudi Arabia. Awareness development among the stakeholders is necessary for restoration and sustainable use of these naturally occurring fungi. Therefore, this paper will outline the importance of survey as well as identification of the truffles and also to develop methods of how to conserve the truffles in Saudi Arabia. We will also consider the impact of desert truffle on Saudi society and economy.

OP 1.7

TRUFFLE CULTIVATION IN GERMANY – CURRENT SITUATION AND PROSPECTS

Ulrich Stobbe[1,2],*, **Ludger Sproll**[1]

[1] *Deutsche Trüffelbäume ,Ludger Sproll, Ulrich Stobbe, Bernd Sproll GbR 78315 Radolfzell, Karl-Bücheler Str. 1, Germany*

[2] *Institute of Forest Botany and Tree Physiology, University of Freiburg, 79085 Freiburg, Germany*

*ulrich.stobbe@fobot.uni-freiburg.de, Telelphone: +49 761 203 3655

Keywords: truffle cultivation, Tuber aestivum, ecological requirements

Truffles (*Tuber* spp.) are subterranean fruit bodies of ectomycorrhizal fungi associated with host plants including oak, beech and hazel. In Germany all *Tuber* spp. are classified as Very Rare or Extinct on the national Red Lists while historical literature described their sporadic existence. Recent studies revealed great abundance of the Burgundy truffle (*T. aestivum*) and suggest ample cultivation potential in suitable habitats. In this talk we present the current status of newly emerged truffle cultivation in Germany, along with particular ecological requirements and the prevailing legal situation. We describe the development of plants and mycorrhizal status of three experimental plantations, which are monitored since their establishment in the year 2011. Preliminary results show a high persistence of *T. aestivum* mycorrhiza in the fine root systems, and indicate good potential for future harvests.

OP 1.8

THE CULTIVATION OF TRUFFLES IN THE SOUTHERN HEMISPHERE – THE PROBLEMS AND THE POTENTIAL

Ian Robert Hall(1),*, **Alessandra Zambonelli**(2)

(1) *Truffles and Mushrooms (Research) Limited and Edible Forest Fungi New Zealand Limited, P.O. Box 268, Dunedin 9054, New Zealand*

(2) *Dipartimento di Scienze Agrarie, Università di Bologna, via Fanin 46, 40126 Bologna, Italy*

*truffle1@ihug.co.nz, Telephone: +64 3 454 3574

Keywords: cultivation, truffle, *Tuber melanosporum*, *Tuber borchii*, *Tuber aestivum*, Southern Hemisphere, New Zealand, methods, problems, potential

It has been 30 years since the start of research on the cultivation of truffles in the Southern Hemisphere, although the idea had been formulated 5 years before that at a conference in Colorado. The methods used to produce plants were eventually found to be little different from those that had been developed in Europe in the 1970s. However, arguably the most important development were the procedures used to modify soil pH and render acidic soils suitable for truffle cultivation. About the same time as the Colorado conference, Francois Picart in California, and later Texas, began selling *T. melanosporum* plants imported from Agri Truffe in France [1]. Some of these were planted in areas much hotter in summer than the traditional areas of France, Italy and Spain, while in New Zealand, and later Australia, plants were grown in areas warmer in winter than anywhere in France, Italy and Spain [2, 3]. The successful production of truffles in acidic soils and areas warmer in winter or summer has significantly expanded the areas that might be used to grow truffles in New Zealand, Australia, Chile, Argentina, South Africa and Uruguay. The knowledge gained from the Southern Hemisphere can now also be applied in non-traditional Northern Hemisphere countries [4].

With the new methodologies came new problems such as trace element deficiencies caused by the application of the very large quantities of lime to some soils, truffle rot in zones with warm winters such as the Western Australia and Bay of Plenty in New Zealand, and oversupply to an unsuspecting and unprimed market. This presentation will review the development of the *T. melanosporum*, *T. borchii* and *T. aestivum* industry in the Southern Hemisphere and the problems encountered since its inception in New Zealand, its spread to Australia in the early 1990s, and later to South America and South Africa.

[1] Picart, F. 1980. Truffle: The Black Diamond. Agri-Truffle, Santa Rosa, California.

[2] Hall, I.R.; Brown, G.; Zambonelli, A. 2007. Taming the truffle: the history, lore, and science of the ultimate mushroom. Portland, Timber Press. 304 p.

[3] Hall, I.R.; Frith, A.; Haslam, W. 2008. A rough climatic comparison of various centres adjacent to or with similar climates to Périgord black, Italian white, Burgundy and bianchetto truffle producing areas. Dunedin, Truffles & Mushrooms Ltd. http://www.trufflesandmushrooms.co.nz/free%20downloads.html

[4] Hall, I.R.; Zambonelli, A. 2012. The cultivation of mycorrhizal mushrooms - still the next frontier! In: J. Zhang, H Wang & M Chen (Eds). Mushroom Science XVIII. Beijing, China Agricultural Press. Pp 16-27.

OP 2.1

HOW MANY FILAMENTOUS FUNGI DO A TRUFFLE?

Simona Ascione, Marco Leonardi, Osvaldo Zarivi, Giovanni Pacioni*

Dept. of Life, Health and Environmental Sciences, University of L'Aquila, 67100 L'Aquila, Italy

* giovanni.pacioni@univaq.it, Telephone: +390862433209

Truffles should not be seen as merely the subterranean fruiting bodies produced by fungal species of the genus Tuber (Ascomycotina), but as microhabitats whose microbiome is quantitatively significant. In fact, by molecular methods it was estimated that in one gram of gleba there are 10 billion of bacteria sl and tens and tens thousand yeasts. Some of these fungicolous microorganisms has been shown that they can inhibit the process of mycorrhization or contribute to the production of volatile sulfur organic compounds. The discovery that also there are filamentous fungi , "hyphomycetes" (Pacioni et al. 2007) inhabiting Tuber ascoma poses the problem of what their function within the truffles.

With this research we wanted to ascertain which species are statistically more spread and how is their quantity inside of truffles. This assessment was conducted with both the isolation of mycelia in culture and molecular methods. We also tried to perform metagenomic analysis directly on truffles, but without success likely due to the huge difference between the amount of Tuber DNA and that of its fungal guests. There are probably 10 billion copies of Tuber DNA vs some hundreds of copies of the different fungicolous species. It has been therefore necessary to use a different molecular approach.

The best strategy seems to be the qPCR real-time. All ITS present in the sample of DNA extracted from a single truffle were amplified with generic primers ITS1-ITS4. Then using specific primers designed for all fungi contaminants, previously isolated in culture, evidence of their presence has been searched within the pool of amplicons of the ITS. And in order to ensure that the fungus detected was that obtained in culture, a sequencing of the amplified obtained in the real-time PCR was performed.

The results of this investigation that contributes to the understanding of the microbiome of the truffle are finally presented and discussed.

OP 2.2

HYPHAL GROWTH AND LIFE CYCLE IN TRUFFLE: NEW RESEARCH PERSPECTIVES AND APPLICATIONS IN THE FIELD

Antonella Amicucci(1)*, Valentina Sparvoli(1), Cinzia Calcabrini(1), Francesco Palma(1), Alessandra Zambonelli(2), Vilberto Stocchi(1)

(1)Department of Biomolecular Science, University of Urbin Carlo Bo, Via Saffi 2, 61029, Urbino (PU), Italy

(2) Dipartimento di Scienze Agrarie, University of Bologna, via Fanin 46, 40127 Bologna, Italy

*antonella.amicucci@uniurb.it, Telephone: +39 0722 305232

Truffles have a complex life cycle involving mycelium reorganization in specialized structures (mycorrhiza and fruiting body), whose accomplishment is affected by endogenous factors as well as biotic and abiotic features.

A better understanding of the complicated morpho-functional, metabolic and gene expression changes in hyphal growth induced by environmental factors is fundamental for trying to modulate truffle life cycle in order to promote mycorrhiza formation and fructification.

Regarding the genes involved in these mechanisms, thanks to the information made available by the recent sequencing of *T. melanosporum* genome, we have identified a large number of proteins involved in these processes, and 149 genes were identified and functionally grouped according to the deduced amino acid sequences [2], so going to describe the hypothetical metabolic pathway.

Furthermore, assuming that the hyphal growth involves the continuous construction of new membranes, we began a study of a protein involved in lipid metabolism. In particular, the gene sequence encoding a perilipin-like protein was characterized in the three main species of *Tuber*, *T. melanosporum*, *T. borchii* and *T. aestivum*, with the objectives to evaluate its role in lipid metabolism, hyphal growth and mycorhization in *Tuber* spp. In fact, recent studies conducted on the ascomycetous *Metarhizium anisopliae*, highlighted that this protein is necessary also for the infection apparatus functionality [3].

Moreover, among the several factors that can influence the phenomenon of polarized hyphal growth, carbohydrates [4] and root exudates [5] were studied, highlighting their positive effect on the development of *T. borchii*.

In conclusion, in the present study, we present a summary of our research carried out on this topic, but many aspects remain to be elucidated in the future, because, certainly, there are many other factors that can influence hyphal growth, mycorrhizae and carpophores development, not yet studied. The knowledge of these mechanisms could provide us new tools to improve cultivation conditions and develop new mycorrhization techniques.

[1] Büntgen U. et al 2012 Nature Climate Change, 2, 827–829

[2] Amicucci A. et al 2012 Fungal Genomics and Biology, 48, 561-572

[3] Wang C. and ST. Leger R. J. 2007 Journal of Biological Chemistry, 282: 21110-21115

[4] Amicucci A. et al 2010 Journal of Molecular Microbiology and Biotechnology, 18, 120-128

[5] Menotta M. et al 2004 CurrentGenetics, 46, 158-165

OP 2.3

HETEROTHALLISM IN *TUBERMELANOSPORUM* AND *TUBER INDICUM*: WARNING AND LESSONS FROM GENOMICS TO PRESERVE IDENTITY AND PROMOTE FRUCTIFICATION OF THE PÉRIGORD TRUFFLE

Andrea Rubini, Beatrice Belfiori , Claudia Riccioni ,Francesco Paolocci*

CNR Institute of Biosciences and Bioresources –Division of Perugia-Via Madonna Alta, 06128 Perugia Italy

*francesco.paolocci@ibbr.cnr.it, Telelphone: +39 075 5014861

Keywords: competition, mating type, black truffles, mycorrhiza, phylogenesis, plant inoculation, *MAT* genes

The last several years have witnessed a leap forward in our understanding of the life cycle and the reproductive biology of truffles [1]. The sequencing of *Tuber melanosporum* genome has permitted the identification of the *MAT* locus, which is the genomic region controlling the reproduction mode of fungi [2]. Since *T. melanosporum* strains harbor either the *MAT1-1-1* or *MAT 1-2-1* gene at this locus, we concluded that this fungus is heterothallic [3,4]. The availability of sequence information for *T. melanosporum MAT* genes gives way to cloning their orthologs in other truffle species. Among these, the Asiatic species *T. indicum* is the closest relative of *T. melanosporum*, and produces black truffles of inferior value with respect to the Périgord truffle. The high rate of polymorphism at morphological and molecular traits within black truffles from Asia prompted several authors to claim the presence of several other species in addition to *T. indicum* [5].

Here, benefiting from the information and genomic tools relative to *T. melanosporum MAT* genes, we aimed to: a) corroborate the occurrence of a mating type dependent spatial segregation of *T. melanosporum* strains on host plants and b) clone the orthologs of these genes from *T. indicum.*

With respect to the first point we collected ectomycorrhizas and soil samples from four *T. melanosporum* grounds located in central Italy and screened them with *MAT* specific primer pairs. These results lend further support to our contention of a spatial segregation, likely due to a competitive exclusion between genotypes, of strains of opposite mating type on their hosts [1,6-8].

Concerning *T. indicum,* here we show that it is also a heterothallic species since its *MAT* locus is organized in two idiomorphs harbored by different strains [9]. By virtue of their high evolutionary rates, the *MAT* genes are good candidates for taxonomic and phylogenetic analyses [10]. On these grounds, the comparative analyses of *MAT* locus from several Chinese specimen point to the presence of at least two cryptic species within the *T. indicum* complex and support the hypothesis of vicariance for explaining the allopatric isolation of *T. melanosporum* from *T. indicum* populations in Europe and Asia, respectively. Notwithstanding, all *T. indicum MAT1-1-1* and *MAT1-1-2* genes show a high rate of similarity with their orthologs from *T. melanosporum*.

The evidence that *T. indicum* is not only the closest relative to *T. melanosporum,* but it is also heterothallic is pragmatically very relevant. Because *T. indicum* and *T. melanosporum* could be sexually compatible, the deliberate or accidental introduction of *T. indicum* in Europe may represent a serious ecological threat for the indigenous populations of *T. melanosporum*. Tools and strategies that span from innovative host plant mycorrhization techniques to methods for certifying species identity and mating type of truffle strains will be discussed.These instruments are fundamental to preserve *T. melanosporum* biodiversity from erosion and counteract gamete limitation that likely occurs in *T. melanosporum* stands, as a important consequence of the spatial segregation of strains with different mating types.

[1] A. Rubini, C. Riccioni, B. Belfiori, F. Paolocci, *Mycorrhiza* (2014) DOI: 10.1007/s00572-013-0551-6.

[2] J.A. Fraser, J. Heitman, *Current Biology* (2003) 13: R792-R795.

[3] F. Martin, A. Kohler, C. Murat et al. *Nature* (2010) 464:1033-1038.

[4] A. Rubini, B. Belfiori, C. Riccioni, E. Tisserant, S. Arcioni, F. Martin, F. Paolocci, *New Phytologist* (2011) 189:710-722.

[5] J. Chen, S. Guo, P.G. Liu, *PloS One* (2011) 6:e14625

[6] A. Rubini, B. Belfiori, C. Riccioni, S. Arcioni, F. Martin, F. Paolocci, *New Phytologist* (2011) 189:723-735.

[7] C. Murat, A. Rubini, C. Riccioni, H. De la Varga, E. Akroume, B. Belfiori, M. Guaragno, F. Le Tacon, C. Robin, F., Halkett, F. Martin. F. Paolocci, *New Phytologist* (2013) 199:176-187;

[8] M.A. Selosse, E. Taschen, T. Giraud, *New Phytologist* (2013) 199:10-13;

[9] B. Belfiori, C. Riccioni, F. Paolocci, A. Rubini, *Plos One* (2013) 8(12): e82353. doi:10.1371/journal.pone.0082353

[10] B.G. Turgeon, *Annual Review of Phytopathology* (1998) 36:115–137)

OP 3.1

THE QUALITY OF COMMERCIAL TRUFFLE FLAVORED OILS ASSESSED BY MEANS OF GC–MS AND ELECTRONIC NOSEW

Lorenzo Cerretani (1), **Angelo Cichelli** (2), **Giuseppe Procida** (3), **Giovanni Pacioni** (4)*

(1) *Pizzoli R&S, Via Zenzalino Nord 1, I-40054 Budrio (BO), Italy*

(3) *Dipartimento di Scienze Chimiche e Farmaceutiche, Università di Trieste, Via Giogieri 1, I-34127 Trieste, Italy*

(2) *DEC, Lab. Merceologico, Università G. d'Annunzio Chieti-Pescara, Viale Pindaro 42, I-65127 Pescara, Italy*

(4) *Dept.MeSVA, Università dell'Aquila, I-67010 Coppito-L'Aquila, Italy*

* giovanni.pacioni@univaq.it, +390862433209

Keywords: Truffles, Olive oil, Flavor, Gas chromatography–mass spectrometer, Head space, Electronic olfactory system.

Truffles are among the most expensive foods and their quality depends on their unique aroma, composed of complex mixtures of lipophilic volatile organic compounds (VOCs). There are many foods flavored with truffle, and oils are particularly common. Using Dynamic Head Space-Gas Chromatography interfaced with a Mass Spectrometer (DHS–GC–MS) and an electronic olfactory system equipped with 6 metal oxide sensors (MOS), 18 samples of olive oil flavored with white and black truffles from the Italian market were subjected to a blind analysis.

Qualitative and quantitative analysis with DHS–GC–MS detected the presence of 63 VOCs, 32 of which can be attributed to olive oil, also defective, and 19 to truffles, while 12 foreign compounds are of dubious origin (synthesis and/or demolition). The data obtained with the e-nose MOS, processed statistically, was able to discriminate the aromas coincident with the three species of truffle declared on the label (the white truffle *Tuber magnatum* and the black truffles *Tuber melanosporum* and *Tuber aestivum*), demonstrating the potential and reliability of this technique, confirming the established malpractice of the use of bismethyl(dithio)methane in black truffles flavorings. In particular, this study showed a higher specificity of different sensors for the three species of truffle:

- sensor number 5 was the most sensitive for the flavored oil obtained with *Tuber melanosporum;*
- sensor numbers 1, 2 and 6 showed a higher specificity for *Tuber magnatum* flavored oil;
- sensor number 3 was the most important in the separation of oil flavored with *Tuber aestivum*.

Wecanthereforeassumethatthe MOSsensorshaveaqualitativesensitivity,and notquantitative, and as a consequence could be "deceived" by the use of synthetic flavors characterised by specific aromas.

On the other hand, the results of DHS–GC–MS allow evaluation of the complexity of flavor determined by the use of real truffles rather than synthetic aromas.

OP 3.2

QUALITY ASPECTS OF THE AUSTRALIAN BLACK TRUFFLE (*T. MELANOSPORUM*)

Garry S.H. Lee(1),*, Kenny Sing Ong Choo(1), Sophiana Benali(2)

(1) *Centre for Forensic Science (M420), University of Western Australia, 35 Stirling Highway, Crawley, WA, 6009, Australia.*

(2) *Université de Brest, 3 Rue des Archives - CS 93837 - 29238 BREST Cedex 3, France*

*Garry.Lee@uwa.edu.au, Telelphone: + 618 64882561

Keywords: Quality, Volatile Aroma, Black Truffle, Sensory

The volatile organic compound profiles of black truffles are distinctive, contributing to the different and unique flavour experiences associated with each truffle. In previous studies, systematic analyses of truffles from Italy and France using gas chromatography mass spectrometric (GCMS) techniques identified some of the important odour active volatiles and provided valuable insights into the chemical basis of perceived flavour differences between truffles of different varieties, cultivars and even from different regions.

Besides the culinary interest, knowledge of the key impact volatiles and their concentrations can be used as a measure of truffle quality. Presently truffle aroma quality is conducted by persons experienced in truffle harvesting. This system is prone to errors, subjectivity and favouritism. Development of a scale underpinned by science will lead to a higher value product.

The Australian Truffle Growers Association (ATGA) has developed a grading standard for Australian truffles based on standards used in Europe. Currently the standard has no provision for aroma as the definition of aromas for each grade of truffle is not known. The ATGA would like to extend their grading standard to include aroma to eliminate errors and ensure that Australian truffles for export are of the highest quality. Because the volatile profiles of truffles vary widely depending on growing and ripening patterns, region of origin and horticultural practices, it is necessary to have accurate data regarding both the chemistry and sensory properties of the truffle and also temporal changes in the concentration of volatiles emitted by the whole undamaged truffle during its growing period. There is a paucity of data in the public domain literature regarding volatile emissions from truffles in relation to quality; most published studies focus on either developing a system for locating truffles or for flavour recreation purposes.

Here we present a combined sensory and chemistry study of the volatiles released during the life span of Australian Black Truffles harvested from 12 growing regions across Australia. The study was conducted over 3 years to account for seasonal differences and investigated truffles from their immature stage through to the onset of rot. A panel of expert handlers as well as Australia top Chefs provided descriptors of high, medium and low quality truffles as defined by the Australian Standard. Chemical spectroscopy techniques including solid phase micro-extraction GCMS, gas chromatography Olfactometry MS and real time volatile detection systems (selected ion flow tube MS) were used to correlate the sensory descriptors to the volatiles.

In total 75 volatile compounds were identified in mature *T. Melanosporum* grown in Australia. Although seasonal differences in the aroma profiles were observed, these were minor compared to regional differences. However, even allowing for these differences, an envelope of 13 major volatiles was identified as characteristic of *T. Melanosporum* grown in Australia. These included formic acid esters which have only been identified as minor components of truffle odour in other parts of the world. Using this information, and the trends observed in the volatile profiles during aging of the truffle, a quality scale based on aroma has been suggested.

OP 4.1

A PATENTED EDIBLE FILM TO IMPROVE THE FRESH TRUFFLE SHELF-LIFE

Enrico Stagnini, Anna Maria Ragnelli, Giovanni Pacioni*

Dept. of Life, Health and Environmental Sciences, University of L'Aquila, 67100 L'Aquila, Italy

* giovanni.pacioni@univaq.it, +390862433209

Keywords: coating, protein film, food quality, conservation.

Truffles, probably the most expensive food in the world, are underground mushrooms which, after harvesting, switch from a microaerobic and protective environment, like the soil, to the air. Because of this stress, they suffer a series of metabolic changes due to both a higher oxygen presence and water loss, becoming extremely perishable with a very short postharvest shelf life. Aromas change, the vital activities are interrupted due to the dehydration or, when they are kept in closed containers, the putrefaction processes start, due to the rich microflora inside which takes over. In order to preserve the vitality and the organoleptic qualities of truffles, a composite edible film has been developed and patented. The formulation of the edible film complies with the regulations given by the EFSA (European Food Safety Authority) on food additives. Colorless and tasteless, based on a specifically refined zein , the film is prepared in water-alcohol solution and it is sprayed on the clean surface of truffles creating a thin coating. The edible film has the triple purpose of slowing the loss of water, of maintaining the characteristic flavors of truffles, by acting as a physical and chemical barrier against VOCs emission and of abating the growth of mold on the peridium surface. Fresh truffles are currently stored in many ways: principally limiting the contact with oxygen and maintain them in a refrigerator at 4-8 ° C; these methods allow a conservation of 10-20 days with a modified flavor. Instead, the fresh truffles treated with the film, can be stored up for more than twice the time allowed so far by other systems hitherto developed. By means of SPME- GCGM analysis, a lower loss of VOCs in treated portions of a same truffle, compared with the portions not treated, was measured. Microbiological tests were performed in order to quantify the microbial population present in the samples treated and untreated with film. The analysis was conducted using analytical methods in accordance with the requirements of the ISO (International Standard Organization), for the control of the microbiological quality of food. The results both after a storage period of 20 days to 40, show an inhibiting action the development of molds and a modest growth of bacterial flora. These evidences give indications about the possibility of extending the shelf life of the product.

ORAL PRESENTATION - SPONSOR COLLABORATION

OPS 4.1

COMPANY LAUMONT S.L. – ESTABLISHMENT, DEVELOPMENT AND LEADERSHIP IN TRUFFLE BUSINESS EITHER FRESH OR PRESERVED

Jordi Serentill,

Deputy CEO Laumont S.L, Tàrrega, LLeida, Spain

Jordi.serentill@laumont.es Telephone: +34 620 905 036

Keywords: fresh sales, know-how, carefully cleaned, observed by several criteria, ISO22000, market leader, delivering best quality.

Established in 1980, company Laumont S.L. implements careful selection and professional manufacturing to supply the end client with the perfect truffle, truffle's products, wild or cultivated mushrooms. Through multiple innovations for the past 34 years the firm establishes a connection between all products from its wide range from rural areas and the final customer. Carefully selected personnel with know-how technology innovations, which follow the latest trends in the industry and under the very strict criteria of food security certificate ISO 22000 obtained in 2012, it has established itself as global market leader regarding to mushrooms and truffles.

This entire chain starts with the harvesting of the highest quality fresh truffles from more than 300 regular suppliers. After receiving the truffles, they are carefully cleaned and selected piece by piece from highly trained staff with over 20 years of knowledge and experience. For each harvesting region are observed several criteria with accuracy to within one gram, such as - the percentage of soil, pieces of truffles, whole above and below a weight of 15 grams, and many others. Based on these data is built in-house evaluation of each region of collection and each provider through which we ensure that the work with them is delivering the best quality raw material that could be found. In average 50% of the truffles go to our production site and 50% are sold as fresh product.

Realization of fresh sales is repeatedly larger and more global compared to 1980's. We are exporting our products to 4 continents and looking for the best partners worldwide, traveling and attending food fairs, to touch the different cultures and to understand exactly what kind of products are the most valuated in each country. The orders of our top customers are processed with individual particularly attention always by the same specialists. This way we insure ourselves that they will be satisfied with their purchase every time. With no less importance for us is the realization of our wide range of products from Tuber Melanosporum. Truffle produced in three different lines - home, professional and industrial - to satisfy even the most pretentious customers.

Company Laumont S.L. and its dynamic and professional team relentlessly look to the future for each potential opportunity for growth and even greater success. Possibilities for increasing production are carefully analyzed, together with future challenges that are currently beyond our scope, such as improved taste, increased durability and fine cuisine.

OPS 4.2

THE FUTURE OF THE TRUFFLES, A PROJECTION OF THE PRESENT

Ismael Ferrer[1,2]**, Mario Cequier**[2]*

[1] *Caspe Escola de Hosteleria , Zaragoza, Spain*

[2] *Trufapasión, S.C Calle San Juan 30, 22423, Estadilla, Huesca, Spain*

*descubre@trufapassion.com, Telephone: +34 649838715

Keywords: trufficulture, truffle product-makers, fresh truffle handling, truffle traceability

TRUFAPASION S.C. is a company dedicated to on-line sales and is located in Estadilla, Huesca, Spain. As part of our service we aim to bring the costumers closer to the passionate world of truffles. We are trufficulturers and truffle product-makers; we offer to costumers an endless range of proposals with the aim of fully enjoying truffles. So, we present tastings (fresh truffles, rice with truffle, truffle pâté, truffle-infused oil, honey, onion jam or truffle liqueur), lectures about the world of truffles (topics such as the handling and care of truffles as they grow or how to use truffles in the kitchen) and trips (truffle orchards, truffle nurseries, fairs in Spain and throughout Europe).

The aim of our presentation is to discuss how truffle is handling nowadays and show the principal aspects of quality in a fresh truffle, in order to establish the state of the art of the truffle quality in our markets and to improve traceability in the truffle sector and authenticity in fresh truffle and its elaborated products.

POSTERS

P 1.1

PRODUCTION OF HIGH QUALITY TRUFFLE PLANTS UNDER ISO 9001 QUALITY LABEL AND THEIR PERFORMANCES IN EDIBLE MUSHROOM ORCHARD®

Robin Bruno, Robin Max, Cammalletti Pierre

ROBIN Pépinières EARL 05500 Saint Laurent du Cros France

info@robinpepinieres.com

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

The ROBIN tree nursery company is involved in large scale production of forest and 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 related to three main goals: reforestation purpose, selected mycorrhizal seedlings for phytoremediation of contaminated area, and establishment of EDIBLE MUSHROOM ORCHARD®

A staff specially trained for mycorrhizal studies manages all steps of production of truffle plants from controls and selections of fruit bodies to final control before delivery 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 using selected batches of seeds and truffles supplied by the customers. Along the growing season the development of the mycorrhizal association is regularly followed. At the end of this season each batch of the production is sampled. Each sample done is observed under stereoscopic microscope 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 focusing on the good distribution of the mycorrhizas of the inoculated truffle and the amount of these mycorrhizas and on the lack of other contaminant fungus along the root system.

Within the framework of European program VERCHAMP®, numerous EDIBLE MUSHROOM ORCHARD® have been set up during the years 2005-2006 in various ecological condition using truffle plants and also plants mycorrhized with *Lactarius* and *Suillus luteus* edible species. These orchards have been followed since their plantations by the Chambre d'agriculture des Hautes Alpes.

Numerous orchards set produce yet truffles and other fungi. The first plants mycorrhizal with *Tuber magnatum* and controlled by molecular tools, have been planted out since 2006 in many locations in France and in Italy. These orchards have been followed using bio molecular tools until their first production. The orchards set up with tree CHAMPION®) give earlier and regular production in many locations in comparison with the normal trees set up in the same time. 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.

P 1.2

SPATIAL-TEMPORAL DYNAMIC OF *TUBER MAGNATUM* MYCELIUM IN NATURAL TRUFFLE GROUNDS

Mirco Iotti (1), Marco Leonardi (2), Enrico Lancellotti (3), Elena Salerni E.(4), Marco Leonardi (1), Giovanni Pacioni 2), Alessandra Zambonelli (1) *

(1) *Dipartimento di Scienze Agrarie, University of Bologna, via Fanin 46, 40127 Bologna, Italy*

(2) *Department of Life, Health and Environmental Sciences, University of Aquila, via Vetoio, Coppito, 67100 L'Aquila, Italy.*

(3) *ipartimento di Agraria, University of Sassari, via A. De Nicola 1, 07100 Sassari, Italy*

(4) *BIOCONNET, BIOdiversity and CONservation NETwork, Department of Environmental Science "G. Sarfatti", University of Siena, via Mattioli 4, 53100 Siena, Italy.*

*alessandr.zambonelli@unibo.it, Telelphone: +39 051 2096579

Keywords: Italian white truffle, real time PCR, mycelium, soil

Tuber magnatum Pico (the Italian white truffle) is the world's most expensive truffle. This truffle, in contrast to the other commercial Tuber species, produces very rare mycorrhizas which are difficult or even impossible to detect in the field. Recently, a real-time PCR assay has been also developed to quantify and to track *T. magnatum* mycelium in soil (Iotti et al., 2012). This technique was proved to be useful to assess the effect of soil tillage on *T. magnatum* in natural truffières (Salerni et al., 2013).With the aim to improve the knowledge on *T. magnatum* soil ecology, this technique was used to study the spatial distribution of *T. magnatum* mycelium in productive patches and its dynamic across seasons.

This study was carried out in four different natural *T. magnatum* truffières located in different Italian regions (Emilia Romagna, Tuscany, Abruzzo and Molise). The mean amount of extraradical mycelium obtained from *T. magnatum* fruiting points ranged between 4 to 7 µg/g of soil. In the productive season the amount of *T. magnatum* mycelium was significantly higher around the fruiting points and decreased going far from these points. *T. magnatum* mycelium was detected in all soil samples taken in correspondence of the ascomata whereas 10% and 22% of the soil samples collected at 100 cm and 200 cm from them gave no detectable amount of mycelium. Moreover, *T. magnatum* mycelium inside the productive patches undergone to seasonal fluctuations probably depending on soil climatic conditions and root development. In spring the amount of *T. magnatum* mycelium was significantly higher than in summer and more uniformly distributed within the productive patches. In summer, probably due to the high temperatures and dry season, *T. magnatum* mycelium significantly decreases, whereas in autumn, during the fruiting season, increases and concentrates in correspondence of future the fruiting points. Studies are in progress to detect the effects of soil moisture and soil temperatures on *T. magnatum* mycelium development.

[1] M. Iotti, M. Leonardi, M. Oddis, E. Salerni, E. Baraldi, A. Zambonelli, *BMC Microbiology* **12** (2013) 93. doi:10.1186/1471-2180-12-93

[2] E. Salerni, M. Iotti, P. Leonardi, L. Gardin, M. D'Aguanno, C. Perini, P. Pacioni, A. Zambonelli, *Mycorrhiza* doi:10.1007/s00572-013-0543-6

P 1.3

CRYOPRESERVATION OF TUBER SPP. MYCELIUM AS A TOOL TO PRESERVE TRUFFLE BIODIVERSITY

Federica Piattoni, Pamela Leonardi, Siham Boutahir, Mirco Iotti, Alessandra Zambonelli

Dipartimento di Scienze Agrarie, Via Fanin 46, 40127 Bologna, Italy

*alessandr.zambonelli@unibo.it, Telelphone: +39 051 2096579

Keywords: cryopreservation, *Tuber borchii*, *Tuber aestivum, Tuber rufum, Tuber melanosporum*

The genus *Tuber* comprises more than 180 species which are worldwide distributed. Some of these form fruiting bodies (truffles) which are highly prized and perhaps they are subjected to continuous harvesting, especially in some European countries. The consequent risk is the irremediable damage of their mycorrhizas and mycelium in the soil. Moreover, although most of them can be successfully cultivated, truffle cultivation often does not take care of the geographical origin of the truffles used as spore inoculum, thus compromising the local truffle biodiversity.

In order to preserve truffle genetic resources, we perfected techniques for long term preservation of *Tuber* strains. To this aim we tested different cryopreservation techniques in liquid nitrogen and in deep freezer (-80 °C). Our results showed that the mycelium of *Tuber borchii* and *Tuber aestivum* can be successfully cryopreserved and that the morphology of the mycelium and of the hyphae (in particular hyphal diameter and hyphal growth unit) is not affected by low temperatures. Preliminary results showed also the possibility to cryopreserve *Tuber melanosporum* and *Tuber rufum* and thus this technique may be applicable to all the cultivable *Tuber* spp.

Our results open up the possibility to collect and preserve *Tuber* strains isolated in different European productive sites to preserve truffle biodiversity.

P 1.4

A NEW CLIMATIC DATASET FOR TUBER MELANOSPORUM FRUITING PARAMETERS FROM SIX CONTINENTS

Paul Thomas[(1),*]

[(1)] *Mycorrhizal Systems Ltd, Unit 4A, Popples Close Farm, HX7 7PG. UK*

*Paul@PlantationSystems.com, Telephone: +44 1422 844095

Keywords: Climate, Orchard, Rainfall, Temperature, Truffle

Tuber melanosporum is highly regarded for its organoleptic properties and is the most widely cultivated of all the Tuber species. Cultivation is employed throughout areas of Europe in which *Tuber melanosporum* occurs naturally and is now also successful in other regions of the world. Cultivation of this species outside of Europe has principally focused on regions perceived to have a similar climatic profile to that of its natural range. Cultivation has been so successful that the only continent not knowingly producing fruiting bodies is Antarctica. Here, data is collated from a range of wild and cultivated *Tuber melanosporum* producing sites, spanning six continents. The compiled data displays a large degree of climatic variation with fruiting sites having a 34x variation in winter rainfall and a 6.6°C variation in annual average temperature. This range is surprising when the classical view of a distinct and narrow ecological niche is considered.

Sites producing *Tuber melanosporum* in countries in which it did not previously naturally exist had significantly higher annual rainfall and lower summer temperatures than those in which there is a natural history of *Tuber melanosporum* populations. This new data set is a useful reference for scientists and truffle cultivators worldwide.

Although the data presented in this study displays a wide range of climatic parameters suitable for cultivation, care must be taken in its interpretation. How such datasets should be applied practically in truffle cultivation and what this means about our understanding of the species, is discussed.

P 1.5

HYPOGEOUS FUNGI OF THE HUNGARIAN CHESTNUT FORESTS

Mihály Csizmár, Zsolt Merényi, Torda Varga, Zoltán Bratek

Eötvös Loránd University, H-1117 Budapest, Pázmány Péter stny. 1/C, Hungary

*csimiz@gmail.com, Telephone: +36 20 9151547

Keywords: *Castanea sativa*, hypogeous fungi, checklist, Hungary

The southwest part of Hungary is under submediterranean climate effect, thus there are lots of sites where the sweet chestnut (*Castanea sativa* Mill.) forms natural forests. This thermophilic tree species has a great tradition for centuries, so many orchards also can be found in Hungary. The sweet chestnut is used by gastronomy, timber industry, apiculture and therapy. Although it is an economically important tree, there has been a decline in its stands due to diseases such as chestnut blight (*Cryphonectria parasitica*). Due to this serious disease thousands of trees, even whole big stands were died out in Hungary. Short summary of the history of its infections in Hungarian stands and of the recently detected occurrence of the natural hypovirulence is given. It is well known that mycorrhizal fungi can strengthen the host plant immune system through physical and physiological effects, enhanced the host vitality. Hence, it is important to investigate the mycorrhizal partner of the chestnut tree. To our knowledge, only a few article deal with the ectomycorrhizal species composition of this forests, and none of them examined hypogeous fungi. In the present work, we summarized the data of the hypogeous fungi of the Hungarian sweet chestnut sites studied in the last eighteen years. We analyzed several dozens of records and showed 18 hypogeous fungal species related to sweet chestnut. The most frequent species were *Tuber rufum* agg., *Tuber aestivum* Vittad. and *Stephensia bombicyna* Vittad. (13%, 10% and 10% of the records respectively). Some of rare hypogaea of acidic soils are listed as well. We also add climatic, pedological, coenological and fungal productivity data of the habitats. Discussion of coexistence of mycorrhiza forming fungi is presented as well. Certain correlations amongst the healthy state of the host tree, the soil characters and the fungal symbionts are already demonstrated. The role of soil texture, pH and $CaCO_3$-content is discussed in details. This study contributes to the knowledge of species composition of *Castanea sativa* communities and tries to describe the specific ecological circumstances of the prosperous cohabitation of *Tuber aestivum* and *Castanea sativa*.

P 1.6

ECTOMYCORRHIZAL DIVERSITY AT A NATURAL *TUBER AESTIVUM* SITE

Yasmine Piñuela Samaniego(1),*, **Gian Maria Niccolo Benucci**(2), **Tine Grebenc**(3), **Hojka Kraigher**(3)

(1) *EUIT Forestal y del Medio Natural, Universidad Politécnica de Madrid. Campus Ciudad Universitaria, Avenida de Ramiro de Maeztu s/n, 28040 Madrid, Spain*

(2) *Department of Applied Biology, University of Perugia Borgo XX Giugno 74, 06121 Perugia, Italy*

(3) *Slovenian Forestry Institute, Vecna pot 2, SI-1000 Ljubljana, Slovenia*

*yasmin.pinuela@hotmail.com, Telephone: +34 626 252 295

Keywords: *Tuber aestivum*, plantations, natural sites, ectomycorrhiza

Here we provided the first ectomycorrhizal community study of natural truffle sites in Slovenia[1], focusing in *Tuber aestivum* communities, based around two frequent ectomycorrhizal plant partners: hornbeam *(Ostrya carpinifolia)* and oak *(Quercus pubescens)*.

The study was performed in two natural *Tuber aestivum* stands that were discovered in year 2007 and regularly checked for the production of this truffle species. The soil samples were taken where the fungus was producing sporocarps. Morphological and molecular approaches were used to identify ectomycorrhiza and reveal the community structures. *T. aestivum* was described in all soil samples and also contained other ectomycorrhizae species. *Tricholoma/ Cortinarius*, *T. aestivum* and *Russula* spp. types of ectomycorrhiza were more commonly present associated with *Q. pubescens*, while several so-called early-stage species were observed associated with *O. carpinifolia,* such as *Tomentella* spp. and *Scleroderma* spp. These different species compositions were consistent with the fact that *O. carpinifolia* is more of a pioneer nature, while *Q. pubescens* usually grows in more stable forest stands; this underlined the influence of the pioneer level of the host plant on the species composition.
We contrasted our results with studies of *T. aestivum* plantations sites in central Italy [2], where the climate (Mediterranean) and soil conditions where similar and the plant partners of the same age (20 – 25 years stands). The number of different types of ectomycorrhiza was comparable among natural stands and plantations. Higher richness of early-stage ectomycorrhiza species was found in hornbeam (as it is shown in our results), but *T.aestivum* was not found in every sample on plantations sites.

Our results, as well as previous studies, indicate the replacement by early-stage species, leading to reduce the initial species as the non-*Tuber aestivum* ECM fungi can represent a competitor for plant roots and nutrients, especially important when truffle production is expected for commercial purposes.

[1] Y. Piñuela, B. Sc. Thesis,Slovenian Forestry Institute (2012)55-56.

[2] G.M.N. Benucci, L. Raggi, E. Albertini, T. Grebenc, M. Bencivenga, M. Falcinelli, G. Di Massimo, *Microbiology Ecology*76 (2011)170-184.

P 1.7

ATTEMPTS TO INTRODUCE BLACK TRUFFLE IN ISRAEL

Ofer Danay[(1),*] Nirit Ezov[(1)], Dan Levanon[(1)], Yoram Luzzati[(2)], Varda Kagan-Zur[(3)], Segula Masaphy[(1)]

[(1)]*Edible Mushrooms and Applied Mycology and Microbiology Departments, MIGAL, P.O. Box 831, Kiryat Shmona IL-11016, and Tel Hai College Upper Galilee IL-12210, Israel*

[(2)]*Kibbutz Bar'am, M.P. Merom Hagalil IL-13860, Israel*

[(3)]*Department of Life Sciences, and The Jacob Blaustein Institute for Desert Research Ben-Gurion University of the Negev, Beer-Sheva IL-841053, Israel*

*ofer@migal.org.il Telephone:+972 545484198

Keywords: *Tuber aestivum,* oak species

At the mid-nineties of the last century, attempts were made to introduce black truffles (*Tuber melanosporum*) in Israel. As part of these efforts fruit bodies were imported from Italy and used to inoculate seedlings: five species of local oaks, four species of introduced oaks and hazelnuts. Inoculated seedlings were planted in five plots along the country from the south to the north. In each plot the seedlings were randomly planted at distances of 3x6 meters. During a period of about 10 years, no fruit bodies of *Tuber melanosporum* were found, and therefore four plots were neglected. Only one plot of about 12 acres in the Upper Galilee - Kibbutz Bar'am at altitude of 700 meters A.S.L was saved. This plot is planted on a lime soil and the average annual rainfall is about 800 mm. The plot received irrigation only during few years after its planting and left arable since then.

At summer 2009, six fruiting bodies were found in this plot. Although the trees were inoculated by *T.melanosporum* they were identified by macroscopic and molecular means as *Tuber aestivum*. There are some explanations to this phenomenon (1). Furthermore there is no information on the existence of *T aestivum* as endemic fungus in the Middle East. Consequently we started monitoring by trained dogs to search for truffles. During the spring and summer of the consecutive 3 years the monitoring continued while improving the search skills. The yields of *Tuber aestivum* during 2013 show that: The highest average yield for a tree was *Quercus boissieri* and second *Q. ithaburensis*. The largest fruit bodies of about 400 and 270 grams were found at trees of *Q. boissieri* followed by *Q. ilex* (190 g) and *Q. libani* (184 g). During summer 2013 we carried out a preliminary study of adding irrigation to parts of the plot. This treatment allowed extension and enlargement of yields at the irrigated regions. The fruiting bodies were sent to known chefs for the review of their sensory characteristics. Their aroma was found to be excellent while their taste was a bit weak. A volatiles profile of the fungus is studied in order to determine the optimal stage for harvest.

[1] T.Turgeman, Y. Sitrit, O.Danai, Y.Luzzati, A.Bustan, N. Roth-Bejerano, V. Kagan-Zur, S. Masaphy. 2012. *Tuber aestivum* replacing introduced *Tuber melanosporum*: a case study. Agroforestry Systems 84: 337–343.

P 2.1

CHARACTERIZATION OF THE GENE OF LYSINE SPECIFIC DEMETHYLASE 1 (LSD1) IN TUBER MELANOSPORUM

Francesco Palma*, **Marselina Arshakyan, Antonella Amicucci and Vilberto Stocchi**

Dipartimento di Scienze Biomolecolari. University of Urbino. Italy.

*francesco.palma@uniurb.it, Telelphone: +39 0722 305 232

Keywords: *Tuber melanosporum*, LSD1, truffle life cycle.

The Périgord truffle (*Tuber melanosporum* Vittad.), also known as black truffle, is an ascomycete found in symbiotic association with roots of deciduous trees, shows a complex life cycle characterized by the alteration between the hyphal life, which leads to the formation of ectomycorrhizae and symbiosis, and the fructification stage, which leads to the formation of fruiting bodies containing sexual spores. During these transitions the mycelium undergoes morphogenetic changes caused by the expression of specific genes, which are triggered by genetics and environmental factors.

Many investigations in different aspects are now devoted for better understanding the mechanism of development of truffle ascoma, trying to identify the genes that are expressed during truffle life cycle stages (free-living mycelium, ectomycorrhiza, and fruiting body), or transitions between them [1-5]. In our research we focused our studies on a gene that encodes the activity of an enzyme, which has a transcriptional role in regulating target genes important for proper formation of fruiting bodies, hence responsible for normal metabolism and morphogenetic changes that occur during truffle life cycle. In this regard, the available annotated *T. melanosporum* genome has been analyzed and characterized the gene which was found is encoding Lysine specific demethylase 1 (LSD1) enzyme activity [6].

The LSD1 is the first histone demethylase that was discovered as a nuclear homolog of amine oxidases. Through a FAD-dependent oxidative reaction, it removes the methyl groups from mono- and dimethylated lysine (Lys) 4 of histone H3 (H3K4me1/2) and Lys9 of histone H3 (H3K9me1/2), reversing methylated state of DNA-associated histone proteins, hence modulating dynamic changes in chromatin structure and play important role in regulating gene transcription and chromatin states. In this research we identified and characterized the gene of LSD1 that can be a direct link between metabolism and transcription, and can have important contribution for understanding the mechanisms involved in morphogenetic changes during truffle life cycle. On the other hand there is no mention about expression of *lsd1* gene in other fungi, and as a nuclear homolog of amine oxidases its expression in *Tuber melanosporum* is another line of evidence that the developmental cycle of truffles is more complicated, thus, need more keys to open the doors of mysteries of this underground delicacy.

[1] A. Amicucci, R. Balestrini et al. *Fungal Genet. Biol* 48 (2011) 561–572.
[2] A. Bolchi, R. Ruotolo et al. *Fungal Genet. Biol* 48 (2011) 573–584.
[3] P. Ceccaroli P, M. Buffalini et al. *New Phytol* 189 (2011) 751–764.
[4] B. Montanini, E. Levati et al. *New Phytol.* 189 (2011) 736–750.
[5] E. Zampieri, R. Balestrini et al. *Fungal Genet. Biol*. 48 (2011) 585–591.
[6] Y. Shi Y., F. Lan et al. *Cell.* 119 (2004) 941–953.

P 3.1

SENSORY DISTINCTIVE FEATURES IN THE AROMA OF TRUFFLE OILS

Amparo Tarrega[(1)], Consol Blanch[(1)]*, Alba Crespi[(1)], Carles Ibáñez[(2)], Míriam Torres[(3)]

[(1)] *Food Science Research Group, Escola Politècnica Superior, University of Vic, C/de la Laura, 13. 08500-Vic, Spain.*

[(2)] *Analytical Research Department, Lucta S.A., C/ de Can Parellada, 28, 08170, Montornès del Vallès, Spain.*

[(3)] *Nutrition & Health Department, Facultat de Ciències de la Salut i del Benestar, University of Vic, C/ Sagrada Família, 7. 08500-Vic, Spain.*

*consol.blanch@uvic.cat, Telephone: +34 881 55 19

Keywords: truffle, oil, aroma profile, trained panel.

Truffles are a highly appreciated food because of their unique aroma, composed of complex mixtures of lipophilic volatile organic compounds. Among the different species of truffles, Tuber magnatum (white truffle), Tuber melanosporum (black truffle) and Tuber aestivum (summer truffle) are the most appreciated and expensive. There are many food products flavored with truffles, and oils are particularly common. Truffle oils are made with real truffles infused in olive oil or sun-flower oil but there are also many commercially-produced truffle oils made by adding some aroma compounds found in truffles, such as thioether (2,4-dithiapentane). The objective of this work was to differentiate between the aroma characteristics perceived in truffle oils of different composition and volatile fraction complexity. Seven commercial samples of truffle oil of different origin (France, Italy and Spain), and one sample obtained from truffles infused in olive oil, were studied. To determine differences in the aroma of the truffle oils, descriptive sensory analysis was performed. To this end, a group of expert panellists (n=10) was trained in the evaluation of truffle oil aromas. First, the descriptors or sensory terms for describing the aroma sensations in truffles were established. Then, panellists were trained in the identification of descriptors and the use of a continuous scale for evaluating the intensity of each descriptor. Finally, the trained panellists evaluated the eight truffle oil samples and the aroma profile of each sample was obtained. Qualitative and quantitative differences between the aromas emitted by the truffle oils were observed and were related with the differences in volatile fraction composition and complexity, determined in a previous work.

P3.2

THE VALUE OF WHITE TRUFFLE: AN INVESTIGATION INTO THE MYSTERIOUS RED SPOT

Valentina Sparvoli[(1)], Elena Barbieri[(1)], Alessandra Zambonelli[(2)], Vilberto Stocchi[(1)] Antonella Amicucci[(1)*]

(1) Department of Biomolecular Science, University of Urbin Carlo Bo, Via Saffi 2, 61029, Urbino (PU), Italy

(2) Dipartimento di Scienze Agrarie, University of Bologna, via Fanin 46, 40127 Bologna, Italy

*antonella.amicucci@uniurb.it, Telephone: +39 0722 305232

Keywords: white truffle, red spot, bacterial spp, 16S analyses

Recently it has been suggested the involvement of bacteria in a peculiar pigmentation of the ascoma, from white to red, found in some specimens of *T. magnatum* Pico. Given the high commercial value of this species of *Tuber* and given that this morphological abnormality also involves a change in sensory level, in the present work the bacterial community of *T. magnatum* Pico samples has been characterized, with the objective of identifying the bacterial spp responsible for this peculiar pigmentation. Experimentally, the truffle samples were analyzed by a culture dependent approach, ARDRA analysis of the 16S rDNA of the bacteria isolated from the pigmented region, and also with a culture independent method, ARDRA analysis of a library of 16S rDNA created from DNA extracted directly from carpophore. The analyses highlighted the presence of bacterial spp commonly found in *Tuber* carpophores (*Proteobacteria* and *Bacteroides*), but at a lower biodiversity. Finally, by mass spectrometry the biochemical characterization of the pigment extracted from the altered tissues was carried out and pigments belonging to the class of carotenoids were identified; this evidence suggested that the bacterial species involved in the peculiar pigmentation, belong to the species *Microbacterium* and *Chryseobacterium*.

P 3.3

ELECTROPHYSIOLOGICAL (EAG) RESPONSES OF *LEIODES CINNAMOMEUS* TO VOLATILES ISOLATED FROM *TUBER MELANOSPORUM*

Antonio Ortiz[1], Guillermo Perez-Andueza[2]*, César Saucedo[2] and Fernando Herrero [2]

See **RT3.1** on page **38**

P 4-1

CHARACTERIZATION OF VOLATILE COMPOUNDS IN TRUFFLE INFUSED-OILS BY HS-SPME-GC-MS ANALYSIS

Consol Blanch[(1),*], **Carles Ibáñez**[(2)], **Albert Hueso**[(1)], **Núria Barniol**[(1)], **Míriam Torres**[(3)]

[(1)]*Food Science Department, Escola Politècnica Superior, University of Vic, C/de la Laura,13. 08500-Vic, Spain.*

[(2)]*Analytical Research Department, Lucta S.A., C/ de Can Parellada, 28, 08170, Montornès del Vallès, Spain.*

[(3)]*Nutrition & Health Department, Facultat de Ciències de la Salut i del Benestar, University of Vic, C/Sagrada Família, 7, 08500-Vic, Spain.*

*consol.blanch@UVic-UCC.cat, Telephone: +34 93 881 55 19

Keywords: truffle, oil, volatile organic compounds, SPME, GC/MS.

As is well-known, the culinary and commercial value of truffles is mainly due to their sensorial properties, such as their aroma, the quality of which clearly gives economic value to this edible fungus. Some culinary preparations are made with this fungus, such as truffle-infused oils. The adulteration of these products must be controlled and prevented due to the high economic cost of natural truffles. The aim of this study is to characterize the volatile organic compounds of some truffle-infused oils, in order to try to determine their authenticity.

Samples were purchased in local (3 samples) and foreign markets (4 samples) and one truffle-infused oil sample was prepared in our laboratory to have a positive authenticity control. Samples were stored at 4ºC and the analysis was done in 24-48h. In our study, volatile compounds were extracted (two replicates of each sample) by headspace-solid-phase-micro-extraction, and separated and identified by gas chromatography-mass spectrometry (HS-SPME-GC/MS) analysis [1-7]. The extraction method was based on SPME static headspace; a 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane coating (DVB-CAR-PDMS) and a 100 µm polydimethylsiloxane coating (PDMS) fibers were applied. Extraction was carried out in 20 mL glass vials, wich are closed with PTFE/Silicone septa using 2g of sample for 30 min at 50ºC to avoid oil oxidation. GC-MS analysis was performed with a Zebron ZB-WAX Plus (30m x 0.25mm x 0.25µm) and a Zebron ZB1 (30m x 0.25mm x 0.25µm) capillary columns and oven programmed temperature. The SPME-extracted volatiles were directly desorbed (10 min) into the split-splitless injector at 250ºC. The mass spectrometer was operated in EI^+ mode. Wiley, NIST'08 and proprietary database libraries were used for mass spectra identification of volatile compounds found in the different studied samples. The GC-MS data showed that only two of the studied oils presented a similar volatile compounds profile as our blank; several mixture of chemical flavourings were found in the others commercial samples. Some considerations about product authenticity will be given, comparing commercial samples with the laboratory truffle-infused oil control sample.

[1] G. Mauriello, *J. Chromatogr. Sci.* **42** *(2004) 299.*

[2] R.E. March, *Int. J. Mass Spectrometry*, **249** (2006) 60.

[3] P. Díaz, *LWT- Food Science and Technology*, **42** (2009) 1253.

[4] L. Culleré, *Food Chemistry,* **122** (2010) 300.

[5] M. Kiss, *Journal of Applied Botany and Food Quality*, **84** (2011) 102.

[6] R. Splivallo, *New Phytologist*, **189** (2011) 688.

[7] G. Pacioni, *Food Chemistry*, **146**, (2014) 30.

AUTHOR INDEX

LIST OF PARTICIPANTS

FAHAD NASSER AL-BARAKAH, KING SAUD UNIVERSITY, RIYADH, SAUDI ARABIA

ABDULAI ABDUL QUDUS, ELOMAK RESEARCH CC, JOHANNESBURG, SOUTHAFRICA

RANA ADAMO, ETSEA, LLEIDA, SPAIN

SARA AHMED, AQUAMED RESEARCH AND EDUCATION, DOHA, QATAR

AMEDEO ALPI, FONDAZIONE AREA, PISA, ITALY

ASMAA ALQARADAWI, AQUAMED RESEARCH AND EDUCATION, DOHA, QATAR

ABDULAZIZ ALQARAWI, KING SAUD UNIVERSITY RIYADH, SAUDI ARABIA

ANTONELLA AMICUCCI, UNIVERSITÀ DEGLI STUDI DI URBINO "CARLO BO", ITALY

HESHAM ATTAR, NATIONAL RESEARCH CENTRE DOKKI, EGYPT

RAFFAELLA BALESTRINI, CNR, TORINO, ITALY

NÚRIA BARNIOL, UNIVERSITY OF VIC, CATALONIA, SPAIN

MIREIA BARTRONS, UVIC, BARCELONA, CATALONIA -SPAIN

JAVIER BERNAL, INVESTUBER SC, SPAIN

CONSOL BLANCH, UNIVERSITY OF VIC, VIC, CATALONIA-SPAIN

JOSE-ANTONIO BONET, UNIVERSITY OF LLEIDA, LLEIDA, CATALONIA-SPAIN

MIQUEL BOSQUED, **RESTAURANT CAL PAGES**, TORELLO, SPAIN

MIGUEL CABRE, THELEMA, CABRILS, SPAIN

CARME CASAS, UNIVERSITAT DE VIC, VIC, CATALONIA- SPAIN

CARLES CASTAÑO, CENTRE TECNOLÒGIC FORESTAL DE CATALUNYA, SOLOSONA, SPAIN

ALEJANDRO CIFUENTES, CSIC, MADRID, SPAIN

CARLOS COLINAS, UNIVERSITAT DE LLEIDA, LLEIDA, SPAIN

ALBA CRESPI, UVIC, CATALONIA-SPAIN

MIHÁLY CSIZMÁR, EÖTVÖS LÓRÁND UNIVERSITY, BUDAPEST, HUNGARY

LAURA CULLERE, UNIVERSIDAD DE ZARAGOZA, FACULTAD DE CIENCIAS, ZARAGOZA, SPAIN

OFER DANAY, MIGAL, KIRAT, ISRAEL

HAUKE DUHM, PILZSCHULE, SALZGITTER, DEUTSCHLAND

ULKA DOERTE ENGELKES, TRUFFLE INSTITUTE, KALEFELD, GERMANY

JOAN MARIA, ESTRADA, INOTRUF SL, SPAIN

ANNA FENOSA, UVIC-, VIC, CATALONIA-SPAIN

MARKO FERK, TRUFFLE INDEPENDENT, LONDON, UK

CHRISTINE FISCHER, CENTRE TECNOLOGIC FORESTAL DE CATALUNYA, SOLSONA, SPAIN

LOTHAR GEHLEN, PRIVAT, OBERHAUSEN, GERMANY

ISABELLA GIANICOLO, ASS. PER IL CENTRO NAZIONALE STUDI TARTUFO, ALBA, ITALY**EVA GÓMEZ MOLINA**, CIET. DIPUTACIÓN PROVINCIAL DE HUESCA, GRAUS (HUESCA), SPAIN

TINE GREBENC, SLOVENIAN FORESTRY INSTITUTE, LJUBLJANA, SLOVENIA

IAN HALL, TRUFFLES AND MUSHROOMS LTD, DUNEDIN, NEW ZEALAND

DIETER HONSTRASS, PILZSCHULE, SALZGITTER, GERMANY

ALBERT HUESO, UNIVERSITAT DE VIC, VIC, CATALONIA- SPAIN

ELENA IBAÑEZ, CSIC, MADRID, SPAIN

CARLOS IBAÑEZ, LUCTA SA, MONTORNES DEL VALLÈS, SPAIN

UWE KUECHENMEISTER, BIOS BIOTECHNOLOGIE SCHÖNOW GMBH, BERNAU, GERMANY

MONIKA KÖRNER, FORSCHUNGSGRUPPE HYPOGÄEN, EINBECK, GERMANY

GARRY LEE, UNIVERSITY OF WESTERN AUSTRALIA, AUSTRALIA

JAUME LLISTOSELLA, UNIVERSITAT DE BARCELONA, BARCELONA, CATALONIA-SPAIN

ALANA MCGEE, TRUFFLE DOG COMPANY & TOIL AND TRUFFLE, EDMONDS, USA

ANTONIETTA MELLO, ISTITUTO PER LA PROTEZIONE DELLE PIANTE, TORINO, ITALY

MICHELE MIRANDA, UNIVERSITY OF L'AQUILA, L'AQUILA, ITALY

MARCOS MORCILLO, MICOLOGIA FORESTAL & APLICADA, VILANOVA I LA GELTRÚ, SPAIN

WILLEM MOSTERT, BELLVILLE, SOUTHAFRICA

GEORGE MOSTERT, PRIVATE, BELLVILLE, SOUTH AFRICA

AMIN MRIDHA, KING SAUD UNIVERSITY, SAUDI ARABIA

CLAUDE MURAT, INRA, CHAMPENOUX, FRANCE

DANIEL OLIACH, FOREST SCIENCES CENTRE OF CATALONIA (CTFC), SOLSONA, SPAIN

ANTONI OLIVERA, UNIVERSITAT DE LLEIDA, LLEIDA, SPAIN

ANTONIO ORTIZ, UNIVERSITY OF JAEN, JAEN, SPAIN

EVELI OTSING, UNIVERSITY OF TARTU, ESTONIA

GIOVANNI PACIONI, UNIVERSITY OF L'AQUILA, L'AQUILA, ITALY

FRANCESCO PALMA, UNIVERSITY OF URBINO, URBINO, ITALY

FRANCESCO PAOLOCCI, CNR-INSTITUTE OF BIOSCIENCES AND BIORESOURCES-PERUGIA, ITALY

XAVIER PARLADÉ, IRTA, CABRILS, SPAIN

GIORGIO PENNAZZA, UNIVERSITY CAMPUS BIO-MEDICO DI ROMA, ROME, ITALY

GUILLERMO PÉREZ-ANDUEZA, UNIVERSIDAD CATÓLICA DE ÁVILA, ÁVILA, ESPAÑA-SPAIN

YASMINE PIÑUELA, SLOVENIAN **FORESTY INSTUTE**, LJUBLJANA, SLOVENIJA

MIKEL QUERALT, UNIVERSITY OF NAVARRA, PAMPLONA, NAVARRA

JOAN REIXACH CURTO, RESTAURANT CAN BAUMES, TAVERTET, SPAIN

NEUS ROQUER JUFRE, RESTAURANT CAN BAUMES, TAVERTET, SPAIN

ALEKSANDRA ROSA-GRUSZECKA, FOREST RESEARCH INSTITUTE, RASZYN, POLAND

HERBERT RUDOLPH, TRÜFFELLAND, VIENNA, AUSTRIA

JOCHEN SCHOETTKER, TRUEFFELFREUNDE, HAMBURG, GERMANY

RICHARD SPLIVALLO, UNIVERSITY OF FRANKFURT, GERMANY

ULRICH STOBBE, UNIVERSITY OF FREIBURG / DEUTSCHE TRÜFFELBÄUME, RADOLFZELL, GERMANY

HANNA SZMIDLA, FOREST RESEARCH INSTITUTE, RASZYN, POLAND

PAUL THOMAS, MYCORRHIZAL SYSTEMS, HEPTONSTALL, UK

MIRIAM TORRES-MORENO, UNIVERSITAT DE VIC, VIC, SPAIN

JAMES TRAPPE, OREGON STATE UNIVERSITY, CORVALLIS, OREGONS, USA

SONJA UNGAR, COMERCIAL TRUFA'S, CHILLAN, CHILE

JORDI VILLA, POLYTECHNICAL SCHOOL OF VIC, CATALONIA, SPAIN

DAVID WERHAHN, TUBERBUAM GBR, KÖLN, GERMANY

MARCO WERTH, TUBERBUAM GBR, GÖTTINGEN, GERMANY

MARCO WOLF, FORSCHUNGSGRUPPE HYPOGÄEN, LENGERICH, GERMANY

ALESSANDRA ZAMBONELLI, UNIVERSITY OF BOLOGNA, BOLOGNA, ITALY

Lucta, servicio global y de confianza

Aromas para alimentación humana,
Fragancias y Aditivos para nutrición animal.

Lucta

Creamos confianza

www.truffleresearch.eu