Young Scientists Meeting

# 11th Young Scientists Meeting 2018

14th – 16th November
in Braunschweig

- Abstracts -

Berichte aus dem Julius Kühn-Institut

200

Julius Kühn-Institut
Bundesforschungsinstitut für Kulturpflanzen

**Kontaktadresse/ Contact**
Dr. Anja Hühnlein
Julius Kühn-Institut (JKI)
Bundesforschungsinstitut für Kulturpflanzen
Informationszentrum und Bibliothek
Erwin-Baur-Straße 27
06484 Quedlinburg
Germany

Telefon +49 (0) 3946 47-123
Telefax +49 (0) 3946 47-255



**Herausgeber / Editor**
Julius Kühn-Institut, Bundesforschungsinstitut für Kulturpflanzen, Braunschweig, Deutschland
Julius Kühn Institute, Federal Research Centre for Cultivated Plants, Braunschweig, Germany

**Vertrieb**
Saphir Verlag, Gutsstraße 15, 38551 Ribbesbüttel
Telefon +49 (0)5374 6576
Telefax +49 (0)5374 6577

**ISSN 1866-590X**
**DOI 10.5073/berjki.2018.200.000**

# Greetings from the President

Dear Young Scientists,

Welcome to the 11th Young Scientists Meeting! Another busy productive year of experiments and research has passed, and again it is time to share your new findings with your fellow scientists. Celebrating the tenth anniversary of the Julius Kühn-Institut makes 2018 a special year. Even more so we are happy and proud that the young scientific community at our institute is vibrant and passionately advancing today's agricultural research questions and discoveries.

The fascination and enthusiasm that has inspired research on cultivated plants since the founding of our predecessor institution 120 years ago is mirrored in the skyrocketing number of registrations for this year's YSM. Almost 90 attendees are expected to make their way to the meeting and use this opportunity to network, to exchange research results and thoughts with their peers, to get inspired and to develop new concepts and ideas. For the first time, the meeting also welcomes attendees from external institutions (universities, botanical institutes, institutes for technology and Leibnitz institutes) who are collaborating with the JKI.

This year's meeting will be held in Braunschweig, where seven of the 17 specialized institutes of the Julius Kühn-Institut are based. Among them they house around 400 staff members including three new young heads of institute (BS, GF and EP) who were appointed over the last two years. In Braunschweig, the JKI shares its premises with several other federal institutions as well as important other research institutions, for example the BVL (Federal Office of Consumer Protection and Food Safety), the TI (Thünen Institute), the FLI (Friedrich-Loeffler-Institute), the PTB (National Metrology Institute), the HZI (Helmholtz Centre for Infection Research) or the TU Braunschweig (Technical University). Thus, Braunschweig may be seen as a hub for agricultural research and legislation.

These days, scientists face multiple challenges. One of the major issues will be addressed by our keynote speaker Prof. Dr. Joachim Schiemann: communicating research results to both the general public and other scientists. Prof. Schiemann, the former head of the Institute of Biosafety in Plant Biotechnology at the JKI, will talk about quality standards for scientific publications and how to overcome "fake science".

We continue the tradition of offering a soft skill seminar as an opener to the scientific meeting. This time it will focus on your future professional life, in particular on self-presenting yourselves during job-interviews and talks.

While talks, workshops and scientific exchange are at the forefront of the meeting, the social and networking aspects are no less important. You can look forward to a get-together featuring a BBQ and a biodiversity gaming night (prices included!) on the first evening as well as a dinner at the Parlament on the second night.

I wish all of you a successful and enjoyable meeting both scientifically and socially. Although I will retire by the end of this year, I will continue keeping an eye out for any prospective meetings and wishing you productive and inspirational discussions now and in the future.

Braunschweig, November 2018

Dr. Georg F. Backhaus, President of the JKI

Foto: Robert Zech

# Content

## Keynote

Quality standards for scientific publications – how to overcome "fake science"? 9
*Joachim Schiemann*

## Session 1

Improvement of quantitative resistance to stem canker in oilseed rape 11
*Dima Alnajar, Andreas von Tiedemann, Birger Koopmann*

Potential of seed transmission of *Verticillium longisporum* in oilseed rape (*Brassica napus* L.) 12
*Xiaorong Zheng, Daniel Teshome Lopisso, Alice Bisola Eseola et al.*

Soil innate microbiome shows the potential to protect crops against human pathogens 13
*Jasper Schierstaedt, Helena J. Barkowski, Sven Jechalke et al.*

Processed biowaste digestates as fertilizer and soil additive in agriculture 14
*Christina-Luise Roß, Karen Sensel-Gunke, Verena Wilken et al.*

Risk assessment with the indicator model SYNOPS based on sugar beet specific pesticide use 15
*Nelia Nause, Jörn Strassemeyer, Nicol Stockfisch*

## Session 2

Verification of a new electronic bee-counting device using video analysis and manipulative trials 17
*Alina Gombert, Sven Bermig, Malte Frommberger et al.*

How can the Beecheck be used in risk assessment to quantify a pesticides impact on bees? 18
*Alina Gombert, Sven Bermig, Malte Frommberger et al.*

Investigating the transfer of acaricides from beeswax into honey, nectar, bee bread, royal jelly and worker jelly 19
*Lara Lindermann, Jakob H. Eckert, Abdulrahim Alkassab et al.*

Reduction of operator exposure during mixing and loading with Closed Transfer Systems 20
*Matthias Kemmerling, Dirk Rautmann, Jens Karl Wegener*

Estimating the effects of climate change on rodent dynamics and associated parasites 21
*Philipp Koch, Martin Pfeffer, Anna Maria Obiegala et al.*

Plant-made putative contraceptive peptides 22
*Khadijeh Ghasemian, Inge Broer, Jana Huckauf*

Self-service traps for common vole (*Microtus arvalis*) predators 23
*Annika Schlötelburg, Alexandra Plekat, Christian Wolff et al.*

## Session 3

Evaluation of Ethiopian barley landraces for drought stress tolerance 25
*Surafel Teklemariam, Kefyalew Negisho, Gwendolin Wehner et al.*

Feed supplementation with β-Asp-Arg dipeptides via stable co-expression cyanophycin and cyanophycinase in plants 26
*Henrik Nausch, Inge Broer*

Development of a robotic solution to detect and fight slugs 27
*Jobst Gödeke, Jens Karl Wegener, Christian Höing et al.*

## Session 4

DNA-free Genome Editing 29
*Janina Metje-Sprink, Thorben Sprink, Frank Hartung*

Accelerating the breeding of carrots (*Daucus carota* L.) by editing the centromeric histone H3 (CENH3) using CRISPR/Cas9 30
*Katharina Unkel, Thorben Sprink, Frank Dunemann*

Insights into molecular breeding of Russian Dandelion 31
*Regina Kölzsch, Katja Thiele, Frank Hartung et al.*

Genetic differences in barley govern the responsiveness to priming agents 32
*Abhishek Shrestha, Ahmed Elhady, Shimaa Adss et al.*

Selection for resistance/tolerance to *Wheat dwarf virus* (WDV) in barley 33
*Sarah Trebing, Antje Habekuß, Frank Ordon*

Do different host species and distinct habitats alter the olfactory host search of the ectoparasitoid *Holepyris sylvanidis*? 34
*Sarah Awater-Salendo, Benjamin Fürstenau*

## Session 5

Stories from One Thousand and Two Secale Samples: Insights into the evolutionary history of domesticated rye and its wild relatives 36
*Mona Schreiber, Martin Mascher*

A candle in the dark: Reference genome assembly for rye highlights the importance of data visualisation and manual editing 37
*M.Timothy Rabanus-Wallace, Nils Stein , Martin Mascher et al.*

Evaluation of the potential of cleistogamous flowering for a sustainable reduction of loose smut infection (*Ustilago avenae* (Pers.) Rostr.) in oats (*Avena sativa* L.) 38
*Sophie Brodführer, Ben Schmehe, Matthias Herrmann*

Suppression of the northern root-knot nematode *Meloidogyne hapla* by soil bacteria 39
*Olivera Topalović, Johannes Hallmann, Holger Heuer*

## Session 6

Efficient induction of inversions in plant genomes using the CRISPR/Cas system 41
*Carla Schmidt, Michael Pacher, Holger Puchta*

High throughput reverse genetic tools for knocking out several genes of the phytic acid pathway in *Brassica napus* 42
*Niharika Sashidhar, Hans-Joachim Harloff, Christian Jung*

Interaction of the Beet necrotic yellow vein virus with the auxin signaling pathway in sugar beet 43
*Sebastian Liebe, Jose Fernando Gil, Heike Thiel et al.*

Towards the high-resolution mapping and isolation of virus resistance / tolerance genes derived from *H. bulbosum* 44
*Julia Kretsch, Dragan Perovic, Antje Habekuß et al.*

Enhanced plant resistance towards phytopathogenic fungi depends on the rhizosphere microbial community composition 45
*Nina Bziuk, Desirée Lauterbach, Søren J. Sørensen et al.*

A systematic map about the available evidence for the application of genome editing in plants 46
*Dominik Modrzejewski, Frank Hartung, Thorben Sprink et al.*

Transcriptional regulation of iron homeostasis of the hemibiotrophic phytopathogen *Colletotrichum graminicola* 47
*Anja Raschke, Jessica Heilmann, Mario Lange et al.*

## Session 7

Novel detected entomopathogenic fungus *Pandora* sp. infects *Cacopsylla* spp. and other phloem-feeding hemipteran insects 49
Louisa M. Görg, Annette H. Jensen, Jørgen Eilenberg et al.

Evaluation of raspberry and strawberry genetic resources for resistance to spotted-wing drosophila (*Drosophila suzukii*) 50
*Eric Fritzsche, Johanna Pinggera, Dora Pinczinger et al.*

Map-based cloning of $Rph_{MBR102}$ conferring resistance to barley leaf rust 51
*Leila Fazlikhani, Dragan Perovic, Frank Ordon*

Identification of markers closely linked to effective leaf rust resistance genes in wheat 52
*Aleksandra Varekhina, Albrecht Serfling, Klaus Pillen et al.*

## Poster

The effect of habitat connectivity on colonisation of forest fragments with rodents 54
*Diana Alexandra Below, Christian Imholt, Hendrik Ennen et al.*

Comparative studies regarding the sensitivity of the honey bee and wild bee species to plant protection products – residue analysis 55
*Anna Wernecke, Tobias Jütte, André Krahner et al.*

Comparative studies regarding the sensitivity of the honey bee and wild bee species to plant protection products – laboratory studies 56
*Tobias Jütte, Anna Wernecke, André Krahner et al.*

Are microRNAs steering the development of the honeybee hypopharyngeal gland? 57
*Monika Krahnstöver, Pooja Gupta, Benedikt Polaczek et al.*

Effects of insecticides and feeding damage on parasitoids in pine forests 58
*Bianca Kühne, Nadine Bräsicke*

Spatial and temporal dynamics of *Trioza apicalis* in organic carrot cultivation 59
*Jasmin Sauer, Holger Buck, Peter Hondelmann et al.*

New strategy for an old problem: Development of a biological control strategy against wireworms in potatoes 60
*Eckhard Immenroth, Dieter von Hörsten, Diedrich Stephan et al.*

Infrared sensors for gap detection in orchards – possibilities and limits 61
*Verena Overbeck, Johannes Günther, Jens Karl Wegener et al.*

Nozzle combinations and arrangements for use of a tramline deactivation on field sprayers 62
*Johannes Bröring, Dieter von Hörsten*

Identification and enhancement of secondary metabolites in medicinal and aromatic plants for potential use as biological pesticides and pharmacologicals 63
*Ali Karimi, Andrea Krähmer, Nadine Herwig et al.*

NANO-PUSH - Development of nanofibers emitting insect repellents as part of innovative push-and-pull strategies for control of fruit tree phytoplasma vectors 64
*Bruna Czarnobai De Jorge, Jürgen Gross*

CHIC: Chicory as a multipurpose crop for dietary fibre and medicinal terpenes 65
*Jochen Menz, Thorben Sprink*

Comparison of different lettuce (*Lactuca sativa* L.) varieties and their quality parameters in three different locations in Lower Saxony 66
*Daniela Schlein, Marcel Naumann, Elke Pawelzik*

Recent efforts in improving the genepool of annual caraway (*Carum carvi* L.) 67
*Daniel Becker, Andrea Krähmer, Claudia Beleites et al.*

Evaluation of two tomato crosses and their parents with a sensory panel and analytical measurements 68
*Larissa Kanski, Mahasin Ahmed, Marcel Naumann et al.*

Naturally occurring flower mutation in off-spring of a large fruited raspberry chance seedling 69
*Dora Pinczinger, Magda-Viola Hanke, Marcel von Reth et al.*

Potato improvement by genome editing 70
*Enikö Lörincz-Besenyei, Thorben Sprink, Janina Metje et al.*

Basil cultivation without sunlight 71
*Jenny M. Köppe, Christoph von Studzinksi, Inga Mewis et al.*

Identifying and analyzing patterns of Phosphorus fertilization 72
*Paul Winklhofer, Sabine Andert, Silke Hüttel et al.*

Towards positional isolation of *Barley mild mosaic virus* (BaMMV) resistance gene *rym15* 73
*Yaping Wang, Antje Habekuß, Dragan Perovic et al.*

Application of next-generation sequencing for simultaneous detection of viruses, viroids and phytoplasmas in grapevine & fruit trees 74
*Kerstin Zikeli, Constanze Berwarth, Dennis Knierim et al.*

Studies on the resistance locus *Rpv12* against downy mildew of grapes (*Plasmopara viticola*) 75
*Sophia Müllner, Reinhard Töpfer and Eva Zyprian*

Using a robotic high-troughput phenotyping method to detect leaf and stripe rust resistances in wheat genetic resources 76
*Ulrike Beukert, Albrecht Serfling, Frank Ordon*

Identification of resistance to stripe rust, leaf rust and Septoria tritici blotch in a multiparental wheat population 77
*Sandra Rollar, Albrecht Serfling, Frank Ordon*

Genome wide association studies for resistance of wheat to the root lesion nematode *Pratylenchus neglectus* 78
*Behnaz Soleimani, Heike Lehnert, Dragan Perovic et al.*

Tomato plants are suitable hosts for *Salmonella* 79
*Azhar Zarkani, Sven Jechalke, Jasper Schierstaedt et al.*

Do bacteria shape the soil structure by EPS production? 80
*Antje Bettermann, Jeroen Zethof, Cordula Vogel et al.*

Weed mapping – a vital component of site specific crop protection 81
*Christina Wellhausen, Henning Nordmeyer, Michael Pflanz*

LC-MS-based metabolite profiling of winter wheat grains 82
*Leslie Tais, Christoph Böttcher, Hartwig Schulz*

# Keynote

# Quality standards for scientific publications – how to overcome “fake science”?

Joachim Schiemann
Julius Kühn Institute, Institute for Biosafety in Plant Biotechnology, Quedlinburg
E-mail of corresponding author: joachim.schiemann@julius-kuehn.de

In the world of academia, getting published in an international research journal is almost the Holy Grail; it helps bump up the CV for hiring and helps in the competition for tenure or promotion. It takes rigorous research, an original contribution, and exhaustive peer or expert reviews.

In times of fake news, science is usually still one of the areas that provide orientation and on which one can rely. The search for truth, the critical discourse - they belong to the foundations of good science. However, these are obviously at risk. A concerted action of science journalists went public on July 19, 2018 with investigations against fake research papers, publishers, and conferences. Unfortunately, several hundreds of ‘predatory journals’ thrive, and cast shadow on quality of faculty and research worldwide.

How the pay-and-publish business works? The predatory publishers' scam works like this: they write to researchers and companies all over the world and recommend a publication in a scientific journal. Then they publish - for a fee - the contributions of the researchers within a few days, often without any significant examination of the contents. Thus even dubious studies receive an alleged seal of science and are in the world.

How to overcome the situation described above and to guarantee a rigid self-control of science? The author will describe his experience as Associate Editor for Frontiers in Plant Science (Plant Biotechnology) and as Topic Editor for the Research Topic ‘Plant Genome Editing – Policies and Governance’ (http://journal.frontiersin.org/researchtopic/7596) aiming at collecting articles on the latest advancements and future targets of genome editing, as well as contributions addressing the regulatory, social and socio-economic aspects, the ethics, risk assessment, management, and biosafety researches.

Frontiers is well aware of the potential impact of published research both on future research and on society and, hence, does not support superficial review, light review or no-review publishing models. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society. Frontiers has a number of procedures in place to support and ensure the quality of the research articles that are published which will be discussed in the talk.

# Session 1

# Improvement of quantitative resistance to stem canker in oilseed rape

Dima Alnajar, Andreas von Tiedemann and Birger Koopmann
Georg August University Göttingen, Division of Plant Pathology and Crop Protection, Göttingen
E-mail of corresponding author: dima.alnajar@agr.uni-goettingen.de

*Leptosphaeria maculans* is one of the most important pathogens on oilseed rape. Using resistant cultivars has been proven to be the best method to manage *L. maculans*. Two types of resistance are generally described, R-gene mediated resistance and quantitative resistance. The latter is known for being more durable and stable.

PhomaDur is a project that aims to improve quantitative resistance and to deepen the understanding of its mechanisms in oilseed rape. Therefore, the objectives of this project are (1) to phenotype a multi parents interconnected mapping population in the field and in the greenhouse in order to identify QTLs, (2) to study blackleg infestation and characterize the range of *Phoma lingam* isolates/races in different regions in Germany and (3) to investigate the quantitative resistance mechanisms. For that, stem canker severity was compared in four regions in Germany in season 2017-2018. Subsequently, the phenotypic assessment was applied to the oilseed rape mapping population from the field with the highest disease severity. Additionally, *L. maculans* was isolated from phoma leave spots from the four studied fields. Preliminary observations indicate that *LepR1* is still very effective in Hadmersleben and Nienstädt, while the resistance genes *Rlm2* and *Rlm9* are 100% broken in both sites. On the other hand, results from two sites showed that *Rlm7*- breaker isolates´ distribution becomes more prominent in some regions in Germany. In order to investigate quantitative resistance mechanisms, different inoculation methods in the greenhouse were tested. Results showed that stem canker severity in greenhouse may vary based on the inoculation method and the placement of the inoculum on different plant tissues.

# Potential of seed transmission of *Verticillium longisporum* in oilseed rape (*Brassica napus* L.)

Xiaorong Zheng, Daniel Teshome Lopisso, Alice Bisola Eseola, Birger Koopmann and Andreas von Tiedemann
Georg August University Göttingen, Division of Plant Pathology and Crop Protection, Göttingen
E-mail of corresponding author: xzheng@gwdg.de

*Verticillium longisporum* is a soil-borne vascular fungal pathogen, which has spread throughout the European oilseed rape cultivation area and recently reached canola fields in Canada. In present study a series of greenhouse and field inoculation experiments using resistant and susceptible cultivars of winter and spring type oilseed rape were conducted to investigate the potential of transmission of *V. longisporum* by seeds of oilseed rape. The identity of the pathogen re-isolated from seeds of greenhouse grown diseased plants was confirmed using a DsRed labeled *V. longisporum* isolate. The fungal colonization from roots to hypocotyls, pods and seeds of diseased plants was further verified by species-specific qPCR. Frequency of recovery of viable colonies of *V. longisporum* from seeds harvested from greenhouse grown diseased plants ranged from 0.08 to 13.3%. Incidence of seed transmission was higher in the susceptible than in the moderately resistant oilseed rape cultivar. Subsequent studies on transmission of the disease into the offspring revealed that only 1.7 to 2.3% of plants showed disease symptoms as confirmed by the formation of microsclerotia in the stems. Different to greenhouse, although low level of *V. longisporum* DNA was found in seeds of both field grown diseased winter and spring oilseed rape, viable *V. longisporum* colonies only yielded from seeds of the spring type but not from winter type plants. Equally, none of these seeds transmit the disease into the offspring. These results strongly suggest that the rate and probability of seed transmission of *V. longisporum* depends on the speed of plant colonization which is significantly faster under greenhouse conditions and in a spring-sown crop compared to autumn-sown oilseed rape.

# Soil innate microbiome shows the potential to protect crops against human pathogens

Jasper Schierstaedt[1,2], Helena J. Barkowski[2], Sven Jechalke[3], Abhishek Shrestha[2], Kornelia Smalla[2], Rita Grosch[1] and Adam Schikora[2]

[1] Leibniz Institute of Vegetable and Ornamental Crops, Department Plant Health, Grossbeeren

[2] Julius Kühn-Institut, Institute for Epidemiology and Pathogen Diagnostics, Braunschweig

[3] JLU Gießen, Institute of Phytopathology, Gießen

E-mail of corresponding author: jasper.schierstaedt@julius-kuehn.de

Foodborne diseases are increasingly associated with fresh fruits and vegetables. Serovars of *Salmonella* were the second most frequent cause in 2015. The biological diversity of soil plays a major role in the establishment of *Salmonella* in the plant production environment.

Antagonists and plant beneficial microbes negatively affect the establishment of *Salmonella* in the rhizosphere. Here we analyzed the tripartite interactions between the human pathogen *Salmonella enterica*, the soil microbiome and the crop plants tomato, lettuce and corn salad grown under greenhouse conditions.

We observed that *Salmonella* persisted in the rhizosphere of lettuce and tomato. In contrast, its numbers declined in the rhizosphere of corn salad. Very important was the observation that reduction of microbial diversity in soil increased the ability of *Salmonella* to persist in this environment. These results clearly show a dependency between the microbial diversity and the potential of *Salmonella* to colonize the rhizosphere as well as the high physiological plasticity of *Salmonella*. In the following, we focused on the impact of resistance induced in crop plants on the establishment in plant production environment and colonization of plants. In greenhouse experiments, crop plants were primed for induced resistance with the bacterium *Ensifer meliloti*. This bacterium produces *N*-acyl-homoserine-lactones, which are known to induce resistance.

Our results show that priming has a negative effect on the persistence of *Salmonella*. Primed plants express the defense-related genes earlier than unprimed plants and are able to close their stomata for longer period. These results indicate the potential of priming for enhanced resistance against *Salmonella*.

# Processed biowaste digestates as fertilizer and soil additive in agriculture

Christina-Luise Roß[1,2], Karen Sensel-Gunke[1], Verena Wilken[1], Kerstin Nielsen[1] and Frank Ellmer[2]
[1] Berlin Humboldt University, Institute for Agricultural and Urban Ecological Projects (IASP), Berlin
[2] Berlin Humboldt University, Department of Agronomy and Crop Science, Berlin
E-mail of corresponding author: roschris@agrar.hu-berlin.de

*Introduction, Material and Methods*

Using biowaste in biogas plants is an established process that will become more important due to the mandatory separate collection of biowaste in Germany established in 2015. Current policy's aim is to recycle valuable compounds in digestates, which are usually composted and, if necessary, further processed.

To test the effects of such biowaste digestates on soil ecology and crop growth a three-year field trial was carried out, which was supplemented by pot experiments, experiments with rhizoboxes and phytotoxicological tests. Furthermore, the carbon mineralization rate of the digestates was determined using a CarbO2Bot® (prw electronics, Germany), earthworm avoidance tests were performed and species and abundance of earthworms were investigated in the field trial.

In addition to agglomeration and pelleting, various additives (meat-and-bone meal (MBM), Calcium ammonium nitrate (CAN), bentonite, straw) were tested to investigate the possibility of influencing the digestates' chemical-physical properties.

*Results*

- The content of readily available nitrogen is low. In the pot experiments, the plants withdrew a maximum of only 12% of the applied nitrogen.
- The digestates contain significant amounts of phosphorus and potassium. These are better plant available than nitrogen.
- After application of the tested products, a significant increase in the soil-pH values was detected.
- In the soil respiration studies, about 20% of the applied carbon was mineralized in the first 100 days.
- The use of composted digestates from biowaste promotes the activity of microorganisms in the soil.
- The investigations in earthworm abundance in the field remained inconclusive. The avoidance tests showed no limitation of the habitat function within the meaning of the ISO standard 17512-1 (2008).
- A phytotoxicity of the digestates existed only in isolated cases and was not dose-dependent, but due to punctual contamination.
- The addition of nitrogen-containing compounds such as CAN or MBM significantly increases the direct fertilizing effect of the products. Depending on the ammonium content, phytotoxic effects were observed. These are larger with the addition of mineral fertilizers than with organic N compounds. Clay minerals partially reduced this phytotoxicity.

# Risk assessment with the indicator model SYNOPS based on sugar beet specific pesticide use

Nelia Nause[1], Jörn Strassemeyer[2] and Nicol Stockfisch[1]
[1] Institute of Sugar Beet Research, Göttingen
[2] Julius Kühn Institute, Institute for Strategies and Technology Assessment
E-mail of corresponding author: nause@ifz-goettingen.de

Even with the appropriate use of pesticides, environmental risks can occur under unfavorable conditions that cannot be estimated within the scope of the approval process.

The research approach of the present project was on the one hand to record the status quo of pesticide use in sugar beet cultivation in Germany and associated environmental risks and the development of crop protection strategies optimized with regard to the environmental risk.

The data on chemical plant protection in sugar beet cultivation in Germany were collected in the years 2010-2015. A number of 2314 randomly chosen farmers were surveyed via questionnaire about the chemical plant protection on their largest sugar beet field, whereby the interviewees changed annually. The farms were distributed over all regions of Germany according to the regional distribution of the sugar beet growing area.

The model SYNOPS-GIS was used for the calculation of possible environmental risks caused by sugar beet specific application of pesticides. The model assesses risks based on agricultural fields derived from spatial land use datasets. Each reported application pattern was combined with a multitude of fields. The environmentally relevant concentrations of active ingredients in the non-target compartments soil, neighboring surface waters and field margins were estimated. The acute and chronic risk indices of the considered application are given as the quotient of the environmental concentration and the toxicity ($LC_{50}$/10, NOEC) for different terrestrial and aquatic reference organisms (exposition-toxicity-ratio, ETR).

The calculated application-specific results were mainly in the (very) low risk category (ETR = 1). In general, the aquatic risk was slightly higher than the terrestrial risk. The aquatic risk was evaluated separately for herbicides, fungicides and insecticides. Medium and high risks were mainly caused by herbicides or insecticides. Herbicides, which are applied on nearly 100% of fields, provide a higher risk reduction potential than insecticides which are used on about 15%.

The identified risks are not founded in the use of specific active ingredients or application patterns. The ETR is not directly determined by the amount of active ingredient applied. The combination of application pattern with field-specific environmental conditions determines the risk.

These risks might be avoidable by implementation of field-specific risk mitigation measures like creation of vegetated filter strips.

# Session 2

# Verification of a new electronic bee-counting device using video analysis and manipulative trials

Alina Gombert, Sven Bermig, Malte Frommberger and Jens Pistorius
Julius Kühn Institute, Institute for Bee Protection, Braunschweig
E-mail of corresponding author: sven.bermig@julius-kuehn.de

Bee counting devices promise new findings in bee research. Since accuracy of existing bee counters is unsatisfactory, the Beecheck was developed. This new device ensures the separation of single bees by small passageways and operates with capacitive sensors providing quantitative information of the object passing the sensor. The sensor readings are transformed to bee exit and entry counts based on an algorithm. Due to variability in bee behaviour, misinterpretation by the algorithm happens occasionally.

In this study, the device counts are verified by empirical methods, enabling the improvement of the algorithm. Videos of the operating device were recorded at varying times of the day and under different meteorological conditions. Subsequently every bee was counted watching the video in the slow-motion mode. In semi-field trials, the counting device was placed in front of bee forage resulting in equal numbers of incoming and outgoing bees. Data from both experiments was compared to the records of the Beecheck.

The capacitance-based sensors accurately detected changes in their electrical field. First results suggest that the behaviour of bees differs according to time of the day and meteorological conditions. Yet, the algorithm has some structural errors leading to the misinterpretation of common situations. However, it is difficult to generalize the sensor readings in a way that the device always correctly decides whether a bee went in or out or turned. The methods described in this study can be used to validate bee-counting devices accuracy so that it can be used in scientific research and risk assessment.

# How can the Beecheck be used in risk assessment to quantify a pesticides impact on bees?

Alina Gombert, Sven Bermig, Malte Frommberger and Jens Pistorius
Julius Kühn Institute, Institute for Bee Protection, Braunschweig
E-mail of corresponding author: alina.gombert@julius-kuehn.de

Standardised field trials are vital for risk assessment of pesticides to bees but are time-consuming and costly. Current practices of monitoring field trials are very labour-intensive and only generate few "snapshots" of parameters of interest, failing to display dynamics over time. An electronic bee-counting device continuously records the in- and outgoing bees, providing additional information but also generating large data sets. This study aims at identifying appropriate target variables and methods of data analysis.
To generate sample data, the Beecheck was placed underneath nine different bee colonies in a common field trial setting in flowering *Brassica napus* and *Phazelia tanacetifolia* with bees being exposed to pesticides. In accordance to the literature, bee activity was associated to meteorological variables and time.

Using the autoregressive integrated moving average (ARIMA) method it may be possible to disentangle the impact of meteorological factors and the disturbances caused by pesticide application, thereby quantifying the pesticide-induced losses of a colony. This would facilitate the comparison of results from field trials in different years and regions with varying weather, being of special interest for risk assessment.

The European Food Safety Authority developed a conceptual model to be used in risk assessment for bee colonies exposed to pesticides. The model simulates the dynamics of a bee colony under different stressors including adverse effects from pesticide exposure. Records obtained with the Beecheck can provide an empirical basis for model parametrisation. Running the model including pesticide-induced losses over several years allows extrapolation from one year to several years of exposure and thus evaluation of long-term risk. Further research is needed to verify the accuracy of the Beecheck.

# Investigating the transfer of acaricides from beeswax into honey, nectar, bee bread, royal jelly and worker jelly

Lara Lindermann[1], Jakob H. Eckert[2], Abdulrahim Alkassab[2], Gabriela Bischoff[2], Robert Kreuzig[3] and Jens Pistorius[2]
[1] Technische Universität Braunschweig, Institute for Geoecology, Braunschweig
[2] Julius Kühn Institute, Institute for Bee Protection, Braunschweig
[3] Technische Universität Braunschweig, Institute of Environmental and Sustainable Chemistry, Braunschweig
E-mail of corresponding author: lara.lindermann@julius-kuehn.de

Beeswax contamination derive from different sources. A main source are acaricides that are used to control the parasite mite *Varroa destructor.* Since it is common practice to recycle wax, acaricides can accumulate in beeswax due to their fat-soluble properties. While many studies focused on the contamination of wax and honey, the purpose of the current study was to directly compare contamination levels in different types of bee products. Special attention was payed to the food of worker and queen larvae (worker jelly and royal jelly). We investigate the transfer pathways of the active substances and compare the influence of properties of the matrices and substances like the water and fat solubility and retention period. Therefore, beeswax without any detectable pesticide residues was used to pour wax foundations with five different acaricide concentration levels. We used a mix of ten different acaricides that had been most frequently detected in commercial beeswax during preliminary testing. The used initial concentration mirrored field-realistic maximum concentrations. In addition, two lower and two larger concentrations were used as further treatments. The poured foundations were processed into honeycombs by bees. Subsequently, the combs were taken from the hives and honey, nectar, bee bread, royal and worker jelly were manually applied to each treated comb. Combs were incubated at in-hive conditions. The duration ranged from a few days for nectar and larval food up to two months for honey and bee bread, mimicking natural processing conditions in a hive. To evaluate the transfer of residues, samples of all matrices, as well as wax, were taken directly from each comb at the start and the end of the trial. To investigate if the process of comb construction from manually poured foundations may have altered the treatment conditions, additional Petri dishes were filled with round pieces of the spiked original foundations. The same matrices were applied to the Petri dishes and incubated accordingly. Samples were analyzed by Liquid Chromatography-Mass Spectrometry (LC-MS/MS).

The results will help to retrace the transfer of acaricide residues from beeswax into bee relevant matrices by taking the differing chemical properties of the active substances and test matrices into account. Ultimately, our results will help modeling the migration of acaricides within the hive and to estimate a possible exposure of adult honey bees and honey bee larvae.

# Reduction of operator exposure during mixing and loading with Closed Transfer Systems

Matthias Kemmerling, Dirk Rautmann and Jens Karl Wegener
Julius Kühn Institute, Institute for Application Techniques in Plant Protection, Braunschweig
E-mail of corresponding author: matthias.kemmerling@julius-kuehn.de

Closed Transfer Systems (CTS) are mechanical systems for plant protection sprayers, which prevent the operator from contamination with the concentrated plant protection product (PPP). They can be used with standard PPP containers with screw cap. To compare different systems, it is necessary to quantify the operator exposure during mixing and loading with sufficient accuracy. Therefore a new measuring method using whole body dosimeters and fluorescent tracer was developed.

In the conventional process the operator opens the cap and the sealing of a PPP container, fills the product into the sprayer and rinses the container with clear water. The fluid path from the container to the sprayer is open in this case. Exposure of the environment and the operator's body can occur easily. A CTS provides a technical improvement to this problem by connecting the container to the sprayer via adaptors. The opening of the sealing as well as the product transfer and rinsing of the empty container happens in a closed system with minimized contamination of the operator.

To measure the human exposure of the operator under realistic conditions, both during conventional filling or filling with CTS, experiments were done with different test persons. Protective clothing consisting of overall, protective gloves and visor was used as dosimeters to collect the fluid. Additionally a layer of long underwear and laboratory gloves was used as inner dosimeters that represent the human skin. In every experiment four containers filled with fluorescent pyranine solution were transferred into the tank of the sprayer and rinsed afterwards. The exposure of all dosimeter types was separately evaluated with fluorescence spectroscopy.

In the first step, the operator exposure for a conventional filling of different container sizes (1 liter, 5 liter and 10 liter) was investigated. The container causing the highest exposure was then used in the second part where different sprayer configurations were compared. At first the experiments were done at the induction hopper of the sprayer, which is the current state of technology. The experiments were done by three different operators with the CTS called easyFlow by agrotop GmbH and without the system. After that the procedure was repeated at the dome shaft of the sprayer tank.

The results confirm a strong reduction of operator exposure with the tested CTS. The protective gloves collect the highest amount of fluid. Also the exposure of the overalls is high in some cases. Other dosimeters like long underwear, laboratory gloves and visors have shown to be less important for the assessment of operator exposure. The measuring method has proved to be sufficiently accurate and a new guideline for testing the operator exposure of different CTS will be created based on it.

# Estimating the effects of climate change on rodent dynamics and associated parasites

Philipp Koch[1,2], Martin Pfeffer[1], Anna Maria Obiegala[1] and Jens Jacob[2]
[1] University of Leipzig, Institute of Animal Hygiene and Veterinary Public Health, Leipzig
[2] Julius Kühn Institute, Institute for Plant Protection in Horticulture and Forests, Münster
E-mail of corresponding author: philipp.koch@julius-kuehn.de

Rodents serve as main food source for a variety of arthropods like fleas, mites and ticks. These arthropods maintain diverse zoonotic pathogens within the rodent population and may transmit them from the rodent reservoir to larger mammals including humans. Well known zoonotic diseases include Lyme borrelioses and FSME. Climatic variation can affect both the population dynamics of rodents and the host-seeking behavior of arthropod vectors. Predicting possible future effects of climate change on rodents, arthropods and vector-borne pathogens is of great public health interest.

This project aims to examine the effect of climate change on the system of common voles (*Microtus arvalis*), ectoparasites and pathogens regarding relevant zoonotic pathogens like *Borrelia* spp. and *Rickettsia* spp.

A large-scale field study will be conducted at the "Global Change Experimental Facility" of the "Helmholtz-Zentrum für Umweltforschung Halle/Leipzig". In half of 50 plots of 400 $m^2$ climate conditions are manipulated to reflect future temperature and precipitation. The other half of plots are un-manipulated experimental controls. Over three years, during the rodent reproductive period, rodents will be live trapped and uniquely marked with a RFID transponder. Blood samples, tissue samples and ectoparasites will be collected, body measurements taken and reproductive activity assessed.

Data will be used to identify potential changes regarding vole breeding and population dynamics, ectoparasite composition/density and pathogen composition/load. This should reveal potential differences between varying temperature and precipitation regimes.

Such empiric systematic assessments of climate change impact on biological systems in the relevant habitat are essential to judge potential consequences for the protection of plants and human health. The findings will be utilized for the development of effective adaptation strategies.

# Plant-made putative contraceptive peptides

Khadijeh Ghasemian, Inge Broer and Jana Huckauf
University of Rostock, Department of Agrobiotechnology, Rostock
E-mail of corresponding author: Khadijeh.Ghasemian@uni-rostock.de

Rodents are the most important group of mammals in terms of the problems they create in agriculture, horticulture, forestry and public health. Fertility is a key factor that determines the population density.

Contraceptive vaccines have been proposed for wildlife population management. Gamete specific proteins may be good candidates for the development of contraceptive vaccines. Sterility can be achieved in mammals via the induction of antibodies against Zona pellucida (ZP) glycoproteins that are located on the surface of the oocyte and mediate the gamete recognition. In addition, a sperm-specific protein, IZUMO, plays a vital role in the sperm-egg fusion process, hence it also may be a potential target for the development of a contraceptive vaccine. In order to prevent non target effects an immuncontraceptive vaccine has to be orally administered and species specific. Specificity might be achieved by the restriction of vaccine to small, species specific peptides.

Plants have shown to be one of the most promising alternative pharmaceutical production platforms that are robust, scalable, low-cost and safe. We tried to establish the transient expression of putative mice-specific contraceptive mZP3, mZP2 and mIZUMO small peptides in *Nicotiana benthamiana*, via viral MagniCON expression system.

Successful production of antigen and improved recombinant protein stability in plant were achieved by fusion of mZP3 antigen to GFP protein. Increasing the protein size by tripling of the antigenic mZP3 epitope also stabilized the antigen and increased the expression levels in *N.benthamiana*.

We also examined the production of mice-specific ZP2 and IZUMO peptides. The antigens were overexpressed as recombinant repeated antigenic peptides using MagniCON system in *N.benthamiana*. Multiplying of the antigenic epitopes can increase antigen-antigenicity as well.

# Self-service traps for common vole (*Microtus arvalis*) predators

Annika Schlötelburg[1,2], Alexandra Plekat[1], Christian Wolff[3], Sonoko Bellingrath-Kimura[2,4] and Jens Jacob[1]

[1] Julius Kühn Institute, Institute for Plant Protection in Horticulture and Forests, Münster

[2] Humboldt-University of Berlin, Division of Land Use Systems, Berlin

[3] Regional Office of Agriculture and Horticulture Sachsen-Anhalt (LLG Sachsen-Anhalt), Department of Plant Protection, Bernburg

[4] Leibniz Centre for Agricultural Landscape Research (ZALF), Institute of Land Use Systems, Müncheberg

E-mail of corresponding author: annika.schloetelburg@julius-kuehn.de

Common voles (*Microtus arvalis*) are a severe pest species in agricultural landscapes, especially during mass outbreaks. Every two to five years, population size can reach up to 2000 individuals per hectare. Then, voles increasingly migrate from undisturbed grassy field margins to farmland. Farmers often use rodenticides to protect their crops. Efficacy of rodenticides can be hampered by alternative food sources, bait shyness, population size and they cannot be used in organic farming.

A new approach of ecologically based rodent management pursues the idea to inhibit migration from primary grassland habitats to secondary farmland habitats. Suitable methods could a ploughed furrow combined with traps. Traps need to be checked and maintained regularly and are therefore not suitable to protect large-scale farmland. But involving efficient vole predators could offer a work- and cost-saving tool for rodent management that is also suitable for organic farming. We tested two types of vole traps that can be emptied by terrestrial predators (e.g. foxes), raptors and other birds. We developed one trap with a triangular shape to fit in a ploughed furrow along field margins. The other trap (standby-box, Andermatt Biocontrol AG, Switzerland) has a lid that can be opened by terrestrial predators to remove captured rodents. In field studies, we tested with camera traps how frequently the two trap types were emptied by predators. Our newly developed trap was emptied more often and by a more diverse group of predators than the standby trap. Only cats (*Felis silvestris f. catus*), racoons (*Procyon lotor*) and foxes (*Vulpes vulpes*) were recorded opening the lid of the standby-box to remove rodents. From the new trap type, voles were additionally removed by stoats (*Mustela erminea*), rats (*Rattus norvegicus*) and a variety of raptors and other birds. Additionally, its opening allows several non-target rodent species to escape. Furthermore, we analysed factors influencing predator access to improve efficacy and animal welfare.

With these improvements, self-service traps could be integrated as large-scale method, so that this new barrier-system can help to manage common voles without rodenticide use and associated risk to wildlife.

# Session 3

# Evaluation of Ethiopian barley landraces for drought stress tolerance

Surafel Teklemariam [1,2], Kefyalew Negisho[2,3], Gwendolin Wehner[2] and Frank Ordon[2]
[1]Ethiopian Institute of Agricultural Research (EIAR), Melkassa Agricultural Research Center, Melkassa, Ethiopia
[2]Julius Kühn-Institut, Institute for Resistance Research and Stress Tolerance, Quedlinburg
[3]EIAR, National Agricultural Biotechnology Research Center, Holetta, Ethiopia
E-mail of corresponding author: surafel.teklemariam@julius-kuehn.de

Drought is the main limiting factor for yield losses in Ethiopia. Barley *(Hordeum vulgare* L.) is the best adapted cereal crop in Ethiopia which is grown from drought-prone areas of an altitude of 1500 meter above sea level up to the highlands of Ethiopia (3400 m) which are characterized by a temperate climate with adequate rainfall. Thus, barley landraces from Ethiopia may be a valuable source to exploit drought stress tolerance. To achieve this, field experiments including 260 Ethiopian barley landraces were conducted in two cropping seasons in Ethiopia (2016 and 2017) at two locations with natural drought stress (Melkassa and Dera) and two locations having adequate moisture conditions (Holetta and Debre Zeit). Additionally, landraces were genotyped with the 50k iSelect chip to identify genomic regions involved in drought stress tolerance by genome wide association studies (GWAS).

The cropping data of 2017 was omitted from further analysis due to the presence of adequate rainfall in drought stress locations. Analysis of variances (ANOVA) revealed significant ($p<0.001$) differences between control and drought stress conditions for total grain yield in 2016. Furthermore, significant ($p<0.001$) effects between the landraces were observed, representing high genotypic variation. Total grain yield at drought stress conditions (Ys) was significantly ($p<0.001$) correlated with days to flowering ($r=-0.68$) and maturity ($r=-0.42$)

Drought indices like stress tolerance index (STI) and stress non-stress production index (SNPI) were used to cluster the landraces, by tolerance to drought stress. Thus, e.g. landraces B191.1 and B222 were clustered as best performing ones under drought stress.

One year field experiments revealed genotypic variation in Ethiopian barley landraces. In a next step, genome wide association studies will be conducted in order to identify genomic regions involved in drought stress tolerance and to develop molecular markers suited to be used in future barley breeding.

# Feed supplementation with β-Asp-Arg dipeptides via stable co-expression cyanophycin and cyanophycinase in plants

Henrik Nausch[1] and Inge Broer[1]

[1] University of Rostock; Department of Agrobiotechnology and Risk Assessment for Bio- und Gene Technology, Rostock

E-mail of corresponding author: henrik.nausch@uni-rostock.de

Livestock diets can be supplemented with dipeptides in order to promote optimal growth and wellbeing. Due to the dual role of arginine as building block for proteins and regulator of physiological functions, pronounced effects were observed after addition of β-aspartate-arginine (β-Asp-Arg) dipeptides to feed. Currently, β-Asp-Arg is generated *in vitro* from the cyanobacterial storage polymer cyanophycin (CGP) via incubation with the cyanophycinase enzyme (CGPase) which are both produced in *E. coli*. Because of the high costs and limited scalability, bioreactor-based production is commonly used for the synthesis of high-value but not for cost-sensitive products such as supplements for animal diets.

Alternatively, recombinant low-value products can be produced in plants in an economic manner using existing agricultural infrastructure and farming practices. We already established the production of CGP in plastids of tobacco and potato, yielding up to 9.4% of the dry weight (dw) in stably transformed plants. We also demonstrated that CGPases can be transiently co-expressed in the cytosol of CGP-producing tobacco via the MagnICON system without affecting the CGP accumulation in intact chloroplasts. Amongst different CGPases, CphE showed the highest yield and was able to degrade CGP in homogenized leaf tissue when the spatial separation of cytosol and plastid stroma was destroyed. Oral administration of feed pellets, which contained purified CGP and CGPase to mice, showed that the released dipeptides were absorbed into the blood.

Now we could demonstrate that CGP degradation is not only possible after transient expression of CphE in the cytosol of CGP-producing tobacco, but also when the CGPase is introduced into these plants via stable transformation. Constitutive expression of CphE in parallel to CGP synthesis does not affect the accumulation of CGP in leaf chloroplasts and degrades CGP completely after decomposition of cells. The transfer of this system to feed plants would therefore provide an easy and cheap system to directly release the β-Asp-Arg dipeptides in the intestine of animals in the digestive process.

# Development of a robotic solution to detect and fight slugs

Jobst Gödeke[1], Jens Karl Wegener[1], Christian Höing[2] and Dieter von Hörsten[1]
[1] Julius Kühn Institute, Institute for Application Techniques in Plant Protection, Braunschweig
[2] University of Kassel, Department of Ecological Agriculture, Witzenhausen
E-mail of corresponding author: jobst.goedeke@julius-kuehn.de

Almost every type of crop can be attacked by slugs, which can lead to significant losses in yield and quality. Ploughless cultivation and the cultivation of intercrops intensify the slug problem, as these factors offer the slugs opportunities to find food and retreat all year round. As a consequence of the mass propagation of slugs, the application of snail baits is carried out as a "routine measure" in the rape growing areas of Europe, but this intervention option achieves only inadequate results. The aim of the project is to develop an alternative robotic solution that detects slugs by sensors and combats them. The use of a robot is expected to increase control success. In addition to the possibility of protecting the environment, operating resources and working time can be saved.

In the first year of the project, a carrier vehicle was built, which navigates autonomously via GPS over an area. In addition, a sensor for slugs detection has been developed that uses digital image processing to detect slugs in the field. In the second project year, the sensor will be used in field trials. It is intended to collect data on the quantitative slug behaviour. In this context, an intensive literature research on slug behavior was conducted, which serves as a basis for the anticipatory control of the robotic solution. In the third year, the individual modules are combined and the functionality of the system is proven. Furthermore, it is checked whether the system can be extended to control other pests (e.g. mice).

KommTek GmbH has developed a robotic platform on which a manipulator arm is mounted.

The Department of Agricultural Engineering at the University of Kassel is working on the development of a detection module for the detection of slugs. Optical properties of slugs and different soils were determined in experiments. It becomes clear that slugs reflect less strongly at a light wavelength of 950 nm than the soils examined. This offers the possibility of segmenting slugs with the help of a filter and making them visible to the robot for targeted control.

As a basis for the navigation of the robot, the Julius Kühn Institute has collected data on slug behaviour within the framework of intensive literature research. It turns out that the life cycle of slugs is strongly influenced by environmental factors. In laboratory tests a suitable tool is developed, which fights slugs safely and energy-efficiently. For reasons of future acquisition costs of the end product and energy efficiency, mechanical tools were first tested for their suitability. The tools to be tested are designed in such a way that various mechanical control principles can be mapped and tested.

# Session 4

# DNA-free genome editing

Janina Metje-Sprink, Thorben Sprink and Frank Hartung
Julius Kühn Institute, Institute for Biosafety in Plant Biotechnology, Quedlinburg
E-mail of corresponding author: janina.metje@julius-kuehn.de

New breeding technologies like CRISPR/Cas systems have become fast, easy and widely used genome editing tools and were entitled „breakthrough of the year 2015“ by Science journal. Typically, RNA-guided endonucleases (RGENs) are delivered into plant cells by transfection with plasmids or by *Agrobacterium tumefaciens* mediated T-DNA transfer. These methods induce stable expression of the CRISPR system in the host, which increases the chance of unwanted off-target effects. Furthermore, the system goes along with a possible integration of recombinant DNA and therefore the existence of transgenic plants [as intermediates]. Removal of that foreign DNA is not always possible e.g. in plants that reproduce asexually. Therefore, new genome editing methods are needed without the introduction of foreign DNA. In a DNA-free genome editing system preassembled Cas9 protein-guide RNA ribonucleoproteins (RNPs) are directly delivered to the plant cell in a vector-free manner. RGEN RNPs targeted mutagenesis is highly efficient. RNPs are demonstrated to act immediately upon delivery and are degraded rapidly in the cells. The short activity period greatly decreased chance for off-target effects. Mutants obtained by this method are completely transgene free and are indistinguishable from naturally occurring genetic variations. DNA-free Genome Editing promise a safer and more precise application of Genome Editing.

# Accelerating the breeding of carrots (*Daucus carota* L.) by editing the centromeric histone H3 (CENH3) using CRISPR/Cas9

Katharina Unkel[1], Thorben Sprink[2] and Frank Dunemann[1]
[1] Julius Kühn Institute, Institute for Breeding Research on Horticultural Crops, Quedlinburg
[2] Julius Kühn Institute, Institute for Biosafety in Plant Biotechnology, Quedlinburg
E-mail of corresponding author: katharina.unkel@julius-kuehn.de

Today`s world is faced with a number of hardships. The climate is changing at an accelerated speed. Droughts, floods and other weather extremes call for a fast-adapting agricultural landscape, while the world's population grows exponentially at the same time. Therefore, we focus on accelerating F1 hybrid breeding to create a wider variety of highly adapted cultivars to use the available natural resources as productive as possible. In our work we focus on carrot (*Daucus carota* L.), a subculture with a high content of secondary metabolites, that supports a colorful and wholesome diet with an additional long history as a model organism.

The production of genetically homogeneous parental lines through several subsequent steps of inbreeding takes up an excessive amount of time and resources. Other *in vitro* (e.g. anther or ovule culture) or *in situ* (e.g. wide hybridization or irritated pollen) techniques to produce haploid or double haploid progeny are inefficient in Apiaceae. We therefore propose to induce targeted mutations in the coding region for the centromeric specific histone H3 (CENH3) using the RNA guided endonucleases (RGEN) technique CRISPR/Cas9.

The equal distribution of chromosomes during cell division depends highly on CENH3, therefore its editing and putative loss of function could provoke uniparental genome elimination of the mutated parent during early embryogenesis when crossed with a plant that has functional CENH3.

We target different regions of the CENH3 sequence and compare mutated lines in their expression and accumulation of CENH3 as well as in their function as putative haploid inducer lines.

We found plants with a chimeric CENH3 genotype and variations of CENH3 accumulation after introducing an expression cassette for CRISPR/Cas9 via the agrobacterium *Rhizobium rhizogenes.* While testing for viable putative haploid inducer lines by self-crossings and crossings with wild type plants, we are establishing transformation systems using *Agrobacterium tumefaciens* to fasten the transformation process and to therefore be able to test additional target regions creating a higher number of putative haploid inducer lines in a shorter time.

We are also working on a DNA-free protocol to induce targeted mutations transiently. Here protoplasts are incubated with preassembled sgRNA and Cas9 proteins, allowing for mutated but non-transgenic lines with compromised CENH3.

# Insights into molecular breeding of Russian Dandelion

Regina Kölzsch, Katja Thiele, Frank Hartung and Joachim Schiemann
Julius Kühn Institute, Institute for Safety in Plant Biotechnology, Quedlinburg
E-mail of corresponding author: regina.koelzsch@julius-kuehn.de

In the framework of the BMBF funded project "EVITA", investigations on the Russian Dandelion *Taraxacum koksaghyz* as a new crop have been conducted. Despite the low rubber yield and unimproved cropping strategies, the plant arouse interest as an alternative source for natural rubber. The projects focus was set on weed management in particular by developing herbicide tolerant plants.

The enzyme acetohydroxyacid synthase (AHAS; old: ALS) is one of the most commonly used and well described targets for herbicide control. Certain amino acid substitutions can confer tolerance against several herbicides of the "ALS inhibitor class". By using unspecific and specific mutagenesis, the *T.* koksaghyz AHAS gene sequence was changed.

The self-incompatible character of *T.* koksaghyz impeded the classical EMS mutagenesis strategy. Therefore, directly after mutagenesis tolerant plants were selected. The survivors were supposed to produce progeny but reproduction was hampered by side effects of the EMS application. Finally, less than twenty plants with herbicide tolerance conferring mutations were obtained, whose propagation systems were not affected, allowing crossing and production of tolerant seeds.

Site specific mutagenesis was performed by using CRISPR/Cas9. Via *Agrobacterium tumefaciens*, plasmid constructs encoding for the single guide RNA and the Cas9 nuclease were transformed into explants of *T. koksaghyz*. Stable transformed plants were regenerated and for some individuals changes in the AHAS gene sequence could be detected. But CRISPR/Cas9 is a dynamic system and only progeny with manifested sequence changes and not carrying the transgene anymore are of interest. Therefore, crossings with *T. koksaghyz* wild type are performed. Sequencing will show what InDels have been passed to the progeny and herbicide trials will reveal if the changes can confer herbicide tolerance, too.

# Genetic differences in barley govern the responsiveness to priming agents

Abhishek Shrestha[1], Ahmed Elhady[1], Shimaa Adss[1], Gwendolin Wehner[2], Christoph Böttcher[3], Holger Heuer[1], Frank Ordon[2] and Adam Schikora[1]

[1]Julius Kühn Institute, Institute for Epidemiology and Pathogen Diagnostics, Braunschweig

[2]Julius Kühn Institute, Institute for Resistance Research and Stress Tolerance, Quedlinburg

[3]Julius Kühn Institute, Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection, Berlin

E-mail of corresponding author: abhishek.shrestha@julius-kuehn.de

During the cultivation of crop plants, priming for enhanced resistance using biocontrol agents is an efficient disease management strategy. Priming results in robust resistance and higher yield. The beneficial effects of the bacterial quorum sensing molecules e.g. *N*-acyl homoserine lactones (AHL) on resistance and plant growth have been shown in different plant species. Presence of AHL influences the transcription of various defense and growth-related genes and modifies the physiology of primed plants. Our study demonstrates the effects of the AHL: *N*-3-oxotetradecanoyl-L-homoserine lactone (oxo-C14-HSL) and AHL-producing bacteria on the priming capacity of different barley genotypes.

Barley is one of the most important crops worldwide and an enhanced resistance against pathogens, such as the powdery mildew-causing fungus *Blumeria graminis f. sp hordei*, is of high importance. We demonstrated that barley, primed with the beneficial bacterium *Ensifer meliloti*, expresses enhanced resistance against *B. graminis.* We also showed for the first time that the capacity to induce priming varies among different barley genotypes. This suggests that appropriate genetic background is required for AHL-induced priming. At the same time, it bears the potential to use these genetic features for new breeding approaches. Further, we assessed physiological phenomena, which are responsible for enhanced resistance in primed barley and presented that it involves stronger activation of the barley ortholog of the AtMPK6 kinase, regulation of defense-related (e.g. *PR1* and *PR17b*) genes and chemical remodeling of the cell wall. The stronger accumulation of lignin upon priming after challenge with chitin was particularly apparent. The global metabolomic changes in barley during priming were though rather specific.

Our results allow the discrimination between *primable* and *non-primable* barley genotypes, a newly introduced classification based on our results, and undeniably open new opportunities for breeding approaches. Furthermore, the use of biological products or beneficial bacteria represents a promising strategy for sustainable plant protection.

# Selection for resistance/tolerance to *Wheat dwarf virus* (WDV) in barley

Sarah Trebing, Antje Habekuß and Frank Ordon
Julius Kühn Institute, Institute for Resistance Research and Stress Tolerance, Quedlinburg
E-mail of corresponding author: sarah.trebing@julius-kuehn.de

*Wheat dwarf virus* (WDV), which is transmitted by the leafhopper *Psammotettix alienus,* causes high yield losses in barley. Due to global warming, insect-transmitted viruses, like WDV, will become more important in the future due to the extended survival time of the vector. Typical symptoms of virus infected barley plants are leaf yellowing, strong dwarfing and mostly dieback of the infected plants. This results in high to complete yield losses. The growing of resistant/tolerant varieties is an environmentally friendly way to avoid respective yield losses. However, up to now little is known about genotypic differences concerning resistance/tolerance to WDV. Therefore, the project aims at the identification of resistant/tolerant genotypes by screening the primary gene pool of barley and to identify quantitative trait loci (QTL) by genome-wide association studies (GWAS).

The last two years (growing period 2016/2017 and 2017/2018) a total set of 500 barley accessions was tested by artificial inoculation using viruliferous leafhoppers in gauze house and greenhouse tests. Until now, half of the genotypes also have been tested under natural infection in the U.K., the Czech Republic, France and Germany. Genotypic differences in the reaction to a WDV infection were observed.

Most barley accessions turned out to be highly susceptible. However, three barley accession showed no symptoms of infection and no virus was detected by DAS-ELISA. Furthermore, nine accessions had in spite of WDV infection still good field performances concerning yield/plant, thousand kernel weight (TKW), plant height and/or number of ears/plant. The promising barley accessions will be retested in 2018/2019. Based on these phenotypic results, a subset of 250 resistant/tolerant and susceptible barley accessions will be selected and genotyped by the 50k iSelect chip (TraitGenetics, Gatersleben). The identification of QTL for WDV resistance and the development of molecular markers are essential to replace the laborious and time consuming resistance tests with WDV-bearing leafhoppers. This will facilitate the integration of breeding for WDV resistance/tolerance into applied barley breeding.

# Do different host species and distinct habitats alter the olfactory host search of the ectoparasitoid *Holepyris sylvanidis*?

Sarah Awater-Salendo[1, 2] and Benjamin Fürstenau[1]

[1] Julius Kühn Institute, Institute for Ecochemistry, Plant Analysis and Stored Product Protection, Berlin

[2] Free University of Berlin, Institute of Biology, Applied Zoology / Animal Ecology, Berlin

E-mail of corresponding author: sarah.awater@julius-kuehn.de

Previous studies showed that the larval ectoparasitoid *Holepyris sylvanidis* uses volatiles emitted by larval feces and host larval cuticular hydrocarbons (CHC) to locate its preferred host, *Tribolium confusum*. Two components, (*E*)-2-nonenal and 1-penta-decene, from the fecal odor of *T. confusum* feeding on wheat grist, are probably key components. Furthermore, host larval CHC mediate host recognition in the parasitoid. However, *H. sylvanidis* attacks larvae of different pest beetle species, infesting diverse stored products. So far, it is unknown whether these behaviorally active compounds are ubiquitously present in the fecal odor blend and on the cuticle of other possible host species living in different host habitats (here: feeding substrates).

Therefore, we studied: (a) the larval CHC composition of four stored product pest beetles (*Oryzaephilus surinamensis*, *Tribolium castaneum*, *T. confusum* and *T. destructor*) and the behavioral response of *H. sylvanidis* towards these possible host species and

(b) the influence of three different feeding substrates (millet, rice or wheat grist) on the fecal odor of *T. confusum* and its effects on the olfactory host search of *H. sylvanidis*.

(a) In contact bioassays *H. sylvanidis* showed typical host recognition behavior when encountering dead and live larvae of the three *Tribolium* species whereas *O. surinamensis* larvae elicited no response. *O. surinamensis* was only attractive when *T. confusum* larval CHC extract was applied onto dead, in *n*-hexane extracted larvae. GC-MS analysis of the larval extracts revealed that CHC profiles of all tested *Tribolium* species were almost identical. The CHC pattern of *O. surinamensis* larvae differed qualitatively and quantitatively, e. g. in the absence of methyl-branched (Me-) alkanes. Since dead and extracted *T. confusum* treated with a fraction of Me-alkanes were recognized by *H. sylvanidis* we suggest that these compounds serve as contact kairomones for host recognition.

(b) In a static four-field-olfactometer we tested the behavioral response of *H. sylvanidis* to larval faces of *T. confusum* feeding on millet, rice or wheat grist. First results indicate that female parasitoids are highly attracted to all feces samples regardless of the feeding substrates. Whether this attraction is mediated by the two key components, (*E*)-2-nonenal and 1-pentadecene, or by other substrate-specific compounds still needs to be analyzed.

Our results might not only enhance the understanding of a parasitoid's host search regarding different host species and host habitat but may also improve the prospective use of *H. sylvanidis* in Integrated Pest Management.

# Session 5

# Stories from One Thousand and Two *Secale* Samples: Insights into the evolutionary history of domesticated rye and its wild relatives

Mona Schreiber[1,2] and Martin Mascher [1,3]
[1] Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben
[2] Johannes Gutenberg-Universität, Institut für Organismische und Molekulare Evolutionsbiologie, Mainz
[3] German Centre for Integrative Biodiversity Research (iDiv), Halle-Jena-Leipzig
E-mail of corresponding author: schreiberm@ipk-gatersleben.de

As a minor crop with only local importance our understanding of the evolutionary history of domesticated rye and the relationship to its wild relatives are still limited. In contrast to wheat and barley, rye is not considered as a founder crop of Neolithic agriculture, even if the wild progenitors of all three cereal crops share the same area of distribution in Southwest Asia. Instead, rye is assumed to be a secondary domesticate that became widely used in Central and Eastern Europe only after its introduction as a weed in the course of the expansion of domesticated wheat and barley. The ability to thrive on poor soils and the high frost tolerance has enabled rye to become a suitable crop under harsh conditions as found in the northern areas of Europe.

Here, we use the combination of genotyping-by-sequencing and whole genome resequencing data on 1002 *Secale* samples mapped to the recently assembled high quality reference genome to study the population history of the small genus *Secale*. Overall, the weak genetic differentiation between wild and domesticated rye points to ongoing gene flow and a fairly recent speciation leading to incomplete lineage sorting and low fertility barriers.

The analysis of the population genomic history of domesticated rye within the complex population structure of the different wild *Secale* species points to the idiosyncratic characteristics of the domestication process of rye. Combining these newly available genomic resources with archaeological and linguistic evidence enables us to explore the differences in use and perception of rye in different geographic areas as a result of the underlying past processes.

# A candle in the dark: Reference genome assembly for rye highlights the importance of data visualisation and manual editing

M. Timothy Rabanus-Wallace[1], Nils Stein[1] , Martin Mascher[1], Andreas Graner[1], Andreas Boerner[1], Andreas Houben[1], Uwe Scholtz[1], Sudharsan Padmarasu[1], Axel Himmelbach[1], Ines Walde[1], Susanne Koenig[1], Manuela Knauft[1], Curtis Pozniak[2], Andy Sharpe[2], David Konkin[2], Faouzi Bekkaoui[2], Jana Cizkova[3], Hana Simkova[3], Jaroslav Dolezel[3], Alex Hastie[4], Heidrun Gundlach[5], Manuel Spannagl[5], Klaus Mayer[5], Eva Bauer[6], Anthony Hall[7], Cristobal Uauy[8], Frank Ordon[9], Berndt Hackauf[9], Viktor Korzun[10], Joachim Fromme[11], André Laroche[12], Stefan Stojałowski[13], Hanna Bolibok-Brągoszewska[14], Monika Rakoczy-Trojanowska[14], Alan Schulman[15], Xuefeng Ma[16], Jessie Poland[17], Hikmet Budak[18], Vijay Tiwari[19], Lynne Reuber[20], Beat Keller[21], Hakan Ozkan[22]

[1] Leibniz-Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany
[2] College of Agriculture and Bioresources, University of Saskatoon, Canada
[3] Centre of Plant Structural and Functional Genomics, Institute of Experimental Botany CAS, Czech Republic
[4] Bionano Genomics, San Diego, USA
[5] Plant Genome and Systems Biology, Helmholtz Zentrum München, Germany
[6] Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt, Technische Universität München, Germany
[7] Earlham Institute, Norwich Research Park, UK
[8] John Innes Centre, Norwich Research Park, UK
[9] Institute for Resistance Research and Stress Tolerance, Julius Kühn-Institut, Germany
[10] KWS LOCHOW, Einbeck, Germany
[11] Hybro Saatzucht, Schenkenberg, Germany
[12] Agriculture and Agri-Food, Ottawa, Canada
[13] Department of Genetics, Plant Breeding and Biotechnology, West Pomeranian University of Technology, Szczecin, Poland
[14] Department of Plant Genetics, Breeding and Biotechnology, Warsaw University of Life Sciences, Warsaw, Poland
[15] University of Helsinki, Helsinki, Finland
[16] Noble Research Institute, Ardmore, USA
[17] Department of Agronomy,Kansas State University, Manhattan, USA
[18] Plant Sciences & Plant Pathology, Michigan State University, East Lansing, USA
[19] Plant Science & Landscape Architecture,University of Maryland, Upper Marlboro, USA
[20] 2Blades Foundation, San Mateo, USA
[21] Department of Plant and Microbial Biology, Zürich University, Zürich, Switzerland
[22] Faculty of Agriculture, Cukurova University, Adana, Turkey
E-mail of corresponding author: wallace@ipk-gatersleben.de

The rye genome poses a major assembly challenge owing to its large size, repetitive content, and fixed heterozygosity. A suite of new technologies (such as molecule-linked reads, chromosome-conformation-capture reads, and optical maps) help to overcome many of these challenges, but integrating the data from very diverse sources of data to produce an assembly is difficult to automate with optimal results. Several years of work by an international consortium of institutions has produced a new reference quality genome for rye, which was completed following the philosophy that the results of automated procedures are best taken as suggestions, to be carefully refined by a human curator with access to an array of intuitive visualisations. Such close curation can markedly increase the quality of a genome assembly, and visually-intuitive representations of a genome assembly (and its relationship to the underlying data), are similarly valuable for those using the genome for downstream applications.

# Evaluation of the potential of cleistogamous flowering for a sustainable reduction of loose smut infection (*Ustilago avenae* (Pers.) Rostr.) in oats (*Avena sativa* L.)

Sophie Brodführer[1], Ben Schmehe[2] and Matthias Herrmann[1]
[1] Julius Kühn Institute, Institute for Breeding Research on Agricultural Crops, Groß Lüsewitz
[2] Landbauschule Dottenfelderhof e.V., Forschung & Züchtung, Bad Vilbel
E-mail of corresponding author: sophie.brodfuehrer@julius-kuehn.de

Loose smut caused by the pathogen *Ustilago avenae* (Pers.) Rostr. is one of the most challenging diseases in organic seed production. The seed-borne disease can be controlled by fungicide seed treatments in conventional farming. In organic oat breeding, integrating resistance genes in modern breeding germplasm is an important goal.

The pathogen's teliospores are dispersed by wind and fall into the opened flower of the host plants during flowering time. Newly formed mycel overwinters with the kernels and infects the seedlings shortly after germination. In infected plants, a 'smutted' panicle with hardly any inflorescence tissue left emerges from the culm at flowering time.

The overall goal of the project is to investigate the role of cleistogamous flowering (where the flower remains closed at flowering time) in preventing the contamination of new inflorescences. The closed hulls might function as a mechanical barrier and could reduce the amount of spores ending up in the flower.

In the project, an oat panel is phenotyped according to flowering traits (rating the degree of open flowering and rating the degree of anther retention) in multiple years and locations. The aim is to identify cleistogamous and chasmogamous phenotypes.

The oat panel consists of 540 current and historical breeding lines, with a mostly european origin. The breeding lines are also phenotyped with respect to their degree of susceptibility to loose smut. To ensure a high infection pressure, the seeds are inoculated with the pathogen's spores before sowing.

In addition, the oat panel is genotyped via genotyping-by-sequencing. Performing a genome wide association scan (GWAS) will help to elucidate the molecular basis of the flowering traits. Furthermore, the GWAS approach will be used to detect regions in the genome that possibly encode resistance genes present in the oat panel.

# Suppression of the northern root-knot nematode *Meloidogyne hapla* by soil bacteria

Olivera Topalović[1], Johannes Hallmann[2] and Holger Heuer[1]
[1]Julius Kühn Institute, Institute for Epidemiology and Pathogen Diagnostics, Braunschweig
[2] Julius Kühn Institute, Institute for Plant Protection in Horticulture and Forests, Münster
E-mail of corresponding author: olivera.topalovic@julius-kuehn.de

Suppression of plant-parasitic nematodes (PPN) is of global importance for the production of human and animal food, since they cause an irreparable damage to plants. Use of nematicides has not only caused environmental disturbances but has often failed to prove its effectiveness on a long term scale. The recent advances in studies on soil suppressiveness have led to the discovery of microbial residents in soil that are able to hamper the performance of PPN on plants to a great extent. The scientists worldwide, ourselves included, aim to fully understand the interactions and existing mechanisms of nematode suppression by defined soil microbial communities. Therefore, we isolated bacteria from soils with a varying degree of soil suppressiveness against the root-knot nematode *Meloidogyne hapla* that attach to the infective stage J2 of the latter, and explored the basic mechanisms behind this attachment. The bacterial isolates that showed the highest degree of attachment to J2 of *M. hapla* belonged to the genera *Microbacterium*, *Brevundimonas*, *Sphingopyxis* and *Acinetobacter*.

These were then used to explore whether they are able to antagonize nematodes in in vitro and in vivo assays. Some of them suppressed J2 and nematode eggs directly or by mediating plant defence responses in plants. We believe that this study helps to better elucidate interactions between PPN and bacteria in soil, and pioneers the consideration of the involvement of nematode-attached microbiome in soil suppressiveness against phytonematodes.

# Session 6

# Novel detected entomopathogenic fungus *Pandora* sp. infects *Cacopsylla* spp. and other phloem-feeding hemipteran insects

Louisa M. Görg[1], Annette H. Jensen[2], Jørgen Eilenberg[2] and Jürgen Gross[1]
[1] Julius Kühn Institute, Institute for Plant Protection in Fruit Crops and Viticulture, Dossenheim
[2] University of Copenhagen, Department of Plant and Environmental Sciences, Denmark
E-mail of corresponding author: louisa.goerg@julius-kuehn.de

The entomopathogenic fungus *Pandora* sp. (Entomophthorales: Entomophthoraceae) was discovered on psyllids in Denmark in September 2016. Reisolated from pear psyllid cadavers, it was possible to cultivate the fungus on solid media. In pathogenicity tests, it was assessed whether this (yet undescribed) species would be able to infect other insects, with special regard to psyllids (Hemiptera: Psyllidae). In the pathogenicity experiments, insects were inoculated with a "conidia shower". For this purpose, mycelia mats were applied to the lid of small plastic cups, using the typical mechanisms of Entomophthorales, which actively eject their conidia and showering on the insects underneath. A not yet quantified but high dosage of conidia was used. After exposure to the conidia, the mortality as well as postmortal symptoms of fungal infection were assessed over a period of 10 days. *Cacopsylla pyri* L. and *C. pyricola* Foerster, which are indigenous pear psyllids in Germany, were successfully infected. Other psyllids, namely *C. picta* Foerster and *C. pruni* Scopoli that cause great damage in fruit production by transmitting phytoplasma diseases to their respective host plants apple or peach, were also susceptible to this fungus. When inoculated with *Pandora* sp. mortality increased and symptoms of the fungal infection on the cadavers were observed 24 hours after onset of mortality. Other psyllids were also susceptible like *C. peregrina* Foerster feeding on hawthorn (*Crataegus monogyna* L.). Furthermore, other phloem-feeding hemipteran insects such as *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae) vectoring several plant viruses, showed decreased survival probabilities after treatment with this entomopathogenic fungus. Regarding these results, the role of *Pandora* sp. as a potential agent for a biological pest control approach is a part of the BMEL-supported research project "PICTA KILL". An innovative formulation should be developed releasing specific attractant volatiles in order to attract the apple psyllid *C. picta* towards the encapsulated fungus (Attract-and-Kill- strategy). Hence, the capsule could increase the specificity of this control approach and thereby decrease the new infestation of apple trees with apple proliferation disease.

# Evaluation of raspberry and strawberry genetic resources for resistance to spotted-wing drosophila (*Drosophila suzukii*)

Eric Fritzsche, Johanna Pinggera, Dora Pinczinger, Marcel von Reth, Thomas Wöhner, Magda-Viola Hanke, Henryk Flachowsky
Julius Kühn Institute, Institute for Breeding Research on Fruit Crops, Dresden
E-mail of corresponding author: eric.fritzsche@julius-kuehn.de

The spotted-wing drosophila (*Drosophila suzukii*), brought in from Asia, is currently considered as the biggest menace for the german cultivation of berry fruits. This drosophila species is ovipositing into almost mature and fully ripe soft-skinned berry fruits, like strawberry, raspberry, blackberry or currant, with her saw-like edged ovipositor. Larvae, developing in infested fruits, are destructing the fabric texture of fruit pulp and are leading to inedible and non-marketable fruits. Damaging the fruit skin with the ovipositor is causing points of entrance for secondary pathogens like fungi and bacteria. Combating *D. suzukii* with insecticides is not possible because of not realizable waiting periods. Currently, covering of total berry fruit plantations with close-meshed nets is the best way to protect the fruits against *Drosophila*. Cultivation of less susceptible cultivars could form a sustained fighting strategy against *D. suzukii*.

Currently, about preferences of *D. suzukii* concerning oviposition into different cultivars within a genus is only little known. This project is investigating oviposition of *D. suzukii* using different cultivars of strawberry and raspberry as a part of choice and no choice tests in a lab. Investigations consists of incubations of ten times three berry fruits of each cultivar with ten female and five male drosophila flies, each. Incubating conditions were a humidity of 70% and a temperature of 23°C. After a 24h incubation with flies, the fruits were incubating without flies for another five days under the same conditions. After that larvae, developing in infested fruits, were counted. Ingredients, fruit skin firmness and berry color will be investigated to explain the different susceptibility between different cultivars.

The results of testing raspberries show, that in 2016 and 2017 ‘Dorman Red’ is the least infested of totally 34 tested floricane raspberries on average. ‘Cascade Delight’ is the average most infested floricane in both years. ‘Autumn Best’ is most feeble infested and ‘Autumn Bliss’ is the most infested of 19 tested primocane cultivar in 2016. In 2017, 29 autumn varieties were tested. ‘Ruby Fall’ shows the slightest and ‘Mapema’ the strongest infestation. The completely evaluation of raspberries tested in 2018 is still pending. The results of five tested strawberry cultivars reveal, that in 2017 the precocious cultivar ‘Darselect’ is most feeble infested and the late maturing cultivar ‘Malwina’ shows the strongest infestation. In 2018 ‘Mieze Nova’ is the least infested and ‘Asia’ the most infested of 14 tested strawberry cultivar.

# Map-based cloning of *Rph*$_{MBR102}$ conferring resistance to barley leaf rust

Leila Fazlikhani, Dragan Perovic and Frank Ordon
Julius Kühn Institute, Institute for Resistance Research and Stress Tolerance, Quedlinburg
E-mail of corresponding author: leila.fazlikhani@julius-kuehn.de

Leaf rust of barley is a serious disease caused by the biotrophic fungus *Puccinia hordei* Otth., which under favorable conditions may cause yield losses up to 62%. So far, twenty-five race-specific leaf rust resistance genes (*Rph1-Rph25*) have been mapped, but no one have been yet isolated. However, some of these have been overcome by new pathotypes of *P.hordei*, indicating the need for introducing new sources of resistance into barley breeding as well as the need for isolating known ones towards deciphering the structure and function offering the possibility of developing functional markers for breeding. In this respect, recent advances in the development of barley genomic resources i.e. 9K and 50K iSelect arrays, genome zipper, POPSEQ, and GBS maps, as well as the barley reference sequence, enhance the possibility of narrowing down the target region harboring respective resistance genes. The *Rph*$_{MBR1012}$ gene previously mapped in the distal region of the short arm of barley chromosome 1H is effective against the highly virulent barley leaf rust (*Puccinia hordei*) isolate I-80. In order to positionaly clone the *Rph*$_{MBR1012}$ gene, a high resolution mapping population (HRMP) was constructed based on the cross "MBR1012 (resistant) x Scarlett (susceptible)". 537 segmental homozygous recombinant inbred lines (RILs) derived from 4775 $F_2$-plants corresponding to a resolution of 0.010% recombination were identified by analyzing the population with two co-dominant flanking SSR markers (QBS94 and QBS113) spanning an interval of 8.0 cM. To down size the target interval, initially 37 SSRs and SNP markers derived from the 9K chip and the genome zipper were mapped at the HRMP, resulting in shortening the target interval to 0.1 cM, flanked by QBS97 and QBS98. Further marker saturation was done by employment of 19 additional SNP markers derived from currently available barley genomic resources i.e. the 50K iSelect arrays and GBS. All selected markers from 50K iSelect and GBS were converted to KASPar markers. The target interval was downsized to 0.01 cM in the window between two KASP markers QBS127 and QBS98. Using BlastN search to the barley genome reference sequence, markers were anchored to the reference barley sequence revealing a physical size of 0.44 Mb. 18 high-confidence and 11 low-confidence genes were detected of which five are related to disease resistance. Allele specific re-sequencing of all 29 candidate genes was conducted to reduce the number of putative candidate genes.

# Identification of markers closely linked to effective leaf rust resistance genes in wheat

Aleksandra Varekhina[1], Albrecht Serfling[1], Klaus Pillen[2] and Frank Ordon[1]

[1] Julius Kühn Institute, Institute for Resistance Research and Stress Tolerance, Quedlinburg

[2] Martin Luther University Halle-Wittenberg, Institute of Agricultural and Nutritional Sciences, Halle (Saale)

E-mail of corresponding author: aleksandra.varekhina@julius-kuehn.de

Leaf rust, caused by *Puccinia triticina*, is the most common and widespread disease of wheat (*Triticum aestivum*) worldwide. Leaf rust causes a reduction of grain quality and yield losses of up to 40%. Epidemics are due to a breakdown of leaf rust resistances (Lr resistances) by virulent rust races. An example for a breakdown is Lr37, which became ineffective within two years in the 2006. One option to avoid epidemics is pyramiding of Lr-genes so that cultivars carry several effective Lr-genes. A prerequisite for pyramiding are closely linked molecular markers. At the moment, more than 80 Lr-genes have been identified, but only a part of these have been deployed in wheat varieties in part due to linkage drag. Resistances showing a high level of resistance in the field are Lr2a, located on chromosome 2DS, and Lr24 on chromosome 3B. In order to get detailed information on their localization and to reduce linkage drag, NILs (near isogenic line) containing one of the Lr-genes were crossed to the susceptible cultivar Monopol. Parental lines and F2 plants were inoculated with leaf rust single spore isolates avirulent to Lr2a and Lr24. The development of fungal structures was analyzed on leaf material of parental lines and of 150 (Lr2a) and 144 (Lr24) F2 plants, each at 72 hours after the inoculation (hai) and uredospore pustule development was scored at 168 hai. First results of the analysis proved the recessive inheritance of Lr2a as a good fit to a 3s:1r segregation ($\chi2=1,5$) and dominant inheritance of Lr24 with 3r:1s segregation ($\chi2=0,39$) were observed. Based on these results kompetitive allele specific PCR markers (KASP) which have been generated based on SNPs detected between near isogenic lines (NILs) carrying the resistances and the susceptible parental line (Thatcher) were genetically mapped and aligned to the reference genome so that candidate genes for Lr2a were identified, already. Next steps will be the analysis of additional F2 populations segregating for effective resistances (e.g. Lr9, Lr19) and pyramiding of these genes.

# Session 7

# Efficient induction of inversions in plant genomes using the CRISPR/Cas system

Carla Schmidt, Michael Pacher and Holger Puchta
Karlsruhe Institute of Technology, Botanical Institute, Karlsruhe
E-mail of corresponding author: carla.schmidt@kit.edu

CRISPR/Cas mediated genome engineering in plants has mainly concentrated on the knockout of genes and the induction of deletions. Sequence inversions occur often during plant genome evolution - also in related cultivars - making the inverted regions inaccessible for plant breeders as a result of fixed linkages. Thus, it is important to set up technologies that allow us to induce inversions within chromosomes in a directed and efficient way in plants.

In the current research, we induced two DSBs in a distance of about 3 - 18 kb using the Cas9 protein from *Staphylococcus aureus* (*Sau*Cas9) for efficient DSB induction on four different loci in the model plant *Arabidopsis thaliana*. The expression of the Cas9 nuclease was controlled by the constitutive PcUbiquitin4-2 promoter from *Petroselinum crispum* (PcUbi4-2) and by the egg-cell specific promotor (EC-P) of *A. thaliana*. The approach, driven by the PcUbi4-2 promotor, was analysed in T1 using digital droplet PCR (ddPCR) to quantify the amount of deletions and inversions in wildtype and *ku70-1* mutants. It was possible to generate deletions with frequencies up to 7 % and inversions up to 2 %. Additionally, we defined via deep sequencing the patterns of junction formation in wildtype and *ku70-1* mutants. Like for deletions, in the majority of cases for inversions re-joining of the cut junctions occurs without further mutations. Surprisingly, in plants deficient in KU70, which is essential for classical non-homologous end joining (NHEJ), inversion induction is enhanced. However, most junctions - that often contain micro homologies - are imperfect with insertions and mainly deletions. Using the egg cell specific expression we were able to induce heritable inversion formation at different loci and at distances between 3 and 18 kb in the percent range. By screening individual lines heritable inversion events up to the 10 % range can be regularly achieved. Most of the events contained the inversion with scarless junctions and without any sequence change within the inverted region making the technology attractive for applications in crop plants.

# High throughput reverse genetic tools for knocking out several genes of the phytic acid pathway in *Brassica napus*

Niharika Sashidhar, Hans-Joachim Harloff and Christian Jung
Christian-Albrechts-University Kiel, Institute of Crop Science and Plant Breeding, Kiel
E-mail of corresponding author: c.jung@plantbreeding.uni-kiel.de

*Brassica napus* L. (oilseed rape) is an important oil crop in the temperate regions. After oil extraction, the seeds are used as a feed for livestock due to their rich protein and balanced amino acid content. However, the meal is not utilized efficiently because of the presence of high quantities of anti-nutritive substances. One of those is phytic acid, accounting for 2-4 % in oilseed rape cultivars. Phytic acid is an important source of inorganic phosphate for plants and is involved in many biological functions. It is still not known to what extent it is required to maintain the basic physiological functions. Identification of low phytic acid mutants may not only provide valuable information for understanding the biological function of the genes involved in the phytic acid pathway, but also result in improved seed quality. However, mutational analyses in oil seed rape is challenging due to polyploidization. Since gene functions are often encoded by several paralogs, more than one gene has to be knocked out to study the underlying effect. In this project, we adopted two different strategies for the mutational analysis. One approach is TILLING by sequencing and the other is genome engineering by using Streptococcus pyogenes Cas9 endonuclease. We chose most of the functional paralogs of seven crucial gene families (ITPK, MIPS, MIK, IMPK, PGK2, MRP5, IPK1) of the pathway. We were able to establish a high throughput mutant screening by sequencing, which resulted in an average mutation density of 1/18 kb in all the targeted genes. Furthermore, by targeting the conserved regions of two subfamilies of the ITPK gene family we obtained gene editing in the spring rapeseed cultivar Haydn by using hypocotyl transformation.

# Interaction of the Beet necrotic yellow vein virus with the auxin signaling pathway in sugar beet

Sebastian Liebe[1], Jose Fernando Gil[2], Heike Thiel[1], Britt-Louise Lennfors[3], Thomas Kraft[3], David Gilmer[4], Mark Varrelmann[1] and Eugene I. Savenkov[2]

[1] Institute of Sugar Beet Research, Department of Phytopathology, Göttingen

[2] Swedish University of Agricultural Sciences, Department of Plant Biology, Uppsala

[3] Maribo Hilleshög Research AB, Landskrona

[4] Université de Strasbourgh, Institut de biologie moléculaire des plantes, Strasbourgh

E-mail of corresponding author: liebe@ifz-goettingen.de

Beet necrotic yellow vein virus (BNYVV) is the causal agent of Rhizomania, a viral disease of sugar beet with high economic importance transmitted by the plasmodiophorid Polymyxa betae. Upon BNYVV infection, plants display massive lateral root proliferation leading to the characteristic symptom of a “root beard” and reduced tap root weight. Auxin as the major plant hormone controls an array of developmental processes including the development of lateral roots. Therefore, it is supposed that BNYVV interacts with the auxin signaling pathway to induce lateral root proliferation but the mechanism responsible for that is still unknown. We identified an Aux/IAA protein (AUX28) from sugar beet as an interaction partner of the pathogenicity factor P25. Aux/IAA proteins are negative regulators of auxin response factor proteins that in turn control the transcriptional activity of auxin response genes. This interaction is crucial for the auxin signaling pathway as it determines the expression of auxin responsive genes involved in lateral root development. P25 and AUX28 interacted in planta as demonstrated by bimolecular fluorescence complementation assay. Domain mapping revealed that P25 is able to interact with domain I and II of AUX28. Subcellular localization showed that P25 localizes to both cytoplasma and nucleus whereas the Aux/IAA protein localizes exclusively to the nucleus. In the presence of P25, the Aux/IAA protein was relocalized to the cytoplasm. This relocalisation must be followed by transcriptional changes of auxin responsive genes. This hypothesis was supported by expression analysis showing that several genes involved in lateral root development are induced upon BYNVV infection. The results provide for the first time evidence that BNYVV interacts with the auxin signaling pathway in sugar beet. A model explaning how BNYVV interacts with the auxin signaling pathway in order to induce lateral root development is presented.

# Towards the high-resolution mapping and isolation of virus resistance/tolerance genes derived from *H. bulbosum*

Julia Kretsch[1], Dragan Perovic[1], Antje Habekuß[1], Viktor Korzun[2], Klaus Oldach[2], Neele Wendler[2], Hélène Pidon[3], Nils Stein[3] and Frank Ordon[1]

[1] Julius Kühn Institute, Institute for Resistance Research and Stress Tolerance, Quedlinburg

[2] KWS LOCHOW GMBH, Bergen

[3] Leibniz-Institute of Plant Genetics and Crop Plant Research, Gatersleben

E-mail of corresponding author: julia.kretsch@julius-kuehn.de

*Hordeum bulbosum* is the only member in the secondary genepool of barley (*H. vulgare*) and hence owns a great genetic potential for barley breeding. This species holds resistances against many pathogens, for example against *Barley mild mosaic virus/Barley yellow mosaic virus* (BaMMV/BaYMV) or *Barley yellow dwarf virus* (BYDV). Both diseases cause high yield losses in barley. Furthermore, the control of the aphid-transmitted BYDV is becoming difficult due to governmental regulations concerning insecticides and the use of chemicals to control BaMMV/BaYMV, transferred by the soil-borne protist *Polymyxa graminis*, is not possible. Thus, breeding for resistance is the only possibility to protect barley against these diseases.

Different *H. bulbosum* introgression lines carry resistance against BaMMV/BaYMV (*Rym16*$^{Hb}$) and *Ryd*$_{203S11}$$^{Hb}$ for tolerance against BYDV on chromosome 2HL. DH lines carrying an introgression containing *Rym16*$^{Hb}$ or *Ryd*$_{203S11}$$^{Hb}$ were identified and characterized using molecular markers. Blasting sequences of these markers against the barley reference sequence allowed anchoring the introgression to the physical map and a size of the introgression fragment of 4.2 Mb for the *Ryd*$_{203S11}$$^{Hb}$ locus and 3 Mb for the *Rym16*$^{Hb}$ locus was calculated. Right now, $F_2$ populations carrying *Ryd*$_{203S11}$$^{Hb}$ or *Rym16*$^{Hb}$ are genotyped by using co-dominant flanking markers to construct a high resolution mapping population. The recombination rate within the introgression was found to be approximately 0.5 %, which is lower than the intraspecific recombination rate within in the barley genome, most likely caused by the incomplete homology between the genome of *H. vulgare* and *H. bulbosum*.

As a basis for isolating the respective genes via a map-based cloning approach, recombinant plants will be selfed, phenotyped and saturated with markers using Exome capture, GBS and Illumina 50K data. A non-gridded BAC library will be utilized to construct a physical map of the target region of *Ryd*$_{203S11}$$^{Hb}$. This map will help to identify candidate genes located in the *H. bulbosum* introgression fragment. In addition, a genotype-specific resistance of *Rym16*$^{Hb}$ will be examined by using resistance gene enrichment sequencing (RenSeq).

The authors thank the German Federal Ministry of Food and Agriculture (BMEL) for funding this project (FKZ 2818201515).

# Enhanced plant resistance towards phytopathogenic fungi depends on the rhizosphere microbial community composition

Nina Bziuk[1], Desirée Lauterbach[1], Søren J. Sørensen[2], Adam Schikora[1] and Kornelia Smalla[1]
[1] Julius Kühn Institute, Institute for Epidemiology and Pathogen Diagnostics, Braunschweig
[2] University of Copenhagen, Department of Biology, Section of Microbiology, Copenhagen
E-mail of corresponding author: nina.bziuk@julius-kuehn.de

The plant's resistance towards phytopathogenic fungi is assumed to be enhanced by interactions with its rhizomicrobiota. It is known that beneficial bacteria in the rhizosphere can trigger a faster and stronger immune response of the plant towards pathogens or abiotic stresses, which can also be called priming. Furthermore, the prokaryotic community compositions of agricultural soils differ depending on the applied management practice. We hypothesize that prokaryotic communities from different agricultural legacy might also influence the ability of the rhizomicrobiota of barley cultivar 'Golden Promise' to enhance the resistance against powdery mildew *Blumeria graminis* f. sp. *hordei*. Therefore, an experimental approach was developed suitable to test the priming ability of the rhizomicrobiota under greenhouse conditions. Detached barley rhizomicrobiota from plants grown in field soil was inoculated to a substrate/sand mixture, which was planted with barley seedlings. Control plants were treated in the same way but with saline solution. At growth stage 13, barley plants were infected with *B. graminis*, control plants were left untreated. The prokaryotic community composition was analyzed by sequencing of 16S rRNA genes amplified from total community DNA directly extracted from rhizosphere soils sampled nine days after infection. The priming efficiency was examined by a detached leaf assay and expression pattern of defense-related genes analyzed by qPCR. Although the resistance against *B. graminis* was not found to be improved, plants treated with the rhizosphere microbiota showed a stronger defense response after the fungal infection. Furthermore, an influence of the rhizosphere inoculant, as well as the presence of the fungal pathogen on the prokaryotic rhizosphere community with several differentially abundant taxa was observed.

Our results suggest a stronger priming ability of the rhizomicrobiota from field soil compared to the substrate community. The developed approach proved to be suitable for testing the abilities of prokaryotic communities originating from soils with different agricultural history to enhance the resistance of barley towards fungal phytopathogens.

# A systematic map about the available evidence for the application of genome editing in plants

Dominik Modrzejewski, Frank Hartung, Thorben Sprink, Dörthe Krause, Christian Kohl and Ralf Wilhelm
Julius Kühn Institute, Institute for Biosafety in Plant Biotechnology, Quedlinburg
E-mail of corresponding author: dominik.modrzejewski@julius-kuehn.de

In order to address **E**thical, **L**egal and **S**ocio-economic **A**spects of **G**enome **E**diting in **A**griculture the **ELSA-GEA** project was established to support an informed public debate and provide science-based input for decision-makers in politics, economics, science and society.

Plant breeding is a developing process and new breeding methods have continuously evolved over time. Within the last decades, genome editing techniques such as Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR associated proteins (CRISPR/Cas), Transcription Activator-Like Effector Nucleases (TALENs), Zinc-Finger Nucleases (ZFN), Meganucleases (MN) and Oligonucleotide-Directed Mutagenesis (ODM) have been developed enabling a precise modification of DNA sequences in plant species like rice, maize, soybean, tomato and many others.

In order to provide a comprehensive overview about the fast growing available evidence for the application of genome editing in plants a systematic map has been conducted. A systematic map is based on a broad review question aiming to identify, collect and evaluate the available academic and grey literature in a systematic and transparent manner. The detailed determination and documentation of the data collection allows a consistent updating and supplementing of the existing literature.

First results of the map identified many market-oriented developments of genome editing, including improved agronomic characteristics, improved food and feed quality, increased tolerance to abiotic and biotic stress and herbicide tolerance.

# Transcriptional regulation of iron homeostasis of the hemibiotrophic phytopathogen *Colletotrichum graminicola*

Anja Raschke[1,2], Jessica Heilmann[1], Mario Lange[1,2] and Holger B. Deising[1,2]

[1] Martin Luther University Halle-Wittenberg, Department of Phytopathology and Plant Protection, Halle (Saale)

[2] Martin Luther University Halle-Wittenberg, Interdisciplinary Center for Crop Plant Research, Halle (Saale)

E-mail of corresponding author: anja.raschke@landw.uni-halle.de

Iron is an essential microelement for all organisms. Due to its low solubility combined with the potential to produce damaging highly reactive oxygen species, a tight regulation of iron uptake and storage is essential for all living cells.

Pathogenic fungi employ several strategies for iron uptake from the host tissue: *(i)* reductive iron assimilation (RIA), *(ii)* siderophore-mediated $Fe^{3+}$ acquisition (SIA), *(iii)* heme uptake, and *(iv)* low affinity iron uptake. As free heme is rare in maize - the host plant of *Colletotrichum graminicola* - this hemibiotrophic fungus mainly applies RIA and SIA. Saprophytic hyphae growth under iron starvation leads to an up-regulation of both RIA and SIA pathways. During the biotrophic stage of infection RIA is highly active, while SIA is specifically suppressed. The subsequent necrotrophic stage is characterized by a reversal in the iron uptake specificity. Maize leaves pretreated with the *C. graminicola* siderophore Coprogen respond with an increased defense reaction including respiratory burst when these leaves were infected later on. In contrast, Coprogen alone did not induce a defense response. This reveals that *C. graminicola* specifically represses the SIA pathway, possibly to evade plant recognition. During the necrotrophic phase such hiding is no longer required. This strategy resembles the specific repression of the synthesis of β-1,3-Glucane, a pathogen associated molecular pattern (PAMP), during biotrophy.

In other fungal species the tight regulation of the SIA und RIA pathways occurs on transcriptional level mediated by two transcription factors SreA and HapX, respectively. However, the so far studied *Aspergillus* spp. were either necrotrophs or saprophytes. Here we report on the identification of *sreA* and *hapX* homologs from the hemibiotrophic fungus *C. graminicola* that were denominated as *CgSRE1* and *CgHAP10*. We showed that both genes are iron-dependent regulated on transcriptional level in saprophytic hyphae. Targeted deletions of these loci led to delayed growth in response to iron availability. Remarkably, the Δ*sre1* strain showed altered hyphal morphology resembling cell wall-deficient mutants. Cell wall deficiency, therefore, could be responsible for the reduced virulence of Δ*Cgsre1*.

Detailed functional characterization of the putative transcription factors CgSre1 and CgHap10 during biotrophic and necrotrophic stages will gain further knowledge of iron acquisition and regulation of iron homeostasis in fungal virulence and provide valuable data to develop novel plant protection strategies.

# Poster

# The effect of habitat connectivity on colonisation of forest fragments with rodents

Diana Alexandra Below, Christian Imholt, Hendrik Ennen[1] and Jens Jacob
Julius Kühn Institute, Institute for Plant Protection in Horticulture and Forests, Münster
E-mail of corresponding author: diana.below@julius-kuehn.de

Habitat fragmentation through anthropological modification/urbanisation can have an impact on the distribution and population abundance of fauna. Increased fragmentation and the presence of landscape elements that block distribution may minimise recolonization of suitable habitat by small mammals after the population crash phase. This is relevant as the human population expands and requires more and more space, which increases fragmentation.

We determined the degree of connectivity of habitat fragments in North-West Germany formally by allocating permeability values to the habitat structures present at landscape scale. These data were related to surveys of the colonisation of forest fragments by rodents to assess relationships between fragmentation and repopulation.

Such information is not only important for the assessment of land use effects but can also contribute to a better understanding of processes driving population dynamics. In addition, risk related to rodent-borne diseases can be considered. First results are presented and discussed.

# Comparative studies regarding the sensitivity of the honey bee and wild bee species to plant protection products – residue analysis

Anna Wernecke, Tobias Jütte, André Krahner, Gabriela Bischoff and Jens Pistorius
Julius Kühn Institute, Institute for Bee Protection, Braunschweig
E-mail of corresponding author: anna.wernecke@julius-kuehn.de

From the registration processes of plant protection products (PPPs) and the associated risk assessment for bees arises an increasing need for experimental data on non-*Apis* pollinators, in order to assess potential side effects of PPPs on this largely understudied set of test organisms. At present, the extent of differences in the reaction of honey bees and wild bee species, especially to PPPs and other factors in our agricultural landscape, remains unclear.

So far, toxicity has only been examined for a few species, mainly the red mason bee *Osmia rufa*/*bicornis* and the bumble bee *Bombus terrestris*. Data on toxicity of active substances to other bee species are limited.

Therefore, we investigated in a series of studies under controlled laboratory conditions the effects on the honey bee (*Apis mellifera* L.) and different wild bee species with various life history characteristics of a pyrethroid insecticide containing lambda-cyhalothrin, classified as harmless to bees, but known for transient effects. To investigate the natural detoxification process of active substances a spray chamber was used to generate a contact exposure by typical field application rates with standard nozzles types used by farmers. After the application living honey bees and individuals of three different wild bee species (bumble bee *Bombus terrestris,* mason bees *Osmia bicornis* and *Osmia cornuta*) were frozen at -20°C half an hour, three days and ten days after exposition, respectively. Redues were analysed using a multi-residue method. The residue level of lambda-cyhalothrin being quantified by use of gas chromatography/mass spectrometry (GC-MS).

This comparative analysis of the residues of active substances in the honey bee and wild bee species investigates to what extent there are differences regarding effects and the metabolism between the honey bee, as representative organism, and other wild bees and which consequences these have for the risk assessment.

While the analysis has not been finalised yet, preliminary results indicate different reactions of the tested bee species. The final results will be presented during the poster presentation.

# Comparative studies regarding the sensitivity of the honey bee and wild bee species to plant protection products – laboratory studies

Tobias Jütte, Anna Wernecke, André Krahner and Jens Pistorius
Julius Kühn Institute, Institute for Bee Protection, Braunschweig
E-mail of corresponding author: tobias.juette@julius-kuehn.de

The registration processes and risk assessment of plant protection products (PPPs) on bees resulted in an increasing need for experiments with non-*Apis* pollinators to assess potential side effects of PPPs on this relatively new group of test organisms. At present, it is still unclear whether and to what extent the sensitivity of honey bees, especially to PPPs and other factors in our agricultural landscape, is comparable to wild bee species.

Currently, active substances have been tested mainly on honey bees and occasionally on other commercially used bee species (e.g. *Osmia bicornis* and *Bombus terrestris*). For the majority of wild bee species toxicity data are lacking.

Therefore, we investigated the effects of a pyrethroid insecticide, containing lambda-cyhalothrin, on the honey bee (*Apis mellifera* L.) and different wild bee species (*Andrena vaga*, *Bombus terrestris*, *Colletes cunicularius*, *Osmia bicornis*, *Osmia cornuta* and *Megachile rotundata*) with various life history characteristics in a series of studies under controlled laboratory conditions. The chosen insecticide is classified as harmless to bees but known for transient effects.

A spray chamber was used to evaluate effects following contact exposure by typical field application rates with standard nozzle types.

After the application mortality and behaviour of bees were monitored for at least 48 h following the OECD acute contact toxicity test (guideline No. 214) and were prolonged up to 6 days (control mortality: ≤10% honey bees; ≤15-20% wild bees).

The aim of the experiments was a comparative analysis of the potential effects of applied PPPs on the mortality of the honey bee and wild bee species. Furthermore, it should be clarified to what extent the extrapolation from data of the honey bee, as representative organism, to other wild bees is possible and which differences in sensitivity exist at the laboratory level.

The evaluation of the results is still in progress, but interim results let assume that the tested bee species show different reactions. The final results will be presented as part of the poster presentation.

# Are microRNAs steering the development of the honeybee hypopharyngeal gland?

Monika Krahnstöver[1], Pooja Gupta[2], Benedikt Polaczek[1], Stefan Börno[3], Ralf Einspanier[1]
[1] Free University of Berlin, Institute of Veterinary Biochemistry, Berlin
[2] Free University of Berlin, Institute of Mathematics, Berlin
[3] Max Planck Institute for Molecular Genetics, Berlin
E-mail of corresponding author: monika.krahnstoever@fu-berlin.de

Honeybee's maintenance and survival relies on the success of reproduction, a function typically associated with the reproductive tract of the queen. Likewise, the workerbee's hypopharyngeal glands represent another important organ for the sustenance of offsprings. Since secretions of these glands (royal jelly) are essential feed components for the bee larvae, amount and composition of the royal jelly is important for the breeding success of the bee colony. Furthermore, some factors are described that may directly influence the performance of these glands, like colony conditions or pesticides.

However, the presence and role of microRNAs, known as superior regulators of the cellular expression, have not been elucidated within the hypopharyngeal gland. The aim of our study is to profile the microRNA pattern and to better understand the effects of microRNAs on the development of the hypopharyngeal glands and its secretion.

Hypopharyngeal glands were isolated in different physiological stages: inactive and active. RNA was extracted and Next Generation Sequencing was used to identify differences in the two sampling groups. First results indicate regulated microRNA candidates and a quantitative validation of these interesting results are on the route.

# Effects of insecticides and feeding damage on parasitoids in pine forests

Bianca Kühne and Nadine Bräsicke
Julius Kühn Institute, Institute for Plant Protection in Horticulture and Forests, Braunschweig
E-mail of corresponding author: bianca.kuehne@julius-kuehn.de

In the Northeastern Lowland pine tree is the most economically important tree species. The majority of the single-layered monocultures grows there on sites characterized by low nutrient and water availability. These imply a high risk for infestations with insect pests. Outbreaks of phyllophagous insects led to disturbances of the ecological equilibrium combined with large economic losses. Complete defoliation in combination with unfavorable weather conditions and infestation with secondary pests can kill large numbers of trees.

As part of the BMEL/FNR research network "Future-oriented risk management for biotic damages in forests to ensure of sustainable forest management", the effects of plant protection products (insecticides applied by helicopter) and feeding damages by different insect pests are investigated on the structure and functionality of the parasitoid community.

Parasitoids are important natural pest antagonists in forest ecosystems. As specialists they can influence the pest population, but delayed in time. Among the parasitoid wasps of highest relevance for biological forest protection are species of Ichneumonidae and Chalcidoidea as well as Scelionidae (suborder Apocrita).

On the investigation area Herzberg (Brandenburg) first results are based on the evaluation of more than 21.300 individuals of the Apocrita, which were collected by pitfall traps (n=6 per study site), ground photo-eclectors (n=3) and flight-intercepting traps (n=4) in 2016. This year an outbreak of *Diprion pini* (L.) (Pine sawfly) was required the application of an insecticide (KARATE® FORST flüssig (pyrethroid)). Furthermore, a test with Mimic® (molt accelerator) took place.

The ichneumonid wasp *Pleolophus basizonus* (Grav.) was the most common species. It is known as the most important cocoon parasitoid of *Diprion pini* (L.).

# Spatial and temporal dynamics of *Trioza apicalis* in organic carrot cultivation

Jasmin Sauer[1], Holger Buck[2], Peter Hondelmann[3], Rainer Meyhöfer[3], Quentin Schorpp[1], Martin Hommes[1], Christian Ulrichs[4] and Ute Vogler[1]

[1] Julius Kuehn Institute, Institute for Plant Protection in Horticulture and Forests, Braunschweig

[2] Öko-Beratungsgesellschaft mbH (Naturland Fachberatung), Visselhöfede

[3] Leibniz Universität Hannover, Institute for Horticultural Production Systems, Hannover

[4] Humboldt-Universität zu Berlin, Thaer-Institute of Agricultural and Horticultural Sciences, Department of Urban Ecophysiology of Plants, Berlin

E-mail of corresponding author: jasmin.sauer@julius-kuehn.de

The carrot psyllid *Trioza apicalis* (FOERSTER 1848) is known in Scandinavian countries as a main pest in carrots and in case of mass occurrence. It can cause devastating damage up to total loss of harvest. Carrots are produced on about 12.500 ha in Germany with up to 1.700 hectares of organically cultivated carrots. Increasing cultivation of a particular crop also increases the risk of culturally specific pathogens. Since the availability of pesticides is limited in organic farming, other plant protection measures are needed to protect the cultivated crops. Here, the carrot psyllid *T. apicalis* is studied in Lower Saxony with a carrot production area of about 500 hectar. We needed to learn more about behavior of the pest and the symptoms of damage on carrot plants. With systematic assessments for leaf damage symptoms and two complementary laboratory experiment in which the sedentary nature of *T. apicalis* was studied, we performed estimates and spatial distribution of the pest in the tracked area. We observed the temporal migration of *T. apicalis* into carrot populations using a monitoring system on 17 plots in 2017 and 14 plots in 2018 in Lüneburg and Weserbergland. The monitoring took place with two yellow traps on each plot, which were changed weekly and evaluated.

The Rating of harmful symptoms took place on 9 plots in 2017 and on 12 plots in 2018. The leaf deformation was measured. To estimate the potential for damage, we carried out a laboratory experiment in which high and low infestation with *T. apicalis* was simulated.

In the experiment of Sedentariness , we measured the potential of *T. apicalis* to move from one to another plant.
It could be shown that 84% and 67% of the insects arrive on the carrot areas between the end of May and the end of June. During this time the carrots are particularly endangered due to their young stage of development.

The monitored areas in the Lüneburg area were more heavily affected in both years of investigation than those in Weserbergland. Due to the very cold and wet weather in 2017 and the very hot and dry weather in 2018, the infestation was very low on all plots.

The high infestation with 5 *T. apicalis* per plant showed clear damage after two days already with symptoms on the leaves, whereas the low infestation with one insect per plant showed no symptoms. The studies are intended to predict the extent to which *T. apicalis* poses a threat to organic carrots cultivation in and serve as a support for decision making for carrot growers whether and to what extent they use pesticides and / or other control measures.

# New strategy for an old problem: Development of a biological control strategy against wireworms in potatoes

Eckhard Immenroth[1], Dieter von Hörsten[1], Diedrich.Stephan[2], Tanja Bernhardt[2], Jörn Lehmhus[3], Maximilian Paluch[3], Helmut Junge[4], Kristin Dietel[4], Fabian Wollny[4] and Helmut Lehner[5]

[1] Julius Kühn Institute, Institute for Application Techniques in Plant Protection, Braunschweig

[2] Julius Kühn Institute, Institute for Biological Control, Darmstadt

[3] Julius Kühn Institute, Institute for Plant Protection in Field Crops and Grassland, Braunschweig

[4] ABITEP GmbH, Berlin

[5] LEHNER Agrar GmbH, Westerstetten

E-mail of corresponding author: eckhard.immenroth@julius-kuehn.de

Wireworms are larvae of the click-beetle and pose a major problem in European agriculture. Especially since the expiry of the approval of chemical control agents, the search and testing of biological alternatives, notably for potato growing, is becoming increasingly important. In the project Agri-Met, which is funded by the BLE, a granulate for spreading and a sprayable formulation for the regulation of the pest need to be developed and tested on the basis of the naturally occurring fungus *Metarhizium brunneum*. The JKI Institute for Plant Protection in Field Crops and Grassland, the JKI Institute for Application Techniques in Crop Protection and the industrial partners ABITEP GmbH and LEHNER Agrar GmbH are involved in the project.

In this project, the Institute for Application Techniques in Crop Protection i. a. has the task of testing the developed granules for their abrasion behaviour as well as the technical conditions of application for a possible application of the liquid suspension. Furthermore, the distribution of the granules in the potato ridge will be determined and an optimal storage with regard to the control of the larvae will be determined. The granules are produced in the so-called coating process and consist of killed millet seeds, which is coated by the biomass of the fungus.

The abrasion was determined using the Heubachtest, which is the standardized method to investigate the abrasion of dressed seed. The first investigations of the granulate have shown that only small amounts of abrasion can be observed. Furthermore, the application of the liquid suspension should be tested. For this purpose, experiments have been carried out with a hand pressure vessel and appropriate nozzle technology. The dry product was dissolved and filtered before application. It has been shown that the different batches of the material sometimes differ significantly in the quality of application. This can be determined by the contamination of the nozzle filters, which varies widely. The granule distribution in the earth dam should be analysed in layers. Here, the existing soil moisture has been found to be a problem because the granules begin to dissolve in the aqueous medium. Sampling the soil and then fast drying could significantly improve the results here. For the next year some trials are planned. One aspect of these trials is the comparison between a pre-treatment with a granulate application in autumn and a regular procedure with an application in spring.

# Infrared sensors for gap detection in orchards – possibilities and limits

Verena Overbeck[1], Johannes Günther[2], Jens Karl Wegener[1] and Tanja Pelzer[1]
[1] Julius Kühn Institute, Institute for Application Techniques in Plant Protection, Braunschweig
[2] Technische Universität Braunschweig, Institute of Mobile Machines and Commercial Vehicles, Braunschweig
E-mail of corresponding author: verena.overbeck@julius-kuehn.de

In agriculture, the use of modern sensor technology becomes popular to detect pathogens and diseases, water and nutrient status of plants and to improve the precision of use of plant protection products (PPP). In the three-year project OLSVA, different sprayers were equipped with infrared sensors for gap detection to adapt the application of PPP on heterogeneous orchards. The heterogeneity is determined by different age of the trees, growth of the tree crown during vegetation, pruning and natural gaps.

During the project term, first results of the gap detection spraying system showed that the quality of leaf coverage was worse compared to conventional spraying. Furthermore, more pathogens could be found in gap area and the top the trees. The first infrared sensors (IRS01), which were used, had detection faults by driving speeds >6 km/h and this could be an explanation of the results. Therefore, a second generation of sensors (IRS02) were developed and used. These sensors had a better detection quality at higher driving speeds. Generally, it is not clear, which parameters (colour, size) of the object the sensors detect but it`s necessary in order to improve the sensor system.

Main focus of the study was on the evaluation of the detection performance of different infrared sensors and the suitability to detect gaps in orchards. Therefore, measurements were done under laboratory conditions to find out which influence driving speed, colour of the object, the distance to the object and sleeves against sunlight on the detection quality had. Especially for IRS01, results showed that faults in detection occurred by increasing distances between sensor and the object and increasing driving speeds. Results of IRS02 were constant during all measured driving speeds and distances. Sleeves against sunlight increased the noise floor of the sensors but did not influence the object detection during driving. The summarized assessment of the results showed that IRS02 is suitable for a gap detection. The biggest disadvantage is the fact that the sensors can´t collect some information about the tree crown volume which could be used for application models depending on the amount of leaves in the different areas of the tree crown.

# Nozzle combinations and arrangements for use of a tramline deactivation on field sprayers

Johannes Bröring and Dieter von Hörsten
Julius Kühn Institute, Institute for Application Techniques in Plant Protection, Braunschweig
E-mail of corresponding author: johannes.broering@julius-kuehn.de

The interests of society and the political commitment are directed to limiting the use of chemical pesticides to the minimum necessary extent and using them more purposefully. First approaches to reduce the pesticide application are the GPS section control as well as the adaptation in section of the output quantity. As a further possibility to reduce the amount of pesticides used, would be the recess of the culture-free tramline during the application. With common practice boom sizes and an exactly recess of the tramlines, plant protection products can be reduced by 3 to 5 %. The amount of savings is influenced by technical and crop cultivation parameters:

- Sizes of boom
- Width of tramline
- Row distance in row crops (maize, sugar beet)
- Scope of application (herbicide, fungicide, insecticide, growth regulator)
- Mode of action of pesticides (systemic or contact effect)

In addition to the savings of pesticides, a recess of the tramline also has ecological advantages such as the reduction of run-off of pesticides, their inputs to soil, surface water and groundwater. However, the technical feasibility by switching off two or four nozzles in the tramline. Consequently an exact recess of the tramline as well as the lateral distribution, comply with the requirements, would not be achieved. The nozzle combinations and nozzle arrangements in this area must be modified in such a way that they can be variably adjusted to common practice tramline widths. Furthermore, edge nozzles are used in the tramline area. By recording distribution patterns of individual nozzles on a test stand for single nozzles (Resolution: 2.5 cm) theoretical nozzle combinations were created, which allow a relatively good recess of the tramlines. Then these variants were projected onto a larger nozzle assembly and subjected to a lateral distribution measurement (Resolution: 10 cm).

First results show that recesses of pesticides in the tramline are possible and that adequate lateral distributions can be achieved. For practicality, qualitative and quantitative spray liquid measurements in field are planned.

# Identification and enhancement of secondary metabolites in medicinal and aromatic plants for potential use as biological pesticides and pharmacologicals

Ali Karimi[1,2], Andrea Krähmer[1], Nadine Herwig[1], Hartwig Schulz[1], Javad Hadian[3] and Torsten Meiners[1]
[1] Julius Kühn Institute, Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection, Berlin
[2] Free University of Berlin, Institute of Pharmacy, Berlin
[3] Shahid Beheshti University, Medicinal Plants and Drug Research Institute, Tehran
E-mail of corresponding author: ali.karimi@julius-kuehn.de

In line with the national policy on bioeconomy this project seeks to provide an important contribution in generating bio-based products supporting the management of the global change from fossil fuels to renewable resources, processes and services. It aims to get a better understanding of mechanisms and processes at the genetic and molecular levels of medicinal plant and secondary compound production and to apply this understanding also to improving individual agricultural processes. Specifically we suggested to

1.) adapt and apply metabolomics approaches to identify secondary plant metabolites as active structures/compounds against agricultural plant infestations;

2.) enhance the content of biologically and pharmacologically active substances by selecting genetic raw materials from extreme environments;

3.) enhance the production of these metabolites by mimicking extreme environmental conditions (global climate change) during cultivation.

It is aimed to obtain plant extracts, which can be used as bio-based plant protection products (pesticides). In the end of the project, the collaboration should provide a selection of new "highly-efficient" plants, which are characterized by optimal quality and high resistance, especially with regard to influences of the global climate change. Research studies are planned to evaluate the individual influence of genetic background of selected plant species and cultivars in Iran with respect to optimal quality, resistance and stress tolerance. In this context suitable (fast) analytical screening methods (e.g. LC-MS, GC-MS, vibrational spectroscopy techniques) will be applied to obtain objective data from the individual metabolic profile. In-vitro and in-vivo bioassays of the antifungal and antibacterial activities of the selected substances will be performed. The metabolite data sets will be correlated with the bioassay data.

Here I will show results of an antifungal assay on 8 Iranian medicinal plants which helped to select the two plant species which will be used as model plants. In summer 2018 14 populations of *Zataria multiflora* and 10 population of *Ferula assa-foetida* were sampled in different regions of Iran. First results from chemical analyses of plant and soil samples will be presented.

# NANO-PUSH - Development of nanofibers emitting insect repellents as part of innovative push-and-pull strategies for control of fruit tree phytoplasma vectors

Bruna Czarnobai De Jorge and Jürgen Gross
Julius Kühn Institute, Institute for Plant Protection in Fruit Crops and Viticulture, Dossenheim
E-mail of corresponding author: bruna.czarnobai@julius-kuehn.de

Fruit trees of the family Rosaceae are seriously affected by phytoplasmas of the apple proliferation group. Phytoplasmas are pathogens of agriculturally important plants, including pears, apples, plums and citrus causing a wide variety of symptoms that range from mild yellowing to death of infected plants. Phytoplasma disease is caused by a group of specialized phloem-limited bacteria and are transmitted between plants, mainly, by insect vectors.

Some of these phytoplasmas are on the list of pests recommended for regulation by the European and Mediterranean Plant Protection Organization (EPPO), like 'Candidatus Phytoplasma mali', 'Ca. P. pyri', and 'Ca. P. prunorum' the most economically important disease in stone fruits, European Stone Fruit Yellows (ESFY) causing crop losses of infected peach and apricot trees.

Insects of the genus Cacopsylla (formerly Psylla) are known as the major responsible vectors and it was shown recently that they use chemical cues for orientation and host identification.

To overcome the spreading of this diseases, especially the transition to healthy plants, several techniques using volatile substances as insects attractive and repellents and also new system for the delivery of such substance are being studied.

Thus, nanotechnology offers great promises for delivery systems as an innovative tool. Nanofibers can be used to deliver both attractive compounds, and insect repellents. Through nanoencapsulation chemicals are slowly but efficiently released to a particular host plant for insect pest control.

This combined application of insect attractant (pull component) and repellents (push component) connected to a practical push-and-pull control method will reduce the use of chemical pesticides and ensure economic, sustainable and long term cultivation of pome and stone fruit in Germany and throughout Europe.

Nanoformulations for push-and-pull systems can prevent the migration and proliferation of psyllids in orchards, resulting in a strong reduction of new infections by phytoplasmas.

Systems for dispensing repellent substances in crop protection are without precedent and the use of nanotechnology in agrobiotechnological applications are in focus of the latest research.

The objective of this study is the development of an innovative push-and-pull strategy for the management of phytoplasma vectoring psyllids by using nanofibers for emitting repellent compounds.

# CHIC: Chicory as a multipurpose crop for dietary fibre and medicinal terpenes

Jochen Menz and Thorben Sprink
Julius Kühn Institute, Institute for Biosafety in Plant Biotechnology, Quedlinburg
E-mail of corresponding author: jochen.menz@julius-kuehn.de

Known for its health beneficial properties since ancient times, root chicory (*Cichorium intybus L.*) is nowadays a rather under-utilized crop in Europe. CHIC is the Chicory Innovation Consortium and will change this in a Horizon2020 funded project. By using New Plant Breeding Techniques (NPBTs), chicory will be established as a multipurpose crop, i.e. by optimizing the production of bioactive and health-related products with clear benefits for consumers. The NPBTs will be used to steer bioprocesses in chicory and mobilize its under-explored potential to produce immunomodulatory prebiotics and medicinal terpenes. Four different approaches within NPBTs are tested to improve chicory in this highly interdisciplinary project together with European scientific project partners, end-users and SMEs.

JKI's focus lies on a DNA-free genome-editing approach with RNA-guided endonucleases like Cas9. Furthermore, the evaluation of the different established NPBT approaches, the identification of potential off-target effects, the evaluation of safety aspects and the regulatory landscape by using these NPBTs fall under JKI's tasks in CHIC. Chicory will be boosted as a robust multipurpose crop, tolerant to adverse environmental conditions from which bioactive compounds can be extracted, contributing to sustainable agriculture and a bio-based economy in Europe.

# Comparison of different lettuce (*Lactuca sativa* L.) varieties and their quality parameters in three different locations in Lower Saxony

Daniela Schlein, Marcel Naumann and Elke Pawelzik
University of Goettingen, Division Quality of Plant Products, Goettingen
E-mail of corresponding author: daniela.schlein@uni-goettingen.de

Lettuce is an annual or biennial plant that is consumed worldwide. In Germany, it is very popular, because of its high content of water, and dietary fiber and its availability throughout the year. However, several of these varieties are negatively affected by the consumer acceptance, because of a high number of bitter compounds in these plant extracts, for example lactucin. In the summer of 2017, we conducted field experiments in three different locations in Lower Saxony (Seevetal, Cappeln, and Göttingen). In each experiment, five leaf lettuce varieties (radicchio, endive, red oak leaf, frisee, and iceberg) were planted with six biological replications. Four of the five varieties were chosen based on a self-implemented consumer survey, rating the most bitter lettuce types known. Iceberg was chosen as a non-bitter lettuce. In general, to characterize some of the important quality traits, the distribution of mineral content, total phenolics and nitrate were estimated. Furthermore, the color of the lettuce was measured with a non-invasive method called 'Electronic Eye' (Iris, Alpha MOS Company, France). The aim of the analyses was to verify the differences in the varieties (variety effects) and additionally, to find the general location effects, for example, in the distribution of the ingredients. The first results showed a significant difference between the potassium and magnesium content in each variety, even between the locations. The same results are shown in nitrate and in the content of total phenolics. Furthermore, the results of the color displayed a significant difference between the different locations. These results should be verified in the course of a second field experiment (2018) to determine a possible correlation between the nutritional composition and the color data set.

# Recent efforts in improving the genepool of annual caraway (*Carum carvi* L.)

Daniel Becker[1], Andrea Krähmer[2], Claudia Beleites [2], Bärbel Zeiger[1], Wolfram Junghanns[3] and Frank Marthe[1]

[1] Julius Kühn Institute, Institute for Breeding Research on Horticultural Crops), Quedlinburg

[2] Julius Kühn Institute, Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection, Berlin

[3] Dr. Junghanns GmbH, Aschersleben, OT Groß Schierstedt

E-mail of corresponding author: daniel.becker@julius-kuehn.de

Caraway fruits (achenes) are used for pharmaceuticals and as a spice. Due to essential oil content they remedies gastrointestinal afflictions. The main components of the essential oil are carvone (≥ 50 %) and limonene (30 – 45 %). According to the European Pharmacopoeia (Ph. Eur.) an essential oil content of ≥ 3 % after distillation is required.

In Europe predominantly biennial cultivars are grown, although annual cultivars were already introduced in the 1990th. Initially, annual cultivars had a low essential oil content, but breeding activities succeeded in developing cultivars fulfilling the requirements of pharmacopoeia. However, annual cultivars fail to reach the level of yield and essential oil content of biennial caraway down to the present date. Therefore, an essential oil content of 5 % and a yield of 1.5 t/ha were set as breeding goals. In addition, the proportion of stalked fruits (stalk-appendix) should be low, because this is an undesirable trait regarding processing and hence marketing.

The breeding material mainly originates from initial crossings between an annual breeding line and essential oil-rich biennial cultivars. In 2018, all breeding lines (nearly 150 lines) reached an inbreeding level of $I_5$.

Here we describe the investigation of 50 lines (including standards):

As most important trait for selection, we analysed essential oil content (including carvone and limonene content). Essential oil content was estimated using non-invasive near-infrared spectroscopy (NIRS). Predictions were based on a reference extraction: Extracts were analysed using gas-chromatography with flame ionization detector (GC-FID). Afterwards extraction values were corrected for distillation as required by Ph. Eur. Distillates also were analysed using GC-FID.

In addition, among other traits we observed stalk-appendix, thousand grain weight (TGW), single plant yield, height and flowering time. Here we will show selected results and correlations between the mentioned traits.

Best lines were selected for propagation to enable estimation of yield in future trials.

# Evaluation of two tomato crosses and their parents with a sensory panel and analytical measurements

Larissa Kanski[1], Mahasin Ahmed[2], Marcel Naumann[1] and Elke Pawelzik[1]

[1] University of Goettingen, Division Quality of Plant Products, Goettingen

[2] University of Khartoum, Faculty of Agriculture, Department of Agricultural Engineering, Khartoum
E-mail of corresponding author: lkanski@uni-goettingen.de

Tomato is the most popular vegetable in Germany and it is one of the most consumed horticultural crops in the world. Tomatoes have high nutritional value as they are rich in vitamins and antioxidants. At the same time, consumers are not satisfied with the taste of fresh tomatoes and complain about their poor flavor. Conventional breeding programs have mainly focused on yield, firmness, and long shelf-life, which may have caused a decrease in flavor acceptance. In recent decades, consumer preferences have changed towards sustainable products with an increased focus on the sensorial quality of the products. The flavor of the tomato is a complex interaction of taste and aroma. Volatile aroma compounds define the typical flavor of the tomato. Major contributors to the taste of tomatoes include sugars and acids. The amounts of sugars and acids not only influence the taste but also the overall flavor of tomatoes and are, therefore, important parameters for consumer acceptance. The concentrations of sugar and acid and their ratio in the fruit are determined by the cultivar and environmental conditions. Within the PETRA$^{q+n}$ project (participatory development of quality tomatoes for sustainable regional production), the goal is to create a scientific basis to breed tomato cultivars with improved quality and optimal adaption for sustainable regional and urban production in Lower Saxony. During the summer of 2018, two tomato crossbred offspring in the $F_4$-generation and their parents were cultivated in an organic low-input production system. The evaluated crosses were combinations of parental cultivars with high yield and good quality parameters, which showed positive results in breeding sensory evaluation and analytical measurements in the first experimental year in 2017. A trained sensory panel evaluated important fruit quality attributes such as color, sweetness, sourness, skin strength, and fruit juiciness. The sensory results were compared with results of the physio-chemical analysis of total soluble solids (TSS), titratable acidity (TA), texture, and color measurements. The present study compares the quality traits of the crosses and their parents with a trained sensory panel and shows whether the results of analytical measurements reflect the results of the human senses and whether the crosses show improved quality attributes compared to their parents.

# Naturally occurring flower mutation in offspring of a large fruited raspberry chance seedling

Dora Pinczinger, Magda-Viola Hanke, Marcel von Reth and Henryk Flachowsky
Julius Kühn Institute, Institute for Breeding Research on Fruit Crops, Dresden
E-mail of corresponding author: dora.pinczinger@julius-kuehn.de

A red raspberry population was established from an open pollination of a large fruited chance seedling. Subsequently, three different floral phenotypes were observed in this population. Type 1 is equivalent to the wild type raspberry flower phenotype with five sepals and petals, with stamens and carpels present. Type 2 has six to eight sepals and petals, with stamens and carpels present. Type 3 has sepaloid and carpeloid structures, but no petals and stamens.

Floral phenotype as well as fruit weight, length and drupelet number was evaluated for this population. Type 1 and 3 fruits are smaller and have a smaller number of drupelets than type 2 fruits.

The ABC-model of flower development suggests that type 3 phenotypes are caused by perturbation of class B genes. In other Rosaceae plant species similar phenotypes are caused by mutation of the *PISTILLATA* (*PI*) gene, as *APETALA3* has several homologs and is thus more robust against impairment. We defined MADS- and K-box containing genes from *Rubus occidentalis* (black raspberry) by Hidden Markov Model search. A neighbor joining tree was produced through amino acid sequence homology. Although no homolog for PI was found initially, a BLAST search detected a non-annotated sequence with high homology to *Arabidopsis thaliana PI*.

A PCR with type 1 and 3 genomic DNA was performed. Since fragments were of the same size, we speculated that the difference between type 1 and type 3 is on the sequence level. Indeed, cloning and sequencing of type 1 and 3 cDNA and type 3 gDNA revealed that type 1 translates into 212 and type 3 into 207 amino acids. Both sequences differ in 54 amino acids. This makes impairment of PISTILLATA in type 3 plants a plausible explanation for type 3 phenotypes.

An RT-PCR on type 1 and type 3 whole flowers and individual whorls was conducted. There was substantial reduction of *PI*-levels in type 3 samples. As expected, whole flower as well as petals and stamens displayed the highest *PI*-levels. Moreover, we plan complementation of the *A. thaliana pi-1* mutant with type 1 and type 3 alleles.

To analyze the impact on fruit size, we crossed type 1 and type 3 plants with two tester cultivars. Fruit size evaluation will be carried out in the $F_1$.

This project will also lead to the development of molecular markers for fruit size, which might benefit current raspberry breeding programs.

# Potato improvement by genome editing

Enikö Lörincz-Besenyei[1,2], Thorben Sprink[2], Janina Metje[2], Uwe Sonnewald[3] and Björn Krenz[1]
[1]Leibnitz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig
[2]Julius Kühn Institute, Institute for Biosafety in Plant Biotechnology, Quedlinburg
[3] Friedrich-Alexander Universität Erlangen-Nürnberg, Department of Biology, Erlangen
E-mail of corresponding author: enikoe.loerincz@julius-kuehn.de

Potato (*S.tuberosum*) is the third most important crop in the world after wheat and rice in terms of human consumption. It is considered as a global security food producing more food per unit than any other major crop. Short days and moderate temperatures promote tuber formation ensuring proper timing of vegetative propagation of the plant. Day length regulates important aspects in plant development such as flowering or tuber formation. In potato a FLOWEING LOCUS T (FT) paralog named SP6A (SELF-PRUNING6A) gene, that respond to different environmental conditions, mediates the tuberization. The day length is sensed by the leaves and SP6A will induce tuberization under short day conditions. Under elevated temperatures, SP6A expression is supressed by a specific-miRNA. This leads to low tuberization efficiency at elevated temperatures.

The climate change is a big challenge in these days for agriculture. Very hot summers have a negative impact on tuberization. DNA-free genome editing *via* CRISPR (clustered, regularly interspaced, short palindromic repeat) /Cas9 (CRISPR associated protein) is widely used to induce site-directed mutagenesis for crop improvement. A mutation will be introduced in the SP6A-specific miRNA with DNA-free genome editing using potato protoplast and RNPs (ribonucleoproteins). We expect that it will affect tuberization at elevated temperatures and allow to ensure tuber yield under conditions of global climate changes. The first step in this study was to choose the best gRNA for genome editing. Thus the *in vitro* efficiency of the gRNAs was tested. Further on, efficient gRNAs will be used for potato protoplast transfection with RNPs.

*The financial support of the Federal Ministry of Education and Research (BMBF) is acknowledged.*

# Basil cultivation without sunlight

Jenny M. Köppe[1,2], Christoph von Studzinksi[3], Inga Mewis[3], Oliver Arnold[4] and Hartwig Schulz[1]
[1] Julius Kühn Institute, Institute for Ecological Chemistry, Plant Analysis and Stored Product Protetction, Berlin
[2] Free University of Berlin, Institute of Pharmacy, Berlin
[3] Humboldt University Berlin, Urban Plant Ecophysiology, Berlin
[4] FutureLED GmbH, Berlin
E-mail of corresponding author: jenny.koeppe@julius-kuehn.de

To enable a high-quality as well as cost-efficient greenhouse production in Berlin and Brandenburg all year round, an LED light system was developed which optimally reflects the sunlight spectrum in the range of the photosynthetically active (400-700 nm) as well as of the ultraviolet A and B (280-400 nm) radiation.

To evaluate the effectivity of these LED lights for the cultivation of certain aroma and medicinal plants, a randomized full-factorial experiment with two different light intensities (PPFD of 200 and 100 µmol/m$^2$/s) and four independent replications with four basil cultivars (*Ocimum basilicum* L. var. *cinnamomum 'Cinnamon'*, *O. basilicum* L. var. *thyrsiflorum* 'Thai Magic', *O. basilicum* L. var. *odoratum* 'Anise' and *O. basilicum* L. var. *purpureum* 'Dark Opal') under the exclusion of natural sunlight was conducted. The weekly assessment of plant height and plant development of 288 individuals per cultivar demonstrates a significantly faster growth of all four basil cultivars when grown under the maximal light intensity of 200 µmol/m$^2$/s in comparison to basil cultivars grown under the lower light intensity of 100 µmol/m$^2$/s. Comparable growth results are achieved two ('*Cinnamon'*), five ('*Anise'*), and seven ('*Thai Magic'*, '*Dark Opal'*) days later for basil plants grown under the lower light intensity. In a second experiment with identical study design, UV-A (315-400 nm) or UV-B (280-315 nm) light were added to the spectrum with the PPFD of 200 µmol/m$^2$/s. The development of the basil cultivars did not differ significantly between both spectral ranges, but was slowed down by five ('*Cinnamon'*), six ('*Dark Opal'*), seven ('*Anise'*) and nine ('*Thai Magic'*) days in comparison to the results found in the first experiment.

Within the short cultivation period of four weeks, 'Cinnamon', 'Anise' and 'Thai Magic' grown under the high light intensity reached a marketability, which is only met under optimal commercial greenhouse cultivation conditions of the region, and takes up to seven weeks in dependence of the season. A PPFD of 100 µmol/m$^2$/s as well as the addition of UV radiation delays the development of all four basil cultivars by a maximum of nine days.

Under all tested light intensities and spectral ranges, the LED system permits an accelerated as well as target-oriented production of basil under the absence of sunlight. However, a comprehensive final evaluation of the applied LED system will be only possible when the composition of the basil leaves has been properly determined by GC-FID and GC-MS and the outcome of an extensive cost-benefit analysis has been calculated in detail.

# Identifying and analyzing patterns of Phosphorus fertilization

Paul Winklhofer[1], Sabine Andert[1], Silke Hüttel [2] and Bärbel Gerowitt [1]
[1] University of Rostock, Department of Crop Health, Rostock
[2] University of Bonn, Institute of Food and Resource Economics, Bonn
E-mail of corresponding author: paul.winklhofer@uni-rostock.de

Phosphorus (P) is an essential nutrient in agricultural crop production. However, in aquatic ecosystems P can lead to environmental damages trough eutrophication. Beside local surpluses of P, global recourses of rock phosphate are limited. Although agriculture is the main consumer of P, information about on-farm fertilisation use is scarce.

Within the joint project InnoSoilPhos[1], we will examine fertilisation strategies of approximately 50 farms in five regions in northern Germany. The regions correspond to administrative districts located on gradients of climatic, soil and structural production conditions. We collect fertilisation and other crop production data at the field-scale for the period 2010-2018. In addition, general production features of these farms are surveyed at the farm-scale.

The first objective of this work is to characterise how farms fertilize regarding quantities, types, allocation to crops and frequencies. We are interested in whether and how strategies can be classified depending on region, site and other farm(er) characteristics. We target at investigating fertilisation patterns by explorative methods such as cluster analysis, ANOVA but also mixed models. Second, our objective is to analyse potential relationships between P fertilisation and other cultivation factors, like crop protection or crop rotation. We plant to apply multivariate methods here.

The results will help to identify and understand factors influencing fertilisation strategies at the farm level and thereby will help to find reduction potentials.

The authors gratefully acknowledge the German Federal Ministry of Education and Research (BMBF) for funding the BonaRes project InnoSoilPhos (No. 031B0509).

[1] Website: www.innosoilphos.de

# Towards positional isolation of *Barley mild mosaic virus* (BaMMV) resistance gene *rym15*

Yaping Wang, Antje Habekuß, Dragan Perovic and Frank Ordon
Julius Kühn Institute, Institute for Resistance Research and Stress Tolerance, Quedlinburg
E-mail of corresponding author: yaping.wang@julius-kuehn.de

Barley is the second most important cereal crop in Europe. *Barley mild mosaic virus* (BaMMV) and *Barley yellow mosaic virus* (BaYMV), which are transmitted by the soil-borne protist *Polymyxa graminis* have a serious impact on barley yield. Although a number of resistance genes are already known, which range from *rym1* to *rym18*, resistance of some genes has been broken by new virus isolates. For example, *rym4* is ineffective against BaYMV-2 and *rym5* turned out to be not effective against a new BaMMV strain. Therefore, developing of closely linked molecular markers and next the isolation of up to now less used resistance genes is a genuine need for sustainable barley production.

In previous studies on doubled haploid (DH) lines derived from the $F_1$ of the cross of the resistant barley accession 'Chikurin Ibaraki 1' to the susceptible winter barley cv. 'Plaisant' *rym15* was located on the short arm of chromosome 6H. However, the study showed that the order of markers is inverted in relation of the genetic map derived from the cross from 'Lina' × *Hordeum spontaneum* 'Canada park'. Therefore, our work aims to construct a high resolution mapping population of the gene *rym15* being effective against BaMMV, to (i) resolve the discrepancy between the two maps, (ii) narrow down the target region and saturate the map, (iii) with the final aim to isolate *rym15.*

Two crosses derived from the resistant barley cv. 'Chikurin Ibaraki 1' and susceptible cultivars 'Uschi' and 'Igri' were used for the construction of a high resolution mapping population of *rym15*. Segregation ratios in 365 and 158 $F_2$ plants from the 'Igri' × 'Chikurin Ibaraki 1' and 'Chikurin Ibaraki 1' × 'Uschi', i.e 85(R) : 280(S) and 30(R) : 128(S), respectively, fit to a ratio of 1r:3s ($\chi2$=0.571, $\chi2$=3.046), suggesting the presence of one recessive resistance gene. Six published SSR markers and 5 KASP markers developed based on the 50K Illumina array data were used for medium-resolution mapping. Genetic maps were constructed, new robust codominant flanking markers were identified and conflicting order of markers was solved. Furthermore, in order to construct the high resolution mapping population of *rym15*, 166 and 158 $F_2$ recombinants were selected by screening 2172 and 3413 $F_2$ plants from the crosses 'Igri' × 'Chikurin Ibaraki 1' and 'Uschi' × 'Chikurin Ibaraki 1', respectively. 86 SNPs between two flanking markers were identified by comparing the 50K Infinium Illumina array of susceptible and resistant bulks. These markers will be used for marker saturation of the target locus. Next, parental lines and bulks will be screened using Genotyping by Sequencing (GBS) for further marker saturation with the final aim to facilitate positional cloning of *rym15*.

# Application of next-generation sequencing for simultaneous detection of viruses, viroids and phytoplasmas in grapevine & fruit trees

Kerstin Zikeli[1], Constanze Berwarth[1], Dennis Knierim[2], Christoph Hoffmann[3], Michael Maixner[3], Stephan Winter[2] and Wilhelm Jelkmann[1]

[1] Julius Kuehn Institute, Institute for Plant Protection in Fruit Crops and Viticulture, Dossenheim

[2] Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig

[3] Julius Kuehn Institute, Institute for Plant Protection in Fruit Crops and Viticulture, Siebeldingen

E-mail of corresponding author: kerstin.zikeli@julius-kuehn.de

Next-generation sequencing (NGS) technologies have now become an integral part of plant health research. In addition, NGS is applied to a greater extent for detection of plant pathogens in the last years. Thus, it is used for diagnostics of viral and virus-associated diseases of grapevines and fruit trees. RNA sequencing combined with metagenomic analysis enables an unbiased analysis of infected plant samples.

Phytoplasma, viral and viroidal diseases cause severe harvest losses in viticulture and orchards.

Grapevine enation disease (GED), causing formation of enations on the underside of basal leaves and growth depression of infected plants, has been reported in Germany in 2006. The etiology of GED still remains unknown, no correlation of reverse transcription-PCR detected virus species and occurrence of disease has been found so far.

Bois noir, European stone fruit yellows, pear decline and apple proliferation belong to the most prevalent and economically important phytoplasma diseases of grapevine respectively of fruit trees in Europe.

In this study, a NGS protocol (Illumina MiSeq platform) was applied for detection of viral and phytoplasmic infections of grapevine and fruit tree samples. Symptomatic as well as asymptomatic samples were analysed and subjected to a NGS pipeline starting from total RNA extract for generating an untargeted metagenome dataset. Therefore, untargeted and unknown pathogens may be identified.

Besides viruses and phytoplasmas detected by PCR, further viruses and viroids were found to be present. This NGS approach enabled the detection of low titer infections in tissues (samples were partially tested negatively by normal PCR assay) as well as parallel detection of phytoplasmas, viruses and viroids in a single grapevine or fruit tree sample.

# Studies on the resistance locus *Rpv12* against downy mildew of grapes (*Plasmopara viticola*)

Sophia Müllner, Reinhard Töpfer and Eva Zyprian
Julius Kühn Institute, Institute for Grapevine Breeding, Siebeldingen
E-mail of corresponding author: eva.zyprian@julius-kuehn.de

*Plasmopara viticola,* a heterothallic obligate biotrophic oomycete, is the causative agent of grapevine downy mildew, a widespread severe disease. In 1878 *P. viticola* was imported from North America to Europe, together with grape phylloxera resistant rootstock vines. Since then, the pathogen has caused considerable yield losses. The pathogen hibernates in leaf debris and soil as sexual oospores. In spring, oospores germinate at a temperature above 11 °C and form macrosporangia. Under wet conditions, macrosporangia liberate flagellated zoospores. With the rain, zoospores are splashed to the leaves onto the lower surface, where they can reach the stomata to cause infection. After 5-9 days yellow lesions called „oil spots“ appear on the upper site of the leaf surface. Under suitable weather conditions (high humidity and 20-25 °C) *P. viticola* sporulates and a secondary infection starts. Because *P. viticola* causes a high crop loss annually, research and breeding of resistant grape varieties is essential for sustainable viticulture. Only with precise knowledge of the resistance mechanisms and the genetic location of resistance factors a targeted breeding it is possible to reduce the annual amount of consumed pesticides. In2013 Venuti *et al.* identified the resistance locus *Rpv12* using QTL analysis of *Vitis amurensis*. *V. amurensis* is native to the cool climates of the Far East (China and Russia) and shows resistance against *P. viticola*. In the early 20th century the asiatic species *Vitis amurensis* ‘Ruprecht’ was crossed with *Vitis vinifera* ‘*Getsh’* to yield ‘Michurinets’. Other interesting cultivars are ‘Kunbarat’ and ‘Kunleany’. They possess resistance characteristics due to *Rpv12.* This locus was detected on Chromosome 14 and is inherited independently of other resistance loci. Within the locus *Rpv12* 12 NBS-LRR genes (coiled coil-nucleotide binding site – leucine rich repeats) have been identified within the reference genome (PN40024). An additive effect with *Rpv3* was detected, since *Rpv12* confers a foliar resistance to strains that are virulent on *Rpv3* cultivars. For identification of the responsible gene for the resistance, we compare susceptible grapevine with resistant cultivars by leaf disc assay and light-, fluorescence- and cryo scanning electron microscopy. The aim is to identify physiological responses of the cell. These investigations should reveal molecular mechanisms and the candidate genes involved, which shall be further evaluated by amplification, comparative sequencing and gene expression analysis.

# Using a robotic high-troughput phenotyping method to detect leaf and stripe rust resistances in wheat genetic resources

Ulrike Beukert, Albrecht Serfling and Frank Ordon
Julius Kühn Institute, Institute for Resistance Research and Stress Tolerance, Quedlinburg
E-mail of corresponding author: ulrike.beukert@julius-kuehn.de

Bread wheat is one of the most important crops for human nutrition worldwide. To ensure food security, wheat production need to be increased by 60% till 2050. Yearly infections with leaf and stripe rust caused by *Puccinia triticina* and *Puccinia striiformis*, respectively, result in significant yield losses. Cultivation of resistant varieties carrying effective resistance genes is the most efficient and environmental friendly solution in order to avoid yield losses. Due to the emergence of virulent races the breakdown of existing resistances was observed in the past so that the identification of genotypes with up to now unknown resistances is an important task.

To achieve this, the wheat ex-situ collection of the IPK Gatersleben is analyzed for disease resistances, whereby precise phenotyping is a prerequisite for mapping of quantitative resistances. In order to characterize genetic resources of wheat, phenotyping of 9,700 winter wheat accessions in field trials and in greenhouse experiments was started. The detection and quantification of resistances is in addition achieved in detached leaf assays using high throughput technologies and digital imaging via the robotic platform Macrobot. Genotypes showing quantitative, race-nonspecific resistances are being detected and will be further characterized using microscopical and molecular techniques.

First results based on 5800 accessions revealed that 12.7% of the genotypes carry qualitative resistance against leaf rust, whereas 48.9% showed quantitative differences in resistance. 4.7% turned out to be resistant against both, *P. triticina* and *P. striiformis*.

# Identification of resistance to stripe rust, leaf rust and Septoria tritici blotch in a multiparental wheat population

Sandra Rollar, Albrecht Serfling and Frank Ordon
Julius Kühn Institute, Institute for Resistance Research and Stress Tolerance, Quedlinburg
E-mail of corresponding author: sandra.rollar@julius-kuehn.de

The emergence of stripe rust (*Puccinia striiformis*) and leaf rust (*Puccinia triticina*) races containing virulence against common resistances genes and the loss of fungicide effectivity against Septoria leaf blotch (STB) increase the demand for wheat varieties with effective resistances to these pathogens. Therefore, 400 genotypes of the multi-parental Bavarian Magic Wheat (BMW-) population generated from eight German elite wheat cultivars were screened for resistance against these pathogens in order to conduct genome wide association studies (GWAS) and to develop closely linked molecular markers suitable for marker-assisted selection.

Phenotyping of the BMW-population was conducted in multi-years field trials at three locations. To ensure a reliable infestation, an inoculation was performed with leaf and stripe rust at one and with STB at all three locations using defined spore concentrations. Respective genotypes were scored two to four times and the area under the disease progress curve (AUDPC), as well as the average ordinate (AO) were calculated. In order to identify already known resistance genes, the parental lines were analysed with differential sets of isolates for all pathogens in detached leaf assays (STB) and whole plant tests (rust fungi).

Ratings of the field trials allowed the identification of quantitative differences and completely resistant genotypes with respect to leaf and stripe rust. Statistical analysis revealed significant differences ($p < 0.0001$) between the 400 lines of the BMW-population for all three diseases and showed a broad variability in the leaf rust (0 % to 64 %) and in the STB (0 % to 42 %) field trials, while most of the genotypes turned out to be resistant to stripe rust. The statistical evaluation of detached leaf assays showed significant impact on infestation for the genotype ($p < 0.0001$), isolate ($p < 0.0001$), and the respective interaction ($p = 0.0120$). Thus, it was possible to differentiate between highly aggressive and less aggressive isolates. In the whole plant test for leaf rust resistance, several effective resistances were detected, which are further characterized with molecular markers.

Based on the collected phenotypic data and the genotypic data available from the 20k iSelect chip, GWAS will be conducted in order to identify major genes and quantitative trait loci (QTL). The detected resistances that are present in the elite parent lines can be used quickly in new varieties to combine and improve resistances.

# Genome wide association studies for resistance of wheat to the root lesion nematode *Pratylenchus neglectus*

Behnaz Soleimani[1], Heike Lehnert[1], Dragan Perovic[1], Gina Capistrano[2], Christian Jung[2] and Frank Ordon[1]

[1] Julius Kühn-Institut, Institute for Resistance Research and Stress Tolerance, Quedlinburg

[2] Kiel University, Plant Breeding Institute, Kiel

E-mail of corresponding author: soleimani.behnaz@julius-kuehn.de

The root lesion nematode (RLN), *Pratylenchus neglectus*, is a migrating endoparasite that is known as an economically damaging pathogen of wheat and many other crop species. Growing of resistant or tolerant cultivars is the most efficient, cost-effective and environmentally-friendly strategy to avoid yield losses caused by *P. neglectus*. The present study aims to identify resistant genotypes showing low infection to *P. neglectus* and to identify QTLs using genome wide association studies (GWAS).

A set of 149 diverse wheat genotypes was subjected for phenotyping by infection with 1000 nematodes at the University of Kiel. In parallel 9556 single nucleotide polymorphism (SNP) selected out of data present for these genotypes from the 15K and the 90K iSelect (SNP) chips turned out to be available for genome-wide association studies. In a first step this marker set was mapped in the IWGSC RefSeq of wheat. After filtering for minor allele frequency (MAF) > 5%, maximum percentage of missing values <10%, heterozygosity (< 12.5%) and SNP imputation 8842, markers were selected for GWAS. The Mixed Linear Model (MLM) approach was applied to detect QTL for the number of nematodes in GWAS analysis employing kinship and population structure (Q matrix) estimated on 2050 informative markers selected on LD data.

First analysis identified five significant marker trait associations (LOD≥ 3) on chromosomes 1A, 2A, 4B, 6B and 6D of wheat genome.

Finally, GWAS will be performed in an extended set of 313 diverse wheat genotypes for which phenotyping is ongoing.

# Tomato plants are suitable hosts for *Salmonella*

Azhar Zarkani[1], Sven Jechalke[2], Jasper Schierstaedt[3], Marlies Becker[1] and Adam Schikora[1]
[1] Julius Kühn Institute, Federal Research Centre for Cultivated Plants, Institute for Epidemiology and Pathogen Diagnostics, Braunschweig
[2] Justus Liebig University Giessen, Department of Phytopathology, Gießen
[3] Leibniz Institute of Vegetable and Ornamental Crops, Department of Plant-microbe interaction, Großbeeren
E-mail of corresponding author: azhar.zarkani@julius-kuehn.de

*Salmonella* is able to cause disease in humans and animals and also to colonize plants. For successful colonization, *Salmonella* needs to attach and adhere to host surfaces. Recent reports show that *Salmonella enterica* is able to colonize a variety of plant species and organs; hence it can cause disease outbreaks and result in severe economic losses.

Salmonellosis outbreaks are increasingly associated with the consumption of contaminated raw fruits and vegetables. Interestingly, contamination of produce can occur along the whole production chain, also during the plant growth.

Importantly, *Salmonella* originating from plants maintains its virulence in animals. Thus, *Salmonella*-contaminated (crop) plants play an important role in its transmission towards animal and human hosts.

As a consequence, plants might be an alternative host for *Salmonella*. However, the knowledge about factors influencing the persistence of *Salmonella* in the plant environment and the associated colonization of plants is still insufficient.

Consequently, we investigated the interaction between *Salmonella enterica* and tomato, chosen as a model for crop plants. We analyzed the survival of *Salmonella* in the soil as well as on and in the plant and monitored the gene expression patterns in tomato plants during the interaction with *Salmonella*. We assessed the immune response of tomato plant to the attack of different strains of *Salmonella* and tested whether the different bacterial genetical factors are differently perceived by the plants.

Additionally, we assessed the role of *Salmonella* motility on its persistence in the host plant.

# Do bacteria shape the soil structure by EPS production?

Antje Bettermann[1], Jeroen Zethof[2], Cordula Vogel[2], Broder Rühmann[3], Volker Sieber[3], Karsten Kalbitz[2] and Kornelia Smalla[1]

[1] Julius Kühn Institute, Institute for Epidemiology and Pathogen Diagnostics, Braunschweig

[2] Technische Universität Dresden, Institute of Soil Science and Site Ecology, Tharandt

[3] Technical University of Munich, Straubing

E-mail of corresponding author: antje.bettermann@julius-kuehn.de

Erosion is considered to be one of the most important processes contributing to soil degradation. Soil microbial communities are shaped by vegetation and plant exudates are known as major drivers of the development of the microbial community composition in the vicinity of the roots. Microorganisms produce diverse extracellular polymeric substances (EPS) that are assumed to contribute to soil aggregation and thus may have important implications for soil erosion. We hypothesize that the amount and diversity of microbially produced EPS is plant- and soil type dependent.

Soil samples were taken at two semi-arid field sites in Almería Province, Spain. Both sites were located on moderately steep slopes, with a micaschist substrate that was either rich or poor in carbonates. Soils at both sites are particularly prone to erosion as under the semi-arid climate conditions plant density is low. At each of the sites, five hydrological disconnected plots were defined. Within each plot, one Anthyllis cytisoides individual and one Macrochloa tenacissima tussock was chosen which are abundant plants at both field sites. Around the plants, a gradient in distance to the stem/outer border of the tussock perpendicular to the hillslope was sampled to a depth of 5 cm, avoiding the sieving crust. The samples were subjected to extraction and characterization of EPS as well as to extraction of total microbial community – DNA and plated onto R2A medium in order to determine colony forming units as well as to isolate randomly picked bacterial colonies. The 400 bacterial isolates were transferred to a biofilm inducing medium. Obviously well growing strains were then screened for the composition of produced exopolysaccharides – an important part of EPS. The isolates were identified by sequencing of the 16S rRNA gene. The composition of the total soil bacterial communities was assessed by PCR-DGGE (denaturing gradient gel electrophoresis) of 16S rRNA gene fragments amplified from total community DNA. DGGE revealed changes in the bacterial communities depending on the distance to the plant as well as depending on the soil type of the respective sites. Among the 225 isolates, 67 were able to synthesize exopolysaccharides, 45 of which could be identified. These exopolysaccharide producers were affiliated to the four major phyla detected in soil: Actinobacteria, Firmicutes, Bacteroidetes and Proteobacteria. Their occurrence was linked stronger to the plant species than to the respective soil.

Linking soil properties to bacterial communities, total EPS and particularly exopolysaccharide production will provide a better understanding of processes leading to soil aggregation and erosion.

# Weed mapping – a vital component of site specific crop protection

Christina Wellhausen, Henning Nordmeyer and Michael Pflanz
Julius Kühn Institute, Institute for Plant Protection in Field Crops and Grassland, Braunschweig
E-mail of corresponding author: christina.wellhausen@julius-kuehn.de

Weed mapping as a component of site specific crop protection allows for a reduced herbicide use and consequently for a more sustainable agricultural production. Site specific weed management requires information on the number and location of different weed species in the field.

Gathering this information requires high-resolution aerial images and automatic weed classification to generate field maps. Aerial images are taken with an unmanned aerial vehicle (UAV), with flights being carried out at a flight altitude of 5 m over a selected field. The altitude and camera parameters result in a ground resolution of 2.5 pix/mm, allowing the differentiation of single weed plants. Besides UAV imagery, ground-truth data is generated through manual weed counting and a second set of photographs taken at about 70 cm above ground.

The ground-truth set of images is visually examined and plants are annotated with ground truthing locations. Sub-images showing single weed plants are then extracted for model building and validation. The sub-image sets are divided into a calibration (train) and a validation (test) set. These sets will be used to train an image classifier using the “bag of visual words” (BoVW) concept. The image classifier can subsequently be used to analyse the UAV images.

With the BoVW models, the occurrences of soil, crop and weed species can be mapped on the field scenes. This detailed information on the distribution of different weed species could then be used to generate maps for site specific herbicide application of different herbicides. So far, these maps were only generated by using manual weed counting data. Automatic weed recognition would expedite this process significantly. Together with new application technologies, e.g. a direct injection system and weed density thresholds, this enables a more precise weed management.

# LC-MS-based metabolite profiling of winter wheat grains

Leslie Tais, Christoph Böttcher and Hartwig Schulz
Julius Kühn Institute, Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection, Berlin
E-mail of corresponding author: leslie.tais@julius-kuehn.de

Wheat was one of the first domesticated food crops and its cultivation reaches far back into history. Today, wheat is one of the major crops and an important source for nutrients like carbohydrates and proteins. Whole grains contain besides starch and protein various phytochemicals from the primary and secondary metabolism. The essential role of wheat as a foodstuff comes along with the indispensability to ensure sufficient and secure crop production for worldwide nutrition.

The AWECOS (**A**ssessment of **w**heat cropping systems from an **eco**nomical, ecological and the **s**ociety's perspective) project aims to assess different breeding strategies for winter wheat. The project identifies advantages and disadvantages of eight different genotypes cultivated with different plant protection strategies at five locations around Germany. The assessment will focus on economical, ecological and socio-economic impacts, as well as on the phytochemical quality of wheat samples.

To investigate environment-specific metabolite changes in winter wheat grains, UHPLC/ESI-QTOF-MS based metabolite profiling studies were carried out. To cover a wide range of metabolites with different polarity, two analytical methods were used to provide information about semipolar and nonpolar compounds. In the chromatographic method specific UHPLC columns (C18 and C8), suited for metabolites of different polarity, were used. The coupling of UHPLC to a high resolution mass spectrometer allows separation, annotation and relative quantification of numerous wheat metabolites. Around 250 semipolar compounds including vitamins, free amino acids, lignans, benzoxazinoids and flavanoids as well as 150 nonpolar phytochemicals like alkyresorcinols, sterols, phospholipids and triglycerides could be annotated.

A non-targeted data analyses approach in positive and negative ion mode combined with analyses of variance were used to investigate metabolite changes. The results show significant environment-specific differences in the metabolite patterns and the discriminability of the profiles on basis of a principal component analyses.

„Berichte aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft“
erscheinen seit 1995 in zwangloser Folge

Seit 2008 werden sie unter neuem Namen weitergeführt:
„**Berichte aus dem Julius Kühn-Institut“**

| | |
|---|---|
| Heft 175, 2014 | NEPTUN-Gemüsebau 2013. Dietmar Roßberg, Martin Hommes, 44 S. |
| Heft 176, 2014 | Rodentizidresistenz. Dr. Alexandra Esther, Karl-Heinz Berendes, Dr. Jona F. Freise, 52 S. |
| Heft 177, 2014 | Siebentes Nachwuchswissenschaftlerforum 2014, 26. - 28. November in Quedlinburg - Abstracts -, 57 S. |
| Heft 178, 2015 | Netz Vergleichsbetriebe Pflanzenschutz, Jahresbericht 2013, Analyse der Ergebnisse der Jahre 2007 bis 2013. Bearbeitet von Bernd Freier, Jörg Sellmann, Jörn Strassemeyer, Jürgen Schwarz, Bettina Klocke, Hella Kehlenbeck, Wolfgang Zornbach, 103 S. |
| Heft 179, 2015 | Fachgespräch: „Kupfer als Pflanzenschutzmittel“ Berlin-Dahlem, 21. November 2014. Stefan Kühne, Britta Friedrich, Peter Röhrig, 56 S. |
| Heft 180, 2015 | Fachgespräch: „Gesunderhaltung von Pflanzen im Ökolandbau im Spannungsfeld von Grundwerteorientierung, Innovation und regulatorischen Hemmnissen“ Berlin-Dahlem, 20. November 2014. Stefan Kühne, Britta Friedrich, Peter Röhrig, 40 S. |
| Heft 181, 2015 | Achtes Nachwuchswissenschaftlerforum 2015, 19. - 21. Oktober in Quedlinburg - Abstracts -, 42 S. |
| Heft 182, 2015 | Netz Vergleichsbetriebe Pflanzenschutz, Jahresbericht 2014, Analyse der Ergebnisse der Jahre 2007 bis 2014. Bearbeitet von Bernd Freier, Jörg Sellmann, Jörn Strassemeyer, Jürgen Schwarz, Bettina Klocke, Silke Dachbrodt-Saaydeh, Hella Kehlenbeck, Wolfgang Zornbach, 42 S. |
| Heft 183, 2016 | Pflanzen für die Bioökonomie – Welche Herausforderungen ergeben sich für die Qualität nachwachsender Rohstoffe? 50. Vortragstagung - Abstracts - , 94 S. |
| Heft 184, 2016 | 23rd International Symposium of the International Scientifi c Centre of Fertilizers Plant nutrition and fertilizer issues for the cold climates. Bearbeitet von/ Compiled by Silvia Haneklaus, Peder Lombnæs, Ewald Schnug. Son (Norway), September 8-10, 2015, 30 S. |
| Heft 185, 2016 | 24th International Symposium of the International Scientific Centre of Fertilizers Plant nutrition and fertilizer issues for specialty crops. Bearbeitet von/ Compiled by Silvia Haneklaus, Eduardo Rosa, Ewald Schnug. Coimbra (Portugal), September 6-8, 2016, 65 S. |
| Heft 186, 2016 | 9th Young Scientists Meeting 2016, 9th - 11th November in Quedlinburg – Abstracts –, 2016, 59 S. |
| Heft 187, 2017 | Handlungsempfehlung zur Anwendung von Glyphosat im Ackerbau und der Grünlandbewirtschaftung der Bund-Länder-Expertengruppe. 11 S. |
| Heft 188, 2017 | 2. Symposium Zierpflanzenzüchtung 13./14. März 2017 in Quedlinburg – Abstracts –, 2017, 48 S. |
| Heft 189, 2017 | Bericht über Erkenntnisse wissenschaftlicher Untersuchungen über mögliche direkte und indirekte Einflüsse des Pflanzenschutzes auf die Biodiversität in der Agrarlandschaft. Bearbeitet von/ Compiled by Bernd Freier, Sandra Krengel, Christine Kula, Stefan Kühne, Hella Kehlenbeck, 2017, 72 S. |
| Heft 190, 2017 | Schlussbericht zum Vorhaben Thema des Verbundprojektes: Untersuchung zur Epidemiologie bodenbürtiger Viren in Triticale mit dem Ziel der Entwicklung von virusresistenten Sorten mit hohen Biomasseerträgen für die Biogas- und Ethanolgewinnung. Ute Kastirr, Angelika Ziegler, 2017, 50 S. |
| Heft 191, 2017 | 25th International Symposium of the Scientific Centre for Fertilizers “Significance of Sulfur in High-Input Cropping Systems” Groningen (Netherlands), September 5-8, 2017. Bearbeitet von/ Compiled by: Luit J. De Kok, Silvia Haneklaus, Ewald Schnug, 2017, 58 S. |
| Heft 192, 2017 | 9th Young Scientists Meeting 2017, 6th – 7th November in Siebeldingen - Abstracts -, 2017, 80 S. |
| Heft 193, 2018 | Sekundäre Pflanzenstoffe – Rohstoffe, Verarbeitung und biologische Wirksamkeiten, 52. Vortragstagung, 2018, 65 S. |
| Heft 194, 2018 | Netz Vergleichsbetriebe Pflanzenschutz, Zwei-Jahresbericht 2015 und 2016, Analyse der Ergebnisse der Jahre 2007 bis 2016. Bearbeitet von/ Compiled by: Silke Dachbrodt-Saaydeh, Jörg Sellmann, Jörn Strassemeyer, Jürgen Schwarz, Bettina Klocke, Sandra Krengel, Hella Kehlenbeck, 2018. |
| Heft 195, 2018 | Abschätzung der Habitatwirkung veränderter Produktionsverfahren auf Indikatorvogelarten der Ackerbaugebiete im Forschungsvorhaben „Maisanbau für hohen Ertrag und biologische Vielfalt“ am Beispiel der Feldlerche (*Alauda arvensis*). Jörg Hoffmann, Udo Wittchen, 2018, 48 S. |
| Heft 196, 2018 | **SPISE 7,** 7th European Workshop on Standardized Procedure for the Inspection of Sprayers in Europe Athens, Greece, September 26-28, 2018. Bearbeitet von/ Compiled by: Paolo Balsari, Hans-Joachim Wehmann, 2018, 302 S. |
| Heft 197, 2018 | Schlussbericht zum Vorhaben Vorkommen und Schadwirkung des *Soil-borne wheat mosaic virus* (SBWMV) in Winterweizen. Dr. Ute Kastirr, Dr. Angelika Ziegler, 2018, 34 S. |
| Heft 198, 2018 | Schlussbericht zum Vorhaben Monitoring zum Vorkommen bodenbürtiger Viren in Weizen, Triticale und Roggen in den wichtigsten Getreideanbaugebieten Deutschlands. Dr. Ute Kastirr, Dr. Angelika Ziegler, Dr. Annette Niehl, 2018, 58 S. |
| Heft 199, 2018 | NEPTUN-Gemüsebau 2017. Dietmar Roßberg, Martin Hommes, 2018, 42 S. |


JKI
Julius Kühn-Institut
Bundesforschungsinstitut für Kulturpflanzen