Abstracts of Papers Presented at

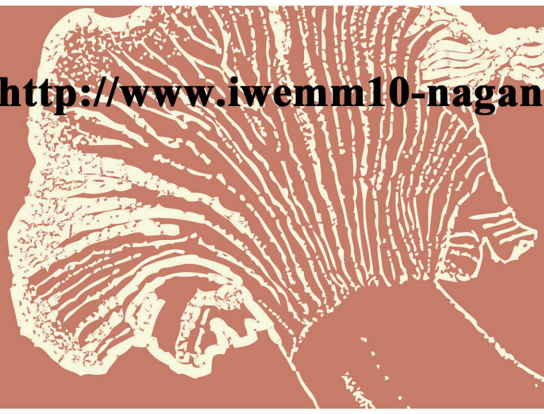
The 10th International Workshop on Edible Mycorrhizal Mushrooms

IWEMM10

Forest Resources for Our Future

20th – 29th October 2019

RAKO Hananoi Hotel, Suwa, Nagano, Japan



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Edited by:

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Forest Resources for Our Future

Welcome to Suwa, Nagano, Japan! We are very happy that families of the international workshop of Edible Ectomycorrhizal Mushrooms (IWEMM) from all over the world will meet again in Suwa. I would like to thank the many generous sponsors and partners in Japan and overseas for their cooperation in organizing IWEMM10.

Japan is a country of forests, where 68% of the land is occupied by forest. This percentage is unusually high among developed countries. Local people, especially those in rural areas, spend their lives connecting closely with the forest for obtaining natural resources. Many species of mushroom have been harvested in the wild and used as food. Of these, matsutake mushroom (*Tricholoma matsutake*) was given a special status in Japan for over a millennium. It seems that matsutake mushroom production is closely related with our lifestyle. In recent days, the production of matsutake mushroom has been considerably decreased in Japan. Since ancient times, many wild mushrooms (e.g., hiratake, shiitake, shoro, and shimeji) have been collected from forest areas. These valuable forest resources should be left for future generations too. Therefore, it is important to promote discussions on the conservation of these resources and the development of their cultivation techniques. The members of the organizing committee believe that IWEMM10 will help you advance your studies and cooperation.

Dr. Takashi Yamanaka
Chairman of IWEMM10 organizing committee
E-mail: yamanaka@ffpri.affcr.go.jp

Edible Mycorrhizal Fungi (EMF) are fascinating symbiotic organisms that underpin the health and productivity of some of the earth' most important ecosystems: mostly boreal and temperate forests, but also tropical and sub-tropical forests, savannas and desert areas. In addition to their critical role supporting plant growth, they provide a unique and delectable source of food. Over the millennia, humans have formed special bonds with these organisms. We harvest the mushrooms they produce for nutrition and income, revere them as exquisite cuisine (Matsutake, Truffles, Caesar's mushrooms) or simply enjoy them as favourite seasonal delicacies. EMF are among the last organisms to be domesticated due to their complex symbiotic biology and the cryptic soil ecosystems where they dwell. The science-based cultivation of truffles started only 42 years ago and is far from being complete. Some species still proudly defy cultivation, including the mighty Matsutake! Further progress with cultivation will likely bring countless economic, social and environmental benefits. But because their harvest still largely relies on collecting from forests and other natural areas, it remains of paramount importance to preserve their biodiversity, their native biomes and the rich but fragile knowledge that ethnic communities

throughout the world have accumulated about these organisms and their habitats. Doing so will also contribute to the mitigation of climate change through economic incentives to retain forest cover. For all these reasons and more, it is a great honour and privilege to present the abstract book of the IWEMM10 held for the first time in Japan, a notorious mycophilic country where inhabitants calls mushrooms “kinoko” or “child of the tree”. On behalf of all participants, I would like to express our gratitude and warmest thanks to the local organizing team for having prepared for us what will certainly be one the most interesting and successful IWEMMs. Enjoy the conference!

Dr. Alexis Guerin-Laguette
Secretary of IWEMM scientific committee
E-mail: alexis.guerin@plantandfood.co.nz

The IWEMM10 organising committee is very grateful to the following members of the international scientific committee who have kindly edited the abstracts whenever required: Shannon Berch, Greg Bonito, Alexis Guerin-Laguette, Dalia Lewinsohn, Anne Mitchell, Xavier Parladé, David Pilz, Raymond Prince, and Andrea Rinaldi.

Dr. Akiyoshi Yamada
Secretary general of IWEMM10 organizing committee
E-mail: akiyosh@shinshu-u.ac.jp

Treasure of nature

Distinguished participants of IWEMM10, welcome to Nagano. I am Ide Eiji, Director General of Forestry Department, Nagano Prefecture. I extend our warm welcome to all of you on behalf of the Nagano Prefectural Government.

I would like to congratulate you on this special occasion of IWEMM10 in Nagano. Nagano is called the Alps in Japan, as well as the Rooftop of Japan because of the surrounding mountain ranges including the Northern, Central and Southern Alps. We are also blessed with Japan's major rivers, such as the Tenryu, Kiso, Chikuma, and Sai Rivers, which flow through the rich natural environment. We are also a world-renowned winter resort, which hosted the Nagano Winter Olympics in 1998.

Nagano is one of the best-known producers of mushroom in Japan. Mushrooms play an important role in providing joy of mountains to us, such as going mushroom hunting in the mountains and enjoying them at home. Particularly, we are Japan's largest producer of the highly sought-after and valuable matsutake mushrooms. In the fall, many tourists visit Nagano to enjoy them. That is why the matsutake industry rejoices and despairs to hear the news of good and bad harvest each year. We are also a proud producer of tasty apples and grapes. I hope you will also enjoy our fruit while you are here.

In closing, I wish all of you continued success and good health.

Thank you

Mr. Eiji Ide
Director general of Forestry Department
Nagano Prefecture

The City of Lake, Firework, and Sake

Welcome to the city of Suwa in Nagano Prefecture. My name is Yukari Kaneko, and I am the mayor of Suwa. I'd like to take this opportunity to tell you a bit about Suwa. Suwa is a lake shore plateau city that's very comfortable all year long with a year-round average temperature of 11 degrees Celsius, total land area of approximately 110 km2, population of around 50,000 people, and elevation of 760 m. Lake Suwa, Suwa Taisha, one of Japan's oldest shrines, and the grassland plateau of Kirigamine are all located here. The city has a lush natural environment, and people have lived here since the ancient paleolithic era. Numerous communities formed during the Jomon period, and Suwa is one of the most prominent sites for Jomon cultural artifacts. Stone tools and earthenware have been discovered in a variety of locations within the city.

In the field of forestry, the mushroom-cultivation forests in Suwa have a total area of approximately 73 km2, comprising roughly 66% of the total area of the city. The elevation ranges from around 800 m in the Kirigamine plateau to about 1,600 m at the top of Mt. Moriya in the west. The forests consist mainly of larch trees planted after the war, and these trees account for about 48% of the total forest area. The jikobou produced by this larch forest is an extremely popular seasonal taste among residents of the city of Suwa and Nagano Prefecture as a whole, used in a variety of different dishes. 25% of the forest is made up of Japanese red pine trees, the species on which a luxury food product called matsutake mushrooms grow. The observation site this time, the Ushiroyama area, is well-known nationwide as a thriving production area for all kinds of mushrooms, including matsutake. Recently, there are reports of insect damage to pine trees in forests all over Japan, and we are also making efforts to prevent these damages in our forests.

Although this international workshop will only last for one short week starting today, we hope that all of the participants will be able to experience our active city with its lush natural surroundings, harmonious atmosphere, and friendly residents, as well as the accumulated industry and culture of Suwa.

In closing, I hope the international workshop held in Suwa this time is extremely fruitful and also a time that all of you will remember fondly in the future.

Yukari Kaneko
Mayor of Suwa City

Topics of the Congress

1. Anthropology
2. Biodiversity
3. Biotechnology
4. Conservation
5. Cultivation
6. Ecology
7. Economic importance
8. Evolution
9. Food security and health
10. Genetics
11. Genomics
12. Global climate change
13. Molecular Biology
14 Mycology
15. Phylogeny
16. Sustainability
17. Taxonomy
18. Traditional knowledge

Scientific Committee Members

- Roberto Flores Arzú (Guatemala)
- Carolina Barroetaveña (Argentina)
- Shannon Berch (Canada)
- Greg Bonito (USA)
- Guillermo Pereira Cancino (Chile)
- Gérard Chevalier (France)
- Simon Egli (Switzerland)
- Alexis Guerin-Laguette (New Zealand/ France)
- Lahsen Khabar (Morocco)
- Dalia Lewinsohn (Israel)
- Fernando Martínez-Peña (Spain)
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- Wang Yun (New Zealand/China)
- Akiyoshi Yamada (Japan)
- Takashi Yamanaka (Japan)
- Yu Fu-Qiang (China)
- Alessandra Zambonelli (Italy)

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Kazato Shiro (Nagano Prefecture Office)
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Michihiko Odate (Suwa City Office)
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Kazuhiro Hayashi (Nagano Prefecture Special Forest Product Promotion Association)
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Ayumi Oda (Shinshu University)

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- Japanese Conference on Mycorrhiza

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- Hokto Corporation, Japan
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- Japan Mushroom Meister Association
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- Minami-majino Production Forestry Association, Japan
- Ooguma Forest Use and Agricultural Cooperation, Japan
- Tatsuzawa Common Land Management Association, Japan
- Han-i Forestry Association, Japan
- Suwa Forestry Association, Japan

Congress Program (1/10)

Overall events of IWEMM10: 20 ~29 October

Date of October	Event
20 (Sunday)	Pre-workshop and registration of IWEMM10
21 (Monday) ~ 23 (Wednesday)	Main congress of IWEMM10
24 (Thursday) ~ 25 (Friday)	Field excursions
26 (Saturday) ~ 29 (Tuesday)	Post meeting field trip

20th October (Sunday):

Pre-workshop - Mushrooms and Our Life (at Suwa City Hall)

Time	Content
11:00 – 11:05	**Opening remarks** by Takashi Yamanaka
11:05 – 11:30	**Lecture 1**: Introduction to the Research on Edible Mycorrhizal Mushrooms by Alexis Guerin-Laguette
11:30 – 11:55	**Lecture 2**: Introduction to Traditional Forest Managements for the Sustainable Matsutake Harvest in Suwa Area by Takashi Kanai
(Break)	
13:10 – 14:10	**Documentary movie show 1**: by Tristan Stock
14:30 – 15:30	**Documentary movie show 2**: by Marion Neumann
16:00 – 16:25	**Panel Discussion**
16:30	**Closing Remarks**

Registration and Welcome Party of IWEMM10 at RAKO Hananoi Hotel from 5:00 PM. Please come to the Suimei (3rd floor).

Congress Program (2/10)

21 October (Monday)

Time	Content	Room
8:30 –	**Registration**	Lobby (1st floor)
9:30 – 10:15	**Opening Ceremony**	Parque (2nd Floor)
(break)		
10:25 – 11:25	**Keynote lecture 1:** The Anthropocene and the underground city of mycorrhiza by **Anna Lowenhaupt Tsing (**University of California)	
(break)		
11:30 – 12:30	**Keynote lecture 2:** What will happen to ectomycorrhizal fungi in fragmented forests? - population genetic implications from endangered or relict forests by **Kazuhide Nara** (The University of Tokyo)	
(Lunch)		
13:30 – 13:50	**Oral Session 1: Anthropology and Mycology** 1-1. Mushrooms and humans: overview of matsutake and other edible mushrooms in anthropology and documentary films (by S Satsuka, J V Dyk, J Pokorn)	
13:50 – 14:10	1-2. "Mushroom people": a documentary about mushroom pickers in the Pacific Northwest (by T Stoch)	
14:10 – 14:30	1-3. The mushroom speaks - what kind of relationships can we imagine between the human and the fungal world? (by M Neumann)	
14:30 – 14:50	1-4. Foraging culture of mushrooms in Japan: background and recent trends (by H Saito)	
14:50 – 15:10	1-5. How the matsutake economy in Southwest China affects the social lives of the Yi people (by M J Hathaway)	
15:10 – 15:30	1-6. The status of matsutake production and trade in Japan (by J Akamine)	
(break)		
15:40 – 16:00	**Oral session 2: Sustainability, Conservation, and Global climate change** 2-1. Patagonia fungi, trails and tastes®. Fostering local development through identity valued wild edible mushrooms (by C Barroetaveña, M B Pildain)	

Congress Program (3/10)

21 October (Monday)

Time	Content	Room
	Oral session 2: Sustainability, Conservation, and Global climate change	Parque (2nd Floor)
16:00 – 16:20	2-2. Multiproductive truffle culture in Spain. The 'Tubersystems' project (by J Parladé, A Rincón, A M de Miguel, C Calvet, A Camprubí, J Pera, M T Martínez-Ferrer, J M Campos-Rivela, M Martín-Santafé, J Sabaté, A Probanza)	
16:20 – 16:40	2-3. Sustainability of edible mycorrhizal fungi and their trees in Ireland (the land of the setting sun!) (by M L Cullen, H F Fox)	
16:40 – 17:00	2-4. Isolation, identification, and utilization of fungi associated with *Tristaniopsis obovata* grown in a heath forest ecosystem, Bangka Island, Sumatra, Indonesia (by M Turjaman, A Hidayat, S A Faulina, A Yani, Aryanto, Najmulah, Helbert, K Nara, Y Tamai)	
17:00 – 17:20	2-5. Environmental niche modelling and climate change scenarios used to predict the likely distribution of truffle species for the period 2071–2100 (by P W Thomas, T S F Silva)	
(break)		
	Oral session 3: Traditional knowledge and Economic importance	
17:30 – 17:50	3-1. Traditional knowledge on *Lyophyllum* species in central Mexico: its contribution to identify new species (by C Burrola-Aguilar, Y Arana-Gabriel, R Garibay-Orijel, A Montoya-Esquivel, M Jaime-Salinas)	
17:50 – 18:10	3-2. The economic importance of possible developments in the U.S. truffle industry (by B Upchurch, O Martin, D Detmer, D Hudson, R Prince)	
18:10 – 18:30	3-3. A coordinated management of forest mushroom collection areas improves the efficiency and sustainability of its mycotourist use (by F Martínez-Peña, J L Minguell, B Rodríguez-Prado, P de Frutos)	
18:30 – 18:50	3-4. Picking edible mycorrhizal mushrooms (EMM) for a living (by Wang Y, Li S, Su K, Zhang X, Li Y)	

Congress Program (4/10)

21 October (Monday)

Time	Content	Room
19:00 – 20:00	**Sake tasting**	Parque (2nd Floor)

22 October (Tuesday)

Time	Content	Room
8:30 – 14:30	**Field work**: managed matsutake forest site, Ushiroyama, Suwa (Guided by Takashi Kanai)	Please gather in the lobby (1st Floor) by AM 8:00
	Oral session 4: Conservation, Biodiversity, and Ecology	Parque (2nd Floor)
15:00 – 15:20	4-1. Wild edible and mycorrhizal fungi conservation programs in Israel (by D Lewinsohn, O Bonneh)	
15:20 – 15:40	4-2. Discovering a new mycogeographic region and new edible mycorrhizal mushroom species in Guatemala (by R F Arzú)	
15:40 – 16:00	4-3. *Halimium* as an ectomycorrhizal symbiont. A new look at Mediterranean ectomycorrhizal communities (by A C Rinaldi, O Comandini, M Leonardi)	
16:00 – 16:20	4-4. Species diversity and utilization of edible ectomycorrhizal mushrooms in Pha Wang Nam Kaew Forest Reserve (by T Kaewgrajang, M Nipitwattanaphon)	
16:20 – 16:40	4-5. Management of Mediterranean *Cistus ladanifer* scrublands and *Boletus edulis* production (NW Spain): what we know after 15 years of study (by P Martín-Pinto, M Hernández-Rodríguez, O Mediavilla, J A Oria-de-Rueda)	
16:40 – 17:00	4-6. The impact of silviculture practices in belowground fungal communities: a case study in Mediterranean *Quercus pyrenaica* forests (NW Spain) (by M Santos-Vicente, J Geml, J A Oria-de-Rueda, P Martín-Pinto)	
(break)		
17:15 – 17:35	4-7. Documenting the ectomycorrhizal fungi of *Quercus garryana* at the northern extent of its range (by S M Berch, T Witte, J Tanney)	

Congress Program (5/10)

22 October (Tuesday)

Time	Content	Room
17:35 – 17:55	**Oral session 4: Conservation, Biodiversity, and Ecology** 4-8. Bacteria and truffle: a complex relationship (by A Amicucci, A Zambonelli)	Parque (2nd Floor)
17:55 – 18:15	4-9. Comparing soil fungal and bacterial communities in truffle-productive orchards of Australia and Europe (by Benucci G M N, A De Miguel, A Deveau, D Donnini, H Eslick, P Marco, G Marozzi, C Murat, S Sanchez, F Le Tacon, G Bonito)	
18:15 – 18:35	4-10. Morphological and microbiome variation of *Tuber pseudobrumale* in southwestern China: widening the repertoire (by M Herrera, D Liu, F-Q Yu, J Pèrez-Moreno)	
18:35 – 18:55	4-11. Isolation and characterization of bacteria from fruiting bodies of *Rhizopogon roseolus* (by S Pramoj Na Ayudhya, Y Ozaki, Y Onda, T Aimi, N Shimomura)	
18:55 – 19:15	4-12. *Streptomyces* sp. A11, an ectomycorrhiza-associated actinobacterium has different effects on various fungal growth but promotes mycorrhizal formation (by L-M Vaario, S Ogawa, M Hara, N Matsushita)	
19:30 – 20:30	**Experience of Japanese traditional tea ceremony** (max. 14 persons can attend)	Kiko (2nd Floor)

23 October (Wednesday)

Time	Content	Room
8:25 – 8:45	**Oral session 5: Japanese government project and its related research theses** 5-1. Researches for the cultivation of *Tricholoma matsutake* in Japan (by T Yamanaka, A Yamada, H Furukawa)	Parque (2nd Floor)
8:45 – 9:05	5-2. Sustainable harvest of matsutake: thirty-eight years chronological data in relation to pine forest management in Nagano, Japan (by H Furukawa, A Yamada, T Yamanaka, K Katagiri, K Masuno)	

Congress Program (6/10)

23 October (Wednesday)

Time	Content	Room
	Oral session 5: Japanese government project and its related thesis	Parque (2nd Floor)
9:05 – 9:25	5-3. Radiation mutagenesis of the ectomycorrhizal fungus *Tricholoma matsutake* (by H Murata, S Nakano, T Yamanaka, T Shimokawa, T Abe, H Ichida, Y Hayashi, K Tahara, A Ohta)	
9:25 – 9:45	5-4. Cultivation study of *Lyophyllum shimeji* in forest conditions (by M Kawai)	
9:45 – 10:05	5-5. Genotypic diversity of an Asiatic black truffle, *Tuber himalayense,* in naturally generated, highly productive truffle grounds (by N Nakamura, J P. Abe, H Shibata, A Kinoshita, K Obase, J R P Worth, Y Ota, S Nakano, T Yamanaka)	
10:05 – 10:25	5-6. Ectomycorrhizal colonization by *Tuber japonicum* and its promotion on the growth of *Pinus densiflora* seedlings in vitro (by S Nakano, A Kinoshita, K Obase, N Nakamura, T Yamanaka)	
(break)		
	Oral session 6: Molecular biology, Evolution and Phylogeny, and Biotechnology	
10:40 – 11:00	6-1. First identification of a hidded gap in the 26s rRNA of desert truffles (by A Navarro-Ródenas, A Carra, Á L Guarnizo-Serrudo, A Morte)	
11:00 – 11:20	6-2. Unravelling whitish truffle (*Tuber borchii*) life cycle in the first truffle orchard established by mycelial inoculated plants (by P Leonardi, C Murat, F Puliga, M Iotti, A Zambonelli)	
11:20 – 11:40	6-3. New species of the genus *Boletus* (porcini mushrooms & allies) in Central America confirmed by phylogenetic analyses (by C P López, R F Arzú, G Wu, F Yu)	
11:40 – 12:00	6-4. State of the art of the ethnomycology, biodiversity and biotechnology of the edible ectomycorrhizal mushrooms of Mexico (by J Pérez-Moreno, M Martínez-Reyes, F Hernández-Santiago)	
(Lunch)		
13:00 – 14:30	**Poster session**	Kiko and Yoko (2nd Floor)
(break)		

Congress Program (7/10)

23 October (Wednesday)

Time	Content	Room
	Oral session 7: Cultivation	Parque (2nd Floor)
14:40 – 15:00	7-1. Success in artificial root colonizations and fruit body formations of *Entoloma clypeatum* with *Pyrus betulaefolia* (by M Shishikura, Y Takemura, N Maekawa, A Nakagiri, K Sotome, N Endo)	
15:00 – 15:20	7-2. Ectomycorrhization of monokaryotic and dikaryotic strains of hedgehog mushrooms (*Hydnum* L.) with pine seedlings in vitro (by R Sugawara, N Maekawa, A Nakagiri, K Sotome, N Endo)	
15:20 – 15:40	7-3. Desert truffle crop depends on agroclimatic parameters during two key periods (by A Morte, A Andrino, J E Marqués-Gálvez, F Arenas, A Navarro-Ródenas)	
15:40 – 16:00	7-4. Fertilizing regime selection and its correlation with mycorrhizal development of *Tuber melanosporum*, *Tuber aestivum* and their hosting oak trees (by S Levy, N Ezov, I Pereman, A Silber, O Danay)	
(break)		
16:10 – 16:30	7-5. The Australian pests and diseases of truffles and their host trees project 2015–2019 (by A Mitchell, S Learmonth, A Mathews, C Linde, A Davey, A Seago)	
16:30 – 16:50	7-6. Recent advances in the cultivation of edible mycorrhizal fungi in New Zealand and China (by A Guerin-Laguette, R Wang, Y Wang, F-Q Yu)	
16:50 – 17:10	7-7. Development status of the truffle industry in Panzhihua, Sichuan, China (by M Yang, C Liu, P Tang, Y Wang, A Guerin-Laguette)	
17:10 – 17:30	7-8. Diversity of commercial wild mushrooms in Yunnan, China and cultivation of the ectomycorrhizal genera *Lactarius* and *Tuber* (by R Wang, A Guerin-Laguette, F-Q Yu, C Colinas)	
17:30 – 17:50	7-9. People & edible ectomycorrhizal mushrooms towards a traditional ecological knowledge based on DNA barcode & antioxidant properties: the case of dry dipterocarp forest, northeastern, Thailand (by C Phosri, W Gunnula, N Prophet, H Kokkaew, N Suwannasai)	

Congress Program (8/10)

23 October (Wednesday)

Time	Content	Room
(short break) 17:55 – 18:25 18:30 – 19:00	 Presentation by candidates of IWEMM11 (vote and discussion by scientific committee members) Closing remarks	 Parque (2nd Floor)
19:30 – 22:00	Gala dinner	Suimei (3rd floor)

24 October (Thursday): Field Excursion (1)

Time	Content	Remarks
8:30 – 10:00 – 11:30 12:00 – 13:30 15:00 – 16:00 17:30	Departure from hotel Matsutake market in Toyooka Village Lunch at the Horikoshi district in Toyooka Village, where we enjoy matsutake dishes as traditional local style Kosumo-nousan, a large mushroom cultivation factory of Nameko mushroom (*Pholiota microspora*) Arrival at RAKO Hananoi Hotel	Please gather in the lobby of RAKO Hananoi Hotel before 8:20.

25 October (Friday): Field Excursion (2)

Time	Content	Remarks
8:30 – 8:50 – 9:30	Departure from hotel Suwa Taisha Shrine (Kamisha Hon-miya), one of the oldest shrines in Japan: we see the traditional architecture of the shrine and learn long history of Shinto (Japanese indigenous religion) estimated for over two thousand years.	Please gather in the lobby of RAKO Hananoi Hotel before 8:20.

Congress Program (9/10)

25 October (Friday): Field Excursion (2)

Time	Content	Remarks
10:45 – 12:15	Matsumoto-jo Castle, one of the oldest castles exist at present in Japan: we see the traditional architecture of the castle at the Sengoku Period (16~17th Centuries)	
12:45 – 14:45	Lunch at Daio Wasabi Nouen Farm. After the lunch, we will visit the cultivated area of wasabi (Japanese horseradish).	
15:00 – 16:00	Azumino Winery	
17:00	Arrival at RAKO Hananoi Hotel	

Congress Program (10/10)

Post Meeting Fieldtrip: 26 ~ 29 October

Date	Time	Content	Remarks
26, Sat	8: 30 – 10:30 – 11:30 12:00 – 15:00 15:30 – 17:00 17:30	Departure from hotel Visit Hokto Corporation Lunch in Obuse Town, and visit Ganshouin Temple Visit Entoku Cultivation Factory of Ogiwara Group to see the large-scale cultivation system of mushrooms Arrival at hotel and check-in (Shiga Highlands, Yamanouch Town)	Please gather in the lobby of RAKO Hananoi Hotel before 8:20.
27, Sun	8: 30 – 9:30 – 11:30 12:30 – 16:00 16:30 18:00 – 19:30	Departure from hotel Visit Wild Snow Monkey Park in Yamanouchi Town Lunch at Nakamise Street (Ichiryu Manpei), and visit Zenkoji Temple Arrival at hotel and check-in (Nagano City) Dinner	
28, Mon	9: 00 – 11:30 12:30 12:30 – 17:00 17:30	Departure from hotel Arrival at JR Tokyo Station Deposit baggage in the hotel Traveling Tokyo Arrival at hotel and check-in	
29, Tue	9:00 – 17:00 18:00 –	Traveling Tokyo Dinner	

Keynote Lectures

(21st October)

10:25 ~ 12: 30 at Parque (2nd Floor)

Keynote Lecture 1

The anthropocene and the underground city of mycorrhiza

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In this time of environmental danger, fungi are both scourge and savior. The environmental simplifications of monoculture plantations have encouraged plagues of pathogenic fungi; the bath of antifungal toxins used against them have encouraged ever-more resistant strains. Fungi, working together with humans, have caused more species extinctions than any other type of organism. At the same time, fungi make the regrowth of plant life possible in the face of human disturbance, restoring forests and other complex ecosystems. The first half of this talk reviews a few of the roles that fungi have played in pressing forward, on the one hand, and holding back, on the other, the worst offenses of this epoch of human-caused environmental damage, the Anthropocene.

The second half of the talk asks how attention to fungi might allow scientists and ordinary people to work together to better appreciate life on earth. Mushroom picking has increased in popularity around the world. But neither lay people or scientists have paid enough attention to the possibilities of experiential learning involving fungal associations with root tips, which show not only species diversity but, even more importantly, interspecies interactions—the basis of the new ecological evolutionary biology. In some environments, such as sand, the diversity and beauty of root tip-fungi associations is accessible to observers without much equipment or special training. This talk argues that natural history experiences with this “underground city” can advance public understanding of the ways all species live only through interactions with each other.

Keynote Lecture 2

What will happen to ectomycorrhizal fungi in fragmented forests? - population genetic implications from endangered or relict forests -

Kazuhide Nara
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Key words: endangered species, population genetics, microsatellite, gene flow, hypogeous sporocarps

Forest fragmentation is becoming a serious issue for the conservation of wildlife inhabiting natural forests through "extinction vortex" that includes genetic factors. When gene flow between small fragmented populations is restricted, inbreeding inevitably increases within the populations, resulting in reduced genetic diversity and decreased fitness of the populations. Such inbreeding depression is one of the main factors threatening animal and plant species, but we know almost nothing about its effect on fungi. To explore fungal conservation genetics, we investigated population genetic structures of *Rhizopogon alpinus* and *Suillus spraguei* in the ice-age relict *Pinus pumila* forests, *Rhizopogon togasawariana* in endangered *Pseudotsuga japonica*, and *Rhizopogon yakushimensis* in endangered *Pinus amamiana* forests. These ectomycorrhizal (ECM) fungal species are host specific, while the host populations have been fragmented since the last ice-age or even much earlier, providing ideal opportunities for studying the effect of long-time fragmentation on ECM fungal populations. ECM roots and soil spore banks were sampled from the fragmented forests throughout the entire distribution range, and genotyped by newly developed microsatellite markers. We found that *Rhizopogon* populations were more genetically differentiated than the host trees in all the examined combinations. Significant inbreeding was confirmed in some *Rhizopogon* populations. In contrast, no genetic differentiation or inbreeding was found in *S. spraguei*. *Rhizopogon* that depends on animals for spore dispersal would be more vulnerable to forest fragmentation than wind dispersal *Suillus*. Moreover, our results indicate that extinction risks of these recently discovered *Rhizopogon* species may be higher than the threatened host species.

Topics: Conservation

Oral Sessions

(21st ~ 23rd October)

at Parque (2nd Floor)

Oral session 1: Anthropology and Mycology

Oral session 2: Sustainability, Conservation, and Global climate change

Oral session 3: Traditional knowledge and Economic importance

Oral session 4: Conservation, Biodiversity, and Ecology

Oral session 5: Japanese government project and its related research theses

Oral session 6: Molecular biology, Evolution and Phylogeny, and Biotechnology

Oral session 7: Cultivation

Oral session 1-1: Anthropology and Mycology

Mushrooms and humans: overview of matsutake and other edible mushrooms in anthropology and documentary films

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Key words: Matsutake, Edible mushrooms, Anthropology, Documentary Films, Society, Environment

As an introduction to the Mushrooms and Humans panel, this paper provides an overview of the recent anthropological studies and documentary films featuring edible mushrooms, with specific focus on matsutake, available in English. While there are extensive case studies on the relationship between humans and animals or humans and plants in anthropology, until recently anthropologists have not paid enough attention to mushrooms. In most of the existing social scientific studies, such as geography, development studies, and forest economics, mushrooms are mainly studied as an object of conservation for resource management. However, in the past two decades, in parallel to the increasing interest in mushrooms in popular literature and art, a growing number of anthropological studies on edible mushrooms have emerged. These studies analyze mushrooms as more than mere "resources" for economic revenue-making or sustainable development. They approach mushrooms as "guides" to explore the complex dynamics in social, cultural, and natural environments, reflecting the concerns of global climate change and the negative impacts of human civilizations accumulated in the past few centuries. These new approaches urge anthropologists to understand mushrooms holistically by learning from natural sciences along with traditional knowledge. The paper suggests productive future directions for anthropology and related social sciences, as well as collaboration across natural and social sciences.

Topics: Ecology, Sustainability, Conservation, Traditional knowledge

Oral session 1-2: Anthropology and Mycology

"Mushroom people": a documentary about mushroom pickers in the Pacific Northwest

Tristan Stoch
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Key words: Pacific Northwest, America, *Tricholoma, Cantharellus, Boletus, Tuber*

Mushroom People is a documentary film that examines human relationships to nature via the practices of mushroom pickers in the Pacific Northwest region of North America. The project began in 2017 and is slated for completion in 2021.The Pacific Northwest is a region conducive to the growth of many culturally and economically important mushroom species. These include, but are not limited to: *Tricholoma murrillianum, Cantharellus formosus, Boletus edulis,* and *Tuber oregonense*. This relative abundance of valuable mushrooms is due to both natural and anthropogenic factors. Structured as an environmental history of the region, the film discusses anthropological changes and their impacts on both wild mushrooms and the humans that gather them. Groups profiled in the film include: indigenous pickers keeping traditional ecological knowledge alive, immigrant groups who brought mushroom picking traditions from their home countries, academic mycologists who study fungi in the Western scientific tradition, mycological societies that encourage "amateurs" to study mushrooms, commercial pickers who sell mushrooms within a global commodity chain, and citizen scientists who are expanding mycological knowledge with the help of information technology. The film's thesis is that human-fungal relationships can foster ideologies where people are neither exploitative of, nor completely removed from, nature.

Topics: Ecology

Oral session 1-3: Anthropology and Mycology

The mushroom speaks - what kind of relationships can we imagine between the human and the fungal world?

Marion Neumann
Independent documentary filmmaker, Germany
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Key words: Film, Art, ecology, Interaction, Human-fungal relationships

In this presentation I will share my findings about possible relationships between the human and the fungal world which I have come across during the research process of my latest documentary in development: THE MUSHROOM SPEAKS. The film tells stories about people who explore the healing qualities of the fungal kingdom and its ability to regenerate, to transform and to re-connect with its environment. We discover the actions of the grassroot movement *Radical Mycology* with Peter McCoy from Portland; we think about possible collaborations between fungal beings and humans in the time of "anthropocene" in a conversation with the anthropologist Anna Tsing at her research site in Denmark; we go foraging with Ursula Weiher, an elder mushroom picker, who shares her extensive knowledge about edible mushrooms and their symbiotic relations in the Germany's Black Forest; and we learn about the potential of a medicinal mushroom with the work of neuroscientist Franz Vollenweider in a Swiss laboratory. These protagonists spread the spores of a growing change and the film traces a poetic journey towards a myco-cultural future, offering a reflection on our relationship to the natural world.

Topics: Ecology, Global climate change, Traditional knowledge, Cultivation

Oral session 1-4: Anthropology and Mycology

Foraging culture of mushrooms in Japan: background and recent trends

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Key words: History, Secondary vegetation (Satoyama), Traditional knowledge, Food culture, Commercial and non-commercial use, Underuse, Succession of knowledge

Japan is known as a "mycophilia" country. However, the species of mushrooms used, their abundance, and their culture varies; this is remarkably localized across Japan. According to an analysis of the mushroom species used in local communities, it appears that mushroom culture has historically been developed from the interactions between humans and vegetation, and most of it has relied on secondary vegetation, the so called "Satoyama." For instance, we can consider *Tricholoma matsutake* as a by-product of forest degradation caused by increasing population or developing industries. Subtle local differences in mushroom culture can be observed, especially in many relatively minor mushrooms. Except for a small number of mushrooms, such as *T. matsutake*, *Lentinula edodes*, and *Glifora frondosa*, most mushrooms are not widely distributed and have been consumed only domestically or locally. These conditions may be a factor of the cultural diversity, reflecting the localization, of mushrooms. However, for some decades, afforestation of Japanese cedar (*Criptomeria japonica*) or Hinoki cypress (*Chamaecyparis obtusa*), which are considered more profitable for commercial timber, and ecological succession have affected secondary forests. Therefore, ecological change is a significant threat to traditional mushroom culture. However, new knowledge about edible mushrooms has been widely distributed to the new generation through publications and the internet.

Topics: Ecology, Traditional knowledge, Anthropology

Oral session 1-5: Anthropology and Mycology

How the matsutake economy in Southwest China affects the social lives of the Yi people

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Key words: Matsutake, Anthropology, Ethnic Minorities, Economic importance, Social change

Matsutake mushrooms, highly valued in Japan for centuries, have since the 1980s become a major force in shaping the lives of groups living in Southwest China. China has become the world's most important source of matsutake, and for a number of people living in matsutake-reach areas, they have devoted themselves to their harvest, with serious impacts on their economy and their daily lives. In this talk, I explore how the matsutake mushroom trade is shaping the lives of one ethnic minority group in China, the Yi people, and how this wealth is fostering new form of social relations and generating new spaces for forms of ethnic resurgence.

Topics: Traditional knowledge

Oral session 1-6: Anthropology and Mycology

The status of matsutake production and trade in Japan

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Key words: Production statistics, Trade statistics, Supply chain, Foodways

Matsutake (*Tricholoma matsutake*) is one of the most expensive mushrooms in the world. Its flavor and texture has long attracted the Japanese, especially the aristocracy, making them feel the autumn aroma. It normally grows in the sunny pine forests throughout the Japanese archipelago. There used to be more than 6,000 tons of domestic matsutake production in the early 1950s. However, since the 1960s, its production has been sharply decreasing, and Japan currently only produces less than 100 tons of matsutake. Imported matsutake fills the gap between supply and demand. For example, from 2012 to 2016, Japan imported matsutake from 9 countries, whereas, its average self-sufficient ratio was only 4.4 percent. Chinese matsutake dominates with about 60 to 70 percent of the total supply in the Japanese market. This paper will explore two statistics, Trade Statistics of Japan and Non-timber Forestry Production Statistics, to provide general information on the matsutake industry in Japan.

Topics: ustainability, Conservation, Economic Importance

Oral session 2-1: Sustainability, Conservation, and Global climate change

Patagonia fungi, trails and tastes®. Fostering local development through identity valued wild edible mushrooms

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Key words: Mycotourism, Mycogastronomy, Non-wood forest products, Multiple purpose forestry

The western region of Patagonia (Argentina) has a wide strip of native subantarctic forest, and a forest-steppe ecotone area with exotic conifer plantations and meadow to the east. Edible wild mushrooms are one of the most diverse and abundant "non-wood forest products", although largely underutilized. Previous scientific and technical studies looking at the whole picture were conducted, dealing with diversity, taxonomy, ecology, nutritional and antioxidants profiles, post-harvest treatments, domestication, ethnomycology, among other topics. The joined production of scientific knowledge with knowledge transfer to harvesters and to the gastronomical and tourism sectors, allowed us to go forward and propose that the successful use of this non-timber forest resource will largely depend on a strategy to promote novel uses for mushrooms, linked to mycogastronomy, functional foods and mycotourism. These goals began to be successfully addressed from Patagonia Fungi, Trails and Flavors®, our technological and scientific platform that received political support from the six Patagonian provinces and the National Science Secretariat through a Bioeconomy grant. Since then, a coordinated strategy to achieve direct transfer to the entire value chain was established, generating outreach material including brochures, booklets, manuals, audio-video material and website publishing, and ICTs for mushroom identification and good harvest and post-harvest practices. Also public lectures, training, workshops, events, setting of mycotouristic trails, surveys among restaurants, and interaction with growers and chefs to develop new recipes and value added products are being performed. These activities involve the multiple use of Patagonia's forest environments, promoting their valuation and conservation.

Topics: Sustainability

Oral session 2-2: Sustainability, Conservation, and Global climate change

Multiproductive truffle culture in Spain. The 'Tubersystems' project

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Key words: Sustainable truffle farming, *Tuber melanosporum*, PGPR, Aromatic plants, Pollinators, Biological control

The TUBERSYSTEMS project started in 2019 with the general objective of developing and applying strategies to design sustainable, multiproductive black truffle (*Tuber melanosporum*) agrosystems in Spain. The proposed management techniques will be based on the knowledge of the ecology of truffle-producing systems (plantations and forest areas), including the dynamics of pollinators and natural enemies of pests, the interactions of the fungus with the companion plants, and the associated microbial communities. Inoculations with selected rhizospheric microorganisms, mix cropping systems with aromatic plants and establishment of refuge plants for beneficial entomofauna will be implemented in experimental plantations. We hypothesize that sustainable multiproductive systems, based on the know-how of the interactions between macro and microorganisms, will provide an improvement of the ecosystem services related to truffle farming, including not only tangible goods as truffles, but also non-material benefits related to the agrosystem regulation and socio-cultural values. The TUBERSYSTEMS project is structured in two complementary sub-projects with three general objectives: i) the study of the diversity of macro and micro-organisms and their interactions in truffle producing systems, ii) the selection and management of these organisms for the establishment of multiproductive truffle plantations with aromatic plants, and iii) the analysis of the ecosystem services associated with different truffle producing systems. Here we present a general overview of the project in the framework of the truffle culture in Spain, a description of the main outlined tasks, and the methods involved. The generated knowledge-based management guidelines will be applied to promote a sustainable truffle culture.

Topics: Sustainability

Oral session 2-3: Sustainability, Conservation, and Global climate change

Sustainability of edible mycorrhizal fungi and their trees in Ireland (the land of the setting sun!)

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Key words: Ecology, Habitats, Conservation, Canagement, Croadleaf woodlands, Conifer forestry

Ireland is a temperate Atlantic island of 84,000km^2 to the west of the Eurasian continent. Only approximately 10% of land is currently under trees and ⅔ of this is commercial, alien species conifer forest that experiences clearfelling in circa 35-40 year cycles. Results from over 20 years of study in Irish broadleaf woodlands and conifer forests are presented. The naturally depauperate flora of Ireland features such mycorrhizal tree genera as *Quercus*, *Betula*, *Corylus* and *Pinus sylvestris*. *Fagus sylvatica*, though not considered native, is important for ectomycorrhizal fungi particularly *Russula*, *Boletus* and *Tuber*. Removal of *Fagus* for pure native woodlands is problematic. Minor non-native conifer trees such as *Larix* can be locally important for Boletales. Main edible mycorrhizal fungi include members of the Genera *Cantharellus*, *Craterellus*, *Hydnum*, *Lactarius*, *Laccaria*, *Tuber* and Order Boletales. Notable interactions between these edible fungi and Irish fauna will be discussed. The history of Irish woodland management precluded traditions relating to fungi being passed down. Land use stability in some colonial estate woods and forgotten pockets of land has meant that ectomycorrhizal communities have survived. Threats to extant and future ectomycorrhizal populations and diversity in Ireland are clearfelling, phytosanitary lapses leading to alien pest and disease introductions, spread of *Armillaria mellea* agg. and *Hedera helix*, use of fertilizers and biocides, as well as lack of conservation. Planting or embellishment of new woods with potential for edible ectomycorrhizal associates may rely on spore and mycelium banks in soil or on sustainable mycorrhization programmes with appropriate local material.

Topics: Sustainability

Oral session 2-4: Sustainability, Conservation, and Global climate change

Isolation, identification, and utilization of fungi associated with *Tristaniopsis obovata* grown in a heath forest ecosystem, Bangka Island, Sumatra, Indonesia

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Key words: Ectomycorrhiza, Edible Mushroom, Heath forest, Inoculation, Shoot cutting

Tristaniopsis obovata (Myrtaceae) associates with ectomycorrhizal (ECM) fungi to produce wild edible mushrooms. Also, their flowers are a source of nectar for forest bees that produce wild bitter honey with high economic value. The current problem in the heath forest ecosystems is the conversion of the forest to oil palm, pepper plantations and open tin mining areas, which have reduced the number of the original forests. The aim of this study was to obtain information on the potential of edible fungi associated with *T. obovata*, as well as the ability of selected fungi to stimulate growth of *T. obovata* seedlings. The studies involved the collection of seeds/seedlings from natural sources, isolation and molecular identification of fungi associated with *T. obovata* through ITS rDNA analyses, seedling propagation trials using shoot cuttings with KOFFCO (Komatsu FORDA Fogging Cooling) System, and inoculations of potentially ECM fungi to improve the growth of seedlings at the nursery. Preliminary results of DNA identification showed that the ECM edible fungus *Heimioporus retisporus* (Boletaceae) was associated with *T. obovata*. Other ECM fungi detected were 4PK1 (Corticiaceae), 17BK2 (Corticiaceae 2), and 24PK4 (*Cortinarius* sp). The ECM inoculation affected plant growth and nutrient contents of *T. obovata* seedlings. The propagation of shoot cuttings through KOFFCO system was effective for *T. obovata* seedlings production, with a survival rate about 50%. The results of this study revealed the basic role of science and technology to conserve and sustain the productivity in heath forests ecosystems.

Topics: Cultivation, Conservation, and Sustainability

Oral session 2-5: Sustainability, Conservation, and Global climate change

Environmental niche modelling and climate change scenarios used to predict the likely distribution of truffle species for the period 2071-2100

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Key words: Environmental niche modelling, Climate change, Cultivation, *Tuber aestivum, Tuber melanosporum,* Temperature, Rainfall, Oaks, Hazel

Truffles are the hypogeal fruiting bodies of fungi from the genus *Tuber*. Held in high regard for their culinary properties, the two most widely cultivated are the Périgord truffle (*Tuber melanosporum*) and the summer or autumn truffle (*Tuber aestivum syn. uncinatum*). Both species are ectomycorrhizal and span Mediterranean and temperate climates where seasons are well defined, but not characterized by extremes. Within the climatic parameters in which these two species occur, there is known sensitivity of fruiting to changes in summer precipitation and summer temperatures, to name just two. Indeed, the sensitivity is such that individual climatic events can significantly impact the annual fruiting quantities in any given year. For the areas in which these species exist, current climate change models combined with historical datasets presents a large reduction in harvests. Although the suitability of current areas in which these species occur may reduce under climate change, the suitability of other areas may increase. Using extensive species distribution datasets here we present high-resolution environmental niche modelling to show the current suitable zones for these species. Incorporation of up-to date climate change models and new data on fruiting of both species at the climate extremes, allows us to present likely distribution maps of both species for the time period 2071–2100. The results will be relevant to those working in the field and will allow those in the industry to help plan for cultivation in a changing climate. Further, the methodology presented here may be applied to other ectomycorrhizal species.

Topics: Global climate change

Oral session 3-1: Traditional knowledge and Economic importance

Traditional knowledge on *Lyophyllum* species in central Mexico: its contribution to identify new species

Cristina Burrola-Aguilar[1], Yolanda Arana-Gabriel[1], Roberto Garibay-Orijel[2], Adriana Montoya-Esquivel[3], Maricarmen Jaime-Salinas[3]
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Key words: Differmia section, Rural communities, Management, Edible mushrooms, Cultural importance

Worldwide, *Lyophyllum* genus, in particular species in the *Differmia* section, are known for their culinary, economic, medicinal and alimentary importance. This section comprises *L. fumosum, L. decastes, L. shimeji* and *L. loricatum*; species able to form facultative ectomycorrhizas or to complete their life-cycle as saprobes. The objective of this study was to compile the traditional knowledge existing in Mexico about *Lyophyllum*, as well as to determine the true taxonomic identity of species with higher cultural importance. We conducted phylogenetic analyses for specimens obtained from markets and temperate forests in central Mexico. It was determined that in Mexico the mushrooms commonly known as "clavitos" and "xoletes" have been reported as *L. fumosum and L. decastes*. These species are highly appreciated by rural communities and have a great cultural importance, although they are not abundant, occurring in *Pinus* sp. and *Abies religiosa* forests. The taxonomic and phylogenetic analyzes placed them in two clades in the Differmia section, an undescribed clade sister to *L. shimeji* and another undescribed clade sister to *L. fumosum*. Thus, both species have been erroneously assigned to Eurasian taxa based solely on their morphology. This type of study allows us to broaden the knowledge of mushroom biocultural resources harvested by people from rural communities and to identify species with cultivation potential, for consumption and marketing purposes.

Topics: Traditional knowledge

Oral session 3-2: Traditional knowledge and Economic importance

The economic importance of possible developments in the U.S. truffle industry

Brian Upchurch [1], Olivia Martin [2], Don Detmer [3], Diana Hudson [4], Raymond Prince [5]
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[2] Immediate past-President of NATGA
[3] Vice-President of NATGA, Professor of Medical Education, University of Virginia
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Key words: Truffles, Imports, Exports, Demand, Domestic growers, USDA

Demand for truffles in the United States has grown as American consumers have increased their appreciation of these mushrooms as a culinary delicacy important to many of the world's finest cuisines. Despite some success by domestic growers, most of the increased consumption of truffles has been satisfied by imports. As a result, the United States has become an important export market for truffle producers in France, Italy, Spain, Australia, New Zealand and other countries.

This situation may be on the brink of change as several commercial farms – along with individuals - are examining the feasibility of producing truffles on a large scale. If successful, American growers could have an important economic impact on growers in other countries.

Currently, little information about the U.S. truffle industry, and any changes that may be occurring, is available. A recent status report by Y. Wang and Y.L. Chen, for example, does not include developments in the U.S. truffle industry such as the production of *T. borchii* and *T. lyonni* (see "Recent Advances in Cultivation of Edible Mycorrhizal Mushrooms"; *Mycorrhizal Fungi: Use in Sustainable Agriculture and Land Restoration*; Springer 2014; Z. Solaiman, L. Abbott, and A. Varma, eds.).

The report for presentation at the IWEMM10 Conference will include:

A. The latest information concerning the current status of the U.S. truffle industry including imports, exports, domestic production and final consumers;
B. Estimates of the trends in domestic supply and demand;
C. Activities of NATGA and other private organizations supporting basic research and the cultivation of mycorrhizal mushrooms in the United States; and
D. United States Department of Agriculture's efforts to improve data on the cultivation and consumption of mycorrhizal mushrooms.

The data will be collected primarily by officials of NATGA from agencies, researchers, importers and individual growers. The purpose of the report is to provide useful information about the U.S. market to growers, importers, and investors inside and outside the United States.

Topics: Economic importance

Oral session 3-3: Traditional knowledge and Economic importance

A coordinated management of forest mushroom collection areas improves the efficiency and sustainability of its mycotourist use

Fernando Martínez-Peña[1, 2], Joaquín Latorre Minguell [2], Beatriz Rodríguez-Prado[3], Pablo de Frutos[3]
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Key words: Wild edible fungi, Forest management, Mycotourism, Forest multifunctionality

Nowadays, forest management aims to diversify multifunctionality including new ecosystem services based on mycological resources as mycotourism. In some European regions as Castilla y León (Spain) wild edible mushrooms represents 10% of total forest production and mycotourism supposes 40% of the total value generated by this wild edible mushrooms (65 million euros/year). The study has analyzed the demand for mushroom collection permits, in each of nine areas regulated by the mycology program of Castilla y León (www.micocyl.es) between 2013 and 2016 in a total regulated surface of 402,893 hectares. The study shows the important change in the way of use of the resource by the collectors, who give more and more importance to the recreational aspects and less to the commercial ones. It is an activity increasingly linked to luxury and more demanded by collectors as their income increases. Secondly, the study shows that shared management of regulated areas is always preferable to the individual management of each of the owners. This result is related to the way in which the collectors of the different collection areas respond to the price at which the collection permit is put up for sale. Finally, it can be considered that there is a significant margin of increase in permit prices in some areas. On the other hand, there are other regulated areas that have almost appropriated the entire Marshallian consumer surplus with their charging system for the collection of edible wild mushrooms and that would not have room to raise those sales prices of the collection license. The evidence found supports the idea that the current permit rate system, differentiated by type of collector, is an appropriate tool to address this duality in the profiles of collectors who continue to coexist in the forests of this region of southwestern Europe.

Topics: Economic importance, Sustainability.

Oral session 3-4: Traditional knowledge and Economic importance

Picking edible mycorrhizal mushrooms (EMM) for a living

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Key words: Harvest, Edible Mycorrhizal Mushroom, Living

With the rapid development of science, technology and industry, human beings lives have changed dramatically. However, modern human beings are not fundamentally different from their barbaric ancestors who made a living from collecting and hunting. That is to say, the food is supreme that has remained unchanged since ancient times. Food comes from planting and picking. The proportion of food picked for human use is now small, but humans still can't live without picking. The wild edible fungi are the most important picking foods until now, of which over 70 % are mycorrhizal. Yunnan Province of China is a good example to illustrate the importance of the EMMs to human beings. Yunnan Provence has 760 species of wild edible mushrooms (WEM), with the most important EMM species, such as *Tricholoma matsutake*, truffles, *Boletus edulis, Cantharellus cibarius, Lyophyllum shimeji* and others. Total natural production of EMM is estimated at 500,000 tons and their harvest is up to 100,000 tons/year. The wild edible fungus market is spread all over the province. Wild edible fungi are important livelihoods for mountain farmers. There are 30,000 families who have harvested wild edible fungi in Yunnan Province. The income from harvesting wild edible fungi can account for (10)-20-70(-90) % of a farmer family's cash income. The per capita annual income of harvesting wild edible fungi is over RMB1,000, and the maximum is more than RMB10,000 (a farmer family). Many farmers rely on the cash income from harvesting wild edible fungi to build up homes, send their children to school, seek medical treatment, and purchase production tools

Topics: Economy

Oral session 4-1: Conservation, Biodiversity, and Ecology

Wild edible and mycorrhizal fungi conservation programs in Israel

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Key words: Wild fungi, Conservation, Red list

Many emerging challenges on mushrooms mycobiota conservation have arisen as a result of climate change, pollution, over-exploitation and habitat destruction. Due to the important ecological roles wild mushrooms play in nutrient cycling, a general awareness towards conservation of wild fungi is globally underway. The declining wild mushroom yields across Europe generated much interest and a national red-Listing project started in 1982 that determined that at least 10% of European macrofungi are threatened with extinction, mainly due to changing land use and increasing nitrogen deposition. The mushrooms mycobiota in Israel is very unique and varied due to the country geographical location. Over 600 wild mushroom species have been reported in Israel, some are endemic and many of them are mycorrhizal. The mushroom mycobiota in Israel is protected only in nature reserves and in National Parks, and it could be that many of the prevailing species are endangered and face extinction. One of the most remarkable successes in nature preservation in Israel was passing the law protecting the wild flowers in Israel that was legislated in 1964. Before 1964 many wild flowers were picked, sold and even exported abroad. These caused a huge damage in wild flower populations. Since 1964 many wild flower populations have recovered as it can be witnessed during the blooming months in the Israel spring. Inspired by this law, we have initiated a project aimed at protecting endangered wild mushroom populations in Israel. The project includes the involvement of scientists, mycological amateurs together with the Jewish National Fund and the Society for the Protection of Nature in Israel. The project aims to identify endangered species and create a red list of species and habitats that need protection. For that we will survey current populations and compare to previous herbarium mushroom fruiting records. The possibilities to protect the unique Israeli mycobiota will be discussed.

Topics: Wild mushrooms conservation

Oral session 4-2: Conservation, Biodiversity, and Ecology

Discovering a new mycogeographic region and new edible mycorrhizal mushroom species in Guatemala

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Key words: Boletales, Russulales, Mesoamerica, pine-oak forests, ectomycorrhizae

Guatemala has often been considered an extension of the distribution area of northeast American mycorrhizal mushrooms, but recent local studies indicate that it is a partial reality. After visiting many locations, particularly pine-oak forests in various parts of the country, we conclude that Guatemala, despite its small extension, has two main mycogeographic regions, both with many endemic mycorrhizal fungal species. The first one is located in the west highlands, with plant and fungal species more related to the northwest of the continent such as *Boletus edulis, Turbinellus floccosus*, *Lactarius aestivus, Rhizopogon ocrhaceorubecens.* The second one, less known, covers the eastern part of the country and could extend to the west highlands of Honduras and El Salvador and contains species such as *Boletus guatemalensis, Aureoboletus singerii*, *Lactarius salmoneus, Tylopilus leucomycelinus.* The origin of these species can be explained by important geological events and geographic isolation since the Cretaceous, as well as the permanence and evolution of Pinaceae and Fagaceae on the different soils in Nuclear Central America. We have found the largest part of undescribed species, especially in Russulales and Boletales (with almost all the recently described genera in Boletales like *Butyriboletus, Caloboletus, Heimiella, Retiboletus, Sutorius, Tylopilus and Xerocomellus*) in the eastern region. Phylogenetic analyses confirm high fungal diversity and endemism in Guatemala, including some new genera. To contribute to the conservation of this surprising diversity, menaced by deforestation and climatic change, we recommend establishing international cooperation projects that include social and environmental benefits, especially those associated to access to water.

TOPIC: Biodiversity

Oral session 4-3: Conservation, Biodiversity, and Ecology

Halimium as an ectomycorrhizal symbiont. A new look at Mediterranean ectomycorrhizal communities

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Key words: Ectomycorrhiza, Maquis shrubland, Mediterranean biome, *Cistaceae*, Molecular phylogeny

Shrublands cover vast swaths of the Mediterranean basin. The plants occurring in this environment characterized by stresses like drought and fire play a critical role in the Mediterranean ecosystem, protecting soil from erosion and desertification, acting as nurse species for tree seedlings and favoring the establishment of late-successional species. To perform such tasks, the shrubs and small trees that make this vegetation system developed adaptations, including the association with a vast number of ectomycorrhizal fungi, mainly associated with *Cistaceae*. While this has been widely recognized for *Cistus*, the allied genus *Halimium* has not received significant attention. The genus, whose centre of diversity is in the western part of the Mediterranean basin, comprises 13 accepted species. *Halimium* species are generally found in open vegetation types, like matorral shrublands and garrigues, or at the verges of woods, in degraded forest areas, abandoned fields, pasturelands, and also on coastal sandy soils. To fill the knowledge gap on *Halimium* mycorrhizal biology we recently described the morpho-anatomical and molecular features of the ectomycorrhizae formed by *Halimium halimifolium* with *Scleroderma meridionale* as collected from coastal dunes in Sardinia (Leonardi et al, Symbiosis 76: 199-208, 2018). We also performed a study of the ectomycorrhizal community in pure *Halimium* stands in south-western Sardinia. Our data, along with a thorough analysis of literature records, allowed us to draw a surprisingly rich picture of *Halimium* associated ectomycorrhizal fungi, with more than 70 recorded species, including uncommon or rare taxa, like *Gyroporus pseudolacteus*, *Cortinarius coeruleopallascens* and *Lactifluus brunneoviolascens*.

Topics: Biodiversity

Oral session 4-4: Conservation, Biodiversity, and Ecology

Species diversity and utilization of edible ectomycorrhizal mushrooms in Pha Wang Nam Kaew Forest Reserve

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Key words: Wild mushrooms, Ethnomycology, Mycorrhizal mushrooms

Pha Wang Nam Kaew Forest Reserve, Nakhon Ratchasima Province has a richness of forest resources, especially wild mushrooms. Local villages located around the forest reserve intensely utilize this source of wild mushrooms. This situation has raised concern about the sustainability of wild mushroom biodiversity for the future. Therefore, the objectives of this research were 1) to study the diversity of wild edible ectomycorrhizal mushrooms (WECM) and 2) to study the patterns of utilization of local communities on the biodiversity of WECM in the forest reserve. The survey was conducted during rainy season in 2015-2018. The questionnaires were distributed to villages and mushroom buyers were randomly selected. Moreover, general observations were made and in-depth interviews with key informants were conducted. The results revealed that 20 species of WECM were found around Pha Wang Nam Kaew Forest Reserve. They were taxonomically classified into 5 genera: *Amanita*, *Astraeus*, *Craterellus*, *Lactarius*, and *Russula*. The edible mushrooms, which had the highest frequency of occurrence was *Russula* cf. *emetica*. The majority of local people usually collected wild WECM in the forest reserve for selling. High prices were realized for prized species, especially the *Amanita,* which ranged from 50 THB and up to 400 THB/kg. The average amount of wild mushroom collected was 16.7 kg/household/year. Overall, local people had positive attitudes towards wild mushroom biodiversity conservation. The majority of local people mentioned that income from wild edible mushroom was an important source of revenue for their families. However, about 60% of local people thought that collecting wild mushroom would not be sustained in the future.

Topics: Biodiversity

Oral session 4-5: Conservation, Biodiversity, and Ecology

Management of Mediterranean *Cistus ladanifer* scrublands and *Boletus edulis* production (NW Spain): what we know after 15 years of study

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Key words: Mediterranean ecosystems, *Cistus ladanifer*, *Boletus edulis*, Edible mushrooms, Fungal diversity, Mycorrhizal fungi, Long-term studies

The Mediterranean *Cistus ladanifer* scrublands in northwest Spain have been traditionally considered unproductive. However, several native plants serve as hosts to highly-valued mycorrhizal mushrooms, such as *Boletus edulis*. Fungal harvesting is an important socioeconomic resource for countryside areas. Rural inhabitants are active collectors and mycotourism is a rising activity. *Cistus* scrublands can enhance the local economy if we control the risk of forest fires, a common contingency that requires proper forest management. During the last fifteen years, our research group has been leading several projects to make an in-depth investigation of the ectomycorrhizal relation between *C. ladanifer* and *B. edulis*, and the drivers for sporocarp production. We summarise here global aims, relevant results and future steps. The first stage was focused on fungal productivity and richness, and their relation with fuel reduction treatments. A 50% clearing was the best treatment for edible mushroom production, particularly *B. edulis*. *Boletus* yields were extremely variable over the years and mycelium dynamics in soil showed a positive correlation with sporocarp production, using qPCR as a prediction tool. We also studied bacterial communities in soil (DNA metabarcoding) to disentangling mycorrhizal relationships. Currently, we are working on optimising an *in vitro* protocol for the mycorrhization of *B. edulis* with *C. ladanifer*, testing the effects of co-inoculation with helper bacteria found in the most productive sites. All these findings bring us closer to controlled *B. edulis* production in wild plantations, the final objective of this long-term project. Plantation management will generate a positive economic impact in rural areas and will place high valued products in the market.

Topics: Economic importance

Oral session 4-6: Conservation, Biodiversity, and Ecology

The impact of silviculture practices in belowground fungal communities: a case study in Mediterranean *Quercus pyrenaica* forests (NW Spain)

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Key words: Forest management, Fungal community ecology, Fungal functional groups, DNA metabarcoding, Illumina sequencing, Edible mushrooms, Mediterranean ecosystem

Quercus pyrenaica is the most abundant deciduous tree in Spain under continental Mediterranean climate. However, as far as we know, studies regarding biodiversity and ecology of fungal communities in relation to forest management are limited at belowground level. We tested the impact of forest management and conservation on the richness and composition of taxonomical and functional groups of fungi in Northwest Spain, with special attention to edible mushrooms. We sampled three stand-structures linked to forest management: (1) young- or medium-size stands, lacking of silvicultural management; (2) coppice woodland under silvicultural intervention in the last 15 years; and (3) old-growth stands with open woodland structure (*dehesa* type) under silvopastoral use. Total fungal richness was similar in all forest types, but differences among taxonomic and functional groups in forest types were detected. Ascomycota shows higher proportional richness in *dehesa* type forests and Basidiomycota are less represented in young stands. Mycorrhizals are the most represented guild, followed by saprotrophs. Ectomycorrhizal and lichenized fungi show higher richness in coppice woodland under silvicultural intervention. With respect to edible mushrooms, total richness was similar in all forest types, but differences in relative abundance values were found. Relative abundance was higher in young forests without management and lower in *dehesa* type forests. Particularly, several high valued edible mushrooms were only found in unmanaged forests (*Boletus edulis*, *B. reticulatus*). In-depth study of fungal communities linked to forest management will help us to propose a science-based management guideline to facilitate the exploitation of edible mushroom resource in rural areas.

Topics: Biodiversity

Oral session 4-7: Conservation, Biodiversity, and Ecology

Documenting the ectomycorrhizal fungi of *Quercus garryana* at the northern extent of its range

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Key words: Garry oak, Oregon white oak, *Tuber* aff. *candidum*, *Tuber beyerlei*

Quercus garryana is native to western North America and its range extends from mid-California in the south to southern British Columbia in the north. Considerable study of *Q. garryana* in the US, especially southern Oregon, by Darlene Southworth and her students and colleagues has detected over 40 species of fungi forming ectomycorrhizas and about 35 species as sporocarps found fruiting with these trees. Until now, very little has been published on the ectomycorrhizal fungi of *Q. garryana* at the northern end of its range on Vancouver Island. In 2016, it was reported that the exotic *Amanita phalloides* forms ectomycorrhizas and fruiting bodies with *Q. garryana* on southern Vancouver Island, raising the possibility of spread of this poisonous mushroom into the local forests. As part of that study, a small number of additional ectomycorrhizal fungi were documented. Since then, we sampled ectomycorrhizas of *Q. garryana* at two additional locations, sequenced their ITS regions, and compared our results with those from Oregon. One of the striking attributes of the ECM community of *Q. garryana* both in BC and the US is the relatively large number of ascomycetes, including species within Pezizaceae, Pyronemataceae, and Tuberaceae. Several species of *Tuber* were detected including *Tuber beyerlei* and an undescribed species in the *candidum* group. Further work to identify the species of ectomycorrhizal fungi of *Q. garryana* in BC will likely require expansion of the molecular database of local macrofungi to improve matches with the sequences from the ectomycorrhizas.

Topics: Biodiversity

Oral session 4-8: Conservation, Biodiversity, and Ecology

Bacteria and truffle: a complex relationship

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Key words: Truffle, Bacteria, Ascocarp development, Aroma, Cultivation

The association between bacteria and truffle was demonstrated by numerous studies. They are present in all phases of the truffle biological cycle and in the surrounding soil. Microbial communities associated to truffles are different depending on *Tuber* species, life cycle stage (mycorrhizas, ascocarps, mycelium) and ascocarp development stage. They also differ depending on the characteristics of the soil and on the geographical area. They therefore should have a key role in any stages of truffle development. It was also demonstrated that they contribute to the truffle typical aromatic bouquet. For these reasons many studies have been done and many projects are underway in order to better understand their roles. Recently, a typical red chromatic alteration of the prized Italian white truffle has been attributed to some bacterial species, which, although not altering the ascocarp aromatic properties, changes their appearance, questioning their commercial value. Various studies are in progress in the field to assess the influence of bacteria on the development of mycorrhizae and fruiting. A research project funded by the Marche region and European funds was set up with the aim of developing new cultivation methods and new strategies to implement the production of natural Italian white truffle grounds through the soil inoculation of selected bacteria.

Topics: Biodiversity

Oral session 4-9: Conservation, Biodiversity, and Ecology

Comparing soil fungal and bacterial communities in truffle-productive orchards of Australia and Europe

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Key words:

Given their irresistible aroma and unpredictable supply, truffles in the genus *Tuber* are one of the most expensive foods. These ectomycorrhizal fungi are also cultivated successfully worldwide. Recently, Australia has become the top producer of *Tuber melanosporum* (black truffle), a species native to Europe. Species may thrive when introduced into a non-native habitats due to a release from natural competition. In this study, we aimed to test the competition release hypothesis by characterizing fungal and bacterial communities in truffle plantations in native (European) and non-native (Australian) habitats. In total, we sampled 650 soils from 27 orchards (n=20-25/orchard). DNA was extracted from soils and the 16S and ITS ribosomal DNA and was sequenced with Illumina MiSeq resulting in 23,860,471 ITS and 30,941,017 16S demultiplexed reads. Sequences were quality filtered and clustered into 13,168 fungal and 16,559 bacterial Operational Taxonomic Units (OTUs) with the UPARSE pipeline. NMDS and PERMANOVA showed that fungal and bacterial communities are structured by country of origin. *Tuber melanosporum* had a higher relative abundance in Australian soils (10.4%) compared to European soils (7.0%), but was the most dominant species under productive trees in both continents. Truffle microbiomes at productive sites in both continents also included the non-mycorrhizal fungi *Fusarium*, *Cryptococcus*, *Mortierella*, and *Ilyonectria* spp. and bacterial (Chloroflexi) and archeal (Nitrososphaeraceae) taxa. Overall, ectomycorrhizal fungi had three times fewer OTUs and a lower relative abundance in Australian soils, consistent with the competition release hypothesis.

Topics:

Oral session 4-10: Conservation, Biodiversity, and Ecology

Morphological and microbiome variation of *Tuber pseudobrumale* in southwestern China: widening the repertoire

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Key words: Black truffle, Excavated truffle, Microbial diversity, Ecotype, Geographic origin

The knowledge of Chinese truffles is far to be complete. *Tuber pseudobrumale* is an excavated black truffle known so far only from its type collection in southwestern China. By studying in detail collections of different geographic origin, a conspicuous macro- and micromorphological variation in this marketed species, including wart shape and size, peridium colour and spore size, was detected. This morphological variation contrasted with the strong phylogenetic consistency shown by ITS analysis. By using high through-put sequencing analysis, the structure and putative function of the microbiome of both peridium and gleba of different collections of *T. pseudobrumale* was also explored for the first time. Bacterial and fungal diversity was higher in the peridium than in the gleba according to Shanon, Chao1 and Simpson diversity indexes. Similarly, with respect to other truffle species, the bacterial community in *T. pseudobrumale* was enriched of and dominated by Proteobacteria, Actinobacteria and Firmicutes, irrespective of geographic origin. However, unlike other *Tuber* species, the gleba was dominated by the bacterial genera *Arthrobacter, Cupriavidus, Enterobacter* and *Pseudomonas*; and by the fungal taxa Saccharomycetes, Tremellomycetes and Sordariomycetes. Principal component analysis and non-metric multidimensional scaling (NMDS) showed a clear trend toward clustering among microbiome communities of peridium and gleba from collections sharing the same geographic origin. Functional differences of the microbiome as for carbohydrate, lipid and amino acid metabolism were shown among collections from different sites. This study presents insights into the variation in inter- and intraspecific truffle morphology, microbiome composition and putative functions related to geographic origin.

Topics: Ecology

Oral session 4-11: Conservation, Biodiversity, and Ecology

Isolation and characterization of bacteria from fruiting bodies of *Rhizopogon roseolus*

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Key words: Bacteria, Ectomycorrhizal mushroom, Fruiting body, *Pinus thunbergii*, *Rhizopogon roseolus,* Shoro

Rhizopogon roseolus (Corda) Th. M. Fr. is an edible ectomycorrhizal (ECM) mushroom. Bacterial strains promoting the development of mycorrhizal symbiosis defined as mycorrhizal helper bacteria (MHB) have been frequently isolated from mycorrhizospheres. Recently, we have isolated certain bacteria from fruiting bodies of *R. roseolus* and showed that the bacteria exhibited stimulation on mycelial growth. However, there have been few studies on bacteria from fruiting bodies of *R. roseolus*. In this study, we isolated a lot of bacteria from fruiting bodies and investigated their effect on mycelial growth of *R. roseolus* using co-culture method. In addition, we examined the effect of combined inoculation with *R. roseolus* and bacteria on mycorrhization and growth of *Pinus thunbergii* seedling. Among nineteen cultivable bacteria isolated from the fruiting body of *R. roseolus*, six bacterial species significantly stimulated the mycelial growth. The combination of inoculum of *R. roseolus* and the bacterial strain GIB024 varied greatly in their ability to colonize *P. thunbergii* roots. Moreover, the bacterial strain GIB024 without *R. roseolus* gave the highest growth of host seedling as root dry mass, total dry mass and Root/Shoot ratio after inoculation for 60 days. These results suggest that bacterial strain GIB024 will be useful for successful production of ectomycorrhizal seedlings.

Topics: Cultivation

Oral session 4-12: Conservation, Biodiversity, and Ecology

Streptomyces sp. A11, an ectomycorrhiza-associated actinobacterium has different effects on various fungal growth but promotes mycorrhizal formation

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Key words: Actinobacteria, Ectomycorrhiza, Growth, Host plant, Interaction

Interactions between mycorrhizal fungi and actinobacteria are particularly common in the rhizospheres of mycorrhizal plants. A better understanding of the roles of mycorrhiza-associated actinobacteria in host plants would open an alternative avenue for exploring new values from forests. In this study, we isolated a mycorrhiza-associated *Streptomyces sp*. A11 strain from the ectomycorrhizal fungus (ECM), *Suillus luteus* growing symbiotically with container seedlings of *Pinus densiflora*. First, we evaluated the antifungal capacity of A11 on various pathogen and ectomycorrhizal fungi growing on agar media. Then we investigated the impacts of A11 on mycorrhizal formation and host growth by quantifying plant growth parameters and nutrient uptake in microcosms. The results showed that A11 inhibited the growth of several pathogenic fungi whereas inhibition of ectomycorrhizal fungal growth was less evident. The inhibitory effect depended on the medium used to cultivate A11 but also the solvent used for the extraction on the agar media. From the microcosm trial, we found that A11 significantly promoted mycorrhizal formation between *S. luteus* and *P. densiflora* seedlings by 4 weeks after inoculation. Moreover, 14 weeks after inoculation, the above-ground biomass of seedlings growing on the substrates containing both *S. luteus* and A11 was significantly greater than those inoculated with only *S. luteus*. The addition of A11, however, seemed to have no influence on nutrient (N and P) uptake by the seedlings. This study suggests that ECM-associated *Streptomyces sp*. A11 can enhance mycorrhizal colonization and host plant growth; possibly even enhancing species competitiveness.

Topics: Biodiversity

Oral session 5-1: Japanese government project and its related research theses

Researches for the cultivation of *Tricholoma matsutake* in Japan

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Key words: Matsutake, Ecology, Physiology

Matsutake, the fruiting body of *Tricholoma matsutake*, has evoked the taste of autumn since ancient times in Japan. Approximately 1200 years ago, a matsutake foray in the vicinity of Nara, an old capital city of Japan, was described in the Man-yo-shu, an ancient anthology of Japanese poems. *Tricholoma matsutake* is an ectomycorrhizal species associated with pine, and the area in which matsutake can be found has increased along with the expansion of Japanese red pine forests. In the early 1940s, about 12,000 Mg of *T. matsutake* was harvested. However, over the years, the production has decreased drastically to less than 100 Mg per year. This decline may be due to the changing life styles and movement of the population to urban areas, leading to inadequate management of pine forests and subsequent infestation with pine wilt disease, which damages pine trees and decreased the population of *T. matsutake*. Accordingly, the price of matsutakes has also increased. Because of the decrease in the domestic harvests, matsutakes have been imported from foreign countries to meet the domestic consumption. Recently, greater than 90% of the domestic consumption has been satisfied with imported matsutakes, whose prices are lower than those of domestic matsutakes. There have been many trials for the cultivation of matsutake in the field. However, no matsutakes were obtained after these trials, except for few cases in which a fruiting body occurred after planting trees infected with *T. matsutake*. We review research on matsutake, its ecology and physiology, and the results of trials for the artificial cultivation of matsutake in the field.

TOPIC: Cultivation

Oral session 5-2: Japanese government project and its related research theses

Sustainable harvest of matsutake: thirty-eight years chronological data in relation to pine forest management in Nagano, Japan

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Key words: Matsutake mushroom, Edible mushrooms, Cultivation, Environment managed, Weather conditions

Management of pine forests has been considered important for the production of matsutake (fruit-bodies of *Tricholoma matsutake*), a highly prized edible mycorrhizal fungus in Japan, but there is still scant scientific evidence supporting this. In the present study, we showed the positive effect of long-term forest management on matsutake production. In 1980, we established a 0.5 ha research plot in a matsutake-productive pine-dominated forest site (ca. 20–30-years old trees) in Toyooka, in the southern part of Nagano Prefecture, Japan. In a half of the site, bush and oak trees were cut and litter was removed from the forest floor (managed plot, MP), while the rest of the site was left undisturbed (control plot, CP). The number of shiro – a matsutake colony – was 9 in MP and 6 in CP back in1980. After that, the number of shiro in MP increased gradually to 26 in 2018. The harvested number of matsutake fruiting bodies in 1990 was 230 in MP and 147 in CP (relative productivity value in CP was ca. 0.6), respectively. Afterwards, the matsutake harvest in MP sustained a stable level while that in CP decreased gradually (relative productivity value in CP in 2018 was less than 0.2). These data strongly suggest that forest management such as thinning and litter removal exert positive effects on shiro development and matsutake production. Besides, we present data on the relationships between meteorological conditions (soil temperature, precipitations) and matsutake production in Nagano, Japan.

Topics: Cultivation

Oral session 5-3: Japanese government project and its related research theses

Radiation mutagenesis of the ectomycorrhizal fungus *Tricholoma matsutake*

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Key words: Agaricomycetes, γ-Ray, Heavy-ion beams, Radiation mutagenesis, Mutants

Ionizing radiation has been widely used to create new agricultural crop cultivars and other mutants. Agaricomycetes have, however, not often been subjected to such mutation breeding. *Tricholoma matsutake* is an ectomycorrhizal agaricomycete that lives in conifer forests and produces prized, but not yet cultivated, mushrooms. Here we report the isolation of mutants of *T. matsutake* NBRC 33136, designated G1 and Ar 59, by irradiating the fungus with γ-ray and argon-ion beams respectively. Both mutants apparently have the same phenotype on PDA containing dye-linked water insoluble substrates. They exhibit mycelial morphology that differs from the wild-type, by higher levels of cellulose- and amylose-degrading activities. G1 also produced protuberances that were composed of non-aerial hyphal tissues while Ar 59 did not. Also, G1 suppressed lateral root development, induced stunting and caused fatal wilting of *Pinus densiflora* seedlings in vitro, while Ar 59 exhibited no harmful effects on the host. Although G1 produced a Hartig net; it was unlike NBRC 33136 and Ar 59 which grew massive hyphae that protruded from thick mycelial sheaths and penetrated the plant's intercellular space, which had deformed cortical cells. As in the wild type, Ar 59 formed a Hartig net that was composed of uniseriate hyphae protruding from thin mycelial sheaths. Thus, mutagenesis by ionizing radiations is useful for producing mutants of *T. matsutake*. The phenotype of G1 suggests the presence of a region in the fungal genome that may act as a switch to change between mutualism and parasitism in this species.

Topics: Genetics and Genomics

Oral session 5-4: Japanese government project and its related research theses

Cultivation study of *Lyophyllum shimeji* in forest conditions

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Key words: *Lyophyllum shimeji*, *Pinus densiflora*, Matsutake, Aseptic seedlings, Air-layered saplings

Honshimeji (*Lyophyllum shimeji*) is a famous edible mushroom in Japan. It is said from the past that "Matsutake has good smell, on the other hand Shimeji has good taste", so *L. shimeji* is traded in expensively in Japan. *L. shimeji* is an ectomycorrhizal mushroom associated with *Pinus densiflora*, *Quercus serrata*, *Q. crispula*, *Q. phillyraeoides*, among others hosts. The cultivation of *L. shimeji* in forest conditions makes it necessary to establish mycorrhizal associations between *L. shimeji* and the host plants. Ectomycorrhizal plants, such as *P. densiflora*, easily form ectomycorrhizas with some fungi when they grow in nursery. For the purpose to obtain non-mycorrhizal saplings lacking ectomycorrhizal roots, I applied air-layering method. Saplings of *P. densiflora* obtained by air-layering were inoculated with spawns of *L. shimeji* in clay pots filled with autoclaved soil. About two months after inoculation, they formed ectomycorrhiza. In one of those pots, fruit bodies of *L. shimeji* arose in the autumn. The inoculation tests in the pots showed that *L. shimeji* formed ectomycorrhizal associations easily. From these results, it was expected that *L. shimeji* formed mycorrhizal associations with saplings as the host plant in forest conditions if mycelial blocks of *L. shimeji* were arranged with thick density around the saplings and mycelia spread in the soil. In the earliest case, fruit bodies of *L. shimeji* occurred eleven months after inoculation in the forest. When *L. shimeji* were inoculated in young and small stands of *P. densiflora* grown along a strip road or in oak forests, fruit bodies of this fungus appeared in autumn. *Q. phillyraeoides* saplings are also appropriate for *L. shimeji* cultivation instead of *P. densiflora*. Also applying this method for *Tricholoma bakamatsutake*, which is within the group of Matsutake mushrooms, resulted in the production of fruit bodies.

Topics: Cultivation

Oral session 5-5: Japanese government project and its related research theses

Genotypic diversity of an Asiatic black truffle, *Tuber himalayense*, in naturally generated, highly productive truffle grounds

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Key words: Mating type, Microsatellite, Sexual reproduction, Simple sequence repeat (SSR), *Tuber indicum* species complex

The ectomycorrhizal genus *Tuber* includes many commercially valuable truffle species such as *T. magnatum* and *T. melanosporum*. Two haploid individuals form a fruiting body: the maternal partner forms the tissues and ascospores, and the paternal partner contributes to the ascospores. Understanding genotypic diversity of each mating partner in a productive truffle ground is key to successful truffle cultivation. However, fine-scale genotypic diversity has not been studied in Asiatic truffles. In this research, the maternal genotypic status of *T. himalayense*, a member of the *T. indicum* species complex, was studied based on a simple sequence repeat (SSR) and mating-type loci analyses. Genomic data of *T. himalayense* obtained with an Illumina Hiseq 2000 sequencer were used to develop 15 new polymorphic SSR markers. In addition, four SSR markers developed for the *T. indicum* species complex were used. From 2004 to 2011, a total of 1244 fruit bodies were collected from two naturally-occuring truffle colonies in plantations of *Castanea crenata* and *Quercus dentata* in Yamanashi Prefecture, Japan. Of these, 80 dried specimens were subjected to molecular analyses. Maternal individuals of both mating types were found to contribute to fruiting bodies, although all belonged to a single multi-locus genotype. Significant spatial segregation of mating types was detected in the *C. crenata* plantation, where a sufficient number of samples was available. Our results suggest a low level of maternal genotypic diversity of *T. himalayense* at our sites, despite sustainable truffle production there.

Topics: Molecular biology

Oral session 5-6: Japanese government project and its related research theses

Ectomycorrhizal colonization by *Tuber japonicum* and its promotion on the growth of *Pinus densiflora* seedlings in vitro

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Key words: Ectomycorrhizal fungi, Physiology, Pure culture, Truffle cultivation, Tuberaceae

A white-colored truffle *Tuber japonicum*, indigenous to Japan, is an ascomycetous ectomycorrhizal (EM) fungus. The fruiting bodies of this truffle occur in Fagaceae and Pinaceae forests, and are promising edibles with pleasant aromas. In this study, we examined the mycelial growth and EM colonization rate of *T. japonicum* on pine, and the influence of EM colonization on the growth of pine. The mycelial growth of seven strains of *T. japonicum* was evaluated by the colony diameter on modified Melin–Norkrans (MMN) agar medium after 30 days of incubation. For EM synthesis, the seedlings of *P. densiflora* were aseptically grown in pumice containing MMN liquid medium with a 10-fold dilution of glucose. Sixty-day-old seedlings were inoculated with each strain and cultivated for further 120 days. There was a significant difference in colony diameter and EM colonization rate among the strains. The EM colonization rates of the strains ranged from 0.2% to 61.7%. The EM colonization rate was positively correlated to the mycelial growth. The growth of *P. densiflora* seedlings was significantly different among the strains. Four of seven strains improved the growth of the seedlings compared with the non-inoculated seedlings, whereas the other three strains did not. The growth of the seedlings was positively correlated to the EM colonization rate. These results suggest that strains exhibiting rapid mycelial growth on agar medium are available for the production of EM plants.

Topics: Cultivation

Oral session 6-1: Molecular biology, Evolution and Phylogeny, and Biotechnology

First identification of a hidded gap in the 26s rRNA of desert truffles

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Key words: *Terfezia, Tirmania,* Desert truffles, Hidden gap, LSU rRNA, Domain D7

Despite the integrity of ribosomal RNA, some desert truffles present a non-canonical profile of rRNA where the 3.3 kb band is absent, the 1.8 kb band is clear and an additional band of 1.6 kb is observed. A similar rRNA profile was identified in organisms belonging to other Kingdoms, with the exception of the Kingdom Fungi. rRNA profiles of desert truffles were analyzed to verify the presence of this non-canonical profile. The RNA of desert truffles and yeast were blotted and hybridized with probes complementary to LSU extremes. RACE of LSU rRNA was carried out to determine the LSU rRNA breakage point. LSU rRNA of desert truffles presents a post-transcriptional cleavage of five nucleotides that generates a hidden gap located in domain D7. LSU splits into two molecules of 1.6 and 1.8 kb. Similar to other organisms, a UAAU tract downstream of the breakage point was identified in some *Terfezia* and *Tirmania* species. Phylogenetic comparison suggests that during fungi evolution mutations were introduced in the hypervariable D7 domain, resulting in a sequence that is specifically post-transcriptionally cleaved in some desert truffles. Financial supports from the projects CGL2016-78946-R (AEI/FEDER, UE) and 20866/PI/18 (FEDER/ Fundación Séneca, Murcia, Spain) are acknowledged.

Topics: Molecular biology

Oral session 6-2: Molecular biology, Evolution and Phylogeny, and Biotechnology

Unravelling whitish truffle (*Tuber borchii*) life cycle in the first truffle orchard established by mycelial inoculated plants

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Key words: Bianchetto truffle, Cultivation, Mycelial inoculation, SSR analysis, Mating types

Tuber borchii Vittad. (Bianchetto) is an edible truffle with a wide geographical distribution in Europe. Although it is less valuable than the precious Italian white truffle (*Tuber magnatum* Pico) and the Perigord black truffle (*Tuber melanosporum* Vittad.) it has recently become very popular. Its cultivation has become widespread in Europe and more recently in New Zealand where this truffle is particularly appreciated by gourmets. *Tuber borchii* cultivation has also been successful in Australia and USA. The cultivation of *T. borchii,* similarly to those of other truffles, is realized by synthetizing mycorrhizal plants in specialized nurseries by spore inoculum and planting them in suitable places. Recently, the first truffle orchard realized with plants inoculated by five different *T. borchii* mycelia (alone and in mixture) began to produce 9 years after planting. This plantation presents a unique opportunity to investigate the *T. borchii* sexual reproduction. To accomplish this, the mating type of each isolate as well as those of mycorrhizas, ascomata and extraradical soil mycelia was determined. Moreover, maternal and paternal genotypes were assessed in 18 ascomata using simple sequence repeat (SSR) markers identified in the T. *borchii* genome. The maternal genotypes of the fruiting bodies as well as the mating type of the corresponding *T. borchii* mycorrhizas and soil mycelium corresponded to those of the inoculated mycelia with only two exceptions. This demonstrates that the inoculated mycelia persisted 9 years after plantation. In regards to paternal partners, only two had the same genotypes as those of the inoculated mycelia, suggesting hermaphroditism. Most of the new paternal genotypes have a signature of recombination of those of inoculated mycelia, suggesting that meiospores are the most important source of paternal genotype.

Topics: Molecular biology

Oral session 6-3: Molecular biology, Evolution and Phylogeny, and Biotechnology

New species of the genus *Boletus* (porcini mushrooms & allies) in Central America confirmed by phylogenetic analyses

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Key words: Endemic species, Pine-oak forests, Central America, ITS, nrLSU, *Boletus edulis*

Several molecular phylogenetic analyses were made with DNA sequences of *Boletus* spp. recently collected in Guatemala to determine their phylogenetic position and identity. For this purpose, we additionally incorporated unpublished sequences from México and California, as well as sequences from GenBank of related species from around the world. Although all phylogenetic trees established new *Boletus* species in Mesoamerica, they were related to North American species. We sequenced and compared the ITS, nrLSU, TEF-1 and RPB2 regions to distinguish cryptic species in Guatemala and possibly Central America. The multi-loci analyses showed that *Boletus edulis s.l.* formed a monophyletic clade, with representatives in Europe, North Africa, Asia, North America and Guatemala. *Boletus pinophilus* also forms a monophyletic clade but with a more limited distribution and more phenotypic variation, particularly in Central America. The ITS region was not suitable to separate species, despite the macroscopic and microscopic differences. The *B. variipes–B. luteoloincrustatus–B. quercophilus* species-complex was separated into six new species in Guatemala, all associated with local oaks. These species differ in their amount of intraspecific phenotypic variations and geographic distribution. *Boletus variipes* var. *fagicola* was found to be polyphyletic and represented by two clades in North and Central America. A new species close to *B. variipes* was found in Mexico. We confirm that similar phenotypic species, despite their origin, tend to form close clades. Future efforts are ongoing to form an international team to analyze the *Boletus* diversity worldwide and establish new *typus* for every species to avoid name misapplication for similar species.

Topics: Evolution and Phylogeny

Oral session 6-4: Molecular biology, Evolution and Phylogeny, and Biotechnology

State of the art of the ethnomycology, biodiversity and biotechnology of the edible ectomycorrhizal mushrooms of Mexico

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Key words: Traditional knowledge, Neotropics, Biodiversity, Biotechnology, Ethnomycology

Mexico ranks fourth worldwide among the 17 recognized mega-diverse countries. Additionally, the country has 68 ethnic groups each one with its own worldview and management of natural resources. As a consequence of these enormous biological and cultural diversities, the consumption of more than 450 species of wild edible fungi has been recorded, 60% of which are ectomycorrhizal. Ethnomycological studies conducted in the country have demonstrated the precision, depth and vastness of traditional knowledge in ethnic groups, related to diversity, biology and ecology of edible species. Thus, the country is one of the most important genetic and cultural reservoirs of edible ectomycorrhizal fungi worldwide. Additionally, the development of biotechnological studies of inoculation mainly in oaks and pines has gained increasing interest. During the last two decades, 118 combinations of native trees and edible ectomycorrhizal mushrooms have been studied. Greenhouse studies have shown positive effects in plants inoculated with these fungi in terms of growth, macro and micronutrient transfer, photosynthesis, chlorophyll and carotene content. Colonization levels greater than 80% have been registered mainly for edible Neotropical species of *Laccaria, Hebeloma, Suillus, Rhizopogon* and *Helvella.* Field studies have shown up to 70% higher increases in survival of inoculated pines and oaks in comparison with non-inoculated plants. The cultivation of *Suillus pseudobrevipes,* associated with native pine trees, has begun, being the first Neotropical edible ectomycorrhizal mushroom to be cultivated. The beginning of sporome formation started three years after transplantation. Despite these advances, the biotechnological development of ectomycorrhizal edible fungi in the Neotropics is still in its infancy.

Topics: Biotechnology

Oral session 7-1: Cultivation

Success in artificial root colonizations and fruit body formations of *Entoloma clypeatum* with *Pyrus betulaefolia*

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Key words: Basidiomata, Edible mushroom, Inoculation, Japanese pear, Pure culture

Entoloma clypeatum is known as an edible mushroom which forms morphologically unique "mycorrhizae" on the roots of Rosaceae plants (Kobayashi and Hatano 2001). In this study, we inoculated *Pyrus betulaefolia* seedlings with *E. clypeatum* cultures to synthesize this "mycorrhizal" symbiosis. Mycelia of five strains of *E. clypeatum* were isolated from basidiospores germinated on MNC agar plates (Shishikura et al. 2019). The strains were then transferred to 100 mL agar slants of MNC, using gellan-gum instead of agar (GMNC), or Hyponex-yeast extract-glucose (HYG). Each agar medium solidified in the bottom of a 300 mL polycarbonate jar. The resultant mycelia were covered with approximately 200 mL of autoclaved andosol or vermiculite containing MNC liquid. After that, an aseptic *P. betulaefolia* seedling was planted on the center of soil surface in each jar. The inoculated seedlings were grown at 15 °C and a photosynthetic photon flux density of 100 μmol/m^2/s. After four months of incubation, fungal sheath formation was observed on the roots for all strains tested. The anatomical structure of the fungus-colonized roots corresponded to the descriptions of "mycorrhizae" reported by Kobayashi and Hatano (2001). Although root colonization was observed in all the substrates, the proportion of colonized root per whole root was higher in andosol than in vermiculite. In this study, one isolate of *E. clypeatum* repeatedly produced mature fruit bodies, when MNC was used for the slant in the bottom of the jar. These results may help us achieve the cultivation of *E. clypeatum* and better understand the ecological function of their "mycorrhizae".

Topics: Cultivation

Oral session 7-2: Cultivation

Ectomycorrhization of monokaryotic and dikaryotic strains of hedgehog mushrooms (*Hydnum* L.) with pine seedlings in vitro

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Key words: Cantharellales, Edible mushrooms, Life cycle, Haploid, Pure culture, Mycorrhizal synthesis

Despite their edibility, mycorrhizal synthesis of *Hydnum* species has never been reported because pure culture isolation has been difficult. Recently, we successfully established pure cultures of monokaryotic and dikaryotic mycelia of *Hydnum* species from their basidiospores (Sugawara et al. 2019). In the present study, we synthesized ectomycorrhizae from monokaryotic or dikaryotic *Hydnum* isolates with pine seedlings in vitro. We tested 23 strains from 8 species of *Hydnum*, including 11 monokaryotic strains of *H.* cf. *albidum*. We inoculated cultured mycelia of each strain onto axenically germinated seedlings of *Pinus densiflora*. These were then planted in a mixture of sphagnum moss and vermiculite moistened with MNC liquid (0.2% glucose) medium. Inoculated seedlings were incubated at 20 °C and under illumination with 100 μmol/m^2/s for six months. Afterwards, the morphology and anatomy of the root systems were inspected under dissecting and DIC microscopes. Ectomycorrhizal formation was observed on 16 strains from 4 species tested. Although all of ectomycorrhizae synthesized in this study produced only diminutive rhizomorphs, 7 strains from 2 species grew fully developed mantles having the ring-like hyphal arrangements (plectenchymatous) known in *H. rufescens* (Agerer 1996). Ectomycorrhizae produced with *H.* cf. *albidum* monokaryotic hyphae also had a typical Hartig-net and a plectenchymatous mantle. These were the same as those of dikaryotic ones but lacked clamp connection on hyphal septa. There was no apparent difference in the rate of ectomycorrhizal formation between monokaryotic and dikaryotic strains of *H.* cf. *albidum*. These results will likely facilitate the cultivation and breeding of hedgehog mushrooms.

Topics: Cultivation

Oral session 7-3: Cultivation

Desert truffle crop depends on agroclimatic parameters during two key periods

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Key words: *Terfezia*, *Helianthemum*, Precipitation, Aridity index, Soil water potential

Desert truffles have become an alternative agricultural crop in semiarid areas of the Iberian Peninsula due to their much appreciated edible value, and their low water requirements for cultivation. Although most studies related to desert truffle production point to the sole importance of precipitation, this work is the first systematic study carried out to characterize whether other important agroclimatic parameters for example evapotranspiration, soil water potential, relative air humidity %, aridity index or air vapour pressure deficit, may have an impact on a desert truffle production in an orchard with mycorrhizal plants of *Helianthemum almeriense* x T*erfezia claveryi* for 15 years from the plantation. The results show that *T. claveryi* production has two key periods, during its annual cycle: autumn (Sept-Oct) and spring (end of March). Agroclimatic parameters can influence the final crop a long time before the desert truffle fruiting season contrary to what happens with other edible mycorrhizal mushrooms. The aridity index and soil water potential seem to be the most manageable parameters in the field and can be easily controlled by applying irrigation during the above mentioned periods. Four different models to manage desert truffle plantations are proposed based on these agroclimatic parameters in order to optimize and stabilize carpophore fructifications over the years. Financial supports from the Projects CGL2016-78946-R (AEI/FEDER, UE) and 20866/PI/18 (FEDER/ Fundación Séneca, Murcia, Spain) are acknowledged.

Topics: Cultivation

Oral session 7-4: Cultivation

Fertilizing regime selection and its correlation with mycorrhizal development of *Tuber melanosporum, Tuber aestivum* and their hosting oak trees

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Key words: Ectomycorrhiza, *T. melanosporum*, *T. aestivum*, Fertilizing regime

Truffles are highly sought after due to their alluring organoleptic virtues which make them a desirable culinary product. It is for this reason and rising demands that the truffle-market holds high economical value worldwide. This international trend has boosted up the efforts to maintain and develop highly productive truffle orchards which will be both profitable and involving minimal risks. Truffle cultivation methodology was initially developed in France and is mainly based on truffle-spores inoculation of oak seedlings planted in soils which are limited in pivotal nutrients essential to the tree. This practice, while contributing to the maintenance of the mycorrhizal interaction, is also causing a delay in the optimal growth of the host tree. Another approach to increase crop yields is by forming densely planted orchards which are being progressively thinned. However this practice further increases initial investments. In this study we sought to determine fertilizing regimes which will enable the efficient development of both fungi and the hosting oak tree in different local soils of northern Israel. In turn, these modified regimes are expected to lead to early truffle fruiting. To this end, we have monitored the mycorrhizal development of *Tuber melanosporum* and *Tuber aestivum* with *Quercus ilex* and *Quercus boissieri*, respectively, in three main soil types: Basalt, Tuff and calcareous soil, in combination with altering fertilizing regimes. Moreover, the addition of crushed limestone to the soils was tested. Our preliminary results throughout the initial years clearly indicate that controlled addition of relatively low concentration of NPK minerals to the plants grown in 10 liter buckets does not inhibit mycorrhizal colonisation in all the soils and fertilization regimes tested. The ramifications of the increments of fertilizer concentrations will be examined in further studies.

Topics: Cultivation

Oral session 7-5: Cultivation

The Australian pests and diseases of truffles and their host trees project 2015–2019

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Keywords: Cultivation, *Tuber melanosporum*, Truffle orchard management, Pests and diseases

The Australian truffle industry began in the early 1990s in Tasmania and the east coast of Australia. It was, and still is, focused predominantly on *Tuber melanosporum*, though more recently trees are being inoculated with *T. borchii*. Production in the early orchards was highly variable but following the development of improved nursery methods in the late 1990s production has steadily increased. Truffle production now occurs in almost all states of Australia and continues to increase.

From 2015 to 2019 Australia conducted a national research project aimed at improving the quality and quantity of truffle produced through improved management of pests and diseases that negatively impact on production. Australian truffle growers were surveyed about the nature of their operation as well as the potential pests and diseases that they had observed in their orchards. A wide range of pests and diseases were observed by growers. The research team based in Manjimup then conducted further research to delineate the role of those pests and diseases in causing damage to either the host trees or to the truffles themselves. Further, the project investigated cultural practices that could minimise the impact of the identified pests and diseases. The project involved growers at all stages of orchard development from initial planting to commercial production. The project team included members across Australia and involved growers across the country and has contributed to developing a somewhat more cohesive industry. The major findings of the project are presented.

Topics:

Oral session 7-6: Cultivation

Recent advances in the cultivation of edible mycorrhizal fungi in New Zealand and China

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Key words: *Lactarius deliciosus*, Saffron milk cap, Truffle, *Tuber* spp., Tree plantation, *Pinus* spp.

New Zealand has pioneered the cultivation of truffles in the southern hemisphere by being the first country to cultivate the Périgord black truffle (*Tuber melanosporum*) in 1993. Nowadays, the truffle industry has grown considerably and over 1 ton of truffles are produced annually, i.e. *T. melanosporum*, *Tuber borchii*, *Tuber aestivum* and *Tuber brumale*. A 23-year-old *T. melanosporum* plantation near Christchurch, Canterbury, has been particularly productive (≈50 kg/ha) for six years consecutively. New Zealand is also pioneering the cultivation of saffron milk cap (*Lactarius deliciosus*). Yields in Canterbury can reach 400 kg/ha under *Pinus radiata* 6 years after planting and up to 1.1 ton per ha under *Pinus sylvestris* 9 years after planting. Furthermore, while canopy closure began under *P. radiata* 7 years after planting, followed by a drastic reduction of yields (115 to 215 kg/ha), no canopy closure has yet occurred under *P. sylvestris* 11 years after planting with yields still hovering at 700 to 800 kg/ha. These results enable us to postulate the future orchard management techniques that could optimize and maintain yields, delivering profitable saffron milk cap orchards. From 2015, in Kunming, China, we successfully conducted mycorrhizal syntheses between *Lactarius* and pine species native to China. We further studied the effects of growth condition, type of substrate and pine/fungus combination on mycorrhizal synthesis. Three hundred mycorrhizal seedlings, some with high mycorrhization rates, have been produced and out-planted, along with control, non-inoculated seedlings in Yunnan and Guizhou provinces. One year later, mycorrhizal seedling growth appears promising under field conditions.

Topics: Cultivation

Oral session 7-7: Cultivation

Development status of the truffle industry in Panzhihua, Sichuan, China

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Keywords: Cultivation, *Tuber indicum*, Mycorrhizae, Oak, Pine

Panzhihua, located in the south of Sichuan Province, China possesses a dry-hot subtropical valley climate. The unique climate supports a diverse range of truffles. Among these truffle species, *Tuber panzhihuanense, T. indicum* and *T. sinoaestivum* have high commercial value. Panzhihua was awarded "Homeland of Truffles in China" by the China Economic Forestry Association. Unfortunately, the yields of wild truffles have reduced sharply from 150t to 30t due to commercial harvesting. The price of truffles has increased by almost 20-fold over the last ten years. In the early 21st century, research on cultivation started in order to protect the truffle resources. Field surveys of truffle diversity and ecology were carried out. Over 16 truffle species, of which one is new to science, have been discovered in five districts of Panzhihua. They grow in oak woods and pine plantations from 1,100 to 2,600 m above sea level with soils of pH 6-7 relatively rich in organic matter. Mycorrhizal seedlings of *T. indicum, T. sinoaestivum, T. panzhihuanense, T. melanosporum* and *T. borchii*with 12 tree species have been successfully produced. More than 10 experimental plantations (over 6,000 trees) have been established with the above truffle seedlings in different regions with a range of climatic and soil conditions. Two plantations have produced their first *T. indicum* truffles. In one of these plantations, *Castanea mollissima* trees produced truffles only three years after planting and 96 % of the 220 truffle trees show obvious brûlés around them. In addition to truffle cultivation progress, a truffle processing industry has quickly developed.

Topics: Cultivation

Oral session 7-8: Cultivation

Diversity of commercial wild mushrooms in Yunnan, China and cultivation of the ectomycorrhizal genera *Lactarius* and *Tuber*

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Keywords: *Lactarius* sect. *Deliciosi, plantation, Tuber melanosporum, Quercus* spp., Mycorrhizal synthesis, Commercial mushroom

Yunnan province has a major tradition of consumption and trade of mushrooms including fungi obtained from the wild. The survey of mushroom markets, to a large extent, could reflect the problems of development and utilization of wild fungi. A thorough investigation of species diversity of wild commercial fungi from a taxonomic point of view is being undertaken, with ethnomycology information being gathered from tropical, temperate and alpine zones of Yunnan. Updated and new information will help us to propose appropriate protection and management measures in different areas. Among mushroom markets, the high-priced and/or popular commercial mushrooms include matsutake, truffle, porcini, chanterelle and milk agaric. Most of them are edible mycorrhizal fungi (EMF) that form a symbiotic relationship with trees or shrubs. We developed methods to cultivate *Lactarius* and *Tuber* species with native tree species and have successfully achieved 13 combinations using five species of *Lactarius* sect. *Deliciosi* with five species of *Pinus*. Since 2017, hundreds of these *Lactarius* mycorrhizal seedlings, including control, non-inoculated seedlings, have been out-planted in several sites in Yunnan and Guizhou provinces. These are currently being monitored in order to demonstrate that *Lactarius* species can be cultivated in China and to determine their economic and environmental potential. For *Tuber* spp. cultivation, three native Chinese oak species formed a stable mycorrhizal symbiosis with *T. melanosporum* and shoot growth was improved in comparison with un-inoculated seedlings. *Tuber melanosporum*-mycorrhized *Quercus mongolica* and *Q. longispica* could be tested for ascocarp production in the field.

Topics: Biodiversity, Traditional Knowledge, Cultivation

Oral session 7-9: Cultivation

People & edible ectomycorrhizal mushrooms towards a traditional ecological knowledge based on DNA barcode & antioxidant properties: the case of dry dipterocarp forest, northeastern, Thailand

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Key words: Antioxidant, Dry dipterocarp forest, DNA barcode, Edible ectomycorrhizal mushroom, Thailand

Wild edible ectomycorrhizal mushrooms have a great ecological and economical value to humans worldwide. They provide a source of proteins, vitamins and amino acids that are important in nutrition, as food and medicine. The aim of this research was to employ the existing traditional ecological knowledge in combination with scientific knowledge. For that, the DNA barcode by using internal sequence transcripts (ITS) was used to study wild edible ectomycorrhizal diversity and distribution. A study on the collection of wild edible ectomycorrhizal mushrooms from a dry dipterocarp forest in northeastern Thailand was conducted. This study further addresses their chemical and bioactive properties of selected wild edible ectomycorrhizal mushrooms. Our study provides important information necessary to characterise the wild edible ectomycorrhizal mushroom populations and define the use of selected species as a source of delicacy food for local consumers in the northeastern part of Thailand. As part of an ongoing project, a special focus on application of ectomycorrhizal inoculation in reforestation programmes have been attempted throughout several parts of Thailand and Southeast Asia.

Topics: Biodiversity, Bioactive compounds, Traditional knowledge

Poster Session

(Afternoon on 23rd October)

at Kiko and Yoko (2nd Floor)

List of Poster Presentations (1/3)

1	Biotechnology	Effects of frozen storage using liquid nitrogen on mycelial growth and ectomycorrhization of *Tricholoma matsutake*	K Obase, T Yamanaka
2	Biotechnology	A qPCR assay that specifically quantifies *Tricholoma matsutake* and *Pinus densiflora* biomass adjacent to fruiting bodies in soil	M Yamaguchi, M Narimatsu, T Fujita, N Matsushita
3	Biotechnology	Hairy root induction on Yunnan poplar and rock rose, and its implications in fungal inoculum production and mycorrhizal synthesis	W Xu, N Tang, H Gong, F Yu
4	Food security and health	The role of edible mushrooms to assure food security in Ethiopia: an innovative project from social participation to ecological research	P Martín-Pinto, W Tadesse, D Agúndez, D Alem, R Alía, Z Gizachew, J Geml, T Dejene
5	Economic importance	Distribution of *Tricholoma matsutake* in Hokkaido, the northernmost island of Japan	S Gisusi, Y Tamai
6	Sustainability	Pre-inoculation of pine-tree seedlings with *Suillus collinitus* as a beneficial silviculture strategy	S Levy, N Ezov, I Pereman, A Eisenband, A Kalev, O Danay
7	Cultivation and Sustainability	Turmiculture project: desert truffle crop against climate change and for rural development	A Morte, F Arenas, J E Marqués-Gálvez, L M Berná, Á Guarnizo-Serrudo, A Gutiérrez, A Rodríguez, A Navarro-Ródenas
8	Cultivation	Exploring characteristic components in matsutake “shiro” using comprehensive material analysis	T Shimokawa, M Yamaguchi, M Hiraide, H Shibuya, S Nakano, H Murata, T Yamanaka
9	Cultivation	Comparisons of the mycelial growth of several strains of *Tricholoma matsutake* at different incubation temperatures in soil medium	M Narimatsu, M Yamaguchi, T Yamanaka, S Gisusi, T Azuma, Y Tamai, T Fujita, M Kawai
10	Cultivation	Mycorrhizal colonization of *Tuber himalayense* inoculated to mature *Quercus acutissima* and *Castanea crenata* trees in Japan	K Noguchi, N Nakamura, S Nakano, H Shibata, K Mizutani, H Furusawa, A Kinoshita, T Yamanaka
11	Cultivation	Establishment of pure cultures of *Lactarius* section *Deliciosi* and its allied taxa from their ectomycorrhizae	T Yamamoto, N Maekawa, A Nakagiri, K Sotome, N Endo
12	Cultivation	Mycorrhization of *Tricholoma matsutake* and pine seedlings planted in the vicinity of mycorrhizal nursery stock produced in vitro	H Kobayashi, M Kindaichi, M Kawai, T Yamanaka
13	Cultivation	A reliable measure of mycorrhization level in truffle orchards of *Tuber melanosporum* Vittad.	L B Falini, G Marozzi, A Onofri, G M N Benucci, E Albertini, D Donnini
14	Cultivation	Effect of LED lightning and soil nitrogen on the growth of pine seedlings associated with *Tricholoma matsutake in vitro*	K Suzuki, S Yamashita, A Yamada

List of Poster Presentations (2/3)

15	Cultivation	Larch forest managements for the mushroom harvests of *Suillus grevillei* in Japan	K Katagiri, H Furukawa, K Kato, K Masuno, A Yamada
16	Cultivation	Nutritional effect of phosphoric acid on the ectomycorrhization of *Tricholoma matsutake*	K Takahashi, A Yamada, N Hirai
17	Cultivation	Attempt to compare the old and the new world of truffles	P Sourzat
18	Cultivation	Impact of the herbicide roundup® on mycelial growth of truffles, morels, and molds	B R Rennick, G M N Benucci, G Bonito
19	Cultivation	Establishing truffle orchards in the Midwest US for research and education	B R. Rennick, G M N Benucci, G Bonito
20	Cultivation	Strain selection of Japanese yellow chanterelle based of the fruiting ability in the pot cultivation	Y Takeda, M Kimura, W Ogawa, S Yamashita, A Yamada
21	Cultivation	Culture and characterization of mycelia of different edible *Lactarius* species in Guatemala for mycorrhization with local pine species	R F Tovar, R F Arzú
22	Cultivation and Molecular biology	*Tuber magnatum* mycelium survival in soil of orchards established with inoculated seedlings	B Robin, P Cammalletti, P Beacco, E Levesque, Z Chen, F Todesco, C Murat
23	Ecology	Inventory and bioecology of terfez in some regions of the northern Algerian Sahara	L Bradai, H Chenchouni
24	Ecology	Soil chemical and physical properties of *Tuber himalayense* and *Tuber japonicum* habitats in Japan	H Furusawa, T Yamanaka, A Kinoshita, S Nakano, K Noguchi, K Obase
25	Ecology	Temporal dynamics of soil fungal communities after partial and total clearcutting in a managed *Pinus sylvestris* stand	J Parladé, M Queralt, J Pera, J A Bonet, C Castaño, F Martínez-Peña, J Piñol, M A Senar, A M de Miguel
26	Ecology and Diversity	The mycobionts, phytobionts and microniches drive the bacterial and fungal structure of Neotropical ectomycorrhizas	M del R Cardoso-Villanueva, B Xoconostle-Cázares, V M Cetina-Alcalá, M E Sánchez-García, J D Martìnez, J J Almaraz-Suàrez, J Pérez-Moreno
27	Diversity	Fungal diversity and endemic species in Sierra de las Minas, Guatemala	G González, R F Arzú
28	Biodiversity	Diversity and new species of *Cantharellus*, *Craterellus* and *Pseudocraterellus* in Guatemala	L Loy, R F Arzú, T Sato, A Yamada
29	Biodiversity	*Tuber* species of Canada based on molecular identification of existing herbarium collections	S M Berch, G Bonito
30	Evolution and Phylogeny	Revisiting Japanese truffle phylogeny and diversity: possibilities for cultivation and edibility	A Kinoshita, H Sasaki, K Yamamoto, M Ohmae, T Orihara, K Obase, T Yamanaka, A Yamada, K Nara
31	Evolution and Phylogeny	Genetic diversity of the genus *Ramaria* in the Patagonian Andes forests	G C González, M B Pildain, S Visnovsky, C Barroetaveña

List of Poster Presentations (3/3)

32	Genetics and Genomics	The draft genome sequence of *Russula griseocarnosa* and its association with mycorrhizal characteristics	Y Liu, Q Wu, H Hu, Y Xie, X Wu, A Wang
33	Taxonomy	Morphoanatomic and molecular characterization of *Laccaria squarrosa* ectomycorrhiza associated with *Quercus* sp.	M Herrera, D R Rendón, F-QiYu, M Martínez-Reyes, F Hernández-Santiago, J Pérez-Moreno
34	Taxonomy	Taxonomy of Japanese *Tricholoma ustale*	W Aoki, A Yamada, H Nagai, T Ito
35	Taxonomy	Taxonomy of Japanese golden chanterelles	T Sato, W Ogawa, A Yamada
36	Taxonomy	*Tuber rugosum* sp. nov.: a new spiny-spored truffle species from North America	B R Rennick, G M N Benucci, Z-Y Du, G Bonito

Poster session 1: Biotechnology

Effects of frozen storage using liquid nitrogen on mycelial growth and ectomycorrhization of *Tricholoma matsutake*

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Key words: Ectomycorrhiza, Edible mushroom, Preservation, Trehalose, Vermiculite

We examined the effects of frozen storage using liquid nitrogen on the mycelial growth and ectomycorrhization of *Tricholoma matsutake* strain Y1 (ATCC MYA-915 or NBRC 33136). Mycelial fragments of strain Y1 developed on particles of autoclaved vermiculite immersed in 0.2 mL Ohta liquid media in a 1.5 mL cryovial after incubating at 23°C in dark for 1 month. The cryovials were loaded with cryoprotectants (to a final concentration of 5% DMSO and 10% trehalose) and then frozen to −80°C at a cooling speed of −1°C/min using a freezing container and a deep freezer. Cryovials were then stored in the gas phase of liquid nitrogen at approximately −100°C in a locator cryogenic storage system for 1, 3, 6, 12, 24, and 36 months. Following frozen storage, particles of vermiculite in the cryovials were rapidly thawed and incubated at 23°C on Ohta agar plates. Regenerated mycelia were subjected to growth tests on the Ohta agar plates and ectomycorrhization testing using pine seedlings in vitro. Regenerated mycelia were obtained from all cryovials following the different durations of frozen storage. There were no marked differences in colony diameters on the Ohta agar plates among treatments of different duration of frozen storage. Additionally, all regenerated mycelia formed ectomycorrrhizae with pine seedlings in vitro. These results indicate that frozen storage with liquid nitrogen is an efficient method for the long and stable preservation of *T. matsutake* strain Y1.

Topics: Biotechnology

Poster session 2: Biotechnology

A qPCR assay that specifically quantifies *Tricholoma matsutake* and *Pinus densiflora* biomass adjacent to fruiting bodies in soil

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Key words: Edible ectomycorrhizal mushrooms, Soil fungal biomass, Root biomass, Matsutake, Species-specific quantitative PCR

Tricholoma matsutake is an ectomycorrhizal basidiomycete that produces prized, yet uncultivatable, “matsutake” mushrooms. The basidiomycete occurs from dense mycelia, called “shiro”, developed in the rhizosphere of *Pinus densiflora* mainly in Japan. Therefore measuring *T. matsutake* mycelium and *P. densiflora* fine root biomass density in soil allows us to determine the kinetics of fungal and root growth before and after fruiting. This is useful for analyzing the conditions of the shiro and its surrounding mycorrhizosphere, predicting fruiting timing, and managing forests to obtain better crop yields. Here, we have developed novel methods to quantify *T. matsutake* mycelia and *P. densiflora* root biomass in soil by quantifying DNA elements that are uniquely conserved within *T. matsutake* and *P. densiflora* but are absent from other fungal and botanical species. Using this technique, we have demonstrated the measuring of *T. matsutake* mycelium and *P. densiflora* fine root biomass at a transect line placed from the center of the *T. matsutake* fairy ring outward, and its underlying area. The distribution of *T. matsutake* and *P. densiflora* biomass was concentrated in areas adjacent to the fruiting bodies. Furthermore, areas of high density of *T. matsutake* and *P. densiflora* biomasses coincided.

Topics: Biotechnology

Poster session 3: Biotechnology

Hairy root induction on Yunnan poplar and rock rose, and its implications in fungal inoculum production and mycorrhizal synthesis

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Key words: Hairy roots induction, Mycorrhizal synthesis, *Populus yunnanensis*, *Cistus*, Tissue culture

A successful ectomycorrhizal synthesis relies largely on the fungal inoculum quality. Current fungal inoculum preparation protocols usually maintain the asymbiotic mycelia which may cause shifts in their symbiotic capability. Therefore, developing a root organ culture (ROC) where the fungus is maintained in a symbiotic state could help stop the symbiotic capability shift/loss.

Due to their compatibility with diverse ectomycorrhizal fungi, *Populus yunnanensis* and several *Cistus* species were tested for hairy root induction and subsequent mycorrhizal synthesis. Poplar axillary buds and young leaves as well as rock rose hypocotyls were excised for infection and co-culture with *Agrobacterium rhizogenes*. Under the condition of 10 min for infection (suspension OD_{600}: 04-0.6) and 3 days for co-culture, our first results showed that all four *A. rhizogenes* strains (K599, A4, C149 and C151) could induce hairy roots on poplar and rock rose hypocotyls but with variable success rates. The next induction conditions to test will include different infection and co-culture duration regimes as well as medium optimization. The capability of hairy roots to form ectomycorrhizae with various fungi such as truffle and milk cap species will be tested to assess the potential of high-efficiency inoculum production and mycorrhizal establishment.

Topics:

Poster session 4: Food security and health

The role of edible mushrooms to assure food security in Ethiopia: an innovative project from social participation to ecological research

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Keywords: Edible mushrooms, Fungal diversity, Non-timber forest products, Ethnomycology, Rural development, Local knowledge, Food security

In Ethiopia, the harvesting of wild mushrooms was a common practice in rural communities in the past. However, the management information available is scarce due to the rapid disappearance of traditional knowledge. The main goal of this innovative project is to recover or re-introduce the utilisation of currently underused edible mushrooms as a supplementary food resource and important economic income in less-favoured areas. Local populations (mainly women and rural inhabitants) have been involved in each stage of the project: civil society, local government and research centres. The initial stage consisted of the exploration and study of the mycological resource in Ethiopia, including a complete checklist of fungi in forest systems and an evaluation of economic and gastronomic potential at the local context. During the second phase, we have worked on training local populations. The traing has focussed on scientific and technical training in mycology, forest management, and cross-curricular education in gender policies, human rights, and environment and climate change. In the third phase, we addressed the sustainable management of mushroom production. We identified the more valuable edible species and designed participatory management plans for sustainable use. We carried out pilot demonstrations and information campaigns in local communities. Finally, we designed communication strategies specially focussed on local and regional levels. The main achievement of this project was to enhance the value of edible mushrooms that were not being harvested at present. Ethiopian forest systems are highly productive, but local people lack the knowledge to take advantage of the mycological resource. In all cases, local populations' attitude towards this innovative project have been absolutely positive.

Topics: Food security and health

Poster session 5: Economic importance

Distribution of *Tricholoma matsutake* in Hokkaido, the northernmost island of Japan

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Key words: *Tricholoma matsutake*, Boreal forest, *Pinus pumila*, *Picea glehnii*, *Abies sachalinensis*

The Japanese archipelago is a long geographical feature that extends from north to south. In this presentation, we will focus on the distribution of matsutake (*Tricholoma matsutake*) in Hokkaido, with particular interest on symbiotic plants. In Honshu, located in the temperate zone, *T. matsutake* mainly occurs in the red pine (*Pinus densiflora*) forest. However, the Japanese red pine hardly grows in Hokkaido, which is located in the cold (subarctic) zone, resulting in *T. matsutake* coexisting with various other coniferous species, such as *Pinus pumila*, *Picea glehnii*, and *Abies sachalinensis*. To date, most of the *T. matsutake* that are circulated from Hokkaido are considered to have been collected from the *A. sachalinensis* forest, which covers the largest area on this island. The symbiosis of *T. matsutake* with the widely distributed Yezo spruce (*Picea jezoensis*), which is closely related to *P. glehnii*, or the widely planted larch (*Larix kaempferi*), which is the main afforestation tree, has not been confirmed. In contrast, *T. bakamatsutake*, which is regarded as a type of *T. matsutake* mushroom in some cases, has been found in a broadleaf forest in the southern part of Hokkaido. Interestingly, we also found a *T. matsutake* mushroom that uses a broadleaf tree as a host.

Topics: Economic importance

Poster session 6: Sustainability

Pre-inoculation of pine-tree seedlings with *Suillus collinitus* as a beneficial silviculture strategy

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Key words: Silviculture, Ectomycorrhiza, *Suillus collinitus*, *Pinus halepensis*, Mediterranean forest*s,* irrigation regime

Ectomycorrhizal symbiosis is considered a key factor in silvicutural management due to it is role in promoting the development and survival of trees, while enriching the forests with edible mushrooms. In the current study we sought development of new methods for effectively producing Mediterranean pine-tree seedlings that were pre-inoculated with edible mushrooms. We tested inoculating *Pinus halepensis*, *Pinus brutia and Pinus pinea* seedlings with *Suillus collinitus*. Mycelial and seedling development was monitored initially in the nursery and then followed after out-planting in two independent model plots consisting of terra-rossa and rendzina soils. Infection of the pine seedlings with *Suillus collinitus* spores increased their growth-rate in the greenhouse. The largest effect was found with *Pinus halepensis* (180% greater than the control), and to a lesser extent on *Pinus brutia* (160%) and 124% in *Pinus pinea*. Molecular analysis of root samples from the two soil types indicate efficient establishment of the mycorrhizal symbiosis in the trees planted in terra-rossa soils. By contrast, ectomycorrhizal symbiosis could not be detected on trees planted in the rendzina soils. These observations clearly demonstrate the beneficial effect of pine-tree pre-inoculation with *Suillus collinitus* on initial growth. Furthermore, these results indicate that the terra-rossa soil type is suitable for trees inoculated with *Suillus collinitus*, whereas rendzina soils might require more frequent irrigation in the initial years after out-planting in order to support the ectomycorrhizal symbiosis.

Topics: Sustainability

Poster session 7: Cultivation and Sustainability

Turmiculture project: desert truffle crop against climate change and for rural development

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Key words: *Terfezia*, *Helianthemum*, Desert truffles cultivation, Crop training, Gastronomy

The Spanish Turmiculture Association (https://trufadeldesierto.com/) is an association of desert truffle growers that was created in October 2017 to carry out the Turmiculture project. The aim of this project is to establish and consolidate the innovative cultivation of desert truffles or "turmas" (Spanish local name for desert truffles) as an alternative crop in the Region of Murcia (Spain) and to highlight the value of the consumption of these edible truffles in order to promote the rural development of the less productive areas of the Region. *Terfezia* cultivation is an ecological crop, without the consumption of fertilisers or phytosanitary products, that uses autochthonous plant and fungal species and it is applicable in dry areas, which are not very productive. The objectives of the project are: 1) Establishment of plantations in three different climatic and soil zones in the Region of Murcia, 2) Training in techniques of plantation management, marketing, enterprise and mycoturism, and 3) Valorisation of the consumption of desert truffles in the Region of Murcia by studying their conservation after harvest and their use in the gastronomy by prestigious restaurateurs. The activities carried out to obtain these objectives, by the different members of this association, are presented. This project is financial supported by the European Agricultural Fund for Rural Development and Consejería de Agua, Agricultura, Ganadería y Pesca de la Comunidad Autónoma from the Region of Murcia (FEADER, CARM).

Topics: Cultivation, Sustainability

Poster session 8: Cultivation

Exploring characteristic components in matsutake “shiro” using comprehensive material analysis

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Key words: *Tricholoma matsutake*, Metabolome, Fatty acid

Tricholoma matsutake is an ectomycorrhizal agaricomycete that exists symbiotically with *Pinus densiflora* in the field. The mycorrizal mycelia with the fine root and soil are referred to as “shiro”. Although cultivation techniques for the generation of matsutake mushrooms have not yet been developed, the hyphae of *T. matsutake* can spread well in artificial media. Thus, the determination of the chemical composition during development of matsutake “shiro” in the field could facilitate the establishment of the cultivation techniques in the future. We attempted to identify components characteristic in “shiro” using a comprehensive analysis comparing the components of “shiro” obtained from the field with those of artificially cultivated mycelia and fruiting bodies. The “shiro” samples were collected in spring, summer, and autumn at a site in Nagano Prefecture and other sites. The number of detected components tended to increase as the mycelia grew to generate fruiting bodies. Fatty acids with higher numbers of carbon were more prevalent in the “shiro” samples collected in autumn than in those collected in spring. In addition, melissic acid was observed in “shiro” and fruiting bodies but not in mycelia grown artificially. Therefore, the presence of fatty acids with higher numbers of carbon seemed to be a potential indicator of artificial mushroom beds when developing appropriate artificial cultivation conditions.

Topics: Cultivation

Poster session 9: Cultivation

Comparisons of the mycelial growth of several strains of *Tricholoma matsutake* at different incubation temperatures in soil medium

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Key words: Edible ectomycorrhizal mushrooms, Linear mycelial growth, Mycelial density, Optimum temperature, Species-specific quantitative PCR

Mycelial growth in *Tricholoma matsutake* is slower than that in some other ectomycorrhizal fungi, and this slow growth hinders efficient experimentation involving *T. matsutake* cultivation. Although mycelial growth rate is known to be affected by temperature in several fungi including *T. matsutake* on agar plate or liquid medium, the optimum temperature for *T. matsutake* growth in soil remains unclear. Therefore, in this study, we aimed to investigate the effect of temperature on the mycelial growth in soil medium for seven different *T. matsutake* strains collected from different areas in Japan, such as Hokkaido, Iwate, Kyoto and Nara prefectures. Linear mycelial growth was measured under a dissecting microscope, and mycelial density was analyzed using qPCR. After 89 days of cultivation in a soil medium, mycelial growth in the seven *T. matsutake* strains was mainly observed at 5–25 °C. The optimum temperatures for linear mycelial growth and increase in mycelial density were 19.6 °C and 17.6 °C, respectively. These values were lower than those reported previously for *T. matsutake* grown on agar media or in liquid medium. Additionally, these optimum temperatures showed no clear relation with the mean air temperature of the strain collection site. These novel results will contribute to improve an artificial cultivation method in soil medium for *T. matsutake*.

Topics: Cultivation

Poster session 10: Cultivation

Mycorrhizal colonization of *Tuber himalayense* inoculated to mature *Quercus acutissima* and *Castanea crenata* trees in Japan

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Key words: Infected seedlings, Liming, Root tips, Soil, Spore suspension

Truffles have been cultivated in some regions such as Europe and Australia, where tree seedlings with truffle mycorrhizae are planted and grown in the field. Another possible approach would be inoculating mature trees with inocula such as spore suspensions, but information on the procedure is still limited. In this study, therefore, we examined mycorrhizal colonization of *Tuber himalayense* by inoculating mature *Quercus acutissima* and *Castanea crenata* trees with three different inocula (truffle-colonized *Q. serrata* seedlings, spore suspensions or the soil from a natural habitat). In April-May 2018, 10-cm deep square holes of 20-30 cm of side were prepared around the host trees. Then, the holes were filled with lime-amended soil, for each type of inoculum. In October 2018, fine roots of *Q. acutissima* and *C. crenata* were harvested from the inoculation plots and mycorrhizal colonization was checked by molecular analyses. In the *Q. acutissima* site, *T. himalayense* mycorrhizae were found in 2 and 3 of the 12 plots inoculated with truffle seedlings or spore suspensions, respectively, whereas they were found in 2 of the 20 plots inoculated with truffle seedlings in the *C. crenata* site. These results suggest that mature trees can be colonized with *T. himalayense* using the inoculation procedures of this study, although mycorrhization efficiency varied with inoculum types and host tree species.

Topics: Cultivation

Poster session 11: Cultivation

Establishment of pure cultures of *Lactarius* section *Deliciosi* and its allied taxa from their ectomycorrhizae

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Key words: Colony establishment, Ectomycorrhiza, Edible mushrooms, Isolation, Laticifer, Molecular identification

Lactarius section *Deliciosi* and its allied taxa include edible mushrooms represented by the ectomycorrhizal fungus *L. deliciosus*. There are no reports of isolating pure fungal cultures from ectomycorrhizal roots for this group. We sampled root systems which were connected with rhizomorphs to the basidiomata of *L. akahatsu*, *L. tottoriensis*, *L. hatsudake*, *L. subindigo* and *L. laeticolor*. Ectomycorrhizal root tips having orangish laticifers were selected under the differential interference contrast microscope. Then, they were washed with 0.01% Tween 80 solution, followed by surface-sterilization with 0.1% aqueous $Ca(ClO)_2$ and rinsing with autoclaved distilled water before inoculating onto MNC agar medium containing antibiotics. Root tip-inoculated plates were incubated at 20 °C in the dark for 2 months. Mycelia that emerged from the root tips were transferred to another fresh MNC medium. The pure cultures were identified by PCR-RFLP and molecular phylogenetic analyses targeting the nrDNA ITS region. As a result of this process, we were able to establish pure cultures of five *Lactarius* spp. More than 50% isolation rates were achieved in all species except one of two samples of *L. akahatsu*. Mycelial colonies of *L. akahatsu*, *L. hatsudake* and *L. subindigo* were light-yellow with greenish-blue patches on MNC, but those of *L. laeticolor* and *L. tottoriensis* were deep orangish and grew slowly. Our procedure proved to be an effective method for obtaining pure cultures of some species of *Lactarius*.

Topics: Cultivation

Poster session 12: Cultivation

Mycorrhization of *Tricholoma matsutake* and pine seedlings planted in the vicinity of mycorrhizal nursery stock produced in vitro

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Key words: Ectomycorrhiza, *Pinus densiflora*, Matsutake, Aseptic seedlings, Air-layered saplings

"Shiro" is a whitish soil aggregate structure containing ectomycorrhizas and mycelia of *Tricholoma matsutake* and fine roots of host plants. Because *T. matsutake* forms its fruiting bodies from shiros, it is an essential process for its cultivation to develop shiros artificially. We have already succeeded to produce mycorrhizal nursery stock with shiros in vitro. After transplanting the mycorrhizal stock from culture bottle in vitro to clay pot in a green house, ectomycorrhizas of *T. matsutake* on the plants have survived for three years, while root elongations have been limited and shiros disappeared finally. In the present study, we examined contact effect of non-mycorrhizal roots on shiro development in mycorrhizal nursery stocks. Mycorrhizal nursery plants prepared in growth chambers were transplanted to autoclaved clay pots filled with soil materials. Then 3-6 aseptic seedlings or 2-3 air-layered saplings were transplanted in the vicinity of the mycorrhizal nursery plants. Half a year, and one and a half years after transplanting, root systems and shiros were observed in the nursery plants, the saplings and the seedlings. The fungal species of ectomycorrhizas morphologically identified as *T. matsutake* were reconfirmed with DNA analyses. Results showed that shiros in nursery plants moved to marginal parts of root systems, and ectomycorrhizas were formed in contacted root systems of saplings and seedlings. This suggests that it might be effective for shiro development to plant non-mycorrhizal plants near the mycorrhizal nursery plants, however ectomycorrhizas formed by other species were also detected. Therefore, further studies are needed to make the structure bigger.

Topics: Cultivation

Poster session 13: Cultivation

A reliable measure of mycorrhization level in truffle orchards of *Tuber melanosporum* Vittad.

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Key words: Ectomycorrhiza, Truffle orchard, Roots, *Tuber melanosporum*, Black truffle

Tuber melanosporum cultivation not always results in optimal production yields for growers, with obvious consequences on the subsequent truffle supply for the market. To know about the development of the inoculated truffles in a truffle orchard and to understand why sometimes production is low, it is first necessary to estimate mycorrhization rate. Root sampling and relative analyses are time-consuming and usually are too expensive for truffle growers. To date, the number of plants that should be sampled for an objective evaluation of the mycorrhization level in a truffle orchard is still unknown. Therefore, it is necessary to identify a representative minimum number of plants to be analyzed. To this aim we sampled five *T. melanosporum* truffle orchards located in Central Italy to define: i) the relationship between the number of sampled plants and the presence of *T. melanosporum* and other ectomycorrhizal fungi; ii) the minimum number of trees (MNT) needed to obtain a reliable mycorrhization estimate and the minimum sampling ratio (MSR) as the percentage of MNT over the total number of trees present in the truffle orchard. We used a generalised linear model to calculate MNT for *T. melanosporum* and other fungi during two sampling campaigns in each truffle orchard. The mean values of the MNT ranged among 12–17 and 17–18, respectively, for *T. melanosporum* and for other fungi. Thus, we suggest that to reliably estimate the mycorrhization level of a truffle orchard a MSR of at least 12% should be considered as a reliable value.

Topics: Cultivation

Poster session 14: Cultivation

Effect of LED lightning and soil nitrogen on the growth of pine seedlings associated with *Tricholoma matsutake in vitro*

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Key words: Ectomycorrhiza, Edible mushrooms, Matsutake, LED light, Nitrogen source

Tricholoma matsutake forms ectomycorrhizas with *Pinus densiflora* both *in-vitro* and *in-situ*. Although *in-vitro* ectomycorrhization of *T. matsutake* with *P. densiflora* host plants has been routinely studied, it takes several months to develop seedlings, which limits mass production of mycorrhizal seedlings. In this study, we assessed impacts of lighting and soil nutrition on *in-vitro* mycorrhization of *T. matsutake* with *P. densiflora* host. To do this, aspetic *P. densiflora* seedlings were inoculated with mycelium of *T. matsutake in-vitro*. Effects of two light conditions (fluorescent light / red LED light + fluorescent light) and two soil nitrogen conditions (intact granite-based mineral soil / soil supplemented with 1g dried yeast per L intact soil) on *in-vitro* mycorrhizal synthesis were assessed. Six months following the incubation, ectomycorrrhization of *T. matsutake* and host pine growth were measured and compared between experimental plots. The combination of red LED light + fluorescent light and supplemented soil showed a significantly larger number of ectomycorrhizal root tips and greatest plant biomass compared to other treatments. Two-way ANOVA indicated that root biomass was promoted by red LED light + fluorescent light treatments, and shoot biomass was promoted by supplemented nitrogen source (dried yeast). The number of ectomycorrhizal root tips increased about six times in the best combination of lighting and soil nutrient compared to the conventional conditions (intact soil+ fluorescent light). These results help to clarify the impacts light and nutrients on the ectomycorrhizal development of *T. matsutake–P. densiflora* symbiosis.

Topics: Cultivation

Poster session 15: Cultivation

Larch forest managements for the mushoroom harvests of *Suillus grevillei* in Japan

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Key words: Forest management, *Larix kaempferi*, Japanese larch, *Suillus grevillei*

Suillus grevillei is a mycorrhizal fungus specifically associated with larch trees (genus *Larix*), and is a popular edible mushroom in Nagano and other provinces in Japan. The mushrooms are sold in local markets for 10,000 yens /kg in the top season. Currently, we are emphasizing to foresters in the Nagano Prefecture the importance of employing forest management practices that increase the harvests of *S. grevillei* mushrooms. Here, we present an example of such forest management practices conducted in the Nagano Prefecture. We set six experimental plots at different larch forest sites, conducted differing forest management practices at these sites, and observed *S. grevillei* fruiting in the summer–autumn season for 5–7 years. Each experimental plot (20m×20m) was divided into four subplots (10m×10m): in three subplots we cut all the shrubs and then inoculated two of the three subplots with *S. grevillei* spores (crushed basidiomata). The fourth subplot received no treatment and served as a control subplot. The combined data from all six experimental plots showed no significant difference in the harvest value (number of fruiting bodies) as a result of differing forest management practices. However, one plot showed much fruiting in the control subplot adjacent to the boundary with a treated subplot (due to probably an edge effect). When we deleted the data from that one plot the cutting treatment in the remaining five plots showed significantly greater fruiting of *S. grevillei* (P=0.039; t-test). This suggests that forest management is effective for the increase of *S. grevillei* mushrooms harvests in larch forests in Nagano Prefecture.

Topics: Cultivation

Poster session 16: Cultivation

Nutritional effect of phosphoric acid on the ectomycorrhization of *Tricholoma matsutake*

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Key words; Ectomycorrhiza, *Tricholoma matsutake*, Cultivation, Nitrogen source, Phosphoric acid source, Fertilization

Tricholoma matsutake forms fairy rings. The subterranean network of mycelium that lies in the mineral soil layer contained within the ring is called “Shiro”. A recently identified antimicrobial substance, an (oxalate) aluminate complex, accumulates in the shiro area. At the formation of this complex, it is solubilizing the phosphate source and releasing phosphoric acid in the soil. In this study, we investigated the effect of fertilized phosphoric acid and organic nitrogen sources on the ectomycorrhization of *T. matsutake* with *P. densiflora* in vitro. Aseptic *P. densiflora* seedlings were inoculated with cultured mycelium of *T. matsutake*. In vitro mycorrhizal synthesis was conducted with the following soil nutrient ingredients (soil volume 250 mL): intact soil (granite-based mineral soil), nitrogen-added soil (1g dried yeast/kg dried soil), phosphoric acid-added soil (0.996g Potassium dihydrogenphosphate/kg dried soil), and co-addition soil. Six months following the incubations, the experimental plots were measured and compared for the degree of ectomycorrhization of *T. matsutake* and host pine growth. Immediately after incubation, several seedlings were transplanted to larger growing pots (soil volume 1 L) and incubated in vitro for six months. As a result, it was revealed that phosphoric acid fertilization greatly promoted the initial growth of plants. Mycorrhizal formation significantly increased in the co-addition plot. We will examine the long-term nutritional effect of phosphoric acid as it applies to the ectomycorrhization of *T. matsutake.*

Topics: Cultivation

Poster session 17: Cultivation

Attempt to compare the old and the new world of truffles

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Key words: Truffle cultivation, Mycorrhizal plant, Climate change, Irrigation, Weight of tradition

The old world of truffles is located in Europe, mainly in Spain, France and Italy. It is in France that the cultivation of the black truffle *Tuber melanosporum* was developed about two centuries ago. The initial period of abundance was followed by a decline after the two world wars. In 1972, the science-based development of the mycorrhizal plant saved truffle production in Europe. The new world of *T. melanosporum* is located outside of Europe where this species has conquered new areas thanks to the mycorrhizal plant. *Tuber* cultivation has spread since the 1970s to the USA, New Zealand, Australia, South and North America, Africa, Middle East. At the start of the XXI century, Asia has become involved with the discovery of *Tuber indicum* and other promising species such as *T. panzhihuanense*. Throughout the world, the number of successfully grown species is increasing: *T. brumale*, *T. aestivum* / *uncinatum*, *T. indicum*, *T. mesentericum*, *T. borchii*, *T. gibbosum*. In France, where the absence of irrigation systems is frequent, climate change strongly penalizes truffle cultivation. In Spain, thanks to irrigation over large areas, cultivation gives attractive results. In the new world, truffle growers plant mycorrhizal trees in soils where the truffle faces less of its natural fungi competitors. New world's growers also irrigate, weed, and prune, most often with innovating techniques. Some exceptional results contrast with those observed today in Europe. If, in the old world, the low production - compared to the past - and the heterogeneous production results always raise questions, the new world opens perspectives to the old one in the absence of the weight of tradition and thanks to a better transparency of truffle yields.

Topics:

Poster session 18: Cultivation

Impact of the herbicide roundup® on mycelial growth of truffles, morels, and molds

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Key words: Ectomycorrhiza, Edible mushrooms, Herbicide, Interaction, Truffle, Morels

Herbicides are frequently used to control weeds in agricultural and residential settings. Little is known about the impact that herbicides have on fungi, such as *Tuber* species, *Morchella* species, or *Mortierella* species. Understanding how weed management tools such as the herbicide Roundup® interact with these fungi may better inform their use in morel or truffle production. To assess impacts of Roundup®, containing glyphosate, on fungal growth *Morchella americana, M. importuna,* six *Tuber* species, and *Mortierella elongata* were each grown in serial dilutions of an herbicide containing media ranging in concentrations above and below the working ratio recommended by the manufacturer. To determine the impact on fungal growth amongst these genera, hyphal growth on agar media and fungal biomass in liquid media was collected. None of the tested fungi grew in media containing a high herbicide concentration, but the fungi did grow unimpeded in the low herbicide concentration. Though the working herbicide concentration is near the point of fungal growth inhibition *in-vitro*, when applied into the environment during typical application little of this foliar spray would be expected to drench the soils where these fungi inhabit. As such, the use of this herbicide at recommended rates would not be expected to interfere with the mycelial growth of the fungi used in this experiment; however, use of these herbicides in higher than recommended concentrations may inhibit mycelial growth and fungal biomass in the soil.

Topics: Cultivation

Poster session 19: Cultivation

Establishing truffle orchards in the Midwest US for research and education

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Key words:

Interest in truffle cultivation is growing in the United States. The wide climactic variation across North America makes certain regions more amenable to growing particular truffle species and host plants. In the Midwest United States, for example, harsh winters are unfavorable for the cultivation of *Tuber melanosporum*. However, many species of native truffles grow in the region, including the commercial species *T. lyonii* and *T. canaliculatum*. To better inform truffle orchard management strategies in the Midwest region, we are establishing a series of truffle orchards in MI for research, education and demonstration. Our first based experimental truffle orchard was established in 2015 in blocks planted with *T. aestivum, T. borchii, T. canaliculatum* and *T. lyonii* inoculated seedlings of pecan (*Carya illinoinensis*), chestnut (*Castanea dentata* x *sativa*), white oak (*Quercus alba*), white pine (*Pinus strobus*) and Norway spruce (*Picea abies*). Our plot was set up as a full factorial design to test the impact of biochar (10%), and weed control strategies on truffle ECM colonization and fruiting. The 60m x 30m plot is a mix of Metea and Conover loamy sand, into which 320 truffle inoculated seedlings were planted (80 trees / quadrate). Within the first year 10% of the seedlings died. Grasses still dominate the plant community on the orchard treatment where Roundup was applied. Sampling is underway to assess fungal and bacterial community shifts in soils beneath individual tree-seedlings sampled across a time-series. A second orchard is being prepared to address questions on biochar, root density and host fidelity.

Topics:

Poster session 20: Cultivation

Strain selection of Japanese yellow chanterelle based of the fruiting ability in the pot cultivation

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Key words: *Cantharellus*, Ectomycorrhizal synthesis, Edible mushrooms, Host associations, Pot culture

We have achieved repeated fruiting of Japanese yellow chanterelle, *Cantharellus anzutake*, with pine (*Pinus densiflora*) and oak (*Quercus serrata*) hosts in a pot cultivation system (Ogawa et al. 2019). In this study, we aimed to develop the system and to select fungal strains for better mushroom production. We used 10 strains of *C. anzutake* (EN-51, EN-52, EN-61, C-2, C-23, C-30, C-85, S-203, S-236, S-244) and synthesized ectomycorrhizas with *P. densiflora* in vitro. Ectomycorrhizal colonization trended higher in the strains isolated from conifer forests against those from broad-leaf forests. However, this trend was not observed when mycorrhizal seedlings were first acclimated in the 250 mL pots, and subsequently grown in up-scaled 1 L pots. In the 4 L pot cultivation for two years, 6 of the 10 tested strains fruited: strains EN-61, C-23 and C-30 fruited repeatedly. We used chanterelle-colonized pine seedlings in 250 mL pots as mother plants to establish oak-chanterelle association. Four strains (EN-51, EN-52, S-203, S-236) were tested: a chanterelle-colonized pine seedling was co-cultured with a non-mycorrhized *Q. serrata* seedling in a 1 L pot. After the chanterelle-colonization on the oak seedling, both mycorrhizal seedlings in a 1 L pot were separately grown in 4 L pots. All four strains fruited on oak hosts, and three on pine hosts in the 4 L pots. These data strongly suggest that *C. anzutake* strains differ in the fruiting property under experimental conditions, which enable selection of suitable chanterelle strains for mushroom cultivation.

Topics: Cultivation

Poster session 21: Cultivation

Culture and characterization of mycelia of different edible *Lactarius* species in Guatemala for mycorrhization with local pine species

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Keywords: Edible wild mushrooms, Inoculum, Reforestation, Biodiversity

Guatemala is considered a hotspot of biodiversity and many undescribed species are being collected in the recent years. Molecular analyses are also confirming the consumption of more species sold under a common name. In order to generate information about the edible *Lactarius* species and their tree symbionts in Guatemala, we selected strains of four *Lactarius* species (*L. indigo, L.* aff *subpurpureus, L.* aff *deliciosus, L.* aff *miniatosporus*) to inoculate four pine species (*Pinus oocarpa, P. pseudostrobus, P. montezumae, P. maximonoi*). For this purpose, fungal strains were selected from a previous study and certified pine seeds were used. With the aim to describe and improve the mycelium growth, we used M+P medium, as successfully used by Wang et al (2019) for *L. deliciosus*, although the rate of growth was lower for the local strains of *L.* aff *deliciosus* and *L. indigo*. Every species showed differences in the color of the colonies: yellow-greenish for *L indigo*, yellow-orangish for *L.* aff *deliciosus* and orangish-reddish for the rest that also presented abundant aerial mycelium. With these results we suggest that the *L. deliciosus* species complex in Guatemala requires more research for inoculum production, *L. indigo* can be used successfully with BAF and the local red-latex milk caps with M+P. Since those fungal species are highly demanded for food by Mayan people in Guatemala and since the pine/oak wood is still the main fuel in rural homes, we advise to experiment the use of mycorrhized plants with *Lactarius* for reforestation in Guatemala highlands.

Topics: Cultivation

Poster session 22: Cultivation and Molecular biology

Tuber magnatum mycelium survival in soil of orchards established with inoculated seedlings

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Key words: Truffle, *Tuber magnatum*, Controlled mycorrhization, Orchards, qPCR

The white truffle, *Tuber magnatum* Pico, is the most famous and appreciated truffle. This species is naturally harvested in Italy, Balkans, and more recently in France and Switzerland as well as in Thailand. In natural fields the ectomycorrhizae of this species are very difficult to find and during many years the controlled mycorrhizal synthesis remained impossible. As part of a joint research project started in 2004, Robin nursery in collaboration with the French National Institute for Agricultural Research (INRA) were able to produce well-mycorrhized plants. Plants are commercialized since 2010 after a DNA control confirming that each plant harbour *T. magnatum*. The aim of this study was to assess the survival of *T. magnatum* in the soil of French orchards that are 3 to 8 years old. In 2017 and 2018, 107 soil samples were harvested in 12 orchards. The sampling was carried out once for each site in April-June. After DNA extraction, the DNA of *T. magnatum* was specifically detected using a nested qPCR protocol. DNA of *T. magnatum* was detected in 5 sites in plantations that were 3 to 8 years old. Due to monthly mycelium dynamic, we cannot exclude that *T. magnatum* is also present in the other plantations. To our knowledge this is the first study of *T. magnatum* orchards established with high quality mycorrhizal seedlings. We demonstrated that *T. magnatum* is able to survive up to 8 years after plantation. Base on these results, these orchards will be monitored until the first ascocarps are harvested.

Topics: Cultivation, Molecular biology

Poster session 23: Ecology

Inventory and bioecology of terfez in some regions of the northern Algerian Sahara

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Key words: Bioecology; Desert Truffles; Distribution; Climate; Soils; Northern Algerian Sahara

This study focusses on the bio-ecological study of of desert truffles collected from the Northern Algerian Sahara. The identification of desert truffle species with a morphometric characterization show three species of the family Terfeziaceae: *Terfezia arenaria* (Moris) Trappe, *Terfezia claveryi* Chatin and *Tirmania nivea* (Desf) Trappe. These hypogeous ascomycetes live in mycorrhizal association with *Helianthemum lippii* (*Cistaceae*). Desert truffles grow in heterogeneous soils of sandy texture, moderately calcareous, slightly alkaline, with low organic matter and low phosphorus levels. The truffles colonize desert depressions "Dayas" and beds of wadis, since these geomorphological units accumulate rainwater, which promotes the development of both truffles and host–plants. Desert truffles are edible hypogenous fungi that are very well adapted to conditions of aridity. The average production was 785.43 ± 743.39 g / ha. Production is closely related to autumnal precipitations occurring during October–December, which is the critical pre-breeding period for both desert truffles and host-plant species.

Topics:

Poster session 24: Ecology

Soil chemical and physical properties of *Tuber himalayense* and *Tuber japonicum* habitats in Japan

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Key words: Base saturation ratio, Gravel, Soil pH, Soil texture, Truffle

The ecological conditions required for the growth of a black truffle (*Tuber himalayense*) and the newly described white truffle (*Tuber japonicum*) are not well understood. We characterized the soil properties of five (black truffle) and four (white truffle) growing sites in Japan. At each site, soil samples (0–15 cm depth) were collected from adjacent plots with or without ascocarps. The chemical and physical properties of the soil samples were determined. In plots with ascocarps, soil pH was significantly lower (pH 5–6) at *T. japonicum* sites than at *T. himalayense* sites (pH 6–8). Exchangeable calcium concentration and base saturation ratio were significantly lower at *T. japonicum* sites than at *T. himalayense* sites. Exchangeable calcium concentration and the sum of exchangeable cations were significantly lower in plots with *T. japonicum* ascocarps than in plots without *T. japonicum* ascocarps. These results suggest that neutral or slightly alkaline soil with a high base saturation ratio may be favorable for *T. himalayense*, whereas *T. japonicum* may be adapted to more acidic and lower nutrient soils. In plots with ascocarps, soil texture varied among sites of each *Tuber* species and did not differ significantly between the two truffle species. At *T. japonicum* sites, the gravel (>2 mm) content was higher in soil with ascocarps than in soil without ascocarps. This suggests that soils with high gravel content, which may increase water infiltration and air permeability, are favorable to *T. japonicum*.

Topics: Ecology

Poster session 25: Ecology

Temporal dynamics of soil fungal communities after partial and total clearcutting in a managed *Pinus sylvestris* stand

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Key words: Clear-cutting, Ectomycorrhizal edible fungi, Forest regeneration, Fungal diversity, High throughput sequencing, Forest multifunctionality

Forest management aimed to maximize timber production might impact soil fungi, especially those symbiotically associated to tree roots. We analysed the temporal dynamics of soil fungi along five sampling years after tree removal in a managed *Pinus sylvestris* stand in northern Spain, where timber production is combined with regular edible mushroom harvesting. Two management methods were tested: total and partial clear-cutting leaving retention trees for seedling regeneration vs. undisturbed, control plots. Fungal communities (phylotypes and ecological guilds) were analysed by Illumina MiSeq sequencing. We hypothesized that (1) ectomycorrhizal fungal communities will decrease after clear-cutting treatments with a concurrent increase of saprotrophs, (2) the abundance and diversity of the ectomycorrhizal guild will be more preserved in partially clear-cut than in total clear-cut plots, and (3) the overall fungal diversity will decrease in the cut plots leading to major losses of ectomycorrhizal species. Soil fungal composition changed across the five years after clear-cutting by decreasing ectomycorrhizal fungi and increasing saprotrophs. However, these changes did not significantly affect fungal diversity and there were taxa-specific responses to tree harvest treatments. *Boletus edulis*, the most abundant edible ectomycorrhizal species fruiting in the study area and a valuable local resource, was negatively affected by either clear-cutting treatments. Our results indicate a strong effect of tree harvest on the relative abundance of ectomycorrhizal fungi along the first years after clear-cutting. However, levels of fungal diversity were comparable to the undisturbed forest, thus suggesting a potential recovery of ectomycorrhizal fungi through the colonization of the regenerated seedlings.

Topics: Ecology

Poster session 26: Ecology and Diversity

The mycobionts, phytobionts and microniches drive the bacterial and fungal structure of Neotropical ectomycorrhizas

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Key words: Ecology, Metagenomics, Neotropics, Biodiversity, Microniches

Few studies have been done investigating the factors that drive the microbial communities associated with ectomycorrhizae in the Neotropics. The influence of two phytobionts (*Pinus patula* and *Pinus greggii*) and two mycobionts (*Suillus pungens* and *Thelephora terrestris*) in the composition of the microbiome associated with their ectomycorrhizae were evaluated. 16S and 18S libraries were built for the evaluation of microbiomes. PCR amplification was performed using the Biometra TProfessional Thermocycler and sequencing was performed on an Ion Torrent sequencer in CINVESTAV, Mexico. The evaluated samples belonged to microniches, for the non-mycorrhized plants the root and rhizosphere were used, and for the mycorrhized plants the ectomycorrhizae and the ectomycorrhizosphere were used. In the bacterial community 8 dominant operational taxonomic units (OTUs) including Bacillales, Gemmatimonadales and 6 unclassified groups comprised about 90%. For the fungal community, 95% of the relative abundance was composed of 3 OTUs included in Hypocreales, Sordariales and Eurotiales. We identified 79 OTUs at the family level for bacteria and 32 OTUs for fungi, which had a relative abundance greater than 1% in at least one of the microniches. It was observed a greater bacterial diversity in *P. greggii* than in *P. patula*, whereas in the fungal diversity an opposite trend was observed. Mycobionts showed differences when associated with phytobionts, for example *Suillus pungens* increased microbial diversity in *Pinus greggii*, while *Thelephora terrestris* favored that of *Pinus patula*. This work demonstrates that the structure of bacterial and fungal communities associated with Neotropical ectomycorrhizae is driven by phytobionts, mycobionts and microniches.

Topics:

Poster session 27: Diversity

Fungal diversity and endemic species in Sierra de las Minas, Guatemala

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Key worlds: Biodiversity, Macrofungi, Boletales, Ectomycorrhizal endemic species

The Sierra de las Minas Biosphere Reserve (SMBR), Guatemala, is the area with the highest diversity of fungal species in the country due to its geographic isolation, elevational gradients and diversity of micro-climates. Local macrofungi have been formally studied for the first time in the area. We conducted a study from March 2018 to June 2019 at 800-2600 m a.s.l. in oak, pine-oak, pine-cypress and mixed forests (liquidambar, oak, alder, pine and fir). We collected 179 species of macrofungi corresponding to 78 genera in 13 orders mainly mycorrhizal Agaricales, Boletales, Cantharellales Gomphales Russulales, Pezizales and Thelephorales. We found new records for the country such as *Aureoboletus singeri*, *Phylloporopsis boletinoides*, *Lactarius lignyotus*, *Suillus spreaguei,* as well as many unknown species of *Amanita, Boletus*, *Butyriboletus, Caloboletus, Craterellus, Cortinarius, Laccaria, Lactarius, Lanmaoa, Leccinellum, Multifurca*, *Rubroboletus, Suillellus*, *Sutorius, Tylopilus, Xerocomellus* and saprophytic species such as *Hericium*, *Macrocybe, Polyporus* and *Podaxis*. The order with the greatest richness was Boletales with 71 species. The communities of *Quercus* are also the richest, especially around 1000-1500 m a.s.l., but very few fruit bodies were found in the climax community -around 2500 m a.s.l.-, with oak trees over 300 years old. Preliminary phylogenetic studies found new species for the *Boletus* and *Craterellus* genera. The project will also study the last natural *Acer* and *Abies* remnant areas in the continent, located in this reserve, as well as their hypogeous mushrooms and ectomycorrhizae, especially those of endemic *Lactarius* including the local edible complexes of *L. delicious* and *L. indigo.*

Topics: Diversity

Poster session 28: Biodiversity

Diversity and new species of *Cantharellus, Craterellus* and *Pseudocraterellus* in Guatemala

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Key words: Cantharellaceae, Pine-oak forest, *Abies*, Edible mushrooms

We show results of macroscopic, microscopic and phylogenetic analysis of Guatemalan samples of these genera that belong to the mycological collection of USAC (MICG). At a macroscopic level there are differences among the species identified as *Cantharellus cibarius, Cantharellus lateritius, Craterellus tubaeformis, Craterellus ignicolor, Craterellus fallax* and *Pseudocraterellus calyculus*. These include differences in colour, cover of the cap surface and habitat. We also found more diversity of chanterelles in oak forests than in *Pinus* or *Abies* forests, especially in the Eastern zone of the country. Microscopic analysis showed similar results, as reported for North America, for the size of basidia and spores, absence of cystidia, thin walled hyphae in the pileipellis and clamp connections in all specimens. However, three species (with the largest fruit bodies) showed basidia up to 130µm, one with very short spores and one with rugose outer epispore. The phylogenetic analysis, with ITS2, showed clear differences between the Guatemalan species and the sequences registered in GeneBank. The best tree indicates several new species with a wide distribution in the country: nine new species for *Cantharellus,* four new species for *Craterellus* and one for *Pseudocraterellus.* The phylogenetic analysis also coincides with the differences found in the local samples. With these results, we suggest focussing research and conservation efforts on the habitats and hosts for the identification of local species and their distribution in Central America. Finally, we point out that *Cantharellus* and some *Craterellus* are appreciated edible mushrooms for Mayan descendant people in Guatemala.

Topics: Biodiversity

Poster session 29: Biodiversity

Tuber species of Canada based on molecular identification of existing herbarium collections

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Key words: Fungal collections, ITS sequences, Native truffles, Introduced truffles

In Canada, the study of truffles has been somewhat limited and knowledge of our truffle biodiversity has lagged. Because of this, keys to local species do not exist and the identification of *Tuber* species found in Canada has depended on the generous input of foreign experts. In this study, we accessed collections of *Tuber* already existing in Canadian fungal collections with the goal of sequencing their ITS region and using this new taxonomic information to re-assess the identification of the collections. We analysed sequence results from just under 60 collections and documented 16 species of *Tuber* using this approach, many of which were mis-labelled. Three are Mediterranean species of *Tuber* being cultivated in Canadian truffle orchards, one is a European species likely spread incidentally on the roots of horticultural trees, four are native species with culinary value, and the other eight are native species that are ectomycorrhizal partners of native trees. Some of the native species are found in truffle orchards and may pose a competitive threat to truffle cultivation. In addition and based on relatively little sampling of ectomycorrhizal roots, mostly in BC, we have detected three undescribed species of *Tuber* so it is likely that similar work both in BC and across the country would detect other *Tuber* species. More trained truffle dog teams would also greatly enhance our exploration of hypogeous fungi in Canada. By correctly identifying and annotating the collections, we are helping clarify the diversity of fungi in Canada.

Topics: Biodiversity

Poster session 30: Evolution and Phylogeny

Revisiting Japanese truffle phylogeny and diversity: possibilities for cultivation and edibility

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Key words: Biogeography, Forest resources, Hypogeous fungi, *Tuber*

Ascomata produced by *Tuber* spp. are well known as truffles, some of which have high global demand as a culinary ingredient. The discovery of new *Tuber* taxa enhances our understanding of fungal diversity and increases our knowledge of new food resources. In our previous phylogenetic work on Japanese *Tuber* specimens, we found 20 phylotypes in Japan that clustered into five previously known clades (Macrosporum, Rufum, Melanosporum, Puberulum, and Maculatum groups). There was also a novel lineage referred to as the Japonicum group, which was formed by two phylotypes (*T. japonicum* and *T. flavidosporum*). Furthermore, the Chao2 richness estimator estimated that there are still another 20 undiscovered *Tuber* species in Japanese forests. Since that work, we discovered several undescribed *Tuber* specimens that can be distinguished from known taxa by morphology alone. We present here an updated molecular phylogeny (including the new specimens) that is based on ribosomal ITS and LSU sequences of Japanese truffles. We detected two new lineages that formed the Excavatum group, an additional new lineage of the Japonicum group, and two new lineages in the Puberulum group. As there is substantial truffle diversity in Japan; we also discuss their cultivation and edibility.

Topics: Evolution and Phylogeny

Poster session 31: Evolution and Phylogeny

Genetic diversity of the genus *Ramaria* in the Patagonian Andes forests

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Key words: Edible mushrooms, *Ramaria patagonica*, *R. botrytis*, Nothofagaceae

Within the wide diversity of fungal species of the Patagonian Andes forests, the mycorrhizal species of the genus *Ramaria* are one of the most attractives. Their representatives stand out for possessing striking fruiting bodies, colored coral-likes basidiomes, and excellent organoleptic characteristics that make them an interesting *gourmet* product. In Patagonia 15 species are reported, being the most common the endemic *R. patagonica* and the cosmopolite, *R. botrytis.* So far, collection and consumption are based only on morphological characteristics. However, the great macro and micro morphological variability that they present, often makes it impossible to differentiate them to the species level. Nowadays, there are no works that have addressed the genetic study of the Patagonian species. Our investigation aims to elucidate the phylogenetic relationships that exist between endemic species present in Patagonia (*R. patagonica)* and the rest of the cosmopolitan species that are present in our forests and in other parts of the world. Also, we identified morphological characters with taxonomic value, relationships with its edibility and associations with Nothofagaceae species. For this, collections of *Ramaria* spp. were obtained from Nothofagaceae forests from Neuquen to Tierra del Fuego provinces in Patagonia, Argentina. The rRNA internal transcribed spacer (ITS) and large ribosomal subunit (LSU) sequences were amplified and analyzed. Macro and micromorphology characterization were performed and host associations were recorded. Preliminary results indicate that *R. botrytis* is directly associated with *Nothofagus dombeyi* forests, with low morphological variation within specimens. On the other hand, in the case of *R. patagonica*, the morphological and molecular analyses suggest that it could be a complex of species, directly associated with hosts and site characteristics.

Topics: Evolution and Phylogeny

Poster session 32: Genetics and Genomics

The draft genome sequence of *Russula griseocarnosa* and its association with mycorrhizal characteristics

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Key words: *Russula griseocarnosa*, Whole genome, Functional gene, Comparative genome, Phylogenetic tree

Russula griseocarnosa, with health benefits for its richness in nutrients and active functional ingredients, is a famous edible and medicinal mycorrhizal mushroom distributed in Southern China, especially in Guangdong, Guangxi and Yunnan Provinces. Pure cultures of *R. griseocarnosa* are extremely difficult to obtain by manual isolations and has not been artificially cultivated yet. Therefore, the demand for this fungus exceeds the supply, and their genetic information and dedicated research are scarce. Herein, we sequenced *R. griseocarnosa* genome by using fruiting body integration of second- and third- generation sequencing technology, followed by a 'de novo' assembly strategy based on the high-throughput sequence data, and using Genemark ES, Blast, CAZy and other databases for gene function annotation. We also constructed a phylogenetic tree with additional different ecological type fungi. Results showed a draft genome comprising 193 scaffolds, 340 Contigs, GC content 49.45% and 48.35Mb in size. *R. griseocarnosa* showed large differences with other mycorrhizal fungi including *Tricholoma matsutake*, *Suillus luteus* and *Tuber melanosporum*, especially in the number and variety of CAZymes which were extremely limited in *R. griseocarnosa*. There were no shared genes with *T. melanosporum*, also supported by the results of phylogenetic tree analysis. No carbohydrate esterase of CAZymes were annotated in *R. griseocarnosa*. Other carbohydrate active enzymes like auxiliary activities and polysaccharide lyases were also limited in comparison to other saprophytic and mycorrhizal fungus, revealing that there may be special metabolic ways to achieve a closer symbiotic relationship with plants to complete its metabolism and growth.

Topics: Genetics and Genomics

Poster session 33: Taxonomy

Morphoanatomic and molecular characterization of *Laccaria squarrosa* ectomycorrhiza associated with *Quercus* sp.

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Key words: Ectomycorrhizal ecology, ITS, Oak forest, Traditional knowledge, Wild edible mushrooms

Laccaria is a genus which includes more than 70 species distributed in both temperate and tropical regions of the world. They establish ectomycorrhizal symbioses with a range of host trees including species of *Quercus.* However, only few works have documented the morphoanatomical and molecular characterization of their mycorrhizas under natural conditions. Besides their ecological importance, species of *Laccaria* are harvested as food, as a source of income around the world. In this work, for the first time, we describe the ectomycorrhiza of *L. squarrosa* previously only known from one single locality in a *Fagus grandifolia* var. *mexicana* forest in eastern Mexico. Soil samples were gathered by excavating below basidiomes of *L. squarrosa* in *Quercus* forest in Piedra Canteada, Tlaxcala, Mexico. The morphotypes found in the soil samples were photographed and characterized and hand-made sections of root tip morphotypes were made for the microscopic characterization. For molecular identification PCR was performed with DNA extracted from ectomycorrhizas targeting the nuc rDNA ITS. In the molecular phylogenetic analyses, our sequences were clustered together with *L. squarrosa* with high values of bootstrap and posterior probabilities supporting identity of the ectomycorrhiza. The sporocarps of this species are harvested as food by two Mesoamerican ethnic groups: Nahua and Mazahua who live nearby the studied *Quercus* forest. This is the first time that an ectomycorrhiza of a species of *Laccaria* is described from natural conditions in Mexico. This is also the first report of edibility of *L. squarrosa* world-wide and increases the distribution range of this species.

Topics: Taxonomy

Poster session 34: Taxonomy

Taxonomy of Japanese *Tricholoma ustale*

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Key words: Ectomycorrhiza, Poisonous mushroom, Taxonomy, Kaki-shimeji, Ustalic acid

Tricholoma ustale (Fr.) P. Kumm. belonging to *Tricholoma* sect. *Genuina* associates with various Pinaceaeous and Fagaceaeous plants. In Japan, *T. ustale* is called *Kaki-shimej*, and known as a poisonous mushroom that contains the toxic compound ustalic acid. One third of mushroom poisoning occurring annually in Japan are caused by this species. However, *Kaki-shimeji* has traditionally been consumed in some local regions in Japan, suggesting that *Kaki-shimeji* might be a species-complex. In fact, *Kaki-shimeji* shows large morphological variation and ecological habitats. *Kaki-shimeji* was first identified as *Tricholoma ustale* (Fr.) P. Kumm. in 1929 by S. Kawamura. In this study, we aimed to clarify the probable species-complex of *Kaki-shimeji* on the basis of molecular phylogenetic analysis and other approaches. We collected 61 basidiomata of *Kaki-shimeji* from various forest sites in Japan, and conducted multi-gene phylogenetic analyses. We focused on ITS and IGS regions of nuclear rDNA, *tef1*, *GAPDH*, *RPB2*, SSU of mitochondrial rDNA, and *ATP6*. As a result, *Kaki-shimeji* consisted of four distinct clades based on any DNA regions tested. Interestingly, all four phylogenetic clades in the *Kaki-shimeji* differed from that of European *T. ustale*. This result suggests that *Kaki-shimeji* is indeed not *T. ustale.* In addition, all four phylogenetic groups of *Kaki-shimeji* showed difference in the sizes of spores and basidiomata. Ustalic acid was detected by LC-MS analysis in the basidiomata of only two phylogenetic groups of *Kaki-shimeji*. These results warrant further in-depth study of the phylogenetic groups of *Kaki-shimeji* to elucidate their taxonomic positions.

Topics: Taxonomy

Poster session 35: Taxonomy

Taxonomy of Japanese golden chanterelles

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Key words: Phylogenetic analysis, *Cantharellus anzutake*, *C. cibarius*

Japanese golden chanterelles consist of al least 4 species including *Cantharellus cibarius* s.s., one of which, widely distributed from Honshu to Okinawa Islands, was recently described as *C. anzutake* sp. nov. (Ogawa et al. 2018). In addition, Japanese *C. cibarius* specimens were only found from Hokkaido Island. In the present study, we conducted multi-gene molecular phylogenetic analyses of Japanese golden chanterelles: 13 dry specimens and 8 cultured strains stored in our laboratory, and 14 specimens newly collected from various parts of Japan in 2018. Six genetic regions, i.e. internal transcribed spacer 2 (ITS2) region of rDNA, ATP synthase 6 (*ATP6*), cytochrome c oxidase subunit 3 (*COX3*), minichromosome maintenance complex component 7 (*MCM7*), glyceraldehyde-3-phosphate dehydrogenase (*GPD*), and RNA polymerase 2 subunit (*RPB2*) locus, were targeted. In the phylogenetic analyses, 40 sequence data of related species registered in NCBI were added. As a result, *C. cibarius*, which was previously confirmed only from Hokkaido, is reported from Mt. Fuji in Honshu Island for the first time. In the analysis of *ATP6*, *C. anzutake* branched into two clades: one includes samples from Nagano and Miyagi Prefectures in Honshu and Hokkaido, respectively, and another one from Nagano and Fukuoka Prefectures in Honshu and Okinawa, respectively. In *MCM7*, specimens from Okinawa showed to form an independent cade from others in *C. anzutake* branch. In *GPD* and *RPB2*, Swedish *C. cibarius* specimens branched off from Japanese *C. cibarius* specimens. Overall, these results suggest that multi-gene molecular phylogenetic analyses are useful for resolving the taxonomy of Japanese golden chanterelles.

Topics: Taxonomy

Poster session 36: Taxonomy

Tuber rugosum sp. nov.: a new spiny-spored truffle species from North America

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Key words: Ectomycorrhiza, Truffle, Phylogeny, Fatty acids, Species description

Truffle species in the genus *Tuber* are ectomycorrhizal (ECM) ascomyceteous fungi that produce hypogeous fruiting bodies, some of which are sought after for their economic and culinary value. Beyond their culinary value, *Tuber* species are ecologically significant to the trees on which they associate. One of the most speciose lineages within *Tuber*, the Rufum clade, is widely distributed throughout Asia, Europe, and North America. This clade has been estimated to consist of more than 43 species, many of which have not yet been formally described. Here, we use phylogenetic and morphological methods to formally describe *T. rugosum* sp. nov., which was previously recognized as *Tuber* sp. 69. We also demonstrate that pure isolates of *T. rugosum* have a unique fatty acid profile compared to the pure isolates of related species *T. texense* and *T. lyonii*. *Tuber rugosum* sp. nov. has been collected in Quebec, CA, Minnesota, and in Michigan, USA, and we confirm that it is ectomycorrhizal with *Quercus* trees. One of the key morphological traits evident in *T. rugosum* is the ever-present rugose regions found across the peridium of the ascocarp; these rugose regions variably exhibit exposed sterile glebal tissue. The aroma and flavor is mild to earthy and as with its sister species *T. spinoreticulatum,* it is not likely to have much economic value.

Topics: Taxonomy

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