20th ISMS CONGRESS

Program and Abstract Book

26-27 February 2024, Las Vegas, USA

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Introduction

Abstracts & Presenter Bios

How to Use This Book

Abstracts in this book have been divided into oral presentations and posters.

Oral Presentations

Abstracts in this book are listed in the same order as they appear in the program - ie the number beside the presentation title in the program corresponds to the same number beside the title in the Book of Abstracts.

Posters

Posters have been loosely grouped into topics and allocated a number primarily for pinning purposes. The number beside the poster title in the Book of Abstracts relates to the order the poster will appear in the poster gallery.

Author Index

Abstracts can also be located via the author index at the end of the book.

Welcome from the ISMS President

On behalf of the ISMS Executive Committee, it is a pleasure to welcome you to the 20th ISMS Congress (ISMSC) in Las Vegas, Nevada in the USA.

This Congress is being held in conjunction with the 26th North American Mushroom Conference (NAMC) to offer delegates a joint event with a seamless smorgasbord of information, inspiration, and networking to deliver a truly memorable experience that will last for years to come.

ISMS Congresses have been held once every four years or so since 1950. The combination of scientists, students, and research workers getting together with growers, traders, and suppliers from all parts of the globe to learn about and discuss the latest discoveries, new technologies, and where the industry is heading, has been a highly successful formula for over 70 years.

The 2024 ISMSC will continue that legacy in Las Vegas with a modern program of oral presentations and poster sessions surrounded by meal breaks and social functions that will provide the all-important delegate interaction opportunities. The diversity of experiences that Las Vegas has to offer will certainly enhance the event.

The Congress program will showcase the work of scientists from 22 countries delivering 60 cutting-edge oral presentations and posters. The *Book of Abstracts* will enable delegates to see what the Congress program has to offer and plan their daily activities. It will also be a handy reference compendium in the office and a practical memento of your time at this potentially life-changing event.

The Congress program includes three high profile and highly entertaining keynote speakers. Thank you in advance to Dr Shiuan Chen, Dr Eoin O'Connor and Dr Lynn Rothschild for giving their time and sharing their knowledge so we can have a greater insight into what is coming down the pipeline.

Planning, organizing and running an event like the ISMSC/ NAMC is a demanding task requiring lots of support from a large cast. ISMS wishes to thank all those who have helped in getting us this far. A special mention for Dr John Pecchia, the Scientific Committee, and Liz Bouzoudis (ISMS Secretariat) for their excellent work in putting the scientific program together. Also to the authors and presenters who have provided such worthy content.

A big shout-out to the American Mushroom Institute for hosting this joint event, particularly the office team of Rachel Roberts, Lori Harrison, and Amy Ducharme.

Thank you to our sponsors and exhibitors who financially underpin this event and provide another source of knowledge and experience for delegates. Make sure you spend plenty of time in the Expo each day. It is amazing who you will meet and what you will learn.

It is also important that I publicly acknowledge the tireless and talented efforts of Elizabeth Bouzoudis in managing the ISMS side of this joint event. Liz is the engine room of the ISMS Secretariat and has put in a truly herculean performance since planning began several years ago.

Before closing, I'd like to take a few moments to reflect on what we as delegates can do to leverage all the hard work that has gone into getting us here today.

No matter where you are from, how long you have been in the industry, or whether you are an ISMS veteran or a first time Congress delegate, you will have the opportunity to be part of a very special "family" experience during your time in Las Vegas and beyond.

The 'international mushroom family' will be important as times become more turbulent - globally and in our worlds at home. Cherish it, nourish it, and respect it and this family will support you for the rest of your life in the industry and even afterwards if you happen to move on.

Experience tells me you will get the most out of your attendance in Las Vegas if you actively participate in all aspects of the program day and night. Attend as many sessions as you can, visit the poster area during the breaks, say hello to as many of your fellow delegates as possible and celebrate the joy of being part of a unique industry at the functions and after parties.

Learn, laugh, and have fun! It's a privilege to be able to do so!

Greg Seymour
President ISMS
26 February 2024

Comments from Scientific Committee Chair

On behalf of the ISMS scientific committee, I am pleased to welcome everybody back, in-person, for the 20th ISMS Congress being held this year in Las Vegas, Nevada, USA.

We've received over 75 abstracts from 23 countries from authors presenting their most recent research findings from traditional topics centered around substrate utilization and disease control to more current and interdisciplinary fields ranging from mycomaterials to the use of biotechnology to better understand fungal growth and utilization.

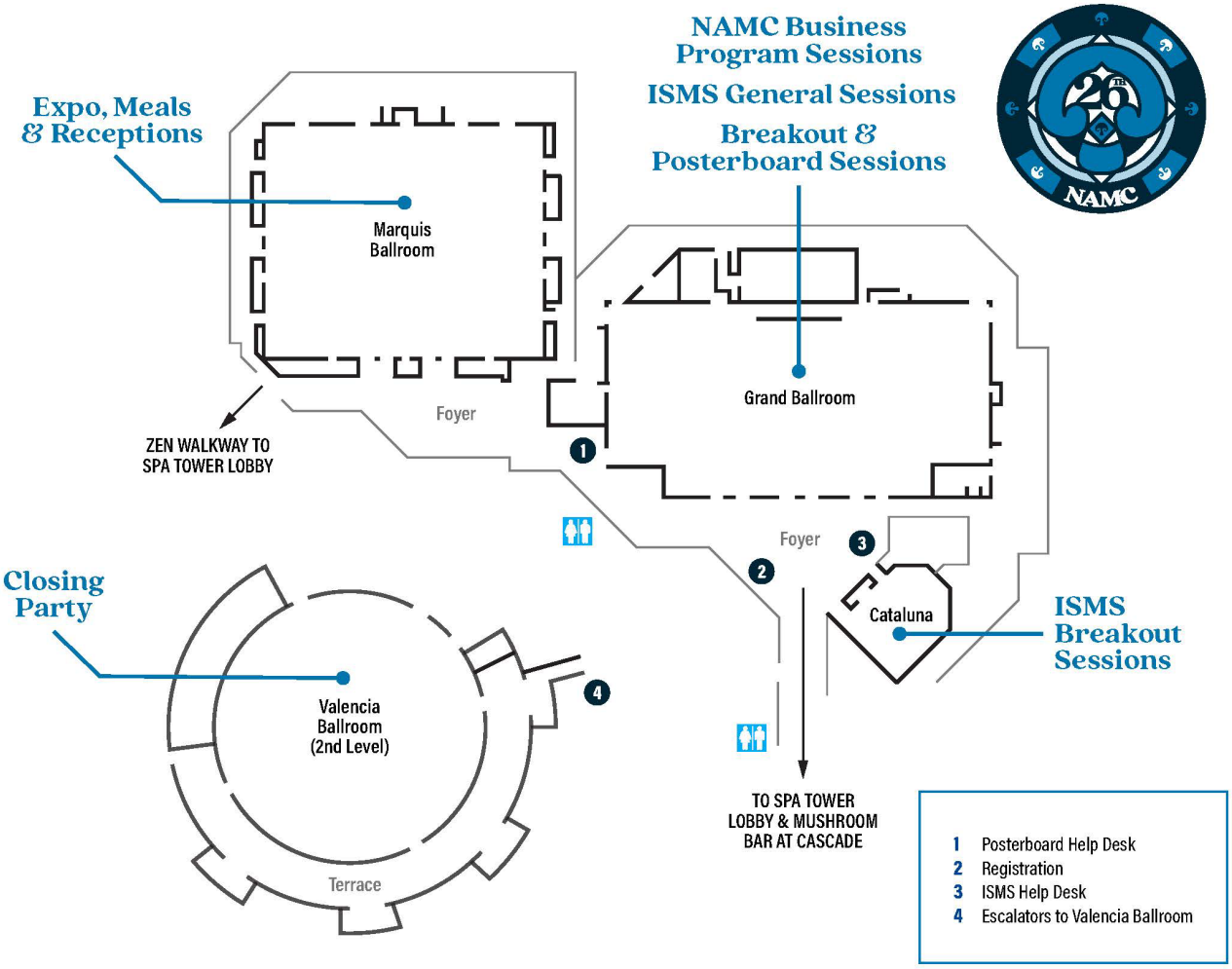
With the ever-growing number of challenges being faced by mushroom growers, it is imperative that we continue to improve our understanding of the science behind mushroom cultivation. Growers are facing mounting issues ranging from labor shortages, increased costs, new diseases and an increased scrutiny surrounding sustainability topics.

We hope participants take this opportunity to highlight their research, as well as network and develop potential collaborations that will strengthen our field moving forward, and build upon the framework laid out by previous scientists as well as our current colleagues.

I'd like to thank members of the scientific committee that helped make this Congress possible: Dr. David Beyer, Dr. Benay Gursoy, Dr. Helen Grogan, Dr. Michael Kertesz, Dr. Carlo Nicoletto, Dr. Eoin O'Connor, Dr. Nancy Pyck and Dr. Fabricio Vieira.

John Pecchia Ph.D.
Scientific Committee Chair

JW Marriott Resort & Spa Venue Map



20th ISMS Congress Program

Sunday, 25 February 2024			
13:45 – 14:00	Cataluna Room AB opens for seminar audience to be seated		Cataluna Room AB
14:00 – 16:00	Future of Casing Report Symposium		Cataluna Room AB
16:30 – 17:30	ISMS General Assembly		Cataluna Room AB
Monday, 26 February 2024			
07:30 – 16:30	Registration Open		Grand Foyer
07:30 – 08:30	**Breakfast**		Grand Ballroom CDE
09:00 – 09:30	Congress Official Opening	Greg Seymour	Grand Ballroom CDE
09:30 – 10:30	**Keynote Address:** Development of white button mushrooms as multitarget anticancer medicine: collective perspectives and insights from our translational research	Dr Shiuan Chen	Grand Ballroom CDE
10:30 – 11:00	**Morning Break**		Grand Foyer
11:00 – 12:00	**Keynote Address:** Profiling the composition and function of the casing devome: manipulating developmental patterns and disease-suppression of blotch in *Agaricus bisporus*	Dr Eoin O'Connor	Grand Ballroom CDE
12:00 – 13:35	**Lunch**		Marquis Ballroom
12:45 – 13:35	**Official Poster Session – Authors in Attendance** (Poster gallery open 12:45 – 17:00)		Grand Ballroom AB
13:40 – 15:00	**Concurrent Scientific Session – Substrate and Casing** Session Chair: Dr Johan Baars		Grand Ballroom CDE
13:40	1. Non-peat casing materials for mushroom production – a systematic review	Ms Gabrielle Young	Grand Ballroom CDE
14:00	3. A novel non-destructive method for monitoring CO2 and O2 concentration during spawn colonization in bag systems	Mr Jan Van Nuffel	Grand Ballroom CDE
14:20	5. Enhancing mycelium production efficiency: a novel approach to evaluate filter materials for growing bag systems	Mr Frederik Cruyt	Grand Ballroom CDE
14:40	7. Feeding the compost: nitrogen supplementation during production of white button (*Agaricus bisporus*) mushrooms	Dr Meghann Thai	Grand Ballroom CDE
13:40 – 15:00	**Concurrent Scientific Session – Genetics, Breeding, Germplasm** Session Chair: Dr Mark Wach		Cataluna AB
13:40	2. Non-GM genome editing and its utilization in science and breeding of mushrooms	Prof Yoichi Honda	Cataluna AB
14:00	4. Transgenes, Anti-sense and CRISPR in *Agaricus bisporus*	Dr Mark Loftus	Cataluna AB
14:20	6. Studies on strain instability in the button mushroom and the grey oyster	Dr Arend van Peer	Cataluna AB

14:40	8. Genomic applications in commercial mushroom breeding	Dr Nolan Bornowski	Cataluna AB
15:00 – 15:30	Afternoon Break		Grand Foyer
15:35 – 16:55	**Concurrent Scientific Session – Substrate and Casing** Session Chair: Dr Christine Smith		Grand Ballroom CDE
15:35	9. The potential of wood-based products as alternative components in mushroom casing	Dr Eoghan Corbett	Grand Ballroom CDE
15:55	11. Optimizing yield and quality of *Pleurotus ostreatus* cultivation through adapted light spectrum and intensity, on an industrial scale	Mr Kristof Gheysens	Grand Ballroom CDE
16:15	13. Colour quality assessment in white *Agaricus bisporus* mushrooms	Dr Andrea Uccello	Grand Ballroom CDE
15:35 – 16:55	**Concurrent Scientific Session – Genetics, Breeding, Germplasm / Nutrition, Bioactive Compounds, Medicinal** Session Chair: Dr John Pecchia		Cataluna AB
15:35	10. Vegetative incompatibility occurs between closely related strains of *Agaricus bisporus*	Dr Johan Baars	Cataluna AB
15:55	12. Applying hybrid plant breeding approaches to an *Agaricus bisporus* breeding program	Dr Nolan Bornowski	Cataluna AB
16:15	14. Metabolite profiling of functional fungi strains of *Hericium erinaceus* & *Cordyceps militaris*: using phenotypes, untargeted metabolomics by mass spectrometry and cell-based assays	Dr Julie Daoust	Cataluna AB
16:35	15. Confirming the food safety of *Agaricus* mushrooms	Dr Jenny Ekman	Cataluna AB
17:00 – 19:00	Expo Reception		Marquis Ballroom
19:00 – 24:00	Mushroom Bar		Cascade
Tuesday, 27 February 2024			
07:00 – 16:30	Registration Open		Grand Foyer
07:00 – 09:00	Breakfast		Marquis Ballroom
08:30– 08:35	Welcome to Day 2	Greg Seymour	Grand Ballroom CDE
08:35 – 09:35	**Keynote Address:** (Synthetic) biology + mycotecture as enabling technologies for space exploration	Dr Lynn Rothschild	Grand Ballroom CDE
09:35 – 09:55	**Scientific Presentation – Pest & Disease**		Grand Ballroom CDE
09:35	16. The effect of metrafenone, prochloraz and Bacillus based biological control agents on the control of cobweb disease in *Agaricus bisporus* mushroom crop trials	Ms Joy Clarke	Grand Ballroom CDE
10:00 – 10:30	Morning Break		Grand Foyer

10:30 – 12:15	**Scientific Session – Pest & Disease** Session Chair: Dr Mark Wach		Grand Ballroom CDE
10:35	17. Investigating biorationals for mushroom integrated pest management	Dr Aimee McKinnon	Grand Ballroom CDE
10:55	18. Microbial perspectives towards mitigation of green mold disease in *Agaricus bisporus* cultivation system	Dr Fabrício Vieira	Grand Ballroom CDE
11:15	19. Development of attract and kill stations to control mushroom phorid flies on farms	Dr Michael Wolfin	Grand Ballroom CDE
11:35	20. Exploring the potential of *Bacillus velezensis* as a bioinoculant for enhanced mushroom cultivation in a post-peat era	Dr William Kay	Grand Ballroom CDE
11:55	21. Mushroom dry bubble disease: novel pathogens, and mycoviruses as potential biocontrol agents	Dr Lorant Hatvani	Grand Ballroom CDE
12:15 – 13:45	Lunch		Marquis Ballroom
12:45 – 13:35	**Official Poster Session – authors in attendance** (Poster gallery open 07:00 – 15:45)		Grand Ballroom AB
13:15 – 15:00	**Scientific Session – Substrate & Casing / Nutrition, Bioactive Compounds, Medicinal** Session Chair: Dr John Pecchia		Grand Ballroom CDE
13:20	22. The impact of recycled mushroom compost casing on the cultivation and yield of *Agaricus bisporus*	Mr Nicholas Gabel	Grand Ballroom CDE
13:40	23. Working towards cultivating morels in the Northeastern United States	Mr Xiangrong Guo	Grand Ballroom CDE
14:00	24. The potential of selected cultivation techniques to shorten the crop cycle of *Agaricus bisporus*	Dr Nancy Pyck	Grand Ballroom CDE
14:20	25. Biofortification of *Pleurotus floridanus* using iron based supplements	Mr Stuart Whitehall	Grand Ballroom CDE
14:40	26. Effect of different light wavelengths on productive and qualitative characteristics of *Pleurotus ostreatus* production	Mrs Marina de Bonis	Grand Ballroom CDE
15:00 – 15:30	Afternoon Break		Grand Foyer
15:30 – 16:50	**Scientific Session – Mycomaterials** Session Chair: Dr Christine Smith		Grand Ballroom CDE
15:30	27. Quality assurance of mushroom products for pets - analysis of 10 products	Dr Robert Silver	Grand Ballroom CDE

15:50	28. Mycelial cell wall: breeding targets for the future mushroom materials	Dr Moriyuki Kawauchi	Grand Ballroom CDE
16:10	29. Aerial mycelium, the fungi world upside down	Dr Bert Rademakers	Grand Ballroom CDE
16:30	30. Bio-processed mycelium-based alternatives to plastic packaging materials	Dr Philippe Amstislavski	Grand Ballroom CDE
16:50 – 17:00	ISMS Program Closing Ceremony		Grand Ballroom CDE
17:30 – 19:00	NAMC Opening Reception		Marquis Ballroom
19:00 – 24:00	Mushroom Pop-up Bar		Cascade

20th ISMS Congress Poster Gallery

No.	Poster title	Presenting author
1	Ergothioneine: science, cultivation and application	Dr Mason Bresett
2	Optimization of parameters for extraction of protein hydrolysate using edible mushroom, *Agaricus bisporus* stems	Mrs Vanessa Grifoll
3	Response surface methodology for the extraction of phenolic compounds and antioxidant activities from edible mushrooms	Mrs Vanessa Grifoll
4	Insect protein in the circular economy	Mr Arpad Mutsy
5	A comparative analysis of secondary metabolites and sensory properties of *Hypsizygus marmoreus* fruiting bodies based on culture period	Dr YounLee Oh
6	Development in chitin bio-based materials used for food biopackaging	Dr Margarita Perez Clavijo
7	Antioxidant activity of polysaccharides from edible mushrooms	Dr Margarita Perez Clavijo
8	Promoting circular economy by the assessment and validation of mushroom industry by-products as sustainable ingredients for diets in swine and fish aquaculture species (GreenBlueCircle)	Dr Margarita Perez Clavijo
9	The potential of mushroom residual flows as a new source of bioactive components	Dr Nancy Pyck
10	Optimizing the application of entomopathogenic nematodes to control sciarid populations	Dr Nancy Pyck
11	Beneficial microorganisms joining forces to fight green mold in mushrooms	Dr Svetlana Milijasevic-Marcic
12	Antifungal activity of peppermint and spearmint essential oils against Trichoderma spp. green mold disease agents of oyster mushroom and shiitake	Dr Ivana Potocnik
13	Zygospore formation in *Syzygites megalocarpus*	Mr Bruce Withey
14	Differential gene expression in *Mycogone perniciosa* and *Lecanicillium fungicola* infecting two genetically closed *Agaricus bisporus* hosts.	Ms María Luisa Tello Martín
15	Cascade strategies for the valorisation of waste streams from common carp pond farming into mushrooms and mealworm	Ms María Luisa Tello Martín
16	Sustainable peat alternatives for casing soil in mushroom (*Agaricus bisporus*) production	Ms María Luisa Tello Martín

17	Cultivation of *Pleurotus ostreatus* and *Pleurotus cornucopiae* in a vertical farming system	Mrs Marina de Bonis
18	A description of the effects caused by temporary atmospheric alteration on *Agaricus bisporus* yield and development	Mr Nicholas Gabel
19	A soft robotic gripper for harvesting delicate produce	Dr Helen Grogan
20	Bacteriophages – unknown viruses of mushroom compost	Prof Michael Kertesz
21	Correlation between yield and enzyme production in *Pholiota nameko* on minimally supplemented wood-based substrates	Ms Swathi Kothattil
22	Correlation between yield and enzyme production in *Pleurotus ostreatus* on minimally supplemented wood-based substrates	Ms Swathi Kothattil
23	An experimental approach to microbiologically manipulate *Agaricus bisporus* developmental patterns	Dr Fabrício Vieira
24	Enabling 'Smart' mushroom agriculture	Dr Steven Haynes
25	Improve awareness of mushroom cultivation and promote human health	Dr Mark Wach
26	Investigation of robotic solutions for button mushroom harvesting	Dr John Pecchia
27	Design and sustainable fabrication of mycelium-based building parts and structures	Dr John Pecchia

Keynote Precis & Presenter Bios

Dr Shiuan Chen, Ph.D., the Lester M. and Irene C. Finkelstein Chair in Biology

Presentation title: Development of White Button Mushrooms as Multitarget Anticancer Medicine: Collective Perspectives and Insights from Our Translational Research

Biography: Dr. Chen received his doctoral degree from the University of Hawaii. He is the Chair and Professor of Department of Cancer Biology and Molecular Medicine, Beckman Research Institute of City of Hope, Duarte, California.

Dr. Chen is recognized for his contributions in the functional characterization of aromatase in breast cancer and in the understanding of aromatase inhibitor (AI) response in breast cancer treatment. Aromatase is the protein that makes estrogen.

Dr. Chen is also known for his research on the demonstration of anti-cancer properties of grape seed extract, mushrooms, pomegranate, and blueberries against breast cancer and prostate cancer. Importantly, he has successfully translated his research findings into several clinical trials, including an NCI-funded phase 2 trial involving mushrooms and prostate cancer. In addition, Dr. Chen's laboratory applies state-of-the-art technologies (single-cell and spatial transcriptomics analyses) to study the immune modulatory mechanism of mushroom intake in prostate cancer. In 2012, he was inducted to the Portrait Gallery of Scientific Achievement for his scientific contributions at City of Hope.

In 2017, Dr. Chen was named as the Lester M. and Irene C. Finkelstein Chair in Biology. He was elected as an AAAS Lifetime fellow in 2022. Dr. Chen has published 272 papers and has mentored 10 graduate students, 38 research and surgical fellows, and several junior faculty members.

Dr Lynn Rothschild, Ph. D.

Presentation title: (Synthetic) Biology + Mycotecture as Enabling Technologies for Space Exploration

Biography: Dr Lynn Rothschild, a research scientist at NASA Ames and Adjunct Professor at Brown University, is passionate astrobiologist focusing on the origin and evolution of life on Earth and elsewhere, while at the same time pioneering the use of synthetic biology to enable space exploration. Her research focuses on how life, particularly microbes, has evolved in the context of the physical environment, both here and potentially elsewhere.

A graduate of Yale, Indiana University and Brown, she has brought her imagination and creativity to the burgeoning field of synthetic biology, articulating a vision for the future of synthetic biology as an enabling technology for NASA's missions, including human space exploration and astrobiology.

From 2011 through 2019 she served as the faculty advisor of the Stanford-Brown iGEM team. Her lab tested these plans in space on in the PowerCell secondary payload on the DLR EuCROPIS satellite. A past-president of the Society of Protozoologists, she is a fellow of the Linnean Society of London, The California Academy of Sciences and the Explorer's Club. She was awarded the Isaac Asimov Award from the American Humanist Association, and the Horace Mann Award from Brown University. She has been a NASA Innovative Advanced Concepts (NIAC) fellow seven times. Lynn was formerly Professor (Adjunct) at Stanford where she taught "Astrobiology and Space Exploration" for a decade. Though late to mycology, she now realizes the error of her ways, and is actively using fungi as a building material and to develop filters for biomining and bioremediation.

Dr Eoin O'Connor, Ph. D.

Presentation title: Profiling the Composition and Function of the Casing Devome: Manipulating Developmental Patterns and Disease-Suppression of Blotch in *Agaricus bisporus*.

Biography: Eoin is a Postdoctoral Scholar at Pennsylvania State University. He holds a doctorate in fungal biology and bioinformatics from Maynooth University and Teagasc (The Agriculture and Food Development Authority), Ireland, and a bachelor's degree in biology from Maynooth University, Ireland. He is currently a postdoctoral scholar in the department of Plant Pathology and Environmental Microbiology at The Pennsylvania State University, in Dr. Kevin Hockett's group and as part of a collaborative project with Drs. John Pecchia and Carolee Bull.

Eoin's research focuses on the use of metaproteomics to understand the composition and function of microbial communities in mushroom substrates and how changes in these communities impact the developmental microbiome (Devome) of mushroom development.

Oral and Poster Abstracts

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Oral Abstracts & Bios

01. Non-peat casing materials for mushroom production – a systematic review

Oral

Ms. Gabrielle Young [1], ***Dr. Helen Grogan*** [2], ***Dr. Ralph Noble*** [3], ***Dr. Saoirse Tracy*** [4], ***Dr. Olaf Schmidt*** [4]

1. *Teagasc/UCD,* ***2.*** *Teagasc,* ***3.*** *microbiotech,* ***4.*** *UCD*

Currently peat is used as the main component of the mushroom (*Agaricus bisporus*) casing layer internationally. Peat functions both as a water reservoir and to induce the formation of fruiting bodies. With many countries aiming to reduce peat use, the industry is under pressure to find effective alternatives. In addition to being sustainable, alternative casing materials must deliver mushroom yield and quality comparable to commercial peat casing. Alternatives must also be economically viable, and easily available. A systematic review of peer-reviewed articles related to mushroom production on alternative casing materials was conducted, with the aim to identify suitable alternative casing materials by exploring the extant literature. This search considered papers published between 1970 and 2023, using the databases Web of Science, CAB Abstracts and SCOPUS. The articles were assessed following predetermined criteria and, where relevant, data was extracted for analysis. It was found that a wide range of alternative materials have been tested with varying degrees of success, with some showing promise. Spent mushroom compost (SMC) was found to be the single, most frequently assessed alternative. Furthermore, it was determined that specific blends of alternative materials may offer superior performance; this was especially true for SMC, vermicompost and coir. Nonetheless, it was observed that there was little consistency in the experimental approach or materials utilised. This was found to be the case for alternative materials and peat controls alike, which varied greatly between studies conducted in different locations and at different times. These discrepancies in reporting and approach indicate the importance of this review in compiling and evaluating the outputs of previous studies. This suggests that there is no one solution for a transition away from peat use in mushroom cultivation; rather that opportunities will vary by geographical location and local production methods. This review explores the performance of a wide range of alternative materials and highlights the limitations previous studies to help inform the design of future casing studies. It is evident further research would benefit from standard characterisations of materials. As industries seek to move away from peat there is a need for methods capable of efficiently assessing the potential performance and characteristics of novel casing materials. Some novel approaches with potential to advance the field will be discussed. In combination, innovative methodologies and standardisation of conventional growth experiments have the potential to identify suitable alternative casings for production of *A. bisporus*.

Presenting Author Biography - Gabrielle Young:
Gabrielle is a PhD student based in University College Dublin and Teagasc in Ireland. Previously she completed her bachelor's degree in biology at the University of Oxford and her master's in ecology at the University of Ab-erdeen. This background in the field of biology broadly has given her a strong interest interdisciplinarity and novel approaches in research. She is particularly interested in sustainability and innovative techniques in agricultural research.

02. Non-GM genome editing and its utilization in science and breeding of mushrooms

Oral

Prof. Yoichi Honda [1]

1. *Kyoto University*

Since we have introduced CRISPR/Cas9 technology for the first time in edible mushrooms, functional analysis of mul-tiple genes can be done easier without preparation of a special strain deficient in non-homologus end joining system and beyond the limitation of number of different selection markers. We used gene disruption with CRISPR/Cas9 to experimentally demonstrate that six lignin-modifying enzyme genes have redundant but essential function in wood lignin degradation by oyster mushroom, *Pleurotus ostreatus* for the first time. Combined with transcriptomic anal-ysis, we also found new genes essential for spore formation and demonstrated that they are new target of breeding for sporeless cultivars using gene disruption with CRISPR/Cas9. Genome editing was also successfully introduced in selective white-rot fungus, *Gelatoporia* (former *Ceriporiopsis*) *subvermsipora* as well as edible and medicinal mush-rooms like shiitake (*Lentinula edodes*), champignon (*Agaricus bisporus*) and Lingzhi (*Ganoderma lucidum*) by our collaborators. In these fungi, new progresses in basic science are expected using similar reverse-genetics strategy we developed in *P. ostreatus*.
Furthermore, new technologies for non-GM (genetically modified) genome editing have been investigated. Using *P. ostreatus*, as a model, we successfully developed several protocols for non-GM genome editing where no ectopic integration of heterogeneous DNA sequence left in the isolated strains. These new technologies can be applied for safe molecular breeding in both edible and non-edible mushrooms to isolate strains for good cultivation or industrial utilization in emerging fields of mushroom materials.

Presenting Author Biography - Yoichi Honda:
Prof. Honda received the PhD degree from Kyoto University, Japan, in 1992. When he has got a position in Wood Research Institute, Kyoto University in 1993, he started to work on mushrooms, especially *P. ostreatus*. Since then, he has been an innovator in molecular genetics of mushrooms. In 1998-99, he also worked in the lab. of Prof. Lorna Casselton at University of Oxford. His achievements include genetic transformation, molecular cloning, recombinant expression and functional analyses of genes using both forward and reverse genetics. Now, he is the Professor of Forest Biochemistry, at Grad. Sch. of Agriculture, Kyoto University. Recently, his research area has been extending to non-GM genome editing technologies for edible mushrooms, breeding of sporeless cultivars, new materials made of mushrooms mycelia etc. He published over 180 peer-reviewed research papers including 35 during the last 5 years. In 2023, he did research in Spain as an OECD CRP fellow.

03. A novel non-destructive method for monitoring CO2 and O2 concentration during spawn colonization in bag systems

Oral

Mr. Jan Van Nuffel [1]

1. *Mycelia*

Efficient gas exchange is crucial for spawn production in closed bag systems where filters regulate CO2 and O2 levels. Despite its importance, no data is available that correlates spawn bag design with internal CO2 and O2 profiles and the resulting spawn quality.
In response, a novel method was developed to assess CO2 and O2 concentration in these closed bag systems during spawn colonization. To fill the forementioned knowledge gap, *Pleurotus* ostreatus was incubated in industry-standard spawn bags with variable filtration surfaces, while monitoring the CO2 and O2 levels. Distinct growth phases based on measured gas concentrations during spawn colonization were observed and visually confirmed on the grain spawn surface.
As a conclusion, the reported findings contribute significantly to the sustainability of the mushroom cultivation industry by allowing optimization of spawn bag designs and incubation times tailored to each species.

Presenting Author Biography - Jan Van Nuffel:
Jan Van Nuffel is a Research Manager with a background in Applied Biosciences. He specializes in the study of gas exchange in spawn and substrate bags, focusing on its influence on spawn quality and exploring crop protection through fungal agents.

Jan's educational journey led him to obtain a Master of Science in Applied Biosciences, equipping him with a strong foundation for his research endeavors.

His primary research interests include designing testing setups to measure gas concentrations during spawn colonization, as well as innovative designs for spawn and substrate bags. Jan is also passionate about optimizing processes and products in mushroom cultivation.

Jan's dedication to advancing knowledge in these areas makes him a valuable contributor to the scientific community. As a Research Manager, he continues to work diligently to improve our understanding of fungal biology and its practical applications.

04. Transgenes, Anti-sense and CRISPR in Agaricus bisporus

Oral

Dr. Mark Loftus [1]

1. *Sylvan BioSciences*

Classical breeding techniques have led to the commercial release of several successful *Agaricus bisporus* cultivars. However, the unusual biology and low recombination rate of *A. bisorus* places limits on what the breeder can achieve. Recombinant Nucleic Acid techniques may offer solutions to these problems. In this presentation, we discuss information on Agrobacterium transformation, RNA Anti-sense technology and additionally consider the potential of CRISPR gene editing. The Agrobacterium-based DNA transformation techniques pioneered by Dr. Peter Romaine and colleagues allow routine, high efficiency addition of foreign DNA to the Agaricus genome. Prior to the discovery of this technique, transformation was a bottleneck; researchers experienced low levels of transformation that were inconsistent. However, Agrobacterium transformants qualify as GMOs, and therefore may find themselves resistant to consumer acceptance. RNA antisense is a technique wherein short runs of RNA (which are opposite strand or Anti-sense) can prevent protein translation. Mushroom DNA is transformed (generally with Agrobacterium) with constructs that are designed to quell or completely silence genes. Earlier work by Dr. Mike Challen and others showed that this technique works well in *A. bisporus*, however it should be noted that RNA Antisense is also considered a GMO method. The method works well in Apples; Arctic Apples ® are GMO with the genes that induce browning in storage greatly reduced in their expression. Other examples of Anti-sense in Agriculture are the Papaya crop in Hawaii, (100% Anti-sense to reduce virus), and the Innate Potato (currently in pre-commercial testing). CRISPR is a technique that is a form of highly specific DNA editing. The technique can be considered “GMO free”, although success in agriculture so far has proven difficult to achieve in many crops. Using CRISPR’s “molecular scissors” it is possible to change the sequence of existing genes, or to add new genes. Early reports by Dr. Yinong Yang in 2016 painted an optimistic picture for the future of CRISPR in *A. bisporus*, however the research lacked peer-review and has proven difficult to repeat. Since 2016 there have been several peer-reviewed papers and at least one patent that report low levels of editing.
In this presentation we will discuss the status of CRISPR in *A. bisporus* in 2024. Which barriers remain, and how might they be overcome? What are the targets for editing in mushrooms? Where will we be in ten years’ time?

Presenting Author Biography - Mark Loftus:
Mark joined Sylvan in 2013 and has been involved in Mushroom Science for over 30 years. He completed Ph. D studies at Manchester University in the UK, with Tim Elliott and David Moore. Mark moved to the states in 1989 with a Post-Doc at UC, Santa Barbara, and has extensive experience in Academia and the US spawn industry.

05. Enhancing mycelium production efficiency: a novel approach to evaluate filter materials for growing bag systems

Oral

Mr. Frederik Cruyt [1]

1. *SacO2*

Efficient gas exchange is crucial for mycelium production in bag systems where filters regulate CO2 and O2 levels. Maintaining optimal levels of CO2 and O2 is critical to prevent oxygen limitations during mycelial growth, which, in turn, directly boosts the yield. Despite the importance of the filter characteristics, existing cross-sector benchmark standards fail to simulate mycelium production conditions adequately because they work with a differential pressure over the filter, which is generally not the case during mycelium incubation. In response, a novel method was developed to assess CO2 and O2 flux through filters in non-pressurized conditions.

To achieve this, a custom machine was engineered featuring standard-sized filters within sealed containers. The containers were flushed with CO2. Next, the filters supported passive gas diffusion with the ambient environment, while CO2 and O2 levels were monitored with central gas sensors.

Through a series of experiments, the filter gas exchange performance could be quantified using a single diffusion coefficient. These coefficients now constitute a comprehensive database, enabling their integration into product specifications and the design of future bag systems.

In conclusion, the novel method addresses the need for a standardized approach to assess filter performance in mycelium production conditions. The resulting database has the potential to lead to the design of more effective bag systems and positively impact mycelium production efficiency.

Presenting Author Biography - Frederik Cruyt:

After his bio-engineering studies, Frederik was inspired by the enormous potential of yeast. He started a journey through Europe to unlock the potential of these single-celled fungi in the beer and wine industries. In 2022, Frederik joined SacO2 and Mycelia as part of a rather young R&D team. He mainly focuses on the development of new filter systems for mycelium growing bags and the production of entomopathogenic fungi for biocontrol applications in agriculture.

06. Studies on strain instability in the Button mushroom and the Grey Oyster

Oral

Dr. Arend van Peer [1], ***Dr. Johan Baars*** [1], ***Dr. Karin Scholtmeijer*** [1], ***Mr. Brian Lavrijssen*** [1], ***Mr. Patrick Hendrickx*** [1], ***Mr. Ed Hendriks*** [2], ***Mr. Corne Vermeer*** [1]

1. *WUR Plant Breeding,* ***2.*** *WUR Plant Research*

The existence and phenotypic expression of strain instability in fungal isolates that are repeatedly sub-cultured is a frequent problem. This is described in the industrial microbiological literature and has also been observed for many years in the mushroom industry. The appearance of morphological changes of mycelium and/or mushrooms during the production of spawn, colonization of substrate, or on mushroom farms, implies that mushroom strains may possess the inherent ability to spontaneously become instable. The biological mechanisms that are associated with instability of mushroom strains are not understood, and a range of different causes have been suggested over time, including stress, genetic deterioration, climate, substrate and spawn quality and pathogens. Instability can remain undetected until the very last stage in the production cycle, i.e. the production of mushrooms at the farm, with severely detrimental crop quality and yields as a result. Theoretically, the earlier that undetected degeneration occurs, the larger the affected part of the production chain and the more serious the economic damage. Strain instabilities however often behave ephemeral, emerging but also again disappearing. This makes studying such instabilities, let alone efficient detection, extremely challenging. In a project co-funded by the Dutch Government, a large consortium of the mushroom industry together with Wageningen University set out to study strain instability in two important mushroom species, *Agaricus bisporus* and *Pleurotus ostreatus*. The project aim was to 1) Define and distinguish different types of strain instability. 2) Isolate instable strains of defined types of instability. 3) Develop toolboxes to reproduce and study important instabilities in the lab and under industrial conditions. 4) Screen for genetic and environmental factors contributing to instability. For two important instability phenotypes, “Cluster” in *Agaricus bisporus* and “Stroma” in *Pleurotus ostreatus*, phenotypes have been characterized, and strains have been isolated from which the phenotypes can be reproduced in the lab. In a commercial production setting, those strains were then tested to see if also under typical industrial conditions Cluster and Stroma could be reproduced. While successful, this testing demonstrated that the whole picture was certainly not complete. From the reproduced Clusters and Stroma, samples for genome and RNA analysis were collected. Currently the data for Cluster is screened for specific mutations, low-present mutations that increase in frequency during development of instable phenotypes, and if presence of viruses could possibly be involved.

Presenting Biography - Arend van Peer:
My mission is to explore and expand the applications of mushroom forming fungi in the bio-based economy, biomaterials, cultivation of existing and new mushrooms, and new applications in medicine. I’m Arend van Peer, team leader of Mushroom Research at Plant Breeding, WUR. Next to the management of my team, my roles include acquisition, project management, education and consultancy, and exchange of information through our various international networks and industries. My interest in fungi started during my Masters and PhD thesis. I soon realized that studies on applications of mushroom forming fungi, other than as crops, were only just emerging. A whole unknown and exiting world was awaiting exploration! The following eight years I dedicated to studies on the genetics and development of mushroom forming fungi in South Korea, Japan and China. At WUR I continue to explore new applications of mushroom forming fungi.

07. Feeding the compost: nitrogen supplementation during production of white button (Agaricus bisporus) mushrooms

Oral

Dr. Meghann Thai [1], ***Ms. Kimberly Tong*** [1], ***Mr. Umberto Calvo*** [2], ***Ms. Sandra J. Evangelista*** [2], ***Dr. Gordon Rogers*** [2], ***Prof. Michael Kertesz*** [1]

1. *The University of Sydney,* ***2.*** *Applied Horticultural Research*

Nitrogen uptake by white button mushrooms from the growth substrate can vary from harvest flushes (or breaks) and substrate depth. Nitrogen is a critically important nutrient in mushroom production and is often lost as ammonia during composting. At cropping, nitrogen is added back into the compost using a delayed-release soy-based supplement. Using this supplement results in excess nitrogen not being taken up directly by the mushroom and potentially being used by competitive microbial pathogens. During cropping, ammonium concentration has been shown to peak in the growth substrate before each flush and drop rapidly during the flush. Organic and inorganic nitrogen supplementation was directly applied to mushroom compost via needle injection, or in-line watering at the interphase of casing and compost, and was added either at casing, or at pin onset prior to each successive flush. There was an increase in total mushroom yield when N was supplemented throughout cropping, in comparison to N supplemented at casing. Nitrogen content in the mushrooms significantly increased for all flushes when N was applied at pinning prior to each flush, compared to when it was applied at casing. Therefore, applying nitrogen supplement directly to the compost during cropping at pinning for each flush could minimise the dependence on soy-based supplements, while also reducing nitrogen losses from the compost.

Presenting Author Biography - Meghann Thai:
Meghann Thai completed her PhD in February 2022 at the University of Sydney. She has a background in Agriculture, specialising in Horticulture. In 2016, she gained valuable knowledge in the mushroom industry, learning how to grow white button mushrooms. Her love of mushrooms has brought her to her current research which looks at the microbial community in *Agaricus bisporus* compost, and how these organisms transform nitrogen throughout the composting process. She is also researching the microbial community in the growth substrate of oyster mushrooms grown on coffee waste as a sustainable waste stream for coffee waste.

08. Genomic applications in commercial mushroom breeding

Oral

Dr. Nolan Bornowski [1]

1. *Amycel/Spawn Mate*

Large-scale commercial crops have long embraced sequencing technology to advance breeding goals. Although the mushroom industry has fewer resources compared to leading agribusinesses, sequencing remains affordable and can provide insight on longstanding challenges in mushroom breeding and production. Here I describe how a modern genomics-focused mushroom breeding program can leverage sequencing and computational tools to improve upon traditional breeding practices. I will highlight transformative applications such as strain fingerprinting, homokaryon selection, and characterization of untapped genetic diversity. We believe that cutting-edge genomic tools will improve our understanding of mushroom biology and accelerate the development of novel varieties.

Presenting Author Biography - Nolan Bornowski:
Dr. Bornowski has a background in plant breeding and bioinformatics and is working to apply quantitative genetics and genomic tools to advance mushroom breeding goals at Amycel/Spawn Mate.

09. The potential of wood-based products as alternative components in mushroom casing

Oral

Dr. Eoghan Corbett [1], ***Dr. Akinson Tumbure*** [1], ***Mr. Brian McGuinness*** [1], ***Mr. Donal Gernon*** [1], ***Ms. Gabrielle Young*** [1], ***Dr. Helen Grogan*** [1], ***Dr. Michael Gaffney*** [1]

1. *Teagasc*

Agaricus bisporus mushrooms are usually grown in conjunction with a peat-based casing layer that is comprised of peat and a neutralising agent (sugar beet lime or chalk). There is increasing environmental pressure to reduce the use of peat in horticulture as peatlands are important carbon reserves from a climate change perspective. Three wood-based products: wood fibre, un-composted bark and windrow-stored bark, were used to reduce the wet-dug black peat content of mushroom casing at rates of addition of 30, 50 and 70% by volume. Their performance in terms of crop yield and mushroom quality was compared to a wet-dug black peat control. The behaviour of water within the casing layer was monitored using high temporal-resolution sensors measuring volumetric water content (%v/v) and matric potential (kPa). The force of suction applied across water-containing pores within each casing treatment was measured using a tensiometer. Treatments containing wood-fibre were not significantly different to the control in respect of Class A mushrooms. They also behaved almost identically in terms of time-resolved suction pressure, exhibiting a sharp negative drop of kPa for each flush. These casing media demonstrated a free availability of water to the developing mushrooms, even under high demand. Conversely, casing treatments containing bark (un-composted or composted) demonstrated a divergence of hydro-physical behaviour (water holding capacity, re-wettability and matric potential) from the control, consistent with pore-space shrinkage, most evident following the first flush. These media also demonstrated a propensity for over-pinning attributed to their more open and less 'sticky' physical structure. As the proportion of bark within these mixes increased, there was a corresponding significant step-wise reduction in the yield of Class A mushrooms, relative to the peat control. Additionally, mushroom colour characteristics (as measured by the ΔE value) for Class A mushrooms in the second flush differed from the controls. The results suggest that the relatively open structure of the two bark-based media did not store adequate available water to produce good yields of high quality mushrooms, while the wood-fibre based treatments performed well, compared to the peat control, both in respect to hydro-physical properties and yields of high-quality mushrooms. It is concluded that wood fibre offers potential as a high-performance peat-alternative in casing media due to its ability to store a deep reservoir of available water to sustain mushroom formation throughout a flush and maintain structural integrity throughout cropping and inter-flush irrigation cycles to support follow-on flushes.

Presenting Author Biography - Eoghan Corbett:
Eoghan Corbett is a Research Officer in Teagasc's Horticulture Development Department in Ashtown, Dublin, Ireland. He is currently conducting research to identify, characterise and assess the agronomic performance of peat-alternative growth and casing materials across the five key sub-sectors of horticulture in Ireland, including mushrooms. He is also interested in transformative processes and emerging technologies which can convert low-value waste or side-stream materials of indigenous industries or land-management practices, into value-added growth media materials. Eoghan completed his PhD in analytical geochronology and geochemistry at Trinity College Dublin in 2020. During this research, he has been involved in projects particularly focussed on developing novel techniques used in the geochronological analysis of minerals, as well as contributing to a greater understanding of the funda-mental physiochemical characteristics of geo-materials. This background brings a new perspective to the challenges of developing peat-alternative growth media.

10. Vegetative incompatibility occurs between closely related strains of Agaricus bisporus

Oral

Dr. Johan Baars [1], ***Mr. Pieter Vervoort*** [2], ***Mr. John Ebben*** [2], ***Dr. Karin Scholtmeijer*** [1], ***Dr. Ben Auxier*** [3], ***Dr. Alfons J.M. Debets*** [3], ***Prof. Duur Aanen*** [3], ***Dr. Arend van Peer*** [1]

1. *WUR Plant Breeding,* ***2.*** *CNC Grondstoffen,* ***3.*** *WUR Laboratory of Genetics*

For a fungal colony, self/nonself recognition is a crucial ability. Nonself recognition leading to somatic incompatibility is commonly used by mycologists to distinguish fungal individuals. When two fungal hyphae meet, the cells fuse and after this identity is assessed. If nonself is recognized, the fusion cell dies and is degraded. We have developed a staining method that allows us to see if cell death occurs in the interaction zone between two fungal colonies. Using this method, we studied vegetative incompatibility between the two genetically very closely related Agaricus bisporus strain Horst U1 and Sylvan A15. Based on their identical DNA sequence and recombination patterns at the ends of their chromosomes we postulate that A15 has been derived from U1 via two subsequent fertile single spore isolates. Using the staining method to see if cell death occurs in the interaction zone between an U1 and an A15 colony, we established that no cell death occurred. We therefore assumed these two strains to be compatible with each other. Based on this we hypothesized that mixing of spawn or colonized compost of these two strains would lead to the establishment of a single contiguous mycelial network that, in contrast to mixing incompatible strains that show cell death in the interaction zone, does not show yield loss. We tested this hypothesis in triplicate trays containing 20 kg of compost each. A 1 to 1 mixture of spawn of the two strains to inoculate the compost resulted in a yield reduction compared to compost that was solely colonized by A15. When compost that was colonized by the U1/A15 spawn mixture was taken out of the tray, mixed an refilled just before casing, yield was lowered even more. Finally, filling the bottom half of the trays with 10 kg of U1 colonized compost and the top half with 10 kg of A15 colonized compost before casing reduced the yield of mushrooms by more than half if compared to trays containing 20 kg of A15 colonized compost. Mixing the two composts before casing reduced yield even further. These results suggest that the phenotype of vegetative incompatibility is more complex than expected.

Presenting Author Biography - Johan Baars:

Johan Baars obtained his Ph.D. in 1996 investigating nitrogen metabolism of *Agaricus bisporus*. From there he moved to the Mushroom Experimental Station in Horst (Netherlands) to join in on a project involving the construction of a genomic map of the white button mushroom *Agaricus bisporus*. In 2001 he became a researcher at the department “Genetics, breeding and bioactive components” of WUR-PPO-Paddenstoelen, where he worked in projects aimed at cultivation and breeding of mushrooms (mainly *Agaricus bisporus* and *Pleurotus ostreatus*). During that period he obtained a plant patent for a sporeless strain of Oyster mushroom which is now marketed in the European Union by the name of SPOPPO. In 2006 he became a researcher at WUR Plant Breeding, Mushroom Research Group. In 2013 he was posted in a part time position as researcher at CNC, one of the largest producers of mushroom substrate in the Netherlands.

11. Optimizing yield and quality of Pleurotus ostreatus cultivation through adapted light spectrum and intensity, on an industrial scale

Oral

Mr. Kristof Gheysens [1], ***Dr. Nancy Pyck*** [1]

1. *Inagro vzw*

This presentation outlines our key findings and ongoing research related to the impact of adapted light spectrum on the fruit body development of *Pleurotus ostreatus* cultivation.
Information from literature indicates the potential influence of blue light stimulation on fruit body development. However, data obtained from trials carried out on an industrial scale is missing to confirm this hypothesis.
In this study we tested the potential of innovative methods to achieve a higher yield. The effect of different light conditions, including light spectra and intensity, was evaluated during fruit body development in *Pleurotus ostreatus* cultivation**. All experiments were conducted using commercial, wheat straw based substrates in fully climatized rooms.** Parameters yield and quality were closely monitored.
The obtained results demonstrate that substitution of conventional white light by an adapted light spectrum had a significant effect on yield, without quality loss. **Application of a light spectrum with 50% conventional white light and 50% blue (450 nm) light resulted in an average additional yield of 4,4% after 2 flushes,** compared to the 100% conventional white light spectrum at a fixed light intensity and lighting time. Interestingly, using different light intensities indicated a less effect on yield than changing the light spectra. Using 100% blue light resulted in a slower fruit body development.
Ongoing research is focused on refining the light recipe to further optimize *Pleurotus ostreatus* cultivation, enhancing yield and/or saving energy.

Presenting Author Biography - Kristof Gheysens:
Ir. Kristof Gheysens holds a master of Bioscience Engineering. He is one of project engineers of the Inagro mushroom team. He has been strongly involved in many trial set up and execution of several privately funded projects or other research trials. He plays also an active role in the knowledge transfer, training and advisory services towards the mushroom sector.

12. Applying hybrid plant breeding approaches to an Agaricus bisporus breeding program

Oral

Dr. Amber Bassett [1], ***Dr. Nolan Bornowski*** [1]

1. *Amycel/Spawn Mate*

Agaricus bisporus has a long history of consumption and cultivation, but a brief history of intentional breeding. Few mushroom hybrids produced via outcrossing have been released and adopted commercially, although those adopted varieties have experienced broad acceptance that has lasted many years. There is limited genetic diversity among commercial varieties, as many are simply derivatives or near copies of each other, but there remains a wealth of untapped diversity among wild strains that could be used to breed button mushrooms with improved traits. Challenges in breeding *Agaricus bisporus* include limited research investment, variety protection, and genetic recombination; however, the life cycle also provides advantages relative to other crops, like the ability to select gametes for hybrid development, limited need for trialing space, and short time to fruiting. Many techniques common in hybrid plant breeding programs have been perfected over time and apply well to *Agaricus bisporus*, especially with the advances in and reduced cost of DNA sequencing that has occurred in the past decade. While there is no substitute for continued investment in breeding over time, applying knowledge from other crops can increase the efficiency of breeding and allow research effort to focus on crop-specific questions. In this talk, I share key techniques and considerations from hybrid plant breeding that can be translated to *Agaricus bisporus* breeding to increase genetic gain and advance our understanding of the genetics of key traits.

Presenting Author Biography - Nolan Bornowski:
Dr. Bornowski has a background in plant breeding and bioinformatics and is working to apply quantitative genetics and genomic tools to advance mushroom breeding goals at Amycel/Spawn Mate.

13. Colour quality assessment in white Agaricus bisporus mushrooms

Oral

Dr. Andrea Uccello [1], ***Mr. Donal Gernon*** [1], ***Dr. Helen Grogan*** [1]

1. *Teagasc*

The assessment of mushroom quality can involve several physical parameters, including density, texture and dry matter content. However, visual assessment in terms of mushroom whiteness is widely adopted by the sector because it similar to the evaluation made by customers at the moment of purchase. An objective assessment of colour characteristics uses the L*a*b* colour space, developed by the International Standardization Organisation (ISO) and the International Commission on Illumination (CIE). This measures three coordinates correlating to lightness (L*), chroma (a*) and hue (b*). As the L* coordinate indicates the lightness degree, it has often been used to assess the mushrooms' colour. Ajlouni (1991) proposed reference values for standard high quality white mushrooms of L* = 97, a* = 2 and b* = 0 and used these to calculate the Euclidean distance (ΔE) to measure how the colour of mushroom samples compared to the proposed standard where high degrees of whiteness result in lower ΔE values. In previous research, we have found that using the Ajlouni ΔE reference vales, ΔE values in the range of 8-12 indicated good quality mushrooms. Mushroom strains and improved standards in mushroom growing have changed since 1991, and so we expect that reference values for L*a*b* values will also have changed. Unfortunately, the Ajlouni (1991) manuscript is no longer in the public domain to provide an insight into the rationale underpinning the L*a*b* reference values. This work characterised the colour of white mushrooms (Sylvan A15 strain) obtained from six mushroom farms over three flushes. The L*a*b* values were measured shortly after harvest using a Konica-Minolta CR-400 chromameter. We analysed the colour variability between growers and between flushes. The results indi-cated that the majority of the samples were of high quality and the whiter mushrooms generally occurred during the second flush. The Ajlouni ΔE ranged from 7.2 to 21.5 and L* ranged from 81.4 to 97.1. Two farms had lower quality mushrooms in the second or third flush. An average value for each colour coordinate was calculated based on a 25% subsample of the dataset with the highest whiteness (L*) values. We propose a new standard set of colour coordinates for high quality mushrooms for the calculation of a new white mushroom reference ΔE. We observed that good quality mushrooms fell within a range 0 to 5 while higher values corresponded to mushrooms that were visibly of lower quality.

Presenting Author Biography - Andrea Uccello:
Dr. Andrea Uccello obtained a Ph.D. in Microbial and Agricultural Biotechnologies from University of Florence (Italy) in 2011. In 2014 he joined Monaghan Mushrooms, where he identified and characterised the internal BioBank of 400+ bacterial and 200+ fungal strains. He was principally focused on the development of a biostimulant that improves the productivity of the mushroom substrate, the scaling up of the production of the biostimulant and its industrial roll up. He contributed in the development of the company's Mycopathogen clinic, which served both internal and external customers. In 2021, Dr. Uccello joined Teagasc to work on the EU funded SoftGrip project, which aims to develop a soft gripper-based robotic system to be employed in the mushroom harvesting. He is currently investigating the mushroom colour assessment, the quantification of mushroom bruising and the identification and quantification of the forces required to generate discolouration in mushrooms during the picking operations.

14. Metabolite profiling of functional fungi strains of Hericium erinaceus & Cordyceps militaris: using phenotypes, untargeted metabolomics by mass spectrometry and cell-based assays

Oral

Dr. Julie Daoust [1]***, Mr. Samuel Andrasko*** [1]

1. *M2 Ingredients*

Mushrooms have a long tradition of use as medicinal and functional ingredients. More recently, fungal ingredients have gained popularity in North America as dietary supplements and ingredients in functional foods. Species such as *Hericium erinaceus* and *Cordyceps militaris* are widely distributed and sought after for their functional benefits. Despite their popularity, there is still a lot of research needed to understand their chemistry, their mode of action in cell models and their efficacy in clinical trial settings. Beyond this, cultivation and processing methods will also affect the secondary metabolites present in functional mushroom products. Selecting the right strain or processing method is currently challenging given the lack of commercially available analytical methods to detect or quantify single secondary metabolites with therapeutic endpoints. Very little is known about the variability of these metabolites between strains of the same species and between identical strains grown in different conditions therefore an untargeted analytical method was needed to adequately assess impact of strain genetics and growing methods for M2 Ingredients cultures of *Hericium erinaceus* and *Cordyceps militaris*.

To guide decision making in strain selection & processing method optimization, an untargeted metabolomics profiling was performed on strains of *Hericium erinaceus* and *Cordyceps militaris* using a Quadrupole TOF mass spectrometer and the Mona, NIST and SCIEX Mass Libraries. The untargeted approach allowed for the identification of bioactive secondary metabolites in each samples and a comparative quantification of these metabolites in each samples served to support strain, growing, and processing method selection. In addition, unexpected matches with molecules that had not been previously reported in the species at hand were obtained. More work is needed to validate the presence of these molecules with other methods.

A comparative analysis of the know bioactive secondary metabolites associated with each set of samples was used to select strains and processing methods for both *Hericium erinaceus* and *Cordyceps militaris*. The selected strains and methods were then tested in cell-based assays to assess their bioactivity. Two cell-based assays were used; a cellular antioxidant protection (CAP-e) assay to assess antioxidant potential and a cell based mitochondrial function assay. In both cases, the metabolomics profiling led to the selection of products that had significant biological activity.

Presenting Author Biography - Julie Daoust:

Julie Daoust, B.Sc., Ph.D.| Chief Science Officer and Head of Business Development at M2 Ingredients

Dr Daoust's expertise in natural product chemistry, product development and clinical research is serving M2 Ingredients to optimize and provide scientific validity to it's functional mushroom product offerings.

15. Confirming the food safety of Agaricus mushrooms

Oral

Dr. Jenny Ekman [1], ***Mr. Adam Goldwater*** [1], ***Dr. Gordon Rogers*** [1]

1. *Applied Horticultural Research*

Mushrooms have a number of features that potentially allow them to carry human pathogenic microbes. These include high moisture content, neutral pH, lack of a protective skin and rapid decay at higher temperatures. Despite scrupulous hygiene procedures, a perception remains that mushroom are a high-risk food in terms of contamination by human pathogens. While facility surveys have occasionally found non-pathogenic species of *Listeria* (including *L. innocua* and *L. welshimeri*) in processing areas and on sliced product, no food safety outbreak has ever been associated with fresh *Agaricus* mushrooms.

To assess risk associated with Australian mushrooms, we tested fresh poultry litter, Phase 3 compost, casing, equipment and facilities for human pathogens over a twelve-month period at four Australian mushroom farms. We also tested fresh mushrooms for *E. coli*, *Salmonella* spp. and *Listeria monocytogenes. Listeria* spp. was consistently the main organism detected at mushroom farms. It was found several times in compost and casing, including Phase 2 compost at the yard and when sampled from the delivery truck. *Listeria* spp. was also found twice in casing where the underlying compost was not positive, suggesting independent contamination events. However, speciation tests confirmed that these were non-pathogenic species; most samples were *L. innocua*, with a few detections of *L. welshimeri* and one detection of *L. seeligeri*. There were no detections of *L. monocytogenes* at any of the farms tested. *E. coli* was detected four times in compost and casing from 82 tests conducted, with populations ranging from 10 to 90 CFU.g^{-1}. *Salmonella* spp. was detected once in Phase 3 compost, but was not found in fresh poultry manure samples or elsewhere in the facilities. Eighty-five samples of fresh mushrooms (whole and sliced) were tested for *E. coli*, *Listeria* spp. and *Salmonella*. One sample was positive for *L. innocua* (not L. monocytogenes). Another sample had detectable *E. coli,* but the populations was below the FSANZ limit of 100 CFU.g^{-1} suggesting it was safe to eat. It is concluded that fresh *Agaricus* mushrooms are extremely unlikely to carry populations of human pathogens sufficient to make consumers sick, even if eaten uncooked.

Presenting Author Biography - Jenny Ekman:

Dr Ekman is a postharvest physiologist and communicator who is passionate about applying science to improve commercial and consumer outcomes. Currently a senior research scientist with Applied Horticultural Research, Dr Ekman has more than 25 years' horticultural experience in Australia, the USA and internationally. While she has worked on everything from broccoli to zucchini, mushrooms have been a more or less constant mycelial strand throughout her research career. She has written reviews and conducted research on mushroom production, postharvest management and food safety. Lately, she has been most focussed on communicating the fabulousness of fungi to the entire supply chain through MushroomLink magazine.

16. The effect of metrafenone, prochloraz and Bacillus based biological control agents on the control of cobweb disease in Agaricus bisporus mushroom crop trials

Oral

Ms. Joy Clarke [1], ***Mr. Brian McGuinness*** [1], ***Dr. David Fitzpatrick*** [2], ***Prof. Kevin Kavanagh*** [2], ***Dr. Helen Grogan*** [1]

1. *Teagasc,* ***2.*** *Maynooth University*

Members of the *Cladobotryum* genus cause cobweb disease on white button mushrooms (*Agaricus bisporus*). Cobweb disease can significantly reduce the yield of healthy mushrooms and cause significant revenue loss for growers. Synthetic fungicides such as prochloraz and metrafenone have been essential for managing cobweb disease on mushroom farms. However as of June 2023, prochloraz can no longer be used on mushroom crops within the European Union (EU). It is expected that the emergence of putative resistant pathogenic strains will increase due to over-reliance on metrafenone, the only remaining approved fungicide within the EU. Mushroom disease treatments in the future will need to rely more on integrated pest management (IPM) schemes. In this work, the use of biological control agents (BCAs) was investigated as an environmentally sustainable alternative to conventional synthetic fungicides such as prochloraz and metrafenone. Prochloraz was shown to still be effective at controlling two different *Cladobotryum* strains, with efficacy values consistently reaching 70% over two replicate trials. Metrafenone was able to control *C. mycophilum* strain 618 (efficacy 90%), which was isolated prior to the introduction of metrafenone to the market, but it failed to control *C. mycophilum* strain 1546, isolated after metrafenone's introduction. This strain should now be classified as resistant. Two further *C. mycophilum* strains isolated in 2019 were also shown to be metrafenone resistant in vitro. The BCA strain *Bacillus velezensis* QST 713, which is the active agent in the commercially available biocontrol product Serenade, was unable to control cobweb disease during this work. The novel BCA strain *Bacillus velezensis* Kos displayed an intermediate level of protection compared to fungicide treatments, with efficacy values reaching 30-40%. Lower inoculum concentrations of the *C. mycophilum* pathogen were also investigated to determine if BCAs performed better at lower disease levels. Lower inoculum concentrations resulted in slightly lower but not significantly different disease levels across all treatments. BCAs have the potential to be a key component of IPM strategies for disease treatment on mushroom crops, however further research into BCAs is required and trials which investigate BCAs in the future will need to look at alternative methods to evaluate efficacy.

Presenting Author Biography - Joy Clarke:
Joy Clarke is a final year PhD student on the Teagasc Walsh Scholarship Programme. She works with Prof. Kevin Kavanagh and Dr David Fitzpatrick at Maynooth University conducting lab-based experiments in proteomics and microbial population dynamics. She also conducts crop trials with Dr Helen Grogan in the mushroom growing unit, Teagasc, Ashtown. Joy works with novel and commercial biocontrol strains and investigates their ability to control both dry bubble and cobweb disease of *A. bisporus*. She is interested in determining how the efficacy of these biocontrol treatments compare to traditional fungicides like metrafenone and prochloraz, and whether they can be an environmentally sustainable alternative to conventional chemical treatments.

17. Investigating biorationals for mushroom integrated pest management

Oral

Dr. Aimee McKinnon [1], ***Dr. Lucy McLay*** [1], ***Dr. John Paul Cunningham*** [1]

1. *Agriculture Victoria*

Lycoriella ingenua Dufour (Diptera: Sciaridae) is a major pest species in commercial mushroom (*Agaricus bisporus*) production worldwide and may also vector mycoparasitic fungi such as *Trichoderma* species. There is a growing demand for alternative control measures for pests and diseases, since control options are limited in many countries and the rise of resistance to available measures continues to threaten production. Biorationals are biologically-based products used for crop protection. The definition is broad and includes formulations that incorporate live organisms, their derivatives (e.g. metabolites), botanical actives, and semiochemicals to control invertebrate pests, inhibit pathogens, or stimulate crop growth. In Australia, the availability of registered biorationals is relatively limited and only few products are currently permitted for use in commercial mushroom growing. We short-listed available biorational agents and formulations thereof, following a review of the scientific literature. We assessed the compatibility of the entomopathogenic fungus *Beauveria bassiana* Bals. (Hypocreales: Cordycipitaceae) isolate PPRI 5339, with *A. bisporus* mycelial growth in mushroom compost bioassays. We then investigated the independent action and/or combined activity of *B. bassiana* with low concentrations of the insect growth regulator, s-methoprene, for their efficacy to suppress *L. ingenua* fly emergence. Results obtained to date indicate the potential to develop an integrated pest and disease management strategy which incorporates biorational options that may work synergistically for commercial mushroom cultivation.

Presenting Author Biography - Aimee McKinnon:
Dr Aimee McKinnon is a research scientist based at Agriculture Victoria for the Department of Energy, Environment and Climate Action (DEECA) in Melbourne, Australia. Aimee has expertise working with fungi as microbial-based biological control agents for crop protection and is currently leading a project investigating non-synthetic alternatives to complement pest and disease management practices in mushrooms.

18. Microbial perspectives towards mitigation of green mold disease in Agaricus bisporus cultivation system

Oral

Dr. Fabrício Vieira [1], ***Dr. John Pecchia*** [1]

1. *The Pennsylvania State University*

Green mold disease is the prevailing 'compost disease' in the *Agaricus bisporus* cultivation system, leading to considerable annual profit losses for mushroom growers in the United States and globally. The disease's progression during the spawn run and casehold phases results in reduced mushroom yields and a potential decline in mushroom quality. The emergence of highly aggressive green mold biotypes, exemplified by *Trichoderma aggressivum* f. *aggressivum*, has led to resistance against some fungicides. Regulatory restrictions have prompted the discontinuation of various chemicals in specific regions. An alternative strategy involves the application of biocontrol agents (BCAs), with *Bacillus* spp. displaying substantial potential in combating green mold. However, the effectiveness of commercial BCAs has exhibited significant variability and inconsistency under cropping conditions. This variability may, in part, be attributed to differences in the biological, physical, and chemical properties of environmental samples where BCAs are applied, impeding the establishment of thriving BCA populations and the attainment of consistent results. Considering that the commercial *A. bisporus* cultivation system relies on intricate ecological relationships with diverse microorganisms within the system (compost and casing), we hypothesize that modifications to compost and/or casing microbiomes through crop management practices or experimental interventions will not only impact mushroom yield but also influence the outcomes of green mold disease. This presentation will explore two distinct instances of microbial community manipulation and their effects on green mold disease outcomes. The first example involves cropping management practices (composting procedures) and BCA application, while the second entails an experimental concept aimed at altering casing microbiomes by blending colonized casing with fresh casing to affect mushroom developmental behavior. In both cases, green mold infestation correlates with observed differences in compost and/or casing microbiomes.

Presenting Author Biography - Fabrício Vieira:
Fabricio Vieira is a Postdoctoral Scholar at Pennsylvania State University and has been working with microbial ecology of substrates for Agaricus bisporus and Pleurotus ostreatus mushrooms with a focus on manipulating the mushroom microbiome towards disease mitigation and nutrient intake.

19. Development of attract and kill stations to control mushroom phorid flies on farms

Oral

Ms. Hannah Martin [1]***, Dr. Nina Jenkins*** [1]***, Dr. Tom Baker*** [1]***, Dr. Michael Wolfin*** [1]

1. *Pennsylvania State University*

The mushroom phorid fly, *Megaselia halterata,* reduces mushroom quality and yield through feeding on the actively growing mycelium and via transmission of pathogenic green mold (*Trichoderma agressivum*). *M. halterata* infestations also plague local communities in the areas surrounding mushroom farms in Kennett Square, PA. We developed and tested novel control methods on mushroom farms to control mushroom fly populations from 2019-2021. Our methods consist of attract and kill stations that target key behaviors in the life cycle of both flies. The first farm to employ this strategy in 2020 reported that *M. halterata* populations on the farm were reduced by 99.99% after one full growing cycle had been completed on the farm. Most notably, mushroom yields increased by 25.66% during the study and are expected to increase further now that fly populations have been reduced to nearly zero. The farm managers were able to extend the cropping cycle from two mushroom harvests per room to three or even four harvests per room and observed significant increases in mushroom quality. Field studies performed at 17 farm sites showed farms using our novel attract and kill stations had significantly reduced fly populations compared to farms not using the methods. Importantly, these field studies were performed from August to December, when MPFs are at their peak populations for the season.

Presenting Author Biography - Michael Wolfin:
Michael is an Assistant Research Professor in the Entomology Department at Penn State University. He has been a member of the Mushroom Fly Research Team since 2017, and his research has led to the development of attract and kill stations to control mushroom fly populations on mushroom farms. He studies the behavior and chemical ecology of mushroom flies and works to apply this research to develop or improve pest management strategies.

20. Exploring the potential of Bacillus velezensis as a bioinoculant for enhanced mushroom cultivation in a post-peat era

Oral

Dr. William Kay [1], ***Prof. Gail Preston*** [2], ***Dr. Jan van der Wolf*** [3], ***Dr. Jaime Carrasco*** [4]

1. *University of Oxford,* ***2.*** *UOXF,* ***3.*** *STICHTING WAGENINGEN RESEARCH (WR),* ***4.*** *CIAF-IRIAF*

Peat is widely used in the cultivated mushroom industry because of traits such as high water retention, low pathogen presence, and affordability. However, due to lack of sustainability, the continued use of this growth medium is likely to be untenable. A primary goal of the BIOSCHAMP project is to develop a microbial product that compensates for any yield losses when using alternative casing materials. Such a product could also help improve the performance of alternative casing materials by controlling potential increases in pathogen presence, especially when using casings with higher nutrient content. To achieve this, we have isolated novel strains of bacteria from the peat casing layer of a commercial crop, and characterised their traits as potential bioinoculants. Some of the most promising strains from this study are a number of strains identified as *Bacillus velezensis*, which show antimicrobial activity against 4 of the major fungal pathogens/competitors of the mushroom crop: *Trichoderma*, *Lecanicillium*, *Mycogone*, and *Cladobotryum*. We have quantitively assessed the localised reduction of pathogen hyphal growth in the presence of these strains, as well as showing inhibition of pathogen spore germination. Genomic analysis show that these strains are closely related, and that all contain loci predicted to be involved in production of multiple secondary metabolites including the antifungal agent Fengycin. Further HPLC work has shown Fengycin to be readily secreted by our bacterial strains. The addition of *Bacillus velezensis* in small scale crop trials has also resulted in decreases in the level of disease when infected with *Lecanicillium fungicola*. Further work surrounds enhancing the durability of these strains in crop, with the ultimate goal of mitigating disease effectively while eliminating the need for further fungicide applications.

Presenting Author Biography - William Kay:
I am a post-doctoral microbiologist who has worked on both fungal and bacterial pathogens. I have worked on crops including button mushrooms, bananas, and wheat.

21. Mushroom dry bubble disease: Novel pathogens, and mycoviruses as potential biocontrol agents

Oral

Dr. Lorant Hatvani [1], ***Dr. Hideki Kondo*** [2], ***Dr. Sándor Kocsubé*** [3], ***Prof. Nobuhiro Suzuki*** [2], ***Dr. Helen Grogan*** [1]

1. *Teagasc,* ***2.*** *Okayama University,* ***3.*** *University of Szeged*

Dry bubble disease, caused by *Lecanicillium fungicola*, seriously affects the production of button mushroom (*Agaricus bisporus*) at a global level. Due to the increasing limitations of chemical pest management, there is high demand for novel, biological means of disease control. Fungal viruses (mycoviruses) have been shown to cause reduced virulence (hypovirulence) in plant pathogenic fungi, as well as *Mycogone perniciosa*, causal agent of wet bubble disease. The objective of the project *'Leca*-VIR' is the search for viruses in fungi associated with dry bubble disease and their evaluation as potential agents to reduce the virulence of *L. fungicola*.

Fifty seven fungal strains originating from dry bubble-affected mushroom crops were identified based on their ITS (internal transcribed spacer) sequences and tested for the presence of virus-associated double-stranded (ds) RNA elements by cellulose column chromatography, which were subsequently identified by rRNA-depletion RNA-sequencing analysis. The fungal cultures were treated with different antiviral agents to eliminate any detected viruses, then the growth and sporulation of the virus-cured derivatives - confirmed by one-step RT-PCR using primers targeting the viral RdRp (RNA-dependent RNA polymerase) sequences - were examined in comparison with the parent cultures.

Most of the examined fungi were confirmed as *L. fungicola*. However, additional strains belonging to the family *Cordycipitaceae*, including *Simplicillium aogashimaense* and *Akanthomyces* spp., were also identified, and whose association with dry bubble disease has not yet been reported.

Viruses were detected in seven *L. fungicola* and three *Akanthomyces* strains and were identified as representatives of the viral families, *Chrysoviridae* (7), *Polymycoviridae* (3) and *Partitiviridae* (1). All were successfully eliminated from the fungal cultures. Virus curing was found not no affect fungal growth however, virus-free derivatives of a *L. fungicola* strain originally infected with a partitivirus and a polymycovirus, produced up to 32-fold higher amounts of conidia compared to the parent culture. The disappearance of the polymycovirus during sub-culturing did not result in altered phenotype therefore, the partitivirus is proposed to play a role in the substantial reduction of conidium production. As abundant sporulation is essential for the infectivity of pathogenic fungi, these findings suggest that this partitivirus may reduce the virulence of *L. fungicola*, and therefore be of interest in future studies targeting the biological control of dry bubble disease.

Lorant Hatvani has received funding from the Research Leaders 2025 programme co-funded by Teagasc and the Eu-ropean Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agree-ment number 754380.

Presenting Author Biography - Lorant Hatvani:

Lorant Hatvani started his scientific career with studying mushroom pathogenic Trichoderma species in 2004 as a PhD student of the University of Szeged/Hungary. During his studies and as a postdoc he visited different institutions (Vienna University of Technology/Austria, University of Pisa/Italy, University of Zagreb/Croatia) as a guest researcher. Currently he is employed at Teagasc/Ireland, the topic of his project "*Leca*-VIR", whose first half was

spent at Okayama University/Japan, is the examination of viruses in the causal agents of mushroom dry bubble disease.

22. The impact of recycled mushroom compost casing on the cultivation and yield of Agaricus bisporus

Oral

Mr. Nicholas Gabel [1], ***Dr. Fabricio Vieira*** [2], ***Dr. John Pecchia*** [2], ***Dr. David Beyer*** [1]

1. *Pennsylvania State University,* ***2.*** *The Pennsylvania State University*

White Button Mushroom (*Agaricus bisporus* cultivation involves a unique process which includes the application of a casing layer. The casing process consists of covering a colonized compost substrate with a soil-like media referred to as the 'casing layer'. The casing layer is a complex environment that has unique physical, chemical, and microbial components that stimulate the transition to sporocarp formation. *Sphagnum* spp. peat moss is commonly used as the casing material for *A. bisporus* cultivation, particularly in North America. Due to unpredictable weather patterns and the enactment of environmental policies to protect peatlands, there are currently fluctuations globally in the price and availability of *Sphagnum* spp. peat moss. Growers and researchers around the world are beginning to use alternative casing materials to alleviate the instability of peat moss supply. Recycled Mushroom Compost (RMC, also known as Spent Mushroom Substrate (SMS, is an material that has historically been used as a peat moss replacement in the casing layer. This study was conducted to determine if the inclusion of 20% or 30% RMC into the casing layer has an effect on sporocarp development and yield. The RMC was sourced from two businesses in Chester County, Pennsylvania. RMC material from one supplier, is generated through actively composting for ~4 months while the other RMC material is generated from passively composting for ~2 years in large piles. The treatments for each alternative casing crop was composed of volume to volume mixes of 20:80 or 30:70 RMC:peat mixes. Mixes were made for each suppliers material resulting in a total of four RMC treatments and a peat moss control. Yield data was calculated from sporocarp number and weight from each treatment. Individual crop yields and total yields were evaluated using ANOVA. The second objective involved the analysis of each mixtures' physical-chemical properties. The properties being examined included water holding capacity, bulk density, particle density, porosity, pH, soluble salts, and the C:N ratio. The data from these physio-chemical analyses were compared to the yield results of each individual treatment using a correlation analysis.

Preliminary results show that inclusion of RMC at low amounts may not have a significant impact on yield. Although, this may depend on the characteristics of the RMC and crop management. This study supports the need for further research on the use and composition of different RMC casing mixes and the effect RMC has on yield and development when compared to peat moss.

Presenting Author Biography - Nicholas Gabel:

My name is Nicholas Gabel, I am a M.S. student in the Department of Plant Pathology and Environmental Microbiology at The Pennsylvania State University. The research that I am conducting is focused on certain areas of White Button Mushroom cultivation. These areas include alternative casing materials and cropping management to address potential limitations in the cultivation process.

23. Working towards cultivating morels in the Northeastern United States

Oral

Mr. Xiangrong Guo [1]***, Dr. John Pecchia*** [2]***, Dr. Gretchen Kuldau*** [2]

1. *Sylvan BioSciences,* ***2.*** *Penn State*

Morels, *Morchella spp*., are edible mushrooms collected globally, prized for their culinary, nutrient, and medicinal attributes. Dried morels fetch prices upwards of $400 per pound in US grocery stores, with a significant portion imported from China. The Chinese morel cultivation industry has seen a rapid expansion since 2012, largely due to the success of an outdoor cultivation method that enhances vegetative mycelial growth through nutrient bag supplementation. Previous research in the Pecchia Lab documented successful indoor fruiting of *M. rufobrunnea*. Building on this success, this study tested the effect of mating on yield by replicating the Chinese nutrient bag technique both outdoors in a shaded structure (a high tunnel and indoors (with controlled environment grow rooms. *Morchella spp*. have mating-type (*MAT* genes, or idiomorphs, that regulate sexual reproduction between opposite mating types (most commonly *MAT 1-1* and *MAT 1-2*. Mating-type testing indicated that *M. importuna* strains appeared heterothallic, while *M. rufobrunnea* strains appeared homothallic. Two species, *M. rufobrunnea* and *M. importuna* – both with a track record of successful fruiting – were inoculated with grain spawn in the indoor and outdoor experiments. The pasteurized nutrient bags consisting of sawdust and grain corn were applied and subsequently removed, and the beds were watered heavily to initiate fruiting. Outdoor cropping experiments were conducted in Pennsylvania and Maine from 2020 to 2022. Indoor cropping experiments were conducted in climate-controlled growing rooms at the Penn State University Mushroom Research Center. As a result, *M. importuna* successfully fruited both indoors and outdoors at a profitable yield, but *M. rufobrunnea* failed to fruit. Therefore its sexual reproductive mode could not be determined. Both *M. importuna MAT 1-1* and *MAT 1-2* strains could be fruited individually and mixed. The yield was higher indoors with *MAT 1-2* individually and outdoors with *MAT 1-1* individually.

Presenting Author Biography - Xiangrong Guo:
Sylvan BioSciences - Research Scientist
Penn State - MS student

24. The potential of selected cultivation techniques to shorten the crop cycle of Agaricus bisporus

Oral

Dr. Nancy Pyck [1], ***Mr. Kristof Gheysens*** [1]

1. *Inagro vzw*

For contemporary *Agaricus* growers, not only the yield is important, but also the rotational frequency of successive cultivation cycles. In recent years, the pressure on this frequency has been intensifying. On the other hand, a faster rotation speed has a positive impact on reducing the infection pressure substantially within a mushroom production site. Therefore, this approach aligns with the principles of integrated pest management.
In this study, the potential of selected cultivation techniques was assessed to shorten the crop cycle of white and brown *Agaricus bisporus*, considering both conventional and organic cultivation. Focus was on minimizing the duration of mycelium growth in the casing soil before airing to accelerate the start of the first flush. Several experiments were set up using phase III compost with different incubation periods. A greater quantity of CAC-ing material was added and cooling down was initiated from 24h to 48h after casing. Next, the effect of different watering regimes was tested, both in the compost at filling and on the casing soil.
Results demonstrated that the timing of the start of first flush until the end of harvest could be reduced by two to three days compared to the standard cultivation method. Notably, there was no negative impact on yield or quality during harvesting.

Presenting Author Biography - Nancy Pyck:
Nancy Pyck is the senior researcher of the research unit edible mushrooms at Inagro, a practically orientated research and advisory institute in Belgium. She holds a PhD and has a strong background in mycology. She is active member of EU minor uses Commodity Expert Group Mushrooms, European Mushroom Growers Group (GEPC) and several committees in the mushroom sector. She participates in several (inter)national projects and has been involved in technical dossiers on phytosanitary issues and many privately funded projects and partnerships. To transfer the knowledge to the sector she also provides advisory and training services to growers and other stakeholders and industry clients in the sector to help to solve problems for the mushroom producers.

25. Biofortification of Pleurotus floridanus using iron based supplements

Oral

Prof. Olutayo Adedokun [1], ***Mr. Jeremiah Odiketa*** [2], ***Mr. Stuart Whitehall*** [3]

1. *University of Port-Harcourt,* ***2.*** *University of Saskatchewan, Saskatoon,* ***3.*** *Nutrigain Limited*

Iron (Fe) biofortified mushrooms can alleviate hidden hunger problems associated with Fe deficiency that causes anemia, which is prevalent in developing countries. The goal of this research was to increase the quantity of Fe in *Pleurotus floridanus* mycelia through supplementation. Treatments consisted of a bulk substrate supplemented with wheat bran and Fe (SB + Fe), wheat bran and positive control (SB + PC), and sawdust only as the control. The control treatment yielded the highest mushroom fresh weight in two out of three flushes. Sawdust supplemented with wheat bran and Fe at a ratio of 1:5 and not 1:10 produced mushroom fruit significantly in the first flush. Results from the statistical analyses showed that the addition of Fe supplements did not increase Fe content in the mushroom fruits due to Fe bioavailability problems. When combined with wheat bran, Fe supplementation increased the biomass of *Pleurotus floridanus*. Future research might investigate how supplement combination can increase Fe bioavailability and how combination with other metals like calcium can improve their availability, the use of a more soluble carrier base and the chemical form (salt) of the Fe compound in the MycroNutrient.

Presenting Author Biography - Stuart Whitehall:
Stuart is the Director of Nutrigain Limited. He has been involved with the mushroom industry since age 9, helping on his family mushroom farm. After his Masters degree in Agricultural marketing in 1986, he joined the family business which focused on sales and technical support for a French spawn company the business represented. The business was also involved in mushroom growing. In the 1990's, the mushroom growing aspect was moved into a research and development unit and much research was conducted in the mushroom sector over the following years.
In 2002, the company changed direction into supplementation as a means of increasing productivity of mushrooms. Much research was carried out on mushroom nutrition. Eventually, a unique liquid supplement base was developed commercially to effectively feed mushrooms during the crop cycle. This principle applies to *Agaricus* and other species. Recent work is on increasing the nutritional content of mushrooms.

26. Effect of different light wavelengths on productive and qualitative characteristics of Pleurotus ostreatus production

Oral

Mrs. Marina De Bonis [1], ***Dr. John Pecchia*** [2], ***Prof. Carlo Nicoletto*** [1]

1. *University of Padova,* ***2.*** *The Pennsylvania State University*

The world of cultivated mushrooms is a potential target for artificial illumination to enhance productive and qualitative characteristics of fruiting bodies. The application of artificial lights in mushroom cultivation has mainly been evaluated in post-harvest treatments and mostly for *Agaricus bisporus*, whereas there is a lack of studies about the possible effect during production phases for other important species. The aim of the research was to evaluate the effect of different light wavelengths to improve the productive and nutritional characteristics of *Pleurotus ostreatus*. Two different experiments were carried out:

- In a mushroom greenhouse: 27 bags of 25 kg each were placed under LED lights during incubation and fruiting stages. The effect of three light wavelengths (red, blue, red+blue) was observed on *P. ostreatus* (P80 mycelium - Italspawn) cultivated with a straw-based formula utilizing commercial substrate provided by three different companies.
- In a growing room, 48 bags of 3 kg each were placed on 4 racks equipped with strip LED lights. The effects of 4 different light wavelengths (blue, red, far red, and extended white) were followed for two flushes of *P. ostreatus* (123 strain - Lambert Spawn) cultivated with two different substrates: straw substrate mainly used in European countries, and cottonseed hulls + straw substrate (75% and 25% V/V respectively, commonly used in the United States).

In both experiments the productive traits as pinheads' appearance, yield (kg/kg), the number of clusters were measured; for each cluster the number of fruiting bodies and the size (diameter and thickness) were also evaluated. During each flush, fruiting bodies were used for qualitative analysis: pH, EC, titratable acidity, and vitamin D_2 content were determined. In the greenhouse, red light increased the speed of primordia initiation compared to other treatments. Different artificial lighting affected morphological traits: blue and red+blue wavelengths increased the diameter of the mushroom body by +19% and +9% respectively. Light treatments did not affect the pH, electrical conductivity, and titratable acidity, but a higher concentration of vitamin D_2 was found in mushrooms grown under blue (+39%) and red+blue light treatments (+34%) compared to control lightning. Further studies could be necessary for determining the application of artificial illumination in mushroom production looking at how radiation's intensity, wavelength and timing of illumination could affect *P. ostreatus* production, nutrition and quality.

Presenting Author Biography - Marina De Bonis:
I'm a Ph.D student from the University of Padova. I'm working in the horticulture research group in the Department of Agronomy, Animal, Food, Natural resources and Environment. My research project includes enhancing edible mushrooms cultivation (both quality and yield) specifically for oyster mushrooms and how to reuse spent mushroom substrate as fertilization for horticulture crop systems.

27. Quality assurance of mushroom products for pets - analysis of 10 products

Oral

Dr. Robert Silver [1]

1. *Chief Veterinary Officer: Real Mushrooms*

Mushroom products for pets are gaining popularity for addressing complex immune system issues like cancer, based on a few published studies. This study's objective was to evaluate label claims of these pet-labeled products by polysaccharide analysis for alpha and beta glucans. Products claiming to be from mushrooms would have high beta glucan content and low alpha glucan digestible starch content. Products with high digestible starch and low beta glucan content are derived from mycelial solid-state fermentation of grain. 60% of the products from mycelium on grain (MOG) were labeled as containing mushrooms, in spite of their analysis indicating high starch and low beta glucan content. Label transparency is important when selecting a product for a serious and potentially life-threatening disease like cancer. Industry standards for accurate labeling are needed to provide the consumer with the information they need for product selection.

Presenting Author Biography - Robert Silver:
Dr Robert Silver is an integrative veterinarian of 40 years practice experience with a special interest in mycopharmacology and its impact on the veterinary patient. Dr Silver is adjunct faculty at both the Chi University and Lincoln Memorial University College of Veterinary Medicine. Dr Silver has contributed 4 chapters in 2 veterinary textbooks on veterinary cannabis therapeutics, and has a peer-reviewed article published on the Endocannabinoid System of Animals. Dr. Silver formulates complex mushroom products for pets for Real Mushrooms and consults with Nammex, one of the largest bulk mushroom extract companies globally.

28. Mycelial cell wall: breeding targets for the future mushroom materials

Oral

Dr. Moriyuki Kawauchi [1]

1. *Kyoto University*

Mushrooms (white-rot basidiomycetes) such as *Pleurotus ostreatus* have multipurpose potential as they can create renewable alternatives for unsustainable materials such as plastics, meats, and leather by utilizing lignocellulosic biomass which is otherwise difficult and unsustainable to recycle. To develop such alternative materials efficiently using mushrooms, it is necessary to understand the structure and biosynthesis of cell walls.

1. Cell wall structure

We characterized the mycelial cell wall structure in *P. ostreatus* using chemical analysis and recombinant fluorescent probe proteins containing carbohydrate binding sites. It was clearly indicated that, in *P. ostreatus*, cell wall contents and structure are drastically different from those reported in ascomycetous fungi. In *P. ostreatus* there is less chitin inner layer, and instead of an outermost layer of α-glucan above β-glucan, the outermost layer largely consists of β-glucan above α-glucan.

2. Hydrophobins

Hydrophobins are small-secreted proteins with both hydrophobic and hydrophilic parts, which help the mycelium to break through the air-medium interface by reducing the surface tension of the medium. We focused on three hydrophobins named *vmh2*, *vmh3*, and *hydph16* as they are dominantly expressed in the vegetative growth stage of *P. ostreatus*. Using a gene knock-out technique, it was demonstrated that *hydph16* strongly affects both cell wall thickness and aerial hyphae formation.

3. Basidiomycete specific chitin synthases (Chsb)

A key structural component of fungal cell walls is chitin which is produced by transmembrane proteins called chitin synthases (Chs). Chitin synthases have been associated with mycelial structure and strength in ascomycetes, however their roles in basidiomycetes are not as well understood. Therefore, evolutionary and molecular genetic analyses were conducted to understand chitin biosynthesis and function in basidiomycetes. Phylogeny of *chs* genes from 9 ascomycete and 6 basidiomycete species supported the existence of 7 previously classified clades and the discovery of 3 novel basidiomycete-specific clades. *P. ostreatus* has 9 chitin synthase genes, 4 of which are basidiomycete specific (named *chsb1~4*). The *chsb1* gene was difficult to disrupt and has not yet been further investigates. The other three *chsb* genes were knocked out, and all disruptants had sparser mycelia, rougher mycelia surfaces and shorter aerial hyphae. Although a significant decrease in cell wall thickness was observed using TEM, no differences in relative percentages of chitin and glucan were found.

These results demonstrate targets to improve hyphal structures for developing mushroom-based alternative materials.

Presenting Author Biography - Moriyuki Kawauchi:

Academic career:

2022- Associate Professor (PI), Laboratory of Environmental Interface Technology of Filamentous Fungi, Grad. Sch. of Agr., Kyoto Univ.

2019-2022 Assistant Professor, Laboratory of Environmental Interface Technology of Filamentous Fungi, Grad. Sch. of Agr., Kyoto Univ.

2018-2019 Postdoc, Grad School of Medicine, Jichi Medical University

2015-2018 JSPS PD, Grad School of Agriculture, The University of Tokyo.
2014-2015 Research fellow MMI, University of Wisoconsin-Madison, USA

Academic education:
2014 Dr. Eng. from Hiroshima University (Engineering
2011 Master. Eng. from Hiroshima University (Engineering
2009 Bachelor. Life. Sci. from Prefectural University of Hiroshima (Life Science

29. Aerial Mycelium, the fungi world upside down

Oral

Dr. Bert Rademakers [1]

1. *Mycelium Materials Europe*

After decades of growing mushrooms and using the fruit-bodies as a harvest a whole new industry is developing in using the mycelium instead of mushroom. In al kind of fields possibilities of mycelium as a living network are investigated . The main directions are; packagings replacement, textile alternatives and meat replacements . All these initiatives are still on a startup business scale but could have an enormous impact when able to scale up. The mushroom industry could radically change and a whole new challenging field of exploration could be possible n the future of fungi. The existing traditional mushroom industry could benefit and even help to explore the field of aerial mycelium. An overview of the existing technology and applications so far could give insight of the practical use as well in the traditional mushroom industry as in a whole new industry of growing aerial mycelium. The author has strong roots in the traditional mushroom industry but is also one of the early adapters of the aerial mycelium technology as well in theory as in practice by his company Mycelium Materials Europe.

Presenting Author Biography - Bert Rademakers:
Bert Rademakers is a mushroom entrepreneur in hart and soul. As a farmers son he has worked on the Dutch Mushroom School and started his own white button mushroom farm in 1991. In 2000 he started Fungi2000 which later became CNC Exotic Mushrooms BV and is now leading in Organic substrate for growing mushrooms in Europe. In 2020 he founded Mycelium Materials Europe as a biotechnology startup. www.linkedin.com/in/bert-rademakers-5b893a6

30. Bio-processed mycelium-based alternatives to plastic packaging materials

Oral

Dr. Philippe Amstislavski [1], ***Mr. Yevhenii Razomasov*** [2], ***Dr. Elena Roik*** [2]

1. *University of Alaska,* ***2.*** *Seven Spruce LLC*

Plastics comprise 90% of all marine debris, with polystyrene from seafood boxes, insulation, and single-use food containers being the most common items in ocean and coastal surveys. Polystyrene foam has low density and resists degradation, thus accumulating in the Arctic over time. Plastics are rapidly colonized by microbes when released in the marine environment. This marine microbial community—plastic debris interaction provides emergent threats to human and environmental health.

These concerns provide impetus for research into bio-based alternatives. Mycelium-based alternatives to synthetic materials provide an opportunity to decrease marine pollution associated with plastics intended for insulation and packaging and extend economic opportunities for many mushroom farms. Substrate from cellulose can be sustainably harvested in many regions. This study explored the effect of introducing the mycelium of several cosmopolitan basidiomycete fungi into foamed fibrillated cellulose. Samples were created by incubating mycelium with foamed cellulose.
Trametes versicolor produced the most robust growth on the cellulose substrate. We characterized the density, microstructure, compression strength, thermal conductivity, water contact angle, compression, and antimicrobial properties of T. versicolor/cellulose samples. Foamed mycelium-cellulose samples had low densities (0.058 – 0.077 g/cm3) and low thermal conductivity (0.03 – 0.06 W/m·K at +10 °C). We observed an unexpectedly high water repellence with water contact angle of mycelium-cellulose foams of 140°.
We also explored how mycelium-cellulose materials produced with four cosmopolitan polypore species affected active virus and common pathogenic microbes showing a significant decrease (over 1,0 log cfu/sample) on the active MS-2 virus during 24 h contact time in all mycelium-cellulose material extracts produced with strains of Irpex lacteus, Fomes fomentarius, T. versicolor, and Fomitopsis pinicola.
These findings indicate that foamed mycelium composites are functionally competitive with plastics and may provide added microbial resistance. Future research needs to address the fish industry and insulation applications.

Presenting Author Biography - Philippe Amstislavski:
Dr. Amstislavski is a mycologist with expertise in mycomaterials, T2M of biomaterials and biopolymer products, and biotechnology workforce training. He is a Professor at University of Alaska.

Profiling the Composition and Function of the Casing Devome: Manipulating developmental patterns and disease-suppression of blotch in Agaricus bisporus

Oral

Dr. Eoin O'Connor [1], ***Dr. Fabricio Vieira*** [1], ***Ms. Isako Di Tomassi*** [2], ***Prof. Carolee Bull*** [1], ***Dr. John Pecchia*** [1], ***Prof. Kevin Hockett*** [1]

1. *The Pennsylvania State University,* ***2.*** *Pennsylvania State University*

The impacts of microorganisms as partners in host developmental and disease-resistant crop phenotypes is an emerging field of study. The term devome (developmental microbiome) describes microbiomes that are necessary or contribute to the development of a host organism. The cultivation of *Agaricus bisporus* involves many changes in the compost and casing microbiome which are crucial for productive mushroom development. The casing layer is host to many *Pseudomonads* and other genera that play a role or are closely associated with the initiation and development of fruiting body production. To identify and characterize microbial communities that are involved in mushroom development, we compositionally and functionally characterized the casing devome using metagenomics and metaproteomics. This involved performing passaging of casing by taking material at the point of pinning (mushroom primordia initiation and adding it to a fresh standard casing substrate (ratio 1:10, a process which was repeated 10 times. Passaged casing triggered early pinning by an average of 4 days when compared to standard casing. Proteomics revealed a remodeling of the casing devome, where bacterial diversity is lower in passaged casing compared to standard casing at day 0 and a sharp increase occurs predominantly of *Gammaproteobacteria* (which contains the genus *Pseudomonas)* leading up to and during pinning. Observations for passaged casing with functional profiles of *A. bisporus* and *Gammaproteobacteria*, in-tandem, reveal dominant host biological processes such as lipid metabolism (potentially linked to fruiting body morphogenesis and high numbers of receptor and cell wall biogenesis proteins in the microbial community. Additionally, passaged casing was assessed for its impact on bacterial blotch disease (*Pseudomonas tolaasii)*. Standard and passaged casing was directly inoculated with *P. tolaasii* (10^6 cells/kg of dried casing, and mushrooms were measured for blotch severity. Non-symptomatic mushroom yield was significantly higher ($p < 0.001$) in passaged casing compared to standard casing. Disease severity was also much higher in standard casing, with the highest level representing 84% compared to 57% of mushroom yield (kg/m^2 in passaged casing. Taken together, our studies involving manipulation of the casing devome offers advantages in shorter cropping cycles (4 days and suppression of the virulence of a common crop disease, bacterial blotch. The overall aim of this work is to capture a microbial cohort from passaged casing that may have benefits commercially. While our understanding of devomes is still in its infancy, their intersection in microbiome sciences and developmental biology offers new prospects into the field of crop production.

Biography - Eoin O'Connor

My research focuses on the use of metaproteomics to understand the composition and function of microbial communities in mushroom substrates and how changes in these communities impact the developmental microbiome (Devome) of mushroom development. I hold a doctorate in fungal biology and bioinformatics from Maynooth University and Teagasc (The Agriculture and Food Development Authority), Ireland, and a bachelor's degree in biology from Maynooth University, Ireland. I am currently a postdoctoral scholar in the department of Plant Pathology and Environmental Microbiology at The Pennsylvania State University, in Dr. Kevin Hockett's group and as part of a collaborative project with Drs. John Pecchia and Carolee Bull.

Poster Abstracts & Bios

01. Ergothioneine: science, cultivation and application

Poster Submission

Dr. Mason Bresett [1]

1. *Chief Science Advisor Real Mushrooms/NAMMEX consultant*

Ergothioneine (ERGO is gaining popularity for its potential use in many health conditions that are supported by clinical and preclinical data. ERGO is also top of mind for many mushroom growers due to an increasing amount of research and demand in the nutraceutical industry. ERGO's role as an analytical marker for fungal quality testing will be discussed. An overview of internal cultivation analytics from top producing ERGO cultivars plus a comprehensive summary on scientific data will be explored in this presentation. By combining internal cultivar analytics with summaries of the current medical research, this presentation will provide practical applications and appreciation for this new "longevity vitamin".

Presenting Author Biography - Mason Bresett:
Dr. Mason Bresett ND is a naturopathic doctor from Canada with a key interest in mycology and mushroom cultivation. Dr. Bresett has a general family naturopathic practice in Sarnia, Ontario. He consults with health care professionals and researchers on the scientific and traditional uses of functional mushrooms. Dr. Bresett is actively working with a group of researchers, medical doctors and oncologists setting up clinical trials using mushroom extracts for integrative health and cancer research. Dr. Bresett has been actively learning from the top ergothioneine experts, mushroom growers and key antioxidant researchers from around the world. Dr. Bresett is the Chief Science Advisor for Real Mushrooms and consults for NAMMEX.

02. Optimization of parameters for extraction of protein hydrolysate using edible mushroom, Agaricus bisporus stems

Poster

Mrs. Vanessa Grifoll [1], ***Ms. Paula Bravo*** [1], ***Ms. Camille Malterre*** [2], ***Ms. Soukaina Hilali*** [2], ***Mr. Job Tchoumtchoua*** [2], ***Dr. Margarita Perez Clavijo*** [1]

1. *CTICH,* ***2.*** *Celabor*

European consumers are increasingly interested in alternative protein food products as viable substitutes for conventional or highly processed foods of animal origin. However, to date, alternative protein food products remain a niche. Worldwide, they represent only 1% of total protein consumption and, if current trends continue, this figure will only reach 5-10% by 2030[1]. In this context, the LIKE-A-PRO EU- project was set to extract proteins from different novel matrices and use them to develop new and appealing food products. In this context, edible mushrooms by-products can be used as an attractive alternative to conventional protein sources, while promoting its valorization. In the present study, the parameters optimisation to produce superior quality protein from mushroom by-products, namely mushroom stems, has been carried out. This residue represents 20% of the harvested mushroom weight. Stems of *Agaricus* mushroom (*Agaricus bisporus* have been selected (obtained from Cultivos Riojal company, as part of The Real Green Food Co, Spain for protein extraction. The mushroom stems are encrusted with peat, which needs be removed. Different methods have been tested, with abrasion being the preferred method. Once the *A.bisporus* stems are clean, they are dried at 40ºC for 72 hours and then ground prior extraction. Protein extraction was optimised by considering different parameters of flour particle size (1mm, 0.56mm and 0.18mm, solute to solvent ratio (1:10, 1:20, 1:30 and 1:40, protein solubilisation and isoelectric precipitation pHs. Protein solubilisation has been performed using a range of alkaline pH (8 to 13 at room temperature with 15 minutes utrasound-assisted-extraction. Isoelectric precipitation process has been carried out using a different pH range from2 to 6. The yields obtained were 34.5-47.8% (1.99 -3.36 g protein/100g dry matter. The results indicate that optimal extraction conditions are obtained when a high solvent to solute ratio was use. The solubilisation pH gave better results at high alkaline values, whereas the Isoelectric precipitation was optimal at low pH values. It was also found that the particle size was not an influencing parameter for the extraction of protein from ground mushroom stems These results show the potential of *Agaricus bisporus* stems in protein concentrates preparation.

[1] Rob Dongoski. (2021. Growth of alternative protein trend and aligning it with consumer needs to stay ahead in the food…EY. Link

This work has received funding from the European Union under Grant Agreement No 101083961.

Presenting Author Biography - Vanessa Grifoll:

Degree in Chemistry and Biochemistry from Rovira i Virgili University (Spain, 2004 and 2005). As a complement to the degrees, I obtained a Master in Food Safety Management (Universidad Camilo José Cela, Spain, 2017) and a Master in Occupational Prevention, specialising in safety, industrial hygiene and ergonomics (Aco2s, Spain, 2007). In 2003, Notthingham Trent University, for the execution of the final degree project “Hydroxytyrosol: Enzymatic sysntesis, properties and detection using High Pressure Liquid Chromatography”. My experience as a researcher started in 2007 atthe Department of Biochemistry and Biotechnology of the Rovira i Virgili University (PTA-2003-02-00786) and at the Technological Centre of Nutrition and Health (CTNS). I have participated in several National and European Research Projects in the field of Nutrigenomics, acquiring experience in cell line assays, animal assays and techniques related to molecular biology. Since 2012, I work at CTICH as a researcher in the area of health and nutrition,

03. Response surface methodology for the extraction of phenolic compounds and antioxidant activities from edible mushrooms

Poster

Mrs. Vanessa Grifoll [1], ***Ms. Paula Bravo*** [1], ***Dr. Margarita Perez Clavijo*** [1]

1. *CTICH*

Phenolic compounds are substances with antioxidant, anti-inflammatory and antibacterial properties. In mushrooms, phenolic compounds are mainly found in the external layer and in the stems. Their content varies according to the species. The phenolic compounds in mushrooms have a number of health benefits. They can help protect cells from oxidative damage, which is a contributing factor in the development of chronic diseases such as cancer, heart disease and Alzheimer's disease. They can also help reduce inflammation and infection. Response surface design was employed to identify the extraction parameters that can improve the phenolic content (TPC from fruiting bodies (FB, stems (S and spent substrate (SS of edible mushroom. Species used in the present study were *Agaricus bisporus*, *Agaricus brunnescens, Agaricus blazei* and *Hericium coralloides.* These parameters included: temperature (A: 50–100°C, time (B: 30–45 min, extraction solvent (C: 60–80% and sample solvent (D: 60–80%. All experiments were done using a pressurized liquid extraction (PLE as a method of extracting samples. Antioxidant activities were determined as Trolox equivalent antioxidant capacity (TEAC, 2,2-diphenyl-1-picrylhydrazyl (DPPH free radical scavenging activity, and ferric-reducing antioxidant power (FRAP to optimised extracts.
Results indicated that the data were adequately fitted into second-order polynomial models for fruiting body, stems and spent substrate. The results showed that the temperature and % sample solvent were the dominating factors that significantly affect the extraction process. Under these conditions, the maximum TPC value found was 8.06 (mg GAE/g extract for *A. blazei* stems. The establishment of such model provides a good experimental basis employing RSM for optimizing the extraction parameters on antioxidants from edible mushrooms extract. Furthermore, ASAP-MS in positive ion mode, ASAP(+-MS, has been used for analysis of phenolic compounds standards and optimised extracts using a Puriflash 5250-MS. Compounds such as chlorogenic acid, coumaric acid, 4-hydroxi-benzoic, rutin, bencene-1,2,3-triol, syringic acid, protocatechuic, catechin, vitamin E and D2 were detected in optimised extracts. The present study results indicate that edible mushrooms can be used as a source of antioxidants and phenolic compounds that can be applied in food and medicine.
This research was financed by Consejería de Economía, Innovación, Empresa y Trabajo Autónomo de La Rioja, Spain, through EXHON Project.

Presenting Author Biography - Vanessa Grifoll:
Degree in Chemistry and Biochemistry from Rovira i Virgili University (Spain, 2004 and 2005). As a complement to the degrees, I obtained a Master in Food Safety Management (Universidad Camilo José Cela, Spain, 2017) and a Master in Occupational Prevention, specialising in safety, industrial hygiene and ergonomics (Aco2s, Spain, 2007). In 2003, Notthingham Trent University, for the execution of the final degree project "Hydroxytyrosol: Enzymatic sysntesis, properties and detection using High Pressure Liquid Chromatography". My experience as a researcher started in 2007 atthe Department of Biochemistry and Biotechnology of the Rovira i Virgili University (PTA-2003-02-00786) and at the Technological Centre of Nutrition and Health (CTNS). I have participated in several National and European Research Projects in the field of Nutrigenomics, acquiring experience in cell line assays, animal assays and techniques related to molecular biology. Since 2012, I work at CTICH as a researcher in the area of health and nutrition,

04. Insect protein in the circular economy

Poster

Mr. Arpad Mutsy [1]

1. *Bio-Fungi Kft.*

Mushroom cultivation is one of the most sustainable agricultural activities, as the mushroom industry uses locally available agricultural by-products to supply healthy food in consistent quantities and of consistent quality all year round, regardless of continent or climate. In addition, all the industry's by-products, such as spent mushroom compost and stems, are 100% natural and recyclable. The fact that we have not yet managed to find a market for these by-products represents a major challenge.

Because of its high water content, volume, and low price, spent mushroom compost can also be used for soil enrichment. The need for further research on how the mushroom content improves soil is worth mentioning in this respect.

Bearing in mind the increasing demand for protein and current global trends, the question arose as to whether spent mushroom compost and stems might be used as an alternative feed in protein production based on insect larvae.

Our aim was to accelerate the natural cycle as a whole by controlled larval breeding, thereby producing larval litter with even higher value added; the larvae themselves are a significant source of protein, which likewise served as a recyclable enrichment during mushroom production, while the litter is also essentially recyclable. However, both products can be used in other, alternative ways, for example as animal feed, in crop production, and in protein supplementation, thereby also replacing other plant-based protein production.

The concept as a whole was inspired by the fact that in nature, mushrooms and larvae represent very important decomposition processes, and this natural technology can be replicated. Because of their protein and mineral content, mushrooms are eaten by worms and their mycelium is damaged by various pests; the same thing happens in mushroom cultivation – think no further than the fungus fly. Another interesting fact is that the larvae are bred on controlled feed material, the benefit of which is that, unlike other species, they are not feeding on refuse, waste, or uncontrolled waste. All of this can now be turned to our advantage.

Presenting Author Biography - Arpad Mutsy:

Owner of Bio-Fungi Ltd., Hungary, a well-known and recognized specialist in mushroom industry.

05. A comparative analysis of secondary metabolites and sensory properties of Hypsizygus marmoreus fruiting bodies based on culture period

Poster

Dr. YounLee Oh [1], ***Dr. Minji Oh*** [1], ***Mr. Jihoon Im*** [1], ***Dr. MinSeek Kim*** [1]

1. *RURAL DEVELOPMENT ADMINISTRATION*

Hypsizygus marmoreus, known as 'Beech mushroom' in English and 'Buna-shimeji' in Japanese, is a mushroom with a sweet and savory flavor. It's not only appreciated for its taste but also for its excellent shelf life and unique mushroom shape. In South Korea, it's gaining popularity, especially among those in their 30s, and in Japan, it's one of the major mushrooms, accounting for 50% of the total mushroom production, alongside Enoki mushrooms. This mushroom is not only used for culinary purposes but is also known for its potential health benefits due to various functional compounds it contains. It's rich in Hypsiziprenol, a unique antioxidant compound with potential anticancer properties. However, cultivating *Hypsizygus marmoreus* requires a longer period, around 90 days, making careful culture management crucial. Additionally, its slight bitter taste, a characteristic of mushrooms, poses a challenge for market expansion. This study aimed to investigate the differences in secondary metabolites and sensory properties resulting from the different culture period. We inoculated 10 strains using solid inoculants, cultivated the mushrooms over a stable growth period of 80 to 90 days at 5-day intervals, and then conducted a secondary metabolite analysis using UHPLC-LTQ-Orbitrap-MS/MS on the harvested fruiting bodies. PLS-DA analysis revealed significant differences in secondary metabolites depending on the culture period, confirming 32 secondary metabolites, including 9 from the fatty acid series, 6 from the lipid series, 3 from the hypsiziprenol series, 2 dipeptides, and 12 unknown compounds. In the case of hypsiziprenol A13, A12, and B12, it was observed that the fruiting bodies cultured for 85 days exhibited higher content of these compounds. To assess taste alongside the secondary metabolite analysis, we conducted sensory evaluations on the same samples, including flavor (mushroom flavor, sweetness, saltiness, umami, bitterness), mouthfeel, and texture. In particular, the 85-day cultured mushrooms exhibited a milder bitterness. We plan to conduct further analysis to explore the correlation between secondary metabolites and taste based on the culture period.

This work was supported by a grant from the Agricultural Policy Support Technology Development Project [Project No. RS-2023-00232566] of the Rural Development Administration (RDA) of the Republic of Korea.

Presenting Author Biography - YounLee Oh:

Oh YounLee currently serves as breeding research on *Agaricus bisporus* and *Hypsizygus marmoreus* as the leader of the Mushroom New Breeding Cultivation Research Laboratory at the National Institute of Horticultural and Herbal Science, Rural Development Administration. She is actively involved in conducting research on the breeding of *Agaricus bisporus* and *Hypsizygus marmoreus*. Her research interests primarily revolve around developing disease-resistant varieties of *Agaricus bisporus* and breeding superior functional varieties of *Hypsizygus marmoreus*.

06. Development in chitin bio-based materials used for food biopackaging

Poster

Dr. Margarita Perez Clavijo [1], ***Mrs. Vanessa Grifoll*** [1], ***Ms. Paula Bravo*** [1]

1. *CTICH*

One of the great interests of science today is focused on the search for biomaterials that free markets from the use of molecules derived from petrochemical sources, promoters of organic compounds from the use of molecules derived from petrochemical sources, promoters of volatile organic compounds or other chemicals with high residual toxicity, both for the environment and for health. In response to this situation, the search for bioplastics that can replace conventional plastics is of major importance in our society. In terms of environmental suitability, biopolymers are environmentally friendly packaging materials. Natural biopolymers come from raw materials are essentially derived from either replenishable agricultural feed stocks (cellulose, starch, and proteins), animal origin (collagen/gelatin), marine food processing industry wastes and mushrooms (chitin/chitosan) and microbial origin (polylactic acid (PLA) and polyhydroxyalkanoates (PHA). Chitin, the world's second most abundant naturally produced biopolymer, is a polysaccharide found in crustaceans, insects and fungi. Fungi offer the advantage that it is not a seasonal product like crustaceans, it is abundant in different regions, and the transformation process to chitosan is much more environmentally friendly in fungi because it does not require aggressive treatments to remove minerals, as is the case when extracted from crustaceans. Biopolymer films are being used for packaging because, in addition to effectively protecting food during storage, they can mitigate the alarming environmental pollution associated with petroleum-based plastics. Although the extraction of chitin and its applications is particularly important in fungi as it is an abundant compound, few studies have been carried out. For this reason, quitimush project was created to research and develop methods for obtaining chitin from edible mushrooms, mycelium obtained by SLF (submerged liquid fermentation) and their by-products (mushroom stems and discarded mushrooms), to develop different prototypes of active packaging, and biofilms containing chitin nanoparticles. Chitin nanoparticles will be evaluated for their physicochemical and morphological characteristics. The following parameters will be evaluated: analysis of the morphology of the nanoparticles by scanning electron microscopy, Fourier Transform Infrared Spectroscopy (FT-IR) analysis, thermal stability characterisation (differential scanning calorimetry and thermogravimetry), characterisation of optical properties (transparency) by UV-visible spectroscopy, structural characterisation by X-ray diffraction and tensile mechanical characterisation of the developed materials (universal testing machine).

This research was financed by Consejería de Economía, Innovación, Empresa y Trabajo Autónomo de La Rioja, Spain, through QUITIMUSH Project.

Presenting Author Biography - Margarita Perez Clavijo:

I have graduated as a Chemist at the University of Zaragoza in 1993. I got a PhD in Analytical Chemistry by the University of Valencia in 2001. I acquired experience in technical analytics focused mainly in food and security in the "Regional Laboratory in La Grajera". From 2004 onwards I have working in ASOCHAMP management and I am the Manager of the Regional Mushroom Technological Research Center in La Rioja (CTICH). During this time I have focused on research management, improving crop productivity, N.I.R (infrared analysis for compost), SMS (spent mushroom substrate) and crop protection. I have participated as technical manager in other National and European funded projects. I have published several papers in international journals and other scientific publications such as the Journal of the Science of Food and Agriculture or the Journal of Functional Foods. Also I have participated in

more than 30 scientific congresses.

07. Antioxidant activity of polysaccharides from edible mushrooms

Poster

Dr. Margarita Perez Clavijo [1], ***Mrs. Vanessa Grifoll*** [1], ***Ms. Paula Bravo*** [1], ***Dr. Josune Garcia-Sanmartin*** [2], ***Dr. Alfredo Martinez*** [2]

1. *CTICH,* ***2.*** *CIBIR*

Mushrooms are edible fungi that possess high nutritional value and many biological activities such as immunomodulatory, hepatoprotective, antitumor, anti-inflammatory, antiviral, hypoglycemic, hypolipidemic and antioxidative. These properties have been demonstrated to be due to their content of bioactive metabolites including polysaccharides, terpenes, phenolic compounds, and many other low molecular weight compounds. In particular, edible mushrooms are interesting as a source of biologically active glucans with a variety of bioactivities, with potential use in the field of biomedical sciences. The antioxidant activity of polysaccharides is of specific interest, as numerous studies have highlighted their ability to reduce oxidative stress induced by excessive production of reactive oxygen species (ROS). The biological activity of polysaccharides has been of increasing interest since in vitro and in vivo studies have demonstrated that they possess neuroprotective activities because of their antioxidant capacity against oxidative stress. In the present study the antioxidant activity of an enriched polysaccharide fraction (EPF) obtained from the fruiting body of edible mushrooms *Agaricus bisporus* (AB), *Agaricus brunnescens* (ABp), *Agaricus blazei* (ABz), *Lentinula edodes* (LE) and *Hericium erinaceus* (HE) were evaluated. The mushroom varieties were harvested and dried, then taken to the laboratory for further analysis. The EPF was extracted by using hot water followed by precipitation with cold ethanol. A previous treatment with ethanol was performed to remove low molecular weight compounds.

Total α- and β-glucans were quantified using the Megazyme International Kit and total carbohydrate was evaluated using phenol sulphuric method. The results showed that the genus Lentinula was the specie with a highest content of polysaccharides with a higher content of (1-3; 1-6)-β-D-glucans. The antioxidant activity was also assessed by a cellular method using cell line SHSY5Y. The cells were treated with the oxidant agent aluminium-maltol and treated with polysaccharides extracts for 3 consecutive days. Overall, the extracted compounds showed high antioxidant activity in polysaccharide extracts as they significantly increased cell survival, suggesting that they are extracts rich in growth factors. In addition, an increase in the proliferative effect is observed with increasing doses of genus *Hericium*. On the other hand, the genus Agaricus showed cytoxicity at the highest concentration tested (1000ug/mL). These findings provided new insights into the possible application of polysaccharides as antioxidant compounds, suggesting that they may help to prevent diseases associated with oxidative damage.

This research was financed by Consejería de Agricultura, Ganadería y Medio Ambiente de La Rioja, Spain, through Project grant nº CT23_04. https://ctich.com/neurofungi-2/

Presenting Author Biography - Margarita Perez Clavijo:

I have graduated as a Chemist at the University of Zaragoza in 1993. I got a PhD in Analytical Chemistry by the University of Valencia in 2001. I acquired experience in technical analytics focused mainly in food and security in the “Regional Laboratory in La Grajera”. From 2004 onwards I have working in ASOCHAMP management and I am the Manager of the Regional Mushroom Technological Research Center in La Rioja (CTICH). During this time I have focused on research management, improving crop productivity, N.I.R (infrared analysis for compost), SMS (spent mushroom substrate) and crop protection. I have participated as technical manager in other National and European funded projects. I have published several papers in international journals and other scientific publications such as

the Journal of the Science of Food and Agriculture or the Journal of Functional Foods. Also I have participated in more than 30 scientific congresses.

08. Promoting circular economy by the assessment and validation of mushroom industry by-products as sustainable ingredients for diets in swine and fish aquaculture species (GreenBlueCircle)

Poster

Dr. Margarita Perez Clavijo [1], ***Ms. María Luisa Tello Martín*** [1], ***Dr. Sonia Ojeda*** [1], ***Mr. Enric Gisbert*** [2], ***Ms. Silvia Torrecilla*** [2], ***Mr. Gerard Verge Mèrida*** [3], ***Mr. Joan Tarradas Font*** [3], ***Mr. Ignasi Sanahuja*** [2], ***Mr. David Torrallardona*** [3], ***Ms. Nuria Tous*** [3]

1. *CTICH,* ***2.*** *IRTA-Aquaculture Program,* ***3.*** *IRTA Mas Bover*

GreenBlueCircle project aims to provide agri-food sector with novel and underexploited sources of raw materials from recycled wastes by: 1)Evaluating the environmental sustainability of using residues from swine and fish production as substrates for mushroom production; 2)Using waste from the mushroom industry (stems and discarded mushrooms) as alternative protein sources to replace conventional soybean and fish meals in swine and fish feeds, and also by considering their potential bioactive functions such as antimicrobial properties; and 3)Assessing the sustainability of the circular strategy proposed (in comparison to current conventional practices) through the life cycle assessment of the production process of various mushrooms, the use of their co-products by farmed fish and piglets, and the upcycling of animal manure for mushroom cultivation. The overall approach is promoting the sustainability and circularity.

To assess the first objective, samples of livestock (chicken manure and solid fraction of pig slurry) and freshwater aquaculture (fish sludge) were collected and analysed to evaluate their potential use as mushroom nutritious substrate ingredient. The following elements and parameters were measured following AOAC guidelines: total nitrogen, organic nitrogen, humidity, pH, conductivity and organic content. Heavy metals and pesticides were also analysed. The selected livestock residues were tested *in vitro* at different inclusion levels to find the optimal nutritious media for ten different edible mushrooms. Petri dishes were prepared with a media including the different residues and agar and inoculated with the mycelium of the mushroom species. Agar medium was used as control. Radial growth was daily measured. Results from the in vitro study showed that waste from poultry farming was the one with the highest potential. Considering these results, 4 species were selected for pilot scale trials (*Pleurotus eryngii, Hericium erinaceus, Agrocybe aegerita* and *Agaricus bisporus var. brunnescens*). To assess the second objective, a digestibility study for four different mushroom species was carried out in piglets of 15-20kg. Seventy-two piglets were offered one of 4 different dietary treatments for 14 days: (T-1) Basal diet; (T-2) Basal diet + *Agaricus bisporus;* (T-3) Basal diet + *Pleurotus ostreatus;* and (T-4) Basal diet + *Lentinula edodes*. Feces were collected between days 12-14 of trial and at day 14 animals were euthanized to collect ileal contents. Apparent ileal and fecal nutrient digestibilities of the different mushrooms were estimated from the digestibilities of the basal and test diets using the difference method.

This work was supported by MCIN/AEI/10.13039/501100011033 and European Union “NextGenerationEU”/PRTR, project no. TED2021-132054B-C22.

Presenting Author Biography - Margarita Perez Clavijo:

I have graduated as a Chemist at the University of Zaragoza in 1993. I got a PhD in Analytical Chemistry by the University of Valencia in 2001. I acquired experience in technical analytics focused mainly in food and security in the “Regional Laboratory in La Grajera”. From 2004 onwards I have working in ASOCHAMP management and I am

the Manager of the Regional Mushroom Technological Research Center in La Rioja (CTICH). During this time I have focused on research management, improving crop productivity, N.I.R (infrared analysis for compost), SMS (spent mushroom substrate) and crop protection. I have participated as technical manager in other National and European funded projects. I have published several papers in international journals and other scientific publications such as the Journal of the Science of Food and Agriculture or the Journal of Functional Foods. Also I have participated in more than 30 scientific congresses.

09. The potential of mushroom residual flows as a new source of bioactive components

Poster

Dr. Nancy Pyck [1], ***Dr. Joran Verspreet*** [2], ***Mr. Kristof Gheysens*** [1], ***Dr. Leen Bastiaens*** [2]

1. *Inagro vzw,* ***2.*** *VITO NV*

Valorisation of residual flows in the mushroom sector is becoming increasingly important in the context of circular economy. The biopolymer chitin, present in fungal cells wall, and its derivative chitosan are renowned as compounds with a broad industrial applicability in diverse sectors such as animal nutrition, agro-industry, cosmetics, and pharmaceuticals. Nowadays the demand for locally produced chitosan of non-animal origin is substantial and for this reason the project CHampITINE was launched to explore the potential of extracting chitin from mushroom by-products, specifically the residual waste generated during harvest of *Agaricus bisporus* and *Pleurotus ostreatus*. Obtained results demonstrated a successful extraction of chitin and conversion to chitosan. Also the potential of the Flemish mushroom supply was assessed and particular logistical aspects were evaluated by all stakeholders involved. The follow-up project VALORCHAMP further examines how mushroom residues can be upgraded as ingredients for food (e.g. spreads, flavour enhancers, protein sources) and other interesting bioactive components (e.g. preservative agents). Focus is not only on chitin and chitosan, but also on other carbohydrates and proteins.

Presenting Author Biography - Nancy Pyck:
Nancy Pyck is the senior researcher of the research unit edible mushrooms at Inagro, a practically orientated research and advisory institute in Belgium. She holds a PhD and has a strong background in mycology. She is active member of EU minor uses Commodity Expert Group Mushrooms, European Mushroom Growers Group (GEPC) and several committees in the mushroom sector. She participates in several (inter)national projects and has been involved in technical dossiers on phytosanitary issues and many privately funded projects and partnerships. To transfer the knowledge to the sector she also provides advisory and training services to growers and other stakeholders and industry clients in the sector to help to solve problems for the mushroom producers.

10. Optimizing the application of entomopathogenic nematodes to control sciarid populations

Poster

Dr. Nancy Pyck [1], ***Mr. Kristof Gheysens*** [1]

1. *Inagro vzw*

The continuous decline of active substances allowed in mushroom cultivation have left growers with limited availability of effective products for the control of the mushroom sciarid fly (*Lycoriella ingenua*). For this purpose the efficacy of applying entomopathogenic nematodes was evaluated in this study. Optimal timing for nematode application during the growing cycle was explored to achieve a greater level of efficacy compared to the standard practices.

The obtained results demonstrated that application at last watering or directly after CAC-ing controlled adult sciarids very efficiently. Combining nematode application in both casing soil and compost did not show a better effect. Also, doubling the standard nematode dose did not significantly improve control of adult fly population when compared to the standard dose.

Next, experiments were conducted to evaluate the mortality capacity of nematodes. For this reason casing soil was sampled on a weekly basis and mealworms were used as test organisms. Results showed that after a period of six weeks nematodes applied were still viable and infectious. Even in the absence of sciarid larvae, the mortality rate of mealworms indicated that nematodes continued to be effective.

In order to achieve maximum performance of applied nematodes, it is essential that some basic and fundamental guidelines are respected. Accordingly, some crucial procedures are recommended.

Presenting Author Biography - Nancy Pyck:

Nancy Pyck is the senior researcher of the research unit edible mushrooms at Inagro, a practically orientated research and advisory institute in Belgium. She holds a PhD and has a strong background in mycology. She is active member of EU minor uses Commodity Expert Group Mushrooms, European Mushroom Growers Group (GEPC) and several committees in the mushroom sector. She participates in several (inter)national projects and has been involved in technical dossiers on phytosanitary issues and many privately funded projects and partnerships. To transfer the knowledge to the sector she also provides advisory and training services to growers and other stakeholders and industry clients in the sector to help to solve problems for the mushroom producers.

11. Beneficial microorganisms joining forces to fight green mold in mushrooms

Poster

Dr. Svetlana Milijasevic-Marcic [1], ***Ms. Jelena Lukovic*** [1], ***Dr. Ljiljana Santric*** [1], ***Mr. Nikola Andjelkovic*** [2], ***Dr. Nikola Grujic*** [2], ***Dr. Tanja Drobnjakovic*** [1], ***Dr. Dejan Marcic*** [1], ***Dr. Ivana Potocnik*** [1]

1. *Institute of Pesticides and Environmental Protection,* ***2.*** *Faculty of Agriculture - University of Belgrade*

The mushroom industry in Serbia is growing rapidly, producing fresh mushrooms of different quality for consumption, processing or export. A lack of effective chemicals for disease control, and pathogen resistance to pesticides, consequentially lead to unsuccessful control of the green mold agent, *Trichoderma aggressivum*. The principles of sustainable use of pesticides require a change in the attitude of composters and mushroom growers towards biological disease control. Altering the microbial comunities in compost and casing soil to stimulate the growth of healthy button mushrooms will hopefully increase mushroom yield and green mold control, and provide a practice-based understanding of the microbial community-pathogen relationships. The objective of the study was to implement two indigenous beneficial microorganisms in the cultivation of button mushroom *Agaricus bisporus*: *Bacillus amyloliquefaciens*, strain B-241, a bacterium with fungicidal activity against *T. aggressivum,* and *Streptomyces flavovirens*, strain A06, an actinobacterium which stimulates mushroom yield. Interrelationship between the two beneficial microorganisms was investigated in an experimental mushroom growing room after inoculation with compost green mold, *T. aggressivum* f. *europaeum,* which was added one day after the spawned compost was placed in boxes (10^6 conidia/mL per m^2). The concentration of beneficial bacterial and actinobacterial suspension was adjusted to 10^8 CFU/mL per m^2 and applied at seven-day intervals. The synergy factor (SF) was calculated as a ratio between the observed and expected impact on yield or effectiveness in suppression of disease symptoms. The results indicated strong synergism in promoting *A. bisporus* productivity in both uninoculated (SF=1.56) and inoculated plots (SF=1.62) after combined application of the two beneficial microorganisms. Regarding efficacy in preventing green mold symptoms, the beneficial bacterium and actinobacterium displayed an additive effect (SF=1.05). Further investigatation is expected to reveal adequate simultaneous application procedure of the beneficial organisms in order to maximize biopesticidal/stimulatory effects, and reduce environmental pollution. This research was supported by the the Science Fund of the Republic of Serbia: Green program of cooperation between science and industry #GRANT No 3/4848 (2023-25) Microbial recipe for edible mushroom production - MICRO-MUSH, and the Ministry of Science, Technological Development and Innovations of the Republic of Serbia: project No 451-03-47/2023-01/ 200214.

Presenting Author Biography - Svetlana Milijasevic-Marcic:

Svetlana is a principal research fellow, bacteriologist, at the Laboratory of Applied Phytopathology, Institute of Pesticides and Environmental Protection, Belgrade, Serbia. Svetlana has been conducting and developing research on plant and mushroom pathogenic bacteria. More recently, her research program has focused on beneficial bacteria as potential biocontrol agents as an alternative to chemical fungicides, especially in *Agaricus bisporu*s and other edible mushroom crops. She participated in several scientific and industrial projects aimed at sustainable crop protection as a work package leader. Svetlana also supervised several masters and one PhD student. In addition to numerous scientific papers, she published two technological solutions regarding substrate disinfection and biopesticide application in the production of edible mushrooms.

12. Antifungal activity of peppermint and spearmint essential oils against Trichoderma spp. green mold disease agents of oyster mushroom and shiitake

Poster

Dr. Ivana Potocnik [1], ***Ms. Jelena Lukovic*** [1], ***Dr. Milos Stepanovic*** [1], ***Dr. Biljana Todorovic*** [1], ***Dr. Tijana Djordjevic*** [1], ***Dr. Rada Djurovic-Pejcev*** [1], ***Dr. Svetlana Milijasevic-Marcic*** [1]

1. *Institute of Pesticides and Environmental Protection*

Production of oyster mushroom (*Pleurotus* spp.) and shiitake (*Lentinula edodes*) is seriously affected by various *Trichoderma* species causing green mold disease. The purpose of the study was to determine the antifungal activity of essential oils peppermint (*Mentha piperita*) and spearmint (*Mentha spicata*), both obtained from plants from Serbia, against *Trichoderma* strains isolated from *Pleurotus ostreatus* oyster mushroom (*Trichoderma pleuroti* KM11 and *Trichoderma pleuroticola* KM12, 2018, North Macedonia) and shiitake substrates (*Trichoderma guizhouense* T59, 2018, Serbia). Air-dried plant material was subjected to hydro-distillation in a Clevenger type apparatus. The obtained essential oils were dried over anhydrous sodium sulphate. Antifungal activity of the oils was tested using *in vitro* methods: contact microdilution by using a pathogen spore suspension, and fumigant macrodilution by applying either pathogen mycelial inoculum or spore suspension. Both oils were lethal for all tested species when the contact microdilution method was used at 12.5 µl mL^{-1}, while no fungicidal effect of either oil was found on any tested strain using the fumigant method. In the contact microdilution bio-assay, both oils inhibited *T. guizhouense* T59 at 3.12 µl mL^{-1}; peppermint oil more strongly suppresed *T. pleuroti* KM11 (3.12 µl mL^{-1}) than *T. pleuroticola* KM12 (6.25 µl mL^{-1}), while spearment oil had an opposite effect. Using fumigation, both oils inhibitied mycelial growth of the tested species at 0.16 µl mL^{-1}, with an exception of spearmint oil, which inhibited *T. guizhouense* T59 at 0.32 µl mL^{-1} of air phase. Inhibition of spore germination of *T. pleuroti* KM11 by fumigation was detected at 0.08 (pepermint oil) or 0.016 µl mL^{-1} (spearmint oil), *T. pleuroticola* KM12 at 0.016 µl mL^{-1} (both oils), and *T. ghuizouense* T59 at 0.32 µl mL^{-1} (peppermint oil) or inhibition was missing (spearmint oil). The most resistant taxon to both oils was *T. guizhoense* from shiitake. Both oils showed similar contact effect, while peppermint oil exhibited a slightly stronger fumigant activity than spearmint oil on the tested strains. Peppermint and spearmint oils could be recommended for further *in vivo* investigation.

This research was supported by the the Ministry of Science, Technological Development and Innovations of the Republic of Serbia: project No 451-03-47/2023-01/ 200214.

Presenting Author Biography - Ivana Potocnik:

Ivana Potocnik is the principal research fellow at the Laboratory of Applied Phytopathology, Institute of Pesticides and Environmental Protection, Belgrade, Serbia, dealing with the pathology of edible mushrooms. Her research focuses on the disease agents of edible mushrooms and implementation of biopesticides based on beneficial organisms and biochemicals. She was a leader or member on several industrial and scientific projects aimed at sustainable crop protection. In addition to scientific publications, she supervised two PhDs and many graduate and master stu-dents. She has two technological solutions concerning ecological disinfection of substrates and the application of biopesticides in the production of edible mushrooms.

13. Zygospore formation in Syzygites megalocarpus

Poster

Dr. David Beyer [1], ***Mr. Bruce Withey*** [2]

1. *Pennsylvania State University,* ***2.*** *The Pennsylvania State University*

This study is to determine the effect of light, temperature, spore deliver method, and addition of mineral salt, potassium chloride on zygospore formation.
An isolate of Syzygites was obtained from the mushroom industry, in Chester County, PA, and maintained in the Mushroom Disease Lab at Pennsylvania State University. Sporangiospores formed easily on PDA media and spore collection was aseptically accomplished by flooding the plate with sterile water with Tween 80, ~15 ml. The spores were liberated by gently dislodging them with a sterile cotton swab. The spore water was transferred to a sterile beaker and the plate was rinsed again to release as many spores as possible. The spore suspension was passed through 4 layers of cheesecloth to filter out the mycelium to a sterile beaker. The filtering step was repeated twice, the spore count was accomplished using a hemacytometer and determined to be 3.75×10^4 spores/ml for the first trial, 4.1×10^4 for the second and 5×10^4 for the 3rd. Each trial was completed on different days of the week with the spore suspension prepared fresh daily. The spore suspensions were placed in a sterile centrifuge tube. Transfer of the sporangiospores to the media was accomplished either as a single point inoculation using 20 µl, 50 µl, 100 µl or dual point using 20 µl/20 µl, 50 µl/50 µl ~3 cm from each other. An additional inoculation method was used where one cluster of aerial sporangiospores was placed in the center of the plate. The plates were then incubated at room temperature (~20º) or refrigerator (~2º) as well as light and dark. To simulate the day light both chambers are equipped with a light strip placed on a timer with 10 hours of light. The plates that were kept in complete darkness (24 hours) were wrapped in aluminum foil. All treatments had three replicates. Zygospore formation was recorded. Liberation of the zygospores from the mycelium has been elusive. The typical way of freeing spores from culture trough gentle agitation doesn't work. The zygospores are firmly attached on two sides as well as being caught in the 3-D matrix of mycelium. Other methods of dislodging the zygospore, using different external forces such as centrifugation, sonication, and homogenizing. Various ages of zygospore and other environmental stresses. A young zygospore culture is translucent turning black as it ages.

Presenting Author Biography - Bruce Withey:
I grew up in Lycoming County where I went to Lycoming College and earned a B.A. in Biology and a minor in Environmental Science. Currently, I am a Plant Pathology and Environmental Microbiology Research Technologist at Pennsylvania State University working at Buckhout Laboratory and the Mushroom Research Center. I have worked with live crops of *Agaricus bisporus* and cultures of its pathogens *Trichoderma*, *Lecanicillium* (*Verticillium*), *LIV*, and *Syzygites*.

14. Differential gene expression in Mycogone perniciosa and Lecanicillium fungicola infecting two genetically closed Agaricus bisporus hosts

Poster

Ms. María Luisa Tello Martín [1], ***Ms. Sioly Becerra Zambrano*** [2], ***Dr. Margarita Perez Clavijo*** [1], ***Prof. Michael Thon*** [2], ***Prof. Ernesto Perez Benito*** [2], ***Ms. Virginia Casado del Castillo*** [3]

1. *CTICH,* ***2.*** *USAL,* ***3.*** *Universidad de Salamanca (USAL)*

The fungal pathogens *M.perniciosa* and *L.fungicola* cause wet bubble and dry bubble disease respectively, being a major threat to *A. bisporus* production worldwide. In order to gain information about the processes involved, and about the genetic factors participating, in the recognition between the organisms participating in the establishment of each interaction, a RNAseq analysis was devised. To this end, the experimental conditions to generate biological samples from the fungus/fungus interactions were optimized. Then RNA was extracted from two genetically closed hosts: white button mushroom (*A.bisporus)* and portobello *(A. bisporus var. brunnescens).* Hosts tissues infected either by *M.perniciosa* or by *L.fungicola* were collected and used for RNAseq analysis. RNA samples derived from mycelium from *M.perniciosa* and *L.fungicola* cultured *in vitro* were included in the analysis. In all cases, three biological replicates were handled. In order to identify *M.perniciosa* and *L.fungicola* genes being specifically expressed during the interaction with both hosts, first the reads generated from the "interaction samples" were mapped to each individual genome and selected. Second, a differential gene expression analysis was carried comparing the expression levels of *M.perniciosa* and *L.fungicola* genes using the reads selected from the interaction samples and the reads derived from the mycelium cultured *in vitro*. Differentially expressed genes (DEGs) analysis between: 1) mycelium from *M.perniciosa* and *A.bisporus* parasitized by *M.perniciosa* (ABMP), 2) mycelium from *L. fungicola* and *A.bisporus* (ABLF), 3) mycelium from *M.perniciosa* and *A. bisporus var. brunnescens* parasitized by *M.perniciosa* (ABPMP) and 4) mycelium from *L.fungicola* and *A.bisporus var. brunnescens* (ABPLF), were performed, obtaining differences in the principal component analysis (PCA) completed with DESeq2 (RStudio). 198 upregulated genes were found to be common in the DEGs analysis of ABMP and ABPMP, while 116 upregulated genes in case of ABLF and ABPLF. In order to obtain information about the nature and functions of the DEGs results, KOALA (KEGG Orthology And Links Annotation) was used. By analyzing the KOALA results of the two groups of upregulated genes, we found 3 KEGG terms present in both lists: K11733 (lysine-specific permease), K08176 (MFS transporter) and K00666 (fatty acyl CoA synthase). Efforts are being done to extend the characterization of these individual genes. Their analysis will provide information about the genetic basis of the interactions between *A. bisporus* and its two main parasitic fungi, so far very poorly characterized. This work was supported by Consejería de Agricultura, Ganadería y Medio Ambiente de La Rioja, under ESTOICO Project nºCT23_03.

Presenting Author Biography - María Luisa Tello Martín:

Ms. Marisa Tello´s experience as a scientist started in the Department of Microbiology and Genetics (University of Salamanca) where she joined the Genetics Group, completing her degree thesis. Thanks to this involvement, she gained extensive knowledge about fungal molecular identification and pathogenicity characterization. She completed her Master thesis joining the Department of Plant Production and Forest Resources in the University of Valladolid. Since 2010 she works at the Mushroom Technological Research Centre of La Rioja (CTICH) in international, national and regional research projects. Thanks to the different projects in which she has been involved, she has expanded her knowledge about mushroom cultivation, mushroom pest and diseases control, mushroom biotechnology, nutritional and medicinal mushroom properties, taxonomy, fungal conservation, and genetics. In

2021, she finished a Master's degree in Advanced Bioinformatics Analysis (University Pablo Olavide). Currently, she is also doing a Ph.D. in Agrobiotechnology (University of Salamanca).

15. Cascade strategies for the valorisation of waste streams from common carp pond farming into mushrooms and mealworm

Poster

Ms. María Luisa Tello Martín [1], ***Ms. Susana Mangado Mangado*** [1], ***Dr. Margarita Perez Clavijo*** [1], ***Dr. Remigiusz Panicz*** [2], ***Mr. Piotr Eljasik*** [2], ***Mr. Slawomir Lisiecki*** [2], ***Mr. Malgorzata Sobczak*** [2], ***Mr. Mateusz Gzyl*** [3], ***Mr. Jose Angel Villoria*** [4], ***Ms. Andrea María Perez*** [4]

1. *CTICH,* ***2.*** *ZUT,* ***3.*** *ICR,* ***4.*** *TEBRIO*

The growing demand of citizens requires an increase in food production by 70%, to supply 2.3 billion more people in 2050[1]. Meeting this demand becomes increasingly complicated in the current climate change scenario. To achieve these objectives, reuse of wastes becomes essential. This study has been carried out as part of the Horizon Europe SAFE project. The aim of the study is to transform waste streams from common carp (*Cyprinus carpio*) pond farming into substrates for mushroom (*Pleurotus ostreatus*) cultivation and reuse the spent mushroom substrate as a substrate ingredient for mealworm (*Tenebrio molitor*) production. To sequestrate solids suspended in the water discharged from drained ponds a multilayer barrier made of straw bricks (rye) was installed in the channel below the common carp farm. After 2 weeks of filtering out suspended solids, straw bricks were removed from the discharge channel and used to formulate substrates in 4 different ratios (25%, 50%, 75% and 100%) and the control (0%). Ten replicate bags were filled of each substrate and sterilised. Bags were inoculated with *P. ostreatus* spawn, incubated, and placed in a cultivation room. From each bag, mushrooms were collected, weighed separately and the biological efficiency (BE) was calculated. After harvesting, substrates were dried and used as ingredients for mealworm production substrate. Mealworm production was carried out by breeding larvae of a known age in laboratory scale boxes filled with the substrates in a defined mass ratio for 4 weeks. Four experimental substrates and 1 control were evaluated. Once a week the excrement, the larvae biomass and the remaining substrate were weighed. The best yield results were obtained in substrate 25% (33.9% BE), followed closely by 50% (29.8%), 75% (29.2%) and 0% (29.1%). Lower yield results were obtained with ratio 100% (15.3%). The use of new substrates containing mushroom wastes showed similar biomass generation rate than the conventional substrate in mealworms. Interestingly, the feed-conversion-ratios (FCR) were lower[RP1] with the new substrates suggesting a better yield for insect biomass production. As the freshwater ponds can accumulate from 0.76 to 3.2 t of sediments per hectare of ponds along the production season, the valorisation of suspended solids in mushroom and worm production is a sound solution to curb release of sediments into river systems and at the same time increase profitability of the freshwater farms[2].

This work was supported by the funding from the European Union's Horizon Europe programme under grant agreement no. 101084549.

Presenting Author Biography - María Luisa Tello Martín:
Ms. Marisa Tello´s experience as a scientist started in the Department of Microbiology and Genetics (University of Salamanca) where she joined the Genetics Group, completing her degree thesis. Thanks to this involvement, she gained extensive knowledge about fungal molecular identification and pathogenicity characterization. She completed her Master thesis joining the Department of Plant Production and Forest Resources in the University of Valladolid. Since 2010 she works at the Mushroom Technological Research Centre of La Rioja (CTICH) in interna-

tional, national and regional research projects. Thanks to the different projects in which she has been involved, she has expanded her knowledge about mushroom cultivation, mushroom pest and diseases control, mushroom biotechnology, nutritional and medicinal mushroom properties, taxonomy, fungal conservation, and genetics. In 2021, she finished a Master's degree in Advanced Bioinformatics Analysis (University Pablo Olavide). Currently, she is also doing a Ph.D. in Agrobiotechnology (University of Salamanca).

16. Sustainable peat alternatives for casing soil in mushroom (Agaricus bisporus) production

Poster

Ms. María Luisa Tello Martín [1]***, Dr. Nancy Pyck*** [2]***, Dr. Jan van der Wolf*** [3]***, Dr. María Sonia Rodriguez*** [4]***, Mr. Fernando López Lasaosa*** [5]***, Dr. Roxanne van Rooijen*** [6]***, Mr. Jeoffrey van den Berg*** [7]***, Prof. Gail Preston*** [8]

1. *CTICH,* ***2.*** *Inagro vzw,* ***3.*** *STICHTING WAGENINGEN RESEARCH (WR),* ***4.*** *IRNASA-CSIC,* ***5.*** *FERTINAGRO,* ***6.*** *KBVB,* ***7.*** *NEWFOSS,* ***8.*** *UOXF*

The mushroom industry plays a key role in the EU's agri-food sector. Nutritionally, it provides a protein-rich alternative to animal products, and it is a key source of vitamin D and selenium. Economically, it was valued at €33.7 billion in 2017 and projected to reach €66.8 billion in 2026 (CAGR of +7.9%). This sector is highly dependent to peat for the casing soil. Peat exists of partially decayed vegetation of organic matter with peat moss as one of the most common components. Due to its extremely low regrowth, it is a depletable resource and as such as "fossil fuel". BIOSCHAMP is an international initiative that counts with the participation of 12 partners from 6 different countries: CTICH (Project coordinator, ES), Inagro vzw (BE), Stichting Wageningen Research (NL), CSIC (ES), Fertinagro Biotech (ES), Ekofungi (RS), Innovarum (ES), EUROCHAMP (ES), Kekkilä-BVB (NL), NF Fibre B.V. (NL), Uprawa Grzybów Łukasz Kiwała (PL) and University of Oxford (UK). In total, the project gathers 5 Research Technological Centres, 3 large companies and 4 SMEs. The BIOSCHAMP project was born with the objective of obtaining an alternative and sustainable peat-free casing for the mushroom industry, contributing to improve the productivity, the sustainability, and the profitability of the European mushroom sector.

In this study, two alternative casing soils were tested in which at least the 50% peat was replaced. Three trials were carried out in semi-commercial growing rooms at CTICH and INAGRO, using black peat as control. At INAGRO, trials were performed using 1.5 m² plots in controlled-climate growing rooms according to standard protocols. Filling weight of ca. 90 kg/m² phase III compost. Seven weeks crop cycle was maintained to harvest two flushes. At CTICH, trials were performed using 3 m^2 plots in Dutch-design climate-controlled rooms, filled with spawned compost (phase II), that was germinated in situ and cased when the compost was fully colonised by the mycelium. Filling weight of ca. 100 kg/m² phase II compost. Seven weeks crop cycle was maintained to harvest two flushes.

In both facilities, 4 cm of casing layer was applied and cac-ing material was added in the casing. The results obtained show that there were no significant differences in the tested casing soils, which supports the possibility of using them as an alternative to the classic peat-based casing soil currently used in mushroom cultivation.

BIOSCHAMP is funded by EU Commission H2020 Programme, topic SFS-04-2019-2020 - Grant Agreement no.: 101000651

Presenting Author Biography - María Luisa Tello Martín:

Ms. Marisa Tello´s experience as a scientist started in the Department of Microbiology and Genetics (University of Salamanca) where she joined the Genetics Group, completing her degree thesis. Thanks to this involvement, she gained extensive knowledge about fungal molecular identification and pathogenicity characterization. She completed her Master thesis joining the Department of Plant Production and Forest Resources in the University of Valladolid. Since 2010 she works at the Mushroom Technological Research Centre of La Rioja (CTICH) in international, national and regional research projects. Thanks to the different projects in which she has been involved, she has expanded her knowledge about mushroom cultivation, mushroom pest and diseases control, mushroom biotechnology, nutritional and medicinal mushroom properties, taxonomy, fungal conservation, and genetics. In 2021, she finished a Master's degree in Advanced Bioinformatics Analysis (University Pablo Olavide). Currently, she

is also doing a Ph.D. in Agrobiotechnology (University of Salamanca).

17. Cultivation of Pleurotus ostreatus and Pleurotus cornucopiae in a vertical farming system

Poster

Mrs. Marina De Bonis [1], ***Prof. Carlo Nicoletto*** [1], ***Prof. Giampaolo Zanin*** [1], ***Prof. Paolo Sambo*** [1]

1. *University of Padova*

Vertical cultivation systems, mainly used for vegetable species, can increase productivity, and reduce soil consumption. About cultivated mushrooms, this technology is mainly applied for *Agaricus bisporus*, but fewer studies exist for the cultivation of *Pleurotus spp*. In this study (funded by PRIN 2020ELWM82 V-FARM), the cultivation of *P. ostreatus* (3253 – Sylvan) and *P. cornucopiae* (3040 – Sylvan) in a vertical farming system using single shelves (SS) and twin shelves (TS) rows layout was considered (December 2022 - March 2023). Shelves were placed in a mushroom cultivation facility and each shelf had 4 cultivation racks: G (ground), 1R (first rack), 2R (second rack) and 3R (third rack). This system quadrupled the typical cultivation density using 12 bags/m^2. For both *Pleurotus* species 25 kg bags were used and filled with a straw-based commercial substrate. Overall, the experiment considered 108 bags for each species. The pinhead formation was monitored and during harvest time mushrooms were collected measuring the yield per bag and the number of clusters. Three clusters per bag were used to evaluate the number, diameter, width, and colorimetric parameters of fruiting bodies. Pinhead's appearance in the first flush showed a faster growth in 3R for both the species and for *P. ostreatus* the SS disposition showed the highest number of pinheads. In the second flush of *P. ostreatus* G and 1R showed the highest production of pinheads (78% and 70%). The yield of the first flush for *P. ostreatus* was higher in G (0.19 kg/kg substrate) and decreased in higher racks (0.15 kg/kg substrate). For *P. cornucopiae*, the yield was higher in 3R (0.04 kg/kg substrate) and lower in G (0.01 kg/kg substrate). During the first flush *P. cornucopiae* had a bigger diameter in G, whereas *P. ostreatus* showed the highest one in 3R. *P. cornucopiae* and *P. ostreatus* can be cultivated in a vertical farming but cultivation height can affect yields and other productive traits. The shelf arrangement (SS or TS) did not influence the production; however, the economic and labor cost consequences need to be evaluated. The thermal gradient detected within the mushroom cultivation facility between G and 3R (±1.5 °C), if not mitigated through air mixing, could be exploited for cultivating both *Pleurotus* species within the same environment, providing a potential combined production. Moreover, arranging differently the bags of the two species on the same shelf could be useful to optimize their yield.

Presenting Author Biography - Marina De Bonis:
I'm a Ph.D student from the University of Padova. I'm working in the horticulture research group in the Department of Agronomy, Animal, Food, Natural resources and Environment. My research project includes enhancing edible mushrooms cultivation (both quality and yield) specifically for oyster mushrooms and how to reuse spent mushroom substrate as fertilization for horticulture crop systems.

18. A description of the effects caused by temporary atmospheric alteration on Agaricus bisporus yield and development

Poster

Mr. Nicholas Gabel [1], ***Dr. John Pecchia*** [2], ***Dr. Fabricio Vieira*** [2], ***Dr. David Beyer*** [1]

1. *Pennsylvania State University,* ***2.*** *The Pennsylvania State University*

White Button Mushroom (*Agaricus bisporus*) cultivation involves covering a fully colonized substrate with a soil-like media termed the 'casing layer'. This layer is a complex environment with a variety of unique physical, chemical, and microbial properties. The casing environment plays an important role in the transition from mycelial growth to sporocarp development. Alternative technology and growing techniques are gaining interest from *A. bisporus* to relieve industrial constraints such as reduced labor, rate of sporocarp production and harvesting rate. Automated harvesting, infrastructure changes, and growing environment management are examples of techniques and technologies being implemented and developed worldwide. "Temporary Atmospheric Alteration" (TAA) was a growing method developed through this research to increase the carbon dioxide concentration in the casing layer head space. The purpose of this study was to describe the effects on sporocarp development caused by increased CO_2 concentrations. This technique consisted of a semi-closed systems to generate a range of carbon dioxide concentrations. The crop design consisted of nine fully spawned Akro-Tubs; 19 x 24-1/2 x 9-1/2 cased with *Sphagnum* peat moss. The experimental design consisted of two different TAA treatments and a control treatment each with three replicates. The CO_2 utilized for this research was naturally produced via aerobic respiration from the developing mycelia of *A. bisporus*. The top portion of the system was comprised of a Akro-tube with a 38 x 24 cm hole cut in the top covered by perforated Modified Atmosphere Packaging (MAP) film to allow limited gas exchange. The semi-closed system was initiated by sealing the top portion to the bottom cased tub with industrial adhesive. Atmospheric data in each treatment was captured using a IOT Monitor. Parameters of the semi-closed system that were examined included timing of TAA initiation and duration of TAA. Data on how the TAA impacted the crop was collected through visual observations, yield results, and environmental monitoring. The yield data of each treatment was processed using ANOVA and the impact of CO_2 on yield was evaluated using a correlation analysis.

Preliminary data indicates the ability to potentially induce a difference in *A. bisporus* development through increasing CO_2 concentration. The most prominent developmental results were observed by initiating a TAA on the 13^{th} day after casing for a timeframe of 36-48 hours. A better understanding of how TAA can improve sporocarp production, may help growers increase their harvest speeds as well as lend itself better to future automatic harvesting technologies.

Presenting Author Biography - Nicholas Gabel:

My name is Nicholas Gabel, I am a M.S. student in the Department of Plant Pathology and Environmental Microbiology at The Pennsylvania State University. The research that I am conducting is focused on certain areas of White Button Mushroom cultivation. These areas include alternative casing materials and cropping management to address potential limitations in the cultivation process.

19. A soft robotic gripper for harvesting delicate produce

Poster

Dr. Helen Grogan [1]***, Dr. Andrea Uccello*** [1]***, Mr. Brian McGuinness*** [1]***, Mr. Donal Gernon*** [1]

1. *Teagasc*

The mushroom sector worldwide is interested in a fully robotic harvester for mushroom crops, particularly in view of the high labour cost associated with hand-harvested mushrooms. In recent years the sector in Europe has been challenged with an extreme shortage of skilled labour for this critical role on the farm. Several interesting technologies have been developed that improve harvesting efficiency, which include semi-automated growing systems that facilitate two-handed picking and conveyor systems that transport, trim and pack harvested mushrooms. Recently, fully integrated robotic harvesting systems have been developed by two companies in Canada. Both are at advanced stages of development and are being trialled at farm scale. They can identify mushrooms to be picked, then harvest, trim and place them in punnets for dispatch. They use either a three-fingered (hard) gripper or a suction cup to harvest the mushrooms. SoftGrip is an EU-funded research project (www.softgrip-project.eu) that is currently nearing completion. Soft robotics uses technologies that focus on imitating living organisms in terms of flexibility and dexterity, such as the human hand. The SoftGrip project has three main objectives: (1) to develop an affordable 'soft' flexible gripper and vision system for the automated harvesting of mushrooms and other delicate produce; (2) to use imitation learning-based control to mimic hand-harvesting and ensure maximum product quality and (3) to use advanced self-healing and recyclable materials to reduce waste. The overall goal is to equip the soft robotic gripper with algorithmic intelligence that will coordinate and control the physical movements of the gripper. The project is also looking into novel functionalised structures and materials that are soft, self-repairable, recyclable, affordable and food safe. At the same time, they should be capable of actuating movement in a controlled manner, extracting insights from sensor data and controlling how the pressure distributes when coming in contact with a mushroom. This way, the soft robotic gripper will be programmed to adjust its grasp to the size of different mushroom caps and to know the precise twisting and pulling moves that can successfully outroot them An advanced prototype has now been developed and will be evaluated at a workshop planned for March 2024.
The consortium includes experts from the BioRobotics Institute, Scuola Superiore Sant'Anna (SSSA, Italy), the Institute of Communications and Computer Systems,National Technical University of Athens (ICCS-NTUA, Greece), the University of Essex (UK), TWI Hellas (Greece), Mitsui Chemicals (Germany), and - the Horticulture Development Department, Teagasc, (Ireland).

Presenting Author Biography - Helen Grogan:
Helen has worked in mushroom research for over 30 years, predominantly in the area of mushroom pathology and disease control. As well as her ongoing work in mushroom pathology, she is currently involved in projects to identify alternatives to peat for use in mushroom casing and evaluating novel robotic technologies for use in harvesting mushrooms. She has had responsibility for a broad portfolio of research projects with national and international funding agencies, working closely with the global mushroom industry to solve issues through targeted research. Helen has published numerous research papers and technical articles, supervised many PhD students and early stage researchers, and presented her work at international science and mushroom conferences around the world. She continues to have a passionate interest in the advances being made in mushroom science that enable the sector remain relevant and sustainable into the future.

20. Bacteriophages – unknown viruses of mushroom compost

Poster

Prof. Michael Kertesz [1], ***Ms. Rebecca Martin*** [1]

1. *The University of Sydney*

Button mushrooms (*Agaricus bisporus*) are a popular and highly nutritious staple of diets across the globe. Commercial cultivation of the button mushroom begins with a controlled, thermophilic composting process in which bacterial and fungal communities transform wheat straw and poultry manure into mushroom growth substrate. The bacterial and fungal communities present in the compost change successively as they break down the start-ing materials, before finally reaching a stable, climax community at the end of phase II. One proposed reason for this succession of bacteria is the presence of bacteriophage in the compost. Bacteriophages are ubiquitous viruses that infect bacteria and are pivotal to ecosystem functioning as they facilitate nutrient cycling through their control of bacterial populations. We have tested the hypothesis that bacteriophage populations contribute to mushroom compost in three ways: isolation of bacteriophages from compost that are specific to either individual bacterial taxa from compost or well characterised laboratory host bacteria; and direct observation of compost extracts us-ing transmission electron microscopy (TEM). Isolated bacteriophages were whole genome sequenced and observed using TEM. Two novel, thermostable bacteriophages were successfully isolated from compost samples, one infecting the compost isolate *Pseudoxanthomonas suwonensis* and the other infecting the laboratory strain *Pseudomonas aeruginosa* PAO1, the latter belonging to the tailed bacteriophage order Siphoviridae. TEM of compost extracts revealed that samples were largely dominated by Siphoviridae, with flexible tails and short tail fibres, and a variety of different head shapes, including icosahedral and elongated. These findings contribute to a larger understand-ing of the mushroom compost microbial community. As bacteriophages might regulate the bacterial community in compost, these results provide a starting point for determining the role of bacteriophages in mushroom composting.

Presenting Author Biography - Michael Kertesz:
Michael Kertesz is an Associate Professor for Soil Microbiology in the School of Life and Environmental Sciences at the University of Sydney. He completed his PhD at the University of Cambridge, and then spent twenty years as a research microbiologist, first at the Swiss Federal Technical University (ETH) and then at the University of Manchester. Michael moved to the University of Sydney in 2009, and his recent research focuses on how bacteria and fungi react to changes in their surroundings, especially the bacterial-fungal-compost interactions that are important in mushroom cultivation. His work has revealed the presence of thousands of unknown bacterial taxa in mushroom compost, explored methods for early detection of mushroom pathogens and investigated the transformations of nitrogen in mushroom compost, while ongoing research is developing a database of compost bio□markers that can be used to maximise mushroom production yield.

21. Correlation between yield and enzyme production in Pholiota nameko on minimally supplemented wood-based substrates

Poster

Ms. Swathi Kothattil [1]***, Dr. John Pecchia*** [1]

1. *The Pennsylvania State University*

This study attempts to understand the correlation between yield and enzyme production in *Pholiota nameko* in wood-based substrates. Four different formulations were inoculated to determine how the substrate affects yield and enzyme production: 1) willow chips, 2) willow chips with cottonseed hulls (3:1),3) willow chips supplemented with millet, rye and wheat bran and 4) Oak sawdust supplemented with millet, rye and wheat bran. *P. nameko* isolate was utilized from the Penn State culture collection (WC 890) and was spawned on sterilized substrate. The sterilization temperature was 121for 30 minutes. Each bag was filled with approximately 2.5 kg (wet weight) of substrate with 3 replicates per treatment. The spawned bags were grown in a climate controlled growing room at the Pennsylvania State University Mushroom Research Center (MRC). During spawn run, air temperature was maintained at 15and the humidity was maintained at 90%. Four samples per bag per treatment were taken on day 30 and prepared for proteomic analysis by mass spectrometry. Samples were collected 1-3 days prior to pinning. The changes in protein expression level on different lignocellulosic substrates were analyzed and compared to the yield. Statistical analysis can reveal the correlation between samples. This information can help in breeding or upregulating genes responsible for enzymatic breakdown of complex carbon molecules.

Presenting Author Biography - Swathi Kothattil:
I am Swathi Kothattil, third year PhD student in the Department of Plant Pathology and Environmental Microbiology, Penn State.

22. Correlation between yield and enzyme production in Pleurotus ostreatus on minimally supplemented wood-based substrates

Poster

Ms. Swathi Kothattil [1]***, Dr. John Pecchia*** [1]

1. *The Pennsylvania State University*

This study attempts to understand the correlation between yield and enzyme production in *Pleurotus ostreatus* in wood-based substrates. Experiments were conducted to assess how the substrate (willow chips, willow chips with cotton seed hull (3:1) or a standard industry wheat straw/cottonseed hull formula) effects yield and enzyme production . A cold weather strain of *P. ostreatus* obtained from North Spore,[55-75°F fruiting temperature] Maine USA, was spawned on pasteurized substrate and grown in climate-controlled growing rooms at the Pennsylvania State University Mushroom Research Center (MRC). The substrate was sterilized at 1for 20 minutes prior to spawning. Each bag was filled with approximately 4.5 kg of wet substrate with 10 replicates per treatment. During spawn run temperatures were maintained at 15 and humidity maintained at 90 percent. Four samples were collected per bag per treatment on day 25 (1-3 days prior to pin formation). Mass[PJA2] spectrometry analysis was used to obtain protein expression profiles. The changes in protein expression level on different lignocellulosic substrates were analyzed and compared with the yield. This information can help in breeding or upregulating genes responsible for enzymatic breakdown of complex carbon molecules.

Presenting Author Biography - Swathi Kothattil:
I am Swathi Kothattil, third year PhD student in the Department of Plant Pathology and Environmental Microbiology, Penn State.

23. An experimental approach to microbiologically manipulate Agaricus bisporus developmental patterns

Poster

Dr. Fabrício Vieira [1], ***Ms. Isako Di Tomassi*** [2], ***Prof. Kevin Hockett*** [1], ***Dr. Eoin O'Connor*** [1], ***Dr. John Pecchia*** [1], ***Prof. Carolee Bull*** [1]

1. *The Pennsylvania State University,* ***2.*** *Pennsylvania State University*

The cultivation of *Agaricus bisporus* relies on a multifaceted interplay of diverse factors, spanning both biotic and abiotic factors. The foundational framework of the cultivation system hinges upon the establishment of an environment rich in organic matter, intricately woven together with a diverse and specialized microbial community. It has been speculated that these microorganisms play pivotal roles within the current cultivation system, yielding outcomes that range from beneficial (e.g., symbiosis) to potentially detrimental interactions (e.g., predation and competition). Nevertheless, while the significance of these communities is undeniable in the current *A. bisporus* cultivation system, their precise impact on mushroom development remains partially unknown. We conceptualized a subtractive approach, which consists of the application of biocides (antibiotics) to compost and casing to provoke shifts in the microbiomes and ultimately, changes in mushroom developmental patterns. Therefore, microorganisms that may associated with mushroom development could be identified by high-throughput sequencing. The antibiotics were chosen based on previous reports targeting their effect on microbial communities in close-related biological niches (soil environment) and for their lack of impact on mycelial growth in compost. A total of 10 antibiotics were tested in crop trials (kanamycin, rifampicin, vancomycin, monensin, carbenicillin, novobiocin, clindamycin, erythromycin, sulfamethazine, doxycycline). In terms of mushroom yield, similar trends were observed when antibiotics were applied to compost or casing, with lower mushroom yield for treatments (antibiotics) that used DMSO (dimethyl sulfoxide) as a solvent (instead of water). The assessment of alpha diversity richness revealed that several treatments exhibited lower diversity indices compared to the standard compost or standard casing. Regarding beta diversity, the global permanova analysis showed significant differences ($p < 0.05$), indicating variations in microbial community composition in compost. However, pairwise adonis comparisons did not yield significant differences ($p > 0.05$). In the casing microenvironment, similar trends in terms of beta diversity index were observed, i.e., significative differences for permanova analysis (global) and not significant for pairwise adonis comparisons. Interestingly, some treatments in casing have their bacterial communities clustered further away from standard casing (PCoA plots) and are correlated with the lowest mushroom yield. Taken together, these preliminary data indicate that mushroom development is affected by certain taxa in both microenvironments (compost and casing). Developing a better understanding of the mechanisms underlying the microbial community's effect on mushroom development will allow us to design crop management tactics within the parameters of the cultivation systems to modulate mushroom growth more precisely in tamed environments.

Presenting Author Biography - Fabrício Vieira:
Fabricio Vieira is a Postdoctoral Scholar at Pennsylvania State University and has been working with microbial ecology of substrates for Agaricus bisporus and Pleurotus ostreatus mushrooms with a focus on manipulating the mushroom microbiome towards disease mitigation and nutrient intake.

24. Enabling 'Smart' mushroom agriculture

Poster

Dr. Ryan Delane [1], ***Dr. Steven Haynes*** [1]

1. *The Pennsylvania State University*

For over six years our team has worked with the Penn State Mushroom Research Center (MRC) on the development of an integrated mushroom agriculture data management suite called *Cropsmarts* (currently in beta test). Based on observations made at several Pennsylvania commercial mushroom farms, we found that much of the crop data collection process is currently performed manually using door charts and other paper-based methods. This means analysis of especially larger data sets is laborious or even impossible.

Our project has involved requirements analysis and usability studies along with software design, development, testing, and validation. Comprised of a mobile application for Android and iOS, and a web application for desktop computers, our application suite is designed to enable efficient data capture and effective data analysis for mushroom agriculture. The *Cropsmarts* suite will allow mushroom growers to perform data analysis over extended timeframes including comparison across mushroom crops. Data analysis features also support summarization of crop data including crop inputs, crop measures (e.g. air and compost temperature, CO2, humidity, etc), and crop outputs. The tool also provides standard crop metrics such as wet-weight efficiency and calculation of cost and revenue information, as well as time-series graphs and other visualizations. These features enable mushroom growers to gain valuable insights about their crops and how changes in treatment affect their yield.

Most recently, we have integrated wireless, automated environmental data capture using commercial-off-the-shelf (COTS) sensor arrays and wireless internet gateways. The architecture allows the onboarding of a wide array of sensors capable of automatically recording a high volume of environmental data directly to the *Cropsmarts* application. We have also developed support for capturing and storing images of mushroom crops to enable future features. In the poster session, we will provide attendees with live demos of the application, and we hope to learn more about the information management needs of the mushroom agriculture industry.

Presenting Author Biography - Steven Haynes:

Haynes is Teaching Professor in the Penn State College of IST. He helped design the Human-Centered Design and Development major and his teaching focuses in this area. He works extensively with both undergraduate and graduate students on the design and development of useful and usable software applications. Haynes' research interests include the design and evaluation of information technologies; design as a research method; design rationale; scenario-based methods; and human-centered design. His research has been supported by the National Science Foundation, the United States Department of Agriculture, the Office of Naval Research, among others. Prior to academia, Haynes worked at a number of technology companies in the United States and Europe including Apple Computer, Adobe Systems, and several software startup companies. In industry he worked as an application software developer, system architect, software development project manager, and application development group manager.

25. Improve awareness of mushroom cultivation and promote human health

Poster

Dr. Mo-Mei Chen [1]***, Dr. Mark Wach*** [2]

1. *University of California, Berkeley,* ***2.*** *Sylvan*

Encourage Cultivation edible & Medicinal Mushroom and full use local best gene pool species to promote the cultivation of edible and medicinal mushrooms.
Most of the mushroom species that can be cultivated are saprophytes, and the mycorrhizae that can be used as mycorrhizae are mostly symbiotic fungi that cannot be cultivated.
Study on Mycological Taxonomic to using the local best gene pool species, is a commendable initiative. Such an endeavor provides myriad benefits, ranging from supporting local biodiversity to health advantages and economic growth. Here's a compelling argument on this:

1. Sustainable Forests & Agriculture: Mushroom cultivation is sustainable. It requires minimal land, water, and energy compared to other crops. In fact, many species can be grown on agricultural waste products like straw, wood chips, or spent coffee grounds, turning waste into food.
2. Biodiversity Conservation: Using local best gene pool species ensures that native and indigenous mushroom species are preserved and propagated. It helps in maintaining the genetic diversity of the local ecosystem.
3. Nutritional Benefits: Edible mushrooms are a rich source of proteins, fibers, vitamins, and essential minerals. They are low in calories and fats, making them a great addition to any diet.
4. Medicinal Benefits: Many mushrooms have been used in traditional medicine for centuries. Modern research supports that species like Reishi (Ganoderma lucidum), Shiitake (Lentinula edodes), and Lion's Mane (Hydum erinaceus) Morel (Morchella spp.) health promoting properties, including boosting the immune system, combating cancer, and supporting neuro health.

Presenting Author Biography - Mo-Mei Chen:

Dr. Chen graduated from the Department of Plant Protection, Beijing Agricultural University. She was Professor and Director of the Forest Pathology Laboratory, Chinese Academy of Forestry, Beijing, China. In 1982, she was invited by University of Wisconsin to work in their Dutch Elm Diseases program. From 1984 until present she has taught and performed research at the Department of Plant Pathology and University Herbarium of the University of California at Berkeley. She has conducted research on West Gall Rust and White Pine Blister Rust and the international quarantine project with the National Science Foundation. Her research interests cover the biogeography of China, North America, and Siberia.

26. Investigation of robotic solutions for button mushroom harvesting

Poster

Dr. Long He [1], ***Dr. John Pecchia*** [1], ***Dr. Daeun Choi*** [2]

1. *The Pennsylvania State University,* ***2.*** *University of Florida*

Pennsylvania accounts for over 60% mushroom production in the United States. However, the mushroom industry has been facing challenges due to increased production costs and continuing labor shortages. Automatic harvesting is an essential solution for the industry. An effective robotic harvesting system needs to equip with a high accuracy computer vision system for mushroom detection and maturity measurement, and a robust mechanism for picking mushrooms from growing beds with minimal damage. In this study, a 3D machine vision system was developed to detect and measure the maturity of mushrooms, which are critical information for robotic harvesting. Prior to design a picking mechanism, a series of dynamic tests were conducted to measure the picking force and picking mo-tion for effectively detaching mushrooms. The test results suggested the combination of bending and twisting is the most effective way to pick mushrooms. Then a vacuum cup type picking end-effector was developed, and a friction coefficient measurement test was conducted to determine the optimal vacuum pressure. At last, the end-effector was tested for mushroom harvesting, and test results showed that the developed mechanism could successfully harvest over 90% of targeted mushrooms. The integration of the machine vision system and harvesting mechanism is in the next step.

Presenting Author Biography - John Pecchia:
John Pecchia is an Associate Research Professor and Director of Graduate Studies in the Plant Pathology & Environmental Microbiology Department at Penn State University. He also serves as the Director of the Mushroom Research Center and Spawn Lab at Penn State and has worked closely with North American mushroom growers for the past 18 years conducting research on mushroom cultivation and disease management issues.

27. Design and sustainable fabrication of mycelium-based building parts and structures

Poster

Dr. Benay Gursoy [1], ***Dr. John Pecchia*** [2]

1. *Pennsylvania State University,* ***2.*** *The Pennsylvania State University*

This poster will present our on-going efforts to design and sustainably fabricate mycelium-based building parts and structures. Among these are an experimental architectural structure designed with 64 unique load-bearing mycelium-based components, mycelium-based acoustic absorbers grown on waste cardboard, and robotic additive manufacturing of mycelium-based panels. The poster will present the cultivation methods, the material tests performed (mechanical tests, acoustic analysis, etc.), and the formal explorations, and discuss the challenges we faced in the projects and our plans moving forward.

Presenting Author Biography - John Pecchia:
John Pecchia is an Associate Research Professor and Director of Graduate Studies in the Plant Pathology & Environmental Microbiology Department at Penn State University. He also serves as the Director of the Mushroom Research Center and Spawn Lab at Penn State and has worked closely with North American mushroom growers for the past 18 years conducting research on mushroom cultivation and disease management issues.

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