Plant Biologicals Network annual symposium 2019

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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 about 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 are 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.

Core Members

Ordinary Members

| Agrolab | Azelis Denmark | Syngenta Nordic | EWH BioProduction |

| Lund Universitet | Ingleby Farms & Forests | Chart Biotechnology |

| Manna Regulatory | BioScience Solutions | UCL University College |

Symposium Programme

Plant Biologicals Network

PBN annual symposium	
13-14 November	
Wednesday 13 November	
12:00	Registration and lunch
13:00	Welcome and opening address
13:10	**Opening Keynote Presentation** *How biology-based technologies can contribute to achieving the Sustainable Development Goals* - **Dr. Morven McLean**, Executive Director, ILSI Research Foundation, USA
14:00	Coffee break
14:25	**Increased plant resilience to climate change** Chair: **Svend Christensen**, Head of Department, University of Copenhagen o *Reducing pest populations by direct and indirect effects using fungal inoculations in horticultural crops* - **Nicolai Vitt Meyling**, Associate Professor, Department of Plant and Environmental Sciences, University of Copenhagen o *Practical experiences with biologicals in organic/biodynamic cropping systems* - **Thomas Harttung**, Organic/biodynamic farmer and entrepreneur at Barritskov and Krogerup o Selected flash talks: *Mitigation of Salinity Stress by Arbuscular Mycorrhizal Fungi* - **Bhoopander Giri**, University of Delhi *Grow the Perfect Seed - Phenotyping with Isotope Ratio Infrared Spectrometry* – **Søren Dalby**, Thermo Scientific

	ABA-mediated modulation of elevated CO2 on stomatal response to drought - **Shenglan Li**, University of Copenhagen
15:30	**Coffee break** **Poster session 1**
16:15	**How to ensure efficacy of plant biologicals** Chair: **Mette Walter**, Head of section, Danish Technological Institute ○ *Learning the difference – Switching your mindset from classical chemical to microbial products* - **Charlotte Klank**, EMEA Plant Health R&D leader at FMC Corporation ○ *Experiences from efficacy testing of Microbial products and alternative chemistry* - **Lise Nistrup Jørgensen**, Senior Scientist, Department of Agroecology, Aarhus University ○ Selected flash talks: *Soil amendment with C sources promotes P biofertilizer traits of Penicillium aculeatum* - **Beatriz Gómez-Muñoz**, University of Copenhagen *Indications of improved germination of sugarbeet pre-washed and primed with protein-based biostimulants* - **Jolayemi Okanlawon Lekan**, Swedish University of Agricultural Sciences *A structured screening approach for identification of fungal endophytes for Septoria tritici blotch control* - **Meike A. C. Latz**, University of Copenhagen
17:20	**Reception** **Poster session 2**
18:00	**Transport to symposium dinner venue (busses will be arranged)**
18.30	**Symposium dinner** **Vestauranten, DGI-Byen, Tietgensgade 65, 1704 Copenhagen V**
21.00	**Optional bus transport from the restaurant back to the symposium venue**

Thursday 14 November

8:30

Biological products for pest and disease control

Chairs: **Niels Kristian Sørensen**, Director Biological Research, FMC

- *Potato disease resistance and towards development of a plant strengthener* – **Erik Andreasson**, Professor in Plant Protection and Head of the Resistance Biology Unit, Swedish University of Agricultural Sciences
- *From Microbiome Analysis to Commercial Product* - **Marcus Meadows-Smith**,– CEO, BioConsortia
- Selected flash talks:

  Bacteriophages as biocontrol agents in agriculture - **Alexander Byth Carstens**, University of Copenhagen

  Studying B. subtilis *in root colonization of different plant species* - **Christopher Blake**, Technical University of Denmark

  Biological control of Fusarium head blight in wheat using naturally occurring endophytes - **Edward C. Rojas**, University of Copenhagen

9:35 Coffee break

10:00

Tomorrow's sustainable agriculture with microbes

Chairs: **Lars Mølbak**, R&D Manager, Chr. Hansen and **Carsten Suhr Jacobsen**, Professor and Head of Department, Aarhus University

Presentation of the Collaborative Crop Resilience Program (CCRP) funded by the Novo Nordisk Foundation.

- *The MATRIX project: Microbiome Assisted Triticum Resilience In X-dimensions* - **Lars Hestbjerg Hansen**, Professor, Department of Plant and Environmental Sciences, University of Copenhagen
- *INTERACT: Decoding the Rhizobiota Interactome for Improved Crop Resilience* - **Mette Haubjerg Nicolaisen**, Associate Professor, Department of Plant and Environmental Sciences, University of Copenhagen
- *The InRoot project of the Collaborative Crop Resilience Program. Molecular Mechanisms and Dynamics of Plant-Microbe Interactions at the Root-Soil Interface* - **Jens Stougaard**, Professor, Department of Molecular Biology and Genetics, Aarhus University
- Selected flash talks:

	Arbuscular mycorrhizal symbiosis is negatively regulated by a plant CLE peptide - **Thomas de Bang**, University of Copenhagen *The rule of natural products in belowground interactions between plant species* - **Hossein Hazrati**, Aarhus University *Characterization of a Novel Bacillus pumilus Strain as a Plant Growth-Promoting Rhizobacteria* - **Asger Ourø Jensen**, University of Copenhagen
11:05	**Coffee break** **Poster session 3**
11:50	**Integrating biology-based and conventional crop protection** Chairs: **Niels Bjerre**, Agricultural Affairs Manager, Bayer and **Troels Toft**, Sector Director Plants, SEGES o *Integrating biologicals in a crop protection schedule: robust and cost effective schedules are prerequisites for successful market penetration* - **Jolanda Wijsmuller**, Value Chain, Biologics & Minor Crops Manager, Bayer o *From pain points to opportunities: Integrating biology-based and conventional crop protection* - **Harry Teicher**, Principal Scientific Consultant, BioScience Solutions o Selected flash talks: *Quercetin; as a promising bioactive compound to alter the concentration of pesticides in honey bees* - **Hamidreza Ardalani**, Aarhus University *Combination of biological control agents and fungicides to control diseases in wheat and reducing the risk of fungicide resistance* - **Birgit Jensen**, University of Copenhagen *Quantifying synergistic effects of combining chemical and immune stressors* - **Nina Cedergreen**, University of Copenhagen
12:55	**Closing remarks**
13:00	**Lunch grab bag**

Plant Biologicals Network members

Symposium sponsors

Opening Keynote Presentation

How biology-based technologies can contribute to achieving the Sustainable Development Goals

Morven A. McLean, Ph.D.

ILSI Research Foundation, USA

When we look at the social, economic and environmental impacts of climate volatility and extreme weather events, it's clear that transformation of our agricultural systems is both a societal and environmental imperative. How can we sustainably increase agricultural production and improve food and nutrition security for populations around the world, and particularly in those areas most vulnerable to the effects of climate change?

Innovations in plant biologicals product development make clear there is a significant role for their application in addressing these challenges. However, realizing the benefits of biologicals for sustainable agricultural production requires an enabling policy environment and a predictable and transparent path to commercialization. Regulation, when considered necessary, should be properly contextualized and commensurate with plausible risks, so that moving from product development to deployment at scale is achievable by public and private sector institutions alike. This presentation will explore the promise of plant biologicals to helping achieve the UN's Sustainable Development Goals (SDGs), including *Goal 2: Zero Hunger*, *Goal 6: Clean Water and Sanitation*, *Goal 12: Responsible Consumption and Production*, *Goal 14: Life Below Water* and *Goal 15: Life on Land*. It will also consider some lessons learned from the regulation of genetically engineered organisms, where asynchronous approaches to risk assessment, regulation and decision-making have constrained innovation and contributed to trade disruptions, and how these experiences may be instructive as more plant biologicals move to market.

Increased plant resilience to climate change

<u>Invited speakers</u>

Reducing pest populations by direct and indirect effects using fungal inoculations in horticultural crops
Nicolai Vitt Meyling, Associate Professor, Department of Plant and Environmental Sciences, University of Copenhagen

Practical experiences with biologicals in organic/biodynamic cropping systems
Thomas Harttung, Organic/biodynamic farmer and entrepreneur at Barritskov and Krogerup and co-founder of Arstiderne and BeyondCoffee, Denmark

<u>Selected flash talks</u>

Mitigation of salinity stress by arbuscular mycorrhizal fungi
Bhoopander Giri, Assistant Professor, University of Delhi, India

Grow the perfect seed – Phenotyping with Isotope Ratio Infrared Spectrometry
Søren Dalby, Thermo Scientific, Denmark

ABA-mediated modulation of elevated CO2 on stomatal response to drought
Shenglan Li, Phd Student, University of Copenhagen

Reducing pest populations by direct and indirect effects using fungal inoculations in horticultural crops

Nicolai V. Meyling

Department of Plant and Environmental Sciences, University of Copenhagen, Denmark

Fungi that infect insects and mites, entomopathogenic fungi, have long been studied and applied for biological control of pests in horticultural production systems by exploiting the unique ability of these fungi to infect and kill their hosts. Benefitting from such direct effects of the fungi may be compromised by a requirement for specific abiotic factors and the challenge of ensuring sufficient contact between fungal propagules and insect hosts. Below-ground application of the fungi against soil-dwelling pests seems most feasible using suitable formulations. While in the soil environment, the fungi can also interact with the plant roots, colonize the plant rhizosphere and in some cases become endophytic. These associations with plants may cause negative effects against pests feeding on the above-ground parts of the plant, but the mechanisms behind these effects are still unresolved. It is hypothesized that the defence system of the plant is modulated by the fungus-plant interaction, indicating an indirect effect of the fungal inoculation on the pest population. Derived benefits such as improved plant growth and crop yield are also reported. Examples of how entomopathogenic fungi can affect pests directly and indirectly when inoculated to horticultural crops will be presented and the mechanisms discussed. Ideally, a single soil application of these fungi would target pests both below- and above-ground through a combination of direct and indirect interactions.

Practical experiences with biologicals in organic/biodynamic cropping systems

Thomas Harttung

Organic/biodynamic farmer and entrepreneur at Barritskov and Krogerup and co-founder of Aarstiderne and BeyondCoffee.

Organic farming can be organized to counter climate change, not only by avoiding the use of fossile fuel based pesticides and artificial fertilizers but by looking at the whole picture of carbon cycles, energy consumption, biodiversity and the social structures between farmers and citizens. In the coming years, the next version of organic agriculture will come into being. This includes new agricultural methodologies and technologies targeted specifically to organic and biodynamic farming. In this talk I will present some of the biological solutions that are being used at the Barritskov and Krogerup farms, and how I see the future development of organic/biodynamic cropping systems.

Mitigation of Salinity Stress by Arbuscular Mycorrhizal Fungi

Bhoopander Giri

Swami Shraddhanand College, University of Delhi, Delhi 110036, India

Soil salinity is a problem of serious concern for sustainable agriculture as it is steadily increasing throughout the world, may be due to fluctuations in climatic conditions, intensive use of chemicals fertilizers and pesticides and industrialization. Excessive concentration of soluble salts in soils or irrigation water adversely affects soil physico-chemical properties, and thereby reduce growth and productivity of plants. In the arid and semi-arid areas, due to low precipitation and high evaporation rates and scanty rainfall, this problem is indeed higher. This planet is inhabited by an array of microorganisms, which do perform a variety of functions. Amongst them, arbuscular mycorrhizal (AM) fungi are most commonly distributed soil organisms, establishing symbiotic associations about 90% of terrestrial plant species. They appear to be most promising soil fungi due to their well-established capability of increasing crop production by helping plants to acquire soil nutrients and combating abiotic stresses like drought and salinity. Our field experimentation with AM fungi revealed that inoculation with mycorrhizal fungi helped host plant in maintaining lower lipid peroxidation and electrolyte leakage, increased activity of reactive oxygen species scavenging enzymes and accumulation of antioxidants. AM fungi had a regulatory effect on the upward movement of Na^+ ions, improve plant's tolerance to salt stress and mitigate injurious effects of toxic ions. The study showed that mycorrhizal fungi mitigate adverse effects of salinity by diminishing ultra-structural aberrations, preventing excessive uptake of Na^+ in plant cells explicitly with increasing levels of soil salinity. This distinctive behavior of AM fungi indicate that they play a constructive role in reducing ion toxicity and minimizing cell damage. Moreover, mycorrhizal fungi have been found to play an affirmative role in improving plants osmotic adjustment under saline conditions.

Grow the Perfect Seed - Phenotyping with Isotope Ratio Infrared Spectrometry.

Søren Dalby

Thermo Scientific

Here we present a novel non-destructive approach to whole plant physiology by observing rapid changes in plant metabolism by simultaneously monitoring carbon and oxygen isotope ratios of carbon dioxide under varying ambient conditions in a plant chamber measured by a field applicable laser-based Isotope Ratio Infrared Spectrometer. Carbon stable ($\delta^{13}C$) isotope composition of CO_2 is an indicator of water use efficiency and response to drought in crops. With traditional methods, the changes in plant canopy were visible after four days by monitoring the ratio of ^{12}C and ^{13}C in CO_2 changes are already visible in 24 hours

ABA-mediated modulation of elevated CO2 on stomatal response to drought

Shenglan Li[1], Xiangnan Li[3,**], Zhenhua Wei[2], Fulai Liu[1,2,*]

[1]Department of Plant and Environmental Sciences, Faculty of Science, University of Copenhagen, Højbakkegaard Allé 13, DK-2630, Taastrup, Denmark
[2]Key Laboratory of Agricultural Soil and Water Engineering in Arid and Semiarid Areas, Ministry of Education, Northwest A&F University, Yangling, Shaanxi 712100, China
[3]Key Laboratory of Mollisols Agroecology, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Changchun 130102, China

Elevated atmospheric CO_2 concentration ($e[CO_2]$) and soil water deficits have substantial effect on stomatal morphology and movement that regulate plant water relations, and $e[CO_2]$ could alleviate the impact of drought stress. Xylem-borne abscisic acid (ABA) plays a crucial role in regulating stomatal aperture serving as first line of defence against drought; whereas $e[CO_2]$ may disrupt this fundamental drought adaptation mechanism by delaying the stomatal response to soil drying. We review the state-of-the art knowledge on stomatal response to drought stress at $e[CO_2]$ and discuss the role of ABA in mediating these responses.

How to ensure efficacy of plant biologicals

Invited speakers

Learning the difference – Switching your mindset from classical chemical to microbial products (preliminary)
Charlotte Klank, EMEA Plant Health R&D leader, FMC Corporation

Experiences from efficacy testing of Microbial products and alternative chemistry
Lise Nistrup Jørgensen, Senior Scientist, Department of Agroecology, Aarhus University

Selected flash talks

Soil amendment with C sources promotes P biofertilizer traits of Penicillium aculeatum
Beatriz Gómez-Muñoz, Assistant Professor, University of Copenhagen

Indications of improved germination of sugarbeet pre-washed and primed with protein-based biostimulants
Jolayemi Okanlawon Lekan, PhD Student, Swedish University of Agricultural Sciences

A structured screening approach for identification of fungal endophytes for Septoria tritici blotch control
Meike A. C. Latz, PhD, University of Copenhagen

Learning the difference – Switching your mindset from classical chemical to microbial products

Charlotte Klank

EMEA Plant Health R&D leader, FMC Corporation

Ensuring reliable efficacy for products often loosely grouped as "biologicals" remains one of the key success factors for their acceptance and more widespread use. In the past, individual products have been introduced to the market where the actual performance in the field did not meet the expectations of the end-users, leading to a skeptical perception of these products. In our talk, we would like to share our experiences with developing microbial products that have a consistent efficacy, highlighting key areas to address, especially with regards to fundamental differences to chemical products. Furthermore, we will address how important aspects to consider when introducing biological products to the market, such as addressing the perception bias that can play a crucial role in the success of a product.

Experiences from efficacy testing of microbial products and alternative chemistry

Lise Nistrup Jørgensen, Thies M. Heick, Peter Hartvig, Louise Hjelmroth

Department of Agroecology, Aarhus University, Denmark

As a result of increasing public concerns about pesticide uses in food production, many activities in the area of testing alternative chemistry have been initiated. The wish is to investigate if more environmentally friendly control measures can replace or complement pesticides currently used. In recent years, small enterprises, as well as large chemical companies, have invested greatly in the development of alternative chemistry and biologicals.

In vitro testing of products will often initially show good potential for disease control, and similarly greenhouse testing often provides significant efficacy. A major challenge, however, remains to provide reliable control under outdoor conditions. The main problems when introducing BCA and alternatives to outdoor conditions are the more variable weather and cropping conditions, which not always support control conditions, and which typically compromise a more persistent effect. The timing of treatments under both greenhouse and field conditions is similarly more challenging, as control often needs to be preventive or early curative, requiring timely and frequent treatments.

Depending on the character of the product, the requirements for documented efficacy is variable and typically less demanding compared with traditional chemistry. Regulation 1107/2009 describe the categories of 'Low-risk' (Articles 22 and 47) and ''Basic substances' (Article 23)

- The EU’s list of basic substances, e.g. baking powder, lecithin, does not require specific GEP trials; however, documentation from peer-review papers, etc. may support an application.
- The EU’s low-risk product list includes substances as *Bacillus subtilis* and laminarin. Principles of efficacy for low risk products are described in EPPO guideline PP1/296. Authorisation of these products require GEP efficacy trials, but the efficacy levels accepted are lower compared with traditional chemistry, and more possibilities for extrapolations are made available.

Experiences from testing of alternative chemistry and microbial products will be presented during the presentation.

Soil amendment with C sources promotes P biofertilizer traits of *Penicillium aculeatum*

Beatriz Gómez-Muñoz[1], Aikaterini Efthymiou[1], Mette Nicolaisen[2], Ole Nybroe[2], John Larsen[2,3]

[1]Section for Plant and Soil Science, Department of Plant and Environmental Sciences, University of Copenhagen, Denmark,
[2]Section for Microbial Ecology and Biotechnology,
Department of Plant and Environmental Sciences, University of Copenhagen, Denmark,
[3]Instituto de Investigaciones en Ecosistemas y Sustentabilidad, Universidad Nacional Autónoma de México, México

Successful application of microbial biofertilizers, such as phosphorus (P) solubilizing fungi to agroecosystems, is constrained from the lack of knowledge about their soil ecology; for example in terms of how they respond an external input of carbon to get established in soil. In soil microcosm experiments we examined the performance of the P solubilizing fungus *Penicillium aculeatum* (Pa) in semi-sterile (irradiated) and nonsterile soil without and with carbon source (starch or cellulose) amendment. The ability of Pa to solubilize sewage sludge ash-P and tricalciumphosphate (TCP) by in semi-sterile and non-sterile low P soil amended with cellulose was also examined. Soil inoculation with Pa resulted in increased fungal population density and microbial respiration, but only in semi-sterile soil amended with cellulose as carbon source. In semi-sterile soil, Pa increased the amount of water extractable P (PWEP), but only when no carbon source was added, whereas in non-sterile soil, Pa increased the amount of PWEP, only in combination with carbon source amendments. Overall inoculation with Pa increased the amount of microbial P (Pmic), with the exception of the treatment with cellulose in non-sterile soil, where Pa reduced Pmic. Inoculation with Pa resulted in increased PWEP in the TCP, sewage sludge ash and nil P treatments, and Pmic was highest in the TCP treatment. In conclusion, amendment of a carbon source markedly improved the performance of Pa in soil in terms of growth and P-solubilization from soil mineral P, TCP and sewage sludge ash both as PWEP and Pmic, though more clearly in semisterile soil most likely due to less competition from other saprotrophic microorganisms.

Indications of improved germination of sugarbeet pre-washed and primed with protein-based biostimulants.

Jolayemi O.L.[1], A.H. Malik[2], T. Ekblad[3], M. Olsson[1], E. Johansson[1]

[1]Swedish University of Agricultural Sciences (SLU), Alnarp, Sweden;
[2]Nelson Seed Development AB, Lund, Sweden;
[3]Maribo Hilleshög Research AB, Landskrona, Sweden

Uniform and rapid germination of sugarbeet seeds after planting remains a major challenge to sugarbeet production. The presence of germination inhibitors found in the seed coats is responsible for the poor germination. Prolonged washing with water has been suggested to reduce the effect of these inhibitors. This experiment aimed to investigate the effect of hydrolyzed wheat gluten (HWG) and potato protein (Pp) priming (priming agents), pre-washed with saline (10 g/l NaCl solution) or tap water on sugarbeet germination. Sugarbeet seeds were pre-washed in either tap water or 10 g/L saline, slightly agitated at 100 r/min for 4 h and re-dried to initial weight. Then, pre-washed seeds were primed with solutions of biostimulant (hydrolyzed wheat gluten- HWG and potato protein- Pp) slightly agitated at 100 r/min for 48 h and re-dried for 48 h to initial weight. There was significant ($p<0.05$) effect of pre-washing on germination, as saline improved germination of sugarbeet compared to tap water and unwashed seeds. The interaction effect of pre-washing and the different concentrations of priming agents was highly significant on germination. Saline pre-washing and milliQ water priming gave the highest germination weight and it is similar to the germination of saline pre-washed seeds primed in 10 g/L HWG and 1 g/L Pp. Only 1 g/L Pp pre-washed in tap water was also similar to the former treatments. Unwashed sugarbeet seeds were generally low in germination across all treatments. Therefore, 10 g/L HWG and 1 g/L Pp priming pre-washed in saline, improved germination of sugarbeet as well as 1 g/L Pp pre-washed with tap water.

A structured screening approach for identification of fungal endophytes for Septoria tritici blotch control

Meike A. C. Latz[1,2], David B. Collinge[2], Birgit Jensen[2] and Hans Jørgen L. Jørgensen[2]

1 Division of Gene Technology, School of Biotechnology, KTH Royal Institute of Technology, Science for Life Laboratory, 17165 Solna, Sweden
2 Department of Plant and Environmental Sciences, Faculty of Science, University of Copenhagen, 1871 Frederiksberg C, Denmark

In a structured screening approach, we identified promising biological control agents (BCAs) against the foliar wheat disease Septoria tritici blotch (STB), with potential for commercial application. STB, caused by the fungus *Zymoseptoria tritici*, is considered the most devastating foliar disease of wheat in the EU. Due to insufficient resistance in commercial cultivars and increasing fungicide resistance in the pathogen population, alternative means of control are much needed.
Since a limitation of many screening programmes is that BCAs with an effect *in vitro* do not necessarily show an effect *in planta*, we relied on *in planta* screening and aimed to optimise host and environmental compatibility of the BCAs by isolating endophytic fungi from the plant, tissue and environment of future application. Furthermore, *in planta* screening was complemented by a small-scale field experiment and *in vitro* characterisation (temperature requirements, sporulation potential and fungicide tolerance) as well as a literature search, to select the most promising isolates for further work. Our approach resulted in identification of two promising fungal strains that significantly and reliably reduced STB by up to 75% under controlled conditions. Significant disease reduction was also observed under field conditions.
The two endophyte isolates have the potential for implementation in integrated disease management strategies using both fungicides and BCAs, because of their fungicide tolerance. Integrated approaches, combining different agents, offer a more sustainable and durable way to control STB. They would allow the reduction of fungicide inputs and thus help reduce the development of fungicide resistance in the pathogen population.

Biological products for pest and disease control

Invited speakers

Potato disease resistance and towards development of a plant strengthener
Erik Andreasson, Professor, Swedish University of Agricultural Science

From Microbiome analysis to commercial product
Marcus Meadows-Smith , CEO, BioConsortia

Selected Flash talks

Bacteriophages as biocontrol agents in agriculture
Alexander Byth Carstens, Postdoc, University of Copenhagen

Studying B. subtilis in root colonization of different plant species
Christopher Blake, Master Student, Technical University of Denmark

Biological control of Fusarium head blight in wheat using naturally occurring endophytes
Edward C. Rojas, PhD Student, University of Copenhagen

Potato disease resistance and towards development of a plant strengthener

Erik Andreasson

Swedish University of Agricultural Science, Alnarp, Sweden

[Abstract pending]

From Microbiome Analysis to Commercial Product

Marcus Meadows-Smith

BioConsortia Inc

BioConsortia's patented "Advanced Microbial Selection" (AMS) R&D platform uses an iterative process of directed selection and evolution of the plant microbiome, aided by microbiome analysis and genomics, to identify individual strains and small teams of microbes that improve plant traits. With additional techniques such as fermentation and formulation optimization, tagging and root colonization robustness, the target is to develop biological biopesticides and biostimulant products with superior efficacy and higher consistency. Examples of successful fungicide, nematicide and yield enhancing biostimulant products will be used to demonstrate the power of this revolutionary platform.

Bacteriophages as biocontrol agents in agriculture

Alexander B. Carstens, Amaru M. Djurhuus, Witold Kot, & Lars H. Hansen

Department of Plant and Environmental Science, University of Copenhagen, Denmark

Plant pathogens are responsible for huge losses in the global food production. As a result, farmers are turning to pesticides and chemical sprays (e.g. copper) for the control of pathogens. However, these treatments often carry negative effects to both human health and the environment. Alternatives to these treatment options are therefore necessary. Biocontrol is often suggested as a promising environmentally friendly alternative to pesticides and chemical sprays. Here, we investigate the potential for using bacteriophages as biocontrol agents against bacterial plant pathogens. Bacteriophages have some unique properties that make them ideal as biocontrol agents. We isolated over 150 bacteriophages acting against nine different species of bacterial plant pathogens. The isolated phages represent 13 previously undescribed bacteriophage genera and at least 40 new species. We sequenced and further characterized the bacteriophages in order to determine the bacteriophages best suited for use as biocontrol agents. Two bacteriophage cocktails each containing six phages were constructed form the isolated phages and applied two independent *in vivo* experiments to treat soft rot in potato tubers. Bacteriophage treatment reduced both disease incidence and disease severity by more than 60% in tubers infected with either *P. atrosepticum* or *D. solani.* These results indicate that bacteriophage biocontrol has potential as an environmentally friendly approach to combatting bacterial plant pathogens.

Studying *B. subtilis* in root colonization of different plant species

Christopher Blake, Mathilde Nordgaard Christensen, Ákos T. Kovács

Department of Biotechnology and Biomedicine, Danmarks Tekniske Universitet, Denmark

The soil bacterium *B. subtilis* is known to supress pathogens as well as promote plant growth. It therefore has a huge potential as natural fertilizer, replacing chemicals traditionally used in agriculture, which are known to cause environmental threats. However, in order to fully exploit this potential of *B. subtilis* we need a better understanding of the interactions between *B. subtilis* and plants. In this study, *B. subtilis* will be examined for root colonization through experimental evolution of the bacterium on different plant species. The evolved strains will be studied for improved root colonization and re-colonization, as well as be re-sequenced to identify genetic alterations. This will allow identification of important genes involved in root colonization of and reveal how *B. subtilis* adapt to different plant species. Furthermore, the importance of chemotaxis and motility in root (re-)colonization will be examined by testing corresponding *B. subtilis* mutants on roots using fluorescence microscopy.

Biological control of Fusarium head blight in wheat using naturally occurring endophytes

Edward C. Rojas, Birgit Jensen, Hans Jørgen L. Jørgensen and David B. Collinge

Department of Plant and Environmental Sciences and Copenhagen Plant Science Centre, University of Copenhagen, Denmark

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 endophyte communities on wheat spikes during FHB infection? 2) Can naturally occurring 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. Fungal taxa such as *Cladosporium*, *Itersonillia* and *Holtermanniella* were negatively correlated to pathogen abundance or were enriched in spikes which remained healthy after pathogen exposure. 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 mode of action of endophyte-mediated biocontrol was studied using RNAseq. The endophyte activates 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.

Tomorrow’s sustainable agriculture with microbes

Invited speakers

The MATRIX project: Microbiome Assisted Triticum Resilience In X-dimensions
Lars Hestbjerg Hansen, Professor, Department of Plant and Environmental Sciences, University of Copenhagen

INTERACT: Decoding the Rhizobiota Interactome for Improved Crop Resilience
Mette Haubjerg Nicolaisen, Associate Professor, Department of Plant and Environmental Sciences, University of Copenhagen

The InRoot project of the Collaborative Crop Resilience Program. Molecular Mechanisms and Dynamics of Plant-Microbe Interactions at the Root-Soil Interface
Jens Stougaard, Professor, Department of Molecular Biology and Genetics, Aarhus University

Selected flash talks

Arbuscular mycorrhizal symbiosis is negatively regulated by a plant CLE peptide
Thomas de Bang, Assistant Professor, University of Copenhagen

The rule of natural products in belowground interactions between plant species
Hossein Hazrati, PhD Student, Aarhus University

Characterization of a novel Bacillus pumilus strain as a plant growth-promoting rhizobacteria
Asger Ourø Jensen, Master Student, University of Copenhagen

Microbiome Assisted Triticum Resilience In X-dimensions

Lars Hestbjerg Hansen

Department of Plant and Environmental Sciences, University of Copenhagen, Denmark

In early 2020 we start a new project funded by the Novo Nordisk foundation called the MA*T*RIX. The project will focus on a full systems description of the wheat flag leaf. The overarching objective is to develop a predictive deep-learning model through mapping of the taxonomic and functional diversity and temporal-spatial dynamics of the wheat phyllosphere microbiome in relation to climate and epidemiological data. The MA*T*RIX model will be used to understand and predict crop yield, microbiome dynamics and disease trajectories in the face of environmental changes and other stresses. In addition, we will use the MA*T*RIX model to examine intervention strategies, including introductions of beneficial hub microbes to increase yield and prevent or suppress diseases.

Comprehensive data collection from large-scale greenhouse and field perturbation experiments with diverse wheat agroecosystems will be used to validate, refine and optimize the developed model.

The consortium will work on wheat in field sites at different agroecological locations in two countries (Denmark, USA). We will integrate metagenomics, metatranscriptomics and metabolomics data, plant traits (physiological, morphological, molecular), imaging and environmental data (soil and climate), epidemiological data and existing mechanistic models into a systems-based deep-learning model. This model should predict the relative contribution of the different variables for wheat resiliency and productivity in the face of biotic and abiotic stresses. This presentation will highlight the raison d'etre behind the project.

INTERACT: Decoding the Rhizobiota Interactome for Improved Crop Resilience – a project under The Collaborative Crop Resilience Program funded by the Novo Nordisk Foundation

Mette Haubjerg Nicolaisen

Department of Plant and Environmental Sciences, Section for Microbial Ecology and Biotechnology, University of Copenhagen, Denmark

During the last decade and along with technological developments, the importance of microbial functioning for plant health and performance has been established, and the potential of using microbial inoculants for improved plant growth by enhanced nutrient acquisition, or by increasing resistance to biotic and abiotic stress scenarios is currently gaining significant interest in both academia and industry. The complexity of the soil surrounding the roots *i.e.* the rhizosphere, both biologically and chemically, however, often hampers translation of beneficial plant-microbe interactions from laboratory testing to biotechnological solutions in real plant production systems, and render predictions of plant responses to specific stress scenarios challenging. Two of the major obstacles for fully exploiting this potential are 1) the lack of deep understanding within microbial ecology of how plant-microbial systems are established and maintained in relation to fluctuating environments during the life cycle of the plant and 2) difficulties inherent to accurate analytics for plant-microbe community characterization under real soil conditions, owing to the complexity of the soil system. Hence, understanding the network of microbial interactions, and how these support plant-resistance to abiotic and biotic stress under constantly changing environmental conditions, will be key to unlocking the enormous biotechnological potential of microbes, leading to sustainable crop productions systems. The overall aim of INTERACT is therefore to decode these important, yet often transient, microbial interactions in the complex soil matrix, in relation to soil biogeochemical status, water stress, and pathogen attack, and to establish the impact of these interactions on plant performance.

The *InRoot* project of the Collaborative Crop Resilience Program. Molecular Mechanisms and Dynamics of Plant-Microbe *In*teractions at the *Root*-Soil Interface.

Jens Stougaard

Department of Molecular Biology and Genetics, Aarhus University, Denmark

The NovoNordisk Foundation has launched a new initiative called “The Collaborative Crop Resilience Program (CCRP)” to address challenges for agricultural productivity and sustainability. InRoot is one of the CCRP subprojects and will focus on the molecular mechanisms and dynamics of plant-Microbe interactions at the root-soil interface using the model legume *Lotus japonicus* (japansk kællingetand) and wheat as experimental systems. To achieve these overarching objectives InRoot will investigate both the plant and the bacterial contributions to crop resilience. Combining expertise from groups at Aarhus University, the University of Copenhagen, the Technical University of Denmark, North Carolina State University, Berkeley, Tohoku University and Utrecht University, InRoot will use interdisciplinary approaches connecting plant and bacterial genetics, protein biochemistry, analytical chemistry and plant physiology with bacterial and plant population biodiversity studies and advanced modeling. The aim and perspective of InRoot is to provide knowledge and tools for evidence-based development of new resilient crops and associated microbial interventions that will improve productivity, reduce the need for fertilizers and pesticides, and alleviate negative environmental impacts that accompany our food production. Plant roots and soil microbes have been associated since the emergence of plants on land. Nevertheless, the mechanisms that coevolved to control and regulate microbiota associations with healthy plants are largely unknown. Improving our understanding of this complex interaction will provide opportunities to reduce the environmental footprint and could also define breeding targets and develop applications through microbial interventions.

Arbuscular mycorrhizal symbiosis is negatively regulated by a plant CLE peptide

Thomas de Bang[1], Karlo M[1], Boschiero C[2], Landerslev KG[1], Blanco GS[1], Wen J[2], Mysore KS[2], Dai X[2], Zhao PX[2]

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Phosphorus (P) is an essential mineral nutrient for plants, but often a limiting factor for growth and crop productivity. The straight-forward solution to this problem has been to apply more P-fertilizer to fields. However, crops only takes up 20% of applied P-fertilizer due to the physicochemical properties of phosphate in soil. Consequently, this practice has led to high P levels in agricultural soils, especially in Europe. Symbiosis with mycorrhizal fungi can increase plant P uptake, but the association is hampered at high P levels, thereby representing a paradox in modern agriculture.

Plants inhibit mycorrhizal colonization by a mechanism called Auto-regulation of mycorrhization (AOM). The AOM mechanism has been known for many years and involves root-to-shoot-to-root signaling; however the molecular details have largely remained unknown. In this work, we identified a small signaling peptide from the CLAVATA3/ENDOSPERM SURROUNDING REGION (ESR)-RELATED (CLE) peptide family as the likely root-to-shoot mobile signal. This CLE peptide is repressed at low P conditions but induced at high P conditions and by mycorrhizal colonization. Overexpression of the CLE peptide reduced overall mycorrhizal colonization, while colonization increased in the mutant.

In perspective, understanding the AOM signaling pathway in detail will enable breeding of crops that will engage in mycorrhizal symbiosis at both high and low P levels, which in turn will reduce the requirements of P-fertilizer.

The rule of natural products in belowground interactions between plant species

Hossein Hazrati, Inge S. Fomsgaard, Bo Melander, Per Kudsk

Department of Agroecology, Faculty of Science and Technology, Aarhus University, Denmark

Plants are consistently releasing root exudates into the rhizosphere which have the potential to influence the growth and development of neighboring plant species. Root exudates contain low and high molecular weight compounds such as sugars, amino acids, secondary metabolites, phytohormones, proteins, enzymes, and polysaccharides. Although aboveground plant interactions through volatile compounds has been extensively studied, only few studies investigated role of the belowground chemical interactions between plant species. The aim of this study is to elucidate the belowground chemical interactions between rye and hairy vetch grown together as cover crop mixture. Rye and hairy vetch were cultivated alone and together in pots filled with micro glasssbeads. Plant were grown for three weeks in climate chamber and their root exudates were collected for the chemical analysis. Targeted analysis with LC-MS/MS was done to identify the changes occurring in secondary metabolite profile of the rye and hairy vetch's root exudates as result of co-cultivation. Quantification results displayed that both hairy vetch and rye are altering their root exudation in response to rye-hairy vetch co cultivation. Hairy vetch significantly increased concentration of root exuded flavonoids such as *Kaempferol-Rha*-Xyl-Gal and biochanin A in response to presence of rye. Concentrations of DIMBOA-Glc in rye's root exudate were increased significantly as result of co cultivation with hairy vetch. The results from this study will increase our understanding about root exuded natural products with growth suppressive effects, which finally leads us to more sustainable plant protection system.

Characterization of a Novel *Bacillus pumilus* Strain as a Plant Growth-Promoting Rhizobacteria

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To secure a stable food production in the future, crop yield and stress resilience must be improved. Numerous reports exist of plant growth promoting rhizobacteria (PGPR) which increase plant growth and stress resistance, but few have included fermentation and seed coating procedures in their studies. Here, a strain closely related to *Bacillus pumilus,* was tested for its PGPR properties when seed coated on cucumber cultivar *Cucumis sativus* var. *fatum*. The *B. pumilus* like strain was cultured with different fermentation strategies using variable pH, temperature and carbon source and tested as PGPR on cucumber from germination to maturity. Application of fermentates demonstrated a significant difference in germination, seedling emergence and flowering 41 days after sowing (DAS), with one fermentate performing significantly better than others. Seed coating additives exhibited a detrimental effect on seedling emergence, but this effect was alleviated from 10 DAS in seeds coated with this fermentate. It furthermore significantly advanced early flowering in cucumber, as treated plants had more flowers compared to treated- and untreated control at 41 DAS. Above- and belowground biomass of 76 days old cucumber plants as well as early root growth was not affected by the PGPR treatments. Root hormonal changes arising from the applied PGPR were analyzed by UHPLC-TQ/MS but further studies are required to conclude upon this. This study suggests that the best performing fermentate has the potential to alleviate the detrimental effect seed coating additives have on germination and that it can advance flowering in cucumber plants. Further studies will show how this strain affects plants under abiotic- and biotic stress.

Integrating biology-based and conventional crop protection

Invited speakers

Integrating biologicals in a crop protection schedule: robust and cost effective schedules are prerequisites for successful market penetration
Jolanda Wijsmuller, Value Chain, Biologics & Minor Crops Manager, Bayer

From pain points to opportunities: Integrating biology-based and conventional crop protection
Harry Teicher, Principal Scientific Consultant, BioScience Solutions

Selected flash talks

Quercetin; as a promising bioactive compound to alter the concentration of pesticides in honey bees
Hamidreza Ardalani, PhD Student, Aarhus University

Combination of biological control agents and fungicides to control diseases in wheat and reducing the risk of fungicide resistance
Birgit Jensen, Associate Professor, University of Copenhagen

Quantifying synergistic effects of combining chemical and immune stressors
Nina Cedergreen, Professor, University of Copenhagen

Integrating biologicals in a crop protection schedule: robust and cost effective schedules are prerequisites for successful market penetration

Jolanda Wijsmuller

Bayer CropScience SA/NV, The Netherlands

In glasshouses in The Netherlands, beneficials and biologicals are very common and are widely used. To make agriculture less dependent on chemical crop protection products, it will be a challenge to use biologicals not only in indoor (high value) crops but also in outdoor crops like vegetables, potatoes, onions, sugar beets, etc.. In these crops the majority of chemical crop protection products are used. If it would be possible to replace chemicals by biologicals in these crops, significant steps could be made to reduce the use of chemicals in agriculture. This will contribute much more than focusing on replacing the still existing uses of chemicals in indoor crops.

For the acceptance of biologicals in outdoor crops it is necessary that growers are provided with a cost effective and robust system in which the final result at harvest is comparable to a system in which only conventional products are being used. It is the challenge and the task of the industry, research stations, and advisors to build these cost effective and robust systems and to build trust and confidence in biologicals. The approach of building systems and implementing the use of biologicals will be discussed.

From pain points to opportunities: Integrating biology-based and conventional crop protection

Harry Teicher

BioScience Solutions, Denmark

Pain points represent opportunities to grow an industry. Conventional pesticide industry pain points include consumer awareness regarding hazards, increasing regulatory requirements, resistance issues due to the lack of new modes of action as well as the declines in registered active substances in the EU.

Biological crop protection industry pain points include low adoption rates, the perception of variable efficacy and slow speed of action, the high specificity of some biopesticides, resistance issues related in part to the evolution of living organisms as well as the need for advanced formulations.

It is from these pain points that significant strategic opportunities within delivery, application, efficacy, safety and resistance management can be anticipated by integrating biological and conventional crop protection – not only between products, but also between the knowledge bases for these strategies.

Driven by legislation as well as consumer and industry demands, Integrated Pest Management (IPM) represents a strategic opportunity, integrating several strategies including chemical, biological & mechanical control, as well as precision technology and decision support tools.

In the current climate of competitive cost structure and R&D funding dilution by corporate acquisition, the coming-of-age of the biopesticide industry and its inclusion in integrated crop protection strategies is seen by many as a sign of a new period of innovation.

For industry leaders, priority is being given to developing Strategic R&D Management frameworks to identify opportunity from pain points. To utilize these opportunities, global corporations are increasingly making use of fourth-generation R&D management models, with the objective of generating intellectual capital via external collaborations.

Quercetin; as a promising bioactive compound to alter the concentration of pesticides in honey bees

Hamidreza Ardalani, Nanna Hjort Vidkjær, Per Kryger, Bente Birgitte Laursen, Inge S. Fomsgaard

Department of Agroecology, Faculty of Science and Technology, Aarhus University, Denmark

Honey bees regularly ingest quercetin, one of the most abundant phytochemicals in plants. Past research suggests that honey and pollen feeding upregulates the bees' detoxification system and that dietary quercetin reduces the toxicity of imidacloprid and tau-fluvalinate. Thus, we hypothesized that dietary quercetin may lead to lower concentration of imidacloprid and tau-fluvalinate by increasing bees' metabolization rates. The aim of this study was to investigate the effect of dietary quercetin on the concentration of three pesticides present in honey bees. Honey bees (n=600) were divided in five groups and fed either quercetin-sucrose paste or only sucrose for 72 h before being exposed to the neonicotinoid imidacloprid (oral exposure), the fungicide tebuconazole or the acaricide tau-fluvalinate (contact exposure). Bees were anesthetized with CO_2, frozen, and extracted with a validated QuEChERS method. Finally, the concentrations of all three above-mentioned pesticides were determined by LC-MSMS. Quantification results of the three pesticides in honey bee samples displayed that there was a significant difference in concentration of imidacloprid ($P=2\times10^{-5}$) and tau-fluvalinate ($P=3\times10^{-2}$) between the bees that were fed quercetin-sucrose paste and bees that were fed only sucrose. However, there was no significant difference in the level of tebuconazole in the group of bees that were fed quercetin-sucrose paste and bees that were fed only sucrose ($P=4.2\times10^{-1}$). The results of current study demonstrate that honey bee intake of quercetin leads to a reduction in the concentration of imidacloprid and tau-fluvalinate but not tebuconazole.

Combination of biological control agents and fungicides to control diseases in wheat and reducing the risk of fungicide resistance

Birgit Jensen[1], Dan. F. Jensen[3], Thies M. Heick[2,], Mukesh Dubey[3], Magnus Karlsson[3], Hans J.L. Jørgensen[1], David B. Collinge[1], Lise N. Jørgensen[2]

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Concerns about pesticide uses in food production call for the identification of alternative and environmentally friendly control measures. Septoria tritici blotch (STB) caused by *Zymoseptorin tritici* is a serious threat to wheat production in Europe. Disease control relies mainly on fungicide application, but excessive use of fungicides has resulted in increasing problems with development of fungicide resistance in the *Z. tritici* populations. Fusarium head blight (FHB) is also an important cereal disease especially since grains infected with *Fusarium* spp. accumulate mycotoxins.
In an ongoing project, we investigate the potential of using the fungal biocontrol agent *Clonostachys rosea* either alone or in combination with the azole fungicide prothioconazole aiming at i) a more sustainable disease control with lower input of chemical fungicides and ii) avoiding or lowering the risk of building-up fungicide resistance in pathogen populations. Field trials carried out in 2018 and 2019 showed that various *C. rosea* spray applications both with and without reduced dosage of prothioconazole significantly reduce STB and FHB. Furthermore, harvest data from 2019 indicates yield increases compared to untreated controls. Grain samples from 2019 show some but not significant reductions in DON following applications of BCA alone.
The effect of *C.rosea* on fungicide resistance development is currently determined based on leaves sampled post-treatment. The sensitivity of *Z. tritici* isolates to increasing concentrations of prothioconazole has been tested and EC_{50}-values determined. Analysis of *CYP51* gene sequences (PacBio sequencing) has identified multiple mutations in *CYP51,* which have been associated with azole resistance. Multiplexing of the DNA samples is underway to enable comparison of frequencies of CYP51 mutations between treatments. Field activities and fungicide resistance analyses continue in 2020.
Data from three years field trial will contribute significantly to our understanding of how biological control can be implemented in sustainable integrated pest management (IPM) strategies.

Quantifying synergistic effects of combining chemical and immune stressors

Kathrine Eggers Pedersen, Brian Lund Fredensborg, Nina Cedergreen

Department of Plant and Environmental Sciences, University of Copenhagen, Denmark

One stressor may render the organism more sensitive to a second stressor. Hence, environmental pollutants may interact with naturally occurring pathogens resulting in effects not readily predictable from neither the ecology of the organism nor the inherent toxicity of the pollutant. When two stressors potentiate the effect of each other the interaction is termed synergistic.

During the last decade, the exploitation of combining traditional pesticides with pathogenic infections has gained increasing interest, mainly to improve methods for controlling pest in agricultural settings and for controlling insect vectors of human diseases. Additionally, understanding the mechanisms behind the (sometimes) synergistic interactions has been a major focus for researchers aiming at reducing mortality of beneficial (and other non-target) insects.

Resultantly, an increasing number of studies claim synergistic effects of combined exposure to pesticides and pathogens. However, the reference model used to define synergy (whether a reference model is even used), vary significantly between studies.

Further, while previous reviews have investigated the frequency of synergistic interactions between mixtures of chemical agents, as good as no studies have reviewed how frequent and under which circumstances synergistic (and/or antagonistic) effects between chemical and pathogenic stressors occur if quantified in a comparable way.

In an ongoing project based on 327 chemical-pathogen combinations (from 90 publications), we aim to quantify the frequency and degree of synergistic and antagonistic interactions between known anthropogenic chemical stressors and natural infections. In addition, if any pattern in chemical/pathogen combination effects can be elucidated, we subsequently wish to define the characteristics of a synergistic combination, as well as a biological or biochemical mechanism behind the synergistic effects.

Poster abstracts

1	Benjamin Perotin	F4028, a novel foliar biofungicide from FMC to protect high value crops
2	Camilla Oskarsson	Increased phosphorus uptake in leek transplants inoculated with arbuscular mycorrhizal fungi in the presence of organic fertilizers
3	Charlotte Klank	Accudo® – microbial biostimulant for achieving higher yields
4	Charlotte Klank	F4018 – a novel soil applied microbial nematicide
5	Charlotte Klank	F4034 – a novel soil applied microbial fungicide
6	Emily Bick	Evaluating mechanisms for diverse plant use in herbivore mitigation
7	Hansol Bae	Automated and Non-disruptive Extraction of Chemicals from Plant
8	Janna Macholdt	Is yield stability of wheat cropping systems becoming more relevant for growers and advisors in the face of climate change?
9	Kourosh Hooshmand	The role of natural products in indirect plant defense against root pathogen
10	Mathilde Nordgaard	*Bacillus subtilis* evolution on plant roots
11	Neel Lindsby & Maja Nielsen	Screening methods for biological control agents of *Fusarium*
12	Shumaila Rasool	Role of endophytic colonization by entomopathogenic fungi on plant growth, cereal aphids and enzyme systems in wheat
13	Thomas Roitsch	Biocontrol of bacterial pathogens by cytokinin producing bacteria and microalgae

P1: F4028, a novel foliar biofungicide from FMC to protect high value crops

Benjamin Perotin, Andréanne Bouchard, Emmanouil Chantzis, Adeline Hechinger, Stavros Kalafatakis, Kimberley Lesniak & Jiangfeng Zhu

FMC Corporation

F4028 is composed of a specific strain of *Bacillus amyloliqufaciens,* FCC1256, which was discovered and developed by FMC's discovery organization at the European Innovation Center, Hørsholm, Denmark. This strain is developed as a foliar biofungicide, to protect fruits and vegetables against key diseases, specifically against botrytis, powdery mildew and bacterial leaf spots.

Results generated at the various steps of its development (in-vitro, whole plant tests, field trials) demonstrate that F4028 outperforms biofungicide standard, shows higher and more consistent activity, and regularly approaches the efficacy of synthetic competitor. When available on the market, F4028 biofungicide will therefore be an important tool to protect high value crops against pathogens limiting agricultural productivity and profitability.

P2: Increased phosphorus uptake in leek transplants inoculated with arbuscular mycorrhizal fungi in the presence of organic fertilizers

Camilla Oskarsson, Siri Caspersen

Department of Biosystems and Technology, Swedish University of Agricultural Sciences, Sweden

Sustainable alternatives to mineral phosphorus fertilizer are urgent, and there are several possible approaches to replace rock phosphate. These include recirculation of waste products used as organic fertilizers as well as inoculation with arbuscular mycorrhizal fungi (AMF). The aim of this study was to investigate the effects of AMF-inoculation on leek (*Allium porrum*) transplants in the presence of organic fertilizers. The effects of inoculation with *Rhizophagus irregularis* on root AMF-colonization, growth, nutrient uptake and root length were examined in a pot experiment conducted in a climate chamber. The experiment was of a completely randomized factorial design with two AMF-treatments (±AMF) and six fertilizer treatments. The latter included mineral fertilizer only, four treatments including organic fertilizers, as well as an unfertilized control. AMF-colonization was successful in all treatments and was not significantly reduced in the presence of organic fertilizers compared to the unfertilized treatment, which was the case in the mineral fertilized treatment. Shoot fresh weight was not significantly affected by AMF, while root fresh weight was reduced. P-concentrations were significantly increased in both shoots and roots, as was the total P-content per pot, in mycorrhizal plants compared to the controls. The P-content was more enhanced in the presence of organic fertilizers than in the mineral fertilizer treatment. AMF-inoculation resulted in a significant reduction of the root length, while the specific root length remained unaffected. In conclusion, the results show the potential for using AMF-inoculation to enhance P-uptake in leek transplants in the presence of organic fertilizers.

P3: Accudo® – microbial biostimulant for achieving higher yields

Charlotte Klank, Grit Torborg, Andréanne Bouchard

FMC Agricultural Solutions

Accudo® is a patented solution based on the proprietary *Bacillus licheniformis* strain RTI184. The product offers valuable benefits along the whole growing cycle, starting at early crop establishment due to its beneficial symbiotic interaction with root and soil, leading to an increased marketable yield in a variety of crops with a focus on high value fruit and vegetables under protected growing conditions. In combination with the product's easy usability (easy to mix, transport and store), the product offers a valuable option for farmers to increase their productivity outlook.

P4: F4018 – a novel soil applied microbial nematicide

Charlotte Klank, Grit Torborg, Andréanne Bouchard

FMC Agricultural Solutions

F4018 is a novel soil-applied bionematicide to protect crops such as potatoes, solanaceous & cucurbit vegetables and sugarcane against nematodes. Using the combination of two microbial active substances, *Bacillus licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002, the product targets cyst nematodes as well as root-knot nematodes through in-furrow and drip & drench application. Due to the favorable product profile, lack of residues and the high likelihood of low risk active substance classification, F4018 will be a valuable tool available to farmer protect their crops against commonly occurring pests in an integrated pest management approach.

P5: F4034 – a novel soil applied microbial fungicide

Charlotte Klank, Grit Torborg, Andréanne Bouchard

FMC Agricultural Solutions

F4034 is a novel soil-applied biofungicide to protect crops such as potatoes and solanaceous & cucurbit vegetables against a suite of soil-born diseases. Using the combination of two microbial active substances, *Bacillus velezensis* strain RTI301 and *Bacillus subtilis* strain RTI477, the product targets diseases such as *Rhizoctonia solani*, *Pythium* sp, *Phytophthora capsici*, *Sclerotinia sclerotiorum* and *Fusarium* sp through in-furrow and drip & drench application. Due to the favorable product profile, lack of residues and the high likelihood of low risk active substance classification, F4034 will be a valuable tool available to farmer protect their crops against commonly occurring diseases in an integrated pest management approach.

P6: Evaluating mechanisms for diverse plant use in herbivore mitigation

Emily Bick[1,2]

[1] University of California, Davis, USA Department of Entomology & Nematology, USA,
[2] University of Copenhagen, Department of Plant and Environmental Sciences, Denmark

Increasing plant diversity in a crop system generally but not always increases yield. Many mechanisms explain how increased plant diversity results in decreased insect crop damage. However, often these mechanisms are not explicitly tested. Knowing said mechanism allows for better implementation of the diverse cropping strategy.

This presentation examines two non-competing mechanisms that may result in the economic advantage of planting diverse crop alfalfa in a California strawberry field. Previous research indicates but does not explicitly test alfalfa acts as a trap crop for economic pest *Lygus hesperus*. However, our findings determined alfalfa is not attractive to *L. hesperus* and therefore does not act as a trap crop. However, there are increased densities of *L. hesperus* nymphs found on alfalfa, indicating the insect either grows faster and / or survives at a greater rate on alfalfa over strawberries. The increased anchored pest density directly results in an increase in both generalist predators and specialist parasitoids. While not directly tested, the increase in beneficial insects is likely one mechanism responsible for the economic benefit of alfalfa placement in a strawberry field.

P7: Automated and Non-disruptive Extraction of Chemicals from Plant

Hansol Bae, Magnus Paludan, Kaare Hartvig Jensen

Department of Physics, Technical University of Denmark, DK-2800 Kongens Lyngby, Denmark

Many high-value compounds can be derived from plants, but their relatively slow growth and limits to cropland hamper industry-level production. Metabolic engineering has the potential to overcome these challenges, with significant attention from both academia and industry. However, downstream extraction processes - which often involves grinding and centrifugation of the entire plant - are extremely inefficient.

In this work, we propose a novel non-disruptive extraction technology, which targets specific cells or tissues where the target molecule is accumulated, thereby maintains the plant biomass without disruption. Therefore, the technology can not only reduce the need for cyclic cultivation and purification but also save time, labor and resources for a new round of cultivation. Utilization of microneedles inspired by insects that feed on sweet sap in plant veins enables the direct extraction of high-value compounds from living cells. Moreover, we established an image analysis-based tissue recognition connected with a micromanipulator to automatize the whole extraction system. We anticipate to utilize the technology in the future, together with the efforts that can accumulate the target compounds in a specific tissue.

P8: Is yield stability of wheat cropping systems becoming more relevant for growers and advisors in the face of climate change?

Janna Macholdt, Bernd Honermeier

Institute of Agronomy and Plant Breeding I, Justus Liebig University Giessen, Germany

Climate change increases the annual yield variability and related yield losses of field crops because of the environmental stress. Against this background, the development of cropping systems with high yield stability and related climate-resilience is necessary to ensure sustainable food production. The aim of this empirical study was to first describe the importance of yield stability as well as currently used practical management strategies that ensure yield stability in wheat cropping systems and secondly, to obtain potential research areas supporting yield stability in the complex system of agronomy. The target groups were German farmers with experience in wheat production and advisors with expertise in the field of wheat cultivation or research. A sample size of 615 completed questionnaires formed the data basis. The study itself provides evidence that the yield stability of winter wheat is even more important than the amount of yield for a large proportion of farmers and advisors. Furthermore, in the view of the majority of the surveyed farmers and advisors, yield stability is a relevant factor in agronomic practice and will gain in importance in the face of climate change. Data analysis showed that site adapted cultivar choice, favourable crop rotations and integrated plant protection are ranked as three of the most important agronomic management practices to achieve high yield stability of wheat. Soil tillage and fertilization occupied a middle position, whereas sowing date and sowing density were estimated with lower importance. However, yield stability is affected by many genetic (G), environmental (E), and agronomic management (M) factors, which subsequently makes it a complex matter. Hence, yield stability should be evaluated based on multi-environmental field experiments and improved in a systems approach by taking G x E x M interactions into account. According to the surveyed farmers and advisors, future research should focus on the agronomic system on the whole rather than on single treatments to develop resilient and yield stable cropping systems under climate change.

P9: The role of natural products in indirect plant defense against root pathogen

Kourosh Hooshmand, Enoch Narh Kudjordjie, Rumakanta Sapkota, Mogens Nicolaisen, Inge S Fomsgaard

Department of Agroecology - Aarhus University, Slagelse, Denmark

Plants at different growth stages continuously synthesize bio-active chemical compounds that serve as direct protection against subsequent infection by detrimental pathogens. Besides, the bio-active compounds may be exuded to the rhizosphere where they facilitate the interaction between the plant and beneficial microbes, which then could contribute to the suppression of soil-borne pathogenic microbes. The present study aimed to investigate the functional role of root exudate (from different host genotypes) in mediating belowground indirect plant defense system. The roots of 3 weeks Arabidopsis thaliana (Col-0 and Ler-0) were challenged with Fusarium oxysporum matthioli pathogen. The root and shoot samples were collected at intervals of 5 days after infection for 25 days, and were subjected to both targeted and untargeted metabolomics analyses as well as metabarcoding (bacteria and fungi community profiling). For targeted analysis, the LC-MS/MS method for identification and quantification of intact glucosinolates, their degradation products, plant hormones, phenolic acids, coumarins, monolignols, lignans, and mycotoxins was developed and fully validated. Phenotypic characterization of the plants displayed pronounced disease symptoms (chlorosis development) for Ler-0 lines at different growth stages whereas Col-0 exhibited strong resistance to pathogen exposure. The disease progression was also evaluated on a molecular level and resulted in the identification of beauvericin in both root and shoot tissues of the susceptible plants which likely was associated with promoting pathogenicity in Fusarium oxysporum. The result from this study will highlight the role of novel natural products in shaping the distinct member of the microbial community leading to disease suppression.

P10: *Bacillus subtilis* evolution on plant roots

Mathilde Nordgaard

Technical University of Denmark

The soil-dwelling bacterium *B. subtilis* is naturally found in soil and known to promote plant growth and protect plants against disease. This bacterium therefore holds a great potential for controlling plant diseases within agriculture, thereby enhancing yields and possibly reducing the application of pesticides. To harvest this potential, we need a better understanding of the complex interplay between *B. subtilis* and plants. In this project, *B. subtilis* root colonization is studied through experimental evolution of the bacterium on *Arabidopsis thaliana* roots. At the end of the evolution, evolved strains will be isolated and re-sequenced to identify the genetic alterations responsible for the improved properties. This project will thereby allow identification of genes involved in root colonization by *B. subtilis* as well as provide novel knowledge about the adaptation potential of *B. subtilis* to plants.

P11: Screening methods for biological control agents of *Fusarium*

Neel Lindsby and Maja Nielsen

University of Copenhagen

The yield and quality of wheat (*Triticum spp.*) and barley (*Hordeum spp.*) can be reduced by various fungal diseases, such as Fusarium Head Blight and Fusarium Seedling Blight. Improving crop yield and quality by reducing the level of diseases, while maintaining minimal environmental impacts, has led to the search for new sustainable strategies for disease control. One such strategy is the use of beneficial microorganisms such as bacterial biological control agents.

For the screening of potential biological control agents with antagonistic effect on fungal phytopathogens *in vitro* assays such as dual cultures are often used. However, such *in vitro* assays focus solely on antibiosis. Thus, potential biological control agent utilising other modes of action are therefore discarded. In addition, the use of an artificial environment, also potentially leads to an overestimation of the ability of the biocontrol agent.

This study focused on comparing the outcome of *in vitro* dual culture assays and *in planta* Fusarium Head Blight assay and Fusarium Seedling Blight assay. The study found that the capabilities of two bacterial strains showed no complete correlation between *in vitro* and *in planta* assays, as one of the strains showed great potential in the dual culture assay but was not able to decrease the level of disease when applied on plant material.

This study therefore emphasizes that the commonly used dual culture can not stand alone in the screening for potential biocontrol agents.

P12: Role of endophytic colonization by entomopathogenic fungi on plant growth, cereal aphids and enzyme systems in wheat

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Recent research has revealed that entomopathogenic fungi can be natural endophytes and inoculations of plants can cause growth promotion and affect herbivore-plant interactions, most likely by altering plant physiological responses. However, the relationship between plant inoculations using entomopathogenic fungi and plant physiological effects has not been investigated. In the present study, effects of seed treatments by three entomopathogenic fungal isolates, *Beauveria bassiana* (KVL 13-39, obtained from BotanicGard) *Metarhizium brunneum* (KVL 04-57) and *Metarhizium robertsii* (KVL 16-38) on plant growth and reproduction of the bird-cherry oat aphid (*Rhopalosiphum padi*) in wheat were evaluated in greenhouse trials over 20 days. At the end of the experiments, plant physiological responses as variability in selected carbohydrate and antioxidant enzymes were also investigated. The three fungal isolates were able to colonize roots and stems of inoculated wheat plants, and the application of the *M. brunneum* isolate significantly increased plant height and biomass compared to plants of the control and *B. bassiana* treatments. Aphid reproduction was also highest in *M. brunneum* inoculated plants. The profile of degrading carbohydrate and specific antioxidant enzymes in relation to observed plant growth promotion and enhanced aphid reproduction will be discussed. The study provides a link between ecological effects and the physiological responses of wheat plants caused by inoculations with entomopathogenic fungi.

P13: 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

P14: Foliar biofungicide induces systemic acquired resistance gene response in *Cucumis sativus* infected with *Podosphaera xanthii*

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As more and more synthetic plant protection products are being eliminated from the market due to regulatory issues, there is an increasing need for the development of biological plant protectants with novel modes of action. However, mode of action studies for biologicals are challenging due to the many-faced effects of microbes on both host and target.

For example, in early microbial screening for biofungicides, the focus is often on the interaction between a specific microbe and a pathogen. Nonetheless one should also consider the microbe-plant interactions as a contributor to the observed antifungal effect.

In this study, we aimed to assess the plant-microbe interaction in *Cucumis sativus* leaves treated with a foliar antifungal microbe during *Podosphaera xanthii* infection. We performed RNA-seq comparing treated and untreated, and infected and non-infected leaves at various time points (4h, 1, 3, 5 and 8 days after infection).

At all timepoints, gene expression in microbe-treated leaves was different compared to untreated leaves. Bifactorial analysis showed that the highest number of differentially expressed genes (that respond to both microbe and pathogen) was observed 3 days after infection. A Gene Ontology Enrichment Analysis revealed that this response is most likely an early induction of the Systemic Acquired Resistance pathway.