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Meeting

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# Keynote

# Sustainable intensification for future crop production

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Current crop production faces multiple challenges. The ever-growing world population along with dietary shifts in large parts of the world are expected to lead to a doubling of world calorie demand by mid-century. At the same time, arable land and freshwater resources for irrigated production are continuously declining. In addition, global climate change leads to an increasing frequency and extent of extreme weather events with negative impact on productivity and yield stability. In European crop production, yield plateaus are recognized for major crops in recent years. Furthermore, farmers find themselves in the dilemma between low producer prices and high societal demands regarding product quality and environmental protection.

In view of the above sustainable intensification of crop production constitutes a promising approach to overcome the described challenges. However, the key question remains how to increase the efficiency of crop production to produce more with less input and reduced environmental impact?

The presentation highlights different research approaches and technological innovations that support sustainable intensification for future crop production.

A key message is that the assessment and understanding of the status quo of farmers' current crop production is crucial to develop viable adaptation options that can successfully be applied in practice in the future.

Accordingly, it is inevitable to evaluate the (historic) impact of climate change on crop production and understand cause-effect relationships to be able to develop promising climate change adaptation strategies.

Finally, potentials and challenges of agriculture 4.0 for sustainable intensification are introduced and discussed. Vast developments in digitalization, sensor technology, remote sensing, robotics, and computer science occurred in recent years. These technological innovations create novel opportunities in cropping systems design. Furthermore, the easy, affordable and fast generation of in-field data of high spatio-temporal resolution generates a great basis for improving the precision of crop management measures. However, to derive meaningful decision support for farmers from such data is still the largest challenge for researchers and commercial actors.

# Session 1:
# Phytopathology

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

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*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. Because of that *P. viticola* 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 in targeted breeding it is possible to reduce the annual amount of consumed pesticides. In 2013 Venuti *et al.* identified the resistance locus *Rpv12* using QTL analysis of *V. amurensis*. *Vitis 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 (nucleotide binding site – leucine rich repeats) have been identified within the reference genome (PN40024).

We have checked the parentage of different *Rpv12*-carrying genotypes to improve the selection of breeding material and support a better SSR-Marker-Analysis. Besides we analyzed differences between *Rpv8,* a locus described on Chromosome 14 in 2011 by Blasi *et al.,* and *Rpv12* by SSR-Marker-Analysis and microscopy.

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.

Using this we detected a dosis effect by homozygous genotypes and an additive effect with *Rpv3*, since *Rpv12* confers a foliar resistance to strains that are virulent on *Rpv3* cultivars. 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, gene expression analysis and functional testing.

# Infection strategies of old and new Yellow rust (*Pucchinia striiformis)* races

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In the last decade, an invasion of new races of the fungus *Pucchinia striiformis* was observed in Europe. This pathogen causes the severe disease Yellow (stripe) rust mainly on wheat, but also on triticale plants. New dominant Yellow rust races like Warrior and Warrior(-) which are named according to the wheat varieties where they caused the first confirmed epidemic outbreaks have nowadays replaced the formerly divers population of pathotypes in Germany.

Although there are reports about the selective advantage of the Warrior strains through temperature adaptation, it remains unclear how they could establish to such great extent. The best mechanism to control the Yellow rust disease is to prevent severe infections by choosing wheat varieties with effective resistance genes. It is known that the new races are highly virulent, and can therefore infect a greater number of wheat varieties.

In order to classify epidemiological bases related to growing conditions and infection severity of new and old Yellow rust races, we conducted germination tests and experiments in growth chambers. We used susceptible as well as resistant wheat varieties and a number of temperature levels and leaf wetness durations. Moreover, we rated Yellow rust infection of different wheat varieties in the field under natural and artificial infection conditions, respectively.

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

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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 common due to a breakdown of leaf rust (*Lr*-) resistances by virulent rust races. An example for a breakdown is *Lr37*, which became ineffective within two years in 2006. One option to avoid epidemics is pyramiding of *Lr*-genes. A prerequisite for pyramiding are closely linked molecular markers and knowledge about the genetic background of *Lr*-genes. Today, more than 80 *Lr*-genes have been identified, but only a part of these have been deployed in wheat varieties due to linkage drag. One of the resistance genes showing a high level of resistance in the field is *Lr24* located on chromosome 3DL. The introgression consisting *Lr24* comprises more than 600 Megabases (MB). Hence no physically closely linked markers are available up to now. In order to get detailed information on its localization and to reduce linkage drag, a near isogenic line (NIL) containing *Lr24* was crossed to the susceptible cultivar Monopol. Parental lines and $F_2$ plants were inoculated with a single spore isolate of a leaf rust isolate avirulent to *Lr24*.The population was rated using the McIntosh scale on leaf material of parental lines and 144 (*Lr24*) $F_2$ plants at 10 days after inoculation. First results of the analysis proved the dominant inheritance of *Lr24* with 3r:1s segregation ($\chi2=0.39$). This result was validated by microscopical analyses of the fungal development 72h after inoculation. Based on these results kompetitive allele specific PCR markers (KASP) have been developed based on SNPs detected between the near isogenic line (NIL-L*r24*) and the susceptible parental line (Monopol). One of 28 KASP markers analyzed in bulked segregant analysis differentiated exactly between susceptible and resistant lines so that a physical region of 10 MB up and downstream from the marker position was aligned to the reference genome. One candidate gene for *Lr24* was identified. The next step will be allele specific sequencing of the candidate gene in order to identify mutations within the exons. SNPs analyses of additional $F_2$ populations segregating for effective resistances (e.g.*Lr27, 31, Lr9, Lr19*) are in progress.

# Reactive oxygen species (ROS) activity of grapevine berry upon anthracnose attack

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Anthracnose an important disease that occurs regularly in in grapevine areas where conditions during the growing season are usually humid and warm with frequent rains during spring months. The pathogen, *Elsinoe ampelina*, infects primarily young tissues, such as shoots and leaves, but also attacks fruits. Most grapevine cultivars grown around the world are susceptible to this pathogen. However, some new bred cultivars of a group denominated PIWI (pilzwiderstandsfähige Rebsorten) show tolerance to anthracnose attack but the mechanism of resistance is not yet clear. Often, the resistance against fungal diseases is linked with ROS (reactive oxygen species).

The aim of this work was to study ROS production linked with grapevine resistance against anthracnose attack of berries. The experiment was designed based on a bifactorial trial (5x2) using five genotypes, ‘Aromera’, ‘Bronner’, ‘Felicia’, ‘Helios’ and GF24 and analyzing berries with or without anthracnose symptoms. Four bunches with symptoms from natural anthracnose infection were collected with three replications, at the phenological stage of berry formation. Disease severity was evaluated using images and the software Quant, measuring the percentage of disease. ROS enzymatic activity, superoxide dismutase, catalase, ascorbate peroxide and guaiacol peroxidase, were measured using skins peeled off from berries. All data were used for bifactorial variance analyses followed by Tukey´s test to analyze the relation between genotypes and berries with and without symptoms. Based on the disease severity observed, the genotypes were classified into different resistance levels. GF24 and ‘Helios’ were grouped as resistant, ‘Aromera’ and ‘Bronner’ as moderately resistant and ‘Felicia’ was considered as susceptible. ROS production showed no interactions between the factors genotypes and berries. Corroborating, the berries with and without symptoms did not show differences for ROS studied. However, resistance and moderately resistance genotypes showed higher ROS activity than the susceptible cultivar. The generation of ROS in mitochondria plays an important role as a signal for HR-induced cell death in plants. These results suggest that the resistance of berries against anthracnose is linked with ROS production. However, the complete ROS route need to be studied in more detail.

# Session 2:
# Environment and Genomics

# Agricultural management influences barley rhizomicrobiota and plant immune response

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Agricultural management practices are assumed to shape soil microbial communities. We hypothesize that the rhizomicrobiota of barley grown in soils under different long-term soil management or fertilization treatment will harbor different prokaryotic communities. Therefore, barley rhizosphere (BBCH55) and bulk soil was sampled from a long term field experiment (LTE) in Bernburg, Germany, with four different agricultural managements (mouldboard plough (MP) and conservation cultivator tillage (CT) with standard N-fertilization and pesticide application (I) or reduced N-fertilization without pesticides (E)). Rhizosphere and bulk soil was investigated by amplicon sequencing of 16S rRNA gene fragments amplified from total community (TC)-DNA. The soil managements MP and CT revealed significant differences in the prokaryotic community composition. However, the fertilization treatments did not show any differences.

In order to test our hypothesis, a greenhouse experiment was conducted with the barley cultivar Golden Promise grown in the four differently managed soils from LTE Bernburg. Additionally, a standard greenhouse substrate was used as control. The plants were grown until BBCH13 and subsequently infected with model pathogen *Blumeria graminis* f. sp. *hordei*. Relative gene expression profiles of the defense-related genes *PR1b* and *PR17b* were determined in barley leaves before and 24 h after infection. The relative gene expression tended to be higher in all infected samples compared to the uninfected samples, with a significant difference for plants grown in MP-I soil. The amount of *B. graminis* fungal hyphae was determined in a detached leaf assay. No differences were revealed for plants grown in field soil which all had significantly lower infection rates compared to the plants grown in standard substrate. Our results indicate that the rhizomicrobiota of barley grown under field conditions was shaped by the agricultural soil management. Whether management dependent differences under greenhouse conditions were less pronounced is presently investigated by amplicon sequencing of 16S rRNA gene fragments obtained from TC-DNA of rhizosphere and bulk soil and will be presented at the meeting. Managing soil microbiomes could be a new approach to enhance crop resistance.

# Establishment and persistence of *Salmonella enterica* in agricultural systems

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In the last years, food-borne diseases were increasingly associated with contaminated fruits and vegetables. *Salmonella enterica* was one of the most frequent recorded causes of food-borne diseases in Europe. The complexity of *S. enterica*'s invasion and persistence in agricultural environments like soil, as well as the colonization of plants is only at the beginning of being understood. However, the elucidation of colonization mechanisms and of biotic and abiotic factors influencing colonization rates would be imperative for the provision of safe fresh produce to the consumer. A multitude of factors influencing the fate of the human pathogen in the agricultural environment and the results of experiments are until now often very contradictory. For example, the persistence of *S. enterica* in soil and the colonization rates of plants differ between soil types and plant species. The interconnection of different influencing factors seems to be multifaceted and might become even more complex when abiotic factors are considered. *S. enterica* adapts to a multitude of environmental habitats which may lead to genetic adaptation in a longer term. To decipher the entire network is a challenging task; however, it might result in efficient prevention strategies and in reduction of food-borne salmonellosis outbreaks.

In our experiments we could show an enhanced persistence of *S. enterica* in loamy compared to sandy soil, and when *Salmonella* was applied to soil together with organic fertilizer (pig manure and chicken litter) compared to *Salmonella* application one month after fertilization. Furthermore, the persistence is prolonged in soil with reduced soil microbial diversity. The colonization rates of lettuce and corn salad were influenced by the soil type, plant species and *S. enterica* strain. Finally, an interaction between *S. enterica* and plants could be observed on transcriptional level.

In summary, our results suggest that successful strategies for the prevention of food-associated disease outbreaks will need to consider the plant production environment as a whole system, including the soil type, the fertilization management practice and maybe most importantly the physiological state of the crop plant.

# Assessment of the impacts of microbial plant protection products on the health and development of the gut microbiome of honeybee (*Apis mellifera*, L.)

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Microbial plant protection products as substitutes for chemical PPPs enjoy an increasing popularity because of their compatibility with integrated pest management. Important are primarily PPPs with different strains of *Bacillus thuringiensis* (B.t.) as active substance due to selective effects on insects of the orders Lepidoptera, Coleoptera and Diptera. Despite the presumed safety for non-target insects, several studies with contrasting results show potential harms of some B.t.-products for insects of other orders like Hymenoptera. Due to the foraging activity of bees, they could be exposed to B.t.-products either acutely after spray application or chronically through ingestion of contaminated pollen and nectar. Recent studies suggest that the gut microbiome of honeybees is a crucial factor for bee health. The most studies were carried out under laboratory conditions and there is still no information about the plasticity of the core microbiome under colony field realistic conditions. Therefore, it is considered as an essential issue to clarify the contribution of the gut microbiome in the development of healthy bees and the relationship to applied microbial PPPs.

In the current study, we evaluated the effect of the product FlorBac® with the active substance *Bacillus thuringiensis* ssp. *aizawai* (strain: ABTS-1857) on adults and larvae of honeybees (*Apis mellifera*) under laboratory and field conditions. Chronic oral toxicity tests on adult bees (OECD guideline 245) and larval chronic toxicity tests (OECD guidance document 239) were therefore conducted. Additionally, possible modifications of the chronic oral toxicity test like additional pollen feeding were assessed. Furthermore, an in-hive feeding experiment was performed to investigate the effects of B.t. on colony development and composition of the gut microbiome in individual bees.

Our results show that the survival of adult bees was affected after chronic exposure to FlorBac® depending on the tested concentrations. Moreover, the exposure duration seemed to play an important role. The mortality of bees arose only after 96 h at the highest concentration, so the acute test duration seems inappropriate for examining the effects of microbial PPPs. Moreover, the feeding with pollen had a significant effect on the survival of the treated bees. Our results demonstrated also a significantly reduced survival of treated larvae at all concentrations. The results of the field study are still pending.

# Usage of highly specific indel mutations for distinguishing Cydia pomonella granulovirus isolates

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Various isolates of the Cydia pomonella granulovirus (CpGV) has been used as an insect pest control agents against codling moth (CM, Cydia pomonella L), a main pest in apple orchards, worldwide since the 1980s. Commercial formulations are based on *in vivo* produced CpGV occlusion bodies (OBs) that are sprayed in aqueous suspensions on leafs and fruits, where they are ingested by CM first larval instar initiating a quick and fatal viral infection. A production of qualitative stable and consistent OB suspensions is important to guarantee the right CpGV isolate composition since isolates, such as CpGV-M, CpGV-S and CpGV-E2, exhibit different virulence to known CpGV resistant field and laboratory CM populations. Furthermore, population of CM were known to host potentially latent CpGV infection, challenging the propagation process. Recently, isolates CpGV-M, -S and -E2 were NGS deep sequenced and characterized for their intra-genetic composition based on single nucleotide polymorphisms. In the present study, the NGS data was used to identify highly isolate specific insertion/deletion (indel) locations that allow the rapid detection and identification of these three CpGV isolates by PCR techniques. Two different open reading frames, namely pe38 and orf47, as well as, an intergenic region with specific indel mutations were chosen for this approach. Indels were not smaller or larger than 19 to 25 bp, respectively. The PCR approach was used for the identification of populations of susceptible and resistant CM populations to detect CpGV latency offering a rapid tool in quality control for CpGV OB production.

# Deciphering the genomic diversity of Bombyx mori nucleopolyhedrovirus isolates from Southeast Asia

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The main source of silk in the wolrd stems from the larvae of *Bombyx mori*, the domestic silkworm. In this report ten isolates of the Bombyx mori nucleopolyhedrovirus (BmNPV) of the family Baculoviridae, genus Alphabaculovirus, from different geographic areas of India and Thailand were deciphered for their genetic composition by analyzing specific single nucleotide polymorphisms (SNPs). An alignment free method was used, where the generation of consensus sequences from the sequencing data was avoided to the greatest possible extent. The quantification of mixtures was done by a so-called quantitative SNP analysis where isolate specific variant positions were used. This method had been successfully applied on isolates of the Cydia pomonella granulovirus, genus Betabaculovirus, and was tested, for the first time, on a member of the genus Alphabaculovirus, namely BmNPV. Based on quantitative SNP analysis of the Indian isolates, a homogenous isolate (BmNPV-My) and two mixed isolates (BmNPV-De and BmNPV-Ja) were identified. The mixtures were about equal parts of BmNPV-My and BmNPV-De/Ja, with the latter two appearing to be highly similar. Thai isolates also included mixtures and pure genotypes, but were harder to decipher. Phylogenetic studies also showed a strong correlation between the distance of BmNPVs and their genetic similarities.

# Session 3:
# Breeding and Plant Physiology I

# A review of heat stress in canola (*Brassica napus* L.)

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With growing importance of canola (*Brassica napus* L.) as a global oil crop, its production increasingly expands beyond temperate regions into warmer climatic zones. Additionally, climate change leads to rising temperatures and a higher risk of heat stress induced crop losses throughout the world. Against this background, we review the status-quo of agronomic research on heat stress in canola. We first evaluate the reported evidence of heat stress induced canola crop losses around the globe. We furthermore review the range of experimental approaches applied in heat stress research in canola, which span from meta-analysis of historic multi-environment trials, and field experiments with sequential sowing, to experiments with induced heats stress in controlled environments. For the latter the largest body of literature exists with heat stress treatments mainly applied during flowering and seed filling stage. The actual effects of different intensities and durations of heat stress treatments are largely investigated via morphological and physiological parameters while most studies determine heat stress effects on yield quantity and quality as well as yield components. Finally, we examine various prospects of alleviating heat stress in canola including genetic and agronomic approaches; we identify existing knowledge gaps and highlight related research demand.

# Effect of species and seedtime on growth, development and yield of blue lupine (*Lupinus angustifolius)*

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Beginning from March 27 in 2018, we created a field trial on the area of the Julius-Kühn-Institute in Berlin Dahlem. The subject of the trial was a comparison of three cultivars of blue lupine (*Lupinus angustifolius*) which were sown on four different times in a weekly interval. The plot design fulfilled the requirements of a two-factorial strip-plot with six replicants. The factors were three cultivars of the blue lupine and the seedtime which differed on a weekly basis. The field trial was carried out under practical conditions on sandy loam using agronomic techniques like deep- and rotary-tilling to prepare a humogen seedbed. Phytosanitary measures were applied to avoid stress by weeds and pest infestation to keep the environment between the subjects of interest as similar as possible.

To quantify differences in the development of the alternatives, we took destructive samples on four determined growing stages using the BBCH-code for identification. To identify the right moment for the harvests there was consecutive observation carried out on at least three days a week on which the BBCH-Code for the 12 alternatives was determined and the plants height and number were analyzed in two defined micro – plots per sample. Once we took the samples, the plants were firstly separated in leaf and stem, and later on the generative parts were separated in legumes and seeds. For better comparability, all samples were drought and weight as dry matter. Another subject of interest to quantify the growing rate was the Leaf Area Index. Starting from May 9 2018, it was determined on a weekly basis using a plant canopy analyzer.

With the collected data we are able to show the speed and length of growth-stages for the different alternatives. Also, we can determine the exact numbers of germinating plants and the amount of yield (including the amount of protein and fat for the seeds).

Besides the characterization of development and growing rate through the visualization of the data collected, the possible impact of cultivar and seedtime on the harvest and yield parameters were statistically verified using a Two-Way Analysis of Variance (ANOVA) using R-Studios.

Using this method, we find significant differences in yield, fat- and protein content in between the alternatives with a different sowing time. Also, the cultivars differ in development rate and yield and show unique reactions on dry conditions regarding the sowing time. One cultivar was superior in adapting to the dry conditions. For the evaluation one has to keep in mind the dry conditions in 2018 leading to differences in the availability of water between the variables.

# Breeding for winter-annual growing of caraway (*Carum carvi*)

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Caraway (*Carum carvi*) is a member of the *Apiaceae* family. It is used as a spice and pharmaceutical easing gastrointestinal inflictions. The effect is attributable to essential oil content. Major components are carvone and limonene. For pharmaceutical use the European pharmacopoeia demands an essential oil content above 3 % by distillation. Besides economic issues, growing of caraway provides benefits in crop rotation for subsequent crops and increases agricultural diversity. In addition, a variety of insects finds nourishment in caraway fields during flowering.

However, as a slow growing crop caraway is particularly affected by extreme environmental conditions. In 2018, yield losses up to 80 % due to summer drought were measured in Saxony-Anhalt. Furthermore, severe infections by powdery mildew were observed in late ripening caraway trials. As one possible solution, we suggest avoiding such conditions by winter annual growing. Sowing in autumn would result in earlier ripening and harvesting in the following year. However, this strategy requires adapted genetic material: Biennial cultivars, which are still widely grown in Europe, need vernalization for flowering induction. The necessary plant size to receive the cold stimulus is usually not reached after late sowing. The newly bred Czech variety 'Aprim' instead lacks a vernalization requirement. Furthermore, annual breeding lines with potential winter-hardiness were found and preselected.

In 2018/2019, 'Aprim' and four preselected lines were tested for winter-hardiness and essential oil content by sowing in parcels of 12 m$^2$ in four repetitions. Essential oil content was estimated using non-invasive near infrared spectroscopy (NIRS). Predictions were based on extraction values as reference, which were measured by gas chromatography flame ionization detection (GC-FID). At last, predicted extraction values were corrected towards distillation by a regression function. Both, extraction and distillation values were gathered in prior trials.

'Aprim' was found to have a sufficient winter survival rate of about 91 % ± 6 % S.E., whereas even the best breeding line showed only a winter survival rate of about 34 % ± 10 % S.E. By contrast for 'Aprim' an essential oil content of 2.1 % ± 0.05 % S.E. was estimated, whereas even the breeding line with the lowest average content reached 4.8 % ± 0.24 % S.E.

For future efforts, it follows that crossings between 'Aprim' and breeding lines of high essential oil content might be the most promising way to gain genetic material for winter annual growing.

# Session 4:
# Breeding and Plant Physiology II

# Differences in the self- and cross-fertilization ratio of raspberry cultivars

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Red raspberry, *Rubus idaeus* L., cultivars are self-fertile. However, wild *Rubus idaeus*, like most other Rosaceae crops as apples or cherries, is self-sterile. Rosaceae self-sterility system is a gametophytically controlled self-incompatibility, with an RNase SI mechanism.

In this study, 20 seedlings from open pollinated mother plants were genotyped with up to 17 SSR markers via capillary electrophoresis to compare the extent of self- and cross-fertilization of 16 raspberry cultivars. The marker profile of the progeny was compared to that of the mother cultivars to determine self- and cross-fertilization in these 320 samples.

There is a range of self- and cross-fertilization in the 16 cultivars assessed, from all progeny self-fertilized in 'Dorman Red' to none of the progeny self-fertilized in 'Rumla'.

Allele mismatches occurred with five markers in the cultivar 'Preußen', where both alleles between progenies and mother cultivar were different. As the samples used for positive controls were taken from the testing station of the Federal Plant Variety Office in Wurzen, not from the original field where the fruit was collected from, a cultivar mix-up or somaclonal variation could have occurred.

These results coincide with a previous study, where six raspberry cultivars of up to six different origins were tested for trueness-to-type by DNA fingerprinting with 16 SSR markers. Nine out of 33 samples turned out not to be true-to-type, which, although a small sample size, still raises awareness of a problem in raspberry trade that could be of a bigger extent than previously assumed.

The two cultivars at the two extremes of the spectrum were hand pollinated to study seed set with self- and cross-fertilization. Seed set of 'Rumla' is significantly higher in cross-pollinated fruit, while with 'Dorman Red', seed set is significantly higher in self-pollinated fruit.

This study delivers new information about the natural propensity of self-fertilization in 16 raspberry cultivars; self-fertilization is tolerated differently from cultivar to cultivar. This can be an important information for growers, as raspberry production increasingly takes place in protected growing.

# Towards Genome-Wide Association Mapping in apple (*Malus × domestica*)

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The Institute for Breeding Research on Fruit Crops in Pillnitz, Dresden is not only a node in the decentralised network of German Fruit Genebank (Deutsche Genbank Obst) but also responsible for coordinating the work between different partners. The institutional Fruit Genebank has an incredibly diverse ex-situ collection of domesticated apples (*Malus* × *domestica*) comprising of 702 cultivars. The oldest cultivar in the collection dates back to the 12th century. These cultivars, along with accessions of wild apple species (*Malus sylvestris*, *Malus floribunda*, etc.) provide a fundamental resource for genetic research and fruit breeding.

This extensive field collection of *Malus* germplasm, although critical for crop improvement, are expensive to maintain and can be unwieldy for research purposes. To improve this situation, Sir Otto Frankel introduced the concept of “Core Collection” in 1984. “Core” is the essential part of the entire collection and is defined as “a limited set of accessions which represents, with a minimum of repetitiveness, the genetic diversity of a crop species and its wild relatives”.

This idea was applied to the apple germplasm collection of the institutional Fruit Genebank. All cultivars were genotyped using 17 SSR markers. The triploids were carefully removed, and the data was fed to different software programs. Finally, a collection of 113 cultivars was selected by PowerCore. Later a ‘Mixed Approach (software findings + expert opinion)’ proposed by Urrestarazau (2019) was adopted and 42 cultivars were integrated to this collection. Ultimately, a “Core Collection” with 155 cultivars was established.

After the successful establishment of the Core Collection, the next step was scoring the phenological events and phenotyping of the cultivars. The future goal will be the Genome-Wide Association Mapping studies to compare the DNA of the plants having varying phenotypes for essential traits like very early and late bud burst etc.

# Accelerating the breeding of carrots (*Daucus carota* L.) via CRISPR/Cas9

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Traditional plant breeding aims to develop plant cultivars that produce a high quality product with a constant yield, but the fast changing climate with more extreme conditions calls for a fast adaptation of crop varieties. While hybrid breeding results in a uniform progeny profiting from the heterosis effect, it is a time consuming challenge to produce homozygous parental lines which are needed for hybrid breeding in cross-pollinated crops like carrot (*Daucus carota* L.). To find a universal tool and to prevent the necessity of homozygous parental lines by back crossings, we aim to accelerate traditional F1 hybrid breeding by using the new plant breeding technique (NBPTs) CRISPR/Cas9.

To achieve (doubled) haploid and therefore completely homozygous parental lines in one generation, we target a gene that is involved in the first cell divisions of the early embryo. During cell division, the microtubules of the spindle apparatus attach to the kinetochore region of the centromere to distribute chromatids equally. Therefore, a compromised function of the centromere specific histone H3 protein (CENH3), as part of the kinetochore, can result in an uneven distribution of chromosomes. In our approach, one parental gamete carries chromosomes with compromised CENH3 due to mutations induced by CRISPR/Cas9 while the other gamete still carries the wild type CENH3. A compromised centromere function of the mutant allows for a uniparental genome elimination of the mutated parent genome when crossed with a wild type plant. The resulting (doubled) haploid progeny can directly be used in hybrid breeding.

We used *Rhizobium rhizogenes* to introduce a CRISPR/Cas9 expression cassette that induces mutations inside the CENH3 coding region. Immunostaining against CENH3 with specific antibodies showed a change in the phenotype, with mutated lines showing a weaker fluorescence compared to the wild type controls.

In order to facilitate this approach, we established a fast, transient and DNA-free method for genome editing in carrot by introducing preassembled sgRNA and Cas9 protein into carrot protoplasts via PEG mediated transformation. Since this complex degrades after around 48 h, the regenerated plant lines carries no foreign genes. A putative lethal effect of induced mutations was visible in a lower regeneration capacity of the protoplasts with only wild type regenerates. To screen for regeneration capacity and viability of mutated carrot lines, we are comparing different target regions inside the CENH3 gene.

# Improvement of virus resistance breeding in barley by the help of *H. bulbosum*

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*Hordeum bulbosum*, the only member in the secondary genepool of *Hordeum vulgare*, holds resistances and tolerances against various 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. 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 carrying resistance against BaMMV/BaYMV ($Rym16^{Hb}$) and tolerance against BYDV ($Ryd_{203S11}^{Hb}$) on chromosome 2HL were characterized. The sizes of the introgression fragments were calculated based on the barley reference sequence and resulted in a size of 4.2 Mb for the locus $Ryd_{203S11}^{Hb}$ and 3 Mb for the locus $Rym16^{Hb}$.

The analysis of 10.000 $F_2$ plants carrying $Ryd_{203S11}^{Hb}$ and 4440 $F_2$ plants carrying $Rym16^{Hb}$ with co-dominant flanking markers resulted in 34 recombinant $F_3$ plants, which will be used to construct high resolution mapping populations. The recombination rate within the introgression is about 14 times reduced compared to 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 used 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, the resistance of $Rym16^{Hb}$ will be analyzed by using resistance gene enrichment sequencing (RenSeq).

# Session 5:

# Modelling and Digitalisation in Agriculture

# Parameterization and evaluation of the GRAMI model for simulation of soybean

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GRAMI is a simple crop model that uses remote sensing (RS) information and few crop growth specific parameters to simulate growth and yield of potential grain crops. This study aimed to extend GRAMI in simulating soybean (*Glycine max*) yield by determining crop parameters required for modeling. Field trials were conducted to calibrate and validate the model in Chonnam National University (CNU), Gwangju, South Korea in 2017-2018 under irrigated and rain-fed condition. Validation data was obtained from another field at CNU. The estimated parameters of radiation use efficiency (RUE), light extinction coefficient (k), and specific leaf area (SLA) were 1.65 g MJ-1, 0.71, and 0.017 m2 g-1, respectively. The GRAMI-soybean model was evaluated for its capability at simulating leaf area index (LAI), above-ground dry mass (AGDM), and yield based on integrating RS data. Simulated LAI and AGDM values agreed with the corresponding measured values with agreeable statistical accuracies and reasonable model efficiencies in both calibration and validation. The model combined with RS data simulated yields in agreement with the measured yields without significant differences in calibration ($p = 0.54$) or validation ($p = 0.92$). The study results demonstrate that the GRAMI-soybean model can reproduce soybean growth and yield using a simple input requirement and RS data.

# Modelling the spatio-temporal distribution of climate-sensitive plant pest species

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**Introduction** Climate-sensitive pests are species whose risk-potential is expected to change significantly as a result of the climatic changes observed to date and predicted for the future. The model's focus on climate-sensitive species closes a gap in the assessment of new pests. Up to now, changes in their long-term risk potential due to climate change have been difficult to assess and were therefore not sufficiently taken into account in pest risk analyses. The prediction of the establishment potential and possible spread of pests and evaluation of different environmental- and management scenarios is of great ecological and economical importance and essential for pest management.

**Concept/Goal** Our goal is to create a simulation model that will allow these predictions and scenario analyses, and to establish in the long term a framework as a standard procedure for supporting plant health risk analyses of climate-sensitive pests. The objective is to create a transferrable, generalized open-source model framework that is easy to extend and easy to couple with individual host plants, current climate data sets as well as new presence/absence data of the pest. The model will predict the probability of survival and establishment as well as forecast the possible spread of the pests based on species-specific spread parameters and distribution properties. The dynamic model consists of different components: The host plant sub-model predicts future cultivation areas and land use changes, taking into account climate change and political trends. The habitat sub-model calculates the climatic suitability of the different areas today and in the future. The dispersal sub-model then generates the probable dispersal of the respective pest, taking into account various dispersal pathways, including those related to humans.

**Results** The results of the model will be presented graphically in the form of distribution- and risk maps. The output will be used in risk analyses to better assess the economic and environmental risks of the species. Additionally, identified high-risk areas for pest establisment can support the planning of survey activities. Furthermore, the model-output could be a decision-support tool for example for plant growers and breeders.

**Outlook** We want to keep the model open source and assure transferability to as many pest groups as possible (fungi, insects, arachnids, nematodes, bacteria) so that the model can be enhanced by different sub-models as well as species-specific functions and parametrization.

# Video portraits of pests in arable land and stored products for an online diagnostic aid

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The almost 20,000 organic farms in Germany, in particular the 400 to 800 annual conversion farms, have a high and special need for information on ecological plant protection. Existing online tools for determining harmful pathogens are either linked to advertising for plant protection products or only cover a limited spectrum of species. An ongoing project is developing an identification aid for organic farming and compiling organic regulation options.

Additionally, video portraits in form of short documentaries are being produced, to illustrate the movements and behaviour of pests in a catchy and memorable way. The learning effect for the user is thereby supported.

For recording insect footage, insects were taken from the environment or from a beneficials-rearing company and reared further in the laboratory.

We used special macro lenses, which are characterized by a high resolution and a low close-up limit. The latter enables taking a very short distance to the object, while still achieving a sharp image. Another requirement is a suitable camera that suits several lenses and records in the codec and colour coding that suits the intended final use of the film.

The data amounted to many gigabytes of film after a single working day. After storing, they were evaluated and labelled for post-production. For producing the voice-over, an informational text was written, to be spoken by a professional speaker over the pre-final-film, followed by a final round of editing.

The current project has so far been published on http://pflanzenschutz.oekolandbau.de for the topics of stock protection, arable farming, weed regulation, fruit and wine growing and hop. The determination aid is designed as a filterable, image-based complete list of harmful organisms that allows any combination of selection options. In addition to characteristics of the organisms, it is also possible to filter for larval characteristics, infested products (storage protection), site conditions (weeds), or infested plant parts and harmful symptoms.

Current research enables more recommendations with regard to regulatory options.

The final film will be exported in a FullHD, SDR format an uploaded to the JKI YouTube channel. This format is the most popular format, although 4K and HDR are also supported. Every film will be linked to the fitting article on the diagnostic aid to get more and specific information. Examples of beneficial insects are also included. Films about the following insects will be produced until the end of the year: colorado potato beetle, dried fruit moth, mediterranean flour moth, drugstore beetle, tobacco beetle, grain weevil (rice weevil, maize weevil), bean weevil, lesser grain borer, flat grain beetle, confused flour beetle, sawtoothed grain beetle, large and small cabbage white.

# Session 6:

# Agricultural and Forestry Management and Regulation

# Uniform seed patterns in wheat cultivation

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For many years, wheat is the most important crop cultivated all over the world. It is grown in various cultivation system from rudimentary hand seed to powerful and expensive seeding machines. Nevertheless, the process of seeding is nearly all the same: the seed is randomly distributed in the field. Especially with modern seeding equipment, there is a big spread between the distances from one plant to another in the row and between the rows. Hence, the distance between the single plants is very unequal.

Due to these space problems, plants are exposed to various stresses. In the narrow plant population there is a moist microclimate, which benefits fungal growth and intensifies plant disease. The tight spaces between the plants in the row and a wide distance between the rows lead to a contrast in water and nutrition availability and the rhizosphere. Because of the limited space for a single plant, it is unusually that plants reach their potential yield maximum. In terms of climate change and stricter nutrition regulations these stresses getting a bigger problem in future agricultural production systems.

A possibility to fight these stresses is to optimize the distribution of each single plant in the field. Many authors described that with uniform seed pattern plants grow more evenly and less plants can gain the same yield compared to ordinary seeding techniques with twice as much plants. The best possible distribution of plants is reached by seeding pattern in an equilateral triangle. In an equilateral triangle, the distance between the single plants in and between the rows is all the same. Because every plant has an optimized space for growing, fewer plants are needed to harvest maximum yield.

In 2018 a field experiment at the Julius Kühn Institute in Braunschweig, Kleinmachnow and Quedlinburg started. Compared are equilateral triangle seed patterns to conventional seeding with common plant density and reduced plant density. The equilateral triangle patterns were sown by hand which took much time and is not practical. Another aim of this project is to develop practical seeding equipment to plant grain in equal seed patterns.

In the field, it is obvious that plants grown in uniform seed patterns develop more regular. Because of more root space, plants are tighter connected to the ground and can be more resistant to weather extremes or mechanical weed control. The ears are nearly twice as long compared to the conventional seeding. Finally yet importantly, the yield is comparable to conventional seeding.

In the common years, more field experiments are planned. It is the aim to get more information about the differences in plant health, finding best genotypes for uniform seed pattern and consider differences in plant requirement in water and nutrients. Maybe it is possible to establish some kind of new cropping system to compete future needs in sustainable agriculture and produce more food for a growing population.

# Preparation of a checklist to evaluate the implementation of the guideline of integrated plant protection (IPP) in the forest

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The National Action Plan on the Sustainable Use of Plant Protection Products (NAPs) provides for the preparation of guidelines on integrated plant protection. As part of the joint project “RiMa-Wald”, subproject 4 was responsible for drawing up the national sector specific guidelines for integrated forest protection in cooperation with key players in forestry and plant protection. Another focus of subproject 4 is to prepare a checklist to assess the implementation of the sector specific guideline in the demonstration operation. During the preparation of the checklist, an online questionnaire was developed and tested in six demonstration operation.

The lecture introduces the method and development of the checklist to the online survey. Moreover, exemplary results of the first online survey are presented.

# Poster

# Using the parasitic wasp *Trichopria drosophilae* (Hymenoptera: Diapriidae) for augmentative biocontrol of Spotted Wing Drosophila (*Drosophila suzukii*; Diptera: Drosophilidae) in Germany

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Spotted wing drosophila (SWD) is a pest of soft-skinned fruit (e.g. Berries, Cherries, Grapes) and originates from South East Asia. Recently emerged in Europe, it poses a high threat for producers.

SWD larvae feed on the inside of ripe fruit. Due to the lack of available control methods (except netting of the crop) as well as insufficient presence of effective natural enemies in the production systems, the resulting damage can take up devastating proportions.

In order to avoid potential unforeseen adverse effects of a palpable classical biological control approach (i.e. importation of natural enemies regulating SWD in its centre of origin) a solution based on suitable *spp.* already present in Germany is preferred.

*Trichopria drosophilae* (TD) is a pupal parasitoid of drosophilids and widely distributed over Europe. Its promising efficacy against DS under laboratory conditions (e.g. Parasitation rate, successful development, longevity) predestine TD as a promising candidate for an augmentative biocontrol product to regulate DS.

In order to adapt an experimental small-scale DS breeding program for mass rearing, (1) an economically feasible quality control management for quantification of relevant population variables is currently established, (2) production efficacy will be improved and (3) possible product formulations will be developed. Consecutively, (4) product performance will be assessed and optimised.

In contrast to pupal parasitoids, larval parasitoids potentially allow earlier (thus better) control of DS populations. DS has been reported to be resistant to larval parasitoids of drosophilids endemic to Germany through encapsulation of the parasitoid inside the host.

In order to use endemic larval parasitoids, host resistance needs to be overcome. The feasibility of this approach will be investigated by adaptive breeding of promising strains on DS under laboratory conditions.

# MAGIC-RESIST: Identification and mapping of effective resistance genes to rust diseases and Fusarium head blight in the MAGIC WHEAT population WM-800

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Basis of the MAGIC-RESIST project is the MAGIC WHEAT population WM-800 which is derived from eight elite wheat varieties (Patras, Meister, Linus, JB Asano, Tobak, Amber, Safari, Julius). The project aims at the identification of effective resistances against leaf rust and stripe rust and Fusarium head blight (FHB), followed by mapping resistance QTLs by genome wide association studies (GWAS). To achieve this, we conduct field tests at two locations in Quedlinburg and Halle over several years using artificial inoculation. Field test results are supplemented by hyperspectral image analyses and high-throughput studies of leaf segments of all genotypes after inoculation with differentiating leaf and yellow rust races. In order to analyze the toxin content of field-harvested grains and to correlate data with scoring results the Deoxynivalenol content will be determined by ELISA. First analyses of the resistance against rusts and FHB showed large differences between the lines in greenhouse and field trials and rust resistant genotypes were detected. Due to the warm and dry weather FHB was difficult to detect in the field. However, it was possible to detect differences in field-harvested grains. Using a potato dextrose agar, *Fusarium* has been detected in suspicious grains and was confirmed by detecting conidiospores in the growing mycelia by microscopy.

Furthermore, morphological data such as plant height, thousand-grain weight, grains per ear and flowering time will be included into the investigations. In the future, genotypes that combine resistance QTLs with desired morphological properties will be included into the breeding programs of the cooperating wheat breeding companies.

# Combined stressors of insecticides, fungicides and pollen quality on honey bee health

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Honey bee health has been reported to be affected by multiple factors including resource quality, exposure to agrochemicals and pressure of pests and pathogens. Currently, concerns are growing about potential interactions between multiple stressors and their impacts on bee health.

Therefore, we performed a semi-field study to investigate the combined impacts of different pollen qualities and exposure to a mixture of agrochemicals. Twenty-four tents with crops with a gradient of different pollen quality for honey bees were realized: Phacelia with high pollen quality, maize with less pollen quality and tents planted with 50% maize and 50% flower mixtures with diverse pollen qualities. Additionally, the variants were sprayed with a tank mixture of the pesticides Mirage 450 EC (Prochloraz) and Biscaya (Thiacloprid). Various parameters were investigated such as mortality of adults, larvae and pupae, activity of detoxification enzymes, weight and longevity of newly hatched bees.

On the other hand, while several studies have reported the synergism between EBI-fungicides and different classes of insecticides, few studies have focused on the effects on honey bee larvae and their activity of detoxification enzymes. Therefore, we aimed to investigate the mortality of larvae in the laboratory fed with artificial diet spiced with the EBI-fungizide Prochloraz, the neonicotinoid Thiacloprid and the novel butenolide insecticide Flupyradifurone (active substances) in single and combined exposure.

Our preliminary results show potential synergistic effects on larval mortality with different magnitudes compared to reported effects on adults. This indicates a lower susceptibility of larvae to tested plant protection products. Activity of the primary detoxification enzymes P450, gluthation-S-transferase and acetylcholinesterase will be analyzed in collected larvae from laboratory as well as semi-field studies.

# Impact of the colonization pattern on beneficial properties of plant-associated bacteria

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Microorganisms have a significant impact on plant growth and health. Plant Growth-Promoting Rhizobacteria (PGPR) and especially those among them, which have endophytic lifestyle, draw increasing attention in the last years. Previous research showed that the soil-borne bacterium *Ensifer meliloti (Sinorhizobium meliloti)* primed *Arabidopsis thaliana* for enhanced resistance against diverse fungal and bacterial pathogens. *Pseudomonas* spp. and *Pantoea* spp., which were isolated from within barley seeds, could also improve barley resistance against fugal pathogens and promote the growth. However, little is known on how the positive influences depend on the colonization pattern expressed by beneficial bacteria while colonizing plants.

In order to assess this question, all three bacteria were first marked with the plasmid pSM1890-GFP, which carries the gene encoding for green fluorescent protein (GFP). We used biparental and triparental mating systems to transform the PGPRs with the pSM1890-GFP plasmid. Assessing the colonization pattern in *Arabidopsis* and barley, our first results revealed that all of three strains (*E. meliloti*, *Pseudomonas* spp. and *Pantoea* spp.) could colonize roots of *Arabidopsis Col*-0 plants however, with diverse patterns. The colonization patterns are being assessed now for the above-ground plant parts (phyllosphere). Our research will further aim at the correlation between the colonization patterns and beneficial properties of plant-associated bacteria.

# Effects of conventional agricultural practices on soil fungal and bacterial communities

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Agricultural intensification has been facilitated by application of agrochemicals and soil tillage, causing environmental and soil health problems such as loss of soil fertility and biodiversity. Since soil microorganisms are the key players of many ecosystem processes, the objective of this study was to compare the fungal and bacterial communities in soils under different long-term agricultural management. To achieve this goal, bulk soils from two different tillage systems (Cultivator Tillage [CT] vs Mouldboard Plough [MP]) with two fertilization levels (Standard N fertilization [INT] vs 50% less N fertilization [EXT]) were compared after harvest of winter wheat. The soils originated from a long-term field experiment established in 1992 with maize/wheat/barley/rapeseed/wheat rotation in Bernburg (Germany). Analysis of microbial community composition based on 16S rRNA genes or internal transcribed spacer (ITS) fragments, for bacteria or fungi, resp., amplified from total community-DNA, were analyzed by Denaturing gradient gel electrophoresis (DGGE). The fingerprints showed that soil microbial communities were affected by tillage system and fertilization level. To analyze in more detail the effect of agricultural management on the community structure of both microbial groups, Illumina Miseq amplicon Sequencing was performed. Similar to DGGE, the PERMANOVA analysis showed a significant effect of the tillage, the fertilization and the interaction between these factors for both fungi and bacteria, but effects were stronger for bacteria and no clear differences between treatments were observed for fungi. Alpha-diversity indices (richness, Shannon and Chao-1´s diversity, Pielou´s evenness) indicated that bacterial communities in CT soils were less diverse, but more evenly distributed compared to MP. In the case of fungi, no significant differences in terms of alpha-diversity were observed between treatments, but CT-EXT tended to have higher diversity and less dominant species. This suggests that soil bacterial communities seem to be more sensitive to agricultural management than fungi. Regarding taxonomy, 36 phyla were found for bacteria, with Proteobacteria, Actinobacteria and Acidobacteria being most abundant. Fungal ITS sequences were affiliated to seven different phyla, being Ascomycota and Basidiomycota the predominant ones. Finally, management-depending responders analysis is still in process for a better understanding about the complex interactions in this agroecosystem. Future work will aim to deepen the identification of microbial taxa that may serve as possible biological indicators for a sustainable crop production management.

# Method for the feasible acquisition of rating data and the use of the data for site-specific plant protection in agriculture – BoniPS

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The monitoring of the occurrence of pests by appropriate methods is an essential target for the use of the chemical pesticides on an adequate level. To use an effective amount of pesticides, it is necessary to control the fields intensively. This is usually done by ratings. These are supported by forecasting models and information from the official advice. However, the regional warnings and recommendations cannot replace the on the field assessment. The required rating is also usually complex and time consuming. Furthermore, the site-specific application of pesticides is hardly used in practice. Based on the rating information and taking into account the thresholds, a decision should be made, if a plant protection measure is being carried out. The aim of the project "BoniPS" is to develop a fully practicable process, from data acquisition onwards to site-specific application of pesticides. This includes also the creation of application maps for winter wheat, winter rape and pea. Therefore, the time required for the rating can be reduced. Data from forecasting systems, alerts from plant protection services and assessments from previous field crossings can be included in the decision-making. In addition, aerial photographs and knowledge of farmers about special characteristics of the fields could be taken into account. The app contains 136 pest profiles for 3 cultures as a help for determination. The characteristics includes the retrieval of time of occurrence (BBCH stage), the injury level, the type of ratings and comparison images. During the rating, the user is also supported with guidance. This leads the user through the complex issue in simple steps. The rating line is a default or manual creation of rating points. The user could be navigated to the rating point via GPS to have an exact position. During the rating, there is a determination of infestation frequency and severity of pests and therefore a geo-referenced determination of the infested areas. The traffic light system is a tool for optimal and site-specific pesticide application. The infestation classes are: red = over / yellow = around / green = below threshold. With the help of the evaluated rating points, a site-specific application map for the application of pesticides can be created. The areas to be treated are marked in the map, but can be changed by the user. It's a simple process to help farmers/contractors to make the best possible decision. The results of the rating are transferred to a suitably equipped plant protection device for carrying out a treatment. The data documentation can be done in real time. BoniPS is the memory of the field through continuous, local data storage for documentation and verification. The site-specific application of pesticides may lead to a reduction of the treatment frequency index and into economical and environmental friendly improvements.

# Methods of monitoring hoverflies (Diptera: Syrphidae) and their fitness conditions in agricultural landscapes in the FInAL-project

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In times of decreasing natural habitats agricultural landscapes become more and more important in supporting populations of insects. They are taking up to 50 % of the land area in Germany and so they have a great impact on the richness of species and the steady declining of insect abundances. Many insects provide important services including pollination, pest control or being a food source for other animals like birds or amphibians.

Adult hoverflies are important pollinators for example of strawberries or fruit trees. Females need pollen for oogenesis and nectar plays a major role for growth and development of adult individuals. 39 % of the larvae are zoophagous and therefore play a major role as biological pest control agents against aphids.

The aim of FInAL (facilitating insects in agricultural landscapes) is to introduce renewable resources into existing cultivation systems which can support local insect populations by providing essential resources, are economically workable and acceptable for farmers. An important role plays the implementing of integrated pest management.

Syrphidae are significant for this project because they are responsible for pollination and pest control. In order to provide these ecological services, it is necessary to research foraging of hoverflies, the abundance and species diversity and how they can be supported by sustainable cultivation systems. The first step is to study the abundance of hoverfly species in the field laboratories before and after planting renewable resources and if this measure is going to have an effect. Therefore, hoverflies are going to be trapped with yellow, blue and white pan and Malaise traps and identified to species level. The second step is to explore several fitness parameters of the hoverfly population in the altered landscape. One factor that we want to study is the use of flower resources provided by the new cultivars by hoverflies and potential effects on fertility and energy resources. Gut analyzes provide information about the acceptance of the new food source and which role it may play in foraging of hoverflies. Pollen analyses will be performed to make statements about how important the cultivars are in proportion to the entire food of hoverflies and how they are going to be accepted as alternative resource. In preliminary tests we are trying to extract the pollen from hoverflies via dissection of the abdomen. This is also possible through examination of fecal pellets, which give us the option of researching the captured individuals alive. Additional analyzes of nutrient, fructose, glucose and lipid levels will give us valuable information about the energy that is available for the hoverflies and which plant is more suitable to support insect populations.

# Value-adding usage of by-products from steam-distillation for essential oil production

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Essential oils derived from medicinal and aromatic plants are widely used in pharmaceutical, cosmetics and food industry. In the process using steam distillation for essential oil production, large amounts of by-products occur that actually have only minor value and/ or are not well defined concerning their composition and potential areas of application.

Plant based products and extracts bear a variety of health promoting, pharmacologically and biologically active compounds. Thus, by-products of plant distillation can add further value to the raw material and enhance the sustainability of the production process. The aim of our project is to evaluate residual fractions of essential oil production for establishment of an economical resource-efficient extraction of plant material.

The main part of by-products of plant distillation comprise the aqueous fraction called hydrolate (or hydrosol) and the distilled biomass (pomace). Hydrolates are present in large quantities and contain volatile and water-soluble compounds, mostly fractions of the essential oil and further plant metabolites. Floral waters such as from roses are used as ingredient of cosmetics and for flavoring food.

In order to support domestic plant distillation industry, plant species, which are important for essential oil production in Germany, have been selected for further investigation within this project.

This poster exemplifies preliminary results on the composition of selected hydrolates and their potential phytosanitary effects. Promising approaches will be pointed out and discussed.

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# FInAL – Innovative integrated cultivation strategies to promote insect diversity

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Insect diversity and abundance are decreasing with negative consequences for ecosystem functioning. Especially changes in cultivation practices, often summarized as intensification, are discussed as major drivers of insect decline. However, agricultural landscapes, covering over 50 % of Germany, have the potential to provide valuable habitats for insects, if managed accordingly.

The FInAL-project aims to develop and demonstrate innovative agricultural cultivation practices that sustain, support and increase insect diversity and biomass as well as ecosystem functions provided by insects. This is to be achieved primarily by the integration of renewable resources and integrated pest management.

Therefore, a large-scale, long-term transformation process will be launched on the landscapes scale within three 3x3 km size landscape laboratories across Germany. In close cooperation between researchers and stakeholders, especially farmers, locally adapted measures to promote insects will be conceptualised in a co-designing process and then implemented. In a multidisciplinary approach, impacts of the landscape transformation on biodiversity, its economic viability, and the social acceptance are going to be evaluated. Monitoring the transformation processes in landscape laboratories for ten years will allow the detection of long-term impacts of the transformation, refine measures to facilitate insects, establish value-chains for alternative crops, and enable comprehensive comparisons with reference landscapes that will be established in the vicinity of the landscape labs.

In the first years of the project, a baseline survey is carried out for all relevant parameters of the study. The Julius Kühn-Institut, Institute for Strategy and Technology Assessment, will investigate the abundance of pests and beneficial insects in-field. It is planned to conduct a monitoring for pests and crop damage in particular in cereal and oil seed rape. In addition, new methods for integrated pest management will be developed which are specially designed to promote the diversity and abundance of insects and will be integrated into the transformed production systems including renewable resources.

As can be imagined, the consistent landscape monitoring in FInAL will generate hundreds of datasets with different research foci. The institute for Strategy and Technology will be responsible to provide a data infrastructure supporting the project consortium in managing their data throughout the entire data life cycle. A joined Database Management System will help to collect, document, process, analyse, share, and publish the research data as to be FAIR – Findable, Accessible, Interoperable, and Re-Usable.

# Marker-assisted selection for *Wheat dwarf virus* (WDV) tolerance in wheat (Triticum aestivum)

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*Wheat dwarf virus* (WDV) is an important pathogen in wheat and other cereals. In many European countries, e.g. Hungary, Spain and Germany, WDV causes high yield losses. WDV is transmitted by the leafhopper *Psammotettix alienus*. Symptoms of a WDV infection on wheat include chlorosis, dwarfing and streaking along with high yield loss. Due to climate change, the incidence of insect-transmitted viruses will become more important worldwide due to the extended survival time of the vector. The absence of insecticides efficient against *P. alienus* renders growing of WDV resistant/tolerant varieties the only effective way to control WDV.

However, little is known about WDV resistance/tolerance sources. Based upon a previous project- in which wheat accessions were screened for WDV resistance/tolerance and quantitative trait loci (QTL) were identified by genome-wide association studies (GWAS), this project aims to use this tolerance in breeding. For this purpose, breeding partners produced single seed descent (SSD) and doubled haploid (DH) populations by crossing tolerant genotypes with suceptible varieties.

This material will be phenotyped for WDV tolerance and agronomical traits and will be genotyped using the 20K Illumina Infinium Chip. Based on the obtained data the WDV tolerance will be mapped and molecular markers (KASP/CAPS) will be developed. 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 wheat breeding.

# Description of the plant health network using the example of *Ralstonia pseudosolanacearum*

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Concerning the international plant health network, Germany is a member state (MS) of the World Trade Organization (WTO) and signed the International Plant Protection Convention (IPPC). It is a MS of the European and Mediterranean Plant Protection Organization (EPPO) at the regional level. The National Plant Protection Organization (NPPO) of Germany consists of the Federal Ministry of Food and Agriculture (BMEL), the Julius Kühn Institute (JKI) and the Plant Protection Services of the Federal Länder (PSD). The aim of all plant health network parties is to prevent and control the introduction and spread of pests of plants and plant products and to ensure safe trade.

If a third country is planning a new law or an amendment to a law regarding sanitary and phytosanitary (SPS) aspects, this is usually communicated to the WTO MS by a SPS notification mostly with a request for technical comments by the impacted countries. For example, New Zealand (NZL) identified nursery stock of *Vaccinium* spp. as a risk pathway for the introduction of *Ralstonia pseudosolanacearum* into NZL and published in a SPS notification the amendment to the Import Health Standard (IHS) regarding nursery stocks to prevent the introduction of this pest. In general, the BMEL notices SPS notifications regarding plant health and assigns the JKI with its scientific examination. In the case of NZL, JKI rated the amendment of the standard as scientifically correct. Furthermore, no trade barriers were seen for the export of *Vaccinium* spp. nursery stock to NZL. If required, the BMEL sends technical comments to the European Union (EU), which in turn submits a summarized technical comment of all EU MS to the notifying country. After the amendment entered into force, the relevant law is updated on the plant health homepage of the JKI. In the case of regular export of the notified commodity to the notifying country of destination, additionally a short notice is made in the “Kompendium” of the JKI summarizing the changes concerning inspectors (e.g. export controls).

# Genetic differences in barley govern the receptiveness to priming agent

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Priming crop plants for enhanced resistance using biocontrol agents is an efficient disease management strategy, since it results in robust resistance and higher yield. The beneficial effects of the bacterial quorum sensing (QS) molecules e.g. *N*-acyl homoserine lactones (AHL) on resistance and plant growth have been shown in different plant species. Here, we present the effects of the AHL, oxo-C14-HSL, on the priming capacity of barley. We demonstrated that barley primed with *Ensifer meliloti*, expresses enhanced resistance against *Blumeria graminis.* We also showed that the capacity to induce priming varies among different barley genotypes. Among a set of barley genotypes, we identified "*primable*" genotypes that had better ability to enhance resistance and "*non-primable*" genotypes that were non-responsive to oxo-C14-HSL and therefore, did not have any ability to enhance resistance. This suggests that appropriate genetic background is required for AHL-induced priming. We further showed that priming for enhanced resistance in barley involves stronger activation of the barley ortholog of the MPK6, regulation of defense-related PR1 and PR17b genes and remodeling of the cell wall structure. Noticeable was the stronger accumulation of lignin upon priming after a chitin challenge. Interestingly, the global metabolomic changes in barley during priming are rather subtle and specific. Identification of these metabolites is important as it opens doors to study the mechanisms and understand the relation between the plant genomic background and the priming agent. This understanding would further increase the efficacy of priming approaches and lead to novel breeding and plant protection strategies.

# Methods to assess host-parasitoid-complexes in meadows as indicators of insect diversity in an agricultural landscape

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Meadows and pastures can act as reservoir habitats for insects in agricultural landscapes. Especially beneficial insects like parasitoid wasps may find better habitat conditions due to food provision by nectar and pollen producing plants, alternative prey and no disturbances evoked by pesticide use or soil cultivation. However, intensification through higher fertilizer inputs and more cuts per year can lead to a decrease in plant species and flower abundance, which in turn can negatively influence the insect community. This loss is more severe for specialized species, which are often important for the natural control of pests.

To test the influence of management practices (fertilizer input, cutting frequency/timing) on the insect diversity and abundance, a number of classical monitoring methods is available (e.g. suction sampling, pan traps). The complexity of multi-trophic interactions - like host-parasitoid-complexes - could act as surrogate for the overall insect diversity. Monitoring of these interactions is less time consuming, more detailed and often produces strong site-specific results.

Especially endoherbivores and their parasitoids, which are dependent on a specific plant organ that they normally do not leave during development, can be highly site-specific, sensitive to habitat change and abundant in agroecosystems. Because of this fact, standardized methods to assess the species richness of endophagous herbivores and their parasitoids could be used to study the complexity of a meadow insect community and influences of management and the surrounding landscape structure thereon.

In preliminary trials, methods to assess the diversity and abundance of two different host-parasitoid-complexes, typically found on meadows in agricultural landscapes, were tested:

1) Centaurea - Tephritidae - parasitoids

Centaurea flower heads were collected from meadows and incubated under controlled conditions. Emerged flies and hymenopteran parasitoids (mainly Chalcidoidea) were extracted every second day. 10% of the flower heads were dissected, larvae/pupae counted, identified and put in gelatine capsules for further development. Together with sweep net samples from the Centaurea patches, the diversity/abundance of the tephritid flies and their parasitoids is analysed and correlated with influencing factors and the overall insect diversity.

2) Capsella - Curculionidae - parasitoids

The cabbage seedpod weevil (*Ceutorhynchus obstrictus*) is an important pest of canola and can use several wild Brassicaceae as alternative hosts. The siliques of *Capsella bursa-pastoris* were collected from meadows and incubated in self-built photoeclectors. Emerged weevils and their parasitoids are identified and their diversity/abundance is correlated to the overall diversity and other factors.

# Illuminating SBWMV-host interaction: Subcellular localization of viral proteins during infection

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Soil-borne cereal viruses cause substantial crop losses and therefore represent an extensive threat for agriculture in Europe, Asia and America. The bipartite Furovirus *Soil-borne wheat mosaic virus* (SBWMV) infects several crop species like wheat, rye or barley and is transmitted by a soil-borne plasmodiophorid, called *Polymyxa graminis*. Resistances against Furovirus infection are barely described, in wheat for e.g. the Sbm1 and Sbm2 genes encode for a translocation resistance, which restricts the infection to the plant roots. The infection of the roots or the translocation of the virus into upper plant tissues depends on the viral movement protein (MP) and coat protein – readthrough (CP-RT) protein.

In this study, we modified a SBWMV cDNA clone to express MP:GFP and CP-RT:GFP fusion proteins to uncover their subcellular localization and illuminate their specific functions during virus infection. Both genes were fused to GFP and the modified cDNA clones were used as templates for RNA synthesis. *In-vitro* produced viral RNA was used for infection of different host plants and the fluorescent proteins were localized by confocal laser-scanning microscopy (CLSM).

Further experiments will aim at identifying host components, which are involved in the infection process. This knowledge could provide new ideas for the development of resistance strategies against soil-borne viruses.

# Investigating the localizations and interactions of *Soil-borne wheat mosaic virus* movement protein and CP-RT protein

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Soil-borne cereal viruses cause substantial crop losses and therefore represent an extensive threat for agriculture in Europe, Asia and America. The Furovirus *Soil-borne wheat mosaic virus* (SBWMV) is transmitted by *Polymyxa graminis*, a plasmodiophorid which produces resting spores containing the virus. These virus-containing resting spores remain infectious in soil for many years. Thus, the only way to combat virus infection by *Polymyxa*-transmitted viruses is to grow virus resistant plants. However, the only known effective resistance locus against Furoviruses encodes for a translocation resistance, i.e. the virus titer is significantly reduced in the shoots of resistant plants, while the roots still become infected. The viral factors determining virus movement from the roots to the shoots and virus transmission by *Polymyxa* are likely the viral movement protein (MP) and viral coat protein – readthrough (CP-RT) protein. To better understand the process of virus movement and virus transmission, we are investigating the subcellular localizations and host interactions of these two viral factors. We expect that a better understanding of host components, with which these two viral proteins associate during infection, will help to develop targets for novel resistance strategies.

# NIRS-based detection of pyrrolizidine alkaloid containing weeds in crop plants after harvest – PA-NIRSort

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The general objective of the project is the development of an efficient sorting system based on hyperspectral near-infrared spectroscopy (NIR) for the detection and separation of impurities by pyrrolizidine alkaloid (PA)-containing plant derived contaminations in cultural plants, e.g. medicinal and aromatic plants. PAs are liver-toxic secondary metabolites in plant defense and are known for causing seneciose in grazing animals. By now, the German Federal Institute for Drugs and Medical Devices (BfArM) has published strict PA maximum contents for phytopharmaceutical products as eg. Teas which can be contaminated with PA-containing weeds such as various types of ragwort, groundsels, common viper's head, common comfrey, common dog's tongue, water-east or borage and thus potentially endanger consumers. Due to these strict limits, four to five PA-containing plants of the species *Senecio vulgaris* may be sufficient to destroy one ton of medicinal drug.

The planned process will analyze fresh and dried plant material on a flat conveyer using hyperspectral NIR spectroscopy to detect impurities in the crop. After identification, contaminants should be removed by a sorting technique, e.g. using compressed air pulses. Similar systems have already been established in plastic waste sorting and quality control, for example for grapes. The aim is to achieve a high throughput of up to five tons of harvested crop within three to four hours. With such an automated sorting technology, the health risks posed by PA-contaminated medicinal plant products could be reduced ecologically and economically efficient for cultivation and processing of medicinal plants. This would also mean safeguarding high-quality and competitive plant derived drug production in Germany.

First results show that a classification of target plant species and contaminating Senecio using NIR spectroscopy is generally possible. In order to be able to carry out such image analyses in real time, the amount of data to be processed will be reduced to the decisive factors by means of multifactorial data analysis.

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# MonViA - Development of a concept for a national trend monitoring of the earthworm community

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The National Monitoring of Biodiversity in Agricultural Landscapes “MonViA” is a joint project of the Julius Kühn Institute, the Thünen Institute and the Federal Agency for Agriculture and Food with the aim of developing a modular monitoring system for agricultural landscapes. A decisive part of this monitoring is the earthworm community.

Intact earthworm communities are essential for high and stable soil fertility. However, earthworms are not evenly distributed in the German agricultural landscape due to natural limiting factors such as texture, moisture and soil pH. Areas where these limiting factors are ideal would be "comfort zones" for earthworms where habitat functions are assumed to be normal and intact. Such “comfort zones” are therefore best suited to observe anthropogenic effects of agricultural use on the diversity and function of earthworms.

In order to characterise agricultural areas, especially arable land, with regard to their relative performance ("comfort zones") for earthworms, a meta-analysis will be carried out using available data from the JKI, TI, existing databases and literature data. The aim is to identify typical earthworm species for the respective site-specific comfort zones. Suitable species must be abundant in the comfort zone, distributed over several comfort zones and easily identifiable.

The results of the meta-analysis are transferred to geomaps, which identify several "comfort zones" in Germany for earthworms via a web GIS application developed in the project. Only these zones will be the focus of a practicable long-term monitoring of the diversity and abundance of earthworm communities under consideration of soil properties and soil cultivation systems. To validate the results, soil analyses and earthworm surveys will be carried out in April and October 2020 and 2021 using standardized methods for the direct and indirect detection of the abundance and activity of earthworms, with a particular focus on the bioindicator species.

Ultimately, all the results achieved in the project are incorporated into the development and implementation of a cost- and personnel-effective yet meaningful monitoring concept for the effects of cultivation measures on the diversity and abundance of earthworm communities and their ecological services. The concept should serve as a basis for a national trend monitoring of biological diversity and identify long-term trends in the biodiversity of earthworm societies in the agricultural areas typical for Germany, for example in the event of a change in management methods.

# Transcriptome-based mapping of anthracnose resistance gene (*Llur*) in yellow lupin (*Lupinus luteus*)

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Yellow lupin (*Lupinus luteus* L.) is a promising legume crop, which is well adapted to poor soils, has a good drought tolerance and higher seed protein content than most legume species. Nonetheless, the yellow lupin has almost disappeared from the fields in Germany due to high susceptibility against the fungal disease anthracnosis caused by pathogen *Colletotrichum lupini*.

A screening for anthracnose resistance of the several lupin cultivars revealed a polish variety with a high level of resistance to anthracnose. Segregation of the anthracnose resistance was assessed in a $F_2$ population (n=200). A 3:1 ratio of resistant to susceptible plants indicates a monogenic dominant inheritance of the resistance locus. The corresponding resistance gene was previously designated *Llur*. Development of resistance linked selection markers shall be accomplished by methodical combination of bulked segregant analysis and NGS-based transcriptomic analysis. Therefore artificially infected leaf segments of resistant and susceptible genotypes of a $F_2$ mapping population were sampled and bulked. RNA was isolated and RNA-Seq was performed to reveal the presence and quantity of RNA in post-inocluated *L. luteus* plant material. Comparative expression studies are aimed at identifying exclusive transcripts or SNPs in the resistant bulk and thus can be used as reliable marker resource for the genetic mapping of resistance gene *Llur* in yellow lupin.

„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“**

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