



INTERNATIONAL SYMPOSIUM

**MICROBE-ASSISTED CROP PRODUCTION –
OPPORTUNITIES, CHALLENGES & NEEDS**

DEC. 2nd – 5th, 2019

*SCHLOSS SCHÖNBRUNN - ORANGERIE, APOTHEKERTRAKT
VIENNA, AUSTRIA*

ABSTRACT BOOK 2019





MICROBE-ASSISTED CROP PRODUCTION 2019

OPPORTUNITIES, CHALLENGES & NEEDS

DEC. 2 – 5, 2019

SCHLOSS SCHÖNBRUNN | ORANGERIE

VIENNA, AUSTRIA

ORGANIZING COMMITTEE: Angela Sessitsch, Alexandra Khassidov, Günter Brader

SCIENTIFIC COMMITTEE: Karen L. Bailey, Gabriele Berg, Günter Brader, Trevor Charles, Kellye Eversole, Philipp Franken, Paolina Garbeva, Heribert Hirt, Michael Ionescu, Jenny Kao-Kniffin, Adam Schikora, Klaus Schlaeppli, Alga Zuccaro & Angela Sessitsch

Table of Contents

GENERAL INFORMATION	3
INTRODUCTION	5
INVITED SPEAKERS	6
SCIENTIFIC PROGRAM	11
DAY 1 MON, 2. DEC. 1^{P.M.} - 8:30^{P.M.}	11
DAY 2 TUE, 3. DEC. 8:30^{A.M.} - 3:55^{P.M.}	13
DAY 3 WED, 4. DEC. 8:30^{A.M.} - 6:30^{P.M.}	15
DAY 4 THU, 5. DEC. 8:30^{A.M.} - 2:00^{P.M.}	18
POSTERS TABLE - POSTERSESSION I	20
POSTERS TABLE - POSTERSESSION II	21
LECTURES	22
OPENING & OPENING LECTURE	23
SUCCESSFUL MICROBIAL APPLICATIONS	24
MECHANISMS MEDIATING HOLOBIONT AND MULTIPARTITE INTERACTIONS	27
PLANT UNDERSTANDING OF INTERACTIONS WITH BENEFICIAL MICROBES	33
MICROBIOME UNDERSTANDING BEYOND PROFILING	40
MORNING KEYNOTE	48
MICROBIAL BIOCONTROL OF PESTS, PATHOGENS AND WEEDS	49
MICROBIAL APPLICATIONS FOR IMPROVING NUTRITION AND ABIOTIC STRESS TOLERANCE	61
REGULATORY ISSUES – SPECIAL SESSION	71
DISRUPTIVE APPROACHES FOR ENGINEERING THE PHYTOBIOME & MICROBIAL DELIVERY	73
CLOSING & CLOSING KEYNOTE	76
POSTER PRESENTATIONS	77
POSTER SESSION 1: SUCCESSFUL MICROBIAL APPLICATIONS	78
POSTER SESSION 1: MECHANISMS MEDIATING HOLOBIONT AND MULTIPARTITE INTERACTIONS	84
POSTER SESSION 1: PLANT UNDERSTANDING OF INTERACTIONS WITH BENEFICIAL MICROBES	91
POSTER SESSION 1: MICROBIOME UNDERSTANDING BEYOND PROFILING	105
POSTER SESSION 1: VARIA	109
POSTER SESSION 2: MICROBIAL BIOCONTROL OF PESTS, PATHOGENS AND WEEDS	113
POSTER SESSION 2: MICROBIAL APPLICATIONS FOR IMPROVING NUTRITION AND ABIOTIC STRESS TOLERANCE	126
POSTER SESSION 2: DISRUPTIVE APPROACHES FOR ENGINEERING THE PHYTOBIOME & MICROBIAL DELIVERY	138
AUTHOR INDEX	142
PARTICIPANT INDEX	150
NOTES	161
IMPRINT	161

General Information

TAXI:

+431 40100 or +431 31300

VENUE ADDRESS:

Schloss Schönbrunn Tagungszentrum | Apothekertrakt | Schönbrunner Schloßstrasse – Entrance | 1130 Vienna, Austria

miCROPe 2019 office at the venue:

The registration desk will be occupied throughout the symposium. Please contact us with any congress related queries in person or by e-mail.

E-mail: office@micrope.org

WiFi is available at the venue with this login data:

WLAN SSID: Meetings
PW: Habsburg

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www.facebook.com/micrope.symposium/

The **language** of the meeting is English.

We reserve the right to use any photograph/video taken at the event without the expressed written permission of those included within the photograph/video.

SHORT TALKS:

Please give your presentation in power point or pdf format to the technician in the break before the session via usb-stick. The program is very tight, so please take care of the prescribed talktime.

POSTER PITCHES:

The uploaded Poster pitches from presenters will be preloaded on the presentation computer.

POSTER SESSIONS:

Posters are only accepted in A0 upright format and in English. The poster sessions are organized into poster sessions I & II and each poster has been assigned to a session. You can see the assignment within the poster table on page 20-21.

Postersession I Part A - Even poster numbers presenting: Monday 2. Dec., 18:30 – 20:30

Postersession I Part B - Odd poster numbers presenting: Tuesday 3. Dec., 12:25 – 14:30

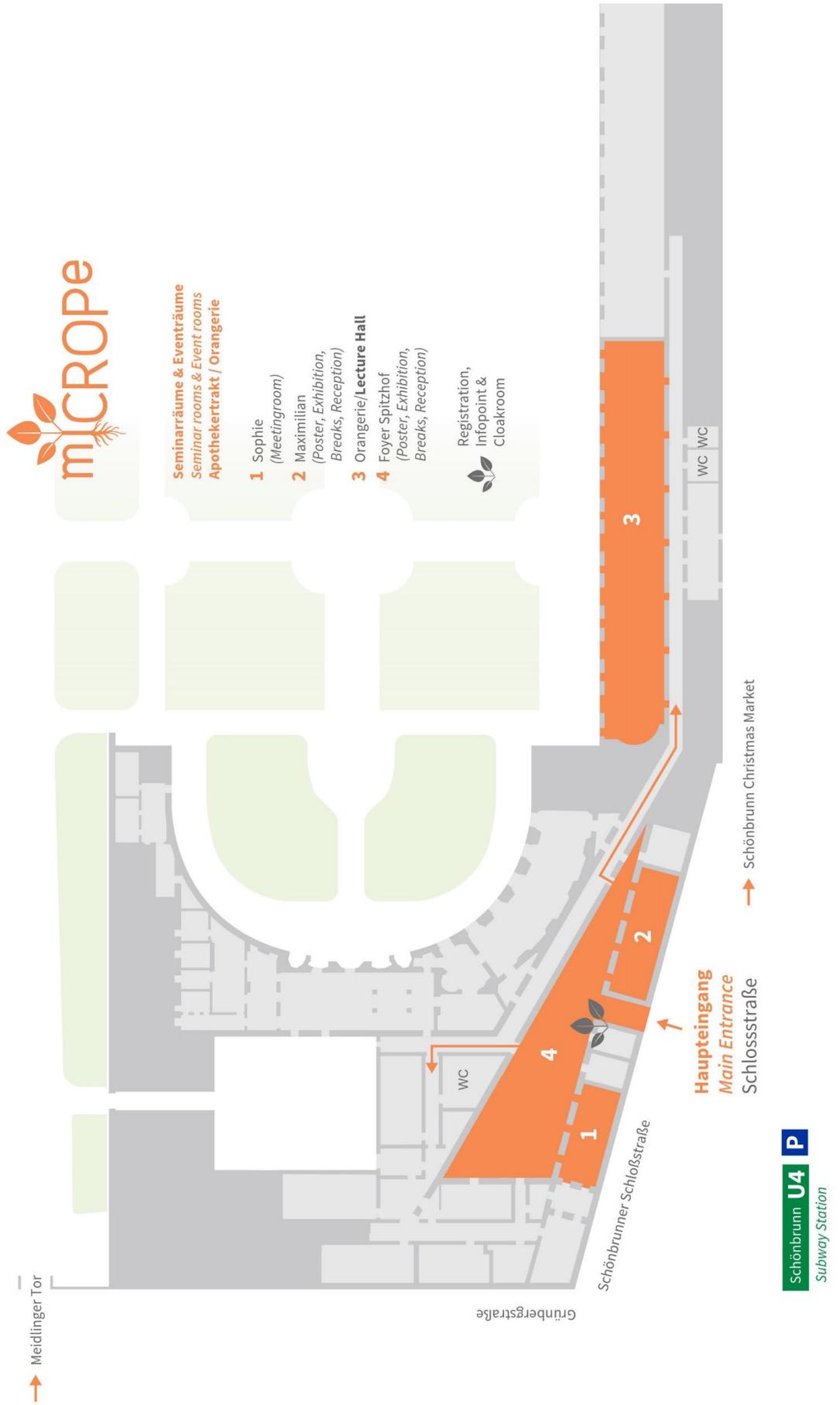
Postersession II Part A - Even poster numbers presenting: Wednesday 4. Dec., 12:40 – 14:30

Postersession II Part B - Odd poster numbers presenting: Wednesday 4. Dec., 16:35 – 18:30

Posters from poster session I have to be removed until the evening of December 3rd.

Five posters will be awarded with Best Poster Awards.

General Information



Introduction

Dear miCROPe attendees,

Our awareness on the importance of naturally occurring microorganisms, frequently referred as to microbiomes, has increased substantially. Plant microbiota are diverse and provide important functions for their host's performance, and mediate functions like nutrient delivery, fitness, stress tolerance, and pathogen or pest control. Current understanding of plant-microbe interactions is helping to develop microbial products, new applications to improve crop production, and create alternatives to chemicals. Microbial ecology is an important asset for understanding the fate of applied microorganisms in a natural environment, and for affecting product development. Greater understanding of microbiome functioning will also lead to new routes of exploration.



The increasing awareness of and interest in plant microbiota is linked to the urgent need to find solutions for current challenges in global crop production such as climate change and global demographic development. Difficulties to be overcome include world-wide population increases, extreme weather events and highly variable weather conditions, emerging pathogens and pests, and diminishing land resources. Furthermore, the use of chemical pesticides poses a threat to human health, animal welfare, and biodiversity. Innovations based on the functioning of plant microbiota have the potential to contribute to combating these challenges.

The symposium "Microbe-assisted crop production – opportunities, challenges and needs" (miCROPe 2019) addresses basic and applied aspects of applying beneficial microorganisms in crop production. Scientific sessions will address mechanistic understanding of holobiont interactions, functional understanding of microbiomes, plant understanding, microbial control of pests, pathogens and weeds, microbial application to improve nutrition and abiotic stress as well as disruptive approaches in microbiome applications. Our aim is to promote innovation as well as implementation of new technologies and to enable discussions between academia and industry.

Around 190 abstracts were submitted by scientists working in academia and industry around the globe. We would like to thank all authors for their valuable contribution to provide new results for further scientific discussion and to make this symposium highly interesting for different stakeholders. This exchange will lead in the future to a better implementation of microbe-based crop production. We would like to particularly thank the scientific committee for their excellent support in organizing a high-quality program. The contributions of all invited speakers are highly appreciated. We furthermore would like to thank all sponsors, partners and supporters of this symposium.

We wish you all an exciting symposium, time to interact with colleagues and friends as well as time to enjoy the Christmas atmosphere in Vienna!

Angela Sessitsch – AIT Austrian Institute of Technology, Austria
on behalf of the Organizing Committee

Invited Speakers

Karen L. Bailey, Agriculture & Agri-Food Canada, CA

Dr. Karen Bailey, Emeritus Research Scientist from Agriculture & Agri-Food Canada, trained as a plant pathologist and applied this expertise to improve plant health by finding solutions to reduce the impact of soil-borne plant diseases and by using fungi for biological control of Canada thistle and other broadleaved weeds. She has more than 300 publications, inventions disclosures and patents, and has received recognition from her peers with awards such as the Queen Elizabeth II Diamond Jubilee Medal, CPS Outstanding Research Award, and CPS Award for Achievements in Plant Pathology. Although having retired from AAFC, she continues to support commercialization activities related to her bioherbicide discoveries.



Gabriele Berg, Graz University of Technology, AT

Gabriele Berg studied biology and biotechnology at the universities in Rostock and Greifswald obtained her Ph.D. in 1995 in microbiology from Rostock University (Germany). In 2003, she got a Heisenberg grant from the DFG (Deutsche Forschungsgemeinschaft), and in 2005 she became a full professor in environmental biotechnology at Graz University of Technology (Austria). Her interests are focused on microbiome research and translation of the results into new biotechnological concepts for our environment as well as for plant and human health. Results have published in more than 200 peer-reviewed papers and in several patents. For her results and developments, she received numerous awards, e.g. Science2Business Award Austria, "ÖGUT Umweltpreis" (2011) and Fast Forward Award Styria (2015). She belongs to the most influential researchers world-wide (top 1, Clarivate Analytics for the category Cross Fields in 2018).



Günter Brader, AIT Austrian Institute of Technology, AT

Dr. Günter Brader studied biology at the University of Vienna and obtained his PhD in plant biology 1997. After gaining post doc experience in the field of molecular biology and plant-microbe interactions at the Institute of Biotechnology and the Department of Genetics of the University of Helsinki, Finland, he was appointed to be Docent in Plant Molecular Biology by the Helsinki University. He is now senior scientist at the Austrian Institute of Technology. Günter Brader is author of 60 peer reviewed publications. The research interests of Günter Brader are in the field of plant-microbe interactions and in understanding and characterizing plant diseases. His work focuses also on the exploitation of beneficial bacteria for biocontrol and nutrient solubilisation and description of the underlying mechanisms.



Trevor Charles, University of Waterloo, CA

Trevor Charles is a bacterial geneticist with a research program in plant-microbe interactions, functional metagenomics, and bacterial genome engineering for bioproducts. Following B.Sc. Microbiology at University of British Columbia, he obtained his Ph.D. in Turlough Finan's lab at McMaster University (symbiotic nitrogen fixation) and did postdoctoral work in Gene Nester's lab at University of Washington (*Agrobacterium*). He held a faculty position at McGill University before moving to his current position at University of Waterloo in 1998, where he is currently director of Waterloo Centre for Microbial Research. He is also co-founder and CSO of the company Metagenom Bio, which applies metagenomic and microbial community analysis to challenges in the agriculture and environment sectors.



Philipp Franken, University of Applied Sciences Erfurt, DE

Philipp Franken started to work on the arbuscular mycorrhizal (AM) symbiosis in 1991 at the MPI of Plant Breeding Research in Cologne and the INRA in Dijon, France. In 1995, Philipp Franken established a working group on AM molecular biology at the MPI of terrestrial Microbiology in Marburg. By the discovery of the plant growth-promoting fungus *Piriformospora indica* in 1998, he became also interested in other groups of beneficial root-colonisers. After his habilitation in Applied Botany and Microbiology at Marburg University, Philipp Franken took over the head of the Plant Nutrition Department at the Leibniz-Institute of Vegetable and Ornamental Crops in 2002. Among other horticultural topics, he further worked mainly on the functions of root-fungus interactions. Since 2019, Philipp Franken is scientific director of the Erfurt Research Centre for Horticultural Crops at the University of Applied Sciences Erfurt. In four research groups, the centre is working on questions of horticultural practice using current methods of biosciences. The scientific work is supported by the Friedrich Schiller University in Jena where Philipp Franken holds a chair of Molecular Phytopathology. The research in his group is aimed towards integrating knowledge about mycorrhizal fungi and other beneficial root colonisers in novel strategies for sustainable plant production systems.



Paolina Garbeva, NIOO-KNAW, NL

Dr Paolina Garbeva is microbiologist by training with strong affinity for Microbial Chemical Ecology. She received her PhD degree from the Leiden University (Netherlands) in 2005. During her PhD, she investigated the significance of microbial diversity on disease suppression in agricultural soil.

After a post-doc at the University of Aberdeen in Scotland, she returned to the Netherlands to work on a personal VENI grant obtained from the Dutch Research Council (NWO). With the VENI project, she discovered that soil bacteria could distinguish among different competitors and fine-tune their strategies to survive. Between 2010 and 2013, Dr Garbeva obtained three personal grants (MEERVOUD, VIDI and ASPASIA) that allowed her to establish a research group within the Department of Microbial Ecology at Netherlands Institute of Ecology. The focus of her research group is to understand the fundamental mechanisms of microbial interactions and communication with particular attention paid to the role of microbial volatiles. This is a novel and unique line of research in the field of Microbial Ecology. Using omics-based tools and novel imaging techniques, her research group would like to further decipher and harness the communicating molecules used by plants and microbes in order to improve plant growth and health.



Heribert Hirt, KAUST, SA

Hirt studied biochemistry at the Univ. of Cape Town and received his PhD from the Univ. of Vienna in 1987. After post-doctoral fellowships at the Univ. of Oxford and Wageningen, he became Professor of Genetics at the Univ. of Vienna. In 2007, he became Director of the INRA Plant Genomics Institute in Paris and in 2014 of the Center for Desert Agriculture at KAUST. His key biological questions are how plants can survive under stress conditions and how microbes contribute to these events by positively or negatively interacting with plants. In the DARWIN21 project (<https://www.darwin21.org>) he searches for beneficial microbes from desert plants with the aim to enhance stress tolerance of plants. A main focus of his research is to identify the microbial and plant genes and pathways that provide stable stress tolerance to plants without interfering with growth and yield. His long term goal is to provide farmers with tailored microbial communities to enhance the performance and protection of crops to specific stress conditions.



Invited Speakers

Michael Ionescu, Lavie-Bio, IL

Michael Ionescu is the VP Research of Lavie-Bio, a subsidiary of Evogene, focused on development of novel ag-biologicals products. For the last 6 years, Michael leads the research and optimization activity of novel microbiome-based Ag-biological to drive food quality and sustainability. His interdisciplinary research team is implementing a rationale biology driven design of microbiome-based products by leveraging a proprietary Computational Predictive Biology (CPB) Platform utilizing genomic and phenotypic big data and advanced informatics, focusing on the discovery and optimization of both Bio-Stimulants and Bio-Pesticides. Michael received his PhD degrees from the Hebrew University of Jerusalem in Life Sciences and Environmental Studies, researching stress response mechanisms in enteric bacteria. He had conducted his postdoctoral research at the University of California at Berkeley in phytopathology, studying cell-cell communication system in plant pathogens with relation to interaction with host plant.

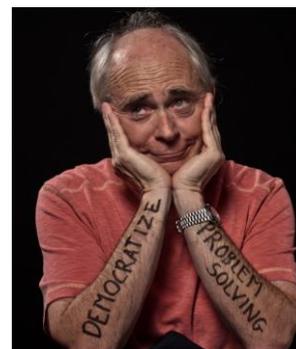


Richard Jefferson, Cambia & QUT, AU

Richard Jefferson is a Professor of Biological Innovation at the Queensland University of Technology (QUT) and founder and CEO of Cambia & The Lens. Richard received a PhD in Molecular, Cellular and Development Biology from University of Colorado at Boulder in 1985, where he developed the glucuronidase (GUS) system as a molecular heuristic tool for transgenesis, developmental and ecosystem studies.

During his NIH postdoc at the Plant Breeding Institute in Cambridge, UK, Richard adapted GUS for agricultural biotechnology and pioneered an open source paradigm by distributing the toolkits to hundreds of labs around the world before publication. This enabled the genetic engineering of virtually all commercial crops, and is now the most cited molecular technology in agriculture. In 1987, with colleagues at PBI, Richard led the world's first field release of a biotech food crop. In 1989 Richard was appointed the first Molecular Biologist for the United Nations FAO/IAEA in Vienna, and in 1991 founded Cambia, an autonomous global social enterprise to democratize science enabled problem solving. Besides Cambia's role in inventing and distributing open source enabling technologies it runs Lens.org, now the longest operating, largest and most comprehensive free, open and secure platform for scholarly and patent discovery, analytics and metrics.

Based on work done on diverse microbial GUS and arylsulfatases since 1980, and their essential role in modulating hormone action in the holobiont, Richard proposed the landmark hologenome theory of evolution in 1994 at Cold Spring Harbor. He was the first to describe the role of the microbiome as the driver of biological evolution, and its role in understanding and optimizing performance of biological systems. In 1997, Richard proposed the concept of ecotherapeutics as a strategy for modulating agriculture and health systems performance by adjusting population structures of microbial constituents. The hologenome theory has profound implications for how we think about ourselves, living systems, the origin of disease, the origins of social behavior and even social institutions in innovation. Richard is an 'Outstanding Social Entrepreneur' of the Schwab Foundation and a regular panelist at the World Economic Forum's (WEF) Davos annual meetings and Summits. Richard served on the WEF Global Agenda Council on Intellectual Property and the Global Agenda Council on the Economics of Innovation. He was named to Scientific American's list of the world's 50 Most Influential Technologists. His work has featured in countless media, including The Economist, New York Times, Newsweek, Red Herring, Nature, Science, Nature Biotechnology.



Jenny Kao-Kniffin, Cornell University, US

Jenny Kao-Kniffin [pronunciation: GAOW-nif-IN] is an Associate Professor at Cornell University's School of Integrative Plant Science. She received her Ph.D. from the University of Wisconsin-Madison in Land Resources, with a specialization in Ecosystem Microbiology. She then served as a Postdoctoral Research Fellow with the National Science Foundation (NSF) investigating landscape-scale patterns of microbial composition near Barrow, Alaska. In 2019, she received the Presidential Early Career Award for Scientists and Engineers from the United States White House for her work on agricultural microbiomes. The research subjects range from crops and model plant species to invasive plants and weeds in agricultural and natural ecosystems, with a major focus on microbiome assembly, modification, and resilience impacting plant traits.



Invited Speakers

Steven Lindow, University of California, Berkeley, US

The Lindow lab focuses on the ecology and management of plant-associated bacteria with a focus on both epiphytic and endophytic bacteria. A thrust of the lab has been on identification of traits that confer fitness and stress tolerance of bacteria on leaf surfaces and their regulation. The contribution of intra- and inter-species chemical communication that mediates expression of cell density-dependent traits in both *Pseudomonas syringae* and *Xylella fastidiosa* are being addressed with the aim of modifying their behaviors to achieve plant disease control. The emigration from and immigration to bacteria to plants via airborne transport is being studied to better understand processes determining the context-dependent assembly of epiphytic communities on leaves.



Jos Raaijmakers, NIOO-KNAW, NL

Jos Raaijmakers received his MSc and PhD degrees from the University of Utrecht (Netherlands), where he studied phyllosphere and rhizosphere microbiology. His PhD work specifically focused on siderophore-mediated iron acquisition by rhizosphere bacteria. He undertook postdoctoral research at USDA and Washington State University (USA) on disease suppressive soils and the antifungal activity of secondary metabolites (phloroglucinols, phenazines) of root-associated bacteria. Upon returning to the Netherlands, he became an associate professor Plant Pathology at Wageningen University working on microbe-microbe/microbe-plant interactions and the diversity & functions of cyclic lipopeptides. Currently he is head of the Microbial Ecology department of the Netherlands Institute of Ecology (NIOO-KNAW) and a Professor at the Institute of Biology of Leiden University. His research program focuses on i) the impact of plant domestication on microbiome assembly, and ii) the role of the plant microbiome in biotic stress tolerance.



Adam Schikora, Julius Kühn-Institut, DE

Adam Schikora studied biology at the Universities of Warsaw, Poland and Göttingen, Germany and received his PhD in mineral plant nutrition. During the post-doctoral trainings in France and Austria, Adam focused on signaling pathways and the cellular responses to diverse stress stimuli, including the response to human pathogenic bacteria. Collaborative action between a host plant and associated bacteria is crucial for the establishment of an efficient interaction. In 2009 he became the leader of the Plant-Bacteria Interaction Group at the Institute of Phytopathology at JL University Giessen, Germany. His group investigates the stimulation of plant immune system by bacterial quorum sensing molecules on one hand, and on the other, how bacteria (e.g. the human pathogen *Salmonella enterica*) manipulate plant defense mechanisms. In 2015 the group moved to Julius Kühn-Institut in Braunschweig, where he continues to study the interaction between crop plants and beneficial as well as human pathogenic bacteria. Currently Adam Schikora lectures plant physiology and microbiology at the TU Braunschweig.



Klaus Schlaeppi, University of Bern, CH

Klaus Schlaeppi studied plant-microbe interactions and obtained his PhD from the University of Fribourg (Switzerland) based on work investigating plant defences against pathogens. As postdoctoral scientist at the MPI for Plant Breeding Research in Cologne (Germany) he contributed to method development and characterization of the commensal root microbiota of the model plant *Arabidopsis thaliana* and related Brassicaceae species. Back in Switzerland, as junior group leader he broadened his research interests to rhizosphere microbial ecology and how the root microbiota could be manipulated to improve agriculture. Today he is lecturer at the University of Bern (Switzerland) and investigates with his team the contribution of the root microbiota to plant growth and how plants communicate to their root microbiota and take influence on their activities. The long-term ambition of his research program is to make use of plant microbiomes in smart and sustainable agriculture.



Invited Speakers

Steven Vandenabeele, Aphea.Bio, BE

Steven Vandenabeele holds a PhD in biotechnology (University of Ghent) and has 20 years of experience in plant biotech (VIB Department of Plant Systems Biology, Rockefeller University, BASF Plant Science). Steven has worked in the ag-biotech industry for more than 10 years: he worked at BASF Plant Science as a group leader Technology Management, as the coordinator for the high-throughput plant phenotyping platform, and as global research manager of the rice yield project. Serving in these functions, he has gained strong experience in people, process and research project management. Three years ago, together with the VIB, he started building the business strategy to develop superior agricultural biologicals based on microbials. Aphea.Bio NV is currently operational since 2017, backed by venture capital. Steven is Aphea.Bio's Chief Scientific Officer and heads a team of 18 expert scientists.



Alga Zuccaro, University of Cologne, DE

Alga studied marine biology at the University of Ancona, Italy before going on to do her PhD at the Technical Institute of Braunschweig, Germany in cooperation with the University of Portsmouth, UK. She held postdoctoral research positions in Braunschweig as well as at Oregon State University. After a period as Group Leader at the Institute of Phytopathology and Applied Zoology in Gießen, Germany and as Research Group Leader at the Max Planck Institute for Terrestrial Microbiology in Marburg, Germany, she became Professor in Ecological Genetics of Microbes at the University of Cologne, Germany in 2014. Research in Alga's group focuses on the mechanisms that enable symbiotic fungi to colonize plants successfully and on the processes accounting for variations in host preferences and fungal lifestyles. The prime models for her studies are the root endophyte *Serendipita indica* (Basidiomycota, Sebaciniales), and the orchid mycorrhizal *Serendipita vermifera*, two beneficial symbionts that colonize the root epidermal and cortex cells of a broad range of plant species, including the dicot model plant *Arabidopsis thaliana* and the agriculturally important monocot *Hordeum vulgare*.



INTERNATIONAL ALLIANCE FOR PHYTOBIOMES RESEARCH

The Phytobiomes Alliance is an international nonprofit industry, academic, and governmental agency consortium

Mission
Establish a science and technology foundation for site-specific, phytobiome-based enhancement of sustainable food, feed, and fiber production

Vision
By 2050, all farmers have the ability to use predictive and prescriptive analytics to choose the best combination of crop/variety, management practices, and inputs for a specific field in a given year taking into consideration all physical (climate, soil...) and biological conditions (microbes, pests, disease, weeds, animals...)

Goals
Identify research gaps and help coordinate projects to address those gaps
Establish national, international, and multi-national public-private projects and networks

www.phytobiomesalliance.org
@phytobiomes
Phytobiomes Alliance

Phytobiomes consists of all organisms living in, on, or around plants (e.g., microbes, animals, other plants), and the environment (i.e., soil, air, water, and climate)

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Scientific Program

DAY 1 | Mon, 2. Dec. | 1^{P.M.} - 8:30^{P.M.}

11:00 - 13:00 Arrival, registration

13:00 - 13:15 WELCOME

13:15 - 14:00 **OPENING LECTURE** Supported by 
Steven Lindow (University of California, Berkeley, US)
 Assembly of epiphytic bacterial communities on plants and their interactions with the plant host: insights for managing the plant microbiome

SESSION 1
 14:00 - 15:00 **SUCCESSFUL MICROBIAL APPLICATIONS**
Session chairs: Kellye Eversole & Angela Sessitsch

14:00 - 14:15 **Steven Vandenabeele (Aphae.Bio, BE)**
 An integrated technology pipeline for the development of superior agricultural biologicals

14:15 - 14:30 **Carolin Schneider (Inoq GmbH, DE)**
 Concept for a reasonable use of mycorrhizal fungi in green business

14:30 - 14:45 **Fabricio Dario Cassan (Universidad Nacional de Rio Cuarto, AR)**
 The successful history of *A. brasilense* Az39 in Agriculture. A metadata analysis

14:45 - 15:00 **Robert Rotter (Multikraft, AT)**
 Market 2 Research 2 Market – The Multikraft Model

15:00 - 15:30 **Coffee break**

SESSION 2
 15:30 - 17:35 **MECHANISMS MEDIATING HOLOBIONT AND MULTIPARTITE INTERACTIONS**
Session chairs: Alga Zuccaro & Paolina Garbeva

15:30 - 15:55 **Alga Zuccaro (University of Cologne, DE)**
 Molecular basis of plant-microbe interaction: know-how and tools for designing microbial communities with beneficial effects on plant growth

15:55 - 16:20 **Paolina Garbeva (NIOO-KNAW, NL)**
 The importance of microbial chemical interactions for plant and soil health

16:20 - 16:35 **Luzia Stalder (University of Neuchâtel, CH)**
 The functional ecology of plant microbiome interactions between the dominant fungal wheat pathogen *Zymoseptoria tritici* and *Pseudomonas* bacteria

16:35 - 16:50 **Ahmed Elhady (Julius Kühn-Institut, DE)**
 Modulation of rhizosphere microbiomes to suppress phytonematodes

- 16:50 - 17:05 POSTER PITCHES:
Rita Grosch (Leibniz Institute of Vegetable and Ornamental Crops, DE)
Long-term organic and mineral fertilization strategies shape the rhizosphere microbiota and performance of lettuce
Riitta Nissinen (University of Jyväskylä, FI)
Soil glyphosate treatment impacts plant endophytic communities in plant species specific manner
Kay Moisan (Wageningen University, NL)
Can soil microbes enhance plant health without direct contact?
Ana Bejarano Ramos (University of Trento, IT)
Wanted: helper bacterial strains enhancing the biocontrol activity of *Lysobacter capsici* AZ78
- 17:05 - 17:20 **Eva Baldassarre Svecova (Institute of Botany of the Czech Academy of Sciences, CZ)**
Effects of plant biostimulant treatments on the root-associated fungi of wheat and barley
- 17:20 - 17:35 **Ole Nybroe (University of Copenhagen, DK)**
Bacterial communities associated with hyphae of plant beneficial fungal biofertilizers
-
- 17:35 – 20:30 **Welcome Reception & Networking**
18:30 – 20:30 **Poster Session I**
-

DAY 2 | Tue, 3. Dec. | 8:30^{A.M.} – 3:55^{P.M.}

SESSION 3 08:30 - 10:10	PLANT UNDERSTANDING OF INTERACTIONS WITH BENEFICIAL MICROBES <i>Session chairs: Heribert Hirt & Adam Schikora</i>
08:30 - 08:55	Heribert Hirt (KAUST, SA) Lessons from desert microbes to enable saline agriculture on arid lands
08:55 - 09:10	Wu Xiong (Utrecht University, NL) Protists within rhizosphere microbiome determine plant health
09:10 - 09:25	POSTER PITCHES: Robert R. Junker (Philipps-University Marburg, DE) Bacteria-flower interactions: bacterial modifications of floral sugar and scent composition result in changes in pollinator behavior and plant reproduction Beatriz Ramos-solano (University San Pablo Ceu, ES) F3H plays a pivotal role of on flavonoid metabolism improving adaptation to biotic stress in blackberry Martina Franchini (University of Nottingham, GB) Transcriptional response of tomato plants to the growth stimulation provided by <i>Gluconacetobacter diazotrophicus</i> Shree Pariyar (Forschungszentrum Jülich, DE) Plant growth promoting bacteria promotes germination and enhances early root traits
09:25 - 09:40	Shalini Kirthi Vasan (Teri-deakin Nanobiotechnology Centre, IN) Understanding molecular, metabolic and phylogenomic events underlying Arbuscular Mycorrhizal Symbiosis: Scope for improving crop productivity
09:40 - 09:55	Carmen Bianco (CNR, IT) Co-inoculation of rice plants with nitrogen-fixing and indole-3-acetic acid (IAA)-producing endophytes: changes in physiological parameters of the host plant
09:55 - 10:10	Sofie Goormachtig (VIB- UGent, BE) Streptomyces as a plant's best friend
10:10 - 10:30	Coffee break
SESSION 3 10:30 - 11:10	PLANT UNDERSTANDING OF INTERACTIONS WITH BENEFICIAL MICROBES <i>Session chairs: Heribert Hirt & Adam Schikora</i>
10:30 - 10:55	Adam Schikora (Julius Kühn-Institut, DE) Genetic differences in barley govern the responsiveness to <i>N</i> -acyl homoserine lactone
10:55 - 11:10	Astrid Forneck (University of Natural Resources and Life Sciences Vienna, AT) Role of Microbes in the Galler-Plant Interaction: <i>Pantoea agglomerans</i> affecting the compatible Grape Phylloxera (<i>Daktulosphaera vitifoliae</i>) - <i>Vitis</i> spp. Interaction

SESSION 4	MICROBIOME UNDERSTANDING BEYOND PROFILING
11:10 - 12:25	<i>Session chairs: Jenny Kao-Kniffin & Klaus Schlaeppi</i>
11:10 - 11:35	Jenny Kao-Kniffin (Cornell University, US) Applying Concepts in Group-level Evolutionary Processes to Assemble Plant Beneficial Microbiomes
11:35 - 11:50	Rafael de Souza (University of Campinas, BR) Synthetic microbial community from the sugarcane core microbiome reveals genetic features for successful plant colonization
11:50 - 12:10	POSTER PITCHES: Lukas Wille (FiBL & ETHZ, CH) Genotype x soil interaction in the composition of root-rot pathogens of pea detected by quantitative PCR Romain Darriaut (INRA, FR) Contrasting soil microbial community profiles in healthy and declined vineyards Gorka Erice (Atens, ES) Changes in soil microbiome can alter peach tree physiology with implications in plant development and in the composition of secondary metabolites Arthur Goldstein (ESPCI Paris, FR) Functional analysis of soil microorganisms for agriculture using millifluidic droplets Birgit Mitter (AIT Austrian Institute of Technology, AT) The bacterial community in potato is recruited from soil and partly inherited across generations Yanyan Zhao (Université catholique de Louvain, BE) Root fungal community structure of <i>Alkanna tinctoria</i> differs with plant developmental stage
12:10 – 12:25	Mette Haubjerg Nicolaisen (University of Copenhagen, DK) A novel microcosm for recruiting phytate-degrading microbial communities under inherently competitive soil conditions
12:25 - 14:30	Lunch break & Poster Session I
14:30 - 14:45	Group Photo
SESSION 4	MICROBIOME UNDERSTANDING BEYOND PROFILING
14:45 - 15:55	<i>Session chairs: Jenny Kao-Kniffin & Klaus Schlaeppi</i>
14:45 - 15:10	Klaus Schlaeppi (University of Bern, CH) Plant responsiveness to soil microbial feedbacks
15:10 - 15:25	Hanna Faist (AIT Austrian Institute of Technology, AT) Evaluating the diversity and functional potential of plant microbiota to improve the selection of potato genotypes able to cope with combined water and nutrient limitations
15:25 - 15:40	Matthieu Barret (INRA, FR) Succession of microbial assemblages during seed development
15:40- 15:55	Steffen Kolb (Leibniz Centre for Landscape Research - ZALF, DE) Volatilome of Wheat Microbiota System under Drought and Flooding: The VolCorn Consortium
16:00	SOCIAL EVENT: Vienna tours (optional)

DAY 3 | Wed, 4. Dec. | 8:30^{A.M.} - 6:30^{P.M.}

08:30 - 09:00 **KEYNOTE**
Richard Jefferson (Cambia & QUT, AU)
 Crops as merobionts: Regenerative agriculture, the microbiome and the climate crisis through the lens of the hologenome theory

SESSION 5 MICROBIAL BIOCONTROL OF PESTS, PATHOGENS AND WEEDS

09:00 - 10:25 **Session chairs: Karen Bailey & Gabriele Berg**

09:00 - 09:25 **Gabriele Berg (Graz University of Technology, AT)**
 Plant microbiome management for sustainable agriculture

09:25 - 09:40 **Michael Rothballer (Helmholtz Zentrum München, DE)**
 The functional relevance of microbe-plant-insect interaction in a cereal crop system

09:40 - 09:55 **POSTER PITCHES:**

Alejandro del Barrio Duque (AIT Austrian Institute of Technology, AT)
Mycolicibacterium strains interact positively with *Serendipita (Piriformospora) indica* for crop enhancement and biocontrol of pathogens

Lara Reinbacher (Agroscope, CH)
 Biological control of wireworms in cover crops

Mei Li (Nanjing Agricultural University, CN)
 Facilitation promotes invasions in plant-associated microbial communities

Williams O. Anteyi (University of Hohenheim, DE)
In vivo localization and role of *Fusarium oxysporum* f.sp. *strigae* and *Bacillus subtilis* against *Striga hermonthica* in an integrated biocontrol system

09:55 - 10:10 **Xingchen Zhao (Ghent University, BE)**
 Behaviour of Bt ABTS-1857 as a biological control agent on spinach plants, cut leaves and spinach juice

10:10 - 10:25 **Gary Felton (The Pennsylvania State University, US)**
 The leaky gut syndrome: insect gut bacteria exacerbate physical and chemical defenses of plants

10:25 - 10:45 **Coffee break**

SESSION 5 MICROBIAL BIOCONTROL OF PESTS, PATHOGENS AND WEEDS

10:45 - 12:40 **Session chairs: Karen Bailey & Gabriele Berg**

10:45 - 11:10 **Karen L. Bailey (Agriculture & Agri-Food Canada, CA)**
 Bioherbicides from creation to commercial success – *What's the problem?*

11:10 - 11:25 **Julia Friman (Wageningen University, NL)**
 Contrasting effects of the rhizobacterium *Pseudomonas simiae* on above- and belowground insect herbivores

11:25 - 11:40 **POSTER PITCHES:**

Leone Olivieri (NIAB EMR, GB)
 Microbial ecology of the European apple canker pathosystem (*N. ditissima*)

Shivani Khatri (Indian Institute of Technology, Delhi, IN)
 Microbiome-assisted management of plant diseases

Daria Rybakova (Graz University of Technology, AT)
 A species-specific crosstalk via volatile exchange between a biocontrol agent *Serratia plymuthica* HRO-C48 and fungal plant pathogens.

Valeska Villegas Escobar (EAFIT University, CO)
 Effect of *Bacillus subtilis* EA-CB0575 on the microbiota, growth development and health of banana plants

- 11:40 - 11:55 **Anne Muola (University of Turku, FI)**
The role of endophytic entomopathogens in modulating plant-microbe-insect interactions
- 11:55 - 12:10 **Friederike Trognitz (AIT Austrian Institute of Technology, AT)**
Plant associated bacteria for the control of *Impatiens glandulifera*
- 12:10 - 12:25 **Alejandro Gimeno (Agroscope and University of Zurich, CH)**
Suppressing *Fusarium graminearum* and mycotoxins by application of microbial antagonists on infected crop residues
- 12:25 - 12:40 **Mohammadhossein Ravanbakhsh (Utrecht University, NL)**
Combining nanomaterials and phages for enhanced bacterial wilt control
-
- 12:40 - 14:30 **Poster session II & Lunch break**
-
- SESSION 5 MICROBIAL BIOCONTROL OF PESTS, PATHOGENS AND WEEDS**
- 14:30 - 15:15 **Session chairs: Karen Bailey & Gabriele Berg**
-
- 14:30 - 14:45 **Christopher Dunlap (USDA-ARS, US)**
Iturinic lipopeptide diversity of the *Bacillus subtilis* group
- 14:45 - 15:00 **Jie Hu (Utrecht University, NL)**
Microbial consortia effectively suppress and prevent infections of *Ralstonia pseudosolanacearum* in *Rosa* sp.
- 15:00 - 15:15 **Sylwia Jafra (Intercollegiate Faculty of Biotechnology UG and MUG, University of Gdansk, PL)**
The plant protecting and plant growth promoting abilities of the synthetic micro-consortium of antagonistic bacterial strains.
-
- SESSION 6 MICROBIAL APPLICATIONS FOR IMPROVING NUTRITION AND ABIOTIC STRESS TOLERANCE**
- 15:15 - 16:35 **Session chairs: Philipp Franken & Günter Brader**
-
- 15:15 - 15:40 **Philipp Franken (University of Applied Sciences Erfurt, DE)**
How plants benefit from root-colonizing fungi: There's more than one way to crack an egg
-
- 15:40 - 16:05 **POSTER PITCHES:**
- Mirjam Seeliger (INOQ GmbH, DE)**
Interactions of arbuscular mycorrhizal fungi and winter wheat in contrasting cropping systems
- Raphael Bousageon (Burgundy University, FR)**
Impact of beneficial microorganisms on strawberry growth, fruit production, nutritional quality and volatilome
- Annamaria Bevivino (Italian National Agency for New Technologies, Energy and Sustainable Economic Development, IT)**
SIMBA: Design, formulation and optimization of plant growth-promoting microbes for their use as microbial consortia inoculants
- Francisco Martin Usero (Arid Zones Experimental Station, CSIC, ES)**
Influence of soil microbial communities linked to organic matter addition on tomato (*Solanum lycopersicum* L.) plant growth under intensive farming
- Mohammed Antar (McGill University, CA)**
Microbial consortia: a way to enhance crop yield under both controlled environment and field conditions
- Shubhangi Sharma (Leibniz-Institut für Gemüse-und Zierpflanzenbau, Großbeeren, Germany, DE)**
Effect of coinoculation of *Rhizoglosum irregulare*, and hyphae attached phosphate solubilizing bacteria on *Solanum lycopersicum*

Scientific Program

- 16:05 - 16:20 **Tania Galindo (The Pennsylvania State University, US)**
Matching root anatomical and architectural phenotypes with soil microorganisms to improve nutrient and water uptake efficiency: a new perspective in plant microbiome research
- 16:20 - 16.35 **Shubham Dubey (IIT Delhi, IN)**
Combating salinity stress with Rhizosphere Engineering: A next-generation approach
-
- 16:35 - 18:30 **Coffee Break & Poster session II**
-
- 19:00 - 23:00 **Conference Dinner (optional)**
-

DAY 4 | Thu, 5. Dec. | 8:30^{A.M.} - 2:00^{P.M.}

SESSION 6	MICROBIAL APPLICATIONS FOR IMPROVING NUTRITION AND ABIOTIC STRESS TOLERANCE
08:30 - 10:25	Session chairs: Philipp Franken & Günter Brader
08:30 - 08:55	Günter Brader (AIT Austrian Institute of Technology, AT) Phosphate fertilization in crops – the contribution of bacteria and fungi
08:55 - 09:10	Klára Bradáčová (University of Hohenheim, DE) Maize inoculation with microbial consortia: contrasting effects on rhizosphere activities, nutrient acquisition and early growth in different soils
09:10 - 09:25	Borjana Arsova (Forschungszentrum Jülich, DE) The impact of beneficial microbes on <i>Brachypodium</i> nutrient uptake under limiting supplies of nitrogen and phosphorus, monitored with non-invasive phenotyping and molecular approaches
09:25 - 09:40	Chanz Robbins (Université de Lausanne, CH) Does genetic variation in single spore progeny of an arbuscular mycorrhizal fungus impact cassava yield
09:40 - 09:55	Sarah Symanczik (Forschungsinstitut für biologischen Landbau, CH) Fertile date palm – a transdisciplinary collaboration project to ameliorate date palm cultivation via microbial inoculation, organic matter management and mixed cropping using nurse plants
09:55 - 10:10	Jaderson Armanhi (University of Campinas, BR) Unraveling plant physiological behavior modulated by a synthetic microbial community using a non-invasive and continuous medium-scale phenotyping platform
10:10 - 10:25	Narges Moradtalab (Universität Hohenheim, DE) Synergistic contribution of microbial consortia, micronutrients, and ammonium fertilization to cold tolerance in maize by regulating phytohormone homeostasis and oxidative stress defence
10:25 - 10:45	Coffee break
10:45 - 11:15	SPECIAL SESSION - REGULATORY ISSUES
10:45 - 11:00	Gianpiero Gueli Alletti (APIS Applied Insect Science GmbH, DE) Registration of biopesticides in the European Union
11:00 - 11:15	Faina Kamilova (Knoell NL BV, NL) Proposal for the application of microbiomes in industry: regulatory challenges and opportunities
SESSION 7	DISRUPTIVE APPROACHES FOR ENGINEERING THE PHYTOBIOME & MICROBIAL DELIVERY
11:15 - 12:35	Session chairs: Trevor Charles & Michael Ionesco
11:15 - 11:40	Trevor Charles (University of Waterloo, CA) Can we tune the microbiome in controlled environment agriculture?
11:40 - 12:05	Michael Ionescu (Lavie-Bio, IL) Harnessing the power of computational genomics to optimize next generation ag-biologicals
12:05 - 12:20	Sascha Patz (University of Tübingen, DE) Lifting the veil of virulence and benefits of plant-associated bacteria by metagenomics approaches

Scientific Program

12:20 - 12:35	Carola Peters (Incotec Europe B.V., NL) Opportunities and challenges of microbial seed application
12:35 - 13:00	Refreshment Break
13:00 - 13:40	CLOSING LECTURE Jos Raaijmakers (NIOO-KNAW, NL) Towards new road MAPs to engineer plant microbiomes
13:40 - 14:00	AWARDS & CLOSURE

Posters Table - Postersession I

Postersession I Monday 2. Dec., 18:30 - 20:30 (Even no.) & Tuesday 3. Dec., 12:25 – 14:30 (Odd no.)

Poster #	Successful microbial applications	Poster #	Plant understanding of interactions with beneficial microbes	Poster #	Poster Session 1: Microbiome understanding beyond profiling
PP1-SA-01	Enrique Gutiérrez Albanchez	PP1-PU-01	Florian Schindler	PF-PU-03	Martina Franchini
PP1-SA-02	Natacha Bodenhausen	PP1-PU-02	Tetard-Jones Catherine	PF-PU-04	Shree Pariyar
PP1-SA-03	Cintia Csorba	PP1-PU-03	Karolin Pohl	Poster #	Poster Session 1: Microbiome understanding beyond profiling
PP1-SA-04	Michele Pallucchini	PP1-PU-04	Jin-Soo Son	PP1-MU-01	Silvia D. Schrey
PP1-SA-05	Andreea Cosoveanu	PP1-PU-05	Johan Meijer	PP1-MU-02	Anurag Chaturvedi
PP1-SA-06	Ryan Sebring	PP1-PU-06	Jemma Roberts	PP1-MU-03	Dagmara Sirová
PP1-SA-07	Isabelle Caugant	PP1-PU-07	Oleg A. Kharchuk	PP1-MU-04	Annika Hoffmann
PP1-SA-08	Katharina Kraxberger	PP1-PU-08	Maximilian Hanusch	PP1-MU-05	Marie Legein
PP1-SA-09	Lisa-Maria Ohler	PP1-PU-09	Michelle K. Carkner		
		PP1-PU-10	Marjo Helander	PF-MU-01	Lukas Wille
Poster #	Mechanisms mediating holobiont and multipartite interactions	PP1-PU-11	Ankita Chopra	PF-MU-02	Romain Darriaut
PP1-MI-01	Pierre-Emmanuel Courty	PP1-PU-12	Frank Waller	PF-MU-03	Gorka Erice
PP1-MI-02	Kari Saikkonen	PP1-PU-13	Barbara Bort Biazotti	PF-MU-04	Arthur Goldstein
PP1-MI-03	Dorota Magdalena Krzyzanowska	PP1-PU-14	Nikoleta Galambos	PF-MU-05	Birgit Mitter
PP1-MI-04	Mason Kamalani Chock	PP1-PU-15	Sa-Youl Ghim	PF-MU-06	Yanyan Zhao
PP1-MI-05	Angelique Rat	PP1-PU-16	Abhishek Shrestha		
PP1-MI-06	Henry Müller	PP1-PU-17	Christoph Lehnen		
PP1-MI-07	Suni Anie Mathew	PP1-PU-18	Stephan Wawra		
PP1-MI-08	Lena Fragner	PP1-PU-19	Romy Moukarzel		
PP1-MI-09	Boyoung Lee	PP1-PU-20	Soo-yeong Lee		
PP1-MI-10	Shruti Pavagadhi	PP1-PU-21	Michael Opitz		
PP1-MI-11	Aditi Buch	PP1-PU-22	Daniela Sangiorgio		
PP1-MI-12	Henry David Naranjo Benavides	PP1-PU-23	Anna Marie Hallasgo		
PF-MI-01	Rita Grosch	PP1-PU-24	Dongmei Lyu		
PF-MI-02	Riitta Nissinen	PP1-PU-25	Muhammad Ahmad		
PF-MI-03	Kay Moisan	PF-PU-01	Robert R. Junker		
PF-MI-04	Ana Bejarano Ramos	PF-PU-02	Beatriz Ramos-solano		

Posters Table - Postersession II

Postersession II Wednesday 4. Dec., 12:40 - 14:30 (Even no.) & Wednesday 4. Dec., 16:35 - 18:30 (Odd no.)

Poster #	Microbial biocontrol of pests, pathogens and weeds	PF-MB-03	Mei Li	PP2-MA-20	Donald Smith
PP2-MB-01	Pierre-Antoine Noceto	PF-MB-04	Williams O. Anteyi	PP2-MA-21	Beatriz R. Vazquez-de-Aldana
PP2-MB-02	Thure Pavlo Hauser	PF-MB-05	Leone Olivieri	PF-MA-01	Mirjam Seeliger
PP2-MB-03	Tomasz Maciag	PF-MB-06	Shivani Khatri	PF-MA-02	Daniel Wipf
PP2-MB-04	Louisa Robinson Boyer	PF-MB-07	Daria Rybakova	PF-MA-03	Annamaria Bevivino
PP2-MB-05	Maria Isabella Prigigallo	PF-MB-08	Valeska Villegas Escobar	PF-MA-04	Francisco Martin Usero
PP2-MB-06	Franz Stocker			PF-MA-05	Mohammed Antar
PP2-MB-07	Gwendolin Wehner	Poster #	Microbial applications for improving nutrition and abiotic stress tolerance	PF-MA-06	Shubhangi Sharma
PP2-MB-08	Hadis Jayanti	PP2-MA-01	Francisco Javier Gutierrez-mañero		
PP2-MB-09	Alessandro Passera	PP2-MA-02	Antoine Persyn	Poster #	Disruptive approaches for engineering the phytobiome & microbial delivery
PP2-MB-10	Lisa Kappel	PP2-MA-03	Allene A. Macabuhay	PP2-DA-01	Lilach Iasur-Kruh
PP2-MB-11	Daniel Uribe	PP2-MA-04	Daniel Buchvaldt Amby	PP2-DA-02	David L. Hallahan
PP2-MB-12	Marco Saracchi	PP2-MA-05	Markus Weinmann	PP2-DA-03	Soon-Kyeong Kwon
PP2-MB-13	Wolfgang Hinterdobler	PP2-MA-06	Yoshinari Ohwaki	PP2-DA-04	Randy Martin
PP2-MB-14	Abhishek Anand	PP2-MA-07	Julian Preiner		
PP2-MB-15	Birgit Jensen	PP2-MA-08	Zhichun Yan	Poster #	Varia
PP2-MB-16	Marta Streminska	PP2-MA-09	Juliya Thomas	PP2-V-01	Jasper Schierstaedt
PP2-MB-17	Rachel Backer	PP2-MA-10	Agnieszka Domka	PP2-V-02	Martina Sauert
PP2-MB-18	Sabine Gruber	PP2-MA-11	Patricia Dorr de Quadros	PP2-V-03	Johannes Ben Herpell
PP2-MB-19	David B. Collinge	PP2-MA-12	Annapurna Kannepalli	PP2-V-04	Tanja Kostic
PP2-MB-20	Leandro Astarita	PP2-MA-13	Md Mohibul Alam Khan	PP2-V-05	Humberto Castillo Gonzalez
PP2-MB-21	Kumar Aundy	PP2-MA-14	Sowmyalakshmi Subramanian	PP2-V-06	Prashantee Singh
PP2-MB-22	Anthi Vlassi	PP2-MA-15	Bong-Nam Chung		
PP2-MB-23	Giovanni Bubici	PP2-MA-16	Xu Cheng		
PP2-MB-24	Eliane R. Santarém	PP2-MA-17	Jakub Jez		
PF-MB-01	Alejandro del Barrio Duque	PP2-MA-18	Przemysław Bernat		
PF-MB-02	Lara Reinbacher	PP2-MA-19	Kerrie Farrar		

LECTURES

miCROPe 2019 - Microbe-assisted crop production opportunities, challenges & needs
Vienna, Austria, December 2 – 5, 2019

Opening & Opening Lecture

Chair: Angela Sessitsch

Supported by



O-01 Assembly of epiphytic bacterial communities on plants and their interactions with the plant host: insights for managing the plant microbiome

Steven Lindow

Plant and Microbial Biology, University of California, Berkeley, United States of America

Aerial plant surfaces often harbor large epiphytic bacterial populations. The size and composition of these communities however are determined by both small-scale interactions of bacteria with each other and with their plant host that determine growth and survival, as well as large-scale features such as the proximity and abundance of other plant species that contribute immigrant inoculum. The maximum population size of epiphytic bacteria is limited by Carbon availability on the plant surface and differs among plant species due to the differing amounts of exudates. These Carbon sources and therefore sites of bacterial colonization on plants are spatially heterogeneous, with the majority of bacteria residing in localized sites harboring relatively large, mixed species cellular aggregates. Cell density-dependent behaviors, often modulated by so-called quorum sensing signal molecules facilitate preferential survival of bacteria at such sites during stressful desiccation conditions. Bacteria also modify the local environment on plant surfaces by their production of hygroscopic biosurfactants that make liquid water more available. Many bacteria also produce compounds such as 3-indole acetic acid (IAA) that apparently facilitate the plant conversion of sucrose to fructose, thus facilitating the growth of epiphytes that typically can consume such monosaccharides at the relatively low concentrations made available by exhibition from plants, but which cannot consume disaccharides at such low concentrations. The composition of epiphytic bacterial communities is only moderately plant species-specific, apparently driven by yet to be determined morphological and chemical features of plant surfaces. Epiphytic bacteria readily escape from the surface of plants and strongly influence the composition of airborne bacteria nearby. Such airborne bacteria are a primary source of immigrant bacteria for the establishment of epiphytic communities on plants that typically harbor few or no resident bacteria early in their development. Because of the differing amounts and types of surrounding vegetation present during the development of new tissues of a given plant species, the composition and size of epiphytic communities on crop species is very context-dependent, and can be strongly influenced by management practices that influence the agro-ecological context of a given crop plant and can be strongly influenced by inoculation of immigration-limited crops by beneficial bacteria.

miCROPe 2019 - Microbe-assisted crop production opportunities, challenges & needs

Vienna, Austria, December 2 – 5, 2019

Successful microbial applications

Chairs: Kellye Eversole & Angela Sessitsch

SA-01 An integrated technology pipeline for the development of superior agricultural biologicals.

Steven Vandenabeele

Aphea.Bio, Belgium

Agricultural biologicals is the broad term for naturally occurring materials such as microorganisms and natural extracts that have the potential to improve the health status and yield of crops. Biologicals can complement or substitute agricultural chemical products and form a cornerstone in the path towards an urgently needed sustainable, integrated agriculture.

Aphea.Bio's mission is '*Applied Nature for Better Agriculture*' and develops novel and superior agricultural biologicals. Aphea.Bio focusses on microorganism-based products that help reducing fertilizer application and controlling fungal diseases for maize and wheat and is in a unique position to deliver novel and powerful solutions to the market because of its innovative research platform. Therefore, it studies and exploits the *in nature* occurring beneficial interactions between plants and soil microorganisms. By identifying amongst the hundred thousands of microorganisms present in the soil those that closely and actively interact with the plant, Aphea.Bio is able to boost plant growth and health by applying the beneficial microorganisms as a seed coating or a sprayable.

Aphea.Bio has built a collection of wheat/maize biostimulant candidate products that are being validated in field trials across the EU. The biostimulant R&D pipeline comprises different steps: a vast microbiome mapping approach across based on wheat and maize rhizo- and endospheres grown in ~100 different low nutrient soils, the proprietary microbial culturing technologies that allows to tap into the 'unculturable' microbial strain pool, the high-throughput phenotypic *in planta* screening in the greenhouse and the initial field trial results will be presented. Besides, in the biocontrol program, the screening of ten thousands of microbial extracts against *Fusarium graminearum*, *Zymoseptoria tritici* and *Puccinia striiformis* forms the basis for a portfolio of microbial strains that significantly reduce disease symptoms *in planta* in the climate chambers. Screening of the lead strains against other major fungal diseases in other crops such as vegetables are performed to test their broad efficacy. In this presentation, an overview of the biocontrol and biostimulant technologies will be discussed.

SA-02 Concept for a reasonable use of mycorrhizal fungi in green business

Carolin Schneider¹, Louis Mercy¹, Eva Lucic¹, Alberico Bedini¹, Alicia Varela Alonso², Philipp Rödel², Stéphane Declerck³, Philipp Franken⁴

¹ Inoq GmbH, Germany

² Institut für Pflanzenkultur, Solkau 2, 29465 Schnega, Germany

³ Université catholique de Louvain, B-1348 Louvain-la-Neuve, Belgium

⁴ Erfurt Research Centre for Horticultural Crops, University of Applied Sciences Erfurt and Friedrich Schiller University Jena, Kühnhäuser Straße 101, D-99090 Erfurt, Germany

There is a discussion ongoing about the management of arbuscular mycorrhizal (AM) fungi in large-scale agriculture. Recent papers reviewed management practices of industrial agriculture (without additional inoculation of AM fungi), their impact on abundance and diversity of the symbionts and on crop yield, but concluded little evidence that mycorrhizal fungi need to be a target of management, at least in wheat. Others found that given the need to feed more people in the world, the yield is not the only parameter to consider, but long-term sustainability and especially yield stability of agroecosystems will become even more important: AM fungi and other soil biota make important contributions to soil aggregation and many other ecosystem functions, e.g. yield stability under changing environmental conditions. Manifold studied improvements in nutrient uptake through AM fungi may reduce the need for fertilizer, whilst achieving an equal yield. Even after harvest, AM fungi can enhance food storage properties. New results of biotisation with mycorrhiza and bacteria in phytopharmaceutical drug production will be highlighted in the presentation.

If everything would be simple and positive, the use of AM fungi inoculum and the awareness of farmers to manage AM fungi and other soil biota would be routine, but this is only the case for a limited number of crops and environments. For sure there are still important questions to answer before we will be able to master the application and predict the effect of mycorrhizal fungi in industrial agriculture, among them the factor implementing plant-fungi interactions in plant breeding. Here new results of the concept of training of AM fungi to subsequent environmental conditions will be presented. This includes the use of root organ cultures for acclimatization to high Pi, and the response of the acclimatized AM fungal strain to different stimuli (strigolactones and different Pi levels) during the pre-symbiotic and the symbiotic phase.

SA-03 The successful history of *A. brasilense* Az39 in Agriculture. A metadata analysis

Belén Rodríguez, Sofía Nieves, Gastón López, Romina Molina, Anahí Coniglio, Verónica Mora, **Fabrizio Cassán**

Laboratorio de Fisiología Vegetal y de la Interacción Planta-Microorganismo, Universidad Nacional de Río Cuarto, Argentina

Azospirillum is one of the most studied bacterial genera in the last 60 years; However, the history of the appearance of biological products formulated with this bacterium began in the 1980s, but intensified in the last 20 years in Argentina, Brazil and the rest of South America. In the case of Argentina, *A. brasilense* Az39 is the strain that has been recommended for more than 40 years for the production of biofertilizers for wheat, sorghum, corn and soybean (co-inoculation). This strain has demonstrated a great capacity to promote plant growth with average yield increases greater than 10.0% and a success rate higher than 70% in different crops in thousand experiments. Despite the immense amount of information available at the agronomic level, until a few years ago very little was known about the molecular basis that determined the ability of this strain to promote plant growth. In 2012, the Laboratorio de Fisiología Vegetal y de la Interacción Planta-Microorganismo of the Universidad Nacional de Río Cuarto conformed an international consortium with the aim to analyze the genome sequence of *A. brasilense* Az39 and *B. japonicum* E109, two of the most used strains for biofertilizer production in South America. Using a combined sequencing strategy, it was established that the Az39 genome has a size of 7.39 Mpb distributed in 6 replicons [1 chromosome, 3 chromides and 2 plasmids]. Through the use of comparative bioinformatics tools, numerous genes and putative proteins involved in the expression of plant growth promotion mechanisms and other related with the rhizosphere lifestyle were identified. The decoding of this information has provided a solid basis for the elucidation of new mechanisms of interaction and growth promotion, as well as some specific components that would determine the agronomic success of this microorganism. In this presentation we will address some of the new biological models recently identified for this bacterium and how they affect their rhizosphere lifestyle.

* BR and SN equally contributed to this work

Financial Support: This work was supported by Consejo Nacional de Investigación Científico-Tecnológica de Argentina (CONICET) and FONCyT through your projects PICT 2012, 2015 and 2017.

SA-04 Market 2 Research 2 Market – The Multikraft Model

Robert Rotter

Multikraft, Austria

How a former stock food company started to use an unknown microorganisms-mix 22 years ago and how this started a new field of successful household and professional animal and plant applications.

How basic EM (Effective Microorganisms)-Technology was transferred into Research based Multikraft-Technology, the main microbial isolates and connecting ingredients of different applications.

In-depth R&D to improve inner properties, quality and effects of this technology AND how research is transferred to market.

How our professional customers learn to act preventative using microbial applications rather than reacting on their problems in conventional ways with less positive effects.

miCROPe 2019 - Microbe-assisted crop production opportunities, challenges & needs

Vienna, Austria, December 2 – 5, 2019

Mechanisms mediating holobiont and multipartite interactions

Chairs: Alga Zuccaro & Paolina Garbeva

MI-01 Molecular basis of plant-microbe interaction: know-how and tools for designing microbial communities with beneficial effects on plant growth

Alga Zuccaro

University of Cologne, Germany

Progress achieved in multipartite interactions allows us now to characterize the mechanisms underlying microbe-plant symbioses in a community context and thus achieve a step change in understanding the functional interconnections between soil, microbiota and plants. Here we address how interaction between the beneficial root endophyte *Serendipita vermifera* and the pathogen *Bipolaris sorokiniana* affects fungal behavior and barley host responses in a microbial community context.

MI-02 The importance of microbial chemical interactions for plant and soil health

Paolina Garbeva

Microbial Ecology, NIOO-KNAW, Netherlands

Microorganisms produce a vast array of secondary metabolites, both soluble and volatile, which have diverse and important biological functions.

The production of microbial metabolites is often triggered by intra- and inter-specific microbial interactions. For example, the antimicrobial volatile compound 2,5-bis(1-methylethyl)-pyrazine is produced as a result of the interaction between the Gram-positive *Paenibacillus* sp. and the Gram-negative *Burkholderia* sp..

In my talk, I will focus on belowground interactions and discuss some of the microbial metabolites involved in microbe-microbe and plant-microbe interactions. I will highlight the ecological importance of microbial chemical interactions for plant and soil health.

MI-03 The functional ecology of plant microbiome interactions between the dominant fungal wheat pathogen *Zymoseptoria tritici* and *Pseudomonas* bacteria

Luzia Stalder¹, Monika Maurhofer², Daniel Croll¹

¹ Biology, University of Neuchâtel, Switzerland

² Group of Plant Pathology, Institute of Integrative Biology, ETH Zurich, Zurich, Switzerland.

Plants are exposed to a wide range of pathogenic fungi and bacteria. It has been shown that the outcome of individual interactions between pathogen and plant cannot be understood in isolation, as the presence of other microorganisms can act synergistically or antagonistically in the disease progression. Yet, factors governing complex (i.e. at least tripartite) interactions are largely unknown. Here, we establish a new microbiome interaction model using tripartite interactions of bacteria, fungi and plants. For this, we focus on wheat, *Zymoseptoria tritici*, the major fungal pathogen of wheat, and the bacteria *Pseudomonas*, a dominant member of the phyllosphere. We characterize how intra-specific variation in a fungal pathogen determines microbial activities in the phyllosphere using genome-wide association mapping. In addition, we will characterize how differential gene expression of the fungus and the bacteria influences the outcome of bacterial-fungal competition. Our results will provide insights into the mechanism of competitive exclusion in the phyllosphere microbiome. We will generate knowledge of the exact loci that fungi evolved as defenses against *Pseudomonas*. The identification of such previously unknown loci will likely reveal previously unknown antimicrobial compounds that could be assessed for agricultural and even human applications.

MI-04 Modulation of rhizosphere microbiomes to suppress phytonematodes

Ahmed Elhady, Johannes Hallmann, Holger Heuer

Institute for Epidemiology and Pathogen Diagnostics, Julius Kühn-Institut (JKI), Germany

In soil, beneficial and pathogenic biota simultaneously colonize the plant rhizosphere. Among the harmful organisms are plant-parasitic nematodes that are economic threats worldwide. They migrate through the rhizosphere to their host plants and live on the cytoplasm of living root cells. Plants influence the microbiome in their rhizosphere and thereby pass a modified microbiome on to the plant subsequently growing in the same soil. In this study, we investigated the effect of plant-soil feedback of different pre-crops rotated with soybean in order to suppress root lesion nematodes (RLN). Transplanting the rhizosphere microbiome from different crops resulted in different degrees of suppressiveness against RLN on soybean roots. The inoculated microbiomes from soybean, Ethiopian mustard and maize significantly reduced the invasion of RLN compared to the microbiomes from bulk soil or tomato rhizosphere. In the analogous experiment with tomato plants and either RLN (*Pratylenchus penetrans*) or root-knot nematodes (*Meloidogyne incognita*), the microbiomes from maize and tomato reduced root invasion of both nematodes compared to the microbiomes from soybean or bulk soil. In a split-root experiment, the suppressive effect of the microbiome on *P. penetrans* was mediated by the plant and depended on the plant species from which the microbiome was transplanted. The DGGE fingerprints of the fungal and bacterial communities of the donor rhizospheres significantly differed among the treatments, as well as the fungal and bacterial communities attached to the surface of RLN that were recovered from those rhizospheres. This implied that attached microbes might antagonized the RLN, directly and/or by signals to the plant. Engineering the plant associated microbiome through pre-, cover- or inter-crops may lead to eco-friendly crop protection.

PF-MI-01 Long-term organic and mineral fertilization strategies shape the rhizosphere microbiota and performance of lettuce

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Belowground plant-microbe interactions are crucial for plant development and health. Although previous studies have shown that soil microbial communities are influenced by fertilization strategies, less is known about the aboveground plant response to the rhizosphere microbiota assemblage shaped by agricultural management strategies. In our study, we aimed to investigate the effects of long-term fertilization strategies across field sites on the rhizosphere prokaryotic (Bacteria and Archaea) community composition and plant performance. We conducted growth chamber experiments with lettuce (*Lactuca sativa* L.) cultivated in soils from two long-term field experiments situated in Therwil, Switzerland and Thyrow, Germany, each of which compared organic vs. mineral fertilization strategies. High-throughput sequencing of bacterial 16S rRNA genes amplified from total community DNA showed a rhizosphere core microbiota shared in all lettuce plants across soils, going beyond differences in community composition depending on field site and fertilization strategies. Firmicutes were enriched irrespective of the field site in the rhizosphere of lettuce grown in organically fertilized soils. When cultivated in organically fertilized soils, a higher expression of several stress-related genes was observed by RT-qPCR analysis in lettuce leaves although plants were visibly free of disease symptoms. Another experiment showed that in presence of the soil-borne model pathogen *Rhizoctonia solani* AG1-IB, the plant productivity (dry biomass) decreased in soils from Thyrow with both long-term organic and mineral fertilization strategies. Moreover, we observed that the expression of genes like *BGlu42* (β Glucosidase), *OPT3* (Iron transporter) and *MYB15* (Transcription factor) were significantly higher in the plants grown in organically fertilized soils in presence of *R. solani*. This could indicate an ISR response via iron-mobilizing phenolics, simulating root iron-deficiency response and changes in iron-homeostasis mechanisms in the rhizosphere, which can be expressed systemically throughout the plant. The ongoing analysis of the rhizosphere microbiome would reveal more information about the suggested mechanism. Taken together, besides effects of fertilization strategy and field site, results of our study under controlled conditions demonstrate the crucial role of the lettuce plant in driving the rhizosphere microbiota assemblage.

PF-MI-02 Soil glyphosate treatment impacts plant endophytic communities in plant species specific manner

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Glyphosate (N-phosphonomethylglycine) is a broad spectrum herbicide, used in weed killing products over four decades, and currently the most commonly used herbicides in agricultural systems and landscaping in EU. Glyphosate is applied before crops are sown, and facilitate better growth of crops by eliminating competing weeds. In soil, glyphosate is quickly adsorbed to soil particles and degraded by soil microbes. Currently, limited information is available on impact of glyphosate on structure and functioning of non target microbial communities, and no information is at hand on impact on plant microbiomes, especially in cold climate agrosystems, where glyphosate degradation might be impacted by cold climate.

In this study, we investigated the impact of soil glyphosate treatment on microbial communities of five different agricultural plant species: faba bean, meadow fescue, oat, potato and hemp. We treated the sample plots (12 replicate plots per treatment) with glyphosate salt or control solution (no glyphosate) twice a year (spring and autumn) for several years. The plants were sown two weeks after glyphosate (or control) treatment, and were harvested in August. Microbial communities were analysed by 16S rRNA gene (bacteria) or ribosomal ITS (fungi) targeted sequencing from community DNA isolated from plant leaves, roots and from bulk soils.

At the time of sampling, no glyphosate was detected in soil or in plant tissue samples, except in potato roots. In agreement with previous studies, we saw no impact of glyphosate treatment on diversity or structure of soil bacterial communities. Plant bacterial communities were primarily impacted by plant species and tissue (leaf, root, nodule). Species richness of faba bean and potato bacterial communities were lower than in other plant species. Glyphosate treatment had no impact on species richness of leaf or root microbial communities. However, bacterial community

structures were impacted in most of the plant species. The community structures in potato roots, and in the leaves of meadow fescue, hemp (OTU level) and oat (family level) were significantly different in plants from glyphosate treated and non treated plots. These differences were driven by significantly lower abundances of several OTUs representing bacterial genus *Pseudomonas* and families *Xanthomonadaceae* and *Rhizobiaceae* in plants grown in glyphosate treated soils.

PF-MI-03 Can soil microbes enhance plant health without direct contact?

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To cope with the different biotic and abiotic stresses that they encounter during their growing phase, plants have evolved different defense strategies that include the recruitment of beneficial soil-borne microorganisms. These microbial partnerships comprise direct colonization and direct protection of the plant tissues, but interestingly also priming of plant defense and growth promotion. Remarkably, it was recently found that in the absence of direct physical contact, plants can still perceive soil microbes *via* volatiles, and respond to these odour cues. Volatiles are commonly produced by microbes, yet the specificity of plant responses to volatiles emitted by microbes of different lifestyles has been overlooked. In this presentation, I will address the effects of volatiles emitted by a range of soil-borne fungi, including plant pathogens and plant mutualists, on plant growth, flowering and resistance. I will discuss how fungal volatiles can influence plant interactions with insect pests, both aboveground and belowground, and affect plant performance. Addressing microbial volatiles as part of the phytobiome opens up new potential applications for crop protection.

PF-MI-04 Wanted: helper bacterial strains enhancing the biocontrol activity of *Lysobacter capsici* AZ78

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Bacterial biocontrol agents (BBCAs) represent a promising strategy for the sustainable management of soilborne plant pathogens. Nonetheless, their efficacy in field often differs from the one achieved in controlled conditions. This might be related to the biological complexity of agricultural soils. Indeed, BBCAs might be considered as invaders that must compete with and integrate in resident soil microbial communities. At the same time, occurring microbial interactions can positively influence the establishment and functioning of a given BBCA. Based on this assumption, the aim of the work herein was to select helper bacterial strains (HBS) boosting the biocontrol activity of the model BBCA *Lysobacter capsici* AZ78 (AZ78) and understand the mechanisms regulating the interaction. A collection of 36 bacterial isolates deriving from tomato rhizosphere was screened for evaluating their impact on the biocontrol activity of AZ78 and further characterized. 16S rRNA gene sequence analysis showed high microbial diversity among the isolates. Out of the 36 isolates, 15 enhanced the activity of AZ78 against *Pythium ultimum* *in vitro*.

The enhancement of AZ78 biocontrol activity might be associated to the establishment of inter-specific interactions based on the release and perception of quorum-sensing (QS) signal molecules between the HBS and AZ78. Interestingly, *Ensifer adherans* strains producing long chain (C6-C12) N-acyl-homoserine lactones (AHLs) QS molecules boosted the production of antimicrobial compounds active against *P. ultimum*. In contrast, a similar positive effect on AZ78 inhibitory activity was not observed in one *E. adherans* strain producing C4 AHLs. However, other bacterial strains belonging to *Achromobacter deleyi*, *Stenotrophomonas tumilicola*, *Variovorax boronicumulans* boosted AZ78 inhibitory activity even if they were unable to produce AHLs. Based on these results, further investigations will be dedicated to understanding how QS signals modulate AZ78 biocontrol activity.

MI-05 Effects of plant biostimulant treatments on the root-associated fungi of wheat and barley

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One of the great challenges the society faces today is to reduce negative impacts of agriculture on the environment while maintaining high crop yields. A promising strategy is to use environmentally friendly plant biostimulants which can enhance crop growth performance, for example, by affecting plant metabolism. However, there has been a lack of knowledge on the effects of biostimulants on the interaction of plants with root-associated fungi which represent important drivers of soil and plant functioning.

In this work, the effects of protein hydrolysate-based biostimulants on field-grown wheat and barley and their root-associated fungi were investigated. Plant biostimulants were foliar-applied in the stem elongation stage (BBCH31) and the plants were sampled 14 and 56 days after the treatments. We aimed to determine putative changes in the root-associated fungal symbiotic communities over time, along with plant growth responses to the biostimulants. Leaf/root dry weight, leaf chlorophyll content and plant height were positively affected by the biostimulant treatments depending on crop species and a type of biostimulating product.

Biostimulants did not affect the root colonization by mycorrhizal fungi in either crop but they significantly increased the abundance of vesicles/spores in comparison with controls in wheat. The metabarcoding of root fungal biodiversity revealed that the community composition was significantly affected by the sampling time, but not by the treatments. Few dominant fungal species differed in the abundance among the treatments. The abundance of *Glomerales* was highest in wheat treated with protein hydrolysates but not statistically different from the controls. Based on our results, protein hydrolysate treatments that promote plant growth do not significantly influence the composition of root-associated fungal communities, though their side contribution to the biostimulants' effect cannot be excluded. More extensive research is necessary to decipher mechanisms of plant-mediated effects of protein hydrolysates on root-associated fungi.

MI-06 Bacterial communities associated with hyphae of plant beneficial fungal biofertilizers

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Fungi from the genus *Penicillium* colonize the rhizosphere and solubilize inorganic phosphate (P), thereby potentially increasing P availability to plants. We have isolated beneficial bacteria from *Penicillium* hyphae that promote fungal growth and P solubilization. Exploiting this positive interaction has high potential for development of biofertilizer consortia for increased plant nutrient use efficiency. However, the main drivers for assembly of hyphae-associated bacterial communities and for their abilities to solubilize or mobilize P in soil remain elusive. We developed a novel baiting type microcosm to study bacterial colonization of hyphae in soil. The approach was used to investigate the impact of soil type on bacterial communities associating with hyphae of two *Penicillium* species. 16S rRNA gene targeted sequencing analysis showed that hyphae-associated communities had lower diversity and less variation in taxonomic structure than soil communities. Besides the hyphosphere effect, the soil type had a large impact on hyphae-associated communities. In particular, soil properties as pH, total carbon, concentrations of P and Mg, as well as soil texture significantly affected the relative abundance of several higher taxa. In contrast, the effect of fungal species was visible only for few discriminative taxa and specific enriched OTUs. qPCR analysis revealed increased abundance of genes involved in inorganic or organic P cycling in several hyphae-associated communities. Taken together, the *Penicillium* hyphosphere represents a unique niche, where soil type and fungal species together orchestrate microbiome assemblage and where fungal as well as bacterial activities may create a hot spot for P turnover. The current study provides a knowledge base important for future development of robust biofertilizer consortia.

miCROPe 2019 - Microbe-assisted crop production opportunities, challenges & needs

Vienna, Austria, December 2 – 5, 2019

Plant understanding of interactions with beneficial microbes

Chairs: Heribert Hirt & Adam Schikora

PU-01 Lessons from desert microbes to enable saline agriculture on arid lands

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A major global challenge of any country in this century is to achieve food security. This is largely hindered by excessive heat, salinity and a lack of water, making up for 60% of annual yield losses. Moreover, 20% of Earth's land surface are made up of desert regions, which are currently considered unfit for agriculture. A simple solution to the above challenge would be to expand agriculture to so far unused land and use abundantly available saline water. Since most crops lack the ability to cope with salinity, major plant breeding efforts are underway to enhance crop tolerance to salt stress. However, these costly and long-term approaches so far provided mostly disappointing results. In contrast, rhizosphere microbes from desert plants showed that various crops can be grown on marginal lands using saline irrigation, making a big step forward towards food security in the future. We show that the certain desert bacterial endophytes can enhance salt tolerance of crops by favourably reducing salt uptake into the shoots. A molecular analysis of the plant-microbial interaction in *Arabidopsis* unravelled a major role of the sulfur pathways in both organisms and a coordinate regulatory role of plant ethylene signalling in this process. These findings open new possibilities for breeding salt-adapted crops and tailoring functional synthetic communities to complement deficiencies in soil, crop and disease resistance.

PU-02 Protists within rhizosphere microbiome determine plant health

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Plant health is intimately controlled by the associated rhizosphere microbiome. Most microorganisms interact positively or neutrally with plants, thereby mitigating negative pathogen-induced effects. However, most microbiome research is focusing on bacteria. As such, it remains unknown if other microbial components, especially the main bacterial predators – protists – are linked to pathogen suppression. In a field setting, we monitored the rhizosphere microbiome throughout the growth of tomato plants, that either developed disease symptoms induced by the bacterial pathogen *Ralstonia solanacearum* or remained healthy. To explore potential underlying mechanism that determine plant performance, we investigated the taxonomic and functional structure of protist communities and linked those with fungi, bacteria, pathogen and bacterial-produced secondary metabolites biosynthesis genes. We show that pathogen development is best predicted by the community structure of protists. In line with bacteria, the community structure of protist consumers (*i.e.*, phagotrophs) at the start of plant differ between later diseased and healthy plants could serve as important indicator of plant health. The relative abundance of phagotrophs negatively correlated with pathogen abundance, suggesting direct predator-prey interactions of protists leading to pathogen declines. In addition, protist community composition was linked with distinct bacterial communities in healthy compared with later diseased plants; this link led to increased expression of secondary metabolite genes that are linked with disease suppression. Therefore, we highlight that protists integrate rhizosphere microbiome structure and functioning throughout plant growth, determining plant performance. This illustrates the potential to both predict plant performance based on initial screening of protist communities and for targeted application of protists to steer microbiome structure and functioning to increase plant performance.

PF-PU-01 Bacteria-flower interactions: bacterial modifications of floral sugar and scent composition result in changes in pollinator behavior and plant reproduction

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Bacteria play crucial roles in plant growth, development, and health and we are slowly beginning to acknowledge their involvement in floral ecology. We focused on two bacterial strains (*Pseudomonas syringae* and *Pantoea agglomerans*) that commonly colonize above-ground plant surfaces including flowers to investigate their effects on floral phenotypes, pollinator behavior and plant reproduction. The genera *Pseudomonas* and *Pantoea* were commonly associated with several plant species along a land-use gradient. Sugar composition of plant surfaces was found to be plant organ and species specific. In lab-experiments we found that *P. syringae* and *P. agglomerans* differently respond to sucrose, glucose and fructose and selectively remove sugars from media containing all three sugars. Thus, flowers may be able to control bacterial community composition by the sugars provided as carbon sources. In turn, bacteria may alter availability of sugars on flower surfaces and nectar. In further experiments, we found similar effects in interactions between bacteria and floral scent compounds. Sugar and floral scent compounds are key in mediating flower-pollinator interactions. Accordingly, we found that *P. syringae* and *P. agglomerans* affect the behavior of honeybees and bumblebees in the lab, and whole flower visitor communities in the field. These effects on pollinator behavior resulted in increased plant reproduction. Our study reveals mechanisms how flowers may control bacterial community composition and how bacteria affect plant reproduction with implications for field applications to increase crop yield.

PF-PU-02 F3H plays a pivotal role of on flavonoid metabolism improving adaptation to biotic stress in blackberry

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The aim of this study is to determine the involvement of the flavonol-anthocyanin pathway on plant adaptation to biotic stress using the *B. amyloliquefaciens* QV15 to trigger blackberry metabolism and identify target genes to improve plant fitness and fruit quality. To achieve this goal, field-grown blackberries were root-inoculated with QV15 along its growth cycle. At fruiting, a transcriptomic analysis by RNA-Seq was performed on leaves and fruits of treated and non-treated field-grown blackberries after a sustained mildew outbreak; expression of the regulating and core genes of the Flavonol-Anthocyanin pathway were analysed by qPCR and metabolomic profiles by UHPLC/ESI-qTOF-MS; plant protection was found to be up to 88%. Overexpression of step-controlling genes in leaves and fruits, associated to lower concentration of flavonols and anthocyanins in QV15-treated plants, together with a higher protection suggest a phytoanticipin role for flavonols in blackberry; kempferol-3-rutinoside concentration was strikingly high. Overexpression of *RuF3H* (Flavonol-3-hydroxylase) suggests a pivotal role in the coordination of committing steps in this pathway, controlling carbon flux towards the different sinks. Furthermore, this C demand is supported by an activation of the photosynthetic machinery, boosted by a coordinated control of ROS into a sub-lethal range, and associated to enhanced protection to biotic stress

PF-PU-03 Transcriptional response of tomato plants to the growth stimulation provided by *Gluconacetobacter diazotrophicus*

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Gluconacetobacter diazotrophicus (Gd) is a non-nodulating endophytic nitrogen-fixing bacterium able to colonise a wide range of crops and to provide beneficial effects to the plant. Besides the production of phytohormones, Gd ability of fixing nitrogen for the plants consumption makes it a good candidate for applications as biofertiliser. The interaction mechanism between Gd and the plant has been partly characterised in sugar cane, but little information is available on interaction mechanisms with other plants.

The aim of this project was to evaluate the effect of Gd inoculation on tomato plants and to elucidate the molecular mechanism of this interaction.

In this study, a wild type (WT) and a nitrogen fixation-impaired strain (*nifD*⁻) of Gd were employed. Tomato seedlings were grown at two different nitrogen levels (0 mM and 2 mM KNO₃) for 10 days and the Gd bacterial suspension was then applied to tomato roots. Four days after incubation, the bacterial suspension was removed and plants were incubated for 14 days before phenotypical evaluations. At 0 mM KNO₃, WT-inoculated plants showed an increase in chlorophyll content and shoot length in comparison to uninoculated (U.T.) and *nifD*⁻-inoculated plants. Comparison between U.T. and *nifD*⁻-inoculated plants showed no difference in chlorophyll content and shoot length. At 2 mM KNO₃, WT-inoculated plants presented an increase in chlorophyll content and shoot length in comparison to both U.T. and *nifD*⁻-inoculated plants. Plant length was positively affected by *nifD*⁻ inoculation in comparison to U.T.. The positive effect provided by *nifD*⁻ inoculation was weaker in comparison to the one provided by WT inoculation. Comparison between U.T. and *nifD*⁻-inoculated plants displayed no difference in terms of chlorophyll content.

Root samples were collected at one and 14 days after inoculation from U.T., WT- and *nifD*⁻-treated plants and subjected to RNA-Seq analysis. Functional annotation of differentially expressed genes is currently in progress in order to identify transcriptional regulations responsible for the early and late response of tomato plants to Gd.

PF-PU-04 Plant growth promoting bacteria promotes germination and enhances early root traits

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Holistic understanding of the relationship of plant trait functionalities (roots and shoots) with beneficial microbes requires advanced technological platforms. In past, various, mostly destructive methods have been used to measure plant microbe interaction, which are not able to quantify the dynamic reaction of plants to microbes. Our major goal was to validate if non-invasive high throughput phenotyping platforms such as *GrowScreen-PaGe* (germination paper based; Gioia et al., 2016) and *GrowScreen-Rhizo* (soil-filled rhizotrons; Nagel et al., 2012) enable quantification of the dynamic effects of plant microbes on root and shoot traits. We monitored the effect of plant growth promoting bacteria (PGPB) on Soybean roots and shoots and quantified the associated microbial abundance on roots and growth media (paper and soil) at three depth layers. Our results show that PGPB promotes early germination and increases root traits on germination paper as well as in soil-filled rhizotrons. The results indicate that both phenotyping approaches were efficient to quantify responses of root and shoot traits during plant-microbe interaction. The results will shed new insights into the dynamics of plant microbe interactions and novel application options for utilizing biologicals for crop improvement.

PU-03 Understanding molecular, metabolic and phylogenomic events underlying Arbuscular Mycorrhizal Symbiosis: Scope for improving crop productivity

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Arbuscular Mycorrhizal Fungi (AMF) are mutualists that colonize more than 80% terrestrial plants. This study uses *in vitro* hairy root cultures to analyze the regulatory control exerted over AMF by the plant hosts and non-hosts by integrating transcriptome, metabolome and phylogenomic analysis. We have screened 21 hairy root cultures using bright field microscopy and ink vinegar staining approach to identify a host (Tomato Roma) and a non-host (Tomato Graftor) of AMF *Rhizophagus irregularis*. Bi-compartmental studies comprising of a mycorrhiza established host (*Daucus carota*) was used to re-confirm non-host status of Tomato Graftor. Comparative transcriptomics of mycorrhized host, blank host and blank non-host revealed >2000 differentially expressed genes (DEGs, FDR 0.01). KEGG pathway analysis of DEGs was used as a reference for complete metabolomic profiling aimed at analyzing AMF-specific early signalling patterns distinguishing a host from a non-host tomato root culture. Top 50 DEG hits were functionally characterized *in silico* and among these DEGs most relevant 12 gene-targets were subjected to phylogenetic analysis in 7 hosts and 4 non-host plant species for identifying the pattern of gene convergence and/or divergence which could trace the evolutionary molecular patterns for adaptations favouring AM symbiosis in hosts. Further, we have proposed the concept of conditional non-host (Tomato Graftor) versus absolute Non-host (*Arabidopsis thaliana*, *Brassica rapa*, *Beta vulgaris* and *Nelumbo nucifera*). Understanding the molecular basis of AM symbiosis distinguishing a host from non-host might provide scope for introducing crops that are modified to attain better nutrient uptake, crop productivity, drought and pathogen tolerance. This study paves the way for application of mycorrhization in agriculturally relevant host plants.

PU-04 Co-inoculation of rice plants with nitrogen-fixing and indole-3-acetic acid (IAA)-producing endophytes: changes in physiological parameters of the host plant.

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To cope with the growing world population an increase in the production of the main crops for human nutrition, including rice, is now urgently needed. To achieve this goal, the expensive and polluting chemical fertilizers have already been overused. Nitrogen (N) is one of the primary nutrients limiting plant growth in agriculture. Biological nitrogen fixation (BNF) by diazotrophic bacteria, which reduce atmospheric N to ammonium using nitrogenase enzyme systems, accounts for 30-50% of the total N in crop fields. The area of BNF research has been expanded by the discovery of N-fixing bacterial endophytes in non-nodulating plants. In the last few years a wide diversity of bacteria associated with cereals have shown to possess the *nifH* gene coding for dinitrogenase reductase. This gene is genetically conserved and thus traditionally used as a marker gene to study the genetic diversity of diazotrophs in nature. To improve plant growth and yield the use of genetically modified diazotrophs or the co-inoculation with nitrogen-fixing and plant growth promoting bacteria has been proposed. We have previously reported that the strain *Enterobacter cloacae* RCA25-64, engineered to produce and release 36-fold more indole-3-acetic acid (IAA) than the wild type *E. cloacae* RCA25, showed increased *nifH* gene expression and nitrogenase activity in liquid cultures and inoculated rice plants. In the present study we analysed the effect of purified IAA on the nitrogen-fixing ability of *E. cloacae* RCA25. Co-inoculation studies were also carried out to test the ability of different wild type IAA-producing endophytes to enhance the *nifH* gene expression and nitrogenase activity in *E. cloacae* RCA25, preventing the use of engineered strains. Our results showed that *Herbaspirillum huttiense* RCA24 performed best. Improvements in nitrogen-fixation and changes in physiological parameters such as chlorophyll, nitrogen content and shoot dry weight were observed for rice plants (*Oryza sativa* L. cv. Baldo) co-inoculated with strains RCA25 and RCA24 in a 10:1 ratio. Based on confocal laser scanning microscopy analysis, strain RCA24 was the best colonizer of the root interior and the only IAA producer located in the same root niche occupied by RCA25 cells. Our data highlight that the assessment of location and distribution of the individual microbial components within the host plant tissues is fundamental to select bio-inoculants containing IAA-producer strains able to enhance nitrogen-fixation.

PU-05 *Streptomyces* as a plant's best friend

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Plant roots release diverse compounds to create a unique environment, the rhizosphere, in which a vast amount of microorganisms find their niche for growth. A subset of these microorganisms, commonly referred to as PLANT GROWTH-PROMOTING RHIZOBACTERIA (PGPR), greatly contributes to plant health and productivity in various manners. Microbiome based studies revealed that *Streptomyces sp.* belong to the root microbiome of many different plant species and that their relative abundances change when plants are grown in changing environments. Indeed, by performing 16S amplicon sequencing and using wheat and maize as examples, we observed that drought causes an increase in the relative abundances of *Streptomyces sp.* of the root microbiome while cold treatment caused a depletion indicating that various abiotic factors cause different changes in the root microbial composition.

These observations triggered us to unravel the molecular interaction between plant roots and *Streptomyces sp.* and to get insights into how the latter can support plant growth. Using wheat but also *Arabidopsis thaliana* as model plants, a detailed phenotypical and molecular insight will be presented into how *Streptomyces sp.* colonize plants and how this colonization provokes plant growth promotion.

PU-06 Genetic differences in barley govern the responsiveness to *N*-acyl homoserine lactone

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Priming crop plants for enhanced resistance using biocontrol agents is an efficient disease management strategy. Enhanced resistance in barley (*Hordeum vulgare L.*) against pathogens, such as the powdery mildew-causing fungus *Blumeria graminis* f.sp. *hordei* (*Bgh*), is of high importance. The beneficial effects of bacterial quorum sensing molecules on resistance and plant growth have been shown in different plant species, including barley. Here, we present the effects of the *N*-3-oxotetradecanoyl-L-homoserine lactone (oxo-C14-HSL) on the resistance of different barley genotypes. Genetically diverse accessions of barley were identified and exposed to either the beneficial, oxo-C14-HSL-producing bacterium *Ensifer meliloti* or the pure *N*-acyl homoserine lactone (AHL) molecule. Metabolic profiling along with expression analysis of selected genes and physiological assays revealed that the capacity to react varies among different barley genotypes. We demonstrate that upon pretreatment with the AHL molecule, *AHL-primable* barley genotype expresses enhanced resistance against *Bgh*. We further show that pretreatment with AHL correlates with stronger activation of barley MAP kinases and regulation of defense-related *PR1* and *PR17b* genes after a subsequent treatment with chitin. Noticeable was the stronger accumulation of lignin. Our results suggest that appropriate genetic background is required for AHL-induced priming. At the same time, they bear potential to use these genetic features for new breeding and plant protection approaches.

PU-07 Role of Microbes in the Galler-Plant Interaction: *Pantoea agglomerans* affecting the compatible Grape Phylloxera (*Daktulosphaira vitifoliae*) - *Vitis* spp. Interaction

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Grape phylloxera (*Daktulosphaira vitifoliae* Fitch) induces histoid leaf galls on susceptible *Vitis* spp. leading to severe host damage symptoms and economic losses in commercial vineyards. In the last years the reports of canopy infestations with leaf-galling *D. vitifoliae* were increasing with particularly high insect populations observed on the foliage of interspecific fungi resistant hybrids (European *V. vinifera* x American *Vitis* spp.). The mutualistic bacterium *Pantoea agglomerans* was detected in leaf galls and attached to the larval integuments of grape phylloxera. In previous work we showed that grape phylloxera associated *Pantoea* spp. are not maternally transmitted. So far *Pantoea* species were shown to play diverse roles for the compatibility of the host-parasite interactions by e.g. breakdown of toxic substance or affecting systemic host plant defences by altering the biosynthesis of volatile metabolites.

Here we aim to assess and compare the volatile metabolomes of grapevine leaves of different hosts under grape phylloxera attack. We hypothesise that *V. vinifera* L. leaves release quantitatively more host defence associated volatiles than interspecific hybrids under grape phylloxera attack. Secondly we hypothesise that the presence of mutualistic *P. agglomerans* results in a decrease of host defence associated secondary metabolites thereby favouring insect development and gall formation.

In total twenty-one single eye cuttings of either *V. vinifera* L. Riesling and *Vitis* spp. Muscaris (Solaris x Gelber Muskateller) are rooted and cultivated in isolated quarantine cages located in climate chambers (26±3°C, 60% rH, 16 h pp) After 2.5 months of incubation insect demographic and host response parameters per plant are determined. Leaf galls, differentiated by 4 insect larval stages vs. not infested leaf tissues are sampled. Subsequent semi-quantitative GC-MS analyses screen the sampled tissues for released host associated defensive VOCs such as MeJA, MeSA, terpenes, aromatic compounds, alcohols and *n*-alkanes.

We expect that the comparative analysis of the generated volatile metabolomes reveals potentially effective secondary volatiles against leaf infesting grape phylloxera, whose biosynthetic and transport pathways are already conserved within the grapevine genome. Furthermore we hope to gain deeper insights in the so far elusive tritrophic interaction between grape phylloxera, *P. agglomerans* and *Vitis* spp.

miCROPe 2019 - Microbe-assisted crop production opportunities, challenges & needs

Vienna, Austria, December 2 – 5, 2019

Microbiome understanding beyond profiling

Chairs: Klaus Schlaeppli & Jenny Kao-Kniffin

MU-01 Applying Concepts in Group-level Evolutionary Processes to Assemble Plant Beneficial Microbiomes

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Group-level processes dominate rhizosphere interactions impacting plant growth and development; however, empirical studies of plant beneficial microbiomes largely focus on single species or strains of microorganisms. In a series of experiments on directed evolution of the rhizosphere microbiome, we aimed to develop microbial communities associated with enhanced seed yield in rapidly cycling *Brassica rapa* and altered flowering time in *Arabidopsis thaliana* using a multi-generation experimental evolution system. We hypothesized that phenotypic plasticity can be modified through selective pressure on the plant trait, while the agents of selection are microorganisms associated with the modified plant traits. Microbiomes were collected from the rhizosphere soil of a subset of plants to be used as inoculants for the subsequent planting generation. After multiple generations of selection for modified plant traits, the composition and function of the rhizosphere microbiome shifted away from the control microbiomes. The microbiomes assembled from a specific trait selection pressure showed the ability to alter the plant traits of novel plant host genotypes or species. The results of the experiments suggest that directed evolution of rhizosphere microbiomes impact the plasticity of plant phenotypes, which could play an important role in commercial plant production systems.

MU-02 Synthetic microbial community from the sugarcane core microbiome reveals genetic features for successful plant colonization

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Recent advances in microbial studies have shown that the microbiome has a profound impact on plant health and development. However, the genetic and molecular mechanisms involved in plant-microbe communication that are responsible for the establishment of the microbial community in plants are largely understudied. Unraveling microbial traits responsible for a successful host colonization is an imperative step towards building biotechnological tools based on microbiome to benefit economically relevant plants. Here we explore these traits by investigating the colonization profile and genome sequence of a synthetic microbial community (SynCom) comprised of representatives from the sugarcane core microbiome that show robust colonization with different plant models. By using culture-independent techniques, we found that the sugarcane is inhabited by a core microbial community comprised of less than 20% of the total microbial richness and that sum up for over 90% of the total microbial relative abundance in plant organs. We created a microbial culture collection comprised of over 5 thousand isolates and selected bacterial representatives to design a SynCom comprised of naturally dominant groups from the sugarcane core microbiome. Inoculation assays and microbial profiling revealed that the SynCom robustly colonized, stimulated root development and tripled maize plant biomass. Curiously, genome sequencing showed that robust colonizers lack commonly investigated plant growth-promoting features such auxin production, nitrogen fixation, phosphate acquisition and ACC-deaminase activity, which might indicate that these features are not deterministic for a successful host colonization. Although robust and non-robust bacterial colonizers showed substantial functional overlaps, we show that significant differences may explain their divergent colonization lifestyle.

PF-MU-01 Genotype x soil interaction in the composition of root-rot pathogens of pea detected by quantitative PCR

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Disease resistance encompasses the mechanisms that allow a plant to withstand or ward off a pathogen. The molecular responses of plants under pathogen attack and the underlying genetics have been extensively studied. However, resistance is not only a trait defined by the warfare between pathogen and host. In fact, resistance is an emergent phenotype of the interactions between the microbial community and the host. Fungal root diseases threaten pea (*Pisum sativum* L.) cultivation, and therefore a valuable protein source and important crop in low-input farming systems. Resistance in current pea varieties against multiple root pathogens is lacking. In order to acknowledge the rhizosphere microbiome as an integral part of the environment, 261 pea genotypes were screened for resistance on naturally infested field soil in a pot-based experiment. Thereof, eight lines with contrasting disease levels were selected and tested on four soils with different disease pressure in a follow-up pot experiment. Along root rot assessments, pea pathogens (*F. solani*, *F. oxysporum*, *F. avenaceum*, *A. euteiches*, *P. ultimum* and *D. pinodella*) and arbuscular mycorrhizal fungi were quantified in diseased roots using qPCR assays. The amount of fungal DNA detected in the roots differed among the pea genotypes and the four soils and a significant pea genotype x soil interaction was evidenced for several pathogen species. For example, the quantity of *F. avenaceum* in the roots mostly depends on the soil (two-way ANOVA, $p < 0.01$) and differs significantly between pea genotypes ($p = 0.013$). *F. oxysporum* and *F. solani* quantities showed significant pea genotype x soil interactions ($p < 0.01$ for both species). Significant correlations were found between *F. avenaceum* and *F. solani* quantity and root rot index ($r_s = 0.38$, $p < 0.01$ and $r_s = 0.56$, $p < 0.01$, respectively). On the other hand, *F. oxysporum* quantity shows no relationship with root rot ($r_s = 0.007$, $p = 0.95$). These results suggest differential roles of the microbes in root rot and highlight the importance of incorporating the complexity of the soil microbiome at early stages of resistance screenings and breeding efforts. Resistance breeding against root rot will be challenged by the fact that soil microbes interact with each other and the plant and that their composition varies between different soils. Further insights into plant-microbe interactions and emerging molecular plant breeding tools will fuel future plant breeding.

PF-MU-02 Contrasting soil microbial community profiles in healthy and declined vineyards

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Grapevine dieback can be defined as a multiannual crop yield loss due to the sudden or progressive vine death which could be associated with biotic or abiotic factors, resulting in a worldwide issue. Grape trunk diseases or viruses are one of the most frequent identified causes of vine dieback. However, a decline is sometimes observed while no disease symptoms or pathogenic causes could be identified. The microbiome at the interface with the root system (rhizobiome) impacts the physicochemical parameters of the soil and consequently influences the adaptation of the vine plant to its environment. Moreover, grapevine associated microbiota is known to be influenced by soil microbiome, therefore microbes from bulk and rhizospheric soils are good bio-indicators for vineyard health status displaying disease asymptomatic decline. VITIRHIZOBIOME project aims to assess the hypothesis that rhizobiome plays a role in the vine development and its impact can explain the contrasting vineyard dieback stages in different soils.

The microbial diversity and activity of 4 different vineyard plots of Bordeaux region during winter and spring periods were investigated. Those plots were selected due to their lack of disease symptoms even though defined dieback areas were detected and unexplained. Subsequently, one relevant plot has been selected, and its healthy and dieback soils were sampled for greenhouse experiment for phenotypical analysis of Cabernet Sauvignon (CS) scion grafted onto low vigour rootstock, *i.e.* Riparia Gloire de Montpellier (RGM) and highly vigour one, *i.e.* 1103 Paulsen (1103P). Plant Growth Promoting Rhizobacteria (PGPR) isolation and screening for further inoculation experiment has been done in parallel.

Significant differences in microbial biomass and activity were found among soils even if those present similar physicochemical characteristics. The results of enzymatic assays distinguished patterns from winter and spring periods with an overall greater activity for healthy soil. However, microbial activity seems to be higher in decline soils compared to healthy ones during spring period regardless the lower quantity of microbial biomass. MALDI-TOF mass spectrometry

allows us to identify the most predominant cultivable strains in both soils of the selected plot, and PGPR screening permit to select the most relevant strain for growth stimulation.

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PF-MU-03 Changes in soil microbiome can alter peach tree physiology with implications in plant development and in the composition of secondary metabolites

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As global population grows in the transition between the current and the goal for future agricultural systems it is mandatory to maximize the food security goals for 2050. We simultaneously need to cope with environmental goals which include the reduction of greenhouse gas emissions, soil biodiversity loss and water pollution. In this new model the management of soil microorganisms can be used as an essential tool to improve plant productivity and fruit quality increasing soil microbiome diversity and water and nutrient utilization efficiency. Within the European Union countries Spain is the first producer of peaches and nectarines reaching more than 40% of the fruit production. The objectives of the high yielding regions include the increase in production with less disease incidence while ensuring the sustainability of the agricultural system. The interaction between plants and soil microorganisms increases the efficiency in nutrient uptake. These soil populations include arbuscular mycorrhizal fungi (AMF) among other fungi and plant growth promoting rhizobacteria. The application of the AMF *Rhizoglyphus irregularis* has been demonstrated to help plants to face abiotic environmental stresses and *Trichoderma* can act both as plant biostimulant and as mycoparasite often used as biocontrol agent of many soilborne plant pathogens. The aim of the study is to reveal the mechanisms of plant metabolism regulation modulated by AMF in the presence of *Trichoderma* related to changes in soil microbial populations. Peach (*Prunus persica* L.) plants were grown in 2 l containers and were inoculated with *Rhizoglyphus irregularis* BEG72 or simultaneously with *R. irregularis* and *Trichoderma koningii* TK7. Control plants were non-inoculated. After 8 months plant growth was measured. Microbial communities have been analyzed through Next Generation Sequencing technologies. Leaf samples were collected to perform metabolomic analysis through ultra-high-pressure liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry.

PF-MU-04 Functional analysis of soil microorganisms for agriculture using millifluidic droplets.

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Introduction : Soil is home to numerous and varied microorganisms. They are useful to crops in many ways: protection from disease, nutrient acquisition, drought resistance... However, while it is every day easier to examine the genome and transcriptome of these microbiotes, it is still laborious to conduct culture experiments on them. Culture provides essential pieces of information on their agronomic functions, though.

Thanks to millifluidics, it becomes possible to conduct automated, high-throughput and quantitative growth experiments. We aim to take advantage of this to assess the qualities and flaws of any given soil's microbiote. We are currently focussing on two functions:

Phosphorus solubilization: we aim to count microorganisms able to make soil phosphorus available to plant. Biocontrol: we are conducting co-culture experiments of pathogens and soil microbes, in order to assess their ability to protect crops from diseases.

Materials and methods We revisit ancient tests and adapt them to Millifluidics, using MilliDrop devices - microorganism culture automatons. They allow us to generate and optically monitor 1000-droplet trains for 48 hours, in a 20 meter long tube. Each droplet is 400nl and can contain either a unique cell or a community.

Microbes are extracted from the soil, and incubated in culture media. We add different reactants to reveal functions of interest.

In the Biocontrol test, the media is co-inoculated with a soil suspension and a pathogen strain.

Results and projections We are able to count viable cells per gram of soil, and to know how many of them are able to solubilize mineral phosphate.

We will soon start applying our protocol to various soil types in France, while continuing to develop tests on biocontrol.

In the future, our method could be applied to other functions such as dinitrogen fixation, or carbon sequestration.

PF-MU-05 The bacterial community in potato is recruited from soil and partly inherited across generations

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Strong efforts have been made to understand the bacterial communities in potato plants and the rhizosphere. Research has focused on the effect of the environment and plant genotype on bacterial community structures and dynamics, while little is known about the origin and assembly of the bacterial community, especially in potato tubers. The tuber microbiota, however, may be of special interest as it could play an important role in crop quality, such as storage stability. Here, we used 16S rRNA gene amplicon sequencing to study the bacterial communities that colonize tubers of different potato cultivars commonly used in Austrian potato production over three generations and grown in different soils. Statistical analysis of sequencing data showed that the bacterial community of potato tubers has changed over generations and has become more similar to the soil bacterial community, while the impact of the potato cultivar on the bacterial assemblage has lost significance over time. However, the communities in different tuber parts did not differ significantly, while the soil bacterial community showed significant differences to the tuber microbiota composition. Additionally, the presence of OTUs in subsequent tuber generation points to vertical transmission of a subset of the tuber microbiota. In summary, we conclude that the bacterial assemblage in potato tubers consists of bacteria transmitted from one tuber generation to the next and bacteria recruited from the soil.

PF-MU-06 Root fungal community structure of *Alkanna tinctoria* differs with plant developmental stage

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Alkanna tinctoria produces alkannin/shikonin (pharmaceutical substances with a wide spectrum of biological properties) and growing evidence suggest to date that endophytes (i.e. bacteria and fungi) are beneficial to plant growth and secondary metabolites (SM) production. Since almost nothing is known about *A. tinctoria* root's fungal community structure, there is a need for a thorough analysis of its fungal community structure per developmental stage, allowing identification and isolation of promising microorganisms for future applications in SM production systems. We characterized the fungal community structure of *A. tinctoria* with Amplicon Sequence Variant (ASV) and diversity (Simpson) index by Illumina MiSeq sequencing based on the ribosomal ITS region. The plants were grown under controlled greenhouse conditions, in a mixture of sterilized substrate (peat moss and perlite) and natural (non-sterilized) soil from two locations in Greece (soil A and B). A control that only comprised the sterile substrate was included. The plants were harvested at four developmental stages (I, II, III and IV), corresponding to peak of growth, flowering, fruiting and dormancy, respectively. Based on ASV data, the fungal community diversity of the control plants was significantly lower and different from the plants grown in the two Greek soils, whatever the developmental stage. Similarly, the total fungal diversity in soil B was significantly higher than in soil A, regardless of stages. Finally, differences were noticed between stages and soils. The fungal communities of plants grown in soil A and B were similar at the stage I and IV, while different at stage II and III. In each stage, more than 30% of the fungi were shared between plants grown in soil A and B. A stable core microbiome (i.e. present at all developmental stages) was identified. In soil A, a total of 45 ASVs (16%) were present at the four stages examined and in soil B, 51 (18%). By merging these two results, 31 ASVs were always occurring in the roots of *A. tinctoria*, regardless the soil and developmental stage. This study reports for the first time the root fungal community of *A. tinctoria*. A wide diversity of fungi was detected in the root system along the plant developmental stages with a stable core microbiome identified throughout the stages. These results open the door to the isolation and testing of promising fungal endophytes to be applied in SM production systems aiming at high yields.

MU-03 A novel microcosm for recruiting phytate-degrading microbial communities under inherently competitive soil conditions

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Rock phosphorus (P), a core component of applied mineral fertilizer, is a finite resource, thus necessitating the development of innovative solutions to maintain and improve the efficiency of P fertilizer use to sustain optimal P nutrition in plants. *Myo*-inositol hexa-*kis*-phosphate (phytate) and its lower order derivatives constitute the majority of identified organic P in many soils and in some cases accumulates in soil with continuous application of P fertilizer. Phytate however is poorly available to plants and in alkaline soils may be precipitated as calcium (Ca)-phytate. Incorporating phytase-producing biofertilizers (i.e., microbial products with capacity to mineralize phytate) into soil for improved plant P nutrition presents a viable and environmentally acceptable way of utilizing P from phytate, whilst reducing the need for mineral P application. A baiting microcosm system consisting of Ca-phytate hotspots placed in low P availability soils was developed and used to recruit microorganisms with distinct taxonomic identities and functional capacities in relation to phytate degradation under natural soil conditions. Treatments containing Ca-phytate showed both direct and indirect evidence for Ca-phytate mineralization *in vitro* and *in vivo*, as well as an increased abundance of *phoX* and *phoD* genes that relate to organic P mineralization. In contrast, genes coding for the well-studied beta-propeller phytase, normally associated with phytase activity in soil, were not enriched in the Ca-phytate hotspots. The microcosms recruited communities with increased proportions of Actinobacteria, Firmicutes, and Proteobacteria, and the genus *Streptomyces* was specifically enriched in the presence of Ca-phytate. Hence, the current baiting microcosm represents a promising approach to isolate and characterize novel phytate degrading microorganisms that are inherently competitive in the soil environment.

MU-04 Plant responsiveness to soil microbial feedbacks

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The root microbiota has important direct functions for plant growth and health as well as indirect impacts as apparent from plant-soil feedbacks or in disease suppressive soils. In indirect health functions, the selective recruitment of beneficial microbiota members to plant roots result in a form of 'soil-borne immune memory' at the benefit of the next plant generation. There is evidence that specific compositions of the complex soil microbiota can prime a 'state of alert' in plants, induce systemic resistance and thereby improve plant health. We found that benzoxazinoids (BX), a class of defensive secondary metabolites that are released by roots of cereals, alter the maize rhizosphere microbial communities. Such a BX-conditioned microbiota impacts the growth of a next generation of maize by increasing jasmonate signaling, plant defenses, and suppressing herbivore performance compared to a non-BX-conditioned microbiota. Similar microbial feedbacks were also in *Arabidopsis* and wheat. Importantly, different plant genotypes varied in their response to the BX-conditioned microbiota and we can now make use of this genetic variation to obtain insights how plants respond to soil microbial feedbacks. Identifying plant loci for positive responsiveness to microbiota feedbacks will open new opportunities to integrate beneficial plant-microbiome interactions into crop breeding programs, which ultimately will enhance sustainability of agriculture.

MU-05 Evaluating the diversity and functional potential of plant microbiota to improve the selection of potato genotypes able to cope with combined water and nutrient limitations

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The H2020 project “SoIACE - Solutions for improving Agroecosystem and Crop Efficiency for water and nutrient use” aims to identify agricultural practices, including the use of microbial inoculants, and plant genotypes of potato and wheat to cope better with water and nutrient limitations. Among 24 European partners, the AIT Austrian Institute of Technology and the Research Institute of Organic Agriculture are assessing how agronomic practices affect soil microbiota and their functions. We evaluated the performance of ten potato genotypes grown with or without the combined stresses of nutrient and water deficit in a field trial at the James Hutton Institute in Scotland. Using amplicon sequencing of phylogenetic marker genes and shotgun metagenomics, we analyzed how plant genotype, plant phenotype and the different stress scenarios affect bacterial and fungal microbiota in the root environment. Generally, Actinobacteria and Sordariales were increased while Proteobacteria, Olpidiomycota, Shannon diversity and richness were reduced in the rhizosphere of potatoes grown under stressed conditions. In the root endosphere under stress, the bacterial, but not the fungal, community changed greatly. Shared, unique and differently abundant microbial amplicon sequencing variants indicated a stress- and genotype-specific recruitment of microbes by potato plants. Metagenome analysis and the analysis of selected bacterial isolates will reveal information on plant growth-promoting potential and functional properties of potato microbiota. Results of this trial will help to identify below-ground traits and select efficient potato genotypes which are best suited to coping with combined stress scenarios while simultaneously supporting beneficial functions of soil and plant-associated microbiota.

MU-06 Succession of microbial assemblages during seed development

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Seeds are involved in the transmission of microorganisms from one plant generation to another and consequently act as a starting point for assembly of the plant microbiome. In the current work, ecological succession of seed microbial assemblages was assessed during key seed development stages including seed filling and seed maturation. Common bean (*Phaseolus vulgaris*) and radish (*Raphanus sativus*) were selected as working models since these two plant species differ in their pollination modes. According to barcoding datasets, pioneer species associated to bean seeds were mostly derived from the vascular system, while primary colonists of radish seeds were either acquired through the vascular system or the floral pathway. In addition, a significant increase in phylogenetic diversity was observed during seed maturation for both plant species probably as a result of external transmission of micro-organisms from fruits. Culture-based collection and subsequent comparative genomics of representative bacterial strains isolated at different seed developmental stages provided some insights on determinants involved in successful seed colonization and persistence. Data generated through this work increase our basic understanding of the governing processes that drive assembly of the seed microbiota. This fundamental knowledge is a first step towards the design of efficient seed microbial inoculants.

MU-07 Volatilome of Wheat Microbiota System under Drought and Flooding: The VolCorn Consortium

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Climate change will increase vulnerability of global food production in Europe by drought and flooding as by associated and frequent weather extremes. The abiotic stressors flooding and drought diminish crop yield and make crop plants more susceptible to pathogens and herbivores. Crop health and yield are stabilized by their beneficial microbiota, especially by mycorrhizal fungi. As a crop plant-microbiota system, both biological components respond jointly to environmental challenges. Crop plant and microbiota emit a complex mixture of volatile organic compounds, the volatilome, which is important in plant-plant communication and plays a role in plant defense to herbivores. Wheat is one of the top three globally produced crops. Hence, we chose it as an experimental model for greenhouse and field manipulation experiments. We consider the volatilome as an integrated signal reflecting key metabolic changes of wheat and its microbiota in response to environmental stresses, and thus their functional interactions. We hypothesize that this volatile-based communication enhances the wheat-microbiota system capability to withstand combined biotic and abiotic threats, such as fungal pathogens or herbivores under drought and flooding. The Leibniz Competition 2019-funded consortium "VolCorn" will reveal abiotic stress-induced volatiles of mycorrhizal or non-mycorrhizal wheat plants and correlate them with functional traits of its microbiota. The central mission of VolCorn is the identification of volatilome components (single or mixtures) that enhance the beneficial microbiota and in turn increase wheat fitness under environmental stress.

miCROPe 2019 - Microbe-assisted crop production opportunities, challenges & needs

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Morning Keynote

MK-01 Crops as merobionts: Regenerative agriculture, the microbiome and the climate crisis through the lens of the hologenome theory

Richard Jefferson

Cambia & QUT, Australia

The climate crisis presents an existential threat to human society and ecosystem resilience. Agriculture is responsible for much of this impact. Systemic, diverse and effective interventions are urgently needed to mitigate the catastrophe.

Hologenome theory asserts that virtually all plants and animals comprise both a scaffold (or 'host') and myriad populations of microbial constituents: its microbiome. The composite organism can be considered a holobiont, in which diverse functions needed to flourish and for the information content to persist over evolutionary time are distributed amongst its genetic contributors.

However, I propose that the development of agriculture has *collapsed the hologenome* in plants, domestic animals and humans to create metastable *merobionts*, through massive inbreeding depression of the microbiome due to recurrent and homogeneous planting and concomitant sedentary lives of associated animals and humans.

In this model, *functional* microbial diversity both *in planta* and in cultivated soils has declined, both through human practice intervention and through a compensatory breeding of the plant 'genome' (typically nuclear and cytoplasmic genomes) at the expense of more agile trait contribution from the microbiome. This leaves the soil reservoir in cultivated areas impoverished of founder population diversity, and rendered the crop vulnerable, and the production system fragile. Free exchange of microbes by the macrobiota involves recruitment, amplification and dissemination of populations. This is not a neutral process in that the composition of sampled microbes does not match that of disseminated microbes. Rather we know that microbes are selectively recruited, differentially amplified and variably disseminated, and thus will have a disruptive, recursive effect on the microbial population structure in the environment, from which the next cycle of recruitment and amplification occurs, beginning a treadmill of population structural change and constriction.

Does this lead inexorably to such apparently disparate phenomena as disease and ecosystem fragility, and a decline in soil microbial diversity and carbon sequestration. I speculate that many of the lessons of resilience that we need to apply to the imperatives of agriculture will be gleaned from studying microbiome and holobiont relationships in minimally perturbed natural systems, not from merobionts in agricultural systems – whether industrial or artisanal.

miCROPe 2019 - Microbe-assisted crop production opportunities, challenges & needs

Vienna, Austria, December 2 – 5, 2019

Microbial biocontrol of pests, pathogens and weeds

Chairs: Karen L. Bailey & Gabriele Berg

MB-01 Plant microbiome management for sustainable agriculture

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The plant microbiome is crucial for growth and health (Berg *et al.* FEMS Microb. Ecol., 2017). Intense agriculture and overuse of chemicals leads to biodiversity loss and resistant pathogens, which are difficult to suppress but cause enormous yield losses. The plant microbiome will be the key to the second green revolution because it provides solutions for sustainable agriculture. To manage or exploit the plant microbiome require a deep understanding of its functioning. The microbiome can be managed indirectly by changing abiotic parameters or directly by microbial treatments or transplants. Seeds are ideal carriers for the latter (Berg & Raaijmakers ISME J, 2018). However, recent studies reveal an unexpected microbial diversity and abundance within seeds, and showed a vertical transmission of an indigenous core microbiome (Adam *et al.* Plant and Soil, 2017). Soil type, climate, geography and plant genotype were identified as main drivers of the seed microbiota. Within millennia of domestication, crops and their seeds underwent traceably many different adaptive trends, allowing rapid speciation and divergence that lead to phenotypic and genotypic distinction to their wild ancestors. During those dynamics, also the microbiomes have secretly co-evolved with the host plants. Interestingly, bacterial endophytes represent the symbiotic component within seeds; in native seeds they form a beneficial network with archaea, while fungi represent an antagonistic component (Wassermann *et al.* Microbiome 2019). To restore microbial diversity important for *one* health issues, tailored bacterial seed treatments can be composed based on the rich diversity of seeds of wild ancestors or other native plants.

MB-02 The functional relevance of microbe-plant-insect interaction in a cereal crop system

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Beneficial rhizobacteria bear a high potential to improve the plant's resistance against biotic stress. However, it is not well-understood how microbial signaling influences the response of the plant against economically important pests. *Acidovorax radialis* N35 and *Rhizobium radiobacter* F4 are a N-acyl homoserine lactone (AHL) producing plant growth promoting rhizobacteria (PGPR), which were tested for their interaction with barley and foliar-feeding aphids within this study. Available AHL negative mutants of these strains will allow to elucidate the role of AHLs in these interactions. We performed several green-house experiments with barley cv. Grace, Chevalier, Scarlett, and Barke growing in commercial gardener's soil. Inoculation of barley seedlings (*Hordeum vulgare*) with *A. radialis* N35 was shown to clearly influence the growth rate of barley positively while the load of foliar-feeding aphids (*Sitobion avenae*) was significantly reduced. However, variations of this effect were observed depending on the cultivar with Barke being the least responsive. NGS Amplicon sequencing analyses of the root associated microbiome revealed a significantly positive correlation between the abundance of reads allocated to *Acidovorax* and plant growth, while a negative correlation was observed for aphid load. Inoculations with *R. radiobacter* F4 showed the same tendency in reducing aphid loads and improving plant growth although not as clear.

Ongoing experiments aim to find out whether indirect signaling via the plant or direct interaction (i.e. antagonism) of the endophytic microbes with aphids are responsible for the current findings. Understanding this inter-kingdom communication provides a promising basis to improve agricultural systems by enhancing crop resistance against herbivorous insects.

PF-MB-01 *Mycolicibacterium* strains interact positively with *Serendipita (Piriformospora) indica* for crop enhancement and biocontrol of pathogens

Alejandro del Barrio Duque, Livio Antonielli, Angela Sessitsch, Abdul Samad, Stéphane Compant

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Serendipita (=Piriformospora) indica is a root-colonizing fungus with the capabilities to enhance plant growth and confer biotic resistance. However, the application of this fungus in the field has led to inconsistent results, perhaps due to antagonisms with other microbes. Here, we studied the impact of single bacteria from the endophytic bacterial community on the *in vitro* growth of *S. indica*. Furthermore, we investigated how combined inoculum of *S. indica* and bacteria influence plant growth and protection against *Fusarium oxysporum* and *Rhizoctonia solani*.

Data showed that, among other taxa, bacterial strains of the genera *Burkholderia*, *Enterobacter* and *Bacillus* negatively affect *S. indica* growth, whereas several strains of *Mycolicibacterium*, *Rhizobium* and *Paenibacillus* stimulate fungal growth. To further exploit the potential of the beneficial interaction, additional experiments were performed with *Mycolicibacterium* strains, as it was the most abundant genus showing positive effects on *S. indica* growth. Some dual inoculations of *S. indica* and *Mycolicibacterium* strains boosted the beneficial effect triggered by *S. indica*, further enhancing the growth of tomato plants, and alleviating the symptoms caused by the pathogens *F. oxysporum* and *R. solani*. However, some combinations of fungus and bacteria were sometimes less effective than microbes inoculated singly.

Genome analysis of four *Mycolicibacterium* strains revealed that these bacteria encode several genes predicted to be involved in the stimulation of *S. indica* growth, plant development and tolerance to biotic and abiotic stresses. Particularly, a high number of genes related to vitamin and nitrogen metabolism were detected. Taking into consideration multiple interactions on and inside plants, we showed in this study that some bacterial strains may induce beneficial effects on *S. indica* and this mutualistic relationship of microbial partners could be an approach to enhance plant growth promotion and tolerance to various biotic and abiotic stresses.

PF-MB-02 Biological control of wireworms in cover crops

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Wireworms cause substantial losses in marketable yield of potatoes but control options are limited, creating a demand for new alternatives. Laboratory and semi-field trials revealed the potential of the entomopathogenic fungus *Metarhizium brunneum* isolate ART2825 against *Agriotes obscurus* and *A. lineatus*, two of the most detrimental wireworm species. In this study we integrate the fungus in the agricultural crop rotation and try to adapt the application method to its ecological and environmental requirements. Application precedes sowing of cover crops in late summer in order to enhance disease development through higher soil temperatures and extend effect duration by the absence of soil disturbance.

During a two-year field trial, we were able to establish the fungus on site and demonstrate the infectivity of the treated soils in laboratory assays. Tendencies to lower potato damages were seen in the first season but damage levels did not significantly differ from the control. To improve plant protection efficacy the application rate was increased in the second year and application time precisely selected. Reasons for the yet pending success were further investigated and potential synergies with selected cover crop species explored.

PF-MB-03 Facilitation promotes invasions in plant-associated microbial communities

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While several studies have established a positive correlation between community diversity and invasion resistance, it is less clear how species interactions within resident communities shape this process. Here we experimentally tested how antagonistic and facilitative pairwise interactions within resident model microbial communities predict invasion by the plant-pathogenic bacterium *Ralstonia solanacearum*. We found that facilitative resident community interactions promoted and antagonistic interactions suppressed invasions both in the lab and in the tomato plant rhizosphere. Crucially, pairwise interactions reliably explained observed invasion outcomes also in multispecies communities, and mechanistically, this was linked to direct inhibition of the invader by antagonistic communities (antibiosis), and to a lesser degree by resource competition between members of the resident community and the invader. Together our findings suggest that the type and strength of pairwise interactions can reliably predict the outcome of invasions in more complex multispecies communities.

PF-MB-04 *In vivo* localization and role of *Fusarium oxysporum* f.sp. *strigae* and *Bacillus subtilis* against *Striga hermonthica* in an integrated biocontrol system

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In view of the variable susceptibility of *Striga hermonthica* to *Fusarium oxysporum* f.sp. *strigae* (Fos) isolates, the combined application of the mycoherbicide *Fusarium oxysporum* f.sp. *strigae* (Fos) and *Bacillus subtilis* (a plant growth promoting rhizobacterium (PGPR)), as an integrated *Striga hermonthica* biocontrol approach was examined. This was to understand the ecological niche (position and role) of Fos and PGPR in the integrated biosystem, in identifying a more effective biocontrol strategy to combat *S. hermonthica*. Localization of Fos isolates (Foxy-2, FK3) and *B. subtilis* (GBO3 strain) within infected *S. hermonthica* was monitored by fluorescent gene expression of transformed Fos and GBO3. Also, Foxy-2, FK3, GBO3, including *Trichoderma viride* (IMB12098 strain) as check, were applied as fungi-bacteria assemblages, and single treatments, to *S. hermonthica* infested rhizoboxes containing sorghum as host crop. Both Fos and GBO3 infected and co-localized diseased *S. hermonthica* shoot. Also, Fos penetration of *Striga* through trichome entry was revealed. Combined treatments of FK3 + GBO3, and Foxy-2 + GBO3, increased sorghum aboveground dry biomass ($P < 0.05$), but not IMB12098 + GBO3. None of the combined fungi-bacteria assemblages significantly suppressed *S. hermonthica* emergence. Single treatments of FK3 and GBO3 increased sorghum aboveground dry biomass ($P < 0.05$), but Foxy-2 and IMB12098 did not. Only FK3 suppressed *S. hermonthica* emergence ($P < 0.05$), but neither GBO3, Foxy-2 nor IMB12098. GBO3 counteracted Fos suppressive activity against *Striga* emergence. Despite GBO3 ineffectiveness in suppressing *S. hermonthica* emergence, it significantly promoted sorghum yield, either when applied alone or in combination with Fos isolates. In the given set-up, the combined application of Fos and GBO3 presented no added advantage in suppressing the emergence of the sampled *S. hermonthica*.

Keywords: *Striga hermonthica*, *Fusarium oxysporum* f.sp. *strigae*, Foxy-2, FK3, *Bacillus subtilis* GBO3 strain, fluorescent gene expression, ecological niche..

MB-03 Behaviour of Bt ABTS-1857 as a biological control agent on spinach plants, cut leaves and spinach juice

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*Bt ssp. aizawai*s strain ABTS-1857 is the active substance of Xentari, which is one of the most widely used commercial BCA in Europe. EFSA document in 2016 reported that dose-response and behavioural characteristics of Bt and field studies after application of Bt biopesticides were quite scarce. Therefore, we studied the behaviour of Bt ABTS-1857 inoculated on plants (pre-harvest), cut leaves (post-harvest) of spinach and spinach juice (food). Spinach plants were growing indoors at ambient temperature and spray inoculated with Bt ABTS-1857 either spores or vegetative cells. Plants were analysed for Bt at day 0 and after 5, 10, 15 and 20 days by plating on MYP agar. Cut spinach leaves were spray inoculated with Bt ABTS-1857 vegetative cells. These leaves were stored at 12 °C for 5 days and daily analysed for Bt by plating on MYP agar. Spinach juice was made from spinach leaves by grinding using an immersion blender, centrifuged and filter sterilized before inoculation. The behaviour of Bt ABTS-1857 was monitored in 20% spinach juice on a daily basis up to 5 days storage at 12 °C and 22 °C. Non-inoculated controls of spinach plants, cut leaves or spinach juice were also plated on MYP agar. At pre-harvest simulation, results showed that the reduction of Bt spores on plants was only 0.48 log after 20 days, while Bt vegetative cells reduced 3.53 log after 20 days. The results indicated that the spores of Bt ABTS-1857 remained present in quite high numbers but did not germinate and grow on the spinach plants and were thus less impacted by the plant's ecosystem than the Bt vegetative cells populations. However, at post-harvest simulation, the inoculated Bt vegetative cells maintained stable on cut leaves and counts on MYP-agar kept at ca. 4 log throughout the 5 days storage. Thus, results showed that the Bt vegetative cells did not grow out on cut spinach leaves during 5-days storage at 12 °C (whereas in BHI Bt ABTS-1857 growth at 12°C occurred). Furthermore, results of growth assessment of Bt ABTS-1857 in 20% spinach juice showed that vegetative cells died from 3.6 to 1.2 log after 5 days at 12 °C, although growth was observed at 22°C in the spinach juice: from 3.6 to 6.95 log. The behaviour of Bt ABTS-1857 is thus very different depending whether spore or vegetative cell but also the physiology, nutrients or competing microbiota of plant, leave, juice (or BHI) and thus case by case experimental data needed to assess behaviour in a particular context.

MB-04 The leaky gut syndrome: insect gut bacteria exacerbate physical and chemical defenses of plants

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Many plant defenses that deter insect herbivory target the attacker's digestive system. We found that plant defenses against the fall armyworm created opportunities for resident gut microbes to penetrate protective gut barriers, invading the body cavity and exacerbating the negative impacts of plant defenses on the insect. These interactions triggered insect immune responses and collectively overwhelmed the insect's ability to cope with multiple stressors. However, the effects varied between bacterial taxa, indicating that variation in the caterpillar microbiome can alter their phenotype. Our results reveal a previously unrecognized, and likely widespread, mechanism allowing the plant to use the insect's gut microbiota against it in collaboration with the plant's own defenses. These results are important for not only understanding the ecological function of plant defenses but also in the rational design and engineering of pest resistance in crops.

MB-05 Bioherbicides from creation to commercial success – *What's the problem?*

Karen Bailey

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The concepts of biological weed control have been around since the turn of the 20th century, but it was not until the 1970s when the thought of employing plant pathogens as biological herbicides emerged. It took until the early 1980s to release the first commercial products, DeVine[®] and Collego[®]. Since then, intensive efforts by researchers around the world have isolated many microbial species that show potential to reduce weed populations (the discovery and proof-of-concept stage). But turning these discoveries into commercial bioherbicide products has been elusive. Since the registration of DeVine[®] and Collego[®], there are only 10 bioherbicides registered by government regulatory agencies worldwide and few have sustained commercial sales for more than a few years. So, why do we find examples of commercial success with other biopesticides, such as those for disease and insect control, but so few examples for weed control? What are the key criteria that must be addressed to allow companies to commercialize future bioherbicide discoveries? The journey from discovery to commercialization of a bioherbicide is a 10-15 years process, so it makes sense to have a clear idea of the stages and steps to logically and swiftly move through the process. This presentation will identify the unique opportunities and challenges encountered with the business model template used to develop the fungus *Phoma macrostoma* as a bioherbicide for broadleaved weed control. Exploring the specific traits of this fungus and the features of this bioherbicide, we will demonstrate the role that science, industry, regulation, consumers and market forces have played in achieving success in urban, horticultural, forestry, and agricultural environments. We also reassess whether the business model template is the most appropriate model for developing bioherbicides and consider alternative approaches to getting bioherbicides into the hands of consumers.

MB-06 Contrasting effects of the rhizobacterium *Pseudomonas simiae* on above- and belowground insect herbivores

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Plant-growth promoting rhizobacteria (PGPR) can enhance plant growth and reinforce plant defense. PGPR, via plant-mediated effects, are generally regarded to have negative impact on the performance of leaf chewing insects. However, few studies include belowground herbivores and the effect of rhizobacteria on their performance is still unclear. To use these bacteria in sustainable agriculture, we need understanding the effects on pest insects interacting with above- and belowground plant tissues.

The aim of this study was to investigate the effects of a PGPR on the performance of an aboveground and a belowground insect pest species. In a greenhouse experiment, we grew white cabbage (*Brassica oleracea* var. Christmas Drumhead) in a sterilized perlite-soil mix, together with the model bacterium in studies of rhizobacterial induced plant defense, *Pseudomonas simiae* WSC417r. After 5 weeks, we infested the plants with larvae of the diamondback moth *Plutella xylostella*, cabbage moth *Mamestra brassicae* or the cabbage root fly *Delia radicum*. Insect weight and plant biomass was recorded. Bacterial inoculation had contrasting effects on the aboveground and belowground herbivores: inoculation reduced the performance of *Plutella xylostella* caterpillars, did not influence *Mamestra brassicae* caterpillars, and enhanced the body mass of adult *Delia radicum*. Thus, the application of rhizobacteria differentially affected insect herbivores and may not result in a uniform protection against insect pests.

PF-MB-05 Microbial ecology of the European apple canker pathosystem (*N. ditissima*)

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European apple canker, caused by the fungus *Neonectria ditissima*, is one of the most important diseases of the apple tree and fruit globally, and is especially destructive in North-Western Europe. Disease management can be problematic because *N. ditissima* is able to produce asymptomatic infections which can last for months or even years. Additionally, as an increasing number of agrochemicals active against European apple canker are being banned in the EU, effective control measures are limited.

N. ditissima has been speculated to initially grow in plant tissues as a component of the endophytic microflora, and later switch to its pathogenic phase. The fungus' interactions with other apple tree endophytes, and their potential action as pathogen facilitators or antagonists, have never been investigated. Together with the host genetics, apple tree endophytes might account for the different levels of disease resistance observed across the different cultivars, and represent an untapped reservoir of biocontrol agents.

In this current project, we are investigating the role of apple tree fungal and bacterial endophytes in the European apple canker pathosystem. We have initially localized the pathogen *in planta* during the asymptomatic phase of the disease. Using high-throughput next generation sequencing techniques (meta-barcoding), we are now carrying out an extensive profiling of the fungal and bacterial endophytic species that co-localize with the *N. ditissima* asymptomatic infection, across different apple cultivars. Our goal is to analyze the correlation between the endophyte profiles characterizing each cultivar and its respective disease resistance level. This will help understand the components of European apple canker field resistance and will inform further research into the development of innovative management strategies, such as the exploitation of endophytes for biological control.

PF-MB-06 Microbiome-assisted management of plant diseases

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Due to ever increasing global food demand, various advancements in agriculture practices are being adopted. The major challenge to maximize crop productivity is the incidence of a variety of plant diseases. Different chemical-based formulations are being used to control them. Although these conventional approaches have enhanced the output, they exert a deleterious impact on environment. An attractive sustainable alternative to this problem is organic farming which, avoids the use of chemicals and is completely based on natural resources. Soils treated with organic manure have enriched microbial diversity which subsequently leads to increased diseases suppressive ability against a number of plant pathogens, thus conferring **general suppressiveness**. The mechanism underlying general suppression of plant pathogens, which is an inherent property of some soil and is due to multitrophic and collective interaction between whole microbial community and diseases causing organisms in the soil ecosystem, is of key interest. In the present study long-term and short-term field experiments of rice–wheat cropping systems under organic and conventional farming practices were chosen to map suppressiveness against a number of common soil-borne pathogen in Indian arable land. The study aims to determine the impact of farming practice on disease suppressiveness. Further, diversity analysis and isolation of potent antagonistic strains along with other desirable PGP properties to suppress the pathogenesis of the plant pathogens in fields. Culture-independent techniques were employed to study the structural and functional diversity in the disease-suppressive soil. Further studies are directed towards employing metabolomic approach to understand the mechanisms and metabolites responsible for suppression of soil-borne pathogens in the soil. The soil treated organically was found to be more suppressive than conventionally treated and bulk soil against all the selected fungal and bacterial plant pathogens. Strains belonging to genera such as *Pseudomonas*, *Bacillus* and *Actinomyces* having antagonistic potential that were isolated from soil treated with organic manure showed significant disease suppressive ability against selected bacterial and fungal pathogens. Strategies to design synthetic microbial community using these selected potent antagonistic strains is the next step of our study. This will prove to be an economical and sustainable solution to control plant diseases.

PF-MB-07 A species-specific crosstalk via volatile exchange between a biocontrol agent *Serratia plymuthica* HRO-C48 and fungal plant pathogens.

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The rhizosphere-associated bacterium *Serratia plymuthica* HRO-C48, commercially used as a biocontrol agent (RhizoStar®, E-nema GmbH Raisdorf, Germany), is able to suppress symptoms caused by soil-borne fungal pathogens. The interaction of HRO-C48 with each of the three tested plant pathogenic fungi *Rhizoctonia solani* AG2 Kühn, *Verticillium longisporum* ELV25 Stark and *Leptosphaeria maculans* MB 158 was assessed by a volatile organic substance (VOCs) assay coupled with a transcriptomic analysis. The selected soil-borne fungal pathogens cause high yield losses in a range of plant crops all over the world. After 72 h of exposure to fungal VOCs, 233 differentially expressed genes were identified among *S. plymuthica* transcripts (94 up- and 139 downregulated). The predicted functions of the common genes that were downregulated due to the exposure of *S. plymuthica* to all three fungal VOCs suggest an unspecific stress response of *Serratia* to the fungal volatiles, as well as an enhanced biofilm formation. The later may be associated with a reduction of cellular motility. The interaction of *S. plymuthica* with the *V. longisporum* VOCs resulted in a significant downregulation of 40 transcripts, while only 20 transcripts were upregulated. The strongest difference in the regulation of genes was found between the treatments of *S. plymuthica* with the VOCs of *R. solani* and *L. maculans*. *R. solani*, which growth was inhibited by bacterial volatiles at strongest, produced in its turn VOCs that resulted in the strongest upregulation of the bacterial transcripts. *L. maculans* VOCs, on the other hand, had exactly the opposite effect on the bacterial gene expression pattern with the majority of the genes being downregulated. Additionally, we found several transcripts putatively involved in antagonistic activity in the HRO-C48 transcriptome. Some of them were upregulated upon the contact with *R. solani* VOCs, and downregulated due to *L. maculans* volatiles. Thus, our data suggests an ongoing strongly species-specific crosstalk via volatile exchange between the fungal and bacterial cells, that may contribute significantly to the antifungal mode of action of the biocontrol agents such as *S. plymuthica* HRO-C48.

PF-MB-08 Effect of *Bacillus subtilis* EA-CB0575 on the microbiota, growth development and health of banana plants

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The artificial introduction of microorganisms has been a widely studied practice to modulate the desired plant responses that emerged as an alternative for biocontrol and handling in agriculture. The inoculation of micropropagated plant tissues has been one of the research fields that has shown improvements in different features of the treated plantlets. This research evaluates the effect of *in vitro* bacterial inoculation on the microbiota of banana plants during their growth stage under greenhouse conditions focusing on plants that showed a reduction in the severity of foliar disease caused by *Pseudocercospora fijiensis*. To this end, a bacterial community characterization associated with the roots of *in vitro* banana plants was initially carried out using a culture-independent approach to identify the main taxonomic groups located in this organ at an early development stage where results indicated that the genus *Stenotrophomonas* was dominant, exceeding 80% abundance in most samples. Following a *screening in planta* carried out with single bacteria and in combinations to determine the effect on plant growth speed and the severity of Black Sigatoka disease after 180 days of *in vitro* bacterial inoculation. The strain *B. subtilis* EA-CB0575 reduced necrotic area by 54% and 33% compared to the control in two experiments carried out at different times. The reproducibility effect obtained with rhizobacteria *B. subtilis* EA-CB0575 led to the third stage of this research focused on evaluating the impact of the inoculation of this strain on the root/rhizosphere microbiota 150 dai, where results indicated a differential enrichment of low abundance taxa between 0.01% and 0.8% out of the total bacterial community with a greater sequence number in the plants treated 24 h before infection with *P. fijiensis* and that was considerably reduced 28 h after infection with the pathogen. Lastly, a comparison of the bacterial community associated with the roots between banana plants *in vitro* with 9 months of development and 14 months old greenhouse grown plants was carried out, confirming that the development stage significantly influences the conformation of the bacterial profile.

MB-07 The role of endophytic entomopathogens in modulating plant-microbe-insect interactions

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During recent years the endophytic entomopathogens, such as *Beauveria bassiana* and *Metarhizium anisopliae*, have been acknowledged to show biocontrol potential against insect herbivores in several different crops. Despite the reported negative effects of endophytic entomopathogens on insect herbivores the exact mechanisms responsible for these effects are still largely unknown. Furthermore, while the harmful side-effects of chemical pesticides on the natural enemies of the pests and pollinators are well known, very little is known about the potential role of endophytic entomopathogens in steering higher trophic level ecosystem services, such as those conducted by the natural enemies of pests or pollinators. Given this, the aim of our ongoing project is to test how endophytic entomopathogens modulate plant-microbe-insect interactions. As a model plant-herbivore-microbe system, we use oilseed *Brassica rapa*, its most important pests (pollen beetle, *Meligethes aeneus* and flea beetles, *Phyllotreta* spp.), and entomopathogenic fungi (*B. bassiana* and *M. anisopliae*) which are known to be able to colonize tissues of oilseed rape plants as asymptomatic endophytes. We are currently running the first set of replicated greenhouse experiments to test the biocontrol potential of the fungi directly as entomopathogens and indirectly via phytohormone signaling pathways of its host plant. Furthermore, at the later stages of the project we will determine if endophytic entomopathogens have bottom-up effects on natural enemies of pests as well as on pollinators.

This project is part of the European Union funded Horizon2020-project 'EcoStack'. The overall objective of EcoStack is to develop and support ecologically, economically and socially sustainable crop production via stacking and protection of functional biodiversity. By designing and implementing integrated systems for ecostacking, the project will contribute to a long-term sustainability of agriculture and food production.

MB-08 Plant associated bacteria for the control of *Impatiens glandulifera*

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Impatiens glandulifera is a highly invasive annual species, which has spread rapidly in many parts of Europe. It was introduced into Europe as ornamental plant in 1839 and later on planted as bee pasture and escaped into nature. Since the introduction in the UK as garden ornamental plant, *I. glandulifera* spreads rapidly. The plant is the tallest annual plant in Europe and reaches 50 to 250 cm in height.

Since 2016 *I. glandulifera* is listed in the EU regulation 1143/2014. Control measures have to be developed to mitigate this plant and reduce the spread. Currently contaminated sites are mostly mowed or mulched. The use of herbicides is mostly prohibited because of the neighboring water resources or destruction of the native vegetation, which makes the site prone for new infestation.

Therefore, a measure targeting only *I. glandulifera* without any negative effect to the environment is needed. The use of biological control agents is one measure to reduce the growth of the invasive plant.

We isolated plant associated bacteria and tested them for herbicidal effects against *I. glandulifera* and other weeds. In in vitro tests most of the bacteria reduced the growth of the weeds depending on the applied concentration. Some bacteria only reduced the growth of certain plant species, but others showed a growth reduction in broadleaved and monocot plants. Further tests are required to test for herbicidal effects in the presence of native soil microorganisms and under more realistic conditions.

MB-09 Suppressing *Fusarium graminearum* and mycotoxins by application of microbial antagonists on infected crop residues

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The orientation towards sustainable agricultural systems requires innovative and integrated methods for control of the fungal disease Fusarium Head Blight (FHB) in wheat to reduce the risk of mycotoxins that contaminate food and feed. Preventive actions against the dominating pathogen *Fusarium graminearum* using biological control agents (BCA) on infected crop residues could contribute to reduced applications of synthetic fungicides. The efforts must focus on microbes with a proven activity against mycotoxin accumulation and a saprophytic lifestyle that is adapted to the environment. Within the Horizon 2020 project MycoKey, we investigated the ability of the fungal species *Clonostachys rosea* and *Trichoderma atrobrunneum* to suppress *F. graminearum* on maize residues and thus to reduce mycotoxins.

At first, we explored the antagonistic activity of *C. rosea* strain 016 on maize stalk pieces infected with *F. graminearum*, either 48 hours before, simultaneously or 48 hours after the treatment. In contrast to other fungal candidates, *C. rosea* strain 016 completely inhibited the formation of perithecia as well as the discharge of ascospores. Investigations on the cellular level using a novel microfluidic platform, the "Fungal-Fungal Interaction device", suggest parasitism behind the observed activity of *C. rosea* against *F. graminearum*. Subsequently, field experiments were carried out in 2016/17 and 2017/18 to compare the efficacy of formulations of *C. rosea* strain 016 and *T. atrobrunneum* strain ITEM908. The collected data included *Fusarium* spore dispersal during the infection period, disease symptoms, mycotoxin content as well as the incidence of *Fusarium* species and *F. graminearum* DNA in harvested grains. The treatments with *C. rosea* strain 016 resulted in significantly lower FHB symptoms and reduced the deoxynivalenol (DON) content in harvested grains by up to 82% in the first and by up to 90% in the second year. Likewise, zearalenone (ZEN) was reduced by up to 80% in the first and by up to 90% in the second year. Treatments with *T. atrobrunneum* ITEM908 showed high variability between years. While no significant reductions were found in the first year, DON and ZEN were reduced by up to 80 and 90% in the second year. The great potential of *C. rosea* to reduce FHB will be further investigated in on-farm experiments.

MB-10 Combining nanomaterials and phages for enhanced bacterial wilt control

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Ralstonia solanacearum is plant-pathogenic bacterium caused bacterial wilt disease in a wide range of plant hosts. We proposed bacteriophage therapy as one of the most promising approaches to control this bacterium in our previous experiments. However, the application of the bacteriophage trophy for plant disease control is currently hindered by pathogen resistance development to bacteriophages and the limitation of movement within plant tissues. The goal of our study is to produce an enhanced virulence synthetic bacteriophage consortium against *Ralstonia solanacearum*, by combining phage trophy and nano-technology. Our results showed that the combination of bacteriophage and silicon nano-particle decreased the ability of bacteria evolving resistant to bacteriophage during the course of evolution, in vitro. We have tested this combination in roses plants under greenhouse condition and showed higher resistance and less disease severity in host plants. We concluded that the combination of nanomaterials and phages can be an effective biocontrol agent to protect the crop plants against phytopathogenic bacteria in agriculture and horticulture.

MB-11 Iturinic lipopeptide diversity of the *Bacillus subtilis* group

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Iturins and closely related lipopeptides constitute a family of antifungal compounds known as iturinic lipopeptides that are produced by species in the *Bacillus subtilis* group. The compounds that comprise the family are: iturin, bacillomycin D, bacillomycin F, bacillomycin L, mycosubtilin and mojavensin. These lipopeptides are prominent in many *Bacillus* strains that have been commercialized as biological control agents against fungal plant pathogens and as plant growth promoters. The compounds are cyclic heptapeptides with a variable length alkyl sidechain, which confers surface activity properties resulting in an affinity for fungal membranes. This study identified 330 iturinic lipopeptide clusters in publicly available genomes from the *Bacillus subtilis* species group. The clusters were subsequently assigned into distinguishable types on the basis of their unique amino acid sequences. The results show some lipopeptides are only produced by one species, whereas certain others can produce up to three. In addition, four species previously not known to produce iturinic lipopeptides were identified. The distribution of these compounds among the *B. subtilis* group species suggests that they play an important role in their speciation and evolution.

MB-12 Microbial consortia effectively suppress and prevent infections of *Ralstonia pseudosolanacearum* in *Rosa* sp.

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Bacterial wilt caused by *Ralstonia pseudosolanacearum* is one of the most destructive bacterial diseases in plants. This pathogen has evolved the ability to infect roses, causing a huge economic loss since its discovery in the EU in 2015. In this work, we seek to increase plant resistance to bacterial wilt by improving the rose microbiome. We discovered that roughly 10% of the cultivable naturally-occurring rose endophytic bacteria can suppress *R. pseudosolanacearum* *in vitro*. We further show that plant inoculation with a consortium of seven pathogen-suppressive strains could prevent bacterial wilt incidence by 50% amount of wilted rose plants compared with the control treatment. Further, the introduced consortia sharply reduced inside rose shoots and roots in asymptomatic plants. We conclude that the plant endophytic microbiome may be a promising target to prevent diseases outbreaks.

MB-13 The plant protecting and plant growth promoting abilities of the synthetic micro-consortium of antagonistic bacterial strains.

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Plant associated microorganisms comprises bacteria possessing plant-growth promoting and plant protecting abilities. Activity of these bacteria results from different mechanisms including the production of a wide range of antimicrobials, e.g. volatile organic compounds (VOCs). These compounds may also influence plant growth. Application of plant-beneficial bacteria is potentially a promising strategy to protect plants against pathogens both during the plant growth and in storage. The mixture of the selected plant-associated bacteria and the individual strain were investigated as potential plant growth promoting and plant protecting rhizobacteria (PGPR). For this, the ability to colonize plant roots and to promote plant growth by the mixture of strains and an individual strain was verified using tomato and *Arabidopsis thaliana* seedlings. The capacity of inhibiting the growth of fungal pathogens (*Rhizoctonia solani* and *Fusarium culmorum*) was analyzed in dual culture of the selected fungus and the individual strain or the mixture. The influence of VOCs produced by the single strain and the mixture on the growth of fungal pathogens was analyzed using the top-bottom culture approach. As the results, we observed that, the strains tested singly are equally effective in tomato roots colonization as the mixture of strains. Three individual strains affected negatively tomato growth after seedling colonization, while this effect was not observed in case of the mixture of strains. Yet, the effect of VOCs on *A. thaliana* growth indicated that VOCs of two strains increased the growth of the rosette area. This effect was not observed in case of the mixture of strains. For fungal growth inhibition, the individual strains were more effective in suppression of fungal growth than the mixture in dual cultures. Yet, the mixture was equally in inhibition of *R. solani* via VOCs, as the individual strains but this effect was not observed in case of *F. culmorum*. In conclusion, the use of the mixtures of the strains for plant protection and plant growth promotion has advantages over the use of individual biocontrol strain.

miCROPe 2019 - Microbe-assisted crop production opportunities, challenges & needs

Vienna, Austria, December 2 – 5, 2019

Microbial applications for improving nutrition and abiotic stress tolerance

Chairs: Philipp Franken & Günter Brader

MA-01 How plants benefit from root-colonizing fungi: There's more than one way to crack an egg.

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Different ecosystem services are provided by root-colonizing fungi, whereof their contribution to nutrient cycling plays an important role for plants. Fungi with saprophytic abilities like sebacinaceous *Serendipita indica* and Dark Septate Endophytes (DSEs) release plant-available phosphate from inorganic and organic compounds. Arbuscular mycorrhizal (AM) fungi are able to transport nutrients along their coenocytic hyphae towards the plant forming a continuum from the source in the soil to the sink inside the root. In case of phosphate, this is reflected by the regulation of nutrient transporters.

Plants also benefit from root-colonizing fungi if environmental conditions are detrimental for their functions. Under drought, many fungi increase the activity of tolerance mechanisms of the plant like the scavenging of reactive oxygen species or the osmotic balance of cells. Due to their hyphal spread outside the roots, AM fungi also impact soil structure providing favorable conditions for plant water uptake.

In order to increase the abilities of fungi to provide ecosystem services, different strategies have been followed. In one approach, an AM fungus was acclimatized to high heavy metal contamination and this increased the tolerance not only of the fungus, but also of the colonized plant. In a second approach, *S. indica* was combined with a bacterium forming biofilms on the hyphae. This led to higher biomasses of the fungus and of the co-inoculated plant under growth-limiting conditions. DSEs are characterized by their high melanin content in their hyphae which has been thought to confer tolerance to different abiotic stressors. The hypothesis concerning the role of melanin in abiotic stress tolerance was tested by molecular, biochemical and genetic approaches.

PF-MA-01 Interactions of arbuscular mycorrhizal fungi and winter wheat in contrasting cropping systems

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The benefits of arbuscular mycorrhizal fungi (AMF) as plant performance improving symbionts with a broad range of plant species have been well documented in research and are more and more recognised in plant producing industry. However, the importance of AMF in agricultural systems has not been defined yet as a beneficial outcome of the sensitive symbiosis of plant and fungi depends on multiple environmental factors, but also the host genotype. This study investigates the interactions between AMF (native vs commercial inoculant), varieties (long vs short straw), fertiliser types (Biogas Digestate, Cow Manure, mineral fertiliser, no fertiliser) and crop protection (conventional vs organic) by using a multifactorial split plot field experiment over two years. In both growing seasons, shoots and roots are harvested for biomass and root colonisation assessment at five key growth stages. First year results show low impact of inoculation on grain yield, plant growth and health, but major effects of fertiliser and host genotype on AMF colonisation of roots. Highest colonisation rates were reached at flowering in non-fertilised wheat plants of the conventionally bred variety while all fertiliser applications decreased AMF abundance significantly. Ongoing experiments analyse the AMF species composition in the harvested wheat roots by distinguishing between native and exogenous AMF strains.

PF-MA-02 Impact of beneficial microorganisms on strawberry growth, fruit production, nutritional quality and volatilome

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Arbuscular mycorrhizal fungi (AMF) colonize the roots of most terrestrial plant species, improving plant growth, nutrient uptake and biotic/abiotic stress tolerance. Similarly, plant growth promoting bacteria (PGPB) enhance plant fitness and production. In our study three different AMF (*Funneliformis mosseae*, *Septoglomus viscosum* and *Rhizophagus irregularis*) were used in combination with three different strains of *Pseudomonas* sp. (19Fv1t, 5vm1K and Pf4) to inoculate plantlets of *Fragaria x ananassa* Duch var. Eliana F1. The effects of the different fungus/bacterium combinations were assessed on plant growth parameters, fruit production and quality, including health-promoting compounds. Uninoculated plants were kept as controls. At harvest, fresh and dry weights of roots and shoots, mycorrhizal colonization and concentration of leaf photosynthetic pigments were measured in each plant. Many fruit parameters were recorded: pH, titratable acids, concentration of organic acids, soluble sugars, ascorbic acids and anthocyanidins; volatile and elemental composition were also evaluated. Data were analyzed with standard statistical methods (ANOVA) and the data obtained from all analyzed parameters were subjected to multivariate statistical methods (PCA and PCA-DA). In general, AMF mostly affected the parameters associated with the vegetative portion of the plant, while the PGPB were especially relevant for fruit yield and quality. The plant physiological status was differentially affected by inoculations, resulting in enhanced root and shoot biomass. Inoculations affected fruit nutritional quality, increasing sugar and anthocyanin concentrations, and modulated pH, malic acid, volatile compounds and elements. In our study, we show for the first time that strawberry fruit concentration of some elements and/or volatiles can be affected by the presence of specific beneficial soil microorganisms. In addition, our results indicated that it is possible to select the best plant-microorganism combination for field applications, reducing chemical inputs, and improving fruit production and quality, also in terms of health promoting properties.

Todeschini V., Ait Lahmidi N., Mazzucco E., Marsano F., Gosetti F., Robotti E., Bona E., Massa N., Bonneau L., Marengo E., Wipf D., Berta B. and G. Lingua (2018) Impact of beneficial microorganisms on strawberry growth, fruit production, nutritional quality and volatilome. *Frontiers in Plant Science*, <https://doi.org/10.3389/fpls.2018.01611>

PF-MA-03 SIMBA: Design, formulation and optimization of plant growth-promoting microbes for their use as microbial consortia inoculants

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The interactions between plant-roots and the surrounding soil, including the resident microbial populations, play an essential role on crop yield. A growing body of evidence demonstrates the potential of various microbes to enhance plant productivity in cropping systems although their successful field application may be impaired by several biotic and abiotic factors. In this context, the activities of Work Package 2 of SIMBA (Sustainable Innovation of MicroBiome Applications in the food system) project were dedicated to exploit the full potential of Plant Growth-Promoting Microorganisms (PGPMs) for sustainable crop production by optimising the efficacy and reproducibility of field applications. In order to identify the PGPMs to be applied as bioinoculants on different crop plants (wheat, maize, potato and tomato) in Italy and Germany, a comprehensive literature survey was performed by examining peer-reviewed articles and results from European related projects. The following functional groups of microorganisms were considered; i.e., phosphate solubilizing microbial strains, nitrogen-fixing bacteria, biocontrol strains, endophytic bacteria. To guarantee the development of compatible microbial consortia, selected PGPMs were preliminary screened *in vitro* for their ability

to coexist and exert a PGP activity. Supported by funding from the EU Horizon 2020 research and innovation programme under grant agreement No. 818431 - SIMBA (Sustainable Innovation of Microbiome Applications in the Food System, <https://simbaproject.eu>).

PF-MA-04 Influence of soil microbial communities linked to organic matter addition on tomato (*Solanum lycopersicum* L.) plant growth under intensive farming

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Intensive greenhouse crop production is one of the most important economic activities in south-east Spain. Intensification has limited the application of organic matter (OM) as a fertilizer in favor of chemical fertilizers, leading to altered soil microbial communities and production loss. We addressed the effects of different greenhouse OM managements on soil microbial communities and their effect on productivity. Greenhouse managements were i) conventional (with addition of chemical fertilizers) without OM application; ii) conventional with OM application, and iii) organic with yearly OM application (without addition of chemical fertilizers). We extracted soil microbial communities from five greenhouses per management type, added the inocula to pots with sterile substrate, and seeded disinfected tomato seeds. Plants grew for 2 months, and at harvest we measured photosynthetic rate, plant growth, and leaf functional traits. Soil microbial diversity and abundance in the original soils did not differ across greenhouse management type. At harvest, plants grew more and had greater photosynthetic rate in pots inoculated with extracts from organic greenhouses than from greenhouses under conventional management without OM application, and potting substrate in the former showed higher bacterial abundance and phylogenetic diversity than the latter. NO₃⁻ and NH₄⁺ content differed across treatments at harvest, but not at the onset of the experiment, showing differences in microbial activity between treatments. Results suggest that soil microbial communities from organic greenhouses had an overall positive effect on crop productivity.

PF-MA-05 Microbial consortia: a way to enhance crop yield under both controlled environment and field conditions

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Root-rhizosphere microbiome associated microbial communities play a key role in the establishment of a plant and contribute to the plant's health and development; plants control this community through root exudates and signal compounds.

This study mainly focuses on the evaluation of specific microbial consortia as microbe coatings for fertilizers, as plant growth promoting agents.

The research evaluates i) field performance and efficacy of the microbial consortia in different soil types and fertility, and ii) how the consortia interact with drought stress under greenhouse conditions.

Corn and potato were grown under field conditions where two different microbial consortia, at various concentrations, were applied at two seeding dates (early and late dates). During the growing season, data were collected for growth variables (plant height, leaf area and biomass) while at the end of the growing season, cobs and tubers were harvested for yield components.

Some of the first year's results show both microbial consortia increased yield in potatoes by 20-23% over untreated plants. The response of corn to the consortia was varied as clay soil resulted in the highest yield, 10-14% greater than the control, while sandy loam showed only a 2.5% increase. On the other hand, the consortia provided potato with tolerance to mild and severe drought stress conditions. Particularly, the consortia promoted root growth and prolonged the shoot growth under greenhouse conditions.

PF-MA-06 Effect of coinoculation of *Rhizoglossus irregulare*, and hyphae attached phosphate solubilizing bacteria on *Solanum lycopersicum*

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Sustainable agricultural practices are needed to improve plant yield and to solve the global crisis of optimal food production in the coming years without further detrimental impact on the environment. Arbuscular mycorrhizal fungi share a symbiotic relationship with the majority of terrestrial plants, playing a key role in improving availability of nutrients and water uptake of plants. AM fungi are barely able to solubilize phosphate in significant amounts but can aid in the transfer of P from the soil to the plants. AM fungal hyphae have been shown to be colonized by a diverse bacterial community. It is important to study the interaction between P-solubilizing bacteria and AM fungi, to unravel if the bacteria can further improve plant P nutrition when AMF are present. It is further important to decipher these interactions in order to exploit the full potential of these microorganisms as bioinoculants. One approach for enhancing the effects of such bioinoculants could be co-formulations.

The aim of the present study was to isolate P-solubilizing bacteria strongly attached to the hyphae of *Rhizoglossus irregulare* using a two compartment pot system (a root compartment and a hyphal compartment), separated by a 30 µm nylon mesh through which AMF hyphae could pass but not the plant roots. *Allium ampeloprasum* (Leek) was used as the host plant inoculated with *R. irregulare*.

A total of 128 hyphae-associated bacteria were isolated, whereof 12 showed stable phosphate-solubilizing activity. Finally, three bacteria belonging to the Pseudomonas family, namely PSB1, PSB11 and PSB18 showed highest potential for inorganic and organic phosphate mobilization.

The three bacteria were further evaluated for their functional characteristics, for interaction with the AM fungus and for their impact as single or co-inoculants on plant growth promotion. We tested the effect of co-inoculation of the bacterial-fungal consortia on *Solanum lycopersicum* and found that plants inoculated with the combination of fungus and bacteria had significantly higher root biomass and improved P uptake compared to the single inoculations.

We conclude that co-formulations of AM fungi and functionally important hyphal colonizers such as P-solubilizing bacteria can be a way to significantly enhance AMF inoculum benefits.

MA-02 Matching root anatomical and architectural phenotypes with soil microorganisms to improve nutrient and water uptake efficiency: a new perspective in plant microbiome research

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Root phenotypes are highly diverse at the architectural and anatomical levels of organization. Specific root phenotypes are associated with better plant growth under low nutrient and water availability. Therefore, root ideotypes have been proposed as breeding targets for crops growing under nutrient and/or water scarcity. For example, roots with phenotypes that correspond to the ideotype *Topsoil foraging* are associated with better plant growth under low-phosphorus stress, and the ideotype *Steep, Cheap and Deep* is linked to low nitrogen/water stress tolerance. We propose that the natural variation in root phenotypes translates into a diversity of different niches for microbial associations in the rhizosphere, rhizoplane and root cortex, and that microbial traits could be synergistic with the beneficial effect of specific root phenotypes. Oxygen and water content, carbon rhizodeposition, nutrient availability, and root surface area are all factors that are modified by root anatomy and architecture and determine structure and functioning of the associated microbial communities. Therefore, the selection of root phenotypes linked to better plant growth under specific edaphic conditions should be accompanied by investigating and selecting the microbial partners better adapted to each set of conditions created by the corresponding root system. Microbial traits such as nitrogen transformation or phosphorus solubilization could have a synergistic effect when correctly matched with promising plant root ideotypes for improved nutrient and water capture. Recent results indicate that root traits that may modify the microbial communities associated with maize are aerenchyma and rooting angle, and root hairs and root class have been studied in other plant species. We will present examples of the effects of anatomical and architectural root phenotypes linked to differential microbial associations as obtained by our research and from recently published results, and propose a model to test hypotheses about the interactive effects of root phenotypes and microbial functions on plant nutrient and water uptake.

MA-03 Combating salinity stress with Rhizosphere Engineering: A next-generation approach

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Plant growth is drastically affected by the salinization of soil. With the ever-increasing mean global temperature and the poor quality of water being used for irrigation, this problem of soil salinization is expected to grow further and will lead to several socio-economic problems. Approximately 50% of the total land under irrigation is adversely affected by salinity. A major push in crop productivity is required to feed the exponentially growing population. The conventional strategy of cultivating salt-tolerant plant varieties has often failed to address this problem effectively. Due to many notable impacts of microorganisms on crops, the use of various microorganisms harboured by crops has gained attention. The area in the immediate vicinity of the root is known as “Rhizosphere” and has been well characterised for its intense microbial activity under the influence of rhizodeposits. Single strains of microbes in the form of inoculants are often ineffective growth of plant and stress adaptability, mainly owing to the competition with the indigenous rhizospheric microbial community and restricted colonization effectiveness. The plant along with its associated microbiome is considered as meta-organism and is known as *holobiome*. We have used the approach of plant-assisted multigeneration approach for acclimatizing the microbiome in which the host plant is allowed to select a microbiome that is beneficial for the growth of the plant. The model system used for the study was *Vigna radiata* (mung bean) owing to its short life cycle. The adapted microbiome helped the plant to better withstand the salinity stress. The plant growth promotion ability and mitigation of salt stress was validated by the enhancement of plant biometric parameters and reduction of plant stress markers. The long-term aim of our research is to develop a synthetic microbial community with the ability to help plant ameliorate salt stress. These innovations will open fresh avenues to capitalize on the rhizosphere microbiome in order to reinforce the tolerance of a plant to salt stress and thus refine agricultural practice under saline circumstances.

MA-04 Phosphate fertilization in crops – the contribution of bacteria and fungi

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Modern agriculture depends on resource-intensive fertilization for optimal nutrition of crops to meet the demand for food and feed. The three main macronutrient components of fertilizers are phosphorus (P), nitrogen (N) and potassium (K). P and K are mined resources and especially mined P (in the form of rock phosphate) is a critical resource for the European Union, where 90% of is imported. In mineral fertilizers P is supplied in form of processed superphosphates. P is often not rare in soils, but not available for many crop plants as it becomes fixed to soil mineral complexes and depending on soil parameters such as pH only a small fraction of P from fertilizers is available for plant uptake.

Bacteria such as Bacilli and Pseudomonads and certain fungi (*Penicillium* and *Aspergillus* spp.) have been long described for P-solubilizing effects and could be applied to substantially reduce the need for application of the limited and energy intense resource P in agriculture. The knowledge on the mechanisms and the contribution of different microorganism on P-uptake in plants and the ability to survive in agricultural soils are crucial factors for improvement of agricultural applications. This and the limitations and the complexity of solutions with living microorganisms for broader applications as P-fertilizers in agriculture will be discussed.

MA-05 Maize inoculation with microbial consortia: contrasting effects on rhizosphere activities, nutrient acquisition and early growth in different soils

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The benefit of plant growth-promoting microorganisms (PGPMs) as plant inoculants is influenced by a wide range of environmental factors. Therefore, microbial consortia products (MCPs) based on multiple PGPM strains with complementary functions, have been proposed as superior, particularly under challenging environmental conditions and for restoration of beneficial microbial communities in disturbed soil environments. To test this hypothesis, the performance of a commercial MCP inoculant based on 17 PGPM strains and seaweed extracts, was investigated in greenhouse experiments with maize on three soils with contrasting pH, organic matter content and microbial activity, under different P and N fertilization regimes. Interestingly, the MCP inoculant stimulated root and shoot growth and improved the acquisition of macronutrients only on a freshly collected field soil with high organic matter content and high background microbial activity, exclusively in combination with stabilized ammonium fertilization. This was associated with transiently increased expression of AuxIAA5 in the root tissue, a gene responsive to exogenous auxin supply, suggesting root growth promotion by microbial auxin production as a major mode of action of the MCP inoculant. High microbial activity was indicated by intense expression of soil enzyme activities involved in C, N and P cycling in the rhizosphere (cellulase, leucine peptidase, alkaline and acid phosphatases) without detectable effects induced by MCP inoculation. Contrastingly, the MCP inoculation did neither affect maize biomass production, nor nutrient acquisition on soils with very little C-org and low microbial activity, although a moderate stimulation of rhizosphere enzymes involved in N and P cycling was recorded. There was also no indication for direct MCP-induced solubilization of Ca-phosphates on a highly buffered calcareous sub-soil supplied with rock-phosphate. The results demonstrate that the MCP strategy, combining large numbers of PGPM strains with complementary properties, not necessarily translates into plant benefits under challenging environmental conditions. Soil properties, such as organic matter content, pH buffering and particle size distribution but also the fertilization regime may crucially influence the plant-microbial interactions. Thus, a better characterization of the conditions determining successful MCP application is mandatory.

MA-06 The impact of beneficial microbes on *Brachypodium* nutrient uptake under limiting supplies of nitrogen and phosphorus, monitored with non-invasive phenotyping and molecular approaches

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In times of increasing global population and decreasing arable land per capita, the understanding of plant nutrient uptake and novel strategies to improve nutrient uptake are of utmost importance. Our work focuses on nitrogen (N) – the second most abundant nutrient in plants and phosphorus (P) – a finite global resource. We present studies where use of plant growth promoting rhizobacteria (PGPR) resulted in improved plant performance under limited N or P in *Brachypodium* – a model plant for cereals. Plant roots were analyzed with the non-invasive root phenotyping platform GrowScreen Page (Gioia *et al.*, 2017), or with the 3D printed EcoFab microcosms (Sasse *et al.*, 2019). The latter was adapted and used in combination with Plant Screen Mobile (Muller-Linow *et al.*, 2019), for non-invasive shoot area estimation, in conjunction with root scanning, over time.

In the case of P limitation, plant biomass was higher in plants inoculated with a PGPR. Time series image-analysis of root phenotype allowed visualization of increased root length and changes in root architecture, pin-pointing the time-window when growth promotion took effect after inoculation. A sand experiment similarly resulted in increased biomass in inoculated plants. Study of the molecular mechanisms behind this whole plant, dynamic phenotype is ongoing and involves metabolomics and lipidomics.

In the case where plants with limiting N supply were inoculated with N-fixing PGPR, an end-point harvest showed that ratio of lateral to primary root length increases. More importantly, N concentration in root and shoot tissue increased, along with greater shoot biomass and leaf area. We complemented this destructive harvest with proteomics to investigate the systemic response of *Brachypodium* constitutively grown under limiting N, to the interaction with the

PGPR. Data analysis revealed that these N-fixing bacteria impact central nitrogen metabolism in *Brachypodium*, and indicate a mode of action that upregulates specific N transporters on the root plasma membrane.

The grass model can thus clearly benefit from PGPR, however the time points, tissue responses and molecular mechanisms were different for organisms and nutrient conditions. Efforts are needed to elucidate plant responses to the microorganisms, addressing molecular and tissue architecture, while taking in context plant developmental stage (Arsova *et al.*, 2019) and time since application.

MA-07 Does genetic variation in single spore progeny of an arbuscular mycorrhizal fungus impact cassava yield

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Arbuscular mycorrhiza fungi (AMF) are prolific soil microbes forming symbiotic relationships with the majority of plant species, including most crops. While crops have witnessed intense genomic modifications through centuries of selective breeding, little has been achieved to exploit desirable traits of the AMF symbiosis, even though it is known that they can increase plant productivity. Recent studies using the model AMF species, *Rhizophagus irregularis*, demonstrated that both mono- and dikaryotic states exist in this mostly asexually propagating species. Being coenocytic in nature, nuclei packaged into spores is posited to be a random process, creating disproportional numbers of nuclei in individual spores and leading to allele frequency changes between single spore progeny. Single spores can be taken from individuals and be used to produce new AMF cultures displaying distinct genetic identities. These 'novel' individuals can then be systematically tested with host plants to begin elucidating dependencies of observed plant traits on AMF genetics. To address these questions, we generated more than 40 single spore cultures originating from one of six parental isolates, consisting of the two karyotic states across the *R. irregularis* phylogeny. A reduced representation ddRADseq protocol was performed and obtained reads were mapped to their respective parental genomes to understand how single spore cultures may vary in allele frequency compared to their parent. We further tested these isolates in cassava cultivation in Colombia where inoculation with different progeny lines caused differences in cassava yield. We report allele frequency changes in progeny and address their potential to increase cassava production.

MA-08 Fertiledatepalm – a transdisciplinary collaboration project to ameliorate date palm cultivation via microbial inoculation, organic matter management and mixed cropping using nurse plants

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Date palm is an important crop in Morocco, Tunisia and other drylands with a high agricultural, economic and cultural value. Harsh environmental conditions of those areas, further accelerated by climate change and the spread of root diseases, threaten date palm cultivation. To overcome limitations in productivity, high inputs of mineral fertilizers and pesticides are applied. However, these external inputs strongly affect the environment and livelihoods.

The project aims at establishing an integrated microbe-assisted fertilization approach, combining the inoculation of native soil microbes, namely arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) during the different date palm growth stages, with adapted agricultural management practices using organic amendments and mixed-cropping in Morocco and Tunisia.

As initial step, we established a culture collection of native microbes, isolated from date palm roots and rhizosphere composed of 24 AMF isolates including eight species from six genera, twelve bacterial endophyte isolates composed of *Paenibacillus*, *Mycobacterium*, and *Achromobacter* species and 34 PGPR isolates. Functional characterization of PGPRs revealed that around 50 % can solubilize phosphorus and potassium and between 9 % and 68 % have the ability to produce siderophores, hydrogen cyanid, chitinase, cellulase, amylase and protease. Consortia of microbes were formed and used for inoculations.

Experiments under nursery conditions revealed that inoculation with AMF and PGPR combined with compost significantly increased growth of date palms as compared to non-amended controls enabling farmers to decrease the time prior to field transplantation. On-farm trials performed in productive date palm groves have shown that PGPR inoculation with or without mixed-cropping with sorghum as nurse plants significantly increase fruit characteristics such as fruit flesh weight as well as fruit length and diameter for up to 14 % and leaf macronutrient concentrations for up to 200 % while in addition enhancing the mycorrhizal potential of the soil.

Our integrated fertilization approach is developed in a participatory approach with key stakeholders in so-called innovation platforms, working at laboratory, on-station and on-farm scale to best tackle farmers' needs in order to facilitate adoption and implementation.

MA-09 Unraveling plant physiological behavior modulated by a synthetic microbial community using a non-invasive and continuous medium-scale phenotyping platform

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The intimate association of plants with bacterial and fungal communities directedly or indirectly modulates physiological behavior and helps plants to acquire nutrients, adapt to drought, heat and salinity stresses as well as facing pathogen attack. Despite multiple studies showing microbiome modulation of plant physiological behavior, our knowledge about the mechanisms governing plant response to beneficial plant-microbe interaction is still limited. Thus, aiming to deeply understand how microorganisms modulate plant phenotype, we designed a non-invasive and low-cost phenotyping platform capable to continuously measure several physiological traits every five minutes. This platform was used to assess the effect of an abundance-based synthetic bacterial community (SynCom) from the sugarcane core microbiome inoculated in maize. The SynCom dramatically increased plant biomass and enhance plant tolerance to drought stress. Observations of mature plants of three different commercial hybrids of maize showed that the SynCom positively impact plant physiology by delaying plants' response to drought stress. Severe water deficit led plants to bend over the ground, a symptom firstly observed in uninoculated plants. We also found that in rehydration inoculated plants showed a faster recovery compared to uninoculated ones, indicating that the SynCom optimizes water usage in the recovering process. The continuous phenotyping revealed that the SynCom positively affected leaf temperature as uninoculated plants strikingly presented a higher temperature compared to those in the presence of the SynCom, may indicating an effective water usage as the increase of plant temperature might be particularly harmful. In a condition of water deficit,

inoculated plants also presented a remarkable increase in yield. Altogether, our findings point to a significant potential of our SynCom in maize development under stressful condition, thus leading to further investigation on key molecular mechanisms involved.

MA-10 Synergistic contribution of microbial consortia, micronutrients, and ammonium fertilization to cold tolerance in maize by regulating phytohormone homeostasis and oxidative stress defence

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Low soil temperature in spring is a major constraint for the cultivation of tropical crops in temperate climates. In this study, we describe the exploitation of synergistic interactions by combined application of micronutrients, consortia of plant growth-promoting microorganisms (PGPM) and the form of nitrogen fertilization (nitrate versus stabilized ammonium supply) on recovery and early growth of maize after two-weeks exposure to low root zone temperatures at 12 °C on a silty loam field soil (pH 6.8). In maize plants with nitrate fertilization, the cold stress increased the necrotic and chlorotic leaves by 133% and impaired acquisition of Zn, and Mn. A pre-selection trial with fungal and bacterial PGPM strains revealed superior cold-protective performance for a combined formulation of *Trichoderma harzianum* OMG16 and *Bacillus spp.* with Zn/Mn supplementation (CombiA), particularly in combination with ammonium fertilization. Compared with nitrate fertilization, stabilized ammonium supply improved Zn and Mn related with moderately increased enzymatic and non-enzymatic detoxification of reactive oxygen species (ROS). The shoot concentration of abscisic acid (ABA) as a key regulator for the adaptive cold stress responses was increased by 33 %. Moreover, ammonium fertilization also increased the root auxin (IAA) concentration (+176 %), associated with increased expression of auxin-responsive genes involved in IAA synthesis (*ZmTSA*), transport (*ZmPIN1a*) and perception (*ZmARF12*). Additional inoculation with the microbial consortium promoted root colonization with the inoculant strain *T. harzianum* OMG16 in combination with ammonium fertilization. Further increased IAA concentrations in the root (+121 %) and shoot tissues (+51 %) and increased *ZmPIN1a* and *ZmARF12* expression resulted in a doubling of root length. An increased ABA/cytokinin ratio and increased concentrations of jasmonic (JA) and salicylic acids (SA) were associated with a further increase in enzymatic and non-enzymatic ROS detoxification in the shoot tissue. Additional supplementation with Zn and Mn further increased shoot IAA, root length and total antioxidants, associated with the highest shoot biomass production (+53 %) and the lowest proportion of oxidative leaf damage. These effects suggest a perspective for synergistic mitigation of cold stress symptoms and induction of stress priming effects by a strategic combination of stress-protective nutrients and selected microbial biostimulants.

miCROPe 2019 - Microbe-assisted crop production opportunities, challenges & needs

Vienna, Austria, December 2 – 5, 2019

Regulatory Issues – Special Session

RI-01 Registration of biopesticides in the European Union

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Biopesticides, comprising microorganisms, plant extracts (botanicals) and semiochemicals (pheromones) are highly increasing in importance and attention on the market of plant protection products. Noteworthy, the reduction in the number of chemical active substances from ca. 1000 to 250 but also the request for sustainable and environmental-friendly agricultural alternatives are fueling the demand for biopesticides. Generally, most biopesticides have little to no effects to human health, non-target organisms and the environment. However, applicants have to overcome several regulatory hurdles for the registration of biocontrol products.

Registration of plant protection products in European Union has increasingly strengthened over the past years. The review program under the previous Directive 91/414 governing the registration process already resulted in fewer active substances. Since 2011 the active substances and products are evaluated according to Regulation (EC)1107/2009. Historically, data requirements for microorganisms are derived from those for chemicals. This is critical, since some data requirements which can be easily covered for synthetic chemicals cannot be applied to microorganisms for technical reasons. In contrast, the major advantage of most biopesticides is that their active ingredients are scientifically well-studied, and humans are familiar with those either through direct use or environmental exposure for a long time. The key information for the selection of experimental data is information on the biology of the organism itself or its compounds, in particular on taxonomy and the mode of action. Here, the process of registration will be presented and data requirements crucial for the evaluation of biopesticides will be critically reviewed based on our experience.

RI-02 Proposal for the application of microbiomes in industry: regulatory challenges and opportunities

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The increasing understanding of synergistic effects between the various microbial components to the benefit of plants suggests that it is attractive to use the microbiome, or a combination of several of its constituents, in agricultural applications. When a promising positive effect of a mixture of a number of microbiome's microorganisms has been spotted, the major constituent(s) responsible for that effect as well as other helping players have to be identified. Today, the industrial-scale manufacturing processes of commercial microbiological product are based on the isolation and production of individual strains, which can then be used as a sole active ingredient or as a mixture of several microbes. Moreover, the mode of action of an individual strain, or a mixture of different strains, does play an important role when deciding on the market-access positioning of a commercial product thus setting the trail for which regulatory approach would be most appropriate.

According to the current EU regulations, each microorganism – used either in plant protection or biostimulant products – must be identified at the strain level ahead of regulatory approval. In addition, its safety for humans and the environment must be demonstrated by means of an individual dossier, i.e. per each microorganism, to be submitted for evaluation at the EU level. Hence, it becomes clear that a registration of commercial products containing a mixture of several microbes is extremely costly, under today's rules, and it will prevent microbiome technologies to reach the market unless new regulatory ideas are put in place quickly.

During this communication, we will be addressing some ideas to improve the present regulatory framework in order to embrace scientific innovations arising from new discoveries in plant-microbe interactions. To this end, we propose to look at the current regulation rules and guidance being used for plant extracts and other complex mixtures. The European Union regulations consider a plant extract as a single active ingredient and the whole extract is evaluated during the review process, even if components are to be identified. We will be exploring the idea to regulate a mixture of microbes in a similar way, by assessing the mixture of microbes as a whole even if the safety dossier will still include the taxonomical profiling at the strain level to exclude potential pathogens.

miCROPe 2019 - Microbe-assisted crop production opportunities, challenges & needs

Vienna, Austria, December 2 – 5, 2019

Disruptive approaches for engineering the phytobiome & microbial delivery

Chairs: Trevor Charles & Michael Ionescu

DA-01 Can we tune the microbiome in controlled environment agriculture?

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¹ Waterloo Centre for Microbial Research, University of Waterloo, Canada

² Metagenom Bio

Controlled environment systems, such as hydroponic greenhouses and vertical farms, offer unprecedented opportunities for ensuring favourable microbiome composition for plant growth, product yield and quality, and resilience against disease-causing microbes. Microbiomic and metagenomic surveys of the recirculating nutrient delivery systems of commercial vegetable greenhouses demonstrate clear, crop-specific effects on microbial community structure. The conditions of the rhizosphere are expected to have microbial community enrichment effects. The ability to degrade 1-aminocyclopropane-1-carboxylic acid (ACC), the immediate precursor to ethylene, is associated with many rhizospheric and endophytic bacteria that have plant beneficial effects. The key enzyme is ACC deaminase, which catalyzes the conversion of ACC to ammonia and α -ketobutyrate. As a result, ACC can serve as nitrogen and carbon source, and the resulting reduction of ACC levels reduces stress ethylene. Of interest is the role that ACC plays in shaping the phytobiome, and how this in turn may influence crop health and productivity. As an initial step towards understanding, we used 16S rRNA gene sequence analysis and shotgun metagenomics to investigate the community dynamics of soil and hydroponic nutrient solution enrichment cultures with ACC as nitrogen source, compared to ammonia. We found that the community became much more constrained on ACC, consistent with ACC metabolism being more of a specialized trait. The ACC-enriched cultures were able to promote plant growth. Metagenome-assembled genomes (MAGs) and genomes of pure culture isolates confirmed the presence of *acdS*, encoding ACC deaminase, and provided insight into the nature and diversity of ACC metabolizing strains in recirculating nutrient delivery systems. These enrichment experiments lay the groundwork to guide strategies for microbiome optimization in operating hydroponic systems.

DA-02 Harnessing the power of computational genomics to optimize next generation ag-biologicals

Michael Ionescu, Galit Kuzntz

Research, Lavie-Bio, Israel

Lavie Bio, a subsidiary of Evogene, focus on discovery, optimization and development of microbiome-based ag-biologicals to improve food quality, health and agriculture sustainability. Product challenges include achieving efficacy, stability (consistency) and commercial viability. Lavie Bio utilizes Computational Predictive Biology (CPB) platform to pre-design and optimize product candidates that can overcome product challenges utilizing the genomic-based prism. By breaking down product challenges to biological challenges (e.g. achieving sufficient shelf life, prolonged colonization of the microbiome), Lavie Bio identify limiting biologies (bottlenecks) that impose challenge on the road to product. Behind each limiting biology are biological functions that are associated with strains that can overcome each challenge. These functions are key tools for *ab initio* design of product candidates. In one example, gram-negative strains with short shelf life are matched based on their genomic content with personalized fermentation and formulation protocols that are predicted to significantly protect them during formulation and stabilize their viability during storage. By utilizing this approach, we already succeeded extending the shelf life of gram-negative product candidates by many folds. Such predictors are enabled by harnessing data from gene level through to the phenotypic level and by genome analyses technologies combining machine learning and comparative genomics. Lavie bio's 'biology driven design' approach and its implementation through the CPB platform for the discovery and optimization of microbiome based ag-biologicals products will be described in the presentation.

DA-03 Lifting the veil of virulence and benefits of plant-associated bacteria by metagenomics approaches

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In the century of climate change and environmental pollution agricultural application of microbes to soil and plants aims to improve plant vitality, resistance and yield. The challenge is to define microbes that provide desired effects under a distinct or multiple different states, such as nutrient deficiency and/or pathogen infestation. Recently emerged innovative high-throughput technologies in molecular biology and informatics allow us to explore microbial interactions in all their detailed perspectives and dependencies. In particular, metagenomics discloses microbial community structures, and gives us the chance to investigate their genomic information and to identify site- and host-specific microbial hubs.

Our approach targets at currently known bacterial genomes stored in publicly available databases. In three major steps we (i) filter single nucleotide and structural genomic variations, (ii) associate specific patterns with their metadata, and (iii) distinguish between plant growth-promoting (PGP) and virulent (human pathogenic or phytopathogenic) genomic variants. We further apply the obtained information to screen metagenomes and respective bacterial strains for those patterns.

Recently, we have established a new ontology for PGP to facilitate the detection of respective functions in genomes and metagenomes, available as implementation in MEGAN6. Hence, it allows to predict PGP potential in metagenomic samples, ideally originating from plants or soil. Noteworthy, the combination with the detection of virulence factors (MEGAN6), implies the potential to differentiate PGP bacteria (PGPBs) from pathogenic strains.

Our great achievement is a tool for mobile screening of PGPBs and pathogens, using long read sequences, that are generated by e.g. a MinION device directly connected to your personal computer. Additionally, we try to predict *in silico* strains that may have the potential (i) to survive in the current microbiome by vacant niche occupation or by revealing versatile competitive traits, (ii) to reduce pathogen abundance, and/or (iii) to improve plant growth by carrying multiple PGP traits.

In conclusion, the novel approach and tool tenders a bunch of application scenarios, like identification of pathogens during pre-/post-harvesting processes, estimation of plant's metagenome virulence and beneficial potential or prediction of applicable PGPBs.

You are interested in my research? I encourage you to talk to me and discuss your remarks.

DA-04 Opportunities and challenges of microbial seed application

Carola Peters

Research & Development, Incotec Europe B.V., Netherlands

Seeds and seedlings face many challenges in the field, either biotic or abiotic. Both stresses have major impact on germination, (early) plant development and yield. Microorganisms have been shown to aid seed and plant to cope with these challenges by improving nutrient availability, relieving drought stress and fighting off hostile microorganisms and insects.

Currently, soil pathogens and pest insects are controlled by chemical plant protection products. The use of many of these products is under review. Therefore, alternative approaches or techniques to fend against environmental challenges are needed.

Nowadays, most microbial products for plants are applied as a soil drench or granulates. The benefit of adding microorganisms already to the seed is that the microbes can grow with the development of the seedling, ensuring rapid colonization of the roots and thus providing nutrients and/or protection at a very early stage of plant development.

Several application methods, such as film coating or pelleting can be used for microbial seed application. For optimal effectivity, a tailor-made solution is needed dependent on the microbial-crop combination. Many microbial species are drought-sensitive and assuring a prolonged microbial survival on seed has been proven challenging. Additionally, seed companies request an integrated approach where the microorganisms, fungicides and insecticides need to be applied to the same seed. Examples will be given on the seed application of several microorganisms.

miCROPe 2019 - Microbe-assisted crop production opportunities, challenges & needs
Vienna, Austria, December 2 – 5, 2019

Closing & Closing Lecture

Chair: Angela Sessitsch

C-01 Towards new road MAPs to engineer plant microbiomes

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Microbial interactions with plants contribute directly or indirectly to plant health and fitness. Of particular interest are interactions that result in the emergence of beneficial plant phenotypes such as disease suppression, improved nutrient acquisition, or drought tolerance. Engineering plant-associated microbiomes to optimize these emergent *microbiome associated phenotypes* (MAPs) is expected to transform the agricultural industry and to lead to more sustainable food production by replacing current unsustainable practices. To achieve this, numerous bottlenecks must be addressed. First, a key challenge will be to develop a quantitative and systematic platform for identifying and prioritizing MAPs. Once prioritized, the second challenge is to unravel the molecular mechanisms for both host and microbiome. Due to the complexity and endless possibilities of confounding ecological interactions, developing an appropriate model to test the importance or particular interactions is essential to develop robust MAPs. In this presentation, a theoretical framework will be presented that can tackle these bottlenecks in this nascent field of engineering MAPs. Next I will present on our BackToRoots work to link the rhizosphere microbiome to host genotype and to discover the ‘missing’ plant microbes and functional traits in the plant microbiome.

POSTER PRESENTATIONS

Poster Presentations

miCROPe 2019 - Microbe-assisted crop production opportunities, challenges & needs
Vienna, Austria, December 2 – 5, 2019

Poster Session 1: Successful microbial applications

PP1-SA-01 A new formulation with bacillus and pseudomonas increases plant fitness, net photosynthesis, yield and precocity in blackberry.

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A plant biostimulant is any substance or microorganism applied to plants with the aim of enhancing nutrition, efficiency, tolerance to abiotic and biotic stress, or improving crop quality. By extension, plant biostimulants also designate commercial products containing mixtures of such substances and/or microorganisms. Biobab R&D S.L. is currently developing effective biostimulants with strain with a solid background. The present study reports effects of 6 potential products in blackberry, a type of crop characterized by their benefits over human health derived from the secondary metabolites present in their fruits.

Experiments were conducted from November 2018 to May 2019 in production greenhouses in Huelva (Spain). Six bacterial combinations made with two bacillus and 3 different pseudomonas strains were defined, two of them with 3 strains, and four with two; treatments were compared with the coformulant (algae) and the negative control. Sixty plants conformed each treatment (20 plants per replicate), treatments were root inoculated once a month (5 applications from transplant to the end of the production period). At flowering, photosynthesis (fluorescence of light reactions and carbon fixation) and leaf photosynthetic pigments were measured. Total phenols, flavonols, and anthocyanins, as well as, nutritional properties of fruits were measured at the peak of production. Yield was recorded along the whole cycle.

Production was significantly increased by 3 bacterial combinations (treatments 4, 5, and 6), and only two (4, 5) of them anticipated fruiting (precocity), an excellent asset for producers, as fruit reaches the market earlier. From these, treatments 5 and 6 significantly increase net carbon fixation and transpiration, while treatment 5, a combination of 3 strains, is the only one able to also increase chlorophylls and carotenes, supporting the active input of energy necessary for carbon fixation and enhanced yield. None of the treatments were able to improve fruit quality, either nutritional or bioactives. Coformulants did not improve any parameter against controls, which demonstrates that results obtained depend exclusively on microorganisms. Treatments performed differently depending on the bacterial combination, so effects depend not only on the effective strains but are improved by certain combinations that are strain specific.

PP1-SA-02 Improving field inoculations with arbuscular mycorrhizal fungi

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Arbuscular mycorrhizal fungi (AMF) form a symbiosis with 80% of plant species, where they receive carbohydrates in exchange for nutrients, in particular phosphate and nitrogen. In addition, AMF reduce nutrient leaching and nitrous oxide emission; therefore, inoculation with AMF can improve soil nutrient cycles and could participate in a more sustainable agriculture. However, field-inoculation with AMF is highly context dependent. Soil fertility, and in particular, N and P fertilization, play an important role. In this project, we performed on farm inoculation experiments in 18 fields with a diverse range of soil properties. Upon maize seeding, the AMF *Rhizoglyphus irregulare* or a non-mycorrhizal control inoculum (carrier substrate) were inoculated. Fertilization was applied manually with all plots receiving standard levels of N and K, but only half of the plots receiving P. We confirmed that AMF inoculation success, as measured by maize biomass, was highly context dependent with enhanced yields being found in 1/3 of the fields. There is no evidence for an interaction effect of AMF inoculation and P fertilizer, suggesting that in the tested agricultural soils, fertilized over many years, phosphate levels were adequate for plant growth. We are currently modeling the relationships between soil properties and AMF establishment for a better understanding of the context dependency of successful AMF inoculations.

PP1-SA-03 Correlation of the bacterial microbiome, genotypic variance and alkannin/shikonin content of wild *Echium vulgare* L., a plant with potential medicinal properties

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Several members of the plant family Boraginaceae are confirmed producers of the secondary metabolites alkannin and shikonin. These compounds bear several biological activities such as anti-inflammatory or antibacterial effects or acceleration of wound-healing. *Echium vulgare* L., a common Boraginaceae species native to Europe, produces alkannins, which are mainly found in the periderm of the root system. As microorganisms have been reported to interact with secondary metabolism of some plants, our aim was to see whether there is a potential link between plant microbiota and secondary metabolite production in *E. vulgare*.

We collected plants at two different growth stages of wild *E. vulgare* at six different sites in Austria to correlate bacterial community patterns, genotypic variance and the production of secondary metabolites. We analysed microbial community composition in different sections of the root system, in the surrounding rhizosphere and bulk soil by next generation sequencing of 16S rRNA genes. Furthermore, we genotyped 64 individuals of *E. vulgare* using 12 microsatellite markers and determined total alkannin/shikonin content of dried root samples by ultra-high-performance liquid chromatography-high-resolution mass spectrometry. The high variance of alkannin/shikonin content in the collected *E. vulgare* roots in our study suggests that factors like the genotypic variance or associated microbiota may influence secondary metabolite production. According to our analysis results we will discuss how microbiota composition and plant population genetic differences correlate with alkannin/shikonin production and which microorganisms might play a role in influencing secondary metabolite production. This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 721635.

PP1-SA-04 Nitrogen-fixation and plant-growth promoting features of *Gluconacetobacter diazotrophicus* on tomato plants

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The endophyte *Gluconacetobacter diazotrophicus* (Gd) is a nitrogen-fixing, plant growth-promoting bacterium (PGPB) originally isolated from sugarcane. Gd colonises plant tissues and establishes close interactions within the inter/intracellular spaces. In contrast to rhizobia, Gd can fix nitrogen under aerobic conditions and establish nitrogen-fixing symbiosis with a wide range of crops.

In this study, the Gd strain AZ0019 was tested in tomato plants grown under hydroponic conditions. The use of fluorescent Gd tagged strains allowed to confirm the colonisation in plant tissues and to detect the plant-bacterial interaction during different colonisation stages. The RNA was extracted from roots at different time points in order to correlate the tomato growth promotion with the modulation of Gd gene markers of colonisation and nitrogen fixation activity.

These data allowed to build a model of Gd mode of action *in planta* throughout different colonisation stages in tomato. Integrating the plant-bacterial interaction imaging with the gene expression will provide a better understanding on how Gd establishes a functional symbiosis and positively affects the plant growth.

PP1-SA-05 Action against *Alternaria*: does *Fusarium* become ally of tomato plants?

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The study pursues the success of fungal endophytic strain HRO8 inoculation (i.e. root apparatus bath and soil application) into tomato plants and its development as innocuous to the culture plants. Biometric measurements considering plants inoculated and not inoculated with HRO8 as well as plants inoculated and not inoculated with pathogenic strain *Alternaria alternata* were performed. Symptoms of alternariosis (i.e. necrosis) provoked by *Alternaria* were evaluated. Overall appreciation: no *Fusarium* wilt stem symptoms were observed when cutting the fragments of the selected samples to proceed with isolation of endophyte and pathogen. No symptoms of fusariosis were observed on leaves, stems or roots during the biometric measurements. The inoculation of the pathogenic strain of *Alternaria alternata* was performed at two timings (i.e. 30 days and 60 days). No significant difference was observed between the two groups of plants ($p > 0.5$) in terms of biometric measurements, pathogen and endophyte isolation and symptoms of alternariosis. When the pathogen was inoculated, the plants in which the endophyte was also inoculated through soil application had an average of 1.1. necrotic leaflets while the plants where the endophyte was inoculated through root bath had an average of 10.4 necrotic leaflets. However, this average of 10.4 is less than half of the average value of control plants inoculated with the pathogen without the endophyte. We might assume that the method of application of the endophyte through roots bath might cause radicular damages which further manifest through plant sensitivity (i.e. in this case higher grade of symptoms caused by the fungal pathogen). These preliminary results indicate that the fungal endophyte HRO8 caused no harm to the tomato plants when directly applied to the soil and also protected the host against *Alternaria alternata*.

PP1-SA-06 Bibb lettuce response to *G. diaz* inoculation under hydroponic growth conditions.

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The objective of this study was to evaluate the effect of bacterial seed inoculation by the nitrogen fixing endophyte *Gluconacetobacter diazotrophicus* over six levels of nitrogen fertilization on root growth and yield of Lettuce. The experiment was conducted in hydroponically managed Kratky jars in a growth chamber at the USDA-ARS Agricultural Research Building on the Penn State University Park campus, from February to April 2019. Treatments consisted of six levels of nitrogen fertilization (60, 82.5, 105, 127.5, 150 and 172.5 ppm N in 800 ml soln) and two levels of inoculation (seed soaked with endophyte, seed soaked without), arranged in a 6 x 2 factorial design with three or four repetitions. The 12 treatments were assigned in a 44 jar completely randomized design, with the highest and lowest nitrogen levels having three replications each and the rest four. The nitrogen source used in the study was nitrate biased to reduce bacterial nif gene suppression by ammoniacal sources, and was supplied by calcium nitrate. Plants were harvested at 31 days post sowing. Bacterial presence significantly influenced the total plant biomass, harvestable plant yield, and root biomass. Aerial tissue production was 13.8% higher ($p < 0.0017$) in the presence of *G. diaz*, and inoculated plants exhibited 9.1% greater root production ($p < 0.0337$). Overall, total plant production was 12.5% greater ($p < 0.0042$) under bacterial inoculation, and yield trends exhibited a nitrogen response plateau at lower applied N levels in the presence of the endophyte.

Key words: *Gluconacetobacter diazotrophicus*, endophyte, Kratky, hydroponics, nitrogen, calcium nitrate, *Lactuca sativa* v. *Bibb*.

PP1-SA-07 Phytobiomes Research for Enhancing the Sustainable Production of Food, Feed and Fiber

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A major paradigm shift in agricultural production is required to meet the demands of a global world population projected to reach 9.7 billion in 2050. We need to sustainably increase crop productivity, while preserving biodiversity, natural resources, and grower income in the context of climate change. To optimize sustainable productivity and profitability on farms, grasslands, and forests, scientists must embrace a holistic, systems-level approach and focus on the complexity within phytobiomes. The term “phytobiome” refers to a plant growing within a specific environment, or biome, and all of the micro- and macro-organisms living in, on, or around it—such as microbes, animals, insects, and other plants—as well as the geophysical environment, which includes soil, air, water, weather, and climate. By establishing a foundation of knowledge on how phytobiome components interact and affect each other, the International Alliance for Phytobiomes Research (www.phytobiomesalliance.org) a non-profit alliance of industry, academic, and governmental partners created in 2016, aims at addressing today’s agricultural challenges. The Alliance facilitates and coordinates international efforts toward expanding phytobiomes research in order to accelerate the sustainable production of food, feed, and fiber for food security.

Current priority areas of the Alliance include filling the gaps in our knowledge of how microbes interact with other phytobiome components in outdoor and controlled environments as well as building a regulatory science foundation to support rapid commercialization of sustainable, microbial based products that increase the productivity and viability of agricultural production systems.

PP1-SA-08 The Multikraft technology & the ability of multi-microbe products to degrade pesticides

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Microorganism based products make an active contribution to climate and environmental protection for the sake of future generations, which forms the company’s central philosophy. The Company works with nature as a role model, promotes its regeneration and supports natural processes. For the intense research on multi-microbe products Multikraft hosts a modern microbiology laboratory, which includes conventional and real-time PCR machines. Close collaborations with international partners such as Barworth Research Ltd in Lincolnshire, SASA (Science and Advice for Scottish Agriculture) in Edinburgh and national partners such as the University of Natural Resources and Life Sciences in Vienna exist. Multikraft is committed to developing and producing its multi-microbe products based on the latest scientific research. To name an example, the main product in the gardening segment for watering and spraying is EM Active, a soil additive containing amongst others *Lactobacillus casei*, *Lactobacillus plantarum*, *Rhodopseudomonas palustris*, and *Saccharomyces cerevisiae*.

Due to the fact that bacteria and fungi have been proven very powerful in degrading chemicals introduced into the environment Multikraft performs intensive research on pesticide degrading bacteria. A study performed in 2016 (Mr. Frank Korting, State Education and Research Center of Viticulture, Horticulture and Rural Development, Rheinland-Pfalz, Germany) showed that by the use of multi-microbe products, accelerated degradation of many active ingredients occur. Actually, there is a project (supported by FFG) which aims at finding pesticide degrading strains and integrating them in the production process to explore if they can survive and multiply within the system. Also, the genomic basis underlying pesticide degradation should be explored within this „Multikraft degraders“ project.

PP1-SA-09 Microbe-assisted vegetation cover to reduce erosion in alpine environments – concept and first results

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Studies on crop species demonstrated the growth-promoting abilities of microbes and also their ability to alter plant traits. We adopted this approach in order to exploit beneficial plant-microbe interactions to reduce sediment remobilisation. Glaciers are facing ongoing and fast retreat due to global warming. The receding ice leaves unvegetated surfaces covered by unconsolidated deposits of sediment, so-called moraines. Sediments remobilised during extreme precipitation and flooding events may have negative effects on natural and anthropogenic structures downstream. It has been shown that high vegetation cover serves as effective protection against erosion, which is also supported by our findings. Apart from cover, our results indicate that plant communities with higher community weighted means in specific functional traits such as root mass and leaf area are more effective in slope protection than plant communities with other functional compositions. Therefore, we tested the effects of naturally occurring microbes on plant growth and trait expressions of the naturally occurring alpine plant species *Campanula barbata* (Campanulaceae) in order to enhance slope protecting abilities of this plant species. A screening of native bacteria collected in the test site identified those that significantly affected seed germination as well as functional trait characteristics in *C. barbata*. In the next steps, we will apply the microbe-assisted seed mixture to the field sites and monitor erosion from experimental plots in the Kaunertal Valley, Austria. Our results provide new insights into plant-microbe interactions in natural ecosystems with implications for a nature-based solution to reduce sediment erosion in high mountain areas.

miCROPe 2019 - Microbe-assisted crop production opportunities, challenges & needs

Vienna, Austria, December 2 – 5, 2019

Poster Session 1: Mechanisms mediating holobiont and multipartite interactions

PP1-MI-01 Resource exchanges in leguminous-gramineous associations: impact of multitrophic associations with beneficial microbes

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Arbuscular mycorrhizal fungi (AMF) and Nitrogen Fixing Bacteria (NFB) have gained an increasing interest as agroecosystem service providers capable of maintaining crop productivity and quality. They can affect the ecosystem productivity by (i) improving plant mineral nutrition, (ii) saving resources, and (iii) having a low environmental footprint. In agroecosystems, one of the objectives is to limit or avoid competition between cultivated plants and to favor "facilitation between plants" for a better productivity. Plant-plant facilitation refers to a "donor" plant that facilitates the growth and development of a "receptor" plant. It is essential to understand how these biotrophic interactions between plants, AMFs and NFBs are established; function, and influence plant growth. This would allow a reasoned soil resources in the context of a productive and sustainable agriculture.

Likewise the great majority of land plants, *Fabaceae* live in a symbiotic associations with AMF, connecting different plant species through common mycorrhizal networks (CMNs). They additionally form a symbiosis with NFBs. Although several studies unveiled the mechanisms of nutrients' exchange between plants and their symbionts in controlled conditions, understanding the terms of trade between the partners of CMNs and rhizobia involved in a complex environment might become experimentally challenging, but of prior importance in order to better picture the exchanges in a crop ecosystem.

To address this question, microcosms containing a pair of test plants (the C3 *Medicago truncatula*, and the C4 *Sorghum bicolor*), connected through a CMN of the AMF *Rhizophagus irregularis* or *Funneliformis mosseae*, with and without the rhizobial interaction with *Sinorhizobium meliloti* (on the leguminous plant), were used.

The differences in ¹³C/¹²C isotope compositions of the photosynthates from the two plants and the administration of ¹⁵N to the microcosm, coupled with RNAseq analysis, allowed us to assess the carbon and N exchanges between symbionts. More specifically we addressed two main questions: i) which is the specific C investments of the two plants species in the AM interaction when cultivated in monoculture (Sorghum-Sorghum and Medicago-Medicago) or mixed culture conditions (Medicago-Sorghum), and ii) how the C/N exchanges in the system might be modified/disrupted when a supplementary symbiotic partner, improving the N nutrition, was introduced.

PP1-MI-02 Complexity of microbe-plant interactions

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Microbes have driven eco-evolutionary adaptations of plants from the origin of life. Here I propose that to understand the importance of microbe-plant interactions to ecosystem functions and services requires understanding microbial versatility, and plants and their associated microbiome should be regarded as co-evolving ecosystems, holobionts. I contend that human perspective and conventional disciplines of life sciences might hamper and/or distract understanding the nature of microbe-plant interactions. For example, research on different microbial taxa and the related scientific disciplines have largely developed separately, and comprehensive community-level studies on bacterial and fungal interactions are lacking. Here, I use *Epichloë* species as examples to demonstrate that plant-microbe interactions are labile ranging from mutualistic to antagonistic, and how these keystone microbial species can modulate microbiota of their shared host plant. I suggest that the next step toward a better understanding of the microbe-plant interactions requires multidisciplinary approaches taking into account complexity and context dependency of these interactions.

PP1-MI-03 Chemical analysis of root exudates to study the interactions between tomato, maize and the plant-associated *Pseudomonas donghuensis* P482

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Plant rhizosphere is inhabited by numerous microorganisms. Plants drive this abundance by secreting a blend of chemical compounds into their root zone. This provides microbes with nutrients the composition of which has a profound effect on the shape and the physiological activity of the microbial community. The profile of compounds present in the plant root exudates depends on many factors. Among other, it varies among different plant species. Despite more and more research is dedicated to deciphering the sophisticated interaction between plant-associated bacteria and their hosts, most studies do not go beyond testing a single bacterium-plant model. Therefore, it remains largely unknown how particular compounds, characteristic for the exudates of certain plant species, are responsible for the host-specific aspects of bacterial interactions with plants.

Pseudomonas donghuensis P482 is a plant-associated bacterium isolated from the rhizosphere of tomato (*Solanum lycopersicum*). Apart from tomato, P482 is also able to colonize other plant species including maize (*Zea mays*). In this study, we aimed to characterize the composition of root exudates of tomato (cv. St. Pierre) and maize (cv. Bejm) and to develop a gnotobiotic plant growth setup, applicable for both plant species, to further investigate differential response of *P. donghuensis* P482 to the compounds secreted by the two plants.

When collecting root exudates for biological assays or chemical analysis, one must take into account the bias introduced by a particular experimental setup. Although the composition of root exudates of tomato and maize has already been reported, the results cannot be easily extrapolated for different culture conditions. Here, tomato and maize were grown for 18 days in sterile conditions from surface-sterilized seeds. The procedure included seed germination on media plates with a composition allowing easy screening of seedlings for potential microbial contamination. Seedlings were transferred to sterile vessels containing ½ or ¼ Hoagland's medium, for tomato and maize, respectively, and sterile gravel as support. For chemical analysis, compounds secreted by 18-days-old plants were harvested into ultrapure water, lyophilized, and their composition was determined by GC-MS and NMR, revealing a set of plant-specific compounds.

PP1-MI-04 Competitive traits of foliar yeasts and their effect on *Pseudomonas syringae* inhibition in tomato

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Fungi living within and on leaf surfaces play an integral role in plant functional processes including resistance to pathogens. The microbial interactions occurring at these interfaces often mediate pathogen protection or facilitation within the plant host. Foliar yeasts are a particular group of unicellular fungi that have been extensively studied for their antagonistic nature towards foliar pathogens. Less studied, however, is the way these yeasts associate with other microbes and persist in the phyllosphere. This knowledge is integral to assessing their effectiveness and viability as biocontrol agents towards plant pathogens over diverse biotic and abiotic conditions. In this study, we characterize various competitive traits of foliar yeasts isolated from tomato, *Solanum lycopersicum*, leaves, including dispersal, *in vitro* antagonism (dual cultural assays), and *in vitro* secretion of volatile organic compounds (VOC). From these traits, we infer how these microbes affect leaf community assembly and ultimately resistance to the foliar pathogen *Pseudomonas syringae* within the plant host.

PP1-MI-05 Activity of root endophytic bacteria isolated from an alkannin/shikonin producing plant: wild *Alkanna tinctoria*

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Endophytes are defined as microorganisms colonizing the internal tissues of plants with no external sign of damage to their host. The colonization *in planta* of these organisms may influence plant secondary metabolite production. Alkannin and shikonin are enantiomeric naphthoquinones produced in the roots of *Alkanna tinctoria*, among other 150 species of the *Boraginaceae* family. These molecules have strong anti-microbial properties and are therefore a source of strong interest for researchers and pharmaceutical companies. Nevertheless, the influence the endophytic bacteria, is not known. To assess the relationship between the production of these compounds by the plant and the endophytic bacteria, the first step was to investigate the diversity of culturable endophytic bacteria in the root. Then, plant-microbes interactions were explored by assessing plant growth promoting activity of the endophytes and the antimicrobial properties of alkannin and shikonin.

Isolation of root endophytic bacteria of wild *Alkanna tinctoria* collected from regions nearby Athens and Thessaloniki, Greece, was performed. Representative strains identified by MALDI-TOF mass spectrometry were then characterized genetically: the 16S rRNA gene was amplified and partially sequenced. Two hundred and eight distinct phylotypes of endophytic bacteria were detected and the most abundant genera were *Pseudomonas*, *Xanthomonas*, *Variovorax*, *Bacillus*, *Inquilinus*, *Pantoea* and *Stenotrophomonas*. These genera were then tested *in vitro* for their plant growth promoting activity (phosphate solubilisation, siderophores production, IAA, ACC deaminase). Among them, *Pseudomonas*, *Pantoea*, *Bacillus* and *Inquilinus* showed interesting properties. Moreover, the interaction between alkannin and shikonin and the bacteria was investigated, especially through resistance and biodegradation assays.

PP1-MI-06 The methylome of plant-beneficial *Stenotrophomonas* and *Serratia* strains

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Plants live in close association with microorganisms that provide beneficial functions. These plant-beneficial bacterial strains have long since been isolated by researchers and employed as inoculum to improve plant growth and health. Here, we shed light on the role of DNA methylation as a regulatory factor for host - and niche specific adaptation of plant-beneficial bacteria. We focused on phylogenetically closely related strains within the genera *Serratia* and *Stenotrophomonas*, which are already well known for their plant-growth promoting and stress protecting capabilities. Despite the high genotypic similarities of our sample strains, the colonization competence and the ability to exhibit beneficial effects *ad planta* were shown to be host-specific. Gene-by-gene comparison revealed that the present repertoire of genomic features alone cannot explain the strain-specificity of the plant-microbe interactions. Though, each strain possesses unique DNA methylases with specific recognition sites resulting in distinct DNA methylation patterns. To elucidate how methylotypes affect gene expression we analyzed the transcriptome of bacterial cultures in response to plant root exudates and nutrient composition. Our results indicate that DNA methylation contributes in controlling gene expression and may represent a significant factor accounting for host plant specificity. The results of this study will advance our understanding of the interplay between the genotype, methylotype and phenotype of plant-beneficial bacteria regarding their interaction with host plants.

PP1-MI-07 Differential impact of winter on endophytic bacterial and fungal communities of same boreal host plants

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Long winters with little solar radiation, low temperatures and snow cover have strongly shaped arctic and subarctic vegetation, and the ability to survive cold winters largely determines plant climatic distribution. Arctic and subarctic plants have evolved various physiological and anatomical modifications to survive the cold winters. Virtually nothing is known about winter microbiomes in cold climate plants, although plant associated microbes, including microorganisms such as bacteria and fungi are known to contribute significantly to plant survival, growth and protection from environmental stresses. In this context, we studied the composition of endophytic bacterial and fungal communities in the key plant species of boreal biome - *Pinus sylvestris*, *Picea abies*, *Vaccinium vitis-idaea* and *Vaccinium myrtillus* - in summer and winter at nine different geographical locations across Finland, with aim to detect putative seasonal fluctuation in the microbiomes. The project was conducted as a citizen science project in collaboration with nine high schools.

Using 16S rRNA gene (bacteria) and ribosomal ITS (fungi) targeted sequencing and sequence analysis, we analyzed the influence of various factors on the composition of bacterial and fungal communities. The bacterial community constituted of 421 OTUs assigned to 111 families. The fungal community constituted 382 OTUs categorised into 67 families. The permutational multiple analysis of variance (PERMANOVA) based on Bray-Curtis dissimilarity matrix of data showed that among the three factors – season, plant and location - plant species had the largest impact on the bacterial community structures, followed by season and site. The clear compositional difference between summer and winter communities were evident from divergent abundances of several OTUs from bacterial genera *Sphingomonas*, *Pseudomonas* and *Granulicella* in all four plants. These season-specific bacterial OTUs were closely related to bacteria from other cold climate plants.

In contrast, fungal communities were mainly shaped by plant species and sampling site, and to clearly lesser extent, season. Further studies on the functions of season specific microbes are needed to unravel their role in plant seasonal fitness.

PP1-MI-08 Metabolomics and machine learning techniques applied to investigate beneficial plant-bacteria interactions

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Endophytic non-pathogenic colonization of plant tissue by bacteria is a well-known and wide spread phenomenon, even expected to be the case for all angiosperms. Symbiotic plant-bacteria interactions comprise various levels of obligations and ecological benefits to at least one of the partners. The beneficial effects and functions for plants are manifold, including enhanced stress resistance, plant growth promotion or capacity for controlling plant-pathogens. In the present work, we focus on obligatory and constant symbioses occurring in the plant families *Rubiaceae*, *Primulaceae* and *Dioscoreaceae*. Highly specialized bacterial symbionts are mainly host-specific, often not cultivable and their absence can lead to dwarf phenotypes of the host-plants. Endophytic bacteria of leaves can be evenly distributed between the mesophyll cells or accumulated in specific leaf areas or in specialized structures.

Most studies focused on genome and proteome analyses suggesting potential alterations of secondary metabolism caused by the presence of beneficial symbionts. However, detailed mechanisms and functions of these highly specialized mutualistic plant-bacteria symbioses are not yet fully understood.

In the present study we investigate alterations in the metabolome of colonized leaf tissue. Primary and secondary metabolites were analyzed by GC-MS and LC-MS respectively, complemented with physiological and morphological data, and analyzed with machine learning techniques. Results indicate distinctive mechanisms of the symbiosis in investigated beneficial plant-bacteria interactions and will be discussed in detail.

PP1-MI-09 Road to reveal the genetic factors of the plant-probiotic rhizobacterium TRM1 contributing to bacterial wilt resistance in tomato

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Tomato is one of the most in-demand vegetables in the world, and bacterial wilt caused by the soil-borne pathogen *Ralstonia solanacearum* is a devastating lethal disease in solanaceous crops. As reported in 2018, a plant-probiotic flavobacterium TRM1 was found to be enriched in the rhizosphere microbiome of the bacterial wilt-resistant tomato variety Hawaii 7996 relative to the susceptible cultivar Moneymaker and also be able to suppress disease development in the susceptible plant. Although TRM1 was shown to reduce bacterial wilt in tomato and its functions were deduced from the genome sequence information, which genetic factors of this plant probiotic are responsible for disease suppression or how the factors affect the plant host or the wilt pathogen remains elusive. As the first attempt to investigate how TRM1 could endow the tomato plant with disease resistance, we examined if TRM1-10 affects the growth of *R. solanacearum* SL341 by an *in vitro* co-cultivation method. The growth of SL341 was inhibited by TRM1-10, while that of TRM1-10 was not affected. This result suggested that inhibiting the growth of the pathogen by TRM1 might be one way of reducing plant disease. For the next step to figure out the genetic factors of TRM1 involved in reducing the tomato wilt disease, we employed transposon mutagenesis and massive sequencing (Tn-seq) expecting to disclose genes contributing to the molecular interactions between the plant-probiotic microbe and the wilt pathogen and also between the plant probiotic and the tomato plant in the rhizosphere, and the findings will be presented.

PP1-MI-10 Live-Exudation Assisted Phytobiome Cultromics System (LEAP-CS) to characterize physiological, molecular and metabolic phenotypes

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Healthy plants are associated with a rich diversity of microbes forming complex microbial consortia that impact their growth and productivity. These plant-associated microbiomes or phytobiomes confer a multitude of benefits to their hosts, developing and engineering them can aid in cultivating climate-resilient, nutrient-efficient and sustainable food crops. In that view, characterizing model rhizosphere phytobiomes, which occupy the niche developed by the gradients of root exudates in the rhizosphere region is of particular interest due to its probable direct role in providing specific factors for plant growth and resilience. However, the lack of a model rhizosphere phytobiomes and its associated metabolic exchanges with the host has restricted the much needed mechanistic understanding of plant-microbe interactions in the rhizosphere. To this end, we have developed a novel Live-Exudation Assisted Phytobiome-Cultromics System (LEAP-CS) which supports development of complex phytobiomes and is suitable for characterizing live host and microbial phenotypes. The foundation of LEAP-CS rests on natural ecological processes underlying the complex and dynamic phytobiome relationships. Modularity of LEAP-CS supports (i) easy manipulation of the phytobiome communities; (ii) chemical complementation assays; (iii) different biological and analytical platforms for phenotyping; and (iv) live, non-invasive plant growth profiling. Our system captures (i) plant-microbe and microbe-microbe interactions; (ii) metabolic exchanges and crosstalk; (iii) microbial community shifts through simultaneous multi-omics analyses at different levels for integrated biological outputs. Consequently, LEAP-CS can be utilized for both mechanistic and translational studies involving plant-microbial interactions and can be used a tool for developing synthetic phytobiomes and consortia for agricultural applications. Integrative omics data from LEAP-CS system for model species (*Arabidopsis thaliana*) will be presented and shared in this paper.

PP1-MI-11 Mining the bacterial inducers for plant defense and shikonin production in plants: an *in-silico* guided approach.

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Several species from the Boraginaceae plant family are used since ancient times for their medicinal properties due to the presence of secondary metabolites such as shikonin (produced in the roots of the plant). Different induction systems for the production of shikonin in plants have been described and the metabolic pathway partially elucidated. However, the role of shikonin in nature has not been fully understood but it is suggested to be part of the plant defense against pathogens and some abiotic factors.

Different plant defense elicitors known as Microbe Associated Molecular Patterns (MAMPs) and some plant endogenous molecules referred as Damage Associated Molecular Patterns (DAMPs) are described in relation to a wide diversity of microorganisms associated with plants. MAMPs and DAMPs are not only related to pathogenic bacteria but also to non-pathogenic symbionts like endophytes.

In the present study we compared the genomes of several endophytic bacteria isolated from the roots of *Alkanna tinctoria* growing in wild conditions in order to mine for MAMPs or DAMPs related to bacteria that could be responsible for the plant defense and shikonin induction.

Based on the genomic comparison, well described MAMPs like flagellin (flg22) and EF-Tu factor are evenly present in the bacterial genomes. Besides, type II, IV and VI secretion systems are also represented in many isolates. Enzymes related with the degradation of pectins from plant cell wall (CAZy PL1, PL3, PL4, PL9, GH28, CE12), that potentially generate DAMP-like molecules known as oligogalacturonides are less represented among the genomes but found to be enriched in some bacterial groups like Chitinophagales, Burkholderiales, Sphingobacteriales and Pseudomonadales. Oligogalacturonides were previously recognized to induce shikonin production in the Boraginaceae plant *Lithospermum erythrorhizon*. In the future, the significance of the bacteria predicted to degrade pectins and other complex polysaccharides from plant origin will be tested *in-vitro* and *in-planta* for induction of shikonin production.

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Poster Session 1: Plant understanding of interactions with beneficial microbes

PP1-PU-01 Metabolomics of the leaf nodulated plant *Ardisia crenata* reveals novel putative cyclic-depsipeptides

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Introduction *Ardisia crenata* belongs to the ethno botanically used genus *Ardisia* in the family Primulaceae, which is rich in biologically active substances with great chemodiversity. The species also lives in obligate leaf endosymbiosis, wherein the plant harbours their bacterial endosymbionts in specialised glands at the leaf margin, referred to as leaf nodules. Expression of the nonribosomal peptid synthetase (*frs*) gene cluster in *Escherichia coli* leads to the synthesis of the cyclic-depsipeptide FR900359, which displays strong and selective inhibition of Gq proteins. This makes it not only a promising drug candidate but also an interesting lead structure for the development of novel pharmaceuticals. A non-targeted metabolomics approach shows substantial differences in secondary metabolite profiles of nodulated and non-nodulated tissues. Examination of MS data revealed novel putative derivatives of FR900359.

Methods Methanolic extracts of nodulated and non-nodulated leaf tissues of *A. crenata* leaves of different developmental stages are analysed by a metabolomics method tailored for secondary metabolites. The sample-components are separated by RP-HPLC and analysed by HRESIMS. The Dataset obtained is processed by the in-house software tool mzFun and subjected to multivariate statistical analysis (PCA) (ANOVA). MS2 fragmentation patterns are analysed to gain structural information of the *m/z* features.

Results and Discussion In the PCA a clear clustering of samples belonging to the same condition occurs, as well as a distinct separation between the clusters. A significant separation of old nodulated tissue from other tissues was found on principal component 1. The *m/z* features, which strongly contribute to this separation exhibit very similar MS2 fragmentation patterns. Besides FR900359, AC-1 and AC-SC, as well as other, previously reported putative cyclic-depsipeptides, 3 novel *m/z* features with similar fragmentation patterns were found. Due to the promising pharmaceutical effects of FR900359, ongoing work focuses on the isolation and structural elucidation of the novel putative cyclic-depsipeptides.

Innovative aspects

- New insights into the bacterial leaf nodule endosymbiosis of *A. crenata* on a metabolic level
- Discovery of novel putative cyclic-depsipeptides
- Convenient *m/z* feature extraction and annotation of LC MS/MS data by new software tool mzFun

PP1-PU-02 Understanding plant glycan interactions with beneficial microbes in the rhizosphere

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Plant roots deposit structurally diverse polysaccharides into the surrounding rhizosphere via sloughed off border and epidermal cells and in the form of root exudates. Evidence from other terrestrial and marine ecosystems has shown that bacteria produce large numbers of carbohydrate active enzymes (CAZymes) with roles in metabolising polysaccharide substrates and thereby accessing energy and glycan building blocks for reprocessing. It has been shown in mammalian gut and algal-based ecosystems that complexity and diversity of polysaccharides drives evolution of diverse microbes with specialised CAZymes, but these processes are poorly understood in the rhizosphere and soil systems. We have developed a transparent soil system for *in-situ* live imaging of roots, polysaccharides and microbes. Our initial data is revealing complex multiscale patterning of polysaccharides in the rhizosphere and microbial responses to this. A deeper understanding of microbial utilisation of root polysaccharides could yield valuable insights into how we can manage a healthy, diverse microbial population to enhance plant production.

PP1-PU-03 Priming for enhanced defense in *Hordeum vulgare*

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Plants can be exposed to one or several stresses, for example harsh environmental conditions and pathogens. Therefore, effective defense strategies are essential. *Priming* can enhance the natural plant resistance, it enables the plant to respond faster and stronger upon challenge. Thus, a primed plant has a fitness benefit if compared to a naïve plant.

One of the inducers of the primed state in plants is the *N*-acyl homoserine lactone oxo-C14-HSL, which is naturally produced by the bacterium *Ensifer meliloti* as a quorum sensing molecule. The knowledge of priming as a part of induced resistance is currently mainly based on the model plant *Arabidopsis thaliana*. In this project we aim to expand the knowledge to crop plants and focused on barley (*Hordeum vulgare*) as one of the most important crop plants worldwide.

In different experimental settings we investigated the biology of priming and genetically-based differences in the priming capacity in a set of 7 diverse barley accessions. This set comprises two reference cultivars (Golden Promise and Morex) and five genetically distant cultivars from the spring barley GENOBAR collection (BCC768, BCC1589, BCC1415, BCC436 and HOR7985). We analyzed gene expression patterns in hydroponic system *via* MACE (massive analysis of cDNA ends) technique and real-time quantitative PCR of primed and unprimed barley before and three days after a challenge with chitin, which mimicked a fungal pathogen. In this context, we aimed to find new priming responsive marker genes and intend to gather new insights in the physiology of priming. Furthermore, we performed a field trial with the barley 7 set and *Ensifer meliloti* as a priming agent. We assessed the infected leaf area, did a qualitative scoring including leaf rusts, powdery mildews and aphids and determined the biomass and yield for primed and unprimed plants. Primed Golden Promise showed a reduced infected leaf area including reduction in chlorosis symptoms and aphid infestation while necrosis was slightly increased. In addition, primed and unprimed barley was challenged with aphids in greenhouse experiments. First results indicate a reduction of aphid biomass and less leaf damage for primed Morex.

In the future our results should help to understand the potential and biological background of priming, in order to improve resistance of barley and other economically relevant cereals and to identify promising breeding targets.

PP1-PU-04 A beech mushroom-derived volatile, Linalool primes plant immunity against bacterial and insect pathogens

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Plants are vulnerable to various biotic and abiotic stresses because they cannot move. Plants overcome these shortcomings by interacting with other living things around them. Specially, plant-associated microorganisms are able to promote plant health by airborne or underground signals. A beech mushroom is an edible mushroom that has been widely cultivated in East Asia for unique flavor and beneficial effects for human health. However, in nature, these mushrooms are often found on woods. In this study, we examined whether the beech mushroom has positive interactions with plants. The wild type *H. marmoreus* Hm 3-10 was isolated from Duk-yu Mountain in Korea. *In vitro* l-plate system, tobacco exposed to fungal volatile blends from Hm 3-10 were significantly reduced disease severity compared with tobacco not exposed to fungal volatiles. Volatile organic compounds (VOCs) produced by Hm 3-10 were extracted and identified using gas chromatography mass spectrometry with solid phase micro-extraction. Total 15 volatile organic compounds (VOCs) was detected and composed of alcohols, aldehydes, and terpenes. Among these VOCs, linalool suppressed disease symptom by inducing jasmonate signaling in tobacco and pepper. Furthermore, it has been found that insects are less accessible to plants treated with linalool. This is first reported that plant-associated beech mushroom primed plant immunities by airborne signaling.

PP1-PU-05 Studies of root traits that support rhizobacterial mediated growth stimulation and stress tolerance of oil crops

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The aim of the study is to identify factors that favor root colonization of Plant Growth Promoting Rhizobacteria (PGPR) that result in improved growth and stress management of *Brassica* oil crops.

Use of PGPR is a promising tool to support more sustainable crop production. In addition to promote growth, many PGPR also prime abiotic stress tolerance and induced systemic resistance to pathogens. The PGPR *Bacillus velezensis* UCMB5113 has been shown to support plants such as oilseed rape (*Brassica napus*), wheat (*Triticum aestivum*) and *Arabidopsis thaliana*. Growth stimulation, improved tolerance to drought, cold and heat stress as well as protection to some pathogens have been shown. UCMB5113 produces phytohormones, lipopeptides and volatile compounds among other substances than the plant benefit from. However, information is poor in general with respect to host plant genotype variation and PGPR efficacy. Such knowledge is important if a PGPR is to be used successfully in agricultural practice.

For that reason we have initiated a screening of several commercial oilseed rape (*Brassica napus*) and rapeseed (*Brassica rapa*) winter and spring cultivars treated with UCMB5113 to study effects on growth, drought and cold tolerance. Sterilized seeds have been treated with different concentrations of *Bacillus* spores and cultivated in standard soil in controlled environment. Drought or freezing stress challenges are given to young plants being more sensitive and mimic cultivation conditions prevalent in middle Sweden. In addition, effects on root system architecture and UCMB5113 colonization efficacy will be analyzed as well as metabolite composition of root exudates. Finally root cell wall composition will be analysed. Using analysis of several variables we expect to find factors that can explain variation in response observed in preliminary experiments conducted in green house. Some results from the ongoing study will be presented.

Beneficial bacteria have great potential in agriculture to replace many chemicals currently used. Since PGPR effects are based on interaction between two very different organisms there will obviously be constraints to develop a successful interaction. Accordingly, identification of factors needed for establishment of beneficial plant-microbe interactions are needed to improve efficacy when applied in crop production. Such knowledge could also be used in breeding to generate plants that maximize the PGPR interaction potential.

PP1-PU-06 Exploiting a mutualist toolkit: using effectors to activate beneficial plant pathways

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The fungal symbiont *Serendipita indica* has shown to confer improved growth and stress resilience to a broad range of host plants, including important crops such as rice, wheat and barley. *S. indica* secretes specific proteins (termed 'effectors') to aid in plant root colonisation. Interestingly, effectors specific to *S. indica* can confer increased stress resilience in crops by targeting and thereby modifying, plant signaling in a highly specific manner. Determining the functions of *S. indica* effectors in increasing crop health, could aid in the development of crops with improved stress resilience and a reduction in the application of agro-chemicals in the future. We have shown increased growth and stress resistance in stably transformed *Arabidopsis thaliana* expressing individual *S. indica* effectors. By employing a high throughput screen of promoter activity, we have also determined how these effectors mediate specific hormonal and immune signaling. Yeast two hybrid screens have shown potential targets, these interactions have been confirmed with co-immunoprecipitation and *in planta* split luciferase assays, in preparation for functional experimentation. In resolving effector structure along with co-expression of target structure, the aim is to develop new stress protection strategies. In applying these methods to beneficial microorganism effectors, we hope to further elucidate how crop hosts perceive and process signals for increased biotic and abiotic resistance.

PP1-PU-07 Soybean nodulation and moldavian bentonites

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Rhizobia inoculants have contributed to increase N₂ fixation and yield in legumes crops. However, most of the inoculants produced world-wide are of poor or suboptimal quality [1]. The nature of soil-borne populations of rhizobia (number, nitrogen-fixing capacity) is determined by factors closely related to physical features of the landscape and the rhizobial status of a soil can be predicted by reference to these physical features [2]. Soybean (*Glycine max* (L.) Merr.) can be inoculated by delivering inoculant mixed with mineral microgranules such as bentonite; effect of granules on soybean nodulation in field experiments was consisted in nodule number (per plant) at R4 20-30 [3]. Granular material must be taken into account to improve the efficiency of this inoculation process [4].

An essential component of bentonites is the layered silicates of the montmorillonite type. In the present work, finely dispersed montmorillonite was obtained from clays of the Prodanesti deposit of Moldova. We have shown that montmorillonite particles are tightly connected to the roots; moreover, the root growth path is determined by the location of montmorillonite microparticles in the soil. The use of aqueous suspensions of highly dispersed clay minerals (both bentonite and pure montmorillonite) together with a suspension of *Bradyrhizobium japonicum* made it possible to obtain a nodule number (per plant) at R4 no less 100.

Conclusion. Natural fine clay minerals obtained on the basis of clay deposits of the Republic of Moldova are a promising material for increasing soybean nodulation

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PP1-PU-08 Successions of microbes associated with below and above ground plant parts in a glacier fore field

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Plant surfaces represent one of the largest habitat for bacteria and fungi where they occur in high diversities. Plant species are characterized by specific microbial communities, but the relative contributions of the plants' phenotype, biotic and abiotic environmental conditions, and dispersal from surrounding habitats to community diversity and composition remain poorly understood. Glacier forefields – large areas of deglaciated substrate, which can be colonized by microorganisms, plants, and animals – provide an excellent opportunity to study several decades of microbial succession over the distance of only a few hundred meters. In order to evaluate the importance of the structuring factors of microbial communities, bacterial and fungal communities of the plant phyllosphere and the soil microbiome, as well as vegetation cover and arthropod communities will be recorded along a temporal gradient spanning over 170 years of microbial primary succession. Additionally, we will test hypotheses generated from field data in microcosm in the lab in order to verify correlational findings in stringent experiments and to gain novel insights into the interdependencies of microorganisms with other taxonomic groups.

PP1-PU-09 The ecology of P capture in organic wheat: Is selection under low P, organic conditions going to get us there sooner?

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Canada's first organic participatory wheat breeding (PPB) program has produced 50 lines bred by organic farmers on their own farms for three years (F3-F6). Many of the lines were selected under environments that have been organic for 20+ years, therefore under low phosphorus (P) or sourced from biological P (manure) for many years. Greater understanding of the impact selection environment may have on AMF colonization and phosphorus uptake in breeding programs is essential as P is a finite, non-renewable, and geographically restricted resource. This work is part of a larger body of work examining phosphorus uptake strategies for farmer selected breeding lines under low P and alternative P sources.

Organic crop production systems create a soil environment very different from conventional systems, and in many cases, lower in P. Many studies have reported that organic farms exhibit greater biological activity and greater AMF colonization in host plants than conventionally managed land. In some cases, like on dryland organic farms in the Canadian Prairie Provinces, this was due to low inorganic P availability, most P was in the organic form unavailable to crops.

Preliminary yield trials comparing PPB breeding lines to conventional registered checks have been conducted at 5 organic sites across the Canadian prairies in 2017 and 2019. In 2017, as a group, farmer lines significantly out-yielded conventional checks by 266 kg ha⁻¹ in the low-yield site and 435 kg ha⁻¹ in the high yield site. The mechanisms resulting in greater yield performance by farmer-selected genotypes will be focus of future research. A preliminary study (Nicksy, unpublished) compared one farmer line "IG" with one conventional cultivar, "Brandon", under a low P environment with synthetic and biological fertilizers (monoammonium phosphate (MAP), compost, frass, and unfertilized). Biomass at Zadoks stage 31 exhibited a cultivar*fertilizer interaction. The "IG" line was higher than "Brandon" in compost, frass, unfertilized by 251, 187, and 248 kg ha⁻¹ respectively, however, "Brandon" biomass was greater than "IG" in under MAP by 227 kg ha⁻¹. Therefore, it appeared the farmer-selected line was better able to respond to biological P sources, while the conventional check line was better able to respond to synthetic fertilizer P. Future studies will investigate a wide range of farmer-selected lines and their interaction with different components and processes within the "soil-plant P ecosystem".

PP1-PU-10 Glyphosate affects mycorrhizal colonization of plants

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Glyphosate, the world's most widely used pesticide, is controlling weeds in agriculture, horticulture, silviculture and urban landscapes. Glyphosate inhibits production of an enzyme in the shikimate pathway of plants and thereby production of some amino acids. Shikimate pathway is found also in microbes and therefore its effects on non-target microbiota important to the ecosystem functions and ecosystem services should not be ruled out. We experimentally studied if glyphosate can remain in the soil and accumulate in a weed grass, (*Elymus repens*) and forage grass (*Festuca pratensis*) in boreal climate. We observed mycorrhizal colonization in the grass roots, and studied if the possible effects of glyphosate on plants and associated mycorrhizal fungi are dependent on biotic and abiotic environmental factors. We detected residues in both target plants and non-target plants in the growing season following the glyphosate treatment. All the plants growing in no-till pots had higher glyphosate residues compared to conspecifics in tilled pots. The glyphosate application significantly reduced the total VA-mycorrhizal colonization and the number of arbuscules of the plants. These results demonstrate negative long-term effects of glyphosate on non-target organisms in agricultural environments and grassland ecosystems.

PP1-PU-11 Plant growth promoting potential and quorum sensing of *Pseudomonas* sp. RTE4 isolated from the rhizosphere of Assam tea.

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Tea rhizosphere is dominated by members of *Bacillus* sp. and *Pseudomonas* sp. Majority of these rhizosphere bacteria are beneficial to plant and are referred as plant growth-promoting rhizobacteria (PGPR). PGPR are mainly responsible for production of phytohormone production, hydrolytic enzymes and antibiotics to combat against phytopathogens. PGPR not only colonise the roots of tea through the biofilm formation but also produce biosurfactants. All these traits are claimed to be regulated by bacterial communication which a density dependent phenomenon is called quorum sensing (QS). QS phenomenon observed in Gram negative bacteria is due to the secretion of signalling molecules which are popularly known as acyl homoserine lactone (AHL). In this study, we isolated gram negative bacterium RTE4 from tea rhizosphere of Rosekandy tea garden, Assam, India. RTE4 induced violacein pigment production in biosensor *Chromobacterium violaceum* CV026. Additionally, RTE4 also induced green fluorescent protein expression in biosensor *E. coli* MT20 (jBA132). AHL was extracted from early stationary phase of RTE4 by acidified ethyl acetate. Reverse phase TLC and LC-MS studies demonstrated the presence of C6-AHL by RTE4. The isolate also showed production of Indole acetic acid production (74.54 µg/ml), phosphate solubilisation (46 µg/ml). The bacterium RTE4 also showed production of protease. Biocontrol studies demonstrated, RTE4 as a promising candidate against two foliar pathogenic fungi namely *Corticium invisium* and *Fusarium solani*. Also secretion of antibiotic phenazine was confirmed by LC-MS. RTE4 formed moderately adherent biofilm which was evident through confocal microscopic images. RTE4 also produced biosurfactant in the presence of dextrose and fructose as carbon source where a sharp decrease in surface tension were observed. Metabolic profiling conducted by BIOLOG GEN III indicated abilities of RTE4 to grow in acidic pH which is ideal for structural stability of AHL molecules. Molecular identification by MALDI-TOF MS and phylogenetic analysis based on 16S rRNA gene sequence confirmed its relatedness with genus *Pseudomonas*. Overall the strain RTE4 showed multiple plant growth promoting activities and also QS molecule, which make it a suitable candidate for the application as biofertilizer.

PP1-PU-12 An improved growth medium for enhanced inoculum production of the plant growth-promoting fungus *Serendipita indica*

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Endophytic colonisation of plant roots can lead to improved growth and enhanced resistance of host plants against abiotic stress and against plant pathogens. Such positive effects have been shown in detailed studies for the basidiomycete fungus *Serendipita indica* (*Piriformospora indica*) (*Sebacinales*), e.g. in Barley, Maize, Poplar, Wheat, Switchgrass, Tobacco, and Arabidopsis. Due to its ease of axenic cultivation and its broad host plant range, it is used as a model fungus to study beneficial fungus-root interactions. *S. indica* and closely related *Sebacinales* fungi were also suggested to be utilized for commercial applications, e.g. to enhance pathogen resistance and crop yield of barley.

Serendipita indica is currently mostly cultivated in a complex Hill-Käfer medium (CM medium) for inoculum preparation, however, growth in this medium is slow, and yield of chlamydospores which are often used for plant root inoculation are relatively low. We therefore tested and optimized growth media for enhanced yield of fungal inoculum. We propose here a vegetable juice-based medium (VJ medium) which was superior to the currently used CM medium with respect to biomass production in liquid medium and fungal growth on agar plates. Using VJ medium, chlamydospore production was more than 20 times higher within the shortened cultivation time of 8 days, compared with CM medium. Interestingly, VJ medium also supported growth and conidiation of other fungi, suggesting its utilization for the propagation of diverse fungi in both research and commercial applications.

The described VJ medium is composed of inexpensive components and is easy to prepare, and is therefore recommended for a streamlined and efficient inoculum production for the plant endophytic fungus *Serendipita indica*.

PP1-PU-13 The diversity and dynamics of plant-associated microbial communities of drought-tolerant *Vellozia* species under different precipitation regimes on *campos rupestres*

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Severe drought is among the most extreme climate events affecting natural and agricultural ecosystems. Natural ecosystems with recurrent periods of drought are considered a rich reservoir of microorganisms that can enhance drought tolerance in plants. This is the case of *campos rupestres* (rupestrian fields), a semi-arid ecosystem characterized by a prolonged dry season and high solar radiation. A dominant monocot plant family, the Velloziaceae, displays adaptive features for drought tolerance (desiccation-tolerant and non-desiccation-tolerant) that make them highly resistant to the seasonal availability of water. During the dry season, desiccation-tolerant species (resurrection plants) drift into a dissection state, with a dead-looking aspect, and return to a hydrated and photosynthetically active state in the rainy season. In contrast, non-desiccation-tolerant species resist dissection and remain evergreen by lowering photosynthetic and respiratory rates during prolonged drought periods. Exploring the microbiome associated with these species displaying different mechanisms of drought tolerance is crucial to understanding the molecular and functional bases of plant adaptation in resource-limited environments. Therefore, we aim to explore the diversity and dynamics of the microbiota associated with four *Vellozia* species, two desiccation-tolerant (*V. nivea* and *V. tubiflora*) and two non-desiccation-tolerant (*V. intermedia* and *V. peripherica*). These species are rocky outcrop vegetation of Serra da Canastra National Park in Brazil. Plants were collected during the dry and rainy seasons allowing us to correlate the microbial diversity with edaphic physicochemical characteristics, plant morphophysiological adaptations and colonization patterns. The microbial profiling is being performed by sequencing and analysis of molecular markers for prokaryotic (16S) and fungal (ITS) communities associated with the substrate and plant organs, accounting for 1,120 prokaryotic and fungal libraries for each species. Aspects of plant-microbe interaction, such as fungal colonization rate and community establishment, will be further investigated by microscopy. We believe that prospecting microorganisms associated with Velloziaceae species will reveal potential functions that can be translated into new biotechnological strategies to enhance yield and performance of agricultural crops under drought stress.

PP1-PU-14 Transcriptomic changes of tomato plants in response to endophytic bacterial strains revealed key pathways of plant-growth promotion in presence of humic acid

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Chemical fertilisers are widely used in conventional agriculture and they cause possible environmental impacts. Plant growth promoting endophytic bacteria can internally colonize plant tissues and promote plant growth without causing damage or eliciting defence responses. Some publications highlighted the synergistic effects of the combined application of endophytic bacteria and humic acid (HA) substances on plant growth. However, there is a lack of knowledge on the molecular mechanisms of their combined interaction with the plant host. The aim of this work was to get insight into the molecular basis of the interaction between endophytic bacteria and tomato plants in the presence of HA, in order to improve the understanding on the mechanism responsible for plant growth stimulation. Three bacterial strains that endophytically colonise tomato plants were selected and they were able to promote tomato shoot length in the presence of HA. Transcriptional changes activated in tomato leaves in response to the endophytic bacteria included the up-regulation of primary metabolic processes, defence responses, growth and development pathways. In tomato roots, genes responsible for defence reaction, transport and oxidative stress were up-regulated by the bacterial strains. Moreover, genes related to secondary metabolism, nitrogen metabolism and hormone signals were activated by the bacterial endophytes only in presence of HA. The presented transcriptome study highlighted also species-specific pathways activated by the three bacterial strains and provided new insights into endophytic bacteria/adjuvant/plant interactions in order to further develop efficient biofertilisers.

PP1-PU-15 Plant growth promoting endophytic bacteria from *Sedum oryzifolium* Makino of Dokdo

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Plant growth-promoting bacteria (PGPB) live in the rhizosphere of plant that help plant growth through the synthesis of auxin, gibberellins and cytokinin like phytohormone, availability of limited plant nutrients and production of siderophore and plant beneficial volatile organic compounds (VOCs). Induced systemic resistance (ISR) was called that plant disease-suppressed resistance mechanism by non-pathogenic rhizobacteria in the plant. ISR is indicated that systemic resistance induced by microorganisms other than pathogens. VOCs are being studied as a new microorganism determinant for enhancement of plant immunity. VOCs indirectly improve plant growth while reducing abiotic and biotic stress. Generally, the case of bacteria produce at least 30 different volatile organic compounds, and that have been reported to cause physiological changes in plant-bacterial, bacteria-bacterial and fungi-bacterial interactions with each other. Some VOCs such as 2,3-butanediol and tridecane were induced plant systemic resistance against pathogens. In this study, plant ISR by endophytic *Erwinia* spp. isolated from *Sedum oryzifolium* Makino has been examined. *Erwinia* is a genus of *Enterobacteriaceae* bacteria, which is closely related to a lot of plant disease. This plant pathogen is causing with a wide host range which carrot, potato, tomato, onion, etc. It is able to cause disease in almost plant tissue. Breaking the stereotype, in this study, induced-resistance by the genus *Erwinia* against *Xanthomonas axonopodis* pv. *vesicatoria* in pepper (*Capsicum annum* L.) plant and against *Pectobacterium carotovorum* pv. *carotovorum* in tobacco (*Nicotiana benthamiana*) has been determined. Also isolated PGPB were investigated the ability of plant growth promotion and induce resistance to plants. As a result, *Erwinia* spp. known as a plant pathogen were existed predominant and has ability of plant growth promoting ability, induced systemic resistance. This indicates that the genus *Erwinia* is in symbiosis with the plant. It was also confirmed that the volatile substances that 2,3-butanediol and acetoin produced by the strain KUDC3020, isolated bacteria of *Sedum oryzifolium* Makino, had a positive effect on the plants.

PP1-PU-16 Specific responses to bacterial quorum sensing molecule are altered in complex interactions

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Gram-negative bacteria primarily produce *N*-acyl-homoserine lactones (AHL) as quorum sensing (QS) molecules. These QS molecules help to coordinate the collective behavior within bacterial populations. In addition, AHL can modulate behavior of the eukaryotic neighbors. Plants, for example, perceive and react to AHL in a diverse manner; often activating specific physiological pathways resulting in augmented growth or/and resistance, phenomenon termed as AHL-priming. Today, AHL-priming for enhanced resistance seems to be an efficient disease management strategy. So far, most of the studies investigated bilateral relationship between a particular AHL molecule and the plant. However, this scenario is highly improbable in the rhizosphere since different bacteria produce different AHL molecules.

In order to examine deeper the impact of AHL on plants, we assessed the impact of five different AHL with varying length of the acyl side chain, from 6 to 14 carbons (oxo-C6-HSL, oxo-C8-HSL, oxo-C10-HSL, oxo-C12-HSL and oxo-C14-HSL) and all their possible combinations on the model plant *Arabidopsis thaliana*. We monitored phenotypic traits like root length and biomass and performed gene expression analyses of four defense-related marker genes to assess the impact on defense due to the potential AHL-priming. Our study confirmed previous results implying that short-chain AHL induce biomass and root growth, whereas the long-chain AHL enhance the immune response. Furthermore, we observed that although single AHL induced specific responses in the plant, the combinations triggered less specific responses. In addition, we observed that defense priming was more prominent in plants that were treated with a combination of more than two AHL molecules, regardless of their structure. Our results therefore indicate that the specific responses to single AHL molecules are altered in interactions with diverse AHL molecules.

PP1-PU-17 Associations and interactions of *Clonostachys rosea* Cr-7 with crop plants

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Clonostachys rosea has extraordinary ability to associate with foliage, flowers, fruits, and roots of a wide diversity of crop plants. In inoculation tests over many years endophytic establishment of the fungus was apparent in foliage of more than 70 diverse temperate and tropical crop species. Conidia of *C. rosea* applied to leaves or flowers were observed to germinate and produce germ tubes from which extremely fine lateral branches penetrated into the host tissues. Evidence indicated that *C. rosea* formed localized micro-colonies, apparently in the apoplast. Inoculated plants lacked visible signs of colonization until the tissues began to naturally senesce, when conidiophores and conidia formed on the tissue surface. Sporulation was preceded by abundant growth and tissue occupation by mycelium of *C. rosea*. Ecologically, *C. rosea* behaved as a pioneer colonizer of senescing plant tissues and was able to preclude growth and sporulation of other leaf- and flower-associated fungi including epiphytes and pathogens. Rapid mycelial growth and tissue occupation in response to attempted tissue invasion by pathogens is considered a principal mechanism by which *C. rosea* is able to control diseases caused by pathogenic fungi. The importance of the endophytic phase of *C. rosea* for disease control prompted tracking of strain Cr-7 in flower and leaf tissues of crop plants treated with the fungus in field and greenhouse trials. Tissue samples were plated on Paraquat-chloramphenicol agar medium which accelerates tissue senescence and thus enables rapid sporulation of *C. rosea*. The fungus sporulated on leaves and flowers of many berry and other crops and sunflower heads. Density of sporulation was related to inoculum density and tissue type. Observations indicated that *C. rosea* Cr-7 established effectively in all crops evaluated, and in several instances enhanced crop growth in the absence of disease. This strain of *C. rosea* is in development by Bee Vectoring Technology Inc. as a bioprotectant applied by bumble bee and honey bee vectoring against diseases caused by *Botrytis*, *Sclerotinia*, *Monilinia* and other pathogens. This application method substantially minimizes waste of the control agent, and in contrast to spray programs, enables daily delivery of the agent throughout the bloom period. Benefits in terms of crop productivity and quality can arise from disease control, biostimulation effects and improved pollination.

PP1-PU-18 Compositional and functional analysis of the β -glucan matrix produced by *S. indica* in planta

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Plants continuously survey their environment for the presence of potentially harmful microbes. During microbial invasion, cell-surface receptor proteins perceive microbe-derived or modified-self ligands and initiate appropriate responses. The recognition of fungal chitin and the subsequent activation of plant immunity are well described. In contrast, the mechanisms underlying β -glucan recognition and signaling activation remain largely unexplored.

Recently, we observed that root colonizing ascomycetes and basidiomycetes surround themselves with a β -glucan matrix during plant colonization using a lectin from the root endophyte *Serendipita indica* (Wawra et al. 2019, New Phytologist). Since β -glucans can act as potent microbe associated molecular patterns that activate the plant immune system, information about matrix composition and effects on the plant immune system are crucial information to understand how fungi establish themselves in this ecological niche. For example, the analysis of the glycan matrix obtained from the root endophyte *S. indica* revealed that the β -glucans present in the matrix are distinct from the ones found in the cell wall. Whether these show different immunogenic activities compared to the ones present in the fungal cell wall is currently under investigation. Furthermore, analysis of the matrix proteome showed, among others, a strong significant enrichment for proteins carrying the β -1,3-glucan binding WSC domain including members that have the potential to act as β -glucan matrix markers. In summary, our current data indicate that comparable to animal pathogenic fungi the *S. indica* polysaccharide matrix has to be treated as distinct 'compartment' rather than just as an extension of the fungal cell wall. Here we will present our latest data on the topic.

PP1-PU-19 Arbuscular mycorrhizal fungal diversity reveals preference in the mycorrhizal colonisation with different grapevine rootstocks.

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Arbuscular mycorrhizal symbiosis is the most widespread type of interaction between plants and microbes in the context of phylogeny and ecology. Arbuscular mycorrhizal fungi (AMF) are regarded as non-specific symbionts, but some AMF fungal groups interact differently in various ecosystems including vineyards. Grapevine plants are normally mycorrhizal and very responsive to AMF colonisation. Although, these fungi have potentially significant applications for sustainable agricultural ecosystems, there is a gap in knowledge regarding AMF-grapevine interactions worldwide and especially in New Zealand. This research focuses on identifying AMF communities colonising grapevines in New Zealand vineyards and investigate the effect of grapevine rootstocks on AMF community diversity and composition.

Root samples were collected from five vineyard sites, each site planted with four to eight different rootstocks. The root samples were used to set up trap cultures for AMF recovery and for molecular identification using denaturing gradient gel electrophoresis (DGGE). AMF spores extracted from pot cultures were identified based on spore morphology and sequencing of the 18S region. Eighty representatives DGGE bands from the surveyed sites were cut, re-amplified and sequenced. Community matrixes with presence/absence data were also generated from DGGE gels and analysed using multivariate analysis.

Six spore morphotypes were extracted from pot cultures and identified as *Ambispora* spp., *Acaulospora* spp., *Glomus* spp. and *Claroideoglomus* spp. Fifty four of the sequenced DGGE bands from root samples were associated with AMF and assigned to *Glomus* spp., *Rhizophagus* spp. and *Claroideoglomus* spp. The AMF community analyses demonstrated that rootstock significantly influences the AMF community composition in all sites. The study showed that for a comprehensive identification of AMF both results from trap culture and molecular work are needed, and that AMF communities colonising grapevine are influenced by rootstock.

PP1-PU-20 Tobacco seed-borne *Bacillus* sp. can role for growth promoting and induced systemic resistance in plant.

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The bacteria are commonly carried on the surface of the seed, but most of seed-borne bacteria cause plant disease. For instance, *Acidovorax avenae* subsp. *citrulli*, *Pseudomonas syringae* pv. *tomato* and *Xanthomonas euvesicatoria* are known as seed-borne pathogenic bacteria causing seedling blight and bacterial fruit blotch of cucurbits, bacterial wilt and canker of tomato, bacterial leaf spot disease, respectively.

In this study, we isolated seed-borne non-pathogenic bacteria from tobacco (*Nicotiana tabacum* L. cv Xanthi). Phylogenetic analyses based on the 16S rRNA gene sequence indicated that seed-borne bacteria formed a phyletic lineage within the genus *Bacillus*. *Bacillus* sp. was most extensively studied as plant growth promoting rhizobacteria (PGPR), various strains of species *B. amyloliquefaciens*, *B. cereus*, *B. pasteurii*, *B. pumilli* and *B. subtilis*, are known as beneficial bacteria in plant. The induction resistance of a plant defined in two ways that systemic acquired resistance (SAR) and induced systemic resistance (ISR). ISR relies on regulated by jasmonic acid (JA) and ethylene (ET) signalling pathway, SAR related to salicylic acid (SA) and pathogenesis related (PR) proteins -chitinase and cellulase.

We show the seed-borne bacteria, *B. aryabhatai* KNUC 0118, which significantly induce the resistance of pepper (*Capsicum annuum* L.) against *X. campestris* pv. *vesicatoria* causing bacterial leaf spot disease. Quantitative real-time polymerase chain (RT-qPCR) analysis showed the expression of *C. annuum* protease inhibitor2 (*CaPIN2*) associated with JA defense signalling in pepper plants treated with seed-borne *Bacillus*. In addition, KNUC 0118-treated pepper plants showed plant growth than that of untreated one. The first discovery of non-pathogenic bacteria from tobacco seeds and their ability to promote plant growth and systemic resistance to plant disease will contribute to further research.

Keywords: Seed-borne bacteria, *Bacillus* sp., ISR, plant growth promoting(PGP)

PP1-PU-21 *Serendipita indica* alters host plants sugar metabolism and the development of *Heterodera schachtii* in systemic *Arabidopsis* roots

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The beneficial root endophyte *Serendipita indica* (= *Piriformospora indica*) is an orchid mycorrhiza that forms mutualistic relationships with many different plants including the model plant *Arabidopsis thaliana*. During this interaction, the endophyte promotes host plant growth and development, increases biomass and seed production. Furthermore, it significantly increases the resistance to abiotic (e.g. drought, salt, toxins and heavy metals) and biotic stresses (e.g. pathogens). Hence, it can be speculated that, similar to arbuscular mycorrhizal fungi, *S. indica* receives carbohydrates (preferably hexoses) from the host in exchange for this service. Therefore, in this study qRT-PCR of *AtSUS* and *AtINV* genes as well as analyses of multiple *sus* and *inv* mutant lines of *A. thaliana* were carried out to assess importance of sugar metabolism during this endophyte-plant interaction. The results show general upregulation in directly colonized roots and initial downregulation followed by an upregulation of *AtSUS* and *AtINV* genes in shoots. Typical growth promotion was only observed in colonised wild type plants, whereas multiple *sus* and *inv* mutant lines showed no such effects indicating the importance of these genes for successful interaction. To elucidate probable effects of *S. indica* root colonization on plant parasitic nematodes, a development assay with the sugar beet cyst nematode *H. schachtii* was carried out. To exclude direct fungal effects on the parasites, the experiment was performed in three-chamber dishes. One half of the plant root system was inoculated with *S. indica*, whereas the other half was infected with nematodes. Interestingly, significant higher numbers of *H. schachtii* females were observed on plants systemically colonised with *S. indica*, whereas syncytia size was significantly decreased in comparison to control plants. These results are supported by downregulation of several genes involved in plant defence (*AtqPDF1.2*, *AtACS6*, *AtBI1*), especially at and shortly after the timepoint of nematode inoculation. This work significantly increases our knowledge on *S. indica*-nematode-plant interaction with special emphasis on sugar metabolism.

PP1-PU-22 Contribution of epiphytic microflora and cultural conditions to raspberry volatilome

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Raspberry (*Rubus idaeus*) is an economically important berry. Raspberry is cultivated in a variety of marginal areas across Europe, and is affected by several biotic adversities. For these reasons, raspberry requires significant inputs, and organic cultivation has not achieved sufficient commercial standards yet.

In addition to flavour and nutraceutical properties, raspberry cultivars are appreciated for their aromatic properties. In this study, the contribution of the cultivation system (conventional/organic) and of the epiphytic microflora of raspberry (cv. Enrosadira) to fruit aroma was investigated. One fruit batch (control) was rinsed with distilled water, while a second batch (sterile) underwent superficial sterilisation. Finally, part of the sterilised fruit was allowed to be recolonised by epiphytic bacteria, obtained from the same fruit before sterilisation.

Surface sterilisation reduced the emission of several compounds, whereas the subsequent microbial recolonisation could restore, at least partly, those emissions. Therefore, fruit epiphytes are likely to contribute to raspberry aroma. However, the set of compounds varied according to the cultivation system. In particular, conventional fruit was richer in terpenes, aldehydes and ketones, generally associated to a flowery-fruity aroma. In contrast, acetate esters and putative fermentation products were mostly found in organic fruit. Thus, differences between fruit from the two cultivation systems possibly arise from the different incidence of environmental stresses; however, such conditions may lead to the selection of a distinct microflora, which would further influence the overall aroma.

This work highlights the role of the epiphytic populations in the formation of raspberry aroma, and, consequently, fruit quality. Future studies will be focused on the characterisation of the main microbial species contributing to volatile emissions, to enhance raspberry quality or prevent post-harvest spoilage.

PP1-PU-23 Co-inoculation of root endophytic *Serendipita* species and arbuscular mycorrhizal fungi affects nutrient contents and arbuscular mycorrhizal root colonisation in tomato plants

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Some of the root endophytic fungal species belonging to the family of Serendipitaceae were recognized to be isolated from spores of arbuscular mycorrhizal fungi (AMF). To date, there are no known studies focusing on their interaction in an AMF-endophyte-plant system. In order to shed light on the interactive effects of AMF and root endophytic fungi on tomato plant development and nutrient status, a greenhouse experiment using tomato plants was conducted with the following factors: (1) *Funneliformis mosseae* (AMF) and (2) *Serendipita* spp. including *Serendipita indica*, *S. williamsii* and *S. herbamans*. Performance of photosystem II was assessed in regular intervals using a chlorophyll fluorometer (PAM 2500). Nine weeks after transplanting, plants were harvested and plant growth parameters and AMF root colonization were determined. Nutrient concentrations of the tomato shoots were analysed by Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-OES, Optima 8300 DV). The results showed that growth and development of tomato plants were unaffected by the combined inoculation with AMF and *Serendipita* spp., except that shoot length was suppressed in the AMF and *S. herbamans* treatment. Furthermore, the performance of photosystem II was also less when AMF and *S. herbamans* were co-inoculated. With regards to the nutrient concentration of tomato shoots, phosphorus was highest when plants were co-inoculated with either *S. williamsii* or *S. herbamans* and AMF. Moreover, the concentration of calcium and manganese increased in *S. indica* inoculated plants and was significantly reduced when AMF was introduced into the endophyte-plant system. The amount of zinc was observed to be lowest in plants inoculated with both *S. herbamans* and AMF. Even though AMF root colonisation was reduced when both AMF and *Serendipita* spp. were combined, the concentration of phosphorus was increased compared to the treatments without AMF. The reduction of other nutrients such as calcium, manganese and zinc might indicate unknown interaction effects and nutrient costs, which need to be investigated in future experiments.

PP1-PU-24 Use of locally isolated PGPR to promote growth in hops (*Humulus lupulus*) and cannabis (*Cannabis sativa*)

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Cannabis production is experiencing renewed consumer demand and there is an increased requirement for producers to develop varieties for a range of end-use applications, as a result of changing legislation around the world. It is now critical to improve cannabis yield and quality (i.e., cannabinoid concentration) for the market. An important challenge in this regard is the need for *Cannabis* producers to achieve higher cannabinoid levels with reduced inputs. Exploitation of the phytomicrobiome is a potential strategy as it is already recognized for its ability to stimulate plant growth and provided pathogen bio-control for many plants.

Hops (*Humulus lupulus*) has been used as a model to develop plant growth-promoting rhizobacteria (PGPR)-based technologies applicable to cannabis, since it is also a member of Cannabinaceae that produces essential oils and can be vegetatively propagated. Hops, unlike cannabis (*Cannabis sativa*) is not a regulated plant. In this project, PGPR isolated from agricultural soils in Southwestern Quebec by our laboratory were screened for plant growth promotion of hops cuttings. Plants were propagated by taking cuttings from mother plants and rooted for two weeks in a misting chamber. Once the cuttings had formed roots, they were inoculated with one of three PGPR strains by soaking the roots in overnight cultures of the strain ($OD_{600} = 0.1$) for 30 minutes. Treated plants were then transplanted into magenta jars containing 50 mL of sterile water. There were five replicates per treatment, arranged in a completely randomized design in a growth chamber maintained at 22 °C/18 °C (day/night) with a 16 h photoperiod. At harvest, plants were sampled for dry biomass production and root length (WinRhizo). The results of this project will be used to develop PGPR-based technologies to be used for plant growth promotion in Cannabis. Based on our initial findings, applying PGPR to rooted hops cuttings results in faster and more vigorous root development in a hydroponic magenta jar growing system.

PP1-PU-25 How can microorganisms modulate alkannin and shikonin production in Boraginaceae plants? An insight from comparative transcriptomics

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Alkannin, shikonin (A/S) and their derivatives, known as red naphthoquinones, are commercially interesting plant secondary metabolites which are produced in the roots of different species of the Boraginaceae family. Specifically, red colour extracts from the roots of *Alkanna*, *Echium* and *Lithospermum* were traditionally used as dyes and in herbal preparations in both Europe and Asia for several centuries. Currently, applications of these extract range from cosmetics, food to pharmaceutical industries, the latter based on their broad-spectrum biological activities such as wound healing, antibacterial, anticancer, and antioxidant.

However, biosynthesis of A/S in Boraginaceae is shaped by specific cultivation regimes which often lead to low yield of these compounds and thus limits their commercial utilization. Recent studies suggest that plants are colonized by a variety of microorganisms which can modulate the biosynthesis of secondary metabolites. Such a system can be exploited for improving A/S production and to meet increasing demand from industry. However, little is known regarding the changes in Boraginaceae species transcriptome in response to with the interacting microorganisms and its relationship with modulation of A/S contents.

In this project, using RNA-sequencing, we aim to explore the underlying molecular mechanisms involved in pathways leading to the enhanced production of A/S. A specifically established *in vitro* system will be used to compare the transcriptome profiles of bacterially challenged and non-challenged plants. For this, clonal plants of Boraginaceae species will be inoculated with several bacterial strains known for shikonin induction and roots will be harvested at different time points. The metabolic and transcriptional profiles of challenged and non-challenged plants will be compared and correlated to understand the potential mechanisms by which bacterial strains modulate the biosynthesis of shikonin.

miCROPe 2019 - Microbe-assisted crop production opportunities, challenges & needs

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Poster Session 1: Microbiome understanding beyond profiling

PP1-MU-01 Tomato plants rather than fertilizers drive microbial community structure in horticultural growing media

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Synthetic fertilizer production is associated with a high environmental footprint, as compounds typically dissolve rapidly leaching emissions to the atmosphere or surface waters. We tested two recovered nutrients with slower release patterns, as promising alternatives for synthetic fertilizers: struvite and a commercially available organic fertilizer. Using these fertilizers as nitrogen source, we conducted a rhizotron experiment to test their effect on plant performance and nutrient recovery in juvenile tomato plants. Plant performance was significantly improved when organic fertilizer was provided, promoting higher shoot biomass.

Since the microbial community influences plant nitrogen availability, we characterized the root-associated microbial community structure and functionality. Analyses revealed distinct root microbial community structure when different fertilizers were supplied. However, plant presence significantly increased the similarity of the microbial community over time, regardless of fertilization. Additionally, the presence of the plant significantly reduced the potential ammonia oxidation rates, implying a possible role of the rhizosphere microbiome or nitrification inhibition by the plant.

Our results indicate that nitrifying community members are impacted by the type of fertilizer used, while tomato plants influenced the potential ammonia-oxidizing activity of nitrogen-related rhizospheric microbial communities. These novel insights on interactions between recovered fertilizers, plant and associated microbes can contribute to develop sustainable crop production systems.

PP1-MU-02 Genomic landscape of a model arbuscular mycorrhizal species *Rhizophagus irregularis*

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Arbuscular mycorrhizal fungi (AMF) form symbioses with the majority of land plant species and play an important role in nutrient dynamics and ecosystem functioning. Multiple isolates of *R. irregularis*, a model AMF species, have been shown to differentially alter the plant growth in greenhouse and field experiments. However, little is known about the genetic and epigenetic basis underlying this response. Three levels of variation in this species (two of which can be genetic) can lead to differential plant growth: 1. Among isolate variation, 2. Within isolate variation in dikaryote isolates; 3. Between clones of the same monokaryotic isolate. The last of these should not be genetic. The major hurdle in understanding the genomic underpinnings is the non-availability of high-quality genome assemblies as well as long read data which can assist in detection of structural variation. In this study, we used long-read Pacific Biosciences data to assemble high quality genomes of six isolates of *R. irregularis* to study degree, nature and function of genomic variation. The results revealed the varying degree of structural variation (SV) in line with the phylogenetic distances among isolates but well within the range observed in other eukaryotes. The prevalence of SV in non-protein coding part of the genome hinted their putative role in gene regulation. We address the different levels of variation observed in the genomes at the three levels of variation. Overall this study provides a comprehensive understanding the putative role genomic variation that can be important for determining physiological processes of AMF, especially phosphorus processing.

PP1-MU-03 Challenges in quantifying bacterial and fungal foliar endophyte colonization by real-time PCR – a seasonal field study

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It has been shown that endophyte behavior and the switch from neutral to a pathogenic or mutualistic plant-microbe relationship can depend to a large degree on the extent of microbial colonization. In soils, the bacteria:fungi ratios represent an important parameter that can indicate dominant biogeochemical processes and nutrient utilization/availability. It is therefore likely that these ratios can be linked to plant host physiology or susceptibility to pathogens and provide information on the nutrient availability within the environment of plant tissues. Yet these types of data are rarely used in studies involving endophytic microbes.

One of the reasons for this may be the difficulty quantifying bulk endophyte colonization in plant tissues. Methods such as microscopy or analysis of organism-specific biomolecules are very laborious and unreliable, often requiring large sample sizes. Real-time PCR (qPCR) targeting 18S rDNA gene is a well-established method for the detection and quantification of fungal biomass in ecological studies and generally works well in plant tissues. There have been several attempts at quantifying the extent of bacterial endophytic colonization by qPCR, however, these studies did not address the key issue of contamination by host organellar (particularly chloroplast) DNA, which can, in our experience, cause an overestimation error of up to several orders of magnitude. This is caused by the high affinity for non-target DNA of the commonly used universal bacterial primer pairs targeting 16S rDNA gene.

Here we present a verified protocol for the quantification of the leaf-associated bacterial endophytes, based on a primer set 335f/769r with high specificity for bacterial DNA. The use of the primer pair was published recently by M. Nakano, who focused on various vegetable products (2018, *Journal of Food Protection* 81: 848–859). We used this approach to assess the seasonal variation in foliar endophytic bacteria:fungi ratios in different plant species along a successional gradient (Sokolov region, Czech Republic). We argue that this high-throughput, relatively low-cost approach can supply additional eco-physiological information significantly improving the interpretation of data on endophyte community composition in many field-based studies.

PP1-MU-04 Who gains the upper hand over the wheat ear?

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The phyllosphere of wheat is full of microorganisms, especially fungi and bacteria. Of great importance are the fungi belonging to the genera *Alternaria* spp. and *Fusarium* spp., since they cause several economically relevant wheat diseases. Furthermore, the fungi produce mycotoxins that are potentially harmful for human health when ingested. A successful colonization depends on the aggressiveness of the fungi and how well it outcompetes the bacteria and fungi that are already present at the wheat ears. Especially the bacteria *Pseudomonas*, which is also commonly found on wheat, is known to have an antagonistic effect on the mentioned phytopathogenic fungi. Therefore, the hypothesis is that some strains of fluorescent *Pseudomonas* might strongly influence the presence of phytopathogenic fungi in an environment, functioning as a regulatory mechanism and therefore actively shaping species composition living on wheat ears. To confirm this, a climate chamber experiment was performed to assess how the appearance of *Pseudomonas* or other phytopathogenic fungi affect the probability of a successful infection by fungal spores. To gain a better understanding in the fungal infection dynamics in cereal crops, 210 flowering wheat ears were inoculated by either *Pseudomonas*, *Alternaria*, *Fusarium* or none as control. After one and two weeks respectively, an antagonist was applied to the same ears, to investigate the fitness of the second player in an already colonized area. At each inoculation point, ears were sampled for each treatment. After three weeks, all remaining ears were harvested and prepared for further analysis. Via qPCR, the temporal development of the infection process can be tracked. We hypothesize that on *Pseudomonas* inoculated plants the development of *Fusarium* or *Alternaria* is inhibited. First results show that *Fusarium* inoculated ears were infected more quickly and thickly and seemed to be less susceptible for the following antagonists.

PP1-MU-05 Characterizing the phyllosphere bacterial communities of greenhouse crops

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The phyllosphere is the above-ground surface of plants and is in contact with the atmosphere. Similar to other plant surfaces, the phyllosphere is occupied by a diverse community of bacteria, fungi and other micro-organisms. Bacteria are the most abundant of these microbes and their cell count ranges between 10^6 to 10^7 cells per square cm of leaf surface. These bacteria interact closely with the plant and can have an effect on plant health and plant growth. Beneficial bacteria can inhibit pathogen growth directly, through microbe-microbe interactions or indirectly, by triggering the plants defence system. Phyllosphere bacteria can also enhance plant growth through the production of plant hormones or by increasing the availability of nutrients.

There is a huge potential in harnessing the phyllosphere microbial community to improve crop production and crop protection while decreasing the negative impacts of pesticides and fertilizers on human health and on the environment. This research aims to gain insights into the taxonomic composition of a healthy phyllosphere microbiome and what factors shape these communities.

In this study, the phyllosphere communities from seven tomato cultivars and three strawberry cultivars were sampled during eight weeks at two research greenhouses. 16s rRNA gene amplicon sequencing was used to determine the bacterial community structure of the phyllosphere and its dynamics over time. The bacterial communities on the leaves were highly variable over time and in space. This is in contrast with the stable greenhouse environment that is highly controlled to minimize biotic and abiotic stresses on the crops. In addition, the diversity of the communities was low. These results suggest that the bacteria on the phyllosphere did not reach a stable community. Interestingly, a high amount of bacteria on the leaves were associated with the insects present in the greenhouse. This indicates that insects play an important role in dispersing bacteria and shaping phyllosphere communities. These insights in the composition and dynamics of bacterial communities are a crucial step towards understanding and modulating phyllosphere communities to improve crop production and crop protection.

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Poster Session 1: Varia

PP1:V-01 Agricultural production systems can serve as reservoir for *Salmonella enterica*

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Fresh fruits and vegetables have numerous benefits to human health. Unfortunately, their consumption was increasingly associated with food-borne diseases in the last years. *Salmonella enterica* was one of the most frequent recorded causes of food-borne diseases in Europe. Agricultural soils and organic fertilizers were postulated to be potential reservoirs of human pathogens, possibly contributing to the contamination of crops during the growing period. Since the competition with indigenous soil microbiota for colonization sites plays a major role in the success of invading species, we hypothesized that the level of diversity will influence the establishment of *Salmonella* in the agricultural environment. Using culture-dependent and -independent techniques, we analyzed the influence of soil bacterial diversity on the survival of three *Salmonella enterica* strains (Senftenberg, Typhimurium 14028s, Typhimurium LT2) in soil microcosm experiments. We demonstrated that the persistence of *Salmonella* was indeed reduced in soil with high diversity of the native microbial community in comparison to the persistence in autoclaved soil. Furthermore, we observed a positive influence of organic fertilization (chicken litter and pig manure) on the persistence of *S. enterica* in soil. Finally, we observed an attachment of *S. enterica* to roots of lettuce plants using CLSM and a colonization of the phyllosphere after inoculation of soil. The results presented here underline the necessity of an integral approach and the importance to preserve a diverse soil microbiome in order to lower the number of disease outbreaks.

PP1:V-02 CORALL – the new whole transcriptome library prep with precise end-to-end coverage is an efficient solution for low input and FFPE RNA

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CORALL is Lexogen's new Stranded Total RNA-Seq Library Prep Kit with excellent whole transcriptome coverage. CORALL enables streamlined generation of Illumina-compatible libraries within 4.5 hours, featuring seamless integration of Unique Molecular Identifiers (UMIs) and exceptional protocol-inherent strand specificity (>99 %). The fragmentation-free protocol uses Lexogen's proprietary Strand Displacement Stop and Ligation technologies to deliver complete transcript representation, including transcription start and end sites. CORALL has been tested with various RNA input types from human, mouse, mini-pig, hamster, plant to bacteria. In combination with Lexogen's RiboCop rRNA Deletion or Poly(A) Enrichment Kits CORALL can be used for RNA input as low as 1 ng as well as degraded and FFPE RNA samples.

PP1:V-03 Lifelong companion or lingering pathogen? – Genome sequence of strain Msb3, a novel *Paraburkholderia* isolate from the phyllosphere

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The genus *Paraburkholderia* is closely affiliated with highly pathogenic *Burkholderia*, yet it describes a variety of environmental species among which we almost exclusively find environmental isolates ranging from beneficial symbionts to free-living metabolically diverse commensals. Nevertheless, the role of this young and rapidly growing genus and the biotechnological application of its members remains a highly controversial topic.

We present the genome of a novel *Paraburkholderia* strain termed Msb3, isolated from leaves of *Dioscorea bulbifera*. We describe the strain as a non-pathogenic keystone species on its healthy greenhouse grown host plants, steadily present over a two-year period of investigation. Its genome was sequenced and an extraordinary variety of genes associated with an endophytic lifestyle were identified. The 8.5 Mbp genome contains genes enabling Msb3 to engage in intimate relationships with a broad spectrum of hosts. It carries complete gene clusters for multiple protein secretion systems (TxSS) including T3SS and T6SS, as well as genes for biological nitrogen fixation and several plant-growth-promotion-pathways, including indole-3-acetic-acid (IAA) production and 1-aminocyclopropane-1-carboxylate (ACC) deamination. Its genetic ability to adapt to multiple lifestyles, e.g. by detoxification of a large set of xenobiotics and degradation of aromatic compounds, make it an ideal candidate for application for agricultural purposes. However, with its genetic repertoire it theoretically also constitutes a risk for human and plant health, displaying many pathogen-like traits. Strain Msb3 represents a perfect organism to study in respect to the potential for biofertilization but also pathogenicity in the genus *Paraburkholderia*.

In an effort to clearly dissect these differences, we also reevaluated current hypotheses concerning the ecology of members of the genus and its sister clades. We found that the proposed lack of pathogenicity in former plant beneficial and environmental (PBE) *Burkholderia* species does not hold true for any genus that originated from this group. These findings emphasize the importance of a clear understanding of genetic as well as physiological processes contributing to the behavior of bacterial species, rather than extrapolating by using phylogenetic relationships and thereby highlight the need for a consensus within the community in respect to characterization of plant growth promoting bacteria related to pathogens.

PP1:V-04 MicrobiomeSupport: Towards coordinated microbiome R&I activities in the food system to support (EU and) international bioeconomy goals

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Knowledge of the potential of microbiomes, throughout the food chains, is seen as a promising means to ensuring the sustainability of our food system. Although a number of relevant European programmes and initiatives are currently running or are being launched, they are largely fragmented, implying a stringent need for coordinated action. This need for joint action should also be regarded at the international level.

MicrobiomeSupport is a Coordination and Support Action uniting 27 partners with the overall objective to establish an international network of experts and stakeholders in the field of microbiome food systems research and assess applicability and impact of the microbiomes on the food system. The objectives of the project are:

- Identification and mapping of microbiome activities, programmes and facilities along the food chain and beyond in the EU and worldwide
- Creation of a platform for scientists, regulatory experts, industry, funding and policy organisations as well as support of the International Bioeconomy Forum to implement the 'Food Systems Microbiome' working group
- Improve use of existing data to allow comparability and improved mining of microbiome data
- Define strategic agendas to enable joint international microbiome applications in the food sector and beyond
- Collaboration and coordination in support of a sustainable bioeconomy in Europe and worldwide, in line with the FOOD 2030 policy goals
- Raising awareness and exchange of knowledge across scientific and political communities, including the International Bioeconomy Forum and the general public

PP1:V-05 Understanding the role of plant secondary metabolites in host - endophyte interactions.

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Plants host a complex internal microbiome from which endophytic fungi represent an important component. This highly diverse group is assumed to have profound impacts on plants; hence, much attention is now being paid to understand the interactions and relationship between endophyte colonization and leaf traits. Characterizing the endophyte communities within a group of plants such as Rubiaceae, a hyperdiverse family that contains several economically important genera, could provide a unique insight into how plants adapt to certain conditions and environments via their microbial community. Thus, this knowledge could shed some light on new approaches to take advantage of the plant's microbiome to equip our crops with the "tools" they need to grow in stressful environments and to fight off pathogens. Through ITS nrDNA metabarcoding (Ion Torrent), we have generated millions of fungal sequences recovered from foliar and bark tissue associated with wild Rubiaceae and coffee plants. We have assessed the diversity of foliar fungal endophytes using a variety of packages in RStudio (i.e., DADA2, phyloseq, CatchAll). As the leaf metabolome is shaped by plant and endophyte metabolites, and by endophyte transformations of those metabolites, the presence of certain fungi is expected to correlate with the plant's chemistry, while others correlate because of their preferences on certain carbon sources or the ability to tolerate toxic metabolites produced by plants. Therefore, diversity composition and function of the community could be linked to the leaf chemical profile. We are proposing ecological network analysis to model, examine and predict if there's a correlation between several taxa and the presence of certain secondary metabolites and what is the effect of certain secondary metabolites on endophytic colonization. The extent to which plant chemical profile shape communities of foliar endophytic fungi is an important question. If there is a link between fungal species and SM diversity, this will support the hypothesis that plants enhance their metabolic diversity and defense by using their own metabolites to select for metabolically diverse endophytes. As abiotic factors induce changes in the metabolome, fungal communities are expected to adapt accordingly, disturbing community balances. The implications of this context reach and impact agricultural management, and climate change science.

PP1:V-06 Regulatory and functional interplay between PhoB and DSF synthase- RpfF in maintenance of iron equilibrium in *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*)

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Bacterial leaf streak in rice caused by the bacterium *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*), amounts to a significant loss of 30% of rice production in India alone. Quorum sensing (cell-cell communication) has been reported to play a pivotal role in the virulence of *Xoc* and is mediated by the signal molecule DSF (Diffusible Signal Factor). DSF is synthesized by RpfF and sensed by its cognate sensor RpfC that further relays the signal to the response regulator RpfG. Additionally, the Pho regulon is essential for phosphate homeostasis and virulence in other *Xanthomonas* species and comprises of the regulator PhoB, the sensor PhoR, the phosphate ABC transporter PstSCAB and PhoU. Previous work from our laboratory showed that low iron condition drastically suppressed the growth of the DSF synthase mutant $\Delta rpfF$; however, $\Delta rpfC$ and $\Delta rpfG$ did not show this phenotype, thereby suggesting the involvement of other sensors and response regulators in iron homeostasis. Peculiarly, overexpression of *phoB* was able to rescue the growth of $\Delta rpfF$ under iron deplete condition by enhancement of siderophore production. This was further affirmed by expression analysis that showed the upregulation of the siderophore synthesis and uptake cluster upon *phoB* overexpression. Study of the insertional mutants of the Pho regulon genes- *phoB*, *phoR*, and *phoU* showed reduced growth under iron deplete condition and the siderophore synthesis and uptake cluster was also significantly downregulated in these mutants. Further, the data suggest that the Pho regulon plays a significant role in exopolysaccharide production, biofilm formation and virulence in *Xoc*. Expression analysis of the PhoBR promoter under iron deplete condition demonstrated that it is significantly upregulated in *Xoc* and downregulated in the $\Delta rpfF$ mutant. Our studies show that PhoB is directly or indirectly regulated by RpfF in *Xoc* and plays a prominent role in the maintenance of iron homeostasis by upregulating the siderophore synthesis and uptake cluster. Further studies are underway to explore the functional and regulatory overlap of Pho regulon and quorum sensing network in the maintenance of iron equilibrium.

Poster Presentations

miCROPe 2019 - Microbe-assisted crop production opportunities, challenges & needs
Vienna, Austria, December 2 – 5, 2019

Poster Session 2: Microbial biocontrol of pests, pathogens and weeds

PP2-MB-01 BIOVINE project: Exploit biodiversity in viticultural systems to reduce pest damage and pesticide use, and increase ecosystems services provision

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Organic vineyards still rely on large external inputs to control harmful organisms. The capacity of plants to increase the ecosystem tolerance to pests and invasive species is a well-known ecosystem service. However, monocultures like vineyards do not exploit the potential agroecosystem services of plant diversity. The BIOVINE project will develop natural solutions based on plant diversity to control pests and reduce pesticide dependence. BIOVINE aims to develop new viticultural systems based on increased plant diversity within (*e.g.* cover crops) vineyards by planting selected plant species for the control of arthropods, soil-borne pests (oomycetes, fungi, nematodes), and foliar pathogens. Through a literature review, candidate plants will be identified and the selected ones will be tested in controlled environment or small-scale experiments. The ability of the selected plants to: a) attract or repel target arthropod pests; b) conserve/promote beneficial organisms; c) control soil-borne pests by mean of biofumigation; d) promote arbuscular mycorrhizal fungi (AMF) to vine root system to increase plant health (growth and resistance); e) control foliar pathogens by reducing the inoculum spread from soil, will be investigated. New viticultural systems able to exploit plant diversity will then be designed based on results of BIOVINE activities, which will then be tested by in-vineyard experiments for a 2-year period. The presentation will mainly focus on the WP4 of the BIOVINE project about "increasing plant health through mycorrhizal fungi". Grapevine like many other plants can develop mycorrhizal symbiosis. Mycorrhiza is a beneficial interaction between plant roots and some fungi. This interaction promotes plant nutrition, and reduces plant stresses and fertilizer needs. Arbuscular mycorrhizal fungi improve access of plants to soil nutrients and water in exchange of a carbon source from photosynthesis. AMF could colonize many plants as well as one plant can be colonized by many fungi. Colonization and the link between plants allow them to communicate in ecosystem and activate plant defence responses. Cover plants give opportunity to improve mycorrhizal interaction in vineyard during winter and possibility to transfer mycorrhizal fungi to grapevine roots. Selected plants are evaluated for their ability to form arbuscular mycorrhizal symbiosis and develop common mycelial network between cover plants and grapevine, thereby promoting their global health.

PP2-MB-02 "MiRA", an EU funded PhD training network studying Microbe induced Resistance to Agricultural pests

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Plants are intimately associated with a diversity of beneficial microorganisms in their root zone, some of which can enhance the plant's resistance to insect pests. Thus, the use of Microbe-induced Resistance (MiR) to reduce pest losses in agriculture has emerged as a promising possibility to improve crop resilience and reduce use of harmful pesticides. However, MiR appears to be strongly context dependent, with reduced benefits under certain biotic and abiotic conditions, and in some crop varieties. Further, it is a challenge to deliver and ensure stable associations of beneficial microbes and plants, and avoid undesired effects on beneficial insects.

In an EU funded Innovative Training Network, "MiRA", 15 early-stage researchers are studying basic and applied aspects of such context-dependency in tomato and potato, including mechanisms, impacts on plant performance and other biocontrol organisms, formulation of microbial inoculants, and economic prospects and constraints for MiR development and uptake in plant production. The PhD students are hosted at 11 academic and private institutions in different European countries and receive additional training and supervision from other members of the consortium during joint trainings and exchange visits.

The MiRA project will greatly improve our future understanding, development and research capabilities in this important scientific and applied field.

This project has received funding from the European Union's Horizon 2020 research and Innovation programme under grant agreement No 765290

PP2-MB-03 Development and evaluation of the synthetic micro-consortium of antagonistic bacterial strains used to protect potato tubers against soft rot under storage conditions

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Potato (*Solanum tuberosum* L.) is the fourth staple food crop worldwide after rice, maize and wheat. The very intensive global potato cultivation together with the international potato tuber market has an enormous impact on the transmission and spread of potato diseases. The diseases impact potato production in any stage of crop growth and under storage and transit. They may affect tubers, above-ground plant parts (foliage), roots or whole plant. From all diseases affecting potato, the ones caused by pectinolytic Soft Rot *Pectobacteriaceae* (SRP: *Pectobacterium* spp. and *Dickeya* spp.): blackleg of stems during field growth and soft rot of tubers in soil and under storage conditions are among the most important bacterial potato diseases recognized in potato production. These led to estimated losses of ca. 10 to 40% crop (even up to ca. 250 million Euro) annually worldwide. No commercial control products to be used against pectinolytic *Pectobacterium* and *Dickeya* in agricultural applications exist. This is due to the fact that during infection the bacteria are readily present in protective niches (inside plant vascular tissues) and the majority of chemicals and physical control measures work only superficially and cannot penetrate to the tissues located inside plants. The PATBIOCON project aimed to use biocontrol measures to protect agricultural and ornamental plants from infections with pectinolytic bacteria. For this, we have selected beneficial bacteria occupying the same niche and antagonistic to *Pectobacterium* and *Dickeya* and evaluated them individually and in micro-consortia against the pectinolytic bacteria under disease-provoking conditions (high temp., high humidity, high pathogen load, hypoxia). Application of a combination of (compatible) beneficial bacteria with different modes of antagonistic action provided significant protection of plant tissues against *Pectobacterium* and *Dickeya* (reduction of tuber soft rot by 46% (p=0.0016) under disease-favouring conditions). The selected antagonists were additionally characterized for features important for their viable commercial applications including growth at different temperatures, resistance to antibiotics and potential toxicity towards *Caenorhabditis elegans*. The implications for control of soft rot caused by SRP with the use of the newly, successfully developed micro-consortium of antagonists will be discussed.

PP2-MB-04 The use of beneficial microbes in commercial horticulture.

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Plants and microbes have co-evolved intimate relationships and the plant-associated microbiome represents one of the key drivers of the overall plant health and productivity. Mycorrhizas, PGPR's and endophytes are well known for their role in plant health. These microorganisms can help plants in alleviating both biotic and abiotic stresses, which are major constraints to agricultural production. Recent advances in sequencing technology and microbiome research have led to a better understanding of the microbial community found in the Rhizosphere.

Commercial horticulture is increasingly facing increasing restrictions from legislation surrounding chemical control, water use and nutrient input. Improving the benefits obtained from soil microbes such as AMF and PGPR may hold an important key to achieving production potentials whilst minimizing input. Research at NIAB EMR has shown that inoculation of horticulture crops with Mycorrhizal fungi increases productivity and confers a greater tolerance of drought.

NIAB EMR are studying the effects of AMF and PGPR on various horticulture crops to study how these amendments affect endophyte composition and plant growth.

Current work focuses how these amendments affect endophyte populations and resistance to European apple canker, caused by *Neonectria ditissima* in apple. European Canker has become the most damaging disease of apple in recent years across many major apple growing regions worldwide. Modern cultivars lack effective resistance to this pathogen and in Europe most efficacious methods of chemical control are no longer available. Cultivars differ in their susceptibility but there is no absolute resistance.

PP2-MB-05 Basidiomycetes are particularly sensitive to volatile organic compounds of *Pseudomonas protegens*

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Volatile organic compounds (VOCs) play an important role in the communication among organisms, including plants, beneficial or pathogenic microbes, and pests. *In vitro*, we observed that the growth of many Basidiomycetes, including *Heterobasidion abietinum*, *H. parviporum*, *Fomes fomentarius*, *Ganoderma lucidum*, *Phellinus pini*, *P. tuberculosis*, and *Rhizoctonia solani*, was inhibited by VOCs of the biocontrol agent *Pseudomonas protegens* strain CHA0. On the other hand, *Daedalea quercina* was the only Basidiomycete that we found resistant. Intriguingly, in taxa outside Basidiomycota, the resistance to CHA0's VOCs seemed a rule with few exceptions. In fact, strains of Ascomycetes (*Aspergillus* sp., *Penicillium* sp., *Fusarium oxysporum* f. sp. *cubense*, *Verticillium dahliae*, and *Pyrenochaeta lycopersici*), Oomycetes (*Pythium aphanidermatum*), and Zygomycetes (*Rhizopus* sp.) were less sensitive or even resistant, with the Ascomycetes *Alternaria tomatophila*, *Botrytis cinerea*, and *Sclerotinia sclerotiorum* being the sensitive exceptions. Therefore, we used the system CHA0/*H. abietinum* and the overlapping plate method to study the mechanisms behind the fungal growth inhibition. Like CHA0, the derivative mutant CHA77, impaired for HCN production, was effective against *H. abietinum*, suggesting that HCN did not primarily participate in such inhibition. Remarkably, CHA0 emitted effective VOCs only when grown on certain media such as Luria-Bertani-agar (LBA), King's B agar, and peptone-agar, but not on potato-dextrose-agar (PDA). VOCs from LBA- and PDA-cultures of CHA0 were sampled using a closed-loop stripping method and analyzed by gas chromatography coupled to mass spectrometry (GC/MS). Comparative analysis of the volatilomes emitted on both media unveiled the candidate molecules very likely involved in the detrimental effect on *H. abietinum* growth. In the overlapping plate system, one colony of CHA0 was enough to drastically limit the growth of *H. abietinum*, and two or more colonies stopped completely the fungal growth, either if a young mycelium plug or a 3-, 5- or 7-day old fungal colony was exposed. Scanning electron microscopy revealed that hyphae exposed to VOCs were severely damaged as they became suddenly shrunk, abnormally branched, and with depressed lesions in the cell wall. It was impressive that such injuries occurred as soon as 15 minutes after the exposure to VOCs, increased in intensity afterward, and 7 days after exposure the hyphae were absolutely unrecognizable.

PP2-MB-06 Biological control of potato diseases guided by plant microbiome approaches

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Plant protection against fungal pathogens is a crucial aspect of modern agriculture and mostly relies on chemical inputs. However, phytopathogens often become resistant against conventional fungicides. Additionally, the microbial diversity in agricultural soils decreases through the application of pesticides, fertilizers and the soil tillage. The aim of the ongoing SusCrop – ERA-NET project 'PotatoMETAbiome' is to establish microbial consortia, which are applicable for potato cultivation to mediate the resistance against soil-borne fungal pathogens (*Verticillium dahliae* and *Rhizoctonia solani*) and support the microbial diversity in soil. For the consortium assembly, pre-selected microorganisms from the strain collection for antagonistic microorganisms SCAM (Institute of Environmental Biotechnology, Graz University of Technology) will be implemented. A total of 111 bacterial strains were already tested comprising the genera *Bacillus*, *Erwinia*, *Paenibacillus*, *Pseudomonas*, *Serratia*, *Streptomyces*, *Ralstonia* and several yet unidentified species. Additionally, fungi of the genus *Trichoderma* were assessed in terms of antagonistic effects. They will be optimized *in vitro* in terms of their efficiency to inhibit the target phytopathogens. The best antagonistic microorganisms will be used for consortium assemblies and their VOCs profiles will be assessed. Established consortia will then be combined with root exudates, which can act as a 'boosters' for the microorganisms. Furthermore, the consortia will be applied in greenhouse experiments and field trials. In a final step, meta-transcriptomic datasets will be obtained to analyze the interaction of the microorganisms in the consortium.

PP2-MB-07 Breeding for priming triggered leaf rust resistance in barley

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Leaf rust (*Puccinia hordei*) is one of the major diseases of barley (*Hordeum vulgare* L.) leading to yield losses up to 60%. Resistance genes *Rph1-Rph26* are known but most of these have been overcome, meanwhile. In this respect, *priming* may offer an opportunity to enhance resistance to *P. hordei*. During quorum sensing in populations of many Gram-negative bacteria, single cells produce *N*-acyl homoserine-lactones (AHL). Those molecules are known to induce resistance in plants. The present study aims therefore at the detection of genotypic differences in the response of barley to AHL, followed by the identification of genomic regions involved in priming capacity of barley, which is one of the most important cereal crops, worldwide.

A diverse set of 200 spring barley accessions was treated with bacteria, i.e. a repaired *Ensifer meliloti* natural mutant strain *expR+ch* producing substantial amount of the AHL oxo-C14-HSL and a transformed *E. meliloti* strain carrying the lactonase gene *attM* from *Agrobacterium tumefaciens* which inhibits AHL accumulation. After three bacterial inoculations, plants at the three-leaf stage were infected with *P. hordei* strain I-80. 12 days after infection scoring of the diseased leaf area and the infection type was conducted and the relative susceptibility was calculated thereof. Results revealed significant effects ($p < 0.001$) of the bacterial treatment indicating a positive effect of priming on resistance to *P. hordei*. Based on the observed phenotypic differences and 23,417 filtered SNPs derived from the Illumina 9k iSelect chip and genotyping by sequencing (GBS), 5 quantitative trait loci (QTL) associated to improved resistance to *P. hordei* after priming with *E. meliloti expR+*, were identified on the short arms of barley chromosomes 6H and 7H. QTL for the *priming* inducibility may be interesting for a pre-selection of *primable* accessions. Finally, KASP markers will be developed, facilitating marker assisted selection of *priming* efficient accessions in barley breeding. Moreover, genes in QTL regions might be interesting candidates for further research on the mechanisms of plant-microbe interactions.

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PP2-MB-08 Assessing the effect of endophyte entomopathogenic fungal combinations on pathogen inhibition

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Entomopathogenic fungi are typically applied in single application strategies; however, a combination of multiple antagonists may improve the control efficacy of herbivores and plant pathogens compared to single antagonist applications. Antagonist combinations might provide protection at different time intervals or under different conditions, occupying different niches and complementing each other. Experiments were conducted as a first step towards the possible advantage of combining various antagonists in planta (as endophytes) enhancing the effectiveness as biological control agents. Experiments were conducted by inoculating strains of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium brunneum*, followed by an inoculation with a plant pathogen (*F. oxysporum*) 24 hour later. These experiments aimed at assessing the ability of the entomopathogenic fungi to; (1) endophytically colonize tomato plant tissues (as a concurrent combination) and (2) inhibit the growth of the plant pathogen. Quantitative PCR was conducted to quantify the presence of each inoculated fungus in different tomato plant tissues. Significant amounts of picogram DNA of *B. bassiana* and *M. brunneum* were found mostly in roots, while the stems were only marginally endophytically colonized by these strains. The inoculated entomopathogenic fungi were able to inhibit the growth of the plant pathogen as compared to single inoculations. We hypothesise that multiple niche occupation by the endophytic fungi reduced the growth of the plant pathogen either by directly blocking pathways or by the production of antagonistic compounds.

PP2-MB-09 Use of bacterial inoculants in the scope of Nutrition-Sensitive Agriculture: an evaluation of biocontrol, nutritional value, and ecological impact

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Nutrition-Sensitive Agriculture (NSA) is a novel concept in agriculture that does not consider only yield, but also nutritional value of the food, sustainability of the production, and the ecological impact of agricultural practices. In accordance with its goals, NSA would benefit from applying microbial-based products as they are deemed more sustainable than their synthetic counterparts.

This study aims to characterize the effect of 3 plant-beneficial bacterial strains (*Paenibacillus pasadenensis* strain R16, *Pseudomonas syringae* strain 260-02, *Bacillus amyloliquefaciens* strain CC2) on the nutraceutical value and performance of romaine lettuce plants (*Lactuca sativa*) and in controlling pathogens. The pathogens used in the trials are *Rhizoctonia solani*, *Pythium ultimum* and *Botrytis cinerea*.

The trial was conducted on plants grown in pots in controlled conditions (greenhouse). After harvest lettuce leaves were subjected to the common practices for the fresh-cut produce production pipeline and stored at 8 °C.

The considered values include photochemical activity of photosystem II, content in antioxidant compounds, as well as some technological characteristics such as firmness, moisture content, tissue integrity, and tint. The effect of the inoculants was also analyzed by characterizing the bulk soil, rhizosphere, and root microbiota in the presence or absence of the inoculants.

The results obtained indicate that strain R16 had a significant ability to reduce symptoms caused by all analyzed pathogens, while the other two strains showed a less efficient biocontrol ability. The performance, nutraceutical, and technological parameters were largely unaffected by the treatments, indicating that the product was equivalent to that obtained without using the bacteria. The only differences observed were related to a beneficial effect on the integrity of the tissues. A lower loss of electrolytes and membrane peroxidation, observed in plants treated with strain R16, might possibly lead to a longer shelf-life. The composition of the microbiota was radically different in the rhizosphere and the root endosphere among treatments, but the bulk soil remained practically unchanged, indicating that the use of these treatments did not have a large-scale ecological effect.

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PP2-MB-10 Chitin and chitosan - key cell wall components in *Trichoderma atroviride* development and mycoparasitism

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Trichoderma atroviride is an effective agent for combating ascomycetes against a variety of plant pathogens, including *Sclerotinia*, *Botrytis*, *Fusarium* and *Rhizoctonia*. The ability to invade host organisms during fungal parasitism requires adaptive remodeling of the cell walls to prevent host recognition and defense reactions. In the mycoparasite *Trichoderma atroviride*, cell wall remodeling plays a central role in the biocontrol of plant pathogens, and chitin and chitosan have been proposed as key elements in this process. Invasive strategies to escape the chitin-triggered host immune system are shared by all plant and human pathogens, but mechanisms in mycoparasites have not been studied. We have identified a series of more than 20 enzymes involved in chitin and chitosan synthesis, and characterized their concerted interplay during mycoparasitic attack and the circumvention of host defense mechanisms. Eight chitin synthases, six chitin deacetylases, additional chitinolytic enzymes (including six chitosanases), transglycosylases and accessory proteins are involved in this complex regulated process. We provide the first complete description of these enzymes associated with glycopolymer synthesis in the cell wall of *Trichoderma atroviride* and show that they are essential for mycoparasitism of significant phytopathogens. In addition, we show first results to combine *Trichoderma atroviride* with exogenous fungal chitosan and demonstrate this powerful application in biocontrol. Our findings critically contribute to understanding the molecular mechanism of chitin and chitosan in mycoparasites during biocontrol with the overarching goal to selectively exploit the discovered strategies.

PP2-MB-11 Functional and genomic potential of the biocontrol capacity of the *Bacillus velezensis* IBUN 2755 strain against *Burkholderia glumae* under field conditions

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Burkholderia glumae is a rice pathogen that causes estimated losses between 40 and 75% of crop yield due to seedling rot and the generation of empty grains towards the end of the crop cycle. Currently, no effective control is known despite its great impact on the crop and its distribution in all rice's producing areas of the world. The strain of *B. velezensis* IBUN 2755, after its evaluation in dual antagonism assays *in vitro* using cell suspension and cell-free supernatants, its activity was tested in rice plants under greenhouse conditions. Besides, the strain was evaluated under field conditions, with natural infection of *B. glumae*, where severity and incidence of the disease and the impact on grain filling were evaluated. Given the biocontroller potential of the strain, sequencing of its genome was carried out through the PACBIO system. Bacterial genome was assembled and the prediction of genes associated with biological control mechanisms was performed through manual search and through the PRISM3 algorithm for antimicrobial compounds. The strain IBUN 2755 was found to be active by the generation of *in vitro* inhibition halos against *B. glumae*, both for cell suspensions and cell-free supernatants. In 7-day seedlings treated with strain IBUN 2755, decreases the severity of the disease, and from 30-day plants up to flowering stage decreases the population density of the pathogen in stem, root and flag sheets in comparison to untreated controls. Besides, a significant decrease of empty grains at harvest time was observed. The results of the field evaluation, showed that strain IBUN 2755 was able to reduce incidence and severity of symptoms caused by *B. glumae*. In addition, grain yield was improved around two tons per hectare in relation to uninoculated control. These results suggest that the strain decreases the symptom of empty grains caused by the pathogen, confirming the results obtained under greenhouse conditions. Genome sequencing showed that strain IBUN 2755 has a circular chromosome of 4,027 Kb, with 4063 coding sequences. Around 7.2% of those sequences are dedicated to the production of antimicrobial compounds, which may be related to its biocontrol capacity against *B. glumae* and other bacterial and fungal phytopathogens towards which the strain has shown antagonistic activity.

PP2-MB-12 Interactions among *Fusarium*, streptomycetes and wheat grains: effects on deoxynivalenol accumulation and fungal growth.

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Streptomycetes have been exploited as biocontrol agents (BCAs) against plant pathogens for their ability to produce different bioactive compounds. They can be used against *Fusarium graminearum*, the main causal agent of Fusarium head blight (FHB) as well as against contamination of grains with deoxynivalenol (DON). In the present research, the effect of four promising *Streptomyces* strains on fungal growth and mycotoxin production on sterilised seeds was assessed. The reciprocal interactions between the pathogen and the four BCAs were assessed directly on sterilized grains using quantitative real-time PCR detection of the two targets (*Fusarium* and *Streptomyces* spp.) and chemical extraction and quantification of ergosterol and DON.

The results indicate that the highest level of DON inhibition (99%) as well as a strong reduction of fungal biomass can be achieved following a simultaneous inoculation of each BCA with the pathogen while late BCA inoculation (3 days post fungal inoculation) did not significantly reduce fungal growth and mycotoxin production.

This research contributes also to the understanding of the mechanisms of action of the four *Streptomyces* strains that act mainly as fungal growth inhibitors. The presence of the fungus influenced differentially the growth of the four strains. This information is important for understanding the fitness of each BCA strain.

Our study confirms the importance of studying the interactions among the grains, the pathogen and the BCA, in order to identify mechanisms of activity of the strains, moving towards their application in real conditions.

PP2-MB-13 Austrian *Trichoderma* spp. impact mycotoxin production of the plant pathogen *Fusarium graminearum*

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Secondary metabolites, including mycotoxins produced by various fungal species have their function in the survival and reproduction in an environment with limited resources. The defense of a colonized food source often leads to the production of toxins protecting the surrounding area against competing fungi, bacteria and insects. Many of these mycotoxins produced on crops or during food storage represent a serious threat for human health. *Trichoderma* species known for their efficient biocontrol abilities against crop pathogens and spoilage fungi are used for decades as biological supplement to pesticides in agriculture.

A screening for antagonistic interaction of diverse *Trichoderma* strains with *Fusarium graminearum* suggested a correlation between antagonistic potential and secondary metabolite production. Here we present that the presence of *Trichoderma* spp. collected from Austrian soils influences the overall secondary metabolite and mycotoxin composition on *F. graminearum*. An HPTLC (high performance thin layer chromatography) screening of 95 strains representing 20 *Trichoderma* species revealed various interaction types between competing strains with a substantial influence on the production of DON (deoxynivalenol) by *F. graminearum*. The presence of several *Trichoderma* strains lead to disappearance of DON production whereas others triggered the production of DON up to 70-fold compared to axenic culture. We also could show that DON overproduction correlates with the presence of several other compounds not produced in axenic culture. Interestingly, the production of ZON (zearalenol) does not correlate with DON but is again influenced by several *Trichoderma* strains.

The altered regulation of secondary metabolism by *F. graminearum* in the presence of *Trichoderma* is likely due to chemical communication between these fungi. Hence we studied the reaction of *Trichoderma* strains to the presence of *Fusarium* as well. We found clear indications for chemical communication which causes production of novel metabolites compared to axenic growth.

In summary we show that a fine-tuned and strain-specific interaction with the crop pathogen *F. graminearum* has considerable influence on mycotoxin production. Moreover, our findings indicate that not only antagonism impacting biomass formation of pathogens, but also an influence on secondary metabolism is worth considering in screenings for biocontrol agents.

PP2-MB-14 Mechanisms underlying the inhibition of *Phytophthora infestans* by cyanogenic *Pseudomonas*

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Phytophthora infestans is the causing agent of late blight disease, which is associated with a loss of approximately 3 billion dollars per year including the control efforts and production losses. The current control methods like the use of synthetic fungicides or copper-based products are not sustainable as well as harmful to nature. In multiple studies, bacteria of the genus *Pseudomonas* were shown to act as biocontrol agents and efficiently inhibit *P. infestans* by producing antimicrobial compounds. Two such antimicrobial compounds are known to inhibit the growth of *P. infestans*: hydrogen cyanide (volatile) and phenazines (non-volatile). The aims of our study are i) to quantify the relative contribution of known determinants of anti-oomycete activity in the overall activity of potato associated *Pseudomonas* strains, ii) to identify novel volatile and non-volatile determinants of this anti-oomycete activity. To this end, we are focussing on two fully sequenced *Pseudomonas* strains and generating mutants of known antimicrobial compounds to confront the bacterial mutants to *P. infestans* at different stages of its life cycle *in vitro* and on the potato plants. Since both strains of interest produce HCN, a potent inhibitor of oomycete development, our current experiments are focused on using HCN mutants to determine: 1) the contribution of HCN to *in vitro* and *in planta* inhibition of *P. infestans* growth by *Pseudomonas* strains and 2) the presence of other novel volatile and diffusible compounds with anti-oomycete activity. Understanding the different modes of action of the anti-oomycete compounds and the molecular mechanisms underlying the inhibition could help us in designing better control strategies against the devastating late blight disease.

PP2-MB-15 A fungal biocontrol agent for Septoria tritici blotch of wheat: from isolation over field trials to elucidating mechanisms in disease control

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Fungal diseases are a frequent cause of considerable yield losses in crops and therefore represent a major threat to modern agriculture. *Septoria tritici* blotch (STB), caused by the fungus *Zymoseptoria tritici* (syn. *Mycosphaerella graminicola*), is considered the most devastating foliar disease of wheat in Europe. Traditional management practices are generally not very efficient in controlling the disease if the weather is conducive for epidemic development and in most years control is heavily dependent on fungicide applications. However, development of fungicide resistance in the *Z. tritici* population is increasing to all commercially available fungicides. Therefore development of innovative and sustainable strategies for effective disease control is urgent.

Endophytic microorganisms, mainly comprising bacteria and fungi, inhabit inner parts of the plant without causing apparent symptoms on their host. Some endophytic strains have shown potential for protection against plant diseases even in different host genotype-pathogen interactions as well as stimulation of plant growth and conferring abiotic stress tolerance. Due to these beneficial effects, there is an increasing economic interest in developing endophytes as biocontrol agents.

The aim of the project was to identify endophytic fungi that efficiently control STB in a reliable manner and investigate their mechanisms of disease control. In a screening approach, fungal endophytes isolated from field-collected wheat leaves were tested for ability to control *Z. tritici* *in vitro* and *in planta*, under both controlled and field conditions. This structured screening approach represents a reliable and robust approach for discovery of new potential biocontrol agents. To understand the protective effect and optimise future application, the mechanisms of selected endophytes in controlling STB were investigated with histology, enzyme assays and RNA-sequencing of both wheat and *Z. tritici* during a time course of the tripartite interaction with the endophyte. We found that the candidate biocontrol agent reduced disease levels through two mechanisms: inhibition of *Z. tritici* spore germination on the leaf surface and induced resistance, resulting in reduced necrotic symptoms and fewer pycnidia in the host. Induced resistance was characterised by differential regulation of hormone signalling and earlier enhanced expression of defence response genes.

PP2-MB-16 Green challenges: practical examples how resilience of horticultural systems can be improved using concepts from soil microbial ecology

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Soilborne diseases, such as *Fusarium* wilt, *Pythium* damping-off, *Rhizoctonia* and *Verticillium* can negatively affect the productivity of different horticultural crops in protected cultivation in the Netherlands. At the same time use of chemical pesticides against these pathogens is being limited due to their adverse effects of human health and the environment. Therefore alternative methods to control soilborne diseases are urgently needed. The project Green Challenges aims to substantially reduce the use of chemical plant protection products against soilborne diseases in greenhouse crops by focussing on the use of products of natural origin and different soil/substrates. We aim to stimulate the natural resilience of the plant- and soil/growing substrate system. In this study, we investigate the effect of natural products that can positively affect plant- and soil functioning via different mechanisms: a) direct disease suppression of disease by introducing antagonistic microorganisms, which can produce enzymes, toxic volatiles or antibiotics that can suppress the growth of the pathogen; b) increase the activity of indigenous bacteria and fungi in the rhizosphere (including disease suppression) by introducing organic materials such as spent mushroom substrate and composts; and c) manipulating induced resistance of plants by inoculation with non-pathogenic micro-organisms, such as mycorrhizal fungi and rhizobacteria.

Effects of application of products of natural origin on disease development was studied in two pathosystems: a) *Pythium aphanidermatum* in cucumber and b) *Fusarium oxysporum* f.sp. *eustomae* affecting lisianthus. Tests were performed in experimental greenhouses (Wageningen University & Research Centre) and in commercial greenhouse production.

PP2-MB-17 Screening for biocontrol of *Cannabis sativa* fungal pathogens with bacteria isolated from soil

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Cannabis is now legal in 25 countries for medical purposes and has been legalized in several jurisdictions for recreational purposes. However, because cannabis cultivation was illegal for most of the 20th century, there is a limited number of pesticides that provide effective control of cannabis pathogens. In addition, this crop is especially susceptible to pathogens because 1) pathogen-resistant varieties have not yet been developed and 2) it is grown indoors under warm, high humidity controlled environment conditions that are favourable for pathogen establishment. In this project, we screened approximately 40 bacteria isolated from soil, with previously demonstrated antibiotic activity, for biocontrol against four common fungal cannabis pathogens (*Botrytis cinerea*, *Fusarium oxysporum*, *Penicillium oslonii* and *Pythium myriotylum*). For initial high-throughput screening, six bacterial isolates were streaked onto a single square petri dishes containing trypticase soy agar (TSA) and grown for one week (two days at 21 °C and five days at 4 °C) to allow for colony establishment and production of secondary metabolites. After one week, sterile water was inoculated with one of the four fungal pathogens and the spore suspension was streaked onto the petri dishes perpendicularly to the bacterial isolates. Plates were incubated at 25 °C for approximately one week, until zones of inhibition could be easily identified. This method allowed for rapid screening using fewer lab materials, compared to classical fungal-bacterial interaction assays used in plant pathology research. In the second round of screening, we used an overlay assay to test individual bacteria against fungal pathogens to confirm suspected antifungal activity. Bacterial isolates were spotted onto TSA in circular petri dishes and grown for one week, as described above. On day seven, a plug of one-week old fungal pathogen was used to inoculate 7 mL of liquid potato dextrose agar (PDA, approximately 40 °C); the spore suspension was then poured onto the plate with the week-old bacterial culture. Plates were incubated for up to one week at 25 °C and the size of the inhibition zone was recorded. Detailed results will be presented. Future work will examine 1) if the strains that test positively for biocontrol activity are able to colonize cannabis plants using green fluorescence protein (GFP)-tagged strains and 2) if the strains provide *in planta* biocontrol against the selected pathogens.

PP2-MB-18 The cell wall as virulence factor in the biocontrol fungus *Trichoderma atroviride*.

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The functional role of chitosan in the cell wall of most fungi is poorly characterized, but its importance for virulence is evident. Chitin to chitosan conversion by specialized deacetylases is a potent escape mechanism of human - and phytopathogenic fungi to avoid activation of the host's chitin-induced immune system. Here we provide first insights into the composition of the *Trichoderma atroviride* cell wall and its role as protective organelle and virulence factor. Chitin and chitosan synthesis have so far only been studied in saprotrophic and phytopathogenic fungi, but not in ascomycetes with a mycoparasitic lifestyle. The elucidation of these mechanisms in mycoparasites to fight pathogens and to identify targets for antifungal drugs is of utmost importance, and we therefore provide first detailed insights into the fungal-fungal system.

Microscopic analysis revealed the intricately regulated interplay of the eight chitin synthases and more than 15 other enzymes - deacetylases, chitinolytic enzymes, and accessory proteins - in the assembly and turnover of chitin and chitosan in the *T. atroviride* cell wall.

How mycoparasitic *Trichoderma* protect their cell wall from their own or hostile hydrolytic enzymes remained largely uncharacterized. Confrontation assays with knock out lines provided the first evidence that chitin and chitosan remodeling is indispensable for virulence and host invasion by the mycoparasites. Our findings further highlight the importance of chitosan in serving as disguise towards hostile chitinases and as ROS scavenger during the mycoparasitic attack. These important insights into cell wall assembly during mycoparasitism enhance our understanding of the biocontrol capabilities of *Trichoderma* spp.

PP2-MB-19 Fungal biocontrol agents for Fusarium Head Blight control

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Fusarium head blight (FHB) is a constraint of wheat productivity worldwide. Wheat spikes infected during flowering show reduced grain yield and quality. Current control measures remain insufficient, especially in a world with increasingly stringent environmental standards. Biological control is a promising albeit little utilised alternative for disease management. Fungal endophytes are microorganisms that colonise plant tissues internally without causing visible symptoms. They have been observed to increase natural stress tolerance, enhance growth and control plant diseases.

Our main objective is to identify adapted fungal endophytes with potential to reduce FHB in wheat. Specifically: 1) What are the dynamics of fungal endophytes population on wheat spikes during FHB infection? 2) Can endophytes isolated from healthy wheat spikes provide biological control against FHB? 3) What is the mode of action when reducing FHB infection?

We have shown that fungal communities in wheat spikes are dynamic during flowering. *Fusarium* infection disrupts the natural processes by reducing fungal diversity inside wheat spikes. Spikes which remained healthy after pathogen exposure harboured specific fungal taxa, e.g., *Cladosporium*, *Itersonillia* and *Holtermanniella*. These results suggest that healthy wheat spikes in areas with high FHB incidence harbour endophytes with biocontrol potential. We recovered 168 fungal isolates from healthy spikes in areas with high FHB. Four fungal isolates that actively reduced *Fusarium* symptoms were identified using a high throughput screening assay *in planta* and the results were validated in the greenhouse. These results confirmed the presence of naturally occurring biological control agents in wheat fields and highlight the need to use *in vivo* systems for efficacy screening.

Finally, the effect of inoculation of a fungal endophyte on wheat spikes during FHB was studied using RNAseq. The endophyte activated plant defence mechanisms at 48h after of inoculation and treated plants responded earlier and stronger to *Fusarium* infection after pathogen inoculation suggesting that biocontrol effect is plant-mediated due to early induced resistance.

Collectively, our study confirms the potential of using naturally occurring endophytes as a reservoir for environmentally friendly disease control agents. Endophyte-based biocontrol solutions would enrich our current integrated disease management practices in a more sustainable manner.

PP2-MB-20 Regulation of defense-related genes in potato plants treated with salicylic acid and bacterial extract

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Potato is a major food crop that can be severely attacked by numerous pests and pathogens. Biotic elicitors represent an alternative tool for management of potato plantations, allowing producers to reduce losses and the amount of chemicals used. An autoclaved extract of *Xanthomonas axonopodis* (XTH elicitor) was found to promote resistance of potato plants (CN102256495A; US8932844B2) against the black leg disease caused by pectolytic bacteria *Pectobacterium carotovorum*, via a poorly understood mechanism. Considering the different signaling pathways involved in plant defense, this work was intended to investigate and characterize the elicitation mechanism of XTH in *Solanum tuberosum* plants by analyzing the expression of *PR-1b*, *PR-2*, *ChtA*, *PAL*, *Pin2*, *JAZ1/TIFY10A-like*, and *ERF1* genes. In order to that, the elicitors (Salicylic acid-SA or XTH) were applied with a delicate brush on a leaflet of a fully expanded leaf (therein named treated leaf). The treated leaf and the immediate upper leaf (systemic leaf) were then removed for analysis. SA induced local and systemic expression of the JA/ET marker genes *JAZ1/TIFY10A* and *ERF1* in potato plants. SA effectively suppressed transcription of the JA-responsive gene *Pin2*. Interestingly, the expression of *Pin2* was upregulated by XTH treatment. Moreover, XTH treatment was able to induce systemic expression of *PAL* gene (Phenylalanine ammonia lyase). Our results suggest that XTH is able to modulate the expression of defense-related genes in potato plants via concomitant activation of the salicylic acid and jasmonic acid signaling pathways. Interestingly, exogenous application of salicylic acid induced the expression of *JAZ1/TIFY10A-like* in potato plants via a putative jasmonate-independent pathway. We envision the use of XTH as a tool for potato crop management.

PP2-MB-21 Comparative spermosphere and phyllosphere microbiome of rice reveals core microbiota with antagonistic potential against foliar pathogens

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Current rice blast and blight control strategies namely host resistance and agrochemicals are not adequate to combat the epidemics. In order to develop an alternative disease management option, bacterial flora naturally occurring on the phyllosphere and spermosphere of rice was explored. Microbiome of basmati and non basmati rice cultivars, PB1 and VLD85, grown in Almora district of Uttarkhand, India were analysed using NGS based metagenomic and cultivation based methods. Microbiome of adaxial and abaxial surface of phyllosphere as well as blast lesion was also deciphered. *Pseudomonas fulva*, *Pantoea agglomerans* and *Methylobacterium platani* were found dominant in the adaxial and abaxial microbiome of phyllosphere. On blast lesion, *Paenibacillus lautus* and *Pantoea septica* were uniquely found. *Pseudomonas oryzae*, *Flavobacterium acidificum*, and *Pantoea ananatis* were found dominant in spermosphere of rice. BOX-PCR based DNA fingerprinting of isolates revealed 104 distinct bacterial isolates among the collection. The isolates were further species identified by comparing 16S rDNA sequences with NCBI, SILVA, RDP and EMBL. Among the 104 species, 36 different species of bacteria were evaluated against *Magnaporthe oryzae* and *Xanthomonas oryzae* pv. *oryzae*. While *Pantoea dispersa*, *Pantoea agglomerans*, *Pseudomonas parafulva*, *Pantoea ananatis*, *Acinetobacter baumannii*, *Enterobacter cloacae*, *Pantoea vagans*, *Pantoea deleyi* were found effective against blast fungus, *Pseudomonas parafulva* BG1, *Chryseobacterium cuculis*, *Pantoea anthophila*, *Enterobacter cloacae*, *Pantoea ananatis*, *Pseudomonas psychrotolerans*, *Pseudomonas monteillii*, *Acinetobacter baylii*, *Pantoea vagans*, and *Pseudomonas stutzeri* were found effective against blight pathogen. This study culminated in identification of potential bacterial communities for microbiome transplantation on rice phyllosphere for mitigation of foliar diseases.

PP2-MB-22 Volatile organic compounds from *Lysobacter capsici* as potential candidates for biological control of soil-borne phytopathogens

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The genus *Lysobacter* includes several bacterial species, which show potential for being used in biological control of plant diseases. Besides their known capability of producing lytic enzymes, *Lysobacter* spp. also synthesize various secondary metabolites. Moreover, a recent study showed that *Lysobacter* spp. were capable of inhibiting the growth of *Phytophthora infestans* *in vitro*, through the production of volatile organic compounds (VOCs) when grown in a protein rich medium [1]. Based on these findings and taking into consideration the need for alternative solutions in crop protection, we used GC-MS in combination with dynamic headspace (DHS) extraction and thermodesorption to investigate the ability of a biocontrol *L. capsici* strain to produce VOCs, which can inhibit the growth of soil-borne phytopathogens (e.g. *Rhizoctonia solani*). The chemical group of pyrazines was among the most abundant in the volatile profile of the tested *L. capsici* strain. We additionally investigated which of the VOCs are more likely to contribute to the inhibitory activity, by analyzing the volatile compounds present in the Petri dish compartment physically separated from the one the bacteria were grown in. Assays with single identified VOCs showed indeed that the tested compounds were able to inhibit the growth of the phytopathogens *in vitro* in a concentration dependent manner. Currently, we are studying further volatile components that presumably contribute to the strain's antifungal activity, with the aim to better understand the mechanisms of VOC-mediated microbe-pathogen communications and to add a steppingstone towards the development of novel biopesticides.

PP2-MB-23 A synthetic microbial community for the control of Fusarium wilt of banana

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Banana is the most produced fruit in the world. More than 150 million tons of bananas and plantains are produced annually for a business affair of 38.5 billion dollars. The entire banana industry has been threatening by *Fusarium oxysporum* f. sp. *ubense* (*Foc*) tropical race 4 (TR4), which is spreading alarmingly in the cultivation area of banana. We evaluated new biological control agents against Fusarium wilt of banana (FWB) to be used in mixture rather than as single strains. *Pseudomonas* spp., *Bacillus* spp., *Streptomyces* spp. and *Trichoderma* spp. were isolated from rhizosphere soils of banana crops in Tenerife island and selected for the *in vitro* antagonism against *Foc*. Effective strains were used to construct two synthetic microbial communities: SynCom1.0, composed of 44 strains (11 per taxon), and SynCom1.1, composed of 7 strains (*Pseudomonas* spp. strains P1A1, P1C1, and PS5, *Bacillus* spp. strains BN8.2 and BT1, *Streptomyces* sp. strain St2AOB1, and *Trichoderma* sp. strain T2C1.4). In a pot-experiment, inocula of SynCom1.0's member strains were produced individually and then mixed equally for the soil inoculation, which was made at transplanting. In the soil, SynCom1.0's member strains proliferated throughout the experiment and, at 35 days post-inoculation, they reduced FWB incidence by 22% and symptom severity by 33% (from a value of 3 to 2 on a 0-4 scale). Also, they mitigated the leaf chlorophyll content depletion due to the disease. With the same application protocol, SynCom1.1 decreased symptom severity by 34% (from 2.9 to 1.9), but it did not affect FWB incidence. Both microbial consortia did not suppress significantly *Foc* in the soil, suggesting biocontrol mechanisms other than the direct antagonism against the pathogen. *In vitro* assays revealed that, besides the antagonism against *Foc*, most SynCom's member strains can antagonize each other, especially at the inter-species level, and that *Foc* can even antagonize the beneficial microbes. However, based on these data, we were able to design a new SynCom (SynCom1.2) composed of three members (*Pseudomonas* sp. strain PS5, *Bacillus* sp. strain BN8.2, and *Trichoderma* sp. strain T2C1.4) that are both cross-compatible and resistant to *Foc*. SynCom1.2 was better than the previous two consortia in controlling FWB, indicating that inconsistent biocontrol obtained with mere combinations of microbes can be significantly improved by designing a tailor-made synthetic microbial community.

PP2-MB-24 *Streptomyces* sp. promotes plant growth and induces defense metabolism of soybean plants against bacterial pustule.

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Plant growth promoting bacteria as *Streptomyces* spp. have become an attractive alternative for increasing the sustainability of agricultural systems. In this study, ten *Streptomyces* isolates obtained from rhizosphere soil of Fabaceae plants were characterized for their plant growth promoting traits such as production of siderophores, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, indole-3-acetic acid (IAA) and phenazines and growth of soybean plants. The role that isolate CLV45 (Stm45) plays on modulating soybean plant defense pathways in response to the phytopathogen *Xanthomonas axonopodis* pv. *glycines* (Xag) was also analyzed. Bacterized seeds were used to evaluate plant growth within 45 days of culture. In order to evaluate the plant defense pathways, soybean seeds from cultivars sensitive and resistant to Xag were: (a) treated with sterile water; (b) microbiolized with Stm45; (c) treated with sterile water and challenged with Xag; and (d) microbiolized with Stm45 and challenged with Xag (Stm45+Xag). The challenge with Xag was performed 15 days after emergence. The modulation of defense metabolism was evaluated by the activity of phenylalanine ammonia lyase (PAL) and the production of phenolic compounds at times 0, 24, 48, 72 and 144 hours after inoculation (hpi) of Xag. The relative expression of plant defense-related genes JAZ, ERF5, PAL and PR1 was determined at 0, 12, 24 and 48 hpi. Results demonstrated that all isolates produced IAA, although CLV45 was the most efficient one, reaching 398.53 mg of IAA g⁻¹ cells. This isolate also showed high activity for ACC deaminase and production of pyocyanin and phenazine-carboxylic acid. Seed bacterization with CLV45 resulted in plants with increased shoot growth (36.63%) and dry mass (17.97%) when compared to the control plants. Compared to control, PAL expression in susceptible plants Stm45+Xag increased 35% at 24hpi, followed by a 4.8-fold increase in PAL activity at 48hpi, although without the corresponding accumulation of phenolic compounds. Increased PAL expression in Stm45+Xag (12hpi) plants resulted in increased activity of this enzyme by 48hpi. In the resistant cultivar, increased expression of ERF5 gene in Stm and Stm45+Xag plants suggests the PGPR-induced ethylene defense pathway. In the sensitive cultivar, the decrease of JAZ gene expression in Stm (0 hpi) and Stm45 + Xag (12hpi) plants could be related to the initial defense by jasmonic acid.

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Poster Session 2: Microbial applications for improving nutrition and abiotic stress tolerance

PP2-MA-01 Formulations containing beneficial bacteria improve strawberry anthocyanin contents and its potential to modify glucose metabolism-related enzymes

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Strawberry is a functional food of high quality due to its high amount of phytonutrients of phenolic nature like anthocyanins, phenols and flavonols, which have demonstrated to have antioxidant, anti-inflammatory and antidiabetic properties. One of the targets of these compounds on diabetes is inhibiting α -amylase and α -glucosidase, involved in the absorption of glucose to the blood. On the other hand, the red color of strawberries relies on anthocyanins which are synthesized along maturation aiming to protect plant seeds; therefore, fruit coloring occurs late in spring and this reduces commercial value of early fruits that hit the market as early as december when produced in southern Spain. As polyphenols are secondary metabolites, and therefore, inducible by different factors, like beneficial bacteria, our working hypothesis is delivering formulations with specific beneficial strains to plant roots to trigger polyphenol metabolism and increase fruit potential health benefits.

In this study, we used L81 (*Bacillus amyloliquefaciens*) and N21.4 (*Pseudomonas fluorescens*), two strains that have already proven to modify strawberry metabolism, to determine if the combination would have a synergistic effect improving the polyphenolic profile of strawberries, and therefore, lowering the IC50 of enzymes related with glucose metabolism. To prove this hypothesis, treatments were delivered 5 times and the concentration of total phenols, flavonoids, anthocyanins, was determined at 5 time points along plant cycle, and IC50 of α -amylase and α -glucosidase under the influence of extracts at one point were determined. It was found that individual bacteria enhanced contents on anthocyanins at the early sampling times better than the combination of both. therefore improving fruit color and quality. However, at the time where a synergistic effect on anthocyanins was detected, this increase was not reflected on IC50; the individual bacteria were more efficient inhibiting α -glucosidase, and α -amylase, and effects were similarly improved by individual strains and combination. This suggests that anthocyanins are not the only compounds responsible of effects on glucose related enzymes, and these bacterial formulations are good to improve fruit quality and market value.

PP2-MA-02 The use of root endophytic bacteria to boost lettuce growth at low temperature conditions

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In Flanders, Belgium, lettuce farmers create a yearly turnover of 37 million euro, making it the regions second most grown vegetable crop. Lettuce growth is optimal at temperatures ranging between 20°C and 25°C, making it possible for farmers to grow it up to a consumable size in six weeks during spring and summer. During the winter time on the other hand, this process takes up to four months. A promising discipline to promote plant growth under stress conditions is the use of plant growth promoting rhizobacteria (PGPR). Through 16S amplicon sequencing, we aim at comparing the rhizo and endomicrobiome of different lettuce cultivars grown at low temperature conditions compared to control conditions to detect the enriched rhizosphere and endophytic bacterial genera in the cold. In addition, we will isolate these PGPR out of the lettuce root and evaluate them for their plant growth promoting potential by adding an overdose of them to the plants. Subsequently, we want to know which plant associated molecular pathways are triggered by these bacteria to promote the plant's growth. The above mentioned experiments will provide us with insights into which bacteria live inside lettuce roots, which of these bacteria can promote lettuce growth and how they influence the plant's molecular pathways to do so. This project will significantly aid lettuce farmers living in temperature climate areas by increasing the crop's turnover rate during the cold season.

PP2-MA-03 THE plant growth promoting rhizobacteria (PGPR) *Paraburkholderia phytofirmans* PsJN improves root morphology and growth dynamics of *Arabidopsis thaliana* under heat stress

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Root systems anchor plants, absorb nutrients and water, and produce and store compounds essential to plant growth and productivity. Experiments and modelling consistently show that plant yield is directly influenced by the architecture, function, and growth dynamics of root systems. An important but understudied area of roots is their response to environmental stimuli including microorganisms in the soil. Root-microorganism interactions involve signaling and communication processes that modify the physiological, morphological, and biochemical properties of plants. High temperature or heat stress is one of the most prominent abiotic stressors due to climate change. We propose that heat will be deleterious to root growth and functions because of its direct effects on cell membrane stability and metabolism, and that harnessing beneficial microorganisms from the soil may reduce the impact of heat stress on roots, plant performance and productivity.

To test if inoculated microorganisms can relieve the negative effects of heat stress on roots, we quantified the effect of *Paraburkholderia phytofirmans* PsJN on the phenotype of the model plant *Arabidopsis thaliana*, under ambient and high temperatures. *P. phytofirmans* PsJN is an endophytic plant growth promoting rhizobacteria (PGPR) reported to provide tolerance and resistance to abiotic and biotic stresses. *Arabidopsis* seeds inoculated with the bacteria were sown on agar petri dishes and grown for a period of 21 days, where they were continuously imaged and phenotyped. Root morphological measurements revealed increases in the lengths and growth rates of the primary and lateral roots, and greater numbers as well as wider branching angles of lateral roots for plants subjected to bacterial inoculation under both temperature conditions. Although heat stress negatively affected root phenotypes, inoculation with bacteria ameliorated this effect. The onset of beneficial interaction was dynamic, and dependent on time, temperature and root traits measured. An advanced platform is now being used to phenotype both roots and transpiring shoots to confirm the benefits of *P. phytofirmans* PsJN on root and plant performance at high temperatures. Metabolomics and lipidomics will then be used to resolve the molecular mechanisms underlying the phenotypic dynamics, and to test the proposal that root membranes are stabilized by PGPR under heat stress.

PP2-MA-04 The plant probiotic AB12 modulates plant water status and root growth in maize

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As the world population and households consumption continues to grow, global demand for food will steadily increase putting an unprecedented productivity pressure on the global agricultural sector. On top of this, increasing agricultural losses due to biotic and abiotic stresses caused by climate change may further threaten food security. A promising strategy to sustain crop productivity under changing climates is the usage of plant probiotics. These are living microorganisms and when administered in adequate amounts, confer a health benefit on the host and have the advantage to assure the plants growth potential and increase the crops stress resilience.

In this study, we characterized the plant probiotic effects of the lead strain AB12 *Bacillus sp.* on maize growth and physiology under well-watered and drought-stressed conditions. The results showed that AB12 treatment increased water use efficiency and root/shoot ratio. Moreover, AB12 treated maize was shown to have an impact on plant metabolism.

Taken together these results, show the potential of plant probiotic rhizobacteria for beneficial influencing plant physiology by increasing plants water use efficiency and providing resilience to drought stress.

PP2-MA-05 Integrative application of plant growth promoting rhizobacteria combined with soil conditioners and arbuscular mycorrhizal fungi for improved mineral nutrition and drought tolerance of wheat plants

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Weather extremes threaten agriculture globally. Stress mitigation, such as of drought, heat, cold and insufficient availability of mineral nutrients, is of increasing importance. To strengthen crop plants by means of microbial agents the EU project SolACE aims to develop integrative application strategies. Therein, the ability of microbial strains to colonize plant roots at sufficient population densities under adverse conditions (rhizosphere competence) is a critical prerequisite. In many instances, however, the numbers of colony forming units of microbial agents decline shortly after soil application. Little is known how the plant and environmental factors influence microbial populations in the exertion of beneficial traits.

The present work investigates the interactive effects of a lignocellulosic soil conditioner (SC), plant growth-promoting rhizobacteria (PGPRs; *Bacillus amyloliquefaciens* FZB42, ABiTEP GmbH, Berlin, Germany; *Pseudomonas jessenii*, Julius Kühn-Institut, Braunschweig, Germany) and arbuscular mycorrhizal fungi (AMF; *Rhizophagus irregularis* MUCL 41833; Université catholique de Louvain, Belgium) in wheat plants exposed to drought stress under greenhouse conditions. Results indicate that the SC not only may improve plant growth due to an increased water holding capacity, but also favorable effects on physico-chemical soil properties. This could provide favorable conditions for root colonization by PGPRs and AMF. In single and combined applications, the influence of these agents to improve root growth, nutrient acquisition, and stress resistance is investigated. Rifampicin resistant selectants of the PGPR strains enable their re-isolation from the rhizosphere to discover colonization patterns under varying conditions. As the rhizosphere competence of *Bacillus* may differ from that of *Pseudomonas*, it is expected that both strains show characteristic responses to the combination with SC and AMF. In turn, also the expression of mycorrhiza helper effects, reflected in improved mycorrhizal root colonization, could be specifically affected by combination with the different PGPR strains.

A better understanding of soil-plant-microbial interactions and specific responses in the population dynamics of rhizobacteria could help to develop improved product formulations and integrative application strategies to support the establishment of intended microbial populations in the rhizosphere and benefit from their multifaceted traits under field conditions.

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PP2-MA-06 Inoculation of endophytic bacteria stimulate the storage root development in sweet potatoes

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Sweet potato (*Ipomoea batatas*) is an important root crop for staple food throughout tropical and warm temperate regions. Because of its rich in carbohydrates, the storage roots of the sweet potato is also being used for the production of ethanol and bioplastics. The physiological process and environmental factors affecting storage root formation have been extensively studied. To date, however, the effects of endophytic bacteria on the storage root development have not been examined. In the previous study, we have isolated and characterized endophytic bacteria from surface-sterilized stems and storage roots of field-grown sweet potato. To evaluate the effects of colonization of endophytic bacteria on storage root development, the strain isolated from storage roots was inoculated to micropropagated sweet potato under the pot condition. The inoculated bacteria were colonized in shoots as well as in roots after being inoculated to the underground part of plants. Inoculation of endophytic bacteria resulted in a promotion of initial thickening growth of storage root after 42 days of inoculation. In contrast, there was no significant differences in the shoot growth and photosynthetic rate between endophyte-inoculated and control plants at this growth period. Increase in storage roots weight upon inoculation of endophytic strain was also recorded in later growth period. The results suggest that the isolated endophytic strain can stimulates the earlier thickening growth of storage root in sweet potato.

PP2-MA-07 Symbiont induced alleviation of tungsten stress in *glycine max* via increased levels of phenolic compounds, polyamines and amino acids

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The transition metal tungsten (W) shares certain chemical properties with the essential plant micro nutrient molybdenum (Mo), and is proposed to inhibit enzymatic activity of molybdoenzymes such as nitrate reductase, by replacing the Mo-ion bound to the molybdopterin co-factor (Mo-MPT). In contrast to nitrate reductase, some studies suggest that the nitrogenase of symbiotically living rhizobacteria, with its primary FeMo co-factor, is able to retain its functionality and that N₂ fixation is indirectly inhibited by a decreased nitrogenase synthesis due to W induced changes in nodule oxygen levels. Additionally, it has been shown that roots and nodules of symbiotically grown leguminous plants exhibit higher levels of proteins involved in hormone and flavonoid biosynthesis in the presence of high concentrations of W. The aim of this study was to clarify if rhizobia symbiosis actually results in an increase in secondary metabolites during tungsten stress and if such a symbiotically induced increase is able to protect nitrogenase from oxidative damage and thus affects the plant's tolerance to tungsten.

Soybean plants inoculated with *Bradyrhizobium japonicum* (Nfix) and a non-symbiotic control supplied with nitrate (Nfed – 10 mM KNO₃) were grown in a semi hydroponic setup. After three weeks, when symbiosis was fully established, plants were exposed to 0.5 mM tungsten (Na₂WO₄) for two weeks and subsequently harvested for metabolomic and proteomic analysis. In order to investigate the tungsten specific response, a zero control as well as a molybdenum control (0.5 mM Na₂MoO₄) was realized.

Our study showed that symbiotically grown plants exhibit a stronger metabolic response compared to their non-symbiotic counterparts in presence of both molybdenum and tungsten. While both Nfix and Nfed treatments showed a decrease in biomass, symbiotically grown soy beans could retain shoot growth and nodule mass/count when exposed to tungsten. We found an increase in phenolic compounds, flavonoids and soluble sugars in Nfix roots and leaves exposed to tungsten which resulted in a higher antioxidant capacity in comparison to Nfed plants. Furthermore, we could show an increase in organic acids, polyamines (i.e. putrescine, spermidine) and amino acids (i.e. Proline, Alanine) in Nfix plants in response to 0.5 mM W. Our results strongly indicate a symbiont induced alleviation of tungsten stress via enhanced radical-scavenging, metal-chelating and osmo-protective capacity.

PP2-MA-08 Long-term effects of nutrient regimes on soil microbiome composition

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Soil microbes have the potential to improve uptake of nutrients by plants. Using such microbiomes could increase the sustainability of agriculture. It seems probably that low input of a certain mineral can enrich for microbes that facilitate. Whether the nutrient composition affects the microbiome has only been studied in short term experiment. However, long term effects of different nutrient conditions on soil microbiome are hardly studied. To study the long term effects of low input we make use of experimental field 'Marwijksoord' in the Netherlands. This field (6 plots, 10 blocks per plot) was started in 1956 and aimed to study the effects on plant performance when one specific mineral was omitted from the fertilizer, for example P, N or K. In addition also the effect of pH (Ca) and salinity (NaCl) is studied. In this study, soil samples were collected from 3 plots in 2 successive years. On these plots different crops were grown which allows to distinguish between the effect of crop and nutrient regime on the microbiome composition. By using meta-amplicon (16s rDNA V4 region) sequencing approach, soil bacterial microbiome were characterized. PERMANOVA shows that long term nutrient regime plays a major role (36.4%, p <0.001) in shaping soil bacterial compositions, causes of location (7.3%, p <0.001) and time (5.6%, p <0.001) were minor. Both core and tailored bacterial microbiome of each nutrient regimes have been determined. Representative strains of these microbiomes will be isolated and their function in facilitating nutrient uptake by plants will be studied.

PP2-MA-09 Understanding the influence of abiotic stress on *in planta* expression of rhizobacterial genes associated with plant growth promotion

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Plant-beneficial microbe interaction studies are of tremendous importance for sustainable and higher crop yield. Molecular interactions between microbes and their plant hosts can be mutual or pathogenic. The mutual interactions are often affected by environmental variables and competitiveness reducing crop yield. Though many PGPR's have been commercialized since from years for enhancing crop yields, problem of **inconsistent** performance and viability of PGPR applied in field conditions is of a major concern. And most research on understanding **plant-microbe interactions** mainly focuses on plant counterpart rather than the bacterial gene expressions. Therefore, a better understanding of environmental influence on ***in planta* expression of bacterial genes associated with PGPR traits** is needed. Unravelling the special bond between the two symbionts at molecular level can help in **designing new strategies** for promoting interactions beneficial to plant host and constituting **bacterial consortia** with essential PGPR traits **unaffected** by various abiotic stress. Few **rhizospheric *Pseudomonas* strains** were tested for their MTC against seven heavy metals. Based on the MTC, heavy metals like **Copper, Nickel, Zinc and Cadmium** were selected. Two model plants ***Vigna radiata* and *Triticum aestivum*** to study the difference in PGPR gene expression under the influence of similar and different metal stress were used. Early interaction factors like **biofilm formation and EPS secretion** under metal stress were quantified. Differential response in phenotype and gene expression of **PGPR traits *in vitro*** under metal stress was explored using **qPCR**. *in vitro* results suggested an increase in few biocontrol related genes at increased concentration and therefore further in order to mimic real conditions, ***in planta* colonized bacterial gene** expression was studied by recovering the bacteria from rhizosphere amended with low and high concentrations of different metals in hydroponics system. **Localization and competitiveness** to colonize under different metal stress was evaluated by **GFP and RFP** plasmid tagging followed by visualization using **Confocal laser scanning microscopy**. **Stress alleviation and gene expression** difference of colonized bacteria in gnotobiotic soil was also assessed. Based on the ***in planta* expression** patterns, selection of traits which were **constitutively** or less unaffected by metal stress can be selected for developing a **consortia** for better growth promotion in **metal contaminated** region

PP2-MA-10 Environment–endophyte–plant crosstalk. How does the environment affect the plant microbiome and how does this affect plant adaptation to the environment?

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Endophytic fungi are cryptic microorganisms, inhabiting internal plant tissues of most, if not all, plant species. The endophytic community structure was suggested to be the resultant of environment and the host genotype. In recent years, the role of these symbiotic fungi in plant toxic metal (TM) stress tolerance has been gaining attention worldwide. However, our knowledge of diversity of endophytic fungi and their role in plant adaptation to the environment is still limited. We hypothesized, that the environment affects the plants endophytic community structure and this in turn has a significant impact on the plants adaptation potential.

We have compared the endophytic mycobiota composition of three *Arabidopsis arenosa* populations – one from the environment polluted with TM of anthropogenic origin, one from the environment, where soils are naturally enriched with toxic metals (serpentine soils) and one collected from reference (unpolluted) environment. Community structure evaluated by metagenomics and by isolation of cultivable endophytic strains was distinct for each of examined populations. The role of endophytic fungi in plant adaptation to metal toxicity are being investigated. We have found, that endophytic fungi isolated from *A. arenosa* population were able to increase significantly plant tolerance to multi-metal toxicity, promote plant growth and affect plant root architecture.

PP2-MA-11 Altering plant microbiome for flavour and nutrition

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It is well known that endophytic and rhizospheric bacteria can stimulate plant growth and resilience through many mechanisms, but their effects on secondary metabolites that impact taste, flavour and aroma remain understudied. Also, for centuries, plant breeders have been improving plant productivity in soil-grown plants - seeds from these plants are increasingly used in soil-less systems such as hydroponics and aquaponics, where they are deprived of their soil microbes' partners. This may lead to plants with depauperate microbiomes, and consequently, producing plants lacking in taste and flavour. Our goal was to investigate the value of inoculating plants in soil-less systems with specific microbes having the genetic potential to improve organoleptic properties. We have a large collection of bacterial endophytes isolated from native herbaceous plants growing well in crude oil-soaked soils in Oil Springs, Ontario, Canada. In this collection, we found abundant microbes with plant growth promotion potential. We tested most of these in *Arabidopsis*, basil, lettuce, bok-choy, and mini bell-pepper plants, and the more efficient were selected after comparison of inoculated and non-inoculated plants. The selected genomes were sequenced in Illumina MiSeq platform and screened for plant growth promoting genes, metabolic pathways related to flavour (carotenoids, polyphenols, and flavonoids). Chosen strains were grown hydroponically with basil, lettuce and mini bell peppers – and evaluated for chlorophyll concentrations, shoot and root biomass, flower and fruit output, vitamin and protein content, and flavour (through a sensory panel and e-nose/ GC-MS). Strain-specific primers were used to evaluate the success of inocula establishment as endophytes in the plant tissue and the effect of inoculum success on the variation in the plant growth responses. Inoculations with single strains were compared with strain mixes designed to provide multiple benefits. Among the selected endophytes, *Plantibacter flavus* strains, *Curtobacterium herbarum*, *Paenibacillus taichungensis*, *Rhizobium selenitireducens*, and *Methylobacterium aerolatum* were found to improve at least one of the evaluated features. We provide details on our results and genetic analyses of the most successful strains.

PP2-MA-12 Microbe mediated nutrient and abiotic stress management in sustainable agriculture

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Root-colonizing non-pathogenic bacteria can improve nutrient uptake by plants and increase plant resistance to abiotic stress factors. Bacteria endowed with plant growth promoting traits as nitrogen fixers, phosphate solubilizers, siderophore and IAA producers have been applied as "Biofertilizers". Some of these PGPR also have shown to increase tolerance against abiotic stresses such as drought, salinity and metal toxicity. Systematic identification of bacterial strains providing cross-protection against multiple stressors would be highly valuable for agricultural production in changing environmental conditions.

We screened large number of isolates for their osmotolerance and ACC deaminase producing ability. Selected isolates, were evaluated for ameliorating moisture deficit stress in soybean and wheat under pot conditions. Bacterial 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity reduced 'stress ethylene' levels within the inoculated plant compared to non-inoculated controls, with improved recovery from water deficiency. An enhanced physiological response was observed in plants. Bacteria mediated plant gene expression studies showed an upregulation of drought-responsive genes such as MRB and WRKY transcription factors. The study indicates the potential of root colonizing bacteria in helping the plant cope with the stress and improve its growth. Such bioinoculants can play an increasingly important role in climate resilient agricultural production systems.

PP2-MA-13 Plant growth-promoting *Pseudomonas pseudoalcaligenes* KB-10 promotes growth and improves salt stress tolerance in coriander plants

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Application of plant growth-promoting bacteria (PGPB) is a promising, feasible and environmentally benign strategy to improve salt stress tolerance in plants. In this study, 25 potential PGPB strains were isolated from the rhizosphere of alfalfa plants growing in salt-affected soils in Jeddah, Saudi Arabia, and tested for their ability to produce indole-3-acetic acid and 1-aminocyclopropane-1-carboxylate deaminase, and to solubilize tricalcium phosphate. Based on phenotypic, biochemical and 16S rRNA gene phylogeny, two promising isolates were tentatively identified as *Pseudomonas pseudoalcaligenes* (KB-10) and *P. putida* (KB-25). A pot experiment was carried out to assess the efficacy of KB-10 and KB-25 treatments in ameliorating salt stress in coriander plants. Coriander plants treated with PGPB strains had significantly higher relative water content, photosynthetic pigment concentrations, peroxidase activity, total biomass, salt tolerance index, and lower salt-induced total phenolic concentration. Strain KB-10 displayed better performance in terms of the aforementioned parameters. Therefore, the genome of KB-10 was sequenced using the HiSeq4000 platform which generated a total of 28 contigs after assembly. The draft genome of strain KB-10 contains 5,241,174 bp with 4921 predicted genes, including 65 tRNA, one tmRNA, and three rRNA genes. This is the first report of a draft genome sequence for a plant growth-promoting *Pseudomonas pseudoalcaligenes*.

Keywords: salt stress tolerance; plant growth-promoting bacteria; *Pseudomonas pseudoalcaligenes*; phytohormones; coriander.

PP2-MA-14 *Bacillus thuringiensis* NEB17 characterization and bacteriocin thuricin 17 as an effective biostimulant on corn seed germination

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Numerous commercial applications of inoculum based beneficial microorganisms are in use worldwide. *Bacillus* strains in particular are preferred as inoculants for their sporulation capabilities and hence are more viable during long term storage. *Bacillus thuringiensis* NEB17 is a soybean root endophyte isolate studied for its potential as plant growth stimulant. It produces a bacteriocin thuricin17 (Th17) which has been found to have a profound effect on corn seed germination. During seed germination, the dehydrogenase activity at 24h, reducing sugars and amylase activity at 72h was significantly higher in Th17 treated corn seeds. Protein profile of Th17 treated seeds at 48h of germination showed up-regulation of aldehyde dehydrogenase, G-quadruplex binding nucleoside diphosphate kinase, cytochrome P450 monooxygenase, auxin binding protein, phosphoenolpyruvate carboxylase, cytosolic orthophosphate dikinase and amylose extender starch branching enzyme.

Apart from the bacteriocin Th17, the cell-free supernatant hormone profile of the bacterium contains high levels of IAA and iP (Cytokinin – isopentenyladenine). The bacterium is also tolerant to NaCl levels up to 700 mM and continues to grow up to 900 mM NaCl with the growth slowing down after 700 mM NaCl. However, as the levels of salt increases, the production of Th17 decreases and the bacterium altogether stops the production of Th17 at 500 mM NaCl. At various salt levels, the likelihood of the bacterium producing various other interesting bio-actives is highly likely and of interest to crop production under abiotic stress.

PP2-MA-15 Long-term subcultured arbuscular mycorrhizal fungal inoculation improves red pepper plant growth and soil glomalin content

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Arbuscular mycorrhizal fungi (AMF) is well known for their ability to improve plant growth and protect plants from harsh environmental conditions. As AMF is an obligate biotroph, storage without losing its efficiency is difficult. This study aimed to analyze the efficiency of long-term subcultured AMF cultures on red pepper plant growth and soil glomalin content. Five AMF cultures propagated and regularly subcultured (*Claroideoglossum etunicatum*, *Rhizophagus* sp., *Funneliformis mosseae*, *Gigaspora margarita* and *Claroideoglossum lamellosum*) for over two years in trap cultures were chosen. Red pepper seedlings were inoculated with different AMF cultures in pots containing natural grass land soil. After 70 days of transplantation, pepper plants were harvested and dry weight, number of fruits, nutrient content and mycorrhizal parameters were determined. Nutrient uptake by pepper plants were analyzed using Kjeldahl and ICP-OES. Mycorrhizal colonization was checked using trypan blue staining method. All mycorrhizal plants showed higher shoot, root length and number of leaves however, the difference was not statistically high. Whereas significantly higher number of fruits were observed in all mycorrhizal plants. Although higher shoot and root dry weights were observed in mycorrhizal plants, they were not statistically different compared to non-mycorrhizal plants. Pepper plants inoculated with *C. etunicatum* and *C. lamellosum* significantly improved fruit dry weight compared to non-mycorrhizal plants by 324% and 352%, respectively. AMFC. *etunicatum* and *Rhizophagus* sp. showed highest colonization efficiency compared to other three mycorrhizal plants. AMFC. *etunicatum* showed highest arbuscules abundance in the whole root system compared to other mycorrhizal treatments. Significantly highest P uptake was observed in plants inoculated with *G. margarita*. Similarly, significantly highest glomalin related soil protein content was observed for *G. margarita*. In conclusion, the use of AMF spores that have been subcultured for an extended period of time can improve plant growth and increase glomalin related soil protein content.

PP2-MA-16 Synthetic bacterial communities of *Indigofera argentea* root microbiome promote plant growth under saline conditions

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Soil salinization negatively affects agriculture around the world. There is increasing evidence that plant tolerance to salt stress is related not only to plant genotype, but also to the root microbiome. Therefore, these microbiomes have the potential to be used to confer salt tolerance to crops. A microbe-based approach has a great potential for agriculture, because it provides a dynamic response to stress and has the potential to be used with a variety of current elite cultivars. However, such microbes fail when introduced as single strains due to competition from the local soil microbiome. It has been proposed that synthetic microbial communities (SynComs) have a much higher chance to be successful within an agricultural setting. In this study, we are going to create robust SynComs with minimal complexity. To do so, we will explore a recently obtained microbiome from the former agricultural fields in the Jizan desert of Saudi Arabia which are scarcely populated with only a single species, the legume *Indigofera argentea*. By using Meta-amplicon (16S rDNA V4 region) sequencing approach, bacterial composition of *I. argentea* was characterized including local soil, rhizosphere and root endophytic compartment. Bacteria were isolated by using selective media and further characterized with various molecular tools, like BOX-PCR, 16S rDNA and housekeeping genes sequencing. Strains belonging to dominant taxa were obtained. Effects of these bacterial strains on growth of native host plant (*I. argentea*) and tomato (MoneyMaker) were tested individually as well as in SynComs under saline conditions.

PP2-MA-17 Simulating global environments, abiotic plant stress conditions and high-throughput plant phenotyping at the Vienna BioCenter (VBC)

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The VBCF Plant Science Facility (Plants) is one out of eleven scientific core facilities forming the Vienna BioCenter Core Facilities GmbH (VBCF). It is a publicly funded non-profit research institute, situated at the Vienna BioCenter (VBC). While servicing in-house plant research, we also offer access to our infrastructure and scientific services to external collaborators.

The VBCF Plant Sciences Facility operates 22 state-of-the-art plant growth chambers (phytotrons) providing highly adjustable environmental conditions i.e. low temperature (-15°C), high temperature (up to 50°C), water logging, different light intensities and spectra (LED: blue₄₀₅, blue₄₅₀, white and red_{660 & 730}) and different gas conditions (e.g. CO₂).

One of these phytotrons hosts a robotic sensor-to-plant camera system for high-throughput plant phenotyping screenings. Phenotyping can therefore be combined with precise environmental simulations across different climate zones and various abiotic stress conditions. Very recently the phenotyping chamber was upgraded with adjustable high-tech LED illumination improving and extending the phenotyping service by high-light stress and adjustable spectral conditions.

Subsequent image analysis runs on LemnaTec OS software, allowing reproducible high-throughput screenings. The software also facilitates analysis of customized phenotyping experiments i.a. (side-view) phenotyping of crop plants, screening of seedlings (agar-plates), root phenotyping, phenotyping of seeds, duckweed and confocal microscopy image analysis.

In 2021, a state-of-the-art, multi-sensor, high-throughput plant phenotyping platform (PHENOPlant, FFG) will go into service facilitating top- and side-view phenotyping of *Arabidopsis* but also crop plants. Sensors will include chlorophyll fluorescence imaging, 3D RGB imaging, thermal imaging and hyperspectral imaging.

To unite the Austrian plant phenotyping community, we have established in 2017 the Austrian Plant Phenotyping Network (APPN.at). This initiative is actively supporting the start phase and future operation of the ESFRI EMPHASIS project.

PP2-MA-18 *Trichoderma harzianum* alleviates 2,4-dichlorophenoxyacetic acid induced oxidative stress in wheat

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2,4-phenoxyacetic acid (2,4-D) is a popular herbicide applied against broad leaved weed species in cereals. However, its application could affect wheat growth. Since 2,4-D is a membrane-active molecule, the interactions of the herbicide with lipids may play an important role in its toxicity mechanisms. On the other hand, it is well known that membrane lipids and lipid derived molecules (e. g. oxylipins - oxidized fatty acids) play a crucial role in the response of plant cells to different kinds of stress including abiotic and biotic factors. The goals of this study were to evaluate the ability of *T. harzianum* to improve the germination and seedling ability in (i) wheat seeds, (ii) seeds stressed with 2,4-D and to determine whether the fungus presence may contribute to lipid peroxidation. To detect any alterations, liquid chromatography (LC) coupled to tandem mass spectrometry (MS/MS) methods were used for bioactive lipid profiling. In the seedlings exposed to the herbicide, increased lipid peroxidation, elevated levels of oxylipins and inhibition of growth were observed. Concurrently, in the seedlings inoculated with *T. harzianum*, growth was stimulated. Interestingly, in wheat seedlings treated with 2,4-D and *T. harzianum*, the level of lipid peroxidation was similar to that in the control and there was no increase observed in oxylipins. In conclusion, it can be said that *T. harzianum* might partly alleviate the toxic effect of 2,4-D on wheat seedlings and could be used for improving wheat germination in the herbicide presence.

Acknowledgment

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PP2-MA-19 Boosting plant performance with bacterial endophytes

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Diverse populations of bacteria live within plant tissues without causing disease. A number of these bacterial endophytes have been demonstrated to promote plant growth and development, and increase host plant resilience to multiple abiotic and biotic stresses.

Nutrients and water are key resources limiting world agricultural production. Nitrogen fertilizers have enhanced crop yields at huge environmental cost, whereas drought has traditionally been managed by irrigation, resulting in ground water depletion. Across the world, many farmers cannot afford to use fertilisers, and in developed countries, perennial energy crops such as *Miscanthus* are largely undomesticated and must produce high annual biomass yields on low-quality land without environmentally costly inputs such as water, fertiliser or pesticides. Developing tolerant plant genotypes involves lengthy and costly plant breeding programs; low-input sustainable alternatives are urgently needed for sustainable agriculture applications.

The Farrar lab has cultured and identified bacterial endophytes from plants growing under abiotic stresses such as salinity and heavy metal contamination, as well as from *Miscanthus* tissues. We have compared the capacity of novel bacterial endophytes to improve plant growth under saline, and limited water and nitrogen conditions, using the model plant *Brachypodium distachyon* in the National Plant Phenomics Centre, Aberystwyth, UK. We have identified plant growth promoting (PGP) and stress-ameliorating strains, and multi-omics analyses are underway to determine the plant and bacterial factors involved in the beneficial plant-endophyte interaction. We aim to apply novel PGP endophyte strains harbouring beneficial traits to improve plant performance under biotic and abiotic stress conditions, including energy crop production and phytoremediation applications.

PP2-MA-20 Hormones of the Holobiont – Technologies for Improved Crop Growth and Climate Change Resilience

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Plants and their associated phytomicrobiome have co-evolved for, on the order of, half a billion years. They assist each other and, as part of this, regulate aspects of each other's gene expression, metabolisms and physiology. The plant plus its associated phytomicrobiome constitute the holobiont, the entity that evolution acts on and that produces biomass and crop yield. We have worked extensively with two microbe-to-plant signal compounds, lipo-chitooligosaccharides (LCO) and the small protein thuricin 17; both greatly improve plant growth and stress tolerance. For instance, thuricin 17 and LCO both increased soybean seed germination rate. Both enhanced the growth of *Arabidopsis* under both optimal and stressful (drought and salt) growing conditions and, at 24 h after treatment, these compounds caused meaningful changes in the levels of phytohormones, and increases in the levels of key proteins (such as those involved in the photosystems and the light harvesting complex). For soybean these signal compounds increased growth, again causing important changes in levels of phytohormones, key proteins and also in specific lipids. Tomato plants treated with these signal compounds had grown better at a stressfully low temperature (15 °C) and had clear changes in root structure. These microbe-to-plant signals are effective at very low concentrations and so constitute hormones of the holobiont. They cause plants, and thus crop production systems, to be more resistant to abiotic stresses, including some of those associated with climate change. The deployment of these signal compounds as low-input, sustainable, biostimulants for use in crop production has the potential to result in more climate change resilient global agriculture.

PP2-MA-21 Improved salinity tolerance on agricultural grasses inoculated with *Diaporthe* endophytes from the halophyte *Festuca rubra pruinosa*

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Festuca rubra subsp. *pruinosa* is a halophytic perennial grass that grows in cliffs of the Atlantic coasts of Europe. An assemblage of 135 different fungal taxa was identified in the roots of this plant species. Seven of these taxa occurring in more than 20% of the plants seemed to belong to the core microbiome of *F. rubra pruinosa*. A *Diaporthe* species was one of these taxa that. Our objective was to test if inoculation with endophytic *Diaporthe* strains could improve the performance under salinity of two important crops: the cereal tritordeum (X *Tritordeum* Acscherson et Graebner) and perennial ryegrass (*Lolium perenne*).

Greenhouse experiments (8 weeks) were carried with tritordeum and perennial ryegrass plants inoculated with *Diaporthe* EB4 strain growing plants under saline (200mM NaCl) and non-saline conditions (0mM NaCl). Inoculated plants of tritordeum increased their aerial biomass production with respect to uninoculated plants by 144% and 75% under saline and non-saline conditions, respectively. Similarly, ryegrass biomass increased by 171% and 70% under saline and non-saline treatments respectively. In addition, the root biomass of EB4- inoculated tritordeum increased by 28% and 55% and under saline and non-saline conditions, with respect to non-inoculated controls. In both plant species the proline content increased in salt-treated plants, but the increase was much greater in plants inoculated with the EB4 strain. Expression of the gene PrP5CS2, involved in proline biosynthesis, was shown to increase in response to *Diaporthe* inoculation under saline conditions up to three times with respect to non-inoculated plant.

The fact that similar results were obtained for tritordeum and ryegrass suggests that *Diaporthe* endophytes are host generalists that could be used to improve the performance of agronomic grasses.

miCROPe 2019 - Microbe-assisted crop production opportunities, challenges & needs

Vienna, Austria, December 2 – 5, 2019

Poster Session 2: Disruptive approaches for engineering the phytobiome & microbial delivery

PP2-DA-01 Seed borne endophytes, from wild *Cicer* species; a potential tool to engineer the microbiome of domesticated chickpea

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Chickpea (*Cicer arietinum*) is the third-largest food legumes worldwide. Chickpea seeds are rich in proteins and nutrients and hence are considered a healthy vegan food. Like many other crops, adaptation and resistance of chickpea to biotic and a-biotic stresses is inferior compared with their related wild species. Therefore, transferring tolerance to environmental stresses from the wild to domesticated species can be achieved by breeding or genetic engineering techniques. However, certain desirable traits relay on plant-microbe interactions. Those interactions may be easily lost under intensive agriculture practices. The art of engineering the plant microbiome in order to re-establish beneficial interactions is a challenging task. Although aspects of improving root microbiome are intensively studied, an impressive breakthrough has not yet been documented.

Wild species of the genus *Cicer* are mostly native to the Near and Middle-East, Central Asia, and East Africa. In Israel, populations of wild *Cicer judaicum* grow in close proximity to domesticated chickpea, often just a few meters apart. In the current study, we describe and compare the composition of seed-borne endophytes of both domesticated and wild *Cicer* spp. populations across Israel. We demonstrate that endophytes, from wild plants, are good candidates for modifying the cultivated chickpea's microbiome, as survival and establishment barriers in related plant species are significantly reduced. The communities of seed-borne endophytes of *C. judaicum* are characterized by high overall diversity, but prevalent species are rare. Among prevalent populations are members of *Bacillus* (69%), *Burkholderia* (54%) and *Sphingomonas* (33%). In contrast, the diversity of seed-borne endophytes in domesticated cultivars of chickpea, have much lower and significantly differed in composition from those of *C. judaicum*, even when the seeds have been collected from neighboring environments. Isolation of endophytic bacteria from the wild and domesticated chickpea seeds yield similar results in terms of diversity and prevalence.

Bacillus isolates, representing the seed-borne populations of *C. judaicum*, show excellent survival rates¹ on seeds of domesticated chickpea and established successfully as endophytes in the root and stem tissue for at least three weeks after planting. In addition, this isolate significantly increased stem height and weight, thus, providing evidences for efficient engineering of chickpea microbiota.

PP2-DA-02 Stabilization of Metarhizium entomopathogen through encapsulation

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Many plant-beneficial microbes are documented in the plant microbiome literature, but relatively few have made it into widespread use in agriculture. Most fall at one or other of the many hurdles between initial proof-of-concept and translation from lab to field. Technical failure modes include feasibility and cost of production, formulation compatibility, and stability. Even if these obstacles are overcome, efficacy requires establishment in the environment with appropriate timing and dose. Entomopathogenic fungi of the genus *Metarhizium* have well-characterized efficacy against a variety of insects. We describe progress in stabilizing this organism for field delivery and control of corn rootworm, *Diabrotica* spp., major pests of corn production in North America and Europe.

PP2-DA-03 Elicitation of the hypersensitive response-like cell death on *Nicotiana benthamiana* by the marine bacterium *Hahella chejuensis*

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Soon-Kyeong Kwon^{1,2}, Jeong-Im Lee³, Boyoung Lee¹, Choong-Min Ryu³, and Jihyun F. Kim¹

¹Department of Systems Biology, Division of Life Sciences, and Institute for Life Science and Biotechnology, Yonsei University, 50 Yonsei-ro, Seodaemun-gu, Seoul 03722, Republic of Korea, ²Division of Life Science, Gyeongsang National University, 501 Jinju-daero, Jinju, Gyeongsangnam-do 52828, Republic of Korea, ³Korea Research Institute of Bioscience and Biotechnology (KRIBB), 125 Gwahak-ro, Yuseong-gu, Daejeon 34141, Republic of Korea Many bacterial pathogens of plants and animals employ type III secretion systems (TTSSs) to deliver effector proteins into the host cell. Among them, plant-pathogenic bacteria typically cause disease symptoms in the compatible hosts while elicit the programmed cell death referred to as the hypersensitive response (HR) in incompatible hosts. The genome of *Hahella chejuensis*, a bacterium isolated from the marine sediment in Jeju Island, Korea, contains two TTSS gene clusters (TTSS-1 and TTSS-2) that are similar to those in the virulence plasmids of the mammalian pathogen, *Yersinia* spp. Transcriptional expression of the two copies each of four TTSS representative genes of *H. chejuensis*, *hctC*, *hctN*, *hctO*, and *hctV* that are homologous to *yscC*, *yopN*, *yscO*, and *yscV* of *Yersinia* spp. respectively was assessed by reverse transcriptase-polymerase chain reaction (RT-PCR) method at different growth stages. Transcript levels of *hctC*, *hctO*, and *hctV* from TTSS-1 were higher than those of TTSS-2. To investigate the function of the predicted TTSSs in *H. chejuensis* *in vivo*, we infiltrated bacterial suspension of *H. chejuensis* on the elicited necrosis similar to that of a typical HR within 24 hours on the leaf of *Nicotiana benthamiana*, which was chosen as a plant model system. It was dependent on the bacterial growth stage; necrosis appeared when bacterial suspension at early stationary phase was infiltrated. A previous study reported that AvrPto1, a pseudomonad effector known for its ability to interact with the resistance protein in tomato, suppressed the HR on the incompatible host plant *N. benthamiana*. *H. chejuensis* containing *avrPto1* suppressed the HR elicitation in *N. benthamiana*. Taken together, these results suggest that the TTSSs of the marine bacterium *H. chejuensis* are functional and responsible for induction of the HR-like necrosis in *N. benthamiana*.

PP2-DA-04 Novel formulations for arbuscular mycorrhizal fungi products: the pathway to biological product adoption in the rhizosphere

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The depletion and degradation of arable soils around the world constitutes a significant threat to maintaining the sustainable food supply that is required to feed a rapidly increasing global population. While intensive agricultural practices have provided increased crop yields, they have also led to a significant increase in soil erosion, reduced water use efficiency, and a disruption to the soil microbiome, which is essential for optimal soil health and plant production. In response to this situation and with pressure from food chain stakeholders, both public and private sector entities are collaborating to conduct scientific research and development initiatives to employ soil microorganisms that naturally replenish and maintain healthy soils, such as arbuscular mycorrhizal fungi. These fungi form hyphae that produce a glycoprotein called glomalin that binds soil particles into stable aggregates, which mitigate soil erosion, improve water infiltration, and restore soil water holding capacity. We will discuss efforts by Valent BioSciences to commercialize arbuscular mycorrhizal fungi with novel, stable product formulations that afford application consistency, and can be easily integrated into current agricultural production practices.

Poster Presentations

Author Index

A

Abbo, Shahal.....	139
Abd El-Daim, Islam A.	136
Abdul-Wahab, Mohd Firdaus.....	89
Achbani, El Hassan.....	69
Adholeya, Alok.....	37
Ahmad, Muhammad.....	80, 104
Ahmed, Aneesh	70
Ait Lahmidi, Nassima	63
Akhtar, Saqib Saleem.....	128
Albrecht, Benjamin	75
Aletti, Consolée	68
Alexandre, Alexandre	59
Al-Garni, Saleh M. S.	133
Al-Ghamdi, Khalid.....	133
Almeida-Trapp, Marilia.....	34
Alonso, Ana Paula	112
Alvarez, J.C.....	56
Alzubaidy, Hanin	34
Ambrosino, Patrizia	63
Amy, Daniel Buchvaldt.....	128
Anand, Abhishek.....	116, 120
Andreozzi, Anna.....	37
Andres-Barrao, Cristina	34
Aneesh, Ahmed	129
Antar, Mohammed	64
Ante, Michael	110
Anteyi, Williams Oyifioda	52
Antonielli, Livio	44, 46, 51, 80
Antonio Lucas, José	79
Armanhi, Jaderson.....	41, 69
Armengol, Josep	114
Arruda, Paulo.....	41, 69, 98
Arsova, Borjana	67, 128
Artyukh, Olga.....	34
Arunachalam, Selvakumari.....	64
Assimopoulou, Andreana	80, 87
Astarita, Leandro	123, 125
Auer, Anneliese	135
Aundy, Kumar	124

B

Babin, Doreen.....	30
Backer, Rachel	103, 122
Bağcı, Caner	75
Bahieldin, Ahmed	133
Bailey, Karen.....	54
Baldassarre Švecová, Eva.....	32
Bannister, Stephanie	110
Bänziger, Irene.....	58
Bárnet, Martin	32
Barret, Matthieu.....	46
Barreto-Hernandez, Emiliano	119
Bárta, Jiří.....	107
Baudry, Jean	43
Beattie, Gwyn A.	82

Becker, Matthias	75
Bedini, Alberico.....	25
Bejarano, Ana.....	31
Belchior Rosendo da Silva, Marcelo	53
Bellaire, Anke	88, 92, 111
Bellastra, Patricia	42
Berg, Gabriele	50, 87, 116
Berger, Andreas	92
Berger, Nils.....	67
Berghage, Robert	81
Bernal Govaerts, Ana	127
Bernat, Przemysław	135
Bernhard Ballhausen, Max	65
Berta, Graziella.....	63
Berthelot, Charlotte	62
Bevivino, Annamaria	63
Bianco, Carmen.....	37
Bianco, Piero	118
Biazotti, Barbara	98
Bibette, Jérôme.....	43
Bilsborrow, Paul	62
Bindeus, Alexander	110
Bisseling, Ton	130, 134
Bitterlich, Michael	47, 62
Blaudez, Damien	62
Bodenhause, Natacha	79
Böhmendorfer, Stefan	120
Bolotin, Oleg	95
Bona, Elisa.....	63
Bonini, Paolo	43
Bonneau, Laurent.....	63
Bonvin, Jérémy.....	68
Boon, Nico.....	106
Bouamri, Rachid.....	69
Boussageon, Raphaël	85
Bradáčová, Klára	67
Brader, Günter	44, 66, 80
Braun-Kiewnick, Andrea.....	93
Breakfield, Natalie W.	82
Brecker, Lothar.....	92
Breeuwsma, Suzanne	121
Brenner, Martin	92
Brunori, Andrea.....	63
Bryant, Ray.....	81
Bubici, Giovanni	116, 125
Buchholz, Franziska.....	44
Budak, Aleksandru	95
Bui, Van Cuong.....	62
Bulone, Vincent.....	122
Bziuk, Nina	93, 117

C

Cabrera, Raimundo	81
Cahill, David	37
Caldara, Marina.....	63
Calvet, Cinta	43
Camprubi, Amelia	43
Carkner, Michelle K.	96
Carrion, Victor.....	76

Author Index

Cartabia, Annalisa	44
Casati, Paola	118
Casieri, Leonardo	85
Cassán, Fabricio	26
Castillo-Gonzalez, Humberto	112
Castro-Moretti, Fernanda R	112
Cellini, Antonio	102
Cernava, Tomislav.....	50, 116
Charles, Trevor	74
Charlton, Megan.....	136
Chatterjee, Subhadeep.....	112
Chaturvedi, Anurag.....	106
Chaverri, Priscila	112
Chebaane, Asma	69
Cheng, JiuJun	74
Cheng, Xu.....	130, 134
Chesneau, Guillaume.....	46
Chinchilla, Delphine	120
Chock, Mason	86
Chopra, Ankita	97
Chowdhury, Soumitra Paul.....	30
Chung, Bong-Nam.....	134
Cid, Geeisy A.....	47
Cirino, Veronica	43
Cocetta, Giacomo	118
Collinge, David B.	121, 123
Colombo, Elena Maria	119
Compant, Stéphane	51, 65
Coniglio, Anahí.....	26
Conlan, Xavier.....	37
Consortium, MicrobiomeSupport.....	111
Cooke, Fiona	136
Cookson, Alan.....	136
Cope-Selby, Naomi	136
Cordovez, Viviane	31, 76
Corella, Joaquim Cruz	106
Cortesi, Paolo.....	119
Cortesi, Paulo.....	38
Cosoveanu, Andreea.....	81
Cottinet, Denis.....	43
Courty, Pierre Emmanuel	63
Courty, Pierre-Emmanuel	85, 114
Croll, Daniel	29
Cruz-Corella, Joaquim	68
Cruz-Ramirez, Carlos.....	119
Csorba, Cintia.....	44, 80
Cusant, Loïc	85
Czajkowski, Robert	60, 115
Czerwicka, Małgorzata	86

D

D.G.M. Franco, Bernadette.....	53
Damasceno, Natalia.....	41
Daneshkhah, Roshanak.....	102
Darrasse, Armelle	46
Darriaud, Romain	42
Darrieutort, Guillaume	42
de Souza, Rafael	41, 69, 98
de Zelicourt, Axel	34
Declerck, Stephane	44
Declerck, Stéphane.....	25
Defez, Roberto.....	37
del Barrio Duque, Alejandro	51

Dewasme, Coralie	42
Dias Carvalho, José João	72
Dicke, Marcel	31, 54
Domka, Agnieszka	131
Doolette, Ashlea	45
Dörr de Quadros, Patricia	132
Drakopoulos, Dimitrios	58
Druille, Magdalena	96
Duan, Jin.....	133
DUBEY, SHUBHAM	66
Duiker, Sjoerd	81
Dunlap, Christopher	59
Dupuy, Lionel	92

E

Eida, Abdul Aziz	34
Eitle, Markus	39
El Hilali, Rania.....	69
Elhady, Ahmed	29
Entwistle, Rebecca	136
Entz, Martin H.	96
Erice, Gorka.....	43
Eversole, Kellye	82

F

Faist, Hanna	46
Falavigna, Vitor	123
Falter-Braun, Pascal	94
Farrar, Kerrie.....	136
Farré-Armengol, Gerard.....	35
Felton, Gary.....	53
Ferhout, Hicham	43
Fernandez Fuentes, Narcis.....	136
Ferrante, Antonio.....	118
Fimognari, Lorenzo	128
Finnegan, Patrick M.	133
Fki, Lotfi.....	69
Forges, Marine	85
Forneck, Astrid	39
Forrer, Hans-Rudolf.....	58
Fragner, Lena	88, 92, 111
Franchini, Martina.....	36, 80
Franken, Carolien	134
Franken, Philipp	25, 47, 62, 65
Frantík, Tomáš	32
Fray, Rupert	36, 80
Frenkel, Omer	139
Friman, Julia	54
Friman, Ville-Petri	52
Froussart, Emilie	38
Fulthorpe, Roberta.....	132

G

Gaber, Dalia	62
Gajewska, Ewa	135
Galambos, Nikoletta	98
Galindo-Castañeda, Tania	65
Gamliel, Abraham	139

Author Index

Ganeshan, Prakash	124
Gange, Alan	55
Garbeva, Paolina.....	28
García-Giraldo, Gisell.....	56
Garcia-Villaraco, Ana	127
García-Villaraco, Ana	79
Gasparetto, Chiara.....	31
Gattupalli, Archana.....	131
Gaube, Paul	35
Geisen, Stefan.....	34
Geistlinger, Jörg.....	30, 70
George, Timothy S.....	45
Geurts, Rene.....	130, 134
Ghim, Sa-Youl	93, 99, 101
Ghodsalavi, Behnoush	32
Giles, Courtney	45
Gimeno, Alejandro.....	58
Giovannetti, Giusto.....	63
Given, Cindy.....	88
Glick, Bernard R.	133
Goldstein, Arthur	43
Goormachtig, Sofie.....	38, 127
Gopal, Selvakumar.....	134
Gopalakrishnan, Subbaiyan	124
Göpel, Yvonne	110
Gorfer, Markus	44
Gosetti, Fabio	63
Govindasamy, Venkadasamy.....	124
Grabenweger, Giselher.....	51
Gray, Neil.....	62
Greiner, Ralf.....	45
Grigoriadou, Katerina	44, 87
Grimm, Maja.....	93, 99
Grosch, Rita	30, 110
Gruber, Sabine.....	118, 122
Grunert, Oliver.....	106
Gu, Yan	34
Gueli Alletti, Gianpiero	72
Guerin-Laguette, Alexis	101
Guilhabert, Magalie	82
Gutierrez Albanchez, Enrique	79
Gutierrez-Albanchez, Enrique.....	127
Gutierrez-Mañero, Fco.Javier	127
Gutierrez-Mañero, Javier.....	79

H

Haeuser-Hahn, Isolde	36
Hage-Ahmed, Karin.....	103
Hajirezaei, Mohammad-Reza	47
Hallahan, David.....	139
Hallasgo, Anna Marie.....	103
Hallmann, Johannes	29
Hanusch, Maximilian	95
Hao, Xiuli.....	32
Hartmann, Martin.....	65
Haselberger, Stefan	83
Hauschild, Rüdiger.....	72
Hauser, Constanze.....	103
Hauser, Thure Pavlo	114
Hawkins, Sarah	136
He, Xie	95
He, Yu-Xi	93, 99, 101
Hegarty, Matthew	136

Hegelund, Josefine Nymark	128
Heikkilä-Huhta, Kati	88
Heil, John.....	74
Helander, Marjo	30, 57, 96
Hernandez-Sanabria, Emma	106
Herpell, Johannes.....	88, 92, 111
Hess, Julia.....	79
Heuer, Holger.....	29
Hill, Phil	36, 80
Hinterdobler, Wolfgang	120
Hirt, Heribert.....	34
Ho Ching, Miko Poh.....	89
Hoffmann, Annika	107
Höglinger, Birgit	70
Holden, Nicola.....	92
Holtgräwe, Tobias	100
Hoover, Kelli.....	53
Hoppe, Christine	93
Horche, Ignacio	79
Horstmann, Juliana	125
Hosaguthi Mallesh, Rathan	129
Hossain, Mohammad Nabil.....	133
Howlader, Mithu	133
Hu, Jie.....	59
Huesgen, Pitter	67
Huisman, Huei Ming.....	121
Huson, Daniel Hamlet	75
Hwang, Ye-Ji.....	99

I

Imperial, Juan.....	41
Ionescu, Michael	74

J

Jablonowski, Nicolai D.....	106
Jabłońska, Magdalena.....	60, 86
Jacquioud, Samuel	30
Jafra, Sylwia	60, 86, 115
Jakubowska, Agata K.	72
Jalal, Rewaa.....	34
Jayanti, Hadis	117
Jechalke, Sven	110
Jędrzejczyk, Roman J.....	131
Jefferson, Richard	48
Jenny, Eveline.....	58
Jensen, Birgit	121, 123
Jez, Jakub	135
Johnson, Alexander	128
Johny, Leena	37
Jones, Asher	53
Jones, Catherine.....	92
Jones, E. Eirian	101
Jørgensen, Hans Jørgen L.	121, 123
Jousset, Alexandre	34, 52, 58
Junker, Robert R.....	44
Junker, Robert R.....	35, 83, 95

Author Index

K

Kabli, Saleh A.	133
Kaczyński, Zbigniew	86
Kägi, Andreas	58
Kamilova, Faina.....	72
Kandeler, Ellen.....	67
Kannepalli, Annapurna	132
Kanukollu, Saranya	47
Kao-Kniffin, Jenny	41
Kappel, Lisa	118, 122
Kehrli, Patrik	114
Keller, Alexander	35
Keller, Beat	58
Khan, Md Mohibul Alam.....	133
Kharchuk, Oleg	95
Khatri, Shivani.....	55
Kim, Jihyun F.....	140
Kim, Jihyun F.....	89
Kinany, Said	69
Kirilov, Aleksandru.....	95
Klink, Sophia M.	50
Klint, Johannes.....	94
Klueken, Michael	36
Knecht, Fionna.....	51
Knox, Paul.....	92
Kolb, Steffen	47
Koller, Robert.....	36
Koskella, Britt.....	86
Kostic, Tanja.....	111
Kostić, Tanja.....	44
Kovács, Gábor M.....	62
Kowalchuk, George.....	34, 58, 59
Kraushaar, Sabine.....	83
Kraxberger, Katharina.....	82
Kreßner, Susanne	62
Krigas, Nikos	44, 87
Krishnappa, Charishma	124
Kruh, Lilach Iasur	139
Krumwiede, Johannes	93, 99
Krychowiak, Marta	115
Krzyżanowska, Dorota M.	115
Krzyżanowska, Dorota M.	60
Krzyżanowska, Dorota Magdalena	86
Kuang, Weiqi	67
Kumar, Mukesh	124
Kunova, Andrea	119
Kurmann, Mario	42
Kuzntz, Galit.....	74
Kwak, Min-Jung	89
Kwon, Soon-Kyeong.....	89, 140

L

L'Haridon, Floriane	116, 120
Lalaymia, Ismahen.....	44
Lalzar, Maya.....	139
Lambert, Joshua	81
Langen, Gregor	100
Langendries, Sarah	38
Latz, Meike A.C.	121
Lauterbach, Desirée.....	93
Lauvergeat, Virginie.....	42

Leach, Jan.....	82
Lebeer, Sarah	108
Lee, Boyoung.....	89, 140
Lee, Jeong-Im	140
Lee, Soon-Jae	106
Lee, Soo-Yeong.....	93, 99, 101
Legein, Marie	108
Lehmann, Silke	94
Lehnen, Christoph	100
Lenka, Sangram.....	37
Lentz, Jonas.....	36
Lentzsch, Peter	107
Li, Guofen.....	120
Li, Mei	52
Li, Weimin	88
Lindow, Steven.....	23
Lingua, Guido	63
Liu, Fulai	128
López, Gastón	26
Lucas, Jose A.	127
Lucas-Barbosa, Dani	31
Lucic, Eva.....	25
Ludewig, Uwe.....	67, 70, 129
Luthe, Dawn	53
Lutz, Lisa.....	57
Lynch, Jonathan	65
Lynch, Michael	74
Lyu, Dongmei	103, 122

M

M, SenthilKumar	132
Macabuhay, Allene	128
Maciag, Tomasz	115
Maciąg, Tomasz	60
Mäder, Paul.....	46, 69
Maloupa, Eleni	44, 87
Mamun, Abdullah Al	129
Manavalan, Lakshmi P.....	36
Mannerucci, Francesco	116
Marchenko, Polina	35
Marcolefás, Evan.....	122
Marengo, Emilio.....	63
Mariappan, Kiruthiga	34
Marmioli, Nelson	63
Marsano, Francesco	63
Martin, Randy	140
Martins, Guilherme	42
Mary, Séverine	42
Masclaux, Frédéric	68, 106
Masneuf-Pomarède, Isabelle	42
Mason, Charles	53
Massa, Nadia.....	63
Mathew, Suni	30
Mathew, Suni Anie.....	88
Maurhofer, Monika.....	29
Mazumder, Pranab Behari	97
Mazzucco, Eleonora	63
Medema, Marnix	76
Meijer, Johan	94
Mercado-Blanco, Jesus	37
Mercy, Louis.....	25, 62
Messmer, Monika M.	42
Mironenka, Julia.....	135

Author Index

Mithoefer, Axel.....	34
Mitter, Birgit.....	44
Mliki, Ahmed.....	69
Moadi, Heba.....	139
Moelbak, Lars.....	128
Moisan, Kay.....	31
Molina, Romina.....	26
Molinaro, Antonio.....	100
Monaco, Stefano.....	37
Mora, Verónica.....	26
Moradtab, Narges.....	70
Moretto, Marco.....	36, 98
Moritz, Thomas.....	94
Moukarzel, Romy.....	101
Mrnka, Libor.....	32
Müller, Henry.....	50, 87
Müller, Marina.....	47, 107
Müller, Martin J.....	97
Müller, Renate.....	128
Müller, Thomas.....	107
Mundy, Dion C.....	101
Muola, Anne.....	57
Muscettola, Isabella.....	86
Mushineni, Ashajyothi.....	124
Muysshondt, Babette.....	108
Mysara, Mohamed.....	106

N

Nagel, Kerstin A.....	36
Naranjo, Henry.....	90
Narraidoo, Nathalie.....	36, 80
Nesler, Andrea.....	124
Nesme, Joseph.....	63, 110
Neuhäuser, Benjamin.....	67
Neuhoff, Daniel.....	63
Neumann, Günter.....	67, 70, 129
Nicksy, Jessica.....	96
Nicolaisen, Mette H.....	32, 45
Nicolay, Gian.....	69
Nievas, Sofía.....	26
Nissinen, Riitta.....	30, 88
Noceto, Pierre-Antoine.....	114
Nussbaumer, Thomas.....	94
Nybroe, Ole.....	32, 45
Nykiel-Szymarńska, Justyna.....	135

O

Oburger, Eva.....	130
Ohler, Lisa-Maria.....	83
Ohwaki, Yoshinari.....	129
Ojira, Ichie.....	99
Okazaki, Keiki.....	129
Olivieri, Leone.....	55
Olsson, Stefan.....	32
Omacini, Marina.....	96
Onofrash, Leonid.....	95
Opitz, Michael.....	102
Ordon, Frank.....	93, 117
Orozovic, Kanita.....	94
Ortolan, Francieli.....	125
Osborne, Rory.....	94

Osman, Mohamed.....	97
Otto, Jan-Christoph.....	83
Ouzine, Mohamed.....	69
Oyserman, Ben.....	76

P

Page, Pierre.....	64
Pal, Vanessa.....	135
Pallucchini, Michele.....	36, 80
Papageorgiou, Vassilis.....	87
Parich, Alexandra.....	124
Pariyar, Shree R.....	36
Park, Hyein.....	89
Pasquali, Matias.....	119
Passato, Stefania.....	63
Passera, Alessandro.....	118
Passovic, Mirza.....	133
Pastore, Chiara.....	102
Patz, Sascha.....	75
Paul, Sangeeta.....	132
Pauwels, Richard.....	47, 62
Pavagadhi, Shruti.....	89
Pedraza-Herrera, Luz Adriana.....	119
Peiffer, Michelle.....	53
Peña-Quemba, Diego Camilo.....	68
Perazzolli, Michele.....	36, 80, 98, 124
Pereira, Eric.....	137
Persyn, Antoine.....	127
Pertot, Ilaria.....	31, 98
Peters, Carola.....	75
Petrásková, Hana.....	107
Pflugfelder, Daniel.....	36
Phytobiomes Alliance, The.....	82
Piazza, Laura.....	118
Pierre, Hohmann.....	42
Pieter, Monsieurs.....	106
Pihlanto, Anne.....	63
Pijnenburg, Rick.....	134
Pineda, Ana.....	54
Pires de Paula, Caio Cesar.....	107
Pittroff, Sabrina M.....	45
Pizzatti, Cristina.....	119
Pohl, Karolin.....	93, 117
Pommier, Thomas.....	52
Posada, Luisa F.....	56
Posada-Vergara, Catalina.....	117
Prasad Sahu, Kuleshwar.....	124
Preiner, Julian.....	130
Prieto, Pilar.....	37
Prigigallo, Maria Isabella.....	116, 125
Puopolo, Gerardo.....	31, 124

R

Raaijmakers, Jos M.....	31, 76
Radl, Kathrina.....	110
Rahi, Praveen.....	97
Rahman, Unjhila.....	133
Rainio, Miia.....	30
Rajashekara, Hosahatti.....	124
Ramos-solano, Beatriz.....	35
Ramos-Solano, Beatriz.....	79, 127

Author Index

Ranca, Aurora	114	Schedl, Andreas.....	47
Rasche, Frank.....	52	Scheiner, Ricarda	35
Rat, Angélique	87	Schierstaedt, Jasper	110
Ravanbakhsh, Mohammadhossein.....	58	Schikora, Adam	38, 93, 99, 110, 117
Ray, Swayamjit	53	Schikora, Marek	99
Raynes, Gareth	136	Schillaci, Martino	67
Reddy, Khedar	132	Schindler, Florian	88, 92, 111
Regan, John	81	Schinnerl, Johann.....	92
Reheul, Dirk	106	Schlaepfi, Klaus	45, 79
Reinbacher, Lara.....	51	Schlüter, Andreas.....	63
Reisinger, Manuel.....	87	Schmidt, Christoph Stephan.....	32
Rempelos, Leonidas.....	62	Schmitz, Lucas.....	134
Revers, Luis Fernando.....	123	Schmoll, Monika.....	120
Richardson, Alan E.....	45	Schneider, Carolin	25, 62
Ridgway, Hayley J.	101	Schneijderberg, Martinus.....	134
Robbins, Chanz	68, 106	Scholda, Julia.....	120
Roberts, Jemma.....	94	Schrey, Silvia	106
Robinson Boyer, Louisa	115	Schuhmacher, Rainer	124
Robles-Aguilar, Ana A.	106	Schweizer, Christian	51
Robotti, Elisa.....	63	Sczyrba, Alex	63
Rödel, Philipp.....	25, 104	Sebring, Ryan	81
Rodić, Nebojsa.....	80	Seeliger, Mirjam	62
Rodriguez Sabina, Samuel	81	Sehr, Eva M.	80
Rodriguez, Alia.....	68	Sehr, Eva Maria	104
Rodriguez, Belén.....	26	Seiler, Réjane	68
Rodriguez-Estrada, Maria T.	102	Seitner, Sebastian	135
Roessner, Ute	67, 128	Seitz, Alexander	110
Rohmann, Philipp	94	Sessitsch, Angeala	57
Roitsch, Thomas Georg.....	128	Sessitsch, Angela.....	44, 46, 51, 80, 82, 104, 111
Rojas, Edward C.	123	Shade, Ashley.....	46
Romano, Silvia	37	Sharif, Lisa	129
Rosikiewicz, Paweł.....	68	Shariyari, Hassan.....	62
Rossi, Vittorio	114	Sharma, Meenakshi	132
Rothballer, Michael	30, 50	SHARMA, SHILPI.....	66
Rotter, Robert.....	26	Sharma, Shubhangi	62, 65
Roux, Christophe	85	Shazhad, Gul-I-Rayna	118
Różalska, Sylwia.....	135	Shen, Qirong	34, 52
Rozpądek, Piotr	131	Shikano, Ikei.....	53
Ruiz, Victoria.....	95	Shrestha, Abhishek	99
Ruppel, Silke	47, 65, 75	Sicher, Carmela	98
Ryan, Matthew J.	82	Silipo, Alba	100
Rybakova, Daria	56	Singh, Prashantee	112
Ryu, Choong-Min.....	140	Singh, Pushplata.....	37
<hr/>			
S		Širca, Saša	114
Saad, Maged	34	Sirová, Dagmara	107
Saikkonen, Kari	30, 57, 85, 96	Sittinger, Maximilian	67
Saloniemi, Irma.....	30, 96	Siwinska, Joanna	115
Samad, Abdul.....	51	Skucha, Anna.....	110
Samokhvalov, Nikolai.....	95	Staba, Mirosława.....	135
Samson, Roeland	108	Smalla, Kornelia	30, 93, 110, 117
Sanders, Ian	68	Smets, Wenke	108
Sanders, Ian R.	106	Smith, Don	122
Sangiorgio, Daniela	102	Smith, Donald.....	64, 103, 133, 136
Sanow, Stefan	67	Smolka, Anna	135
Santarém, Eliane.....	123, 125	Sommermann, Loreen	30
Saracchi, Marco	119	Son, Jin-Soo.....	93, 99, 101
Sartor, Tiago	123	Song, Ju Yeon	89
Satpute, Surekha	97	Song, Yuqi	34
Sauert, Martina.....	110	Sørensen, Soren	63
Savioli, Stefano	102	Sørensen, Søren J.	30, 110
Saxena, Gourvindu	89	Spinelli, Francesco.....	102
Schäfer, Patrick.....	94	Srivastava, Divya	37
		Stalder, Luzia.....	29
		Staniczenko, Phillip	112
		Steccari, Irene	130

Author Index

Steinkellner, Siegrid	102, 103
Stewart, Ethan	135
Stigloher, Christian	97
Stocker, Franz	116
Strauß, Sina.....	35
Streminska, Marta	121
Studer, Bruno	42
Suazo, Gerardo	100
Subramanian, Sowmyalakshmi	133, 136
Sun, Xiaoliang	88, 92
Sutton, John C.....	100
Swarup, Sanjay	89
Symanczik, Sarah	46, 69

T

Tabacchioni, Silvia	63
Tchuisseu Tchakounte, Gylaine Vanissa	75
Tedford, Sherri.....	100
Temperton, Vicky M.	106
Tenbohlen, Tim.....	129
Tenhaken, Raimund.....	35
Terakado-Tonooka, Junko	129
Theppan, Dhivya.....	132
Thomas, Juliya	131
Thomas, Varghese	36
Tietz, Katharina.....	67
Tischler, Sonja.....	92
Tiwari, Subhangini	131
Toderash, Vasili	95
Todeschini, Valeria	63
Torres-Cortes, Gloria	46
Trognitz, Friederike.....	46, 57
Tsopmo, Appolinaire	132
Turnau, Katarzyna.....	131
Turska, Agata.....	60
Tyagi, Swati.....	132

U

Uddin, Shahab	129
Ulrich, Andreas	47
Uribe-Vélez, Daniel	119
Usero, Francisco Martin	64
Uyttendaele, Mieke	53

V

V, Govindasamy.....	132
Vacchini, Violetta.....	118
Valè, Gianpiero	37
van Dam, Nicole M.	47
van der Heijden, Marcel	79
Van der Linden, Inge.....	53
van der Salm, Caroline.....	121
van Labeke, Marie-Christine.....	106
van Loon, Joop J.A.	54
van Tuinen, Diederik.....	85, 114
Vandekerckhove, Tom G. L.....	106
Vandenabeele, Steven	25
Varela Alonso, Alicia	25, 104

Vasan, Shalini K.	37
Vazquez-de-Aldana, Beatriz R.	137
Venail, Julien	94
Viaene, Tom	38
Vidal, Stefan	117
Villegas-Escobar, Valeska	56
Vincent, Bryan.....	44
Vlaeminck, Siegfried E.....	106
Vlassi, Anthi.....	124
Vogelgsang, Susanne.....	58
Vosátka, Miroslav.....	32
Vrhovsek, Urska	39
Vrieze, Mout De	120

W

Walch, Magdalena	39
Walker, Frank.....	70
Walker, Robert.....	128
Waller, Frank.....	97
Wang, Jianing	52
Wanke, Alan.....	100
Wassermann, Birgit.....	50
Wastin, Jules	42
Watt, Michelle	36, 67, 128
Wawra, Stephan.....	100
Ważny, Rafał	131
Weckwerth, Wolfram.....	88, 92, 111
Wehner, Gwendolin	93, 117
Wei, Zhong.....	34, 52
Weinhold, Alexander	47
Weinmann, Markus.....	129
Weisskopf, Laure.....	116, 120
White, Philip.....	46
Whyte, Lyle	122
Wieczorek, Krzysztof.....	102
Wienkoop, Stefanie.....	130
Wiest, Steve	140
Wiktorin, Gregor	110
Will, Torsten.....	93
Willats, William	92
Wille, Lukas	42
Willems, Anne	87, 90
Wipf, Daniel	63, 85, 114
Wuyts, Karen.....	108

X

Xiong, Wu.....	34
Xu, Xiangming	55, 115
Xu, Yangchun	34, 52

Y

Yakti, Wael	62
Yan, Zhichun.....	130, 134
Yang, Tianjie.....	34
Yeap, Yoon Ting.....	89

Author Index

Z

Zabalgogazcoa, Iñigo	137	Zeiner, Niklas.....	94
Zaikova, Elena	74	Zhao, Xingchen.....	53
Zampieri, Elisa.....	37	Zhao, Yanyan.....	44
Zeevi, Adi	139	Zheng, Qi	134
		Zhu, Yong-Guan.....	32
		Zuccaro, Alga.....	28, 100
		Zytynska, Sharon E.	50

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