Plant Biologicals Network annual symposium 2018

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Content

About Plant Biologicals Network

The Plant Biologicals Network has been formed by a number of key players in Southern Scandinavia with the purpose of facilitating knowledge exchange on research, innovation, regulation, and education within the field of plant biologicals. Additionally the network aims at creating awareness on the future for these promising new technologies.

Southern Scandinavia houses a number of leading academic institutions prominent in this area of research as well as headquarters or R&D activities for a number of commercial players. The region has the preconditions to become a globally significant technology hub within the development of plant biologicals.

Our overall purpose is to make sure biologicals is a part of the world communities focus on how the agro industry can adapt to the societal challenges and boundaries within environment and food. The network contribution to this is in relation to agricultural production and the environment by generating new business opportunities, improving agricultural production through knowledge exchanges, and by attracting talent to a growing business area.

Progamme for symposium

Plant Biologicals Network

PBN annual symposium

14-15 November 2018

Wednesday 14 November

12:00	**Registration and lunch**
13:00	**Welcome and opening address**
13:10	**Bio fertilizers and better use of nutrients** Chairs: **Ass. professor Simona Radutoiu**, Aarhus University & **Head of Technology Sourcing and External R&D Agriculture Svend Kaj Petersen**, Novozymes ○ Legume-Rhizobia symbiosis: from field to lab and back: achievement and challenge **Prof. Marco Bazzicalupo** - Department of Biology, University of Florence, Italy ○ Root microbiota assembly and Rhizobium symbiosis, two concomitant processes developing at the legume root-soil interface **Ass. prof. Simona Radutoiu** - Department of Molecular Biology and Genetics, Aarhus University, Denmark ○ Plants and mycorrhizal fungi: a perspective beyond the lab **PhD Alessandra Salvioli Di Fossalunga** - Department of Life Sciences and Systems Biology, Università degli Studi di Torino, Italy ○ Selected flash talks - Lesley Maher – Era Sustainable Australia Pty Ltd - Ass. Prof. Dorette Müller-Stöver – University of Copenhagen - Ass. Prof. Beatriz Gómez Muñoz – University of Copenhagen
14:40	**Coffee break**

15:10	**Plant strengtheners and resilience** Chair: **Prof. Jean W.H. Yong**, Swedish University of Agricultural Sciences ○ Protection against your friends; Plant genes essential for Trichoderma symbiosis **Prof. Allan Rasmusson** - Department of Biology, Lund University, Sweden ○ Bio2Bio – From Organic Waste Streams to Biostimulants and Biopesticides **Postdoc Lin Xu** - Department of Plant Production, Faculty of Bioscience Engineering, Ghent University, Belgium ○ Real possibilities of cytokinin stimulants use in agriculture **Junior Researcher Radoslav Koprna** - Faculty of Science, Palacký University, Czech Republic ○ Assessing plant hormones and secondary metabolites in relation to plant growth **Ass. Prof. Petr Tarkowski** - Department of Biochemistry, Palacký University, Czech Republic ○ Selected flash talks - Ass. Prof. Stephan Wenkel – University of Copenhagen - PhD student Okanlawon Lekan Jolayemi – Swedish University of Agricultural Sciences - Postdoc Lorenzo Fimongnari – Chr. Hansen A/S
16:40	**Short break**
17:00	**The microbiome of wine** Chair: **Prof. Carsten Suhr Jacobsen**, Aarhus University ○ New Developments in flavor science in context of wine yeast **Prof. Dr. Ulrich Fischer** - Institute for Viticulture and Oenology, Neustadt, Germany ○ Microbial evolution on grapevine leaves under copper and biocontrol fungicide treatments **PhD Alex Gobbi** - Department of Environmental Science, Aarhus University, Denmark
17.40	**Reception in the foyer of Copenhagen Plant Science Centre**
18:45	**Buses leaving for dinner venue (if you go on your own see directions)**
19:00	**Symposium dinner at Vestauranten (participation is free of charge)** **At 21.15 and 22.15 buses will be leaving for Copenhagen Plant Science Center**

See you tomorrow at 8.30

Thursday 15 November

8:30	**Regulatory framework for plant biologicals** Chairs: **Regulatory Expert Lise Christina Deleuran**, Novozymes and **Prof. Jørgen Eilenberg**, University of Copenhagen o EU Regulation of Microbial Pesticides and Biostimulants **Henrik Frølich Brødsgaard** - Danish Environmental Protection Agency o Framework for the evaluation of low-risk products **Prof. Per Kudsk** - Department of Agroecology, Aarhus University, Denmark o Benefits of good regulation for plant biologicals **Vice-President Roma Gwynn** - IBMA Global (International Biocontrol Manufacturers Association) AISBL o Selected flash talks - Prof. Nina Cedergreen – University of Copenhagen - Jakob Thomas Damkjær – unaffiliated researcher
10:00	Break
10:30	**From lab to field** Chair: **Ass. professor Mette Haubjerg Nicolaisen**, University of Copenhagen o Microbial solutions for plants are going to be part of the green conversion from conventional pesticides and fertilizers to natural integrated solutions in tomorrow's agriculture **PhD, R&D Manager Lars Mølbak** - Plant Health, Chr. Hansen, Denmark o Isolation of niche-compatible microbial inoculants **Ass. prof. Mette Haubjerg Nicolaisen** - Department of Plant and Environmental Sciences, University of Copenhagen, Denmark o Biostimulants in agriculture – the challenge of assessing effects under natural conditions **Consultant Kim Wendelboe** - Danish Technological Institute, Denmark o Selected flash talks - Swathi Vurrakula – independent researcher - MA student Asger Ourø Jensen – FMC & University of Copenhagen - PhD students Meike Latz – University of Copenhagen

12:00	Closing remarks
12:10	Lunch grab bag/lunch buffet

Date: 14-15 November 2018 (12-12 meeting)

Location: University of Copenhagen, Copenhagen Plant Science Centre, Bülowsvej 21a,
1871 Frederiksberg, Denmark

Public transportation: Nearest metro is Forum, see directions from metro to venue

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Bio fertilizers and better use of nutrients

Invited speakers

Legume-Rhizobia symbiosis: from field to lab and back: achievement and challenges
Professor Marco Bazzicalupo, Department of Biology, University of Florence, Italy

Root microbiota assembly and Rhizobium symbiosis, two concomitant processes developing at the legume root-soil interface
Associate Professor Simona Radutoiu, Department of Molecular Biology and Genetics, Aarhus University, Denmark

Plants and mycorrhizal fungi: a perspective beyond the lab
PhD Alessandra Salvioli di Fossalunga, Department of Life Sciences and Systems Biology, University of Torino, Italy

Selected flash talks (see abstracts under Poster abstracts)

Lesley Maher, Era Sustainable Australia Pty Ltd

Dorette Müller-Stöver, University of Copenhagen

Beatriz Gómez Muñoz, University of Copenhagen

Legume-Rhizobia symbiosis: from field to lab and back: achievement and challenges

Professor Marco Bazzicalupo, Department of Biology, University of Florence, Italy

Legumes crop rotation is an ancient practice to improve fertility of exhausted soils. The reason why legumes were beneficial to cereals growth was discovered in 1883 by Hellriegel and Wilfarth in Germany, who for the first time demonstrated that bacteria inside the root nodules of legumes are responsible for the reduction of atmospheric nitrogen into nitrogen compounds utilized by the plant. After that discovery, the research on nitrogen fixation started together with the inoculation practices, with legumes becoming some of the major crops for feed and fodder.

At present, symbiotic nitrogen fixation represents a unique example of a technological progress with potential far reaching beneficial effects on two of the major planetary challenges: the shortage of food supply for a growing world population and the unrestrainable increase of pollution to which industrial nitrogen fertilizers are highly contributing.

Researches in microbiology, plant molecular biology and genetics, during the last 30 years, produced impressive advancements in the knowledge of molecular mechanisms of symbiotic nitrogen fixation with the added value of contributing to the development of the studies on host-microbe interactions.

Despite these tremendous achievements, two practical objectives of the research on symbiotic nitrogen fixation have been constantly pursued but are still to be reached. The improvement of the legume-rhizobia symbiosis and the transfer of the symbiotic abilities to cereal crops.

Both these areas are actively investigated and many recent results suggest that scientists are on the right track to succeed. One of the crucial aspect of these researches is the ability to exploit genomic sciences and natural genetic diversity to make plant and microbes suitable for high productivity with the minimum of chemical input.

Root microbiota assembly and Rhizobium symbiosis, two concomitant processes developing at the legume root-soil interface

Ass. Professor Simona Radutoiu, Department of Molecular Biology and Genetics, Aarhus University, Denmark

The complex microbial communities that associate with healthy plants in nature (microbiota) have emerged as a tremendous, untapped resource for biostimulants (microbials) in sustainable agriculture. Numerous advances in recent years have deepened our understanding of these complex plant-associated microbial communities, but the principles guiding microbiota assembly and stability are unknown. Microbes form communities where they engage in complex networks of microbe-microbe and microbe-host interactions. Can these complex inventories of plant-associated microbes be untangled, understood and manipulated? Can we predict function from taxa or gene composition? Addressing such complex interactions at the level of fundamental and predictive understanding requires integrated systems approaches where tailored communities are analysed and improved by intertwined empirical and theoretical approaches.

Legumes, unlike most other plants, have the capacity to grow on poor soils. This is because they benefit from recognition of a broad spectrum of beneficial soil microbes e.g. nitrogen-fixing Rhizobium bacteria inducing root nodule symbiosis (RNS). Recently, we have demonstrated that RNS and microbiota assembly are interconnected, as host genes involved in Rhizobium-induced nodulation drive the assembly of root- and rhizosphere-associated microbes in the model legume Lotus japonicus. Remarkably, our findings from Lotus-associated microbiota provided evidence that Rhizobium acts as a bacterial 'hub' facilitating associations of other bacterial taxa in the rhizosphere. In order to understand how root microbiota affects and contributes to RNS we have obtained a sequence-indexed culture collection and use this as a basis for designing tailored communities that are currently investigated for direct effect on Lotus growth. Results from our investigations will be presented and discussed.

Plants and mycorrhizal fungi: a perspective beyond the lab

PhD Alessandra Salvioli di Fossalunga, Department of Life Sciences and Systems Biology, University of Torino, Italy

The current need of safer and environmentally friendly agricultural practices has boosted the use of beneficial microorganisms as biofertilizers. Under this respect, arbuscular mycorrhizal (AM) fungi are widely acknowledged as one of the most promising tools to improve plant nutrition, and support plant tolerance to environmental stresses.

With a long-lasting research on the effects of AM fungi on plants, our group has contributed to shed light on the biological bases of the bio-fertilizing potential of AM fungi, also providing novel insights on impact of the symbiosis on tomato fruit quality. However, plants and AM fungi are not alone in the soil, but interact with a plethora of microbes, overall constituting the so-called root microbiota. Consequently, the positive outcome of AM interactions recorded in laboratory, under controlled and simplified conditions, might not reflect the actual complexity of the "real world".

In this scenario, we recently decided to broaden our perspective and apply the current knowledge on AM interactions in open field experiments, exploring the complex interactions among plant roots, AM fungi and the other members of the root microbiota. Here I will present an overview of our most relevant projects, which include field trials on forage species such as Medicago sativa. Our final aims are to verify the effectiveness of AMF as biofertilizers, and to assess how agricultural practices - including the use of micorbial inocula - can influence the native soil mycorrhizal potential.

Plant strengtheners and resilience

Invited speakers

Protection against your friends; Plant genes essential for Trichoderma symbiosis

Professor Allan G. Rasmusson, Dept. Biology, Lund University, Sweden

Bio2Bio – From Organic Waste Streams to Biostimulants and Biopesticides

Postdoc Lin XU, Horticell, Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent University, 9000 Ghent, Belgium

Real possibilities of cytokinin stimulants use in agriculture
Junior research Radoslav Koprna, Palacký University, Faculty of Science, Centre of the Region Haná for Biotechnological and Agricultural Research, Department of Chemical Biology and Genetics, Czech Republic

Assessing plant hormones and secondary metabolites in relation to plant growth

Ass. Professor Petr Tarkowski, Department of Genetic Resources for Vegetables, Medicinal and Special Plants, Centre of Region Haná for Biotechnological and Agricultural Research, Crop Research Institute, Czech Republic

Selected flash talks (see abstract under Poster abstracts)

Stephan Wenkel, University of Copenhagen

Okanlawon Lekan Jolayemi, Swedish University of Agricultural Sciences, Alnarp

Lorenzo Fimognari, Chr-Hansen A/S

Protection against your friends; Plant genes essential for Trichoderma symbiosis

Professor Allan G. Rasmusson, Dept. Biology, Lund University, Sweden

Trichoderma treatments of plants are used to promote faster growth (biofertilization) and management of pathogens (biocontrol). The latter is carried out by Trichodermal release of antibiotic substances that attack other microorganisms and elicitors of plant defence that immunize the plants. However, the effect of Trichoderma is highly variable and dependent on the plant genotype. Consistently, Trichoderma-specific antibiotic peptides can also damage plant cell membranes. However, plants induce resistance against these peptides when pretreated with cellulase from the same fungus. The resistance depends on previously unstudied genes in the domains of cell wall synthesis, signalling and membrane lipid modification. We are presently investigating the Arabidopsis genes that are essential for the resistance to the antibiotic peptides. Further we investigate the polymorphism of these and other Trichoderma-associated genes in sugarbeet inbred lines that vary in their growth response to Trichoderma afroharzianum.

Bio2Bio – From Organic Waste Streams to Biostimulants and Biopesticides

Postdoc Lin XU, Horticell, Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent University, 9000 Ghent, Belgium
Maaike Perneel and Danny Geelen, CropFit IOF Consortium, Faculty of Bioscience Engineering, Ghent University, 9000 Ghent, Belgium

The quest for replacing conventional agrochemicals drives the development of alternative approaches and more eco-friendly solutions that includes the use of biostimulants and biopesticides. These latter products originate from organic matter and are used to enhance plant growth and improve resistance to abiotic and biotic stress. The Bio2Bio project (2017-2021, FWO-SBO) aims to contribute to the discovery of new biostimulants and biopesticides. As a source, material from organic waste streams and by-products from food industries in Flanders are explored. Bio2Bio has created a library of formulated extracts that are being characterised by means of a screening platform, which is important to assess the potential of an extract as biostimulant or biopesticide. The screening platform gathers several Flemish research partners who are experts in phenotypic analysis of plant performance and disease tolerance either in vitro, greenhouse and field tests. The consortium also hosts the expertise and capacity to determine the bioactive ingredients and to perform mode of action studies. The impact of the project lies in the discovery of potentially new bioactive compounds of natural origin. The research output of the project will link academia with the industry, providing valuable leads for the agrochemical companies to develop new products, benefiting different target groups.

Real possibilities of cytokinin stimulants use in agriculture

Radoslav Koprna[1], Palacký University, Faculty of Science, Centre of the Region Haná for Biotechnological and Agricultural Research, Department of Chemical Biology and Genetics, Czech Republic

Miroslav Strnad[2], Karel Doležal[1,2], Jaroslav Nisler[1,2], Vlasta Matušková[1,2], Marek Zatloukal[1], Magdaléna Bryksová[1], Lucie Plíhalová[1,2]

1- Palacký University, Faculty of Science, Centre of the Region Haná for Biotechnological and Agricultural Research, Department of Chemical Biology and Genetics, Šlechtitelů 241/27, 783 71 Olomouc, Czech Republic

2- Laboratory of Growth Regulators, Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany ASCR and Palacký University, 783 71 Olomouc, Czech Republic

Plant hormones influence plant growth and their development during vegetation period. Raising plant hormones content in plants may lead to yield increase, or the increase of tolerance against abiotic stress after the exogenous treatment. Increased yield was observed via tillering and branching support, higher development of number of flowers and generative organs and senescence delay. Compounds for seed coating (cytokinin antagonists and karrikines), N-9 substituted purine derivatives and urea derivatives for foliar treatment were tested. These substances influencing the level of plant hormones were tested in field plot experiments on winter wheat, spring barley, winter rapeseed, silage maize and vegetables. In most cases, increase in tillers number, root biomass, number of ears, grain yield and plants biomass was observed after compounds application. On average, yield was 3 – 8 % higher than the non-treated control. The greatest effect of substances application was observed during the vegetation seasons with water stress than in optimal periods. Most of the tested compounds have a great potential of use in agriculture despite their low concentration in application solutions. Final concentrations up to 25 μMol are also environment-friendly.

Assessing plant hormones and secondary metabolites in relation to plant growth

Ass. Professor Petr Tarkowski, Department of Genetic Resources for Vegetables, Medicinal and Special Plants, Centre of Region Haná for Biotechnological and Agricultural Research, Crop Research Institute, Czech Republic

Research and utilization of plant genetic resources has a long tradition in the Czech Republic which dates back to the early 20th century. The systematic collecting, study and conservation of plant germplasm is supported by the Ministry of Agriculture within the National Programme on Conservation and Utilisation of Plant Genetic Resources and Agrobiodiversity. In the Section of Applied Research of Vegetables and Special Crops of the CRI in Olomouc, broad collections of genetic resources of vegetables (more than 10, 000 accessions), medicinal, aromatic and culinary plants (MAPs, nearly 1, 000 accessions) traditionally grown in the Central Europe, and a collection of fungi are kept. Regarding to their extent and species representation the collections have a great importance in the European context as potential sources of new traits for breeding (e.g. phenological characteristics, resistance genes, content of nutritionally important substances and secondary metabolites). Development of new analytical methods for natural products analysis include the following activities: Identification and quantification of plant secondary metabolites using UHPLC-MS/MS and GC-MS; Study of biological activity of known and new natural substances; Secondary metabolites screening of selected germplasm accessions with focus on biologically active metabolites; Nutritionally important compounds assessment in selected accessions of vegetable germplasm. Besides secondary metabolites, two groups of phytohormones, cytokinins and strigolactones are molecules of our interest.

The microbiome of wine

Invited speakers

New Developments in flavor science in context of wine yeast

Professor Ulrich Fischer, Institute for Viticulture and Oenology, DLR Rheinpfalz, Neustadt, Germany

Microbial evolution on grapevine leaves under copper and biocontrol fungicide treatments

PhD Alex Gobbi, Lars Hestbjerg Hansen, Department of Environmental Science, Aarhus University, Denmark

New Developments in flavor science in context of wine yeast

Ulrich Fischer, Institute for Viticulture and Oenology, DLR Rheinpfalz, Neustadt, Germany

In the last decade the wine industry has experienced a paradigm shift with regards to its appreciation of yeast which were traditionally viewed as the facilitators of desired alcoholic fermentation and in some cases also as the source of unwanted deterioration of wine due to microbial derived off-flavors and faults. Current and ongoing research however goes beyond these paths, investigating the role of single or mixed yeast strains in enhancing the varietal character of wines. Liberating aroma compounds such as monoterpenes, C_{13}-norisoprenoids or powerful thiols from their non-volatile and thus odor-inactive glycosylated or cysteinylated precursors, certain yeast strains can strongly contribute to more pronounced and attractive wines.

According to the idea that “sensory diversity is the new synonym for wine quality”, which I postulated during a conference discussing the merits of terroir for wine in Australia in 2016, the wine industry tries to broaden the range of microbial genera used for winemaking. For example, non-saccharomyces yeast strains are selected, tested and implemented in the winemaking process to increase the sensory diversity of wine in order to cope with changing consumer preferences.

In my talk I will address the genuine aroma formation of yeast, their ability to liberate different grape derived bound aroma compounds and the role of spontaneous fermentation of a dynamic range of wild yeast on the wine aroma.

Microbial evolution on grapevine leaves under copper and biocontrol fungicide treatments

PhD Alex Gobbi, Lars Hestbjerg Hansen, Department of Environmental Science, Aarhus University, Denmark

Since long, winemakers have been using copper as fungicide on grapevine. However, the potential of copper to accumulate on soil and affect the biota, poses a challenge to achieve sustainable agriculture. Recently, the use of biocontrol agents to replace or complement traditional methods became an option. Here, we carried out a field-experiment in South Africa; two blocks of the vineyard were periodically treated, on leaves, with copper sulphate or sprayed with *Lactobacillus plantarum MW-1* as biocontrol agent. We evaluated the impact, of the two treatments, on bacterial and fungal community following their evolution along the growing season. To do this we combined NGS with quantitative strain-specific and community qPCRs. Our results show the progression of the microbial communities along the season and how the different treatments affect the microbiota. Bacteria appear to be relatively stable at the different time-points and the only taxa that systematically change, between treatments, is *Lactobacillacea*, which includes reads from our biocontrol agent. We detected cells of *Lactobacillus plantarum MW-1*, only on treated leaves using strain-specific qPCR and its amount spans from 10^3 to 10^5 cells/leaves. The period conversely, mostly shapes the fungal community through a succession of different dominant taxa along the months. Between treatments, only few fungal taxa appear to change significantly and the number of ITS copies is also comparable. In this regards, the two treatments seems to affect similarly the microbial community revealing the potential of our biocontrol strain to be further investigated, to become a valid alternative among sustainable fungicide treatments.

Regulatory framework for plant biologicals

Invited speakers

The EU Regulation of Microbial Pesticides and Biostimulants

Henrik F. Brødsgaard, Danish Environmental Protection Agency Denmark, Unit of Pesticides and Biocides

Framework for the evaluation of low-risk products

Professor Per Kudsk Dept. of Agroecology, Aarhus University, Flakkebjerg, Slagelse, Denmark

Benefits of good regulation for plant biologicals

Vice-President Roma Gwynn, International Biocontrol Manufacturers Association, Belgium

Selected flash talks (see abstract under Poster abstract)

Nina Cedergreen, University of Copenhagen

Jakob Thomas Damkjær, unaffiliated researcher

The EU Regulation of Microbial Pesticides and Biostimulants

Henrik F. Brødsgaard, Danish Environmental Protection Agency Denmark, Unit of Pesticides and Biocides

The EU regulation of "plant biologicals" sensu the definition by the Plant Biologicals Network is currently not harmonized for all agents.

In Denmark, "plant biologicals" are currently regulated by either the Regulation (EU) 1107/2009 (micro-biological control agents [bacteria, fungi & viruses], biologically derived products [plant extracts & bacterial metabolites]), the national Nature Protection Act no 1122 of 03/09/2018 (arthropods, nematodes & other macro-biological control agents), or the national Executive Order no 862 of 27/08/2008 for soil improvers not claiming a pest control action (e.g. mycorrhizas or Trichoderma spp.).

Presently, there is an ongoing process on a revision of the fertilizer Regulation (EC) No 2003/2003. The aim is to include plant "biostimulants" in the new regulation. However, before the present draft text may be adopted, the plant protection regulation also needs to be revised in order to eliminate uncertainties related to the two regulations.

To-day, it is often the actual claims by the manufactures that determine what regulation will be enforced on a particular "plant biological", and thus, which data demands are needed to be met in order to obtain a marketing authorization of the product. This is due to the somewhat unclear definitions of the relevant regulations, and the different modes of action of the same beneficial organisms.

Framework for the evaluation of low-risk products

Professor Per Kudsk, Dept. of Agroecology, Aarhus University, Flakkebjerg, Slagelse, Denmark

Abstract: In the EU, the term low-risk active substances was introduced with EC Regulation 1107/2009 that went into force in 2011. Low-risk active substances are a substance classified as having low risk to human and animal health and the environment and therefore do not require specific mitigation procedures. Hence, low-risk products are considered an integral part of an IPM strategy. Typical low-risk products are micro-organisms, botanicals, semiochemicals and bacloviruses but chemical substances may also be classified as low-risk products. Currently only 13 substances are listed as low-risk active substances in the EU. Efficacy has been a bottleneck for placing low risk products on the market because the requirements have been the same as for conventional pesticides. However, low-risk products are more variable in their properties and modes of action than conventional pesticides and for some time it has been widely recognized that a different approach for efficacy evaluation is needed. Therefore, EPPO took on the task to develop a standard for low-risk plant protection products, which is now published as EPPO Standard PP1/296 (https://pp1.eppo.int/). The standard provides guidance on the various steps in the efficacy evaluation. A key point is to describe the mode of action of the low-risk product as this will determine the possibilities for extrapolation and thus the type and amount of efficacy data needed to support registration. The principles laid out in EPPO PP 1/296 are presented and discussed.

Benefits of good regulation for plant biologicals

Vice-President Roma Gwynn, International Biocontrol Manufacturers Association, Belgium

Food security will be one of the defining issues of the 21st Century. The UN Sustainable Development Goals aim to end poverty, protect the planet and ensure prosperity for all: a healthy and productive environment is needed to support this. Agricultural intensification puts pressure on the environment and increases threats to human health. The EU Sustainable Use of Pesticides Directive promotes an approach to agriculture that balances the need for good quality food for an increasing population, with minimising harm to human health and the environment. There is strong scientific evidence that effective plant protection manages the health and productivity of crops best by considering them in an ecosystem context. To support farmers and effectively implement the SUD using this new approach, the EU needs an extensive range of innovative plant protection measures: bioprotection technologies are such vital tools for modern agriculture.

Bioprotection technologies provide effective and innovative plant protection for modern agriculture. This makes them a vital tool to support farmers and effectively implement the Sustainable Use of Pesticides Directive. However, the current lack of a specialist bioprotection regulatory body means that the EU is not fully reaping the benefits of this rapidly growing, predominately SME-based, industry. IBMA therefore propose that by 2020 the EU establishes a bioprotection-specific body that produces a short and precise timeline for the evaluation process, with evidence-based procedures and data requirements.

This new approach for regulation would benefit human health, the environment, biodiversity and the bio-based circular economy and, therefore, society as a whole.

From lab to Field

Invited speakers

Microbial solutions for plants are going to be part of the green conversion from conventional pesticides and fertilizers to natural integrated solutions in tomorrow's agriculture.

PhD, R&D Manager Lars Mølbak, Chr. Hansen Plant Health

Isolation of niche-compatible microbial inoculants

Assciate Professor Mette H Nicolaisen, Section for Microbial Ecology and Microbiology, Department of Plant and Environmental Sciences, University of Copenhagen, Denmark

Biostimulants in agriculture – the challenge of assessing effects under natural conditions

Consultant Kim Wendelboe (Mette Walter, Karina Vincents Lohmann, Søren Kjærgaard Boldsen & Philipp Trénel), The Danish Technological Institute

Selected flash talks (see abstracts under Poster abstracts)

Swathi Vurrakula, Independent Researcher

Asger Ourø Jensen, FMC & University of Copenhagen

Meike Latz, University of Copenhagen

Microbial solutions for plants are going to be part of the green conversion from conventional pesticides and fertilizers to natural integrated solutions in tomorrow's agriculture

PhD, R&D Manager Lars Mølbak, Chr. Hansen Plant Health

The use of microbial based products for improved crop production is an emerging biotechnology with several products on the market. However, for many products, a major challenge is the product stability and their variable performance under different field conditions. It is therefore essential to link the microbes and plant health in order to develop robust and reliable products, which live up to the expectations of the farmer's community. One way of establishing this knowledge is by understanding how plants are responding to microbial products under controlled greenhouse conditions and extrapolating these results to the field. This talk will focus on the benefits and use of microbial solutions for crops in the agricultural industry plant, their potential way of working and how greenhouse trials can be used to design microbial based products for agriculture in the future.

Isolation of niche-compatible microbial inoculants

Ass. Professor Mette H Nicolaisen, Section for Microbial Ecology and Microbiology, Department of Plant and Environmental Sciences, University of Copenhagen, Denmark

Microorganisms from natural environments can improve plant health and growth by enhancing nutrient uptake and combat diseases. The potential for exploiting this natural resource for inoculants in agricultural biotechnology is huge, but currently hampered by low translational power from lab and greenhouse studies to field performance.

Fungal pathogens can cause severe damage to plants, whereas other fungal species can act as biofertilizers and enhance crop performance. In nature, bacteria colonize fungal hyphae. Based on the basic hypotheses that 1) these hyphae-colonizing bacteria can influence fungal growth and physiology, and 2) niche-compatible isolates will have a higher translational power than classically isolated inoculants, we developed two baiting systems to isolate inoculants that were adapted to the ecological niche in which they have to perform – one system used for isolating a biocontrol agent towards sheath blight in rice, and the other used for isolating helper bacteria to increase the performance of the fungal biofertilizer Penicillium bilaii.

The translational power for obtaining biological control agents towards sheath blight was high. Only ten strains were assessed, with the best performing lowering the disease incidence by 40% and at the same time increasing yield with 7% in field testing.

Fungi from the genus Penicillium colonize the rhizosphere and solubilize phosphate (P) for the benefit of plant growth. We isolated helper bacteria from Penicillum hyphae that significantly promote fungal growth and P solubilization. The combination of helper bacteria and biofertilizers are currently being assessed for the development of new biofertilizer consortia, which are able to enhance inoculant robustness, and hence increase crop nutrient use efficiency across environmental conditions.

In conclusion, we found that knowledge on microbial ecology in the isolation procedure is paramount for reducing the number of strains needed to screen, and at the same time improving the robustness of microbial inoculants when transferred from lab studies to field tests.

Biostimulants in agriculture – the challenge of assessing effects under natural conditions

Consultant Kim Wendelboe (Mette Walter, Karina Vincents Lohmann, Søren Kjærgaard Boldsen & Philipp Trénel), The Danish Technological Institute

Agricultural biostimulants are applied to seeds, plants or soils to enhance/benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress and crop quality. The effects of biostimulants are investigated during screening by the producers and under controlled laboratory conditions. The denominator for these tests is that the conditions are controlled to isolate and test for a specific parameter/effect. The challenge is then: Do biostimulants show the same effects in field under natural conditions and how can this be tested to achieve valid and reliable data? Biostimulants are biological products and their effects are therefore depending on the natural - in field - conditions. Standard small plot field testing does not fully account for the complexity of natural conditions, which impact the usefulness of the efficacy data on the biostimulants. New technologies are needed to fully investigate the effect and potential of biostimulants under natural conditions. A method shown to provide useful and valid data is large scale field plots in combination with a collection of georeferenced sensor data. This method provides several layers of data with high resolution, and the method can be customized to test for a specific effect or parameter under natural conditions. Furthermore, this approach helps to understand the interaction between biostimulants, crops and natural environments.

Poster abstracts

1	Adriana Garcia	Mining the rhizosphere of Christmas trees (*Abies nordmanniana*) for plant growth promoting bacteria
2	Akos T Kovacs	Division of labor during biofilm matrix production and plant colonization
3	Andrea Manzotti	How do phytohormones influence the composition of fungal endophyte communities in tomato roots?
4	Anne Winding	Fate of plant beneficial *Bacillus* spores after ingestion by protist grazers
5	Asger Ourø Jensen	Effect of Seed Inoculation with a Known *Bacillus* Plant Growth Promoter on Seed Germination and Plant Growth of Cucumber.
6	Beatriz Gómez Muñoz	*Penicillium bilaii* can increase plant growth and reduce the effect of cold stress in maize plants depending on soil phosphorus availability
7	Dorette Müller-Stöver	The effects of phosphorus (P) solubilizing fungi on P availability from ash or biochar derived from sewage sludge
8	Edward C. Rojas	Healthy wheat spikes endophytes reduce *Fusarium* spp. caused diseases in wheat
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16	Niels Agerbirk	New plant defenses in a multiresistant dead-end trap crop: *Barbarea vulgaris*

17	Nina Cedergreen	Implications of sequence and timing of exposure for synergy between the pyrethroid insecticide alpha-cypermethrin and the entomopathogenic fungus *Beauveria bassiana*
18	Okanlawon Lekan Jolayemi	Biostimulant enhance sugar beet growth and development
19	Peter Stougaard	Mechanistic studies of molecular bacterial-fungal interactions
20	Stephan Wenkel	MicroProteins, versatile tools to influence plant growth and development
21	Swathi Vurrakula	Machine Learning as a tool for biologicals field trials
22	Thomas Roitsch	Biocontrol of bacterial pathogens by cytokinin producing bacteria and microalgae
23	Thure Pavlo Hauser	Microbe induced Resistance to Agricultural pests

P1: Mining the rhizosphere of Christmas trees (Abies nordmanniana) for plant growth promoting bacteria

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Abies nordmanniana is a major Christmas tree species in Europe, but prolonged growth hamper their production, and early root development is important for improved plant growth. The rhizosphere represents a diverse source of plant growth promoting bacteria that may influence root development. This study aimed to characterize bacterial communities associated with roots of A. nordmanniana at the nursery stage, and isolate rhizosphere bacteria able to regulate plant growth. Composition of the bacterial communities from bulk soil and rhizosphere of A. nordmanniana at different sampling sites was compared by 16S rRNA gene sequencing. There were clear differences in community composition between rhizosphere and bulk soil, and significant effect of sampling site on both rhizosphere and bulk soil communities. Proteobacteria, Actinobacteria, Acidobacteria and Bacteriodetes dominated in the rhizosphere. We tested 22 bacterial isolates isolated from the rhizosphere for effects on plant growth. Among the strains we found a few that produced auxins in pure culture and were able to improve seed germination, and increase development of secondary roots and root hairs in both laboratory and greenhouse trials. We currently investigate their effect on plant hormone levels. These results suggest that plant growth promoting bacteria are associated with the rhizosphere of A. normanniana and could enhance growth of their root system.

P2: Division of labor during biofilm matrix production and plant colonization

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Colonization of plant root by Bacillus subtilis and therefore biocontrol depends on the ability of the bacterium to produce the biofilm matrix [1]. Here, I will present experimental and computational approaches to investigate potential benefits arising from division of labor during biofilm matrix production in B. subtilis. In this species, biofilm matrix consists of two major components; EPS and TasA. We observed that clonal groups of B. subtilis phenotypically segregate into three subpopulations composed of matrix non-producers, EPS-producers, and generalists, which produce both EPS and TasA. This incomplete phenotypic specialization was outperformed by a genetic division of labor, where two mutants, engineered as specialists, complemented each other by exchanging EPS and TasA. The relative fitness of the two mutants displayed a negative frequency dependence both in vitro and on plant roots, with strain frequency reaching a stable equilibrium at 30% TasA-producers, corresponding exactly to the population composition where group productivity is maximized. In addition, we show that division of labor observed under laboratory conditions is also detected during plant root colonization suggesting a valuable strategy for plant biocontrol.

[1] Gallegos-Monterrosa et al 2017 Microbiology

[2] Dragoš et al 2018 Current Biology

P3: How do phytohormones influence the composition of fungal endophyte communities in tomato roots?

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Endophytes are microbes capable of colonizing the inner part of different plant tissues without causing disease symptoms. In some cases, they have beneficial effects for the host plant such as biotic and abiotic stress resistance and plant growth promotion. For this reason, the use of these microbes could have a major impact on agriculture worldwide. However, the plant-endophyte interaction involves very complex mechanisms starting from the recruitment of the microorganisms to the colonization of the surface of the plant tissue and then the inner part, with the need to escape the plant immune system. All these processes are regulated by different plant and endophyte signalling molecules necessary for the establishment of the plant-endophyte interaction. Phytohormones are among the signalling compounds known to play a significant role in this interaction, but little is known about the specific ways by which they influence recruitment and colonization of the host tissues. The aim of the current project is to go deeper into the role of these signalling compounds in plant-endophyte interactions.

A community analysis (endophyte isolation and amplicon sequencing) of endophytic fungi was conducted on roots of tomato (Solanum lycopersicum) mutants impaired in synthesis of specific phytohormones (specifically ethylene and jasmonic acid) in order to understand how these compounds influence the composition of the endophytic communities. After the characterization of the endophytic communities, fungal isolates whose root-colonization frequency appears to be influenced by the presence/absence of specific phytohormones were selected. In order to obtain a deeper understanding of the role of these compounds in the plant-endophyte interaction, the selected isolates are currently being screened by confocal microscopy and qPCR in order to identify candidates whose colonization rate is critically affected by the phytohormones of interest.

A transcriptomic analysis of tomato plants inoculated with the isolates selected from the screening will provide further clues as to which physiological mechanisms, associated with endophyte recruitment, are influenced by phytohormones.

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P4: Fate of plant beneficial Bacillus spores after ingestion by protist grazers

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The aim of this study is to understand the symbiosis between bacterivorous protists and plant beneficial bacterial spores, in order to gain insight on survival and dispersal of plant beneficial bacteria in the environment. It has been hypothesized that the spore stage of Bacillus protects against digestion by predating protists.

We report how diverse protist grazers grow on both vegetative cells and spores of B. cereus and how the bacteria survive ingestion and digestion, and even proliferate inside the digestive vacuoles of ciliated protists. The survival ability of B. cereus was investigated in microcosms inoculated with pure cultures of the protists Acanthamoeba castellanii (amoeba), Tetrahymena pyriformis (ciliate) and Cercomonas sp. (flagellate) as grazers. Individual protist cultures were fed with fluorescently labelled B. cereus spores or vegetative cells as the only food source.

I)The presence of fluorescently labelled intracellular bacteria confirmed that B. cereus spores as well as vegetative cells were ingested by protists and appeared intact.

ii) B. cereus digestion and protist growth were determined by qPCR and protists appeared to grow on spores as well as on vegetative cells.

iii) B. cereus spore germination was observed inside the ciliate T. pyriformis which seems contradicting to the observed protist growth on spores.

These observations indicate that protists might act as a survival niche and potential breeding ground for B. cereus with some loss of bacteria to support growth of the protist. This indicates tight symbiosis between bacteria and protist grazers which can have potential for increasing survival of introduced plant beneficials microbes in the rhizosphere.

P5: Effect of Seed Inoculation with a Known Bacillus Plant Growth Promoter on Seed Germination and Plant Growth of Cucumber.

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The development of new plant growth promoters to increase crop yield and early vigor in plants requires screening of high numbers of candidates. In this study, we test different screening methods of a specific Bacillus strain, which is a known plant growth promoting rhizobacteria (PGPR), with the objective of developing a more high-throughput screening process for future PGPR.

Cucumber seeds are coated with a Bacillus strain that has been cultivated under eight different fermentation conditions. All eight treatments will be tested in vitro and in planta experiments and parameters such as germination rate and efficiency, root architecture, early flowering, as well as early fruit development on seed-treated cucumber plants will be measured. To get a better understanding of the mode(s) of action, quantitative production of Indole-3-acetic-acid (IAA) and Acetoin from the Bacillus strain will hereafter be measured on the most and least effective treatments. These metabolites are known to be produced in high amounts by Bacillus strains associated with PGPR properties.

These experiments will first and foremost test whether seed-coating can be used as a high-throughput technique for screening PGP in the future, while simultaneously acquiring further data of the tested Bacillus strain and its efficacy as PGPR. This research can furthermore make it more apparent whether in vitro data correlates to in planta and which parameters are important to measure in the laboratory- and greenhouse steps of a PGPR screening.

P6: Penicillium bilaii can increase plant growth and reduce the effect of cold stress in maize plants depending on soil phosphorus availability

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Penicillium bilaii is a soil fungus reported to increase plant growth and yields of different crops. This response has mainly been attributed to higher soil phosphorus (P) availability, because the majority of these trials were conducted under P limitation. However, we have shown that similar plant growth promotion can occur with no P limitation, suggesting that yield increase in plants inoculated with Penicillium bilaii might be due to other factors that indirectly improve plant P uptake in addition to the possibility of direct P solubilisation.

The objective of this study was to investigate the interaction between soil fertility level, especially available soil P, and effect of Penicillium bilaii on plant growth, and how inoculation may reduce the effect of cold stress in maize plants.

The influence of inherent soil fertility P and application of other nutrients to soil on the ability of Penicillium bilaii to increase plant growth and reduce the effects of cold stress (10°C) in maize plants was studied in pot experiments.

A positive interaction was found between the effect of Penicillium bilaii and available P when other nutrients were applied, where Penicillium bilaii increased root growth that led to greater shoot biomass and nutrient uptake in maize plants. This effect was not observed when other nutrients were not supplied, where root growth was decreased in inoculated plants. Similarly, the inoculation of maize plants with Penicillium bilaii increased shoot biomass in plants subjected to cold stress, but only when the plants were grown in fertile (high P availability) soils.

P7: The effects of phosphorus (P) solubilizing fungi on P availability from ash or biochar derived from sewage sludge

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In total, eight different fungal strains were tested for their ability to increase phosphorus (P) availability from biochar or ash derived from sewage sludge. P solubilization was measured either in liquid culture in comparison with other insoluble P sources or in a micro-scale solid state incubation system. To test for possible mechanisms involved, organic anion production was determined in the liquid cultures. Selected strains were afterwards tested in pot experiments with spring wheat in a semi-sterile P-depleted soil. Penicillium bilaiae 20851 and P. aculeatum were equally efficient in solubilizing P from biochar in the liquid cultures and both biochar and ash in the solid state system. However, when glucose as a carbon source was replaced with sucrose, the P. bilaiae strains (in particular DBS-5) became much more efficient in solubilizing P especially from ash, whereas P solubilization by P. aculeatum was not affected by the carbon source used. P. bilaiae 20851 showed a high ability to solubilize Ca-P – accompanied by excretion of gluconic acid - while P. aculeatum solubilized a larger amount of Fe-P, excreting mainly citric acid. Both strains showed a similar degree of substrate acidification. The effects of fungal inoculation in the pot experiments with ash/P. bilaiae DBS-5 and biochar/P. aculeatum were generally small and not always consistent. Positive effects on shoot P concentration and uptake could sometimes be observed which were not always reflected in an increased shoot biomass, possibly indicating a slow P solubilization not matching the plant's early P demand. Overall, the tested approach to increase the P fertilizer value of thermally-treated sewage sludge products appears to be of low practical relevance, in spite of the significant in vitro P solubilization in specific strain/material combinations.

P8: Healthy wheat spikes endophytes reduce Fusarium spp. caused diseases in wheat

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Fusarium head blight (FHB) is one of the most important diseases in cereals worldwide and is caused by several species in the genus Fusarium (Rojas et al. 2018). New environmental standards require farmers to adopt new control measures. Biological control is a highly promising alternative in disease management. Endophytes are microorganisms that colonise plant tissues asymptomatically. They have been associated with increased stress tolerance and disease resistance. They can represent a new source of biocontrol agents (Collinge et al. 2019).

We have isolated 25 different fungal species from healthy wheat spikes in areas with high FHB levels. We hypothesize that some of these organisms can provide biological control against FHB. We have examined endophyte performance against Fusarium spp. Using detached spikelets in vitro screening we found six strains that effectively reduced F. graminearum symptoms from 20 to 80%. Additionally, in planta assays showed that some endophytes can reduce severity of Fusarium crown rot (caused by F. culmorum) in seedlings and some of these can also reduce significantly FHB severity in spring wheat spikes. Additionally, we have shown that biocontrol effect is lost when detached spikelets are chemically surface sterilized. Thus, indicating that biocontrol effects are plant mediated.

These results confirm the need to use in vivo systems when screening for biocontrol agents as direct antibiosis is not the only mechanism involved in biological control (Latz et al. 2019).

Similarly, we have confirmed the potential of using naturally occurring endophytes as a reservoir for environmentally friendly disease control mechanism in agriculture.

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P9: Phosphite protects against late blight in field-grown potato and tomato in Ethiopia

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Late blight caused by the oomycte Phytophthora infestans is a destructive plant disease affecting both potato and tomato cultivation worldwide. Therefore, production depends on high fungicide use. In this study, field trails were carried out over three consecutive years in Ethiopia to investigate the efficiency of potassium phosphite, an inorganic salt which is directly toxic at the same time as it acts as a plant resistance inducer.

The study included one moderate resistant and one susceptible potato as well as a moderate resistant tomato cultivar. Plants were treated weekly with recommended or doubled dose of phosphite or Ridomil as well as combinations of either 50:50% or 75:25% of the recommended dose of phosphite and Ridomil. Late blight disease was monitored and at maturity, yield was recorded. In a separate experiment we tested the phosphite sensitivity for seven European and Ethiopian P. infestans strains in vitro.

The results showed that phosphite combined with a reduced dose of Ridomil suppressed potato and tomato late blight as well as the full recommended dose of Ridomil. In the moderate resistant potato cultivar, Belete, phosphite alone gave adequate foliar protection. In tomato, phosphite was as effective as the recommended dose of Ridomil. In potato, treatment with only phosphite led to lower yield than when applying the recommended dose of Ridomil or using combinations. In tomato, however, the yield obtained by phosphite treatment was virtually the same as with Ridomil. In vitro plate assays showed small but significant differences in the sensitivity against phosphite between P. infestans strains, something that should be studied further in field conditions.

These findings suggested that phosphite could be used against potato and tomato late blight in tropical highlands. The amount of fungicide used could be reduced by 75 percent, lowering the costs for smallholder farmers.

P10: Endophytic fungi entering Plant Wars

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Alternative practices are urgently needed to transform current agriculture to more sustainable and environmentally friendly systems. Endophytes, a diverse group of bacteria and fungi growing asymptomatically inside plant tissues, are often associated with enhanced plant growth and tolerance to abiotic and biotic stresses, indicating their potential for improving sustainability in agricultural systems. However, the exact mechanisms and effects of the complicated plant-endophyte interactions remain poorly understood and characterized.

Serendipita indica (syn. Piriformospora indica) is an endophytic fungus with several promising agricultural and biotechnological applications. The fungus can colonize the root cortex of a wide range of plants, enhancing plant growth and modulating plant specialized metabolism. Tomato (Solanum lycopersicum) is an important crop, often challenged by fungal pathogens and insect pests. The wide variety of specialised metabolites produced by the plant, and especially terpenes, plays a crucial role in plant defence, helping in repelling possible enemies.

This project involves establishing a balanced interaction between the fungal S. indica and tomato plants, providing a model system for studying general plant-endophyte interactions. However, our main focus is on the mechanisms used by the fungus to induce host specialized metabolism. Preliminary data suggesting that colonization by S. indica results in increased production of specific volatile terpenes in fungus-colonised tomato plants encouraged us to study the induction of these metabolites at transcription level. RNAseq analysis on fungus-associated and fungus-free plant tissues is currently ongoing to provide a more-in-depth view on the actual mechanisms underlying the S. indica-tomato interaction.

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P11: Risk factors in the agricultural production of biopharmaceutical source material

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The goal of this project was to develop a technology platform that allows us to monitor the effects of stress on pollen development would ensure the production of high-quality pollen as source material for allergy vaccine production. The project could lead to agricultural intervention strategies and allow assessment of quality prior to harvest and ultimately underpin work to identify more stress tolerant pollen producing plant lines.

Most biopharmaceutical production uses recombinant organisms grown in controlled environments that produce a highly consistent product. In contrast, source material produced in an open field agricultural production is subject to an array of abiotic stress factors. In particular, short-term drought stress triggers adaptive responses that profoundly influence the polysaccharide composition of the pollen. This adaptive response can potentially influence the process performance and quality of allergy vaccine production.

In this project, a novel microarray-based high-throughput screening platform has enabled the characterisation of polysaccharide composition of the adaptive responses that occur in pollen. The polysaccharide profile combined with a detailed field sensor dataset enables us to link the differences in polysaccharide profile to hourly abiotic stress profiles using a multivariate analysis.

The multivariate analysis has revealed that pollen source material is susceptible to drought stress in highly specific time intervals. Drought stress in those intervals can trigger an adaptive response that profoundly influence the polysaccharide composition of the pollen.

P12: Applying Strategies of Regenerative Agriculture and Biostimulants to improve Efficiencies for Water and Nutrients, and Reversing Land Degradation in the Wheatbelt of Western Australia.

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The soils of Western Australia are inherently sandy, fragile and low in organic matter; such soils have traditionally relied on heavy applications of fertilizer for productivity. Mode of operation is continuous cropping with little fallow or pasture in the 5 year rotation cycle of wheat, barley and canola.

State Department of Agriculture reports that as a result of this style of farming, over 30% of Western Australian agricultural soils suffer from degradation and are losing fertility at the rate of 1% per annum; farmer response has been to increase fertilizer application rates.

Dominant crop is winter ripened wheat, relying only on rainfall for moisture. Rainfall patterns are changing and water usage is becoming a critical factor for planting strategies.

Fluctuating commodity prices, increasing production costs and unpredictable rainfall mean that only 1 in 5 seasons is profitable for most farmers who face a severe cost; price squeeze.

From 2012 to 2017, 16 farmers encompassing 40,000 hectares of broadacre cropping participated in trials to measure the impact on productivity of introducing regenerative agricultural strategies, biostimulants and biological inoculants into their farm management.

Key chemical, biological and physical qualities were tracked over the 5 year trail period to measure soil response to the change in management.

Fertilizer application rates were calculated using agronomy that allowed for efficiency to be delivered by increasing soil biological activity. Crop yield response was monitored; water usage and nutrient efficiency calculated at the end of each season.

This case study of 1 of the participating farmers looks at the efficiencies achieved across 7000 hectares of the family farm, identifying correlations between changing biological soil profile, including DNA markers, and improving efficiencies.

Key words: water usage efficiency; nutrient efficiency; regenerative agriculture; biological inoculants; biostimulants.

P13: 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. Probiotics are "live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO Guidelines for the Evaluation of Probiotics in Food), and have the advantage to assure the plants growth potential and increase the crops stress resilience.

In this study, we characterized the 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 a significant impact on plant metabolism.

Taken together, these results show the great economical potential of probiotic rhizobacteria for influencing plant physiology to increase the plant water use efficiency and providing resilience to drought stress without a potential yield penalty.

P14: Fungal endophytes in biological control of Septoria tritici blotch

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Wheat diseases are a frequent cause of considerable yield losses and therefore represent a major threat in modern agriculture. Septoria tritici blotch (STB), caused by the fungus Zymoseptoria tritici (syn. Septoria tritici), is considered the most devastating foliar disease of wheat in the EU. Fungicides are currently the primary control method of STB, since all commercially available wheat cultivars are susceptible to some extent. The increasing resistance of Z. tritici to all common fungicides therefore necessitates the development of innovative and sustainable strategies for effective disease control. Endophytic microorganisms, mainly comprising bacteria and fungi, inhabit inner parts of the plant without causing apparent symptoms on their host. Some endophytes have even been shown to have beneficial effects in different pathosystems, including conferring abiotic stress tolerance, stimulation of growth and decreasing disease severity. Due to these beneficial effects, there is an increasing economic interest in developing endophytes as biocontrol agents. The aim of the project is to identify endophytes that efficiently control STB in a reliable manner and investigate their mechanisms of disease control. In a screening approach, fungal endophytes isolated from wheat were tested for ability to control Z. tritici in vitro and in planta, under both controlled and field conditions. To optimise the application, the mechanisms of selected endophytes in controlling STB are investigated.

P15: Seed treatments with the entomopathogenic fungi Metarhizium robertsii and Beauveria bassiana contribute to spider mite population control above-ground.

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The fungal genera Metarhizium and Beauveria are considered as both entomopathogens and symbionts of plants; i.e. besides causing mortality of economically important arthropod pests, these fungi are also able to colonize a wide variety of plants. The overall aim of this research was evaluating the potential of Brazilian entomopathogenic fungi as inoculants of beans for plant growth promotion and for suppression of population growth of the spider mite Tetranychus urticae. Seed inoculations by two isolates of M. robertsii and B. bassiana individually and in combinations were studied in bean plants, Phaseolus vulgaris. Effects on plant biomass and flowering, and on population growth of T. urticae feeding on inoculated plants were evaluated. In addition, behavioural responses of the predatory mite Phytoseiulus persimilis searching for prey on fungal inoculated plants and feeding on spider mites of these plants were assessed to evaluate potential conflicting effects of the fungal inoculations on overall pest control at higher trophic levels. The results showed a significant reduction in the population growth of T. urticae and larger plants when these were inoculated with M. robertsii and B. bassiana. The production of string beans was higher in inoculated than in non-inoculated plants. Regarding the predatory mite P. persimilis, no difference was observed in the predation rates on T. urticae from treated and untreated plants. The results bring a new perspective on the use of Metarhizium and Beauveria as plant associates revealing that the use of entomopathogenic fungi as inoculants may be a promising strategy beyond direct insecticidal effect.

P16: New plant defenses in a multiresistant dead-end trap crop: Barbarea vulgaris

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A trap crop serves to trap pest insects from a co-cultivated crop. A dead-end trap crop furthermore kills the pest. The dead-end trap crop concept was developed with the multi-resistant wild crucifer Barbarea vulgaris for control of the world's most devastating pest on cruciferous crops: the diamondback moth (1). We are an informal community of five laboratories worldwide that are currently characterizing the ecology, agronomy and evolution of this system (1), along with the chemical, biochemical and genetic basis of defenses (2-5).

The number of antagonists sensitive to the B. vulgaris defenses is considerable, and includes several pests and diseases (1). A non-resistant genotype has been discovered, which is widely distributed in Eastern Europe and enables genetic studies (2, 3, 5). Three groups of metabolites play key roles. In relation to the diamondback moth, glucosinolates stimulate female moths to lay eggs, and saponins deter newly hatched larvae until they die. In relation to another insect, glucosinolates are deterrent or toxic. Finally, in relation to a number of fungal diseases, non-indole phytoalexins inhibit growth. The biosynthesis of saponins and glucosinolates is well-understood (3), and glucosinolates were recently shown to be precursors of non-indole phytoalexins (4). Transcriptomic (3) and genomic (5) resources and methods for genetic modification (3) are being developed and refined (3). The knowledge obtained may allow optimization of the dead-end trap crop properties or use of biosynthetic genes in crops or modified microbial production systems.

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P17: Implications of sequence and timing of exposure for synergy between the pyrethroid insecticide alpha-cypermethrin and the entomopathogenic fungus Beauveria bassiana

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Abstract: Combining low doses of chemical insecticides with entomopathogens constitutes a sustainable pest control method, but significance of timing and sequence of exposures needs clarification. We studied lethal effects of combinations of the entomopathogenic fungus Beauveria bassiana (KVL03-122) and the pyrethroid alpha-cypermethrin on the beetle Tenebrio molitor under varying timing and sequence of exposure. Synergy over time was evaluated in relation to the model of Independent Action (IA). We expected that an increased disease progression by B. bassiana would make beetles more susceptible to the insecticide, leading to enhanced synergy.

Synergistic effects between B. bassiana and alpha-cypermethrin were observed when B. bassiana was applied first, but only when the interval between applications was >48 h. With 72 h between exposures mortality had increased to 100% after 8 days contrary to the 60% mortality expected. No synergy was observed when the insecticide was applied prior to fungal exposure within 24 h.

Sequence and timing of exposure do matter to achieve synergistic mortality of combining B. bassiana and alpha-cypermethrin, and the IA model proved to be a strong tool to evaluate the interactions of the two stressors over time. Pest control strategies could include B. bassiana followed by low dose exposures of alpha-cypermethrin after 2-3 days.

P18: Biostimulant enhance sugar beet growth and development

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Rapid and even establishment of sugar beet seedlings after planting is important for maximizing yield potential. Additives that improve vigour and early growth of young plants would be of interest in seed enhancement technologies in particular if they are sourced from sustainable and abundant resources. Protein hydrolysates have recently been used as biostimulants for enhancing crop growth. Hydrolyzed wheat gluten (HWG) has been used broadly in cosmetics and the brewery industry but not as a crop growth enhancer. The current work aims to investigate the potential of HWG as seed growth enhancer. Three varieties of sugar beet were sown in soil treated with different concentrations (0-20 g/kg soil) of HWG placed 1.5 cm below the seed. Data was collected weekly for five weeks on growth parameters and biomass. Seed emergence was significantly delayed under higher concentrations of HWG (>1 g/kg) across the three varieties. However, there was significant increase in plant height (cm) and leaf area (cm2) of all three varieties with 1 g/kg HWG, compared to other HWG treatments. It appears that 1 g/kg HWG supported more growth of sugar beet compared to other treatments including control. Higher concentrations of HWG than this tended to have a negative effect on plant performance.

P19: Mechanistic studies of molecular bacterial-fungal interactions

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Plant diseases are a major threat to global food security. Intensive use of pesticides and fungicides for controlling microbial diseases has dramatically increased microbial resistance in agricultural systems and negatively impacted biodiversity. One promising alternative for sustainable disease management are microbial biological control agents (mBCAs). Using culture-based approaches, the psychrophile P. fluorescens In5 with antagonistic activity against phytopathogens was isolated from the potato soil microbiome. A combination of molecular genetics and genomics coupled with matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) imaging mass spectrometry (IMS) identified a large genomic island encoding the two non-ribosomal peptides nunapeptin and nunamycin, which are key components of the antifungal activity of In5. Molecular mechanistic studies of bacterial-fungal interactions uncovered a complex interaction whereby nunamycin appears most active against Rhizoctonia solani with no antimicrobial effect against the oomycete Pythium aphanidermatum. In contrast, nunapeptin was most potent against P. aphanidermatum and Fusarium sp. In order to use such biocontrol agents for the management of plant diseases understanding how antifungal compounds are regulated and synthesized is critical. We have recently identified a key regulator of nunamycin and nunapeptin production, which is upregulated in response to carbon sources indicating the presence of a fungus. This suggests that environmental elicitors play an important role in influencing the peptide production required for the growth inhibition of phytopathogens. Understanding the complex mode of action underpinning the antagonism of In5 against phytopathogens is necessary if effective microbial biocontrol agents (mBCAs) for the management of soil-borne plant diseases are to be successfully developed.

P20: MicroProteins, versatile tools to influence plant growth and development

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MicroProteins are small proteins that contain a single protein domain and are related to larger, often multi-domain proteins. At the molecular level, microProteins act by interfering with the formation of higher order protein complexes. In the past years, several microProteins have been identified in plants and animals that strongly influence biological processes. Due to their ability to act as dominant regulators in a targeted manner, microProteins have a high potential for biotechnological use. I will present how microProteins function, what processes they regulate and how we can use them in a targeted manner.

P21: Machine Learning as a tool for biologicals field trials

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The vast majority of expenditure in introducing a new chemical control to the market is spent on field trials, testing its efficacy in different locations, different crops and on different pests. While major agrochemical companies spend around $50 million on in-field testing of chemical controls, biological control products cost around $10 million for market introduction. Inferring that biologicals are not being tested to the same extent in field-trials, this could be at the root of the skepticism in the farming community behind their in-field efficacy (They work sometimes, but not always).

Field trials remain a resource-intensive yet necessary expense for introducing new biologicals. It is here that technology can play a pivotal role in reducing the number of field trials and hence the overall costs. Machine learning is introduced as a tool to conduct in silico analyses to generate insights from massive amounts of field-trial data and to predict the performance of a biological in untested environments. Results from the analyses of a sample data set using Azure Machine Learning will be presented as an alternative, cost-effective field trial tool.

As big data is gathered through automatic sensors in the field, information about soil conditions, atmospheric conditions in the genetic backdrop of multiple genotypes will be leveraged to provide “precision-medicine” for plants in future farming scenarios.

P22: Biocontrol of bacterial pathogens by cytokinin producing bacteria and microalgae

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Considering future demands in plant protection and restrictions in the use of classic pesticides, the development of alternative strategies is a major goal. Biological control of plant diseases by beneficial microbes offers therein a high potential for integrated plant disease management.

Cytokinins are phytohormones that are known for long time to regulate development and physiology, but have only recently been shown to modulate also plant immunity. Cytokinins enhance resistance against the virulent hemibiotrophic pathogen Pseudomonas syringae in tobacco and Arabidopsis. This resistance involves the induction of the two major antimicrobial phytoalexins in tobacco, scopoletin and capsidiol. The integration into the defense network is evident from the involvement of salicylic acid and the negative interference of abscisic acid.

The mechanism of cytokinin-triggered immunity was also shown to be the basis for the biocontrol activities of a beneficial P. fluorescence strain that had been identified based on its growth promotion. Complementary gain- and loss-of-function approaches with the host plant and the biocontrol strain identified the microbial cytokinin production as a key determinant of the interkingdom protection of Arabidopsis and tobacco from P. syringae (*).

The cytokinin mediated biocontrol ability was shown to be also operational in cytokinin producing pro- and eukaryotic microalgae. Both living algae and extracts protect Arabidopsis and tobacco from infection by P. syringae. The analyses of cytokinin deficient Chlamydomonas reinhardtii insertion and genome editing mutants substantiated the role of cytokinin production by the alga for the pathogen protection. These finding provides a proof of concept that it is possible to combine the cytokinin mediated biocontrol ability with the natural bio-fertilizer ability of microalgae.

Based on the known effect of cytokinins to improve also drought tolerance it is expected that the cytokinin mediated biocontrol ability could be the basis to engineer a biologicals based abiotic and biotic cross tolerance for practical application in agriculture.

(*) Großkinsky et al. (2016) Sci. Rep. 6: article 23310, doi:10.1038/srep23310

P23: Microbe induced Resistance to Agricultural pests

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Plants are intimately associated with a diversity of beneficial microorganisms in and around their roots, 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 plant production has emerged as a promising possibility to improve resilience and reduce harmful pesticide use. European companies have therefore started to develop and market such beneficial microbes. 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 ongoing EU-funded Innovative Training Network, MiRA (miraitn.eu), we train 15 PhD students in basic and applied research on context-dependency of MiR, mechanisms, and impacts on plant performance and other biocontrol organisms. We use this to improve our ability to predict effectiveness of MiR, select plant and microbial strains with improved context-stability, and develop better methods for formulation of microbial inoculants and application in agriculture. The PhD students are trained within a consortium of research institutions and companies from seven European countries, including microbial inoculant producers and agricultural advisors.

At University of Copenhagen, three PhD students will study how insect resistance, induced by mycorrhiza in tomato, is affected by simultaneous attack by plant pathogens, compatibility between resistance-inducing mycorrhiza and root-associated entomopathogenic fungi, and mechanisms of bacteria-induced insect resistance and its moderation by drought.

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