10th Young Scientists Meeting 2017

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- Abstracts -

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Greetings from the President

Dear Young Scientists,

This fall we celebrate the 10th anniversary of the Young Scientists Meeting. For the first time it will be held in Siebeldingen, where the Institute for Grapevine Breeding and the Institute for Plant Protection in Fruit Crops and Viticulture of the Julius Kühn-Institut are located.

The high registration count of 72 attendees reflects the popularity of this eagerly anticipated meeting and its importance for the young scientists' networking. That a wine tasting of the JKI's own wine creations will take place in the experimental wine cellar, might have contributed to these high numbers, if only a bit.

The tasting will be accompanied by a lecture on wine quality held by Prof. Dr. Jochen Bogs (University of Applied Sciences Bingen). In a second keynote, Dr. Katja Herzog (Julius Kühn-Institut) will provide insights in sensor-based phenotyping of grapevine. In addition, Prof. Dr. Holger Puchta's (Karlsruhe Institute of Technology) lecture will revolve around CRISPR/Cas, a revolutionary technology in genetic engineering which earned their discoverers the 2015 Breakthrough Prize in Life Sciences.

Very valuable, especially for young scientists at the starting point of their careers, will be Dr. Ulrike Stahl's (Julius Kühn-Institut) talk on how to publish data and why. After Open Access for textual publications and Open Source for software are widely accepted, now also the Open Data movement is gaining momentum. To stay abreast of these changes and actively contribute to their progress, the JKI adopted a new data policy earlier this year.

Another recent development is the increasing use of social media among scientists to communicate and collaborate. In the run-up to this year's meeting, the organizers have tested several of these channels for their potential to raise interest in modern plant science among the general populace. To complement this, there will be a panel discussion with colleagues from the Public Relations Unit of the JKI to discuss the opportunities and risks the use of social media brings for governmental research organizations.

As every year, there will also be a soft skill seminar in advance of the scientific meeting, and fitting to the conference topic, it will be on scientific communication.

I wish all of you a successful meeting with many fruitful discussions and a pleasant stay in the heart of the Palatinate wine region and return home enriched by the experience and inspired both scientifically and socially.

Siebeldingen, November 2017

Dr. Georg F. Backhaus
President of the JKI

Foto: Robert Zech

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Keynotes

"A great wine begins in the vineyard" Environmental and genetical influences on grape quality

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Many fruit crops are grown with an emphasis on appearance and yield. In contrast, premium wine grapes are one of the few crops that are still grown primarily for their taste and fla-vor. Over the last years, there has been an emphasis by the wine industry to improve quality through reducing yield. However, grape composition and quality is the result of many complex interactions that occur in the field throughout the growing season and yield is only one part of the equation. Hereby, various environmental and genetical factors, which can be influenced by a whole range of viticultural practices determine grape quality and composition. This talk will give an overview on the compounds determining the taste, aroma and quality of grapes and the genetic diversity of grapevine cultivars leading to different wine styles. Further, the influences of environmental factors like light, temperature, water availability and nutrition on biosynthesis of important grape metabolites including sugars, acids, aromatic compounds and polyphenols will be highlighted and their influence on grape and wine quality discussed.

High-throughput plant phenotyping using sensors and automation

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Phenotyping is the quantitative analysis of morphological and physiological features of organisms in a given environment and it is one key technology in the scope of plant research and breeding. In general, phenotypes will be scored manually by visual estimations using common established classification systems, e.g. BBCH scale. Methods often are destructive, labor intensive, time consuming, the number of samples is limited and phenotypic data are subjective with unpredictable error variations.

Valid, objective and precise phenotypic data from large experimental sites are a prerequisite to increase breeding efficiency and to improve functional genetic research. Therefore, sensor based techniques are a supporting novel approach currently under development. In particular for breeding applications phenotyping is largely done under field conditions rather than in controlled environments. As a consequence, non-invasive assessments of physiological traits (e.g. rate of photosynthesis); incidence of diseases or fruit quality (e.g. metabolic compositions of grapes) requires the application of robust sensor based methods.

Numerous sensors are established for (non-invasive) plant phenotyping purposes (e.g. 2D image-based sensors for structure analysis and hyperspectral sensors for physiological traits or disease detection) in labs, greenhouses or the field. The integration of such sensor techniques with RTK-GPS (orientation and data management) on robots, tractors or unmanned air vehicles (UAV) will facilitate automated, non-invasive field phenotyping of plants with high-throughput. Independent of automated or manual sensor usage, efficient data management, automated analysis with user friendly graphical user interface (GUI) and modeling permits the acquisition of objective phenotypic data from a large number of plants with a high precision. Sensor based phenotyping is thus, a promising opportunity to open up the phenotyping bottleneck: it will (i) increase the number of samples and repetitions; (ii) improve quality of recording; (iii) minimize error variations; and (iiii) enable retro specific evaluations.

Double-strand break induced genome engineering in plants: Past, Present, Future

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Sequence-specific nucleases can be used to induce double-strand breaks (DSBs) in plant genomes. In the past we could show that thus gene targeting (GT) by homologous recombination (HR) can be enhanced and targeted mutagenesis can be achieved by error-prone non-homologous end joining (NHEJ). Moreover, by inducing several DSBs, sequences can be deleted out of the genome or chromosome arms exchanged. In the last years CRISPR/Cas became the major tool for targeted mutagenesis. We were able to demonstrate *Streptococcus pyogenes* (Spy)Cas9 nuclease induced, NHEJ mediated, heritable targeted mutagenesis in *Arabidopsis thaliana as well as homology dependent in planta GT*. Off-target effects might be avoided using two sgRNAs and a Cas9 protein that was transformed from a nuclease to a nickase, to induce adjacent single strand breaks (SSBs) in opposite strands. This "paired nickase" strategy has a mutagenic potential at the target site comparable to the nuclease. Interestingly; sequence duplications are a prominent outcome of this approach, hinting to the possibility that the repair of adjacent SSBs is a major cause of sequence duplications during genome evolution of plants. Recently we applied the Cas9 orthologues from *Streptococcus thermophilus* (Sth1Cas9) and *Staphylococcus aureus* (SauCas9) for NHEJ-mediated targeted mutagenesis in *A. thaliana* with efficiencies at least comparable to those of SpyCas9. We were also able to show that the SauCas9 and SpyCas9 proteins only work in the presence of their species-specific single guide (sg) RNAs and show no inter-species interference. Thus, the Cas9 proteins of *S. pyogenes* and *S. aureus* should be appropriate for simultaneously addressing different sequence motifs with different enzyme activities in the same plant cell. The simultaneous use of different Cas9 orthologues will offer the opportunity to control genetic information of plant cells on more complex levels than before and will lay the basis for future synthetic approaches in plant biology.

Our work on genome engineering in plants is funded by the European Research Council [Advanced Grants "RECBREEED" (2011-2016) and "CRISBREEED" (2017-2022)].

Research data publication – why, how and where?

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Research data can be the result of a scientific working process or will arise throughout it. Research data appears in many different formats and encompass, among others, observations and measurements, images, program codes, computer simulations, audio recordings, geo-data, descriptions, results of surveys as well as physical objects of collections and samples of experiments. Thus it is one of the most valuable commodities produced, since arguments, theories, tests or hypothesis are based on it and it forms finally the basis of scientific publications. To guarantee transparent, efficient and high quality research and allow validation of results, research data should be made publicly available and citable as well as properly archived and documented. Funders, such as the DFG and the EU within their Horizon 2020 programs, require these criteria requesting an obligatory data management plan. Also Journals, such as *PLOS ONE* and *Nature,* set up their own data policies where they require authors to make all data promptly and fully available without restriction that underlies the findings in their submitted manuscripts. The JKI passed its own Data Policy in March 2017 which regulates the data management, documentation, publication and responsibility as well as who supports and helps. The JKI supports, promotes, pushes and recognizes any effort of its scientists and PHD students to manage their data properly and make them publicly available as soon as possible. The publication of research data is an important step to make them **FAIR** – namely **F**indable, **A**ccessible, **I**nteroperable and finally **R**e-usable for others. The **FAIR** Data Principles are the most accepted guidelines to date for data management and are also recommended by the JKI Data Policy. They were designed and endorsed by a diverse set of stakeholders representing academia, industry, funding agencies, and scholarly publishers and are intended for all wishing to enhance the reusability of data. However, not every way of data publication fulfills these criteria. There are big differences between publishing your data on your website, as supplement of an article, in a repository or in a data journal as "data paper". The JKI maintains the Open Access repository OpenAgrar which is suited for publication of research data according to the **FAIR** principles. In OpenAgrar a permanent digital object identifier (DOI) and metadata will be assigned to your data, which make them **F**indable, citable and **A**ccessible. Together with a linkage to your research paper and an open license your data would be **I**nteroperable and **R**eusable. The library of the JKI provides practical advices on what you need to do when publishing your data in OpenAgrar or in another **FAIR** repository.

Food Safety

Persistence of *Salmonella enterica* in the plant environment

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In the last years, foodborne zoonotic infection outbreaks were increasingly associated with fruits and vegetables. *Salmonella* has been reported as the second most abundant cause of foodborne diseases in Europe in 2015. This indicates that plants are suitable vectors for *Salmonella enterica*. Contamination of produce can occur along the whole production chain. While the post harvest contaminations can be avoided by hygiene specifications, the possible contamination at production site (e.g. in field) is a more complex issue. *Salmonella* can contaminate soil and plants *via* irrigation water or organic fertilizers. Therefore, it is essential to understand the persistence of *Salmonella* in soil in order to gain insight into its ability to colonize plants and the subsequent hazard of foodborne diseases. Today, the knowledge about factors influencing the persistence of *Salmonella* in soil and plant environment is scarce, and the question whether *Salmonella* colonizes plants as opportunistic bacterium or if it uses plants as alternative hosts is still controversially discussed.

We analyzed the influence of soil fertilization and soil sterilization on the survival of *Salmonella*. We observed an adaption and enhanced persistence in soils with reduced diversity. While fertilization with pig manure had a positive effect on the survival of *Salmonella* in soil, chicken manure had no influence. Sterilization of soil by autoclaving led to a drastic reduction of the abundance of soil bacteria. Denaturing Gradient Gel Electrophoresis (DGGE) as well as Illumina sequencing data revealed changed communities in the autoclaved soils. *Salmonella* was able to survive in this soil up to 6 months and seemed to adapt to this environment. These results indicate strong influence of the indigenous bacterial community in the agricultural soil on the survival ability of *Salmonella.* As an invader in this environment it likely competes for resources with the native community. Despite an initial decline, our data indicated a long-term survival of *Salmonella* in agricultural soil. In another experiment we localized *Salmonella* in the rhizosphere of crop plants using a GFP marked strain. By means of confocal laser scanning microscopy we showed that *Salmonella* moves towards plant roots.

Together, our results indicate that *Salmonella* can persist in soil for extended times. Adaptation to the soil environment may enhance the risk of contamination of fresh produce. The fact that *Salmonella* may use plants as alternative hosts strongly suggests that plants represent a much larger reservoir for animal pathogens than so far estimated.

Beneficial bacteria prime crops for enhanced resistance against human pathogens

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Outbreaks of food-borne diseases, related to contaminated raw vegetables and fruits, indicate that plants are suitable hosts for human pathogens (HP). *Salmonella enterica* is able to colonize plants and use them as alternative hosts. Nonetheless, plants perceive HP and induce their defense responses. A method to increase those immune responses is priming for enhanced resistance. An efficient strategy to control plant pathogens is priming of plants with *N*-acyl-homoserine-lactones (AHL).

The aim of this study was to evaluate whether crop plants are "primable" against *Salmonella*. To this end, rhizospheres of corn salad, tomato and lettuce were inoculated with the AHL-producing strain *Ensifer meliloti* $_{expR}^{+}$, the AHL-negative *E. meliloti* $_{attM}$ strain and as positive control with beta-aminobutyric acid. Plant leaves were then sprayed or infiltrated with *salmonella*. The persistence of *Salmonella* in plants was quantified over 14 days. Furthermore, changes of transcription level of defense related genes in primed and non-primed plants were measured *via* quantitative PCR. In addition, physiological responses as the production of reactive oxygen species (ROS) and stomatal closure were assessed.

Our results revealed that *Salmonella* is able to persist in plant leaves for at least 14 days. Priming has a negative effect on the persistence of *Salmonella*. Primed plants are able to express defense related genes earlier than unprimed plants, produce a higher amount of ROS and are able to keep stomata closed. These results indicate the potential of priming for enhanced resistance against *S. enterica*.

Salmonella survival in different host plants, be careful with what you eat

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The Gram-negative *Salmonella* genus can cause diseases ranging from enteritis to typhoid fever. The variety of *Salmonella* serotypes reflects the adaptation to diverse hosts. Ubiquitous serotypes, such as *Salmonella enterica* serotype Enteritidis or Typhimurium, generally cause gastrointestinal infections in humans but can induce other diseases in animals. For example, they can cause typhoid-like infections in mice but an asymptomatic intestinal colonization in chickens and pigs. In order to provoke a disease in mammals, *Salmonella* must survive the harsh conditions of the gastric tract after being ingested with the contaminated food or water. The two Type III Secretion Systems (T3SS) encoded within the Salmonella Pathogenicity Islands 1 and 2 (SPI-1 and SPI-2) are essential for *Salmonella enterica* virulence. T3SS-1 and T3SS-2 are responsible for the secretion and translocation of a set of bacterial proteins into the host cells, those proteins are named effectors. The aim of effectors is to alter the host cell physiology, preparing host cell for bacterial entry. Emerging evidence indicates that these effectors modulate immune-related proteins and are utilized by bacteria to de-activate intracellular signalling pathways, not only in animals or humans but also in plants.

Salmonella usually enters agricultural environments, in which it may colonize plants, *via* animal faeces. Animal faeces can directly contaminate plants or surface water used for irrigation or during processing. Recently, an increasing number of reports linked *Salmonella* contaminated raw vegetables and fruits with food poisoning. *Salmonella* is able to adjust to different external conditions such as low pH or high temperature, permitting its survival outside the animal organism. Before active colonization of the interior of different plants and suppression of their immune systems, *Salmonella* needs to attach and adhere to plant surfaces. Additionally, *Salmonella* originating from plants maintains its virulence in animals. Hence, plants might be an alternative host for *Salmonella* and can play a role in its transmission towards animals. Nevertheless, the targets of effector proteins in plant cells are not well characterized. Therefore, the aim of our work was to investigate the impact of chosen effectors on the plant immune system, as well as their role in *Salmonella* survival inside the different plant hosts.

We found that *Salmonella* can survive until 14 days post inoculation inside the plant without a significant change in the plant phenotype. Our results give also rise to a hypothesis suggesting the presence of redundant effectors. *Salmonella* might use them to compensate the deficiency of particular (other) effectors. However, this hypothesis requires further experimental tests. In addition, in this work we developed tools, which will be useful to investigate further effectors.

Resistance and Pest Control I

Microsporidia against Spotted Wing Drosophila?
Antagonistic potential of underestimated pathogens in biological control

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Microsporidia are obligate intracellular parasites infecting many organisms up to vertebrates. Mostly, infection starts by peroral ingestion of spores from the environment followed by invasion of host gut tissue where replication occurs. Then, the pathogen spread monotrophic or polytrophic, often leading to systemic host infections. Microsporidia infections often occur chronically with low mortality rates, but fertility and fecundity mostly decrease significantly. Retarded developmental time and reduced fitness of host individuals are also recognized regularly. For biological control, these characteristics can be advantageous if a pest insect has rapid reproduction time and plentiful offspring or when control of the pest is complicated by preference of hardly accessible habitats.

The spotted wing drosophila (SWD, *Drosophila suzukii* MATSUMURA) shows additional problems for effective biological control as oviposition and development of larvae and pupae occur inside ripe fruits causing massive crop losses. Moreover, SWD is polyphageous and not all habitats, possible host fruits and overwintering strategies are known already. SWD samples obtained from USA showed microsporidian infections of unknown species, why we focused in introducing a microsporidia infection into our German breeding line of SWD.

Microsporidia were isolated from SWD and molecular markers based on SSU rDNA were amplified by PCR using universal primer pairs. Sanger sequencing of the PCR fragments suggested that the isolated microsporidium belonged to the genus *Tubulinosema*. With the molecular marker RpB1 (largest subunit of RNA polymerase II) a differentiation between closely related and genetically very similar species of one genus is possible, such as for *Nosema* and *Tubulinosema*. For further description of this species, tissue tropism and developmental stages are examined by light and electron microscopy. Infection experiments to evaluate median lethal concentration (LC50) as well as possible impacts on developmental times are ongoing.

Trichopria drosophilae - a potential candidate to control *Drosophila suzukii* in Germany?

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Ongoing research aims to identify native parasitoids of Drosophilidae in Germany and tries to assess their ability for natural control of the invasive fruit pest *Drosophila suzukii*. In the years 2015 and 2016, samplings of native parasitoids took place in North, South and Central Germany. We captured seven different local parasitoid species in Germany. For the first time, the wasp species *Trichopria drosophilae* PERKINS (Hymenoptera: Diapriidea) was recorded in Germany. It develops as endoparasitoid in *Drosophila* pupae and is currently under investigation in other regions of the world invaded by *D. suzukii*. The biology and ecology of this species in Germany is completely unknown. Therefore, we started to examine important biological attributes of two German strains to evaluate the suitability of *T. drosophilae* as potential biocontrol agent for pest management of *D. suzukii* in Germany. After confirmation that *T. drosophilae* is able to parasitize the target pest successfully, we explored the acceptance and suitability of different developmental stages of *D. suzukii* pupae as host. *T. drosophilae* females accepted all, except very old, pupae during their metamorphosis which lasted seven days at 23 °C. For an effective breeding regarding sex ratio, success of progeny development and size of parasitoids, two to four day old pupae were most suitable as host. In addition, lifetime fecundity, progeny production and sex ratio of the progeny during the life period of female parasitoid were investigated in the two German populations. Females accomplished their full egg-load with mature eggs within four days after hatching. Furthermore, there was a positive relationship between size of females and number of mature eggs. Females and males of both strains lived on average more than 30 days. The number of eggs produced over the whole lifespan was higher in the Central German population (85,5 ± 2,7 eggs/female) than in the South German population (80,7 ± 3,4 eggs/female). Compared to this the female offspring was slightly superior in South German population (61 %) than in Central German population (46 %). Progeny development usually lasted about three weeks in both strains.

The knowledge of these basic values of the reproductive biology of the German strains of *T. drosophilae* allow a first assessment of their potential for natural regulation of *D. suzukii* and also for potential use in mass-production and release programmes. Of course, many other questions on phenology, habitat and host location, host preference etc. need to be addressed and to be tested in further experiments, especially in the open field.

Hymenopterous parasitoids of codling moth: Performance of their ecosystem service in the matter of plant protection - an overview

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During the last years, codling moth (*cydia pomonella*), a key pest in apple, became resistant to several plant protection products. Therefore it is of particular importance to search for alternatives or additional plant protection strategies. Here we focus on the ecosystem service provided by hymenopterous parasitoids in pome fruit production. The aim is to develop strategies for practical and sustainable use of functional biodiversity. Therefore basic knowledge on abundance and diversity of hymenopterous parasitoids in different regions, their food web interactions and temporal occurrence as well as their basic biology is necessary.

For getting information about the actual status of biodiversity in apple orchards samples have been taken in three main apple growing regions which are located in North, Centre and South of Germany. In all regions arthropod sampling took place in plantations of integrated and organic production and additionally in orchards without any plant protection measure.

There was taken an analysis of occurrence of parasitoids and other insects during the growing season 2015, 2016 and 2017 using methods as sweeping net, beating sampling, collecting attacked apples, installing and again collecting corrugated cardboard. These data were used to detect diversity and abundance of hymenopterous parasitoids of all developmental stages of codling moth in all management systems and regions.

To monitor the natural hatch of Parasitoids, an 'outdoor insectarium' has been installed comprising eclectors which contained all collected corrugated cardboards of one growing season. These data will give us the opportunity to give advice to practitioners regarding a gentle plant protection management and to realize parasitoids' full potential.

Selected parasitoids which hatched during the season were used to establish rearings for further investigations on their biology, interaction and reaction on plant protection strategies.

Acknowledgement:

The project is supported (Mai.2015 - Mai.2018) by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the Federal Programme for Ecological Farming and Other Forms of Sustainable Agriculture.

Effect of temperature on growth and sporulation of the Brazilian strains *Beauveria bassiana* and *Metarhizium anisopliae* for control of the Bronze Bug *Thaumastocoris peregrinus*

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The bronze bug *Thaumastocoris peregrinus* Carpintero & Dellapé, 2006 (Hemiptera: Thaumastocoridae) is an eucalyptus pest of Australian origin, but it has causing damage to eucalyptus plantation in Brazil since 2008, year of introduction into the country.

The symptoms caused by this pest are leaf silvering, leaf bronzing and defoliation in susceptible Eucalyptus species or clones. The success for control of *T. peregrinus* involves the Classical biological control with an egg parasitoid *Cleruchoides noackae* Lin & Huber, 2007 (Hymenoptera: Mymaridae), introduced from Australia, and the inundative biological control using entomopathogenic fungi. Since, these microorganisms show advantages, because these are not closely related to the development of the insect, and can infect via contact.

Among the entomopathogenic fungi already reported infecting and causing mortality of the bronze bug, *Beauveria bassiana* and *Metarhizium anisopliae* present good and promising levels of control. And considering that the temperature is limiting factor for fungi development and sporulation, the purpose of this study was to determine of the optimal temperature condition for the radial growth and sporulation of the two brazilian's strains, *B. bassiana* and *M. anisopliae*, for following mass production, and aiming the field application. For this Petri dishes were set up with MPA medium and each strain was incubated at 15, 20, 25, 30 and 37° C.

Both strains showed a higher radial growth at the temperature of 25° C. Sporulation of *B. bassiana* at low temperatures (15, 20 and 25° C) was significantly different of the high temperatures, and for *M. anisopliae* the highest temperatures can be considerate the optimal. The isolates did not grow at 37° C.

Further results on mass production and formulation will be discussed.

Comparative efficacy of four entomopathogenic nematode isolates against the tomato leafminer *Tuta absoluta* in laboratory leaf bioassay

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The efficacy of four entomopathogenic nematode isolates against the tomato leafminer *Tuta absoluta* (Lepidoptera: Gelechiidae) were investigated in laboratory bioassays. The four nematode isolates were *Steinernema carpocapsae* (Weiser) [BA2 isolate], *S. abbasi* Elawad, Ahmad & Reid, *S. feltiae* (Filipjev), and *Steinernema* sp. [J7 isolate] and originate from different regions (Egypt, The Sultanate of Oman, Germany, and Germany, respectively). The nematodes face some challenges because of the special habitat of *T. absoluta* larvae which feed and develop inside galleries they made in leaves. These challenges for nematodes are to find the mines and to penetrate the mines and infect the host inside. Based on these particularities, a leaf bioassay was developed to evaluate the efficacy in a comparable manner.

The different nematode isolates were applied in different concentrations (15, 30, 60, 125, 250, 500, and 1000 IJs/ml) against the 4th instar larvae in tomato leaflets. The results were used to calculate median lethal concentrations causing 50, 90, and 95% larval mortality (LC50, LC90, and LC95) and their confidential limits for the four isolates. The values of LC50, LC90, and LC95 were 44, 306, and 592 IJs/ml for *S. carpocapsae* BA2, 87, 751, and 1565 IJs/ml for *S. abbasi,* 113, 1179, and 2621 IJs/ml for *S. feltiae*, and 103, 3599, and 12055 IJs/ml for *Steinernema* sp. J7, respectively. Based on these values *S. carpocapsae* BA2 was clearly the most virulent isolate in this study. As a next step, semi-field studies considering efficacy of this isolate under more natural conditions and also focusing on best formulation and application techniques are underway.

Small mammal behavior at rat bait boxes: Minimizing the risk to non-target species

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During a rodent infestation rodenticides are the method of choice to manage the problem. The effectiveness of anticoagulant rodenticides (ARs) is related to high toxicity and persistence in the organism, which can also pose an environmental risk. Therefore, their application is permitted only as long as adequate measures are used to minimize those risks. One of these measures is the application of bait in suitable bait boxes that exclude most bird and mammal species from consuming bait. However, non-target mammals and birds up to the size of rats that occur in the vicinity of bait boxes may access bait, which can lead to pri-mary exposure to ARs. One option to minimize this threat is to use a bait box design that limits access of small non-target species to bait.

In our project we pursued this idea and attached bait at a height of 280 mm in the bait box. We assumed that only rats can reach this bait because of their size and their ability to raise their body towards the bait. Most small non-target species should be excluded from reaching the bait and therefore primary exposure should be minimized.

We tested this system in semi-natural enclosures from May until July 2017. The target species was the Norway rat (*Rattus norvegicus*). We also tested access to bait by non-target species: the Wood mouse (*Apodemus sylvaticus*), which is a good climber and the Common vole (*Microtus arvalis*), which cannot climb well as both species can occur in the farm environment where bait is used. Eight individuals of each species held the enclosure for one week. Their behaviour at and in the bait box and their consumption of non-poisonous bait was monitored by video cameras.

Results indicated that only the common vole cannot reach bait. The Norway rat as well as the Wood mouse were able to eat it. While the target species raised its body to reach it the Wood mouse jumped at the bait. Furthermore, the results showed that 280 mm is reachable just by tall rats but not by smaller ones.

EcoOrchard: Boosting agro-biodiversity in European apple orchards

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Research institutes and universities of nine European countries are involved in the research project EcoOrchard to develop appropriate strategies to promote functional agro-biodiversity (FAB) in organic pome fruit production.

To figure out the current stand of FAB and to identify specific differences depending on the national context, a wide survey was conducted among advisors and farmers in 2015. This study revealed that a majority of farmers were using FAB at various levels and were interested in a ´monitoring-tool` to assess FAB in their orchards. Therefore four easy-to-use methods have been tested and modified from participative farmers in 2016 and 2017.

For sharing information on how to enhance functional biodiversity a web-based platform has been created in the transnational context: the EBIO-Network (http://ebionetwork.julius-kuehn.de).

It offers practical, comprehensive knowledge, e.g. technical leaflets, a voluntary stakeholder EU map, as well as a literature database on functional agro-biodiversity.

On the scientific part, synchronized field trials have been performed at different sites in seven countries in 2015, where flower strips were sown into the interrows of the orchards. Natural antagonists of pests like Syrphidae, Coccinelidae and parasitoids of codling moth are supposed to be promoted with these additional floral resources.

To monitor the prevailing pest pressure as well as the state of biodiversity, various monitoring methods were applied in a standardized scheme: visual control, beating sampling, corrugated cardboard bands, sentinel prey cards and assessment of fruit damage.

In order to optimize the plant composition of flower strips, additional studies on effects of flowering plants on main pests and beneficials are carried out. Their requirements for food resources, particularly nectar and pollen, have also been investigated in adjoining laboratory and field experiments at JKI Darmstadt, Institute for Biological Control.

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Resistance and Pest Control II

Marker saturation of the *Rph*$_{MBR1012}$ locus confering resistance against *Puccinia hordei* in barley using the 50K iSelect chip and Genotyping by Sequencing (GBS)

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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 facilitate enhanced identification of resistance genes. The resistance gene *Rph*$_{MBR1012}$ previously mapped on the short arm of barley chromosome 1H is effective against the highly virulent barley leaf rust (*Puccinia hordei*) isolate I-80. In order to isolate *Rph*$_{MBR1012}$ using a map-based-cloning approach, marker saturation of the target region and construction of a high resolution mapping population were undertaken in parallel based on the cross "MBR1012 (resistant) x Scarlett (susceptible)". 492 segmental homozygous recombinant inbred lines (RILs) derived from 4775 F_2-plants were identified by analyzing the population with two co-dominant flanking markers, separated by 8.0 cM. For marker saturation 35 SSRs and SNP markers from the genome zipper, and 9K iSelect chip converted to PCR-based markers, were used. Using this approach the target interval was shortened to 0.15 cM. Using data from the 50k iSelect chip and genotyping by sequencing 19 additional markers were identified and the target interval was further delimited to 0.095 cM. By blasting these markers to the reference sequence, the physical size of the interval was determined at 0.4 Mb. Five disease resistance like genes were identified in the target interval and are now re-sequenced.

Biological and molecular characterization of Baculoviruses breaking CpGV resistance

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Codling moth (CM, *Cydia pomonella* (Lepidoptera)) is a serious insect pest of apples, pears and walnut. Since many years, isolates of Cydia pomonella granulovirus (CpGV) are used as effective agents to control this pest. But in 2005, the first CM larvae, resistant to the commercial used virus CpGV-M (family Baculoviridae), were found and mean-while more than 40 orchards in Europe with CpGV resistance were identified.

Further studies showed that there are three types of CpGV resistance. Type I resistance is inherited in a dominant and Z-chromosome linked way and directed against CpGV-M, whereas the type II resistance is dominant and autosomally inherited. It is directed against CpGV-M and CpGV-S. A third type has a mixed Z-chromosomal and autosomal inheritence.

There are only few CpGV isolates, such as CpGV-E2, which are able to break the different forms of resistance. Therefore, it is important, to search for other resistance breaking Baculoviruses. Recently, a novel Alphabaculovirus CrpeSNPV, which was isolated from the litchi moth *Cryptophlebia peltastica*, had been shown to be effective against CM larvae.

To test the efficacy of CrpeSNPV against resistant CM strains, full range bioassays were performed and the LC_{50} value was calculated. In addition, time mortality response was recorded to compare the speed of killing between CrpeSNPV and other CpGV isolates.

Furthermore, experiments with the *C. pomonella* cell line Cp14R were conducted to obtain more information about the in vitro replication capacity of this new Alphabaculovirus.

A transcriptome-based approach for development of molecular markers for bacterial wilt resistance in perennial ryegrass (*Lolium perenne* L.)

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Bacterial wilt is a severe disease of major forage grasses such as perennial ryegrass (*Lolium perenne* L.), inducing considerable yield losses. The disease is caused by the gammaproteobacterium *Xanthomonas translucens pv. graminis* (*Xtg*) which occludes xylem vessels and causes wilting symptoms and necrosis of the leaves. The overall purpose of this study is directed to the development of selection markers for bacterial wilt resistance for directly use in plant breeding programs. Hence, a BC1 mapping population (n=286) segregating for bacterial wilt resistance was used for phenotyping of host susceptibility by (I) standardized artificial dip-inoculation with *Xtg* and visual observation (II) a DNA-based real-time PCR assay for *in planta* determination of bacterial proliferation. Thence, a next generation based massive analysis of cDNA ends (MACE) transcriptome profiling is currently performed by using infected and non-infected plants for (I) identification of closely linked markers (II) elucidating host defense responses.

Therefore bulks of 10 unambiguously defined resistant and susceptible phenotypes were sequenced for a differential analysis of transcriptional profiling. Various bioinformatical tools facilitate the detection of differential SNPs and transcripts that were exclusively expressed in the resistant bulk (ETRs). Selection of marker candidates is thereby highly focused on over-expressed ETRs. Bulks of resistant vs. susceptible genotypes were used for detection of polymorphic and informative markers. In a second approach transcriptomic data will be used for the characterization of disease resistance mechanism and for the potential prediction of the major QTL *LpXtg1* affecting resistance to bacterial wilt in *Lolium perenne* L..

Development of *Asparagus virus 1* resistant hybrids between *Asparagus officinalis* and its wild relative *A. amarus*

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A. officinalis (2n = 2x = 20) is cultivated worldwide and is an economically important species. Because of its perennial growth, *A. officinalis* is infected by several diseases. The virus *Asparagus virus 1* (AV-1) is the most common one and is transmitted by aphids (*Myzus persicae*) or mechanically. 70 to 100 % of the asparagus fields in Germany are infected. The effects of AV-1 are a reduction of yield and a decrease of the spear quality. AV-1 infection cannot be prevented with insecticides; breeding for AV-1 resistance is the best solution. Due to the restricted breeding, the genetic diversity is relatively limited and resistant cultivars aren't available.

In a resistance evaluation study 44 different *A. officinalis* cultivars and 34 different asparagus wild accessions were tested regarding their AV-1 resistance. All cultivars were susceptible to AV-1; however plants from 29 different wild accessions showed high resistance to AV-1.

The AV-1 resistant wild relative *A. amarus* is hexaploid (2n = 6x = 60). Its origin is the Mediterranean coast. Using interspecific hybridization AV-1 can be transferred into the genetic background of *A. officinalis*. Interspecific hybridization has a lot of advantages but some problems like pre- and post-zygotic barriers can occur. We used manual crosses and embryo rescue to overcome the crossing barrier.

In total 109 crosses were carried out between *A. officinalis* and *A. amarus*. All crosses using diploid *A. officinalis* were unsuccessful. Crossing with tetraploid *A. officinalis* resulted in four embryos which were rescued in vitro. During embryo rescue, the plants were partially cloned so that 252 plants were transferred into soil. For resistance evaluation all generated hybrid plants were infected with AV-1 using viruliferous aphids. Six weeks later, DAS-ELISA was carried out to analyze the infected hybrid plants. 22 hybrid plants from two different crossing occurrences showed high resistance to AV-1.

Backcrossing of the resistant F_1 plants with tetraploid *A. officinalis* was successful, 22 embryos were rescued. In the BC_1 generation 79 AV-1 resistant plants from 22 different crossing occurrences were identified. To establish the next backcrossing generation, crossings with diploid and tetraploid *A. officinalis* plants will be carried out. The hybridity of all progenies were analyzed using cytological and molecular methods.

Genetic diversity of new Chinese Cydia pomonella granulovirus isolates

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Cydia pomonella granulovirus (CpGV) is an efficient biological agent to control codling moth in pome fruit orchards. Different geographic CpGV isolates originating from Mexiko, Canada and elswhere have been commercialized since 1980s to control CpGV-susceptible and resistant codling moth populations. Five genome groups (termed as group A, B, C, D and E) representing different phylogenetic lineages, were proposed according to analyses of full genome sequences of different CpGV isolates. Isolates from these groups have differing biological activity against different types of CpGV resistance. In the light of resistance management, it is important to determine the genetic diversity of naturally occurring CpGV isolates.

Seven Chinese CpGV isolates (ZY, JQ, ALE, KS1, KS2, ZY2, WW) were completely sequenced by Illumina next generation sequencing (NGS), using a self-assembled workflow in the galaxy server of the Julius Kühn-Institut to assemble sequencing reads and determine single nucleotide polymorphisms (SNPs). Based on specific SNPs of genome A to E, SNP markers of each new isolate were classified into different groups which provide information to determine composition of each isolate. We found isolate ZY, KS1 and KS2 are composed of genome group A and E with 76.8% and 22.7%, 69.2% and 30.3%, 85.8% and 13.8% respectively. Isolate ALE and WW are nearly composed of genome group D (92.5%) and E (99%), respectively. Both JQ and ZY2 are comprised of genome group C with 82.6% and 18%, meanwhile JQ and ZY2 containing 17% genome group E and 18% genome group C, respectively. In addition, we found 54, 167, 93, 42, 45, 133 and 38 of non-genome group SNPs in the Chinese isolates. Combining the ratio of different genome group in isolate with its efficacy against different types of resistant codling moth, it point out the way how to optimize the components ratio of CpGV genome group.

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Environment, Nutrition and Morphology

Water use in wheat production in Iran: A comparative analysis of irrigated and rainfed production in Golestan Province

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Iran is a water-scarce country and agricultural use of water resources needs to be strategically planned to ensure a sustainable relationship between the economy, society and the environment. Trade-offs between higher yield outcomes, blue water application and agricultural input must therefore be studied and carefully weighted. This study investigates on regional agricultural water use in Golestan province using the water-driven crop modelling programme AquaCrop in combination with field data from 540 Iranian wheat producers.

In the cropping seasons 2011-2014 actual water productivities range between 0.802 kg/m³ for the producer population which cultivates under rainfed conditions (PG_{rf}; n=277) and 0.951kg/m³ for producers who reported use of irrigation (PG_{irri}; n=260). As a result of numerous simulations, stress rates of 55% (PG_{rf}) and 50% (PG_{irri}) were applied to align simulation results with actual yields for each producer. Based on this model calibration, the following scenarios were developed to simulate and compare different irrigation plans with assumed actual production conditions: (i) no irrigation (ii) supplemental irrigation (iii) full irrigation. Supplemental irrigation increased yields by 16% and 6% for PG_{rf} and PG_{irri}, respectively, full irrigation 19% and 8%. Yet, increased water applications decreased WP_{ET} by on average 4%. Results indicate that current limiting growth conditions other than water availability inhibit desired WP_{ET} increases. Only changing irrigation management does not lead to the necessary improvements in regional water use. The simulated partitioning of evaporation and transpiration throughout crop development indicates great potential to decrease the non-productive evaporation from the production process to increase WP_{ET}. In the region, maximum ratios of transpiration to total evaporation were simulated as high as 85% achieved under optimal production conditions under supplemental irrigation reducing the non-productive share of water use to the maximum. Minimum ratios for PG_{rf} and PG_{irri} are as low as 32% when simulating actual production conditions under a full irrigation schedule.

Under simulated optimal production conditions, water use could be optimized and water productivities were almost twice as high for given climatic and soil conditions in the region (PG_{rf}: 1.499kg/m³; PG_{irri}: 1.698kg/m³). With the same amount of water, 96% (PG_{rf}) and 98% (PG_{irri}) more output could be produced. To combine actual yield information with a crop modelling programme produced valuable site-specific data with the explanatory power to describe current and potential water use situations in the region.

Comparison of selenium biofortified green pea in calcareous chernozem and sandy soils

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Currently, it is estimated that the deficit of micronutrients in food affects several hundred million people worldwide. Selenium (Se) is a micronutrient that is usually ingested in lower amounts than the daily dose prescribed by the Food and Agriculture Organization. Insufficient intake of Se increases the risk of several diseases. Se enters the food chain through plants, and the Se concentration of plants varies according to available soil Se concentration, its bioavailability for uptake into plant roots (which depends heavily on redox equilibria in the soil, but also on several other factors) and species of plants. Another important factor to consider is that the window of Se intake from deficiency to toxicity is rather narrow. Green pea is a valued protein source for the nutritional quality of its seeds for animal feeds and human consumption, while its pods and shoots can be used as forage, too.

The greenhouse pot experiment was performed with calcareous chernozem and sandy soils. Se (as two forms of sodium selenite (Na_2SeO_3; active form: Se^{IV}) and sodium selenate (Na_2SeO_4; active form: Se^{VI}) in two concentrations 0 (control) and 30 mg kg^{-1}) was manually sprayed and supplemented to the soil as an aqueous solution. Green Peas (*Pisum sativum* L.) were sown in separate experiments with three replications and the bi-factorial trials were arranged in a randomized complete block design. At the third stage of growing (the third true leaf has unfolded at the third node), immature plants were removed so that eight intact and mature plants remained in every pot. Growing period lasted 50 days in May and June and plants were harvested at maturity. Morphological traits, relative chlorophyll content (SPAD level), chlorophyll fluorescence parameters, malondialdehyde content, peroxidase (POX) activity, total soluble protein content, and quantification of total Se of green pea were measured. Meanwhile, biotransformation of inorganic Se was evaluated using HPLC-ICP-MS for Se-species separation in the above ground parts of green pea grown in both soils.

Due to high doses Se toxicity, especially in Se^{VI} treatments, 30 mg kg^{-1} Se^{VI} samples didn't grow. Whereas compared to the control, 30 mg kg^{-1} Se^{IV} decreased the growth biomarkers. Also, SPAD level, chlorophyll fluorescence parameters, POX activity of leaves and total protein content showed significant decrease in both soils but this decrease in sandy soil's samples was more. Meanwhile, membrane lipid peroxidation increased but in sandy soils increased more. The total Se content in all of the green pea plant's organs increased with increasing Se^{IV} in both soils, whereas Se uptake in sandy soil's samples were less. The main selenocompound in all samples was selenomethionine. Increasing the Se supplementation lead to higher selenomethionine concentration. Although, these amounts were less in samples grown in sandy soil.

Direct drift during the application of biocidal products?

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Biocidal products are required to protect human or animal health and to protect natural or manufactured materials against harmful organisms. They are used for various purposes, which comprise the disinfection of drinking water, preservation of wood quality, control of rats and insects, among others. Beside the benefits of biocidal products adverse effects are to be expected as they can be harmful to the environment and to human and animal health.

An important component of the environmental exposure assessment is the estimation of the unwanted entry of biocidal products into adjacent environmental compartments as a result of the application, which is also referred as direct drift. An estimation of direct drift requires, however, detailed knowledge on the way of application of the biocidal products.

In Germany a large proportion of the approximately 30.000 biocidal products on the market are currently under review due to existing transitional regulations. For these assessments data about the exposure of non-target areas are needed. But still knowledge about the way in which biocidal products are applied is very limited. The aim of the project is to close existing gaps in knowledge about the application of biocidal products and to identify applications in which direct drift is to be expected.

Based on a comprehensive research an overview of all application areas in which biocidal products are applied by spraying, fogging, misting or comparable application forms was obtained. In the next step all these application areas with expected drift potential are to be evaluated in field experiments.

Investigating the presence of pesticide residues in royal jelly, worker jelly and honey bee larvae under semi-field conditions

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Residues of pesticides have been found in several bee products such as honey, beeswax and bee bread. However, little is known about the quantities of residues in the food of honey bee larvae (worker jelly), in the food of queen bees (royal jelly) and subsequently in the larvae themselves. Since larval food is processed by nursing bees and contains 10-20 % protein that derives mostly from pollen, a contamination seems possible.

The aim of this study was to investigate this route of exposure by evaluating and measuring the pesticide intake in the course of a worst case scenario by confining honey bee colonies in tunnels to restrict their flight area. Flowering phacelia as a highly bee attractive crop was applied with a mixture containing 240 g/L thiacloprid (BISCAYA®), 200 g/L boscalid and 200 g/L dimoxystrobin (Cantus Gold®). For exposure detection, samples of phacelia flowers, honey sacs and pollen loads were taken. Worker jelly was sampled directly from combs in the brood nest containing first and second instar larvae. In order to ensure the production of larvae with a same age, queens were confined in their own colony in an exclusion cage containing an empty comb. Royal jelly was sampled from queen-less colonies using rearing frames and queen-cells.

Samples will be analysed by Liquid Chromatography-Mass Spectrometry (LC-MS/MS) and the results will help to retrace possible exposure routes by taking into account the different chemical properties of the test chemicals. Furthermore, it will become apparent if the active ingredients transfer into the larval food of worker bees and queen bees or even the larvae themselves. If not, currently applied larval test systems would not address a relevant way of exposure.

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

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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 sepals and petals, with stamens and carpels present. Type 3 has sepaloid and carpeloid structures, but no petals and stamens.

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

The cause of the mutation is thought to be a category B MADS-box gene, most likely PISTILLATA (PI), as APETALA3 has several homologs in other Rosaceae members, thus making it more robust against impairment. MADS- and K-box containing genes from Rubus occidentalis (black raspberry) were defined by Hidden Markov model search. A relationship tree was produced through amino acid sequence homology. Although no homolog for PI was found initially, a BLAST search found a non-annotated sequence with high homology to *Arabidopsis thaliana* PI.

An expression study was conducted on type 1 and type 3 whole flower and whorl cDNA with primers created based on Rubus occidentalis PI homolog sequence. Fragments amplified only for type 1 samples, in whole flower and in whorl 2 (petal) and 3 (stamen) samples, which indicates a defect category B MADS-box gene in type 3 phenotypes.

A further PCR with type 1 and 3 genomic DNA as template showed no visible fragment size difference, making a transposon insertion implausible and pointing to a possible SNP, or a disturbance in the promoter region as cause.

Currently, there are type 1 and type 3 mother plants crossed with two raspberry cultivars for fruit size evaluation on the resulting populations.

In the future, sequencing will be conducted on type 1 and type 3 genomic DNA for the PI region to find the possible sequence difference. Additionally, a silencing of PI in type 1 raspberry is planned as proof that the affected gene is indeed PI. The development of markers suitable for marker assisted breeding following progress made in this project could be beneficial in breeding aimed at large fruited raspberry cultivars.

Nutritional value improvement in soybean

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Soybean (*Glycine max*) is one of the most important crops in the world. Soybean serves as a high quality source of protein for the human diet and feed for livestock, as well as an oil seed crop and as a source for biofuel. Soybean lectins are one of the main anti-nutritional factors. In rat feeding studies, raw lectins were shown to be resistant to digestive enzymes and bind to the small intestinal brush boarder, causing increased weight of the small intestine and pancreatic hypertrophy. The main lectin is known as soy bean agglutinin (SBA). Together with the INIA (International Agricultural Research Institute) in Uruguay, CRISPR/Cas9 shall be used to knock-out SBA. Another target are genes which are affecting the seed size of the plant to improve the soybean yield.

Conventional stable expression of the CRISPR/Cas9 system will be compared to a new DNA-free system of preassembled CRISPR/Cas9 guide-RNA complexes. Using the DNA-free system will avoid the production and possible integration of recombinant DNA and therefore the existence of transgenic plants as intermediates. For screening and thorough evaluation of off-target effects, soybean gene orthologues from *Arabidopsis thaliana* will be addressed in the same way by stable and DNA-free transformation. Off-target effects are genetic modifications which occur unintended and outside from the target site and therefore might have an impact on a potential risk assessment of plants produced by Genome Editing.

Phenotyping in Viticulture

Evaluation of an automated 3D based phenotyping pipeline for grapevine bunches to determine bunch architecture traits

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In viticulture *Botrytis cinerea* (*B. cinerea*) is responsible for bunch rot infestations and can cause severe damage during warm and wet periods close to harvest. As *Vitis vinifera* does not show an active defense response, classical resistance breeding is difficult and therefore grapevine breeding put focus on physical barriers *e.g.* berry characteristics like tight berry skin and bunch architecture.

Loose bunch architecture is associated with an increased resilience against *B. cinerea* which results in unfavorable conditions for its growth.

For breeding purposes and research high numbers of plants have to be evaluated during the season *e.g.* for the development of genetic markers for marker-assisted selection. Bunch architecture is a complex trait (bunch volume in relation to berry number, total berry volume). Phenotyping of all these architecture related parameters is very time consuming and labor intensive. The aim of the present study was the development and validation of an automated phenotyping pipeline in order to characterize the mentioned traits for bunch architecture with high-precision and high-throughput.

Therefore, the optical 3D sensor Artec® Spider was used to generate dense 3D point clouds. For an efficient analysis of the 3D sensor data, the software '3D-Bunch-Tool' with a user friendly graphical interface was developed to analyze acquired 3D data automatically. Finally, a list of all selected traits (Number of Berries, Berry Diameter, Berry Volume, Total Berry Volume, Convex Hull Volume, Bunch Width and Bunch Length) is exported.

The pipeline was applied to phenotype 75 bunches of four different varieties and a set of 150 F1 plants of a segregating population. For validation, the results were compared with ground truth data. High correlations and a considerably faster data acquisition compared to the reference data could be achieved.

Artec® Spider was further applied in the field for direct and non invasive phenotyping of grape bunch morphology parameters.

Grapevine berry wax: One trait supporting resilience to *Botrytis cinerea*

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The necrotrophic fungus *Botrytis cinerea* is the causal agent of grey mould and causes serious quality and yield losses in viticulture. Due to the high economic importance of this pathogen, the resilience to *B. cinerea* plays an important role in grapevine breeding. Though there are no genetic resistance mechanisms known, different physical properties of the berry skin and the berry wax layer are known to be responsible for differences in susceptibility to *B. cinerea*.

The epicuticular wax as the outer layer of berries forms in combination with the cuticle a hydrophobic surface of berries. Cultivars with a thick epicuticle wax layer are known to be widely resilient to *B. cinerea*. Among the amount of wax, the composition and the ultrastructure of the wax layer determine the resilience to *B. cinerea.*

Phenotyping of the wax layer for breeding purposes are time-consuming. Therefore a fast and reliable method is needed to screen a high number of plants. The objectives of this project are the development of a sensor-based method to (1) quantify the wax layer and (2) classify the quality of wax relating to susceptibility to *B. cinerea*.

A set of different cultivars has been used to determine differences between cultivars. Therefore, visual assessments in the field have been made, as well as an infection test with Botrytis spores under controlled conditions in the laboratory. Additionally, the effect of the wax layer has been tested by using one set of berries of each cultivar with a removed wax layer. The infection tests were observed for 14 days by visual assessments and RGB images were acquired. To reference the influence of the wax layer, extractions of the berry waxes were made for all of the tested cultivars, followed by an ongoing gas chromatographic analysis of the waxes. As the chemical analysis is complex and time-consuming it should eventually be replaced by a non-destructive method for further phenotyping of higher number of plants.

Visually collected phenotypic data of a segregating F1 progeny were used to do a quantitative trait locus (QTL) analysis of the trait “wax layer”. Due to the subjective character of the assessments, future screenings should be done by a reliable and time-saving method. To develop a sensor based method, datasets of the described analysis were recorded by two hyperspectral cameras which covered the reflection in the spectral range between 400 and 2500 nm. The further research aims at finding wavelengths to describe differences between the wax layers of different genotypes.

Detection of grapevine characteristics using hyperspectral sensors

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Grapevines (*Vitis vinifera*) are large perennial plants for which phenotyping is predominantly bound to the field. However, phenotyping is time consuming, subjective and often destructive. New sensor technologies can help solve this problem. They should be significantly faster, more objective and, above all, non-invasive, so that a feature (trait or subtrait) can be observed over an extended period of time. They provide a level of information, thus being appropriate for plant phenotyping.

As a new field phenotyping platform, the 'Phenoliner', was constructed, which acts as a moveable tunnel and allows the acquisition of phenotypic data in the field under standardized conditions. It is equipped with a geo-referenced (i) multicamera-system and (ii) hyperspectral sensor system.

The hyperspectral sensors detect the visible light as well as the near infrared and short wavelength infrared region (400 – 2500nm). They record the reflection of objects. Because of the characteristic features of the reflectance spectra it is possible to discriminate between water, soil, plants, etc. Furthermore, different soil types as well as plant varieties can be distinguished from each other. With plants they are mainly used for the detection of diseases and the determination of ripeness. In the case of vines, there are many possibilities for the application of these sensors.

Several hundred grapevine cultivars worldwide are in use. It can be assumed that they differ in their spectral data. The aim of this work is to record these differences as baseline for further experiments.

Therefore, reflection spectra of twelve nationally important grapevine cultivars were compared. The ground-based hyperspectral records were accompanied by airborne multispectral measurements performed by an unmanned aerial vehicle (UAV).

At first, only leaves were observed, but in the course of the growing season grapes were also recorded. At the same time, the development stage and the incidence of diseases were assessed. Content of chlorophyll, anthocyanins, sugars, acids, etc. were measured as references.

The results of this work serve as a basis for further experiments, which focus on the early detection of grapevine diseases.

Poster

Plant protection products in the mix - semi-field studies on effects on honey bees

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In a series of screening laboratory experiments on tank mixtures in a spray chamber, synergistic effects on the mortality of adult honey bees were observed for some combinations of pesticides when exposed by contact exposure.

While in the laboratory studies higher numbers of different combinations are screened, to understand the potential additive or synergistic mode of action for different substances and to identify products and combinations of concern, further studies are needed to assess the potential risks for bee colonies. Therefore, higher tier studies in a semi-field setup were conducted to assess effects of these mixtures to honey bees under more realistic conditions, thereby including the effects from both contact and oral exposure.

To assess the risk on honey bees in a worst case scenario, honey bee colonies were confined in tents with flowering phacelia as a highly bee attractive crop. Different field-realistic mixtures of PPPs were sprayed during bee flight. The influence on adult mortality, behaviour, flight activity, and colony development was investigated.

Our results showed that the combination of thiacloprid and an EBI-fungicide cause significant adverse effects on adult mortality, behaviour, and flight activity. These effects last from the day of application until two days after application.

Our results suggest that the application of this mixture poses a possible risk on the honey bees foraging on treated plants under semi-field conditions. Therefore, it is necessary to check whether the application of this mixture harms bees on colony level in a field realistic scenario, too.

Gene expression in midgut cells of type II resistant *Cydia pomonella* larvae exposed to resistance breaking/non-breaking Cydia pomonella granulovirus isolates

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The codling moth (CM, *Cydia pomonella* L.) is a major pest affecting worldwide pome fruit production. Besides chemical agents, biological agents like Cydia pomonella granulovirus (CpGV) are being used to effectively control CM and prevent production loss. Fruits can be sprayed with viral occlusion bodies (OBs), causing infections once eaten.

A type I termed resistance against the widely used Mexican isolate CpGV-M has been observed since 2005. Target of this resistance is a repetitive 24 bp insertion in the viral gene *pe38*. Therefore, new resistance breaking CpGV isolates were needed. Resistance breaking isolates include the English isolate CpGV-E2 and the Canadian isolate CpGV-S, both of which are being used to circumvent type I resistance.

A novel type of resistance was documented for a field derived laboratory strain of CM named CpR5M. This type II resistant CM strain is resistant to CpGV-M and CpGV-S but remains infectious for CpGV-E2. Interestingly, a recombinant CpGV-M (bacCpGVΔpe38$_M^{pe38S::GFP}$) with its *pe38* replaced by the *pe38* of CpGV-S breaks this type II resistance. The mechanism of this novel type of resistance is expected to be a midgut-related blockade, as CpR5M is not affected by oral ingestion of CpGV-S but shows high mortality when injected into the hemolymph.

To study the unknown mechanisms involved in type II resistance on transcription and replication levels, a series of infection experiments were conducted on CpR5M larvae with the baculovirus isolates CpGV-S, -M, -E2 (resistance breaking, positive control), as well as bacCpGVΔpe38$_M^{pe38S::GFP}$. Three days post infection (dpi) midguts of the infected larvae were isolated in order to perform RNA extractions from individual tissue samples. Afterwards, the quality, quantity and integrity of the extracted RNA are going to be determined via RNA Integrity Number (RIN).

Some of the RNA will be reverse transcribed and submitted to qPCRs to quantify the gene expression levels of selected early, late and very late expressed viral genes. The high quality RNA samples are intended for RNAseq with the goal of performing an extensive analysis of host cell transcriptomes to gain a better understanding of the type II resistance.

Genetic diversity of Ethiopian durum wheat landraces differing in drought stress tolerance

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Globally drought is a serious abiotic factor challenging wheat production and quality. In Ethiopia, wheat production is fully dependent on rainfall, which is unpredictable. Therefore, drought stress tolerance is an important breeding goal in Ethiopia.

In this respect, e*x-situ* conserved Ethiopian durum wheat landraces (*Triticum turgidum var. durum,* 2n = 4x = 28) were invistigated in field trials under contrasting moisture teatments (stress and non-stress), represented by two different locations, i.e. Dera for stress and Holeta for non-stress. Furthermore, landraces were tested in growth chambers under stress (20% maximum soil water capacity (SWC) starting at flowering) and control (70% SWC) treatment. Data were analysed using R. Coefficients of correlation were calculated between drought-tolerant criteria and drought indices, as well as grain yield at stressed (Ys) and non-stressed (Yns) variants.

Analysis of variance revealed significant ($p<0.001$) difference between landraces for grain yield in the two variants in field and growth chambers, indicating a huge genotypic variation. Yns was positively correlated with all other traits except days to grain filling (DGF). The correlation between Ys and traits like spike length (SL), days to heading (DH) and days to maturity (DM) showed negative values. Those traits that revealed a positive correlation with grain yield at both stressed and non-stressed conditions may contribute to select genotypes suited for both environments. PCA1 and 2 at Holeta and Dera accounted for 49% and 67% of the variation, respectively. There was a strong and negative correlation of Ys with the drought susceptibility index (DSI) and the tolerance index (TOL), indicating that landraces with higher values are susceptible to drought and are not recommended for respective growing areas.

The positive correlations of Ys and Yns with the stress tolerance index (STI), mean productivity and others, showed that these drought indices are good indicators for yield potential and drought tolerance. According to results from field experiments, genotypes DW072, DW184, DW097-1, Top-66, Werer, Megnagna, DW188, among others can be selected as promising landraces for drought prone areas.

Overall, drought significantly reduces grain yield and other traits. In the study panel, there is a huge genetic variation

for drought tolerance that could be exploited by detecting QTL for drought stress tolerance via genome wide association studies using the 90k iSelect Chip.

Development of a soil granule and a sprayable formulation of the entomopathogenic fungus *Metarhizium sp.* to control wireworms

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Within the BMEL funded project "Agri-Met" three institutes of the JKI and two industrial partners recently started to develop a biocontrol strategy for wireworms in potato.

The involved JKIs are the Institute for Application Techniques in Plant Protection, Brunswick, the Institute for Plant Protection in Field Crops and Grassland, Brunswick and the Institute for Biological Control, Darmstadt. The two industrial partners are ABITEP GmbH, Berlin and LEHNER Agrar GmbH, Westerstetten.

Wireworms are larvae stages of *Elateridae*. Some genera are carnivorous. But there are also those who feed on roots and seedlings. These species are a great threat to agriculture. They feed on potatoes, carrots, corn, asparagus, salad and much more.

Only some pesticides are registered under §53 of the regulation (EC) 1107/2009. Beside the use of these pesticides current combat strategies against wireworms are mechanical machining of the soil, a special crop rotation or the cultivation of special plants (legumes) before the actual sowing. These methods alone are not sufficient. In several studies, the entomopathogenic fungus of the genus *Metarhizium* has proven to be effective against wireworms. Therefore, entompathogenic fungi seem to be a promising alternative for chemical pesticides.

Our part at the Institute for Biological Control is to develop soil granules and sprayable formulations of the entomopathogenic fungus *Metarhizium sp.*.

First, it will be tested which strain of *Metarhizium sp.* is effective against the three common Agriotes species *A. lineatus, A. obscures* and *A. sputator*. For these strains liquid fermentation protocols will be developed to produce submerged spores and biomass. By means of fluid bed drying the fungal biomass will be formulated as granule.

First results of the bioassays and of this production and formulation strategy will be discussed.

Genomics-based exploitation of wheat genetic resources for resistance to leaf rust and stripe rust

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To meet the raising wheat demand worldwide wheat production needs to increase by about 60% until 2050. Yearly infections of leaf and stripe rust caused by *Puccinia triticina* and *Puccinia striiformis* result in significant yield losses. The cultivation of resistant varieties carrying effective resistance genes is an efficient and resource-saving solution 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.

Therefore, the overall goal of GeneBank2.0 is to analyze the wheat ex-situ collection of the IPK Gatersleben by applying an integrated concept including cutting-edge genomics, phenomics, biodiversity informatics, and precision (pre)breeding.

Within this concept reliable phenotyping is a prerequisite for mapping of resistances against fungal diseases.

It is intended to characterize the entire wheat collection plus accessions from the secondary gene pool, i.e. in total around 22,000 genotypes, within a project time of 9 years with the aim to identify novel resistances which are not present in wheat elite cultivars.

In a first step 9,700 winter wheat accessions will be phenotyped in field trials and in greenhouse experiments with regard to leaf rust and stripe rust tolerance. (i) In order to detect and quantify resistances against rusts high throughput techniques (robotic platform Macrobot) will be used for the analysis of detached leaf assays. (ii) Genotypes showing quantitative resistances will be further characterized using microscopical and molecular techniques. (iii) Phenotypic data will be used for mapping the resistances and (iv) available markers will be used in order to detect resistances which are present already in cultivars.

Rhizosphere microbiome as possible inducer of enhanced resistance in barley

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The rhizosphere microbial community is known to harbor a multitude of diverse microbes. Therefore the plant microbiome is also called the second genome of the plant. The microbiome of a plant consists of beneficial, neutral and pathogenic bacteria. Beneficial bacteria may have strong influences on plants via mutualistic associations. The beneficial microbes play an important role in plant growth and health and provide protection against plant pathogens.

One of these protective mechanisms is priming for enhanced resistance. Priming through rhizosphere microorganisms can be achieved by specific compounds released by the microorganisms e.g. *N*-acyl homoserine lactones (AHLs) or other compounds. Defense responses to biotic and abiotic stresses of primed plants are faster and stronger, if compared to unprimed (naïve) plants.

Plant diseases are responsible for about 20 % yield loss worldwide. A better understanding of priming mechanisms would lead to the ability to develop breeding strategies for more resistant crops. Unfortunately, until now the mechanisms of priming are mainly investigated in the model plant *Arabidopsis thaliana.* Therefore, it is our aim to investigate priming in monocotyledonous crop plants.

This project aims to examine the ability of the rhizosphere microbial community of barley (*Hordeum vulgare*) to enhance the resistance against the powdery mildew-causing fungus *Blumeria graminis* f.sp. *hordei*. Cultivation-dependent and cultivation-independent methods are used to gain insights into the structure of the bacterial community in the barley rhizosphere. They also allow answering the question whether soil-specific members of the community are enriched in the barley rhizosphere. Future studies using next generation sequencing techniques will allow the identification of rhizosphere bacterial community members with high abundance.

To investigate the potential of rhizosphere microbial communities to prime barley, a standardized, greenhouse-based experiment was designed. The rhizosphere bacterial community of two barley cultivars grown in different soils was extracted. Subsequently, barley seedlings grown in a substrate/sand mixture were drenched with the extracted microbial fraction. The bacterial community composition associated with the barley roots was analyzed by Denaturating Gradient Gel Electrophoresis of 16S rRNA gene amplicons from total community DNA. The primed state of the barley plant was determined by monitoring the infection with *B. graminis* and analyzed by expression pattern of defense-related genes. The potential ability of different rhizosphere microbial communities to induce priming is assumed to offer great potential for new plant breeding strategies and should be the long-term achievement of this project.

Anchoring the genetic map of near-isogenic introgression lines carrying wild emmer QTL-fragments for drought tolerance to the physical map of *Triticum diccocum*

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Until now wheat breeders had to rely on genetic maps due to the lack of the genome sequence. However, genetic and physical distances may differ significantly from each other, especially in centromeric and telomeric regions. This biased picture might lead to incorrect assumptions and estimations about the size and complexity of QTL-regions, the selection of suitable molecular markers and their suitability to transfer respective QTLs into near-isogenic lines (NILs). The rising availability of cereal genomic sequences and their annotations delivers a first standard that offers the possibility to dissolve this distorted picture and to get a better idea about the physical size of QTL-regions and the number of genes involved.

Suitable free open software packages that allow the comparison of genetic and physical data of NILs from their respective parents to our best knowledge doesn´t exist. We therefore developed a very simple Java-program that can be executed as a "*.jar*" file so that scientists without any programming-skills will be able to use it. Furthermore, the source code of the program will be published so that the code can easily be copied or implemented into other software packages.

We tested the algorithm on two promising NILs that carry QTL-regions for drought tolerance from wild emmer (*T. diccocoides*) on chromosome 2BS and 7AS. Both NILs suffer from linkage drag and were previously established with SSR markers that flank the regions of interest. Recently, these NILs, their respective recurrent parents and F_6 descendents of the original mapping population were genotyped using the 15k-iSelect Illumina chip to improve the resolution of the previously calculated genetic map. This data and the availability of the physical wild emmer genome finally allowed us to get detailed information on these introgressions. The analysis revealed, e.g. that the centromeric regions of the wild emmer chromosomes were transmitted into both NILs. The physical segments of wild emmer cover significantly more than half of the corresponding physical size of the chromosomes. This may partly explain the linkage drag in both NILs.

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Entomopathogenic fungi in apple orchards

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Entomopathogenic fungi (EPF) infect and kill insects. Some of those insects are major pests in agricultural ecosystems, such as apple orchards. Here, one of the most important pest insects worldwide is the Codling moth, *Cydia pomonella*. In this study we examine the natural occurrence of EPF in soils of apple orchards in Germany and their virulence against *C. pomonella*. The samples are collected in three of the main apple growing regions in Germany: “Altes Land” in the northern, Kraichgau in the central and Lake Constance in the southern region. Beside the regional distribution, the seasonal occurrence is a matter of particular interest.

To evaluate this, we take soil-samples in spring, summer and autumn and isolate EPF of the genera *Beauveria*, *Isaria* and *Metarhizium*. The previous results indicate, that there is no clear seasonal influence on the occurrence of EPF in apple orchards but a regional difference. *Metarhizium* is much more abundant in the south than in the other regions. *Beauveria* were isolated more often in the north and in the centre than in the south. *Isaria* is less abundant and occurs only in a few of the northern orchards.

Breeding research on Russian Dandelion (*Taraxacum koksaghyz*) as a rubber producing crop

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Russian Dandelion (*Taraxacum koksaghyz*, TKS) has the ability to produce and store high quality rubber in its roots. With the lack of alternative sources for natural rubber next to the Para rubber tree (*Hevea brasiliensis*), whose cultivation is problematic due to economical and ecological reasons, TKS has turned out as the most promising new resource crop for natural rubber demanding industries.

Since TKS shows high diversity and relatively weak growth, it is still considered a wild type. This work aims to gain insights into the genetic background of this plant and provide important information for breeding programs, in order to develop a new rubber producing crop.

As part of a network of different research institutions and private companies, the JKI Institute for Breeding Research is part of a value chain from breeding up to the finished product made of dandelion rubber. In close cooperation with a breeding partner, the comprehensive genetic variability of TKS shall be used for the development of new varieties with high level and quality of rubber.

On that account, different agronomic traits, such as the formation of a large, clear tap-root with high contents in rubber, early and uniform flowering time, improved tillering in the first year of cultivation, as well as different disease resistances have been defined as breeding objectives.

In order to support these objectives, the genetic diversity of available germplasm of TKS was analyzed and first genetic maps were constructed based on a mapping population segregating for rubber content. By the application of a Genotyping-by-Sequencing (GBS) approach and combination with other molecular marker sources, a relatively high-density genetic map of TKS could be drafted.

This genetic map represents the basis for mapping QTLs regarding rubber content and other traits. Consequently, field trials with the cloned mapping population have been initiated over three years at three different locations for the exact quantification of rubber contents. Furthermore, transcriptomic sequence data (RNAseq, MACE) will be used for annotation of rubber related genes and inclusion in the genetic map. Combined, this information shall be used for the development of selection markers in marker assisted breeding approaches.

Nematicidal effects of fungal metabolites on *Meloidogyne incognita*

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Plant-parasitic nematodes are the cause of annual monetary losses of more than 100 billion US$. In order to sustainably substitute toxicologically and environmentally harmful chemical nematicides, novel and recurrent secondary metabolites of fungal origin have currently attracted much attention.

In this study 12 fungal metabolites of various origin and composition have been tested *in vitro* on second-stage juveniles of the root-knot nematode *Meloidogyne incognita*. The metabolites were dissolved in water or 2% DMSO. Water and 2% DMSO were used as negative controls and the nematicide Nemathorin® (active ingredient fosthiazate) as positive control. After 24 hours of incubation, the percentage of inactive juveniles was recorded. Juveniles were then washed in tap water and exposed to fresh water for another 24 hours. Finally, juvenile activity was again determined. One-Way-ANOVA followed by Tukey post-hoc test was conducted to measure significant differences between various treatments before and after exposure to fresh water. The results of the experiment showed that *4-methyl-3-penten-1-ol* caused 96.6% mortality, followed by *linoleic acid* with 92.8% mortality. Those two compounds exhibited the strongest nematicidal action of all metabolites tested. Notably, they were significantly more effective than the positive control *fosthiazate* with 85.0% mortality. Among four samples extracted from *Arthrobotrys oligospora*, "*ME CMD with C. e.*", showed 36.5% mortality rate. The lipoaminopeptide leucinostatin from *Purpureocillium lilacinum* displayed up to 30.8% mortality on *M. incognita*.

According to our results, the fungal metabolite *4-methyl-3-penten-1-ol* is currently the most promising compound for biocontrol of the root-knot nematode *M. incognita*. Consequently, this secondary metabolite will be considered for further investigations.

Occurrence of the plant-parasitic nematode *Pratylenchus sp.* in cereal fields in Germany

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The root lesion nematode *Pratylenchus* belongs to the most damaging plant-parasitic nematodes worldwide. Root lesion nematodes are able to infest a wide range of different crops. They penetrate the roots of the host plant and feed of them, thereby destroying the root tissue. The infested plants suffer from a reduced uptake of nutrients and water and also from secondary infections by other pathogens, leading to reduced yields. In the past decade, increasing damage in German crop production has been recorded and this pest is regarded by farmers and advisors as a major threat for cereal production. However, precise data about *Pratylenchus* infestations in the fields are lacking. Reasons for the broad occurrence of *Pratylenchus* are seen in narrow crop rotations, early sowing dates, mild winters and the lack of nematicides. Breeding resistant cereal varieties could be a proper way to reduce the infestation rate of *Pratylenchus* in the field.

Based on promising results achieved with barley, the project NEMARES ("Importance of root lesion nematodes in German crop production and strategies to breed resistant varieties") aims to identify new resistant wheat genotypes. It is funded by the Federal Ministry of Education and Research and is a collaboration between the University of Kiel, Julius Kühn Institute, Federal Plant Variety Office, Leibniz Institute of Plant Genetics and Crop Plant Research and the breeding company Nordsaat. Within this consortium our part comprises a Germany-wide monitoring, field and greenhouse experiments of susceptible and resistant genotypes for *Pratylenchus* reproduction, host-plant interaction studies and the specific effect of the genotype on the plant microbiome.

Here, the first results of the monitoring are presented. To determine the occurrence of *Pratylenchus* and other plant-parasitic nematodes in German crop production, 122 soil samples from different geographical regions in Germany were investigated. Due to the patchy pattern of the nematode distribution, each sample consisted of 30 cores per hectare from the upper soil layer (0-30 cm), following a zigzag line through the field. The nematodes were extracted by the centrifugal flotation method. All plant-parasitic nematodes were identified to the genus level.

Pratylenchus was found in 99 % of the samples, followed by *Tylenchorhynchus* (96%). *Paratylenchus* was detected in 63% of the samples. The number of *Pratylenchus* ranged from 4 to 936 nematodes/100 ml soil with an average of 164 ± 180 nematodes/100 ml soil. Those results confirm that *Pratylenchus* is widely spread and can reach very high numbers which will damage the cereal crop. Therefore, there is a high interest by the farmers to control *Pratylenchus* such as by resistant varieties.

Using dropleg technique during flowering of oilseed rape to avoid pollinator exposure

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Spraying of insecticides and fungicides during the flowering stage of oilseed rape can be necessary to control insect pests (*Ceutorhynchus obstrictus* and *Dasineura brassicae*) and pathogens (*Sclerotinia sclerotiorum*). In contrast to conventional application technique dropleg nozzles are kept below the flowering canopy. So the exposure of pollinators to active ingredients is reduced and it was demonstrated that chemical residues in honey decreased significantly.

The JKI tested the efficacy of different insecticides applied with dropleg technique compared to conventional technique against insect pests in field trials conducted in the area of Braunschweig from 2014-2017. Furthermore the effects of the dropleg technique on parasitoids of oilseed rape pests were examined.

The crop was treated at full flowering (BBCH 65) with insecticides. After application until harvest adults and larvae of oilseed rape pests were collected using water trays at soil level of each plot. The emerging of new generation beetles was recorded by photoeclectors, closed at BBCH 78. At least two times during each season oilseed rape plants were cut and the pod damage by *D. brassicae* was examined.

The new technique showed similar efficacy compared to conventional spraying technique. The number of larvae of

D. brassicae dropping to the ground was reduced and effects on the pod infestation with pod midge were observed for about 3 weeks after application. An explanation for the effects of dropleg technique might be that insect pests also hide in the vegetation layer that shelters against unfavorable weather conditions. Nevertheless pest abundance, especially of cabbage seed weevil was not always sufficient to get clear results. In all years yields did not differ significantly regarding the use of conventional or dropleg application technique.

Dissection of pollen beetle larvae from 2015-2017 for eggs of *Tersilochus heterocerus* showed very high parasitism rates up to 90%. There are hints that parasitism rates were higher in the first days after application using dropleg technique compared to conventional spraying. Further studies and a closer look at other parasitoids will be necessary for the future.

Transfection of *Taraxacum koksaghyz* protoplasts with CRISPR/Cas9

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Russian Dandelion (*Taraxacum koksaghyz)* is an upcoming new crop. It is able to produce a cis-1,4-polyisoprene (natural rubber) which is equivalent to the material conventionally obtained from the rubber tree (*Hevea brasiliensis)*. The focus of the BMBF funded "EVITA" project is to introduce a herbicide resistance into *T. koksaghyz* for better weed management during cultivation.

Classical as well as new breeding technologies have been used in order to change the DNA sequence of the gene encoding for the acetohydroxyacid synthase (AHAS). The AHAS enzyme possesses an essential function in amino acid synthesis and is therefore a target for weed control in crops.

EMS mutagenesis of *T. koksaghyz* seeds did result in tolerant plants, though the plants did not carry tolerance conferring mutations in *AHAS1*.

For direct mutation of specific target sites in *AHAS1*, different CRISPR/Cas9 approaches have been set up. *Agrobacterium tumefaciens* mediated transformation of *T. koksaghyz* explants with plasmids encoding for the Cas9 enzyme as well as the single guide RNA (sgRNA) have been performed, but did not result in plants with favored mutations, so far. An *in vitro* cleavage assay to test the cleavage capability by using the specific sgRNAs and Cas9 was successful by showing induction of double strand breaks in *AHAS1*.

As *Agrobacterium* mediated transformations did not lead to transformants up to now, transfection of protoplasts was done. Regeneration of plants from protoplasts is difficult and was therefore not the primary goal of this approach. The objective is to proof the CRISPR/Cas9 system being able to work not only *in vitro* but also *in vivo*.

Therefore, two days after transfection of protoplasts with purified *Streptococcus pyogenes* Cas9 and sgRNA, protoplasts have been harvested and genomic DNA was isolated. Out of the DNA, *AHAS1* was amplified, cleaved *in vitro* (cleavage assay) and sequenced subsequently.
Sequencing results will show, if and to what extent the CRISPR/Cas9 system did perform *in vivo* in *T. koksaghyz* protoplasts.

Resistance and tolerance in different sugar beet genotypes against the beet cyst nematode *Heterodera schachtii*

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Integrated management of the beet cyst nematode *Heterodera schachtii* in sugar beet at the current state is mainly focusing on variety choice. Susceptible, resistant and tolerant (partial resistance) varieties are available to the farmers and used depending on the level of nematode infestation. Although increasingly cultivated, host-parasite interactions between *H. schachtii* and tolerant sugar beet genotypes have so far not been intensively investigated. Moreover the tolerance trait at the present stage is tested in field conditions but not in the greenhouse or in combination with a resistance test.

First part of the study focused to determine the penetration, development and reproduction of *H. schachtii* in a set of 12 sugar beet genotypes (4 susceptible, 3 resistant, 5 tolerant) and *Beta maritima* (*B. maritima* is the genetic source to partial resistance). Plants were cultivated in folded boxes in a climate chamber and harvested at 2, 5 and 7 weeks after inoculation with *H. schachtii* juveniles. Acid fuchsin staining followed by microscopic evaluation was used to determine the developmental stages of nematodes inside the roots. Results indicate that nematode penetration did only marginally differ between genotypes but female-male ratio was reduced in resistant and to lower extent in tolerant sugar beet genotypes and in *B. maritima*. Reproduction rate in tolerant genotypes and *B. maritima* was lower than in susceptible genotypes and almost zero in resistant genotypes.

Second part of the study was targeted to determine the degree of nematode resistance and tolerance at different inoculation levels under greenhouse conditions. Therefore, the same set of genotypes was cultivated in pots filled with 400 ml loess soil and exposed to five densities of nematode inoculum (0, 2,000, 8,000, 20,000, 35,000 juveniles/pot). Plant reaction to different inoculation levels was measured by repeated photographing of each plant followed by digital image analysis. Growth curves were derived on the basis of images taken at an interval of four days prior inoculation until the end of first nematode generation. Results indicate that plant growth was highly reduced in susceptible genotypes followed by tolerant and resistant with increased inoculum level. Plant growth in *B. maritima* was in between susceptible and tolerant genotypes. Shoot and root mass data support this observation. Reproduction rate was mainly determined by genotype but decreased with increasing inoculation level.

High resolution mapping of virus resistance genes derived from *Hordeum bulbosum*

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To prevent yield losses of barley due to viruses there are in general two approaches, i.e. control of vectors or breeding for resistance. The control of the aphid-transmitted *Barley yellow dwarf virus* (BYDV) is becoming difficult due to governmental regulations concerning insecticides. The use of chemicals to control the *Barley mild mosaic virus*/*Barley yellow mosaic virus* (BaMMV/BaYMV), transferred by soilborne *Polymyxa graminis*, is not possible. As there is no complete resistance in the primary gene pool of *H. vulgare* against BYDV and the resistance against BaMMV/BaYMV may be overcome, search for new sources of resistance in *H. bulbosum*, the only member in the secondary gene pool of barley, is of prime importance. The *Hordeum bulbosum* introgression line 203S11 carries resistance against BaMMV/BaYMV (*Rym16*) and Ryd_{203S11}^{Hb} for tolerance against BYDV located on chromosome 2HL. After backcross with the barley cultivar 'Emir' two DH lines carrying the shortest introgression containing either *Rym16* or Ryd_{203S11}^{Hb} for BYDV tolerance were identified and characterized using a set of 31 molecular markers. Blasting sequences of these markers allowed anchoring the introgression to the physical map of barley. A size of 5 Mb for the Ryd_{203S11}^{Hb} locus and 2.2 Mb for the *Rym16* locus were calculated. Up to now, 320 F_2 plants carrying the Ryd_{203S11}^{Hb} were genotyped and out of these four recombinant plants were detected by using co-dominant flanking markers developed with help of the 50K Illumina chip array. In a next step, 1200 F_2 plants carrying the *Rym16* locus will be screened for recombinations in the target interval. Based on such recombinant plants, respective intervals will be saturated by using markers derived from the 50K Illumina chip array, as a basis for isolating respective genes via a map based cloning approach. A non-gridded BAC library will be used to identify candidate genes located in the *H. bulbosum* introgression fragment. The authors thank the German Federal Ministry of Food and Agriculture (BMEL) for funding this project (FKZ 2818201515).

Double trouble! Tank mix of thiacloprid and EBI-fungicide - field study on effects on honey bees

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In a series of laboratory experiments on tank mixtures of plant protection products (PPPs), strong effects on the mortality of adult honey bees were observed when they were exposed to a mixture of prochloraz (ergosterol-biosynthesis-inhibiting (EBI) fungicide) and the neonicotinoidal insecticide thiacloprid. Our aim was to investigate, whether effects on mortality on single bees from laboratory studies will result in adverse effects on bee colonies in a field realistic scenario.

In the field study, the recommended application rate (tank mixture of 72 g thiacloprid/ha (BISCAYA®) and 675 g prochloraz/ha (MIRAGE 45 EC®) was applied on flowering phacelia during bee flight. Four honey bee colonies were placed next to each field with 2 control fields and 2 tank mix fields in a paired setup. The fields were located in two federal states (Lower Saxony and North Rhine Westphalia) in Germany. Several parameters like adult mortality, flight activity and colony development of three colonies were investigated. The forth colony was used for residue analysis in diferent matrices (forager bees, nectar, pollen, honey, bee bread).

Our results showed adverse effects on adult mortality and flight activity. At colony level, a slight reduction in the number of adults per colony compared to the control was found as well as a decrease of the brood area in the treatment groups in both setups. The results from the residue analysis are pending.

As EBI-fungicides are known to affect the mechanisms of detoxification in honey bees when combined with pyrethroid insecticides, this seems to apply for the combination with the neonicotinoidal insecticide thiacloprid, too. Therefore, the results suggest that this mixture poses a possible risk to foraging honey bees and colonies under field conditions when applied during bee flight when bees are actively foraging. Further studies on the magnitude of the effects including the risk on bumble bees and solitary bees will be conducted in 2018; furthermore, the effectiveness of further risk mitigation measures, such as restriction to application after daily bee flights, will be investigated.

DNA-free genome editing in potato

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DNA-free genome editing via CRISPR (clustered, regularly interspaced, short palindromic repeat) /Cas9 (CRISPR associated protein) is a new technique in agronomical important crop improvement. In this research an efficient genome editing method, via potato protoplast transfection using CRISPR/Cas9 ribonucleoproteins (RNPs) was established.

As a target gene for genome editing, the MSH2 gene was selected. The MSH2 gene is implicated in MMR (mismatch repairing system). The MMR system is highly conserved in Eukaryotes and it is involved in correction and reorganization of mispaired nucleotides to prevent homeologous recombination. For DNA free genome editing six gRNAs with predicted lower ``off target`` effect were designed.

To evaluate the efficiency of the gRNAs an in vitro cleavage assay was performed. With the efficient gRNAs the in vivo genome editing in protoplasts using purified Cas9 protein was performed.

The established protocol will be useful to breed resistant potato plants toward pest and diseases and to reduce pesticide consumption. Using pesticides have a negative effect on the environment and it is not cost efficient. The regenerated MMR deficient potato plants could be utilized in breeding programs to achieve resistant potato plants from wild relatives where the resistance genes are still yet not known and the conventional breeding is limited by sexual incompatibility.

The genome edited potato plants have a good prospect to be commercialized because they do not contain foreign DNA.

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

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Plasmopara viticola is the causative agent of grapevine downy mildew, a widespread severe disease. The heterothallic obligate biotrophic oomycete *P. viticola* was imported to Europe in 1878 from North America, 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 good 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 a sustainable viticulture. Only with precise knowledge of the resistance mechanisms and the genetic location a targeted breeding is possible to reduce the annual amount of consumed pesticides. 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 a resistance against *P. viticola*.

In the early 20th century the asiatic *Vitis amurensis* ‘Ruprecht‘ was crossed with *Vitis vinifera* ‘*Getsh*‘ (‘Michurinets‘). Other interesting cultivars are ‘Kunbarat‘ and ‘Kunleany‘. They posses resistance characteristics due to *Rpv12.* This locus was detected on Chromosome 14 and is inherited independently of other resistance genes. Within the locus *Rpv12* 13 CC-NB-LRR genes (coiled coil-nucleotide binding site – leucine rich repeats) have been identified within reference genome. An additive effect with *Rpv3* was detected. It 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 microscopy. The aim is to identify physiological responses of the cell. These results should reveal molecular mechanisms and the candidate genes involved, which shall be later evaluated by amplification, comparative sequencing and gene expression analysis.

Association mapping for resistance to Net Form of Net Blotch (*Pyrenophora teres* f. *teres*) and Spot Blotch (*Cochliobolus sativus*) in a diverse barley set

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Net Form of Net Blotch (NFNB) and Spot Blotch (SB) belong to the most important diseases of barley and are present in all barley-growing regions. Under optimal conditions both pathogens can cause high yield losses of 10% to 40% and also reduce grain quality. The most cost effective and environment-friendly way to prevent losses is growing resistant cultivars. In order to identify sources of resistance, more than 10,000 barley accessions were screened for resistance to NFNB and SB under greenhouse and field conditions. Out of these, 450 barley accessions expressing different levels of resistance were selected. The set comprises landraces and commercial cultivars from the centres of diversity.

Seedling resistance was assessed by conducting greenhouse experiments with two NFNB and SB isolates, respectively. For this, three week old plantlets were inoculated with a spore suspension of 5,000 spores/ mL and symptom assessment was carried out 14 dpi based on the rating scales by Tekauz (1985) and Fetch and Steffenson (1999). Additionally, field trials were conducted in Russia, Belarus and Germany. Disease severity was scored three times during the growing season to calculate the area under disease progress curve (AUDPC) and the average ordinate (AO). For both pathogens genotypic differences concerning resistance were observed.

Genotyping of the accessions was done with the Barley 9k iSelect chip. Marker data were filtered for a minor allele frequency (MAF) >5%, missing data <10% and heterozygosity <12.5% resulting in 5373 markers for conducting genome wide association studies (GWAS). On a reduced marker set of 508 markers kinship with a Modified Roger's Distance and population structure were calculated. The software STRUCTURE showed two sub-populations, i.e. two- and six-rowed genotypes. GWAS was carried out using the software GAPIT with a compressed mixed linear model (CMLM) including population structure and kinship. In order to get information on the reliability of the marker-trait associations, a cross validation with 1,000 runs was conducted. Detection rates of >20% were considered as reliable associations and detection rates of >50% were considered as particularly robust marker-trait associations. Regions associated with NFNB resistance were identified on all chromosomes. Regions associated with SB resistance were identified on chromosomes 1H, 2H, 3H, 4H, 5H, and 7H.

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Important technical components for a working gap detection system in orchards

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Gap detection in orchards seems to be a good possibility to improve the precision of the application of plant protection products on target area and to minimize potential inputs into the environment.

In the two projects LADUS and OLSVA, funded by the Federal Ministry of Food and Agriculture, three different sprayer prototypes were developed. These prototypes were equipped with different fans and different allocations between sensors and nozzles. For testing the novel application system under different climatic conditions and pathogenic potentials, the prototypes were distributed at different fruit growing regions in Germany.

The results of the field trials showed that the use of the gap detection systems resulted in high saving potentials of plant protection products depending on the age and the structure of an orchard. At the same time, drift reduction was possible, which is an advantage to protect the environment.

For achievement of market maturity of these sprayers, different technical components had to be tested for their suitability during the project period. First of all, the primary used infrared sensors showed inaccuracies in application due to higher driving speeds (> 6 km/h), which is out of step with common practice and could be one explanation for worse results of the biological scoring in the first project year. Therefore, novel infrared sensors were developed, that work with higher scanning frequencies and can improve the detection of an object.

Magnetic valves were other important components, of which the efficiency of the gap switching depends on. The problem was that the grower cannot apply plant protection products if the magnetic valves do not open the nozzles. Measurements in the laboratory showed that the coating of the valves was vulnerable to corrosion and resulted in sticking together of individual parts of the valves. Based on the knowledge gained, novel magnetic valves were developed with nano-coating as protection against corrosion.

The sensors as well as the magnetic valves highly influence the efficiency of the gap detection system. The results of year 2017 will show whether the developed gap detection system with the new technical components was successful.

Three plant protection agents against *Aculops lycopersici* on tomato

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In recent years, *Aculops lycopersici* (Tryon) (Acari: Eriophyoidea) has occurred more frequently in tomato cultivation throughout Germany. If infestation of tomato greenhouses occurs *A. lycopersici* can cause devastating damage. At present, there are no beneficials available that show satisfying results when used against *A. lycopersici* on tomato and there also are only few acaricides available against this mite. In order to investigate plant protection agents which potentially could be used against *A. lycopersici* in tomato, a greenhouse trial was conducted between May and August 2017. In this trial the acaricide "Vertimec Pro" (Abamectin, Syngenta), "PREV-AM" (orange oil, Oro Agri) and the entomopathogenic fungus *Beauveria bassiana* formulated as "Naturalis"(e-nema) were compared in their efficacy against *A. lycopersici* on tomato. After inoculation with *A. lycopersici* the population densities and the symptoms caused by *A. lycopersici* on tomato plants were monitored frequently throughout the whole experiment. This allowed assessment of the direct and lasting effects of all three plant protection agents. Abamectin showed good results, *B. bassiana* showed a slight reduction of symptoms and the orange oil showed no effect and performed similar to the water treatment.

This study is part of the SmartIPM project within the C-IPM initiative and is funded by the German Federal Office for Agriculture and Food (FK: 2816ERA01L)

PrimedPlant: Priming for increased disease resistance in *Hordeum vulgare*

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The primed state is a unique physiological state, induced in plants upon a priming stimulus. Subsequently, if compared to a naïve plant, a primed plant is able to respond faster and stronger to a challenging stress. Therefore, priming is an efficient strategy for the plant to protect itself against pathogens.

The knowledge of priming and induced resistance is currently based mainly on the model plant *Arabidopsis thaliana*. Our *PrimedPlant* project's aim is to expand the expertise on priming in barley (*Hordeum vulgare*), as one of the economically most important cereals.

Different stimuli are able to cause and promote the primed state of a plant: *e.g.* chemical compounds or beneficial microorganisms. Previous research showed that bacterial quorum sensing molecules, like the *N*-acyl homoserine lactone (AHL) oxo-C14-HSL, are natural inducers of the primed state. The Gram-negative bacterium *Ensifer meliloti* which is known as root-nodule symbiont in legumes produces oxo-C14-HSL.

Here we investigate the ability of barley to face the challenge against the powdery mildew causing fungus *Blumeria graminis* f. sp. *hordei* upon priming induced by oxo-C14-HSL, produced by *E. meliloti*.

In this setting the priming capacity of different spring barley cultivars is investigated. For this purpose, two reference cultivars (Golden Promise and Morex) and based on their genetic distance, five cultivars from the spring barley GENOBAR collection (BCC768, BCC1589, BCC1415, BCC436 and HOR7985) were selected.

The infection rate and priming capacity was assessed phenotypically, employing a detached leaf assay. The selected cultivars showed differences regarding their resistance to powdery mildew and responded differently to the priming stimulus. Additionally, in order to localize and analyze the plant defense reaction, the production of reactive oxygen species in the leaves was visualized *via* DAB staining. As a second approach, a luminometer-based assay was used to quantify the generation of reactive oxygen in leaves.

Furthermore, we plan to investigate the changes in gene expression upon priming and a triggering stimulus *via* MACE (Massive Analysis of cDNA Ends). This approach should result in detailed understanding of the priming process in barley. In addition, these data will be used to identify marker genes for priming in barley, to further analyze the cultivar-dependent impact of priming on the transcriptional level *via* qPCR.

In the future this information should be used to improve resistance of barley and other economically relevant cereals and to identify promising breeding targets.

Genetic and phenotypic diversity of the *Vitis vinifera* L. teinturier varieties

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The teinturier grapevines (also dyers) are characterized by a more or less intense red coloration of the complete habitus. The main feature regarding wine making, in contrast to classical red wine cultivars, is the colored pulp leading to a higher concentration of anthocyanins in the musts and therefore to wines much darker in color. Originally used to enhance the color of weaker red wines, some cultivars today are also used for wine making without blending.

The origin of these varieties lies probably in the area of the middle Loire (France), where they were already mentioned in the 17th century in the region around Orléans. Because of the lower wine quality of the ancestor `Teinturier´, Louis Boushet, a French grapevine breeder, already started 1824 with the breeding of new teinturier cultivars bearing improved viticultural traits. Based on the work of his son Henri, the teinturier variety with the worldwide highest acreage of approximately 19.398 hectares today is `Alicante Henri Boushet´. Despite the French breedings, German breeders started in the 20th century with crossings in Geisenheim, Weinsberg and Freiburg leading to the known teinturier varieties grown in Germany `Dakapo´, `Cabernet Mitos´ and `Dunkelfelder´, respectively.

The anthocyanin biosynthesis in colored varieties is controlled by two MYB-related transcription factor genes, *VvmybA1* and *VvmybA2*, located adjacent on chromosome 2 at around 14.2 Mb. In contrast, due to loss-of-function mutations in both *VvmybA1* and *VvmybA2*, white cultivars lack the ability to produce anthocyanins in the berry skin during ripening.

The most observed color mutations in grapevine are from white to red. However, the mutation from black to gray is also a common phenomenon giving rise to periclinal chimeras with two genetically different cell layers. Although the mutation leading to the teinturier phenotype is unknown, there are evidences for the involvement of an ectopic *VvmybA1* overexpression. Nevertheless, already French ampelographes from the last century described `Teinturier´ clones only differing in the intensity of the red coloration leading to the conclusion of existing clonal diversity among clones of the ancestor `Teinturier´.

This study focuses on the molecular background of the teinturier-specific mutation at the berry color locus and the influence on the phenotype based on clonal variation.

Screening of a wheat MAGIC population for resistance to stripe rust, leaf rust and Septoria leaf blotch

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Stripe rust (*Puccinia striiformis*), leaf rust (*Puccinia triticina*) and Septoria leaf blotch (*Zymoseptoria tritici)* are important fungal pathogens in wheat and cause yield and quality losses. In addition, the emergence of highly aggressive races with altered virulence patterns and the loss of effectiveness of fungicides increase the demand for wheat varieties with effective resistances. In this respect, the multi-parental Bavarian Magic Wheat (BMW-) population comprising 8 German elite wheat cultivars and being selfed six times was screened for resistance to these pathogens. In order to identify major resistance genes, a differential set of isolates consisting of different virulence/avirulence patterns for each of the three fungal pathogens has been used in detached leaf assays. Differences in the degree of resistance between the parental lines depending on the isolate used were observed.

To get additional information on quantitative resistances, field trials are performed at four different locations. At one of the locations genotypes are artificially inoculated with leaf rust, stripe rust and *Zymoseptoria tritici.* Respective genotypes were scored four times and the AUDPC, as well as the average ordinate (AO) were calculated. The 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 (0 % to 55 % in leaf rust trials). Based on phenotypic data and genotypic data available from the 20k iSelect chip, genome wide association studies (GWAS) will be conducted in order to identify major genes and quantitative trait loci (QTL).

AmphiMove: Moving patterns and microhabitat selection of European anurans in agricultural landscapes

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Researchers worldwide are concerned about the current amphibian decline on global and local scales. Application of plant protection products (PPP) are suspected to be a major reason for decreasing amphibian populations. More and more studies underline the severe effect of PPP for aquatic life-stages of amphibians. Data on risk by PPPs for terrestrial life-stages are rare but required to develop protection strategies towards amphibian entire life-stages.

A guidance document on risk assessment for birds and mammals published by EFSA (European Food Safety Authority) already exists, but is lacking for amphibians. Contemporary EFSA released the first version of a "scientific opinion" on the risk assessment of PPP for amphibians. It is emphasized that detailed ecological data of especially terrestrial amphibians is still under-represented.

The aim of AmphiMove is to fill the gap of data on terrestrial amphibians with focus on movement behavior and microhabitat selection of European anurans in agricultural landscapes. At two study sites individuals of common toads (*Bufo bufo*) and common frogs (*Rana temporaria*) were caught at and around their breeding ponds and tracked via radio-telemetry. Locations, biotic and abiotic parameters of the selected microhabitats were recorded daily. Here, we show preliminary results of the first data collecting period from March to September 2017.

Sustainable management of common voles (*Microtus arvalis*)

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The common vole (*Microtus arvalis*) is the most abundant mammal in European agrarian landscapes. Its population dynamics can vary spatially and seasonally. Every two to five years outbreaks can occur and population densities can reach 1000 individuals per hectare. Especially during these outbreaks, common voles migrate to farmland and damage crops like grain cereals or rapeseed with monetary damage amounting to several million Euros. Farmers often use rodenticides as management method of choice. Its efficacy can decrease if voles emigrate from their primary grassland habitats or if a better food source is available. Furthermore, there may be risks for non-target species and chemical rodenticides are no option for organic farmers. An ecologically based management including expert knowledge of the target species and combining different methods could help organic farmers to protect their crops.

In our BMEL funded project we pursue the idea of inhibiting migration of common voles to farmland by a barrier system. A furrow at the field margin is combined with repelling odorous substances or traps emptied by predators. If a vole wants to reach secondary farmland habitat, it comes across a furrow designed to lead the vole towards a trap. Avian and terrestrial predators learn to patrol these traps and to remove voles. We observed with camera traps if a vole was removed within 12 hours and which predators visited the traps. We could also confirm that rodents of the family *Muridae* were able to jump out of the trap avoiding non-target captures. But because of its opening the trap is quite light and voles often prefer darker surroundings. Increasing trappability can be accomplished by an attractive bait. In the laboratory, we screened natural substances, selected a suitable grain base and created three new bait types. Under semi-natural conditions we tested trappability in four populations with these new baits.

The second approach follows the idea of repellent natural substances placed in the furrow. In T-maze trials we screened different odors. Three essential oils significantly reduced residence time in the treated box of a T-maze. These oils were tested further in enclosures with eight vole populations. One combination of oils significantly reduced oat consumption and may have potential as common vole repellent.

We present our results of three years research in sustainable common vole management.

Development of an image-based phenotyping system for fast investigation of grapevine root architecture

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In recent years, objective, sensor-assisted and automated phenotyping techniques have been developed in order to evaluate above-ground plant traits, e.g. grape bunch architecture or growth characteristics. The investigation of root architecture related traits, like the total root length or amount of secondary roots, is important with regard to adventitious root formation, drought stress tolerance and other abiotic and biotic stress factors in viticulture. One major aim within the project 'MureViU' is the establishment of an image-based phenotyping system for fast acquisition of grapevine root architecture related traits from several hundreds of plants.

In order to elucidate the growing root system, rhizotrons were developed following the example of the automated rhizotron phenotyping platform established at Forschungszentrum Jülich GmbH.

Dormant wood cuttings were planted in rhizotrons filled with dark potting soil and set in a slanted position with the roots growing along a transparent site. Usage of rhizotrons allows the non-invasive observation of the root system with high throughput and over time. By means of this method, several root characteristics, e.g. root length, root branches and growth rate can be measured.

Therefore, following preliminary experiments have started this year: First, a cost-effective rhizotron prototype setup was developed and several genotypes including rootstock varieties were screened. Second, the same set of genotypes was phenotyped with the established system in Jülich. And third, determination of root biomass (total dry weight per plant) as ground truth.

Furthermore, and in the course of establishing the new rhizotron system, phenotypic data from a mapping population of V3125 (*Vitis vinifera* 'Schiava grossa' × 'Riesling') and rootstock cultivar Börner (*V. riparia* × *V. cinerea*) will be collected. After repetition of this experiment in the following years, the data will be finally used for QTL analysis in order to identify gene loci associated with root system characteristics within this mapping population.

Selection of a diverse set of wheat genotypes for conducting genome wide association studies for nematode resistance

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Root lesion nematodes (RLN) of the genus *Pratylenchus* are known as pathogens on many crop species. The economic damage in crop production by RLN throughout the world is well documented. Two species of *Pratylenchus* (*P. neglectus* and *P. penetrans*) are also gaining importance on wheat in Germany causing high yield losses. The best way to avoid damage of RLN and reduce yield losses is the use of resistant or tolerant varieties. The goal of this study is to get information on the variability concerning the reaction of wheat to *P. neglectus* and *P. penetrans* infection and to identify QTL involved in resistance using a genome wide association genetics approach (GWAS). In this respect, the creation of a genetically diverse panel of wheat genotypes is a prerequisite. To achieve this, two different grouping approaches based on similarity and dissimilarity, i.e. K-medoids and Principal coordinate analyses (PCoA) were used for selecting 300 out of 890 genotypes. Due to different platforms (15K and 90K) on which the 890 genotypes were analysed, 12,896 markers common in all sets were identified in a first step. Next, filtering was conducted for minor allele frequency (MAF) > 5%, maximum percentage of missing values <10%, and maximum percentage of heterozygous SNPs <12.5%. At the end, 10,979 markers were obtained, which were used for the analysis of genetic diversity, i.e. calculation of Rogers' distance (RD). Based on the RD result, the clustering of the 300 out of 890 genotypes was conducted using the K-medoids method. The result of the heatmap and PCoA confirmed that the 300 genotypes selected represent the maximum genetic diversity present in the 890 genotypes.

The set of 300 selected genotypes will be tested for nematode resistance and genome wide association studies will be conducted.

Exposure by nesting material? – Method development of a suitable design for higher tier studies with solitary bees

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The registration processes and risk assessment of plant protection products on bees resulted in an increasing need for experiments with non-apis pollinators to assess potential side effects of plant protection products on this relatively new group of test organisms. Recently, numerous studies have been performed but there is still a wide range of ongoing challenges. One of the challenges is the risk from insecticide exposure to solitary bees, especially at their larval stages, by contaminated nesting material (e.g. mud partitions – mason bees).

In 2017, an experiment was performed under modified field conditions with the horn-faced mason bee *Osmia cornuta* (Hymenoptera, Megachilidae) with 6 replicates per treatment group at two comparable locations in Southeast Lower Saxony, Northern Germany. The aim of the experiment was to develop a suitable test method for higher tier risk assessments with solitary wild bees exposed to treated nesting material. The potential effect of the insect growth regulator (IGR) Diflubenzuron to bees and their brood was examined. The IGR was applied at two concentrations (T1: 1ppm, T2: 5ppm) directly into the pollen mass and on the mud wall. The results in both treatments were compared to a water treated control (C).

The reproduction capacity and brood termination rate were observed in the study as endpoints. Furthermore, hatching success and flight activity were recorded as additional information at several occasions. Other observations and surrounding flowering plants in the nearby environment were also documented and considered.

The present results provide no evidence that the exposure by Diflubenzuron has an effect on the development during the larval stages of *Osmia cornuta*, neither in pollen mass nor in the nesting material. It remains to be seen whether the results can be confirmed at the end of our experiment in next spring, when further parameter like completion of development, phenotypical changes, hatching and fitness will be evaluated.

The developed method initially seems to be suitable but it is still prone to various sources of errors which have to be excluded by some modifications. For excluding those errors and uniting laboratory regulations and natural conditions of the field experiments, probably semi-field experiments might be more appropriate. Semi-field test designs would investigate the exposure of adult bees as well as their brood to field-realistic pesticide quantities and ensure a collecting only of contaminated pollen/nectar and nesting material.

The role of scale insects as vectors of grapevine viruses in German viticulture

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Grapevine leafroll is one of the most widespread and economically important virus diseases of grapevine worldwide. It is caused by different Grapevine leafroll-associated viruses (GLRaV). Typically, symptomatic leaves of vines show a progressing interveinal discoloration and frequently roll downwards. Infected vines have often lower sugar content and the yield can be severely reduced. Scale insects are vectors of these viruses. The following species are known vectors and present in German viticulture: *Phenacoccus aceris, Heliococcus bohemicus, Pulvinaria vitis* and *Parthenolecanium corni.*

Healthy vines can be infected through feeding by scale insects if the vectors had previously acquired GLRaV from the phloem of infected vines. Nymphs of the mentioned species are more efficient vectors than adult females because they are more mobile than adults. Scale insects have a great sexual dimorphism and adult males of the four species cannot suck from phloem and are no vectors.

The role of scale insects as vectors in German viticulture needs to be checked through epidemiologically analyses. Different vineyards are tested for infection by the most widespread scale insect transmissible viruses in Germany. These are Grapevine leafroll-associated virus 1 and -3 (GLRaV-1; -3) and Grapevine virus A (GVA). Additionally we investigate the occurrence of GLRaV-2. For this virus the vector and spread is unknown. The results of this survey will be used to reconsider the risk posed by scale insects as virus vectors to German viticulture.

In some winegrowing regions a high density of scale insects was found in vineyards. To investigate possible reasons for increasing scale insect populations, we aim to test the side effects of plant protection products on scale insects and their natural enemies. We use different fungicides, herbicides and insecticides in the lab. Since the native species Phenacoccus *aceris* is difficult to rear, the obscure mealybug *Pseudococcus viburni* is used as a model organism for these studies. Additionally a non target- organism (Parasitoid) will be checked as well. Products with direct or indirect side effects on our model organism *Pseudococcus viburni* will later be checked on the native scale insect *Phenacoccus aceris* in the same way, but with field sampled insects.

Identifying resistance/tolerance for *Wheat dwarf virus* (WDV) in barley

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Due to global warming, insect-transmitted viruses, like the leafhopper transmitted *Wheat dwarf virus* (WDV), will become more important in barley and other cereals in the future. Typical symptoms of virus infected barley plants are leaf yellowing and strong dwarfing, which in most cases result in high yield losses. Growing of resistant/tolerant cultivars is an environmentally friendly way to control WDV. 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).

In 2016/2017, a set of 260 barley accessions was tested by artificial inoculation using viruliferous leafhoppers of the species *Psammotettix alienus* in gauze house and greenhouse tests. Genotypic differences in the reaction to a WDV infection were observed. Most barley accessions turned out to be highly susceptible. However, 13 barley accession showed no or low infection rates (0 to 29 %) and/or a low virus titer. Furthermore, 20 accessions showed, despite a high virus titre, a high level of tolerance, i.e. good performance concerning yield/plant, TKW, plant height and/or number of ears/plant relative to the non-infected control variant. The promising barley accessions will be re-tested in 2017/2018 and in addition 240 accessions selected from different gene banks will be tested. On the basis of these phenotypic results, a subset of 250 resistant/tolerant and susceptible barley accessions will be selected and genotyped by the 50k iSelect chip (TraitGenetics). 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.

Targeted modifications of centromeric histone H3 (CENH3) by using CRISPR/Cas9 in carrots (*Daucus carota* L.)

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Plant breeding needs to evolve constantly to address the growing demands concerning not only the yield but also the biotic and abiotic behavior of crops. At the same time it is facing climate change and a decrease in arable land area. While a lot of studies about improving breeding strategies focus on major crops like cereal or maize we bring attention to accelerate the breeding of carrot (*Daucus carota* L.), a subculture with a high content of secondary metabolites which makes it a great addition to a colorful and wholesome diet.

The main breeding method of carrot is F_1 hybrid breeding. However, the production of genetically homogeneous parental lines through several subsequent steps of inbreeding takes up a lot of time and resources. The production of haploid parental lines by tissue culture techniques has been proven to be inefficient in plants of the family of *Apiaceae.* Interspecific hybridization is yet unknown to achieve haploids in *Daucus* species. We therefore propose to apply the RNA guided endonucleases (RGEN) technique CRISPR/Cas9 to modify the kinetochore specific centromeric histone H3 (CENH3) which is crucial for the proper segregation of chromosomes during cell division.

In eudicots CENH3 consists of a highly conserved C-terminal histone fold domain (HFD) and a N-terminal tail that varies between species in its length and sequence. Modifications and possible loss of function of CENH3 to provoke uniparental genome elimination during early embryogenesis has been proposed as a new plant breeding technique (NPBT) for haploid induction.

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.

The introduction of an expression cassette for CRISPR/Cas9 by agrobacterium-mediated plant transformation via *Rhizobium rhizogenes* resulted in a high number of transgenic hairy root lines. We therefore screened hairy root lines for mutations induced by the non homologous end joining (NHEJ) pathway in the target region prior to somatic embryogenesis to identify highly mutated lines. Changes in the accumulation of CENH3 in mutated lines were visualized by staining with a specific antibody in cytogenetic studies.

We found changes in the geno- and phenotype of CENH3 in transgenic hairy root lines and produced transgenic plants carrying a variety of mutations in the targeted region inside of the CENH3 sequence.

High-resolution and -density mapping of *Barley mild mosaic virus* (BaMMV) resistance gene *rym15*

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Barley is the second most important cereal crop in Europe. Besides others, *Barley mild mosaic virus* (BaMMV) which is transmitted by the soil-borne protist *Polymyxa graminis* has a serious impact on barley yield. Although a number of BaMMV resistance genes were already identified, 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* has been shown 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 double 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 high resolution mapping population of BaMMV resistance gene *rym15*, to (i) resolve the discrepancy between the two maps, (ii) narrow down the target region and saturate the map, (iii) with final aim to isolate *rym15*.

Two crosses derived from resistant barley cv. 'Chikurin Ibaraki 1' and susceptible cultivars 'Uschi' and 'Igri' comprising 2260 and 5671 F_2 grains, respectively, will be used for the construction of the high resolution mapping population of *rym15*. The reaction to BaMMV of homozygous recombinant plants concerning the target interval will be assessed by artificial inoculation and DAS-ELISA in green house tests. The Next-generation sequencing (NGS) techniques such as exome capture, and Genotyping by sequencing (GBS) based bulk segregant analysis (BSA) will be used for the detection of polymorphisms followed by the development of KASPar markers.

Until now, the resistance test of BaMMV in 365 F_2 plants originating from 'Chikurin Ibaraki 1' × 'Igri' cross showed segregation of 85 resistant : 280 susceptible. The segregation fitted a ratio 1:3 ($\chi 2=0.571$), suggesting the presence of one recessive resistance gene. We genotyped a set of 365 F_2 plants by using 6 SSR markers and 5 KASP markers developed based on the 50K Illumina array data. Genetic map was constructed and new robust co-dominant flanking markers were identified. Our plan is to screen about 5,000 F_2 plants with flanking markers, to develop a high density and resolution map of *rym15* gene in order to facilitate positional gene isolation.

Priming to enhance resistance to leaf rust in barley

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Leaf rust (*Puccinia hordei*) is one of the major diseases of barley (*Hordeum vulgare* L.) leading to yield losses up to 60% besides a reduction of malting quality. Resistance genes *Rph1-Rph24* are known in barley but most of these have been overcome meanwhile and the primary gene pool of barley is to some extent depleted for new resistance genes. Priming of barley may offer a new opportunity to enhance resistance to *P. hordei*.

Bacterial communities such as the soil bacteria *Ensifer meliloti* are known to prime resistance in plants. By quorum sensing N-acyl homoserine lacton (AHL) is produced, which leads to systemic signalling in plants. Up to now knowledge on this phenomenon, which has been observed in *Arabidopsis thaliana*, in barley is limited. The present study therefore aims at the detection of genotypic differences concerning priming capacity.

For this purpose a diverse set of 200 spring barley accessions is analysed in greenhouse pot experiments for priming efficiency regarding leaf rust resistance. The plants are treated with bacteria, i.e. repaired *E. meliloti* natural mutant *expR+ch* overexpressing AHL and transformed *E. meliloti* carrying the lactonase gene *attM* from *Agrobacterium tumefaciens* which inhibits AHL production and acts as a control. Plants are treated three times with a bacteria suspension and are infected with *P. hordei* strain I80, 16 days after sowing. 12 days after infection, scoring of fungal growth and infection type, as well as biomass production is conducted.

First results showed significant effects ($p<0.001$) of the bacterial treatment indicating a positive effect of priming on *P. hordei* resistance. Besides this genotypic differences concerning the effect of priming were observed. In a next step genome wide association studies will be conducted in order to identify genomic regions involved in priming efficiency and develop molecular markers suited to be used in future barley breeding.

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Genetic loci determining the flowering time phenotype in grapevine

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In contrast to genetic studies on resistance, studies addressing phenological traits of grapevine such as bud break, flowering and ripening are still in their infancy. The fact, that these traits are highly affected by environmental determinants like light intensity and temperature makes it difficult to investigate their genetic background and respective knowledge is still limited. Therefore, we are interested in detecting loci and underlying genes that are relevant for control of flowering time in grapevine.

Phenotyping of flowering time and subsequent QTL analysis were carried out for several years using a mapping population derived from a cross of the early-flowering breeding line GF.GA-47-42 and the late-flowering cultivar 'Villard Blanc' consisting of 151 F1 individuals. Results show QTLs for time of full bloom on seven chromosomes with a major QTL on chromosome 14. For the purpose of refinement and verification of the QTL regions the mapping population was expanded to about 1000 F1 individuals and first QTL analyses were conducted.

In a previous work putative flowering time candidate genes were identified by a global approach using sequence information of model organisms and the grapevine reference genome sequence, resulting in a list of about 400 candidate genes spread over the whole grapevine genome.

Yet for a detailed investigation the major QTL region on chromosome 14 was screened for candidate genes combining knowledge about the physical QTL position, known conserved domains and RNA-Seq data from time series covering inflorescence development. This strategy led to promising candidate genes with potential roles in the flowering time control network of grapevine, which needs to be verified in the future.

Additionally, time of bud break was phenotyped in the same mapping population to check whether early or late flowering time is dependent on the time of bud break. However, QTL analysis with data from three years detected a single locus on chromosome 7 that does not overlap with any QTL for flowering time. Next steps will be fine mapping of the QTL region on this chromosome and screening for candidate genes that control the time of bud break.

Morphology of *Paratylenchus projectus* and its host plant spectrum in widely cultivated crops of Germany

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Paratylenchus projectus did achieve little attention in the past due to their minor economically importance. However, the high numbers of *P. projectus* reported recently initiated further research on this species. *P. projectus* was reported as an ecto-parasite of pasture grasses, cereals, soybean etc., but little is known about its host status on other crops. In this study we described the morphological characteristics of *P. projectus* and its host spectrum among widely cultivated crops in Germany. This work will provide information on the different developmental stages within a suspension and its possible role in parasitizing process. Besides, the host spectrum test will improve our understanding on the damage potential of *P. projectus*.

P. projectus was originally isolated from a potato field in Neu-Eichenberg near Witzenhausen, Germany, and maintained on ryegrass (*Lolium annuum*) in the greenhouse. Nematodes were extracted by the modified Baermann technique. The nematode suspension was examined under the microscope distinguishing among second-stage (J2), third-stage (J3), and fourth-stage juveniles (J4) plus female. Twelve crops were selected for the host spectrum test: potato (*Solanum tuberosum* cv. Desiree); tomato (*Solanum lycopersicum* cv. Moneymaker); bell pepper (*Capsicum annuum* cv. Yolo wonder); wheat (*Triticum aestivum* cv. Ozon); maize (*Zea mays* cv. Ronaldino); celery (*Apium graveolens* cv. Balena); carrot (*Daucus carota* cv. Bolero); soybean (*Glycine max* cv. Custer); pea (*Pisum sativum* cv. Grandera); parsley (*Petroselinum crispum* cv. Halblange); *Beta maritima* and cucumber (*Cucumis sativus* cv. Centrido). Each crop had 10 replicates and each pot was inoculated with 100 *P. projectus*/100 ml soil. After 9 weeks, the experiment was terminated and number of *P. projectus* determined.

P. projectus is a small nematode with a long stylet. The vulva is located in the posterior, head conical, tail broadly conoid. The J2 and J3 of *P. projectus* differ from the female by their smaller body size and the absence of vulva. The J4 is strikingly different with other stages, i.e. the stylet is reduced, short and slender, and the valve of median bulb is much less refractive.

The host spectrum test showed that only soybean is a good host for *P. projectus* with a multiplication rate of 2.76. *Beta maritima* and cucumber also allowed reproduction of *P. projectus* with multiplication rate of 1.58 and 1.38, respectively. All other crops caused a reduction in *P. projectus* numbers, which makes them non-hosts for *P. projectus*.

AAA+ ATPase AP460 – A virulence factor of apple proliferation disease?

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´*Candidatus* Phytoplasma mali`, a wall-less phytopathogenic bacterium of the class *Mollicutes* and the agent of apple proliferation (AP) disease, causes specific symptoms (undersized fruits, witches'-brooms, rosetting, enlarged stipules) and nonspecific symptoms (foliar reddening, yellowing, stunting, decline) on apple plants. ´*Ca.* P. mali` colonizes phloem sieve elements inducing impairment of phloem function (e.g. callose deposition on sieve plates and phloem necrosis).

However, symptom expression of AP disease is highly variable, ranging from non-symptomatic to mildly, moderately and severely affected trees. Additionally, differences in disease development of infected trees (no symptoms at all, temporary or permanent recovery) were monitored in long-term observations. These variations in severity and variability of symptom expression are due to differences in the virulence of AP phytoplasma accessions (phytoplasma population in a tree). They are phenotypically classified into avirulent, mildly, moderately or severely virulent. Molecular analyses of AAA+ (ATPases Associated with various cellular Activities) protein genes from infected trees of different symptom expression revealed the existence of single and multiple strain accessions. Multiple infections are usually composed of differently virulent strains and shifts in the population may alter symptomatology. This research focuses on the membrane-associated AAA+ ATPase AP460. Since virulence-specific, as well as suppression-related clustering, was previously identified in the AP460 gene, there is indication that AP460 plays a significant role in phytoplasma pathogenicity. The current study examined 147 full-length deduced AP460 protein sequences of single- and multiple-strain accessions of different virulence. In sequence alignments two relevant regions were detected: region 1 in the N-terminal part possessed conserved substitutions associated with suppression of virulence. The more diverse region 2 in the C-terminus contained conserved substitutions, not only associated with two different groups of virulence but also with suppression of virulence. Three of these suppression-related substitutions, occuring next to key residues of ATPase motifs, seem to affect ATPase function. Membrane topology prediction programs (Phobius, PolyPhobius) indicate an extracellular C-tail orientation of AP460, essential for being a virulence factor. An immunohistochemical localization procedure will be employed for confirming the predicted orientation. A purified recombinant C-terminally 6xHis-tagged AP460-ΔTM protein (transmembrane do-main deleted) will be used as an antigen to generate polyclonal antisera.


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