Nachwuchswissenschaftlerforum
Young Scientists Meeting

Fünftes Nachwuchswissenschaftlerforum 2012

4. - 6. Dezember
in Quedlinburg

- Abstracts -

Berichte aus dem Julius Kühn-Institut

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Bundesforschungsinstitut für Kulturpflanzen

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Greetings from the President

Dear Young Scientists,

I cordially welcome you to this year´s Young Scientist`s Meeting of the Julius Kühn-Institute which is being held at its head quarters in Quedlinburg. Being the 5th Young Scientists Meeting, we may already claim that this meeting has become a successful JKI tradition. Being a higher federal authority and a federal research institution with a broad variety of research areas and scientific topics, the exchange of knowledge within the JKI and between the specialized institutes is a key for regularly defining our overall direction. So what comes more naturally to you who work for three years in a row with one specialized topic as to share your knowledge and skills and then step back from the puzzle piece you are working on to get the greater picture.

During the next three days you will process the scientific exchange by presenting and discussing your research results. You will also promote the scientific exchange between the different JKI research areas because you represent all the different institutes that exist within JKI.

Therefore I am convinced that all participants will personally benefit from the NWF. Firstly by expanding your knowledge, secondly by increasing your presentation skills. This is, as you all know, very important for your future professional life in science, development or industries.

This year, for the first time, all your contributions will be held in English. So it is a practical training for international conferences to come in your future.

In addition, you get the chance to meet young fellow scientists from all 15 JKI-Institutes, probably an important base for networking in your future professional life. During two joint evenings you will have time to get to know each other. So please get to know each other’s work and personality, start to weave networks and try to keep contact as long as your stay at the JKI lasts. We have a very copious and well-founded knowledge at the JKI. Make good use of this benefit. It is an easy way to improve your profession and by doing so you simultaneously improve the research quality of the JKI. It is tradition that the NWF is self-organized by you, the Young Scientists of JKI. Thus, you already contribute to our high research quality.

At this point, I wish to express my gratitude to the members of the organization team, who invested their precious time to organize this year’s “Young Scientists Meeting”, continuing the excellent tradition for the benefit of us all. I hope you all will enjoy your stay at Quedlinburg and return home enriched by the experience and impressions of this picturesque town in the very heart of our country.

Quedlinburg, December 2012

Dr. Georg F. Backhaus
President and Professor

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Section 1

Environmental impacts of agriculture I

Detection of antibiotics and antibiotic resistance genes in manures from different pig producing systems and in biogas plants

Wolters, B.[1], Hartung, S.[2], Widyasari, A.[2], Kreuzig, R.[2], Smalla, K.[1]

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Antibiotics are extensively used in animal livestock and are often excreted unchanged or as active metabolites by treated animals. These antibiotics lead to a fitness advantage of resistant bacteria present not only in the intestine of the animals, but also in soil to which the manure is applied as fertilizer. In turn the abundance of resistant bacteria and of corresponding antibiotic resistance genes (ARGs) in the microbial community is assumed to increase. Via mobile genetic elements these genes can spread even among phylogenetically distant bacteria and new resistant genotypes of pathogens may emerge.

The aim of our project is to monitor antibiotics and ARGs in manures in order to develop mitigation strategies and to minimize the risk of resistance transfer to pathogens.

To identify parameters influencing the degradation of antibiotics and the reduction of ARGs during storage of manure or its treatment in biogas-plants we sampled 16 different pig producing facilities and 9 biogas plants (fermenters are fed with pig manure) at different process steps. The samples were analyzed for the presence and content of antibiotics (via LC/MS/MS), ARGs and mobile genetic elements (via PCR, Southern blot and quantitative real time PCR).

Changes in the composition of the microbial communities in manure from different farms and during the fermentation process of biogas-plants were analyzed by using DGGE of 16S rRNA gene fragments amplified from total community DNA.

We were able to detect plasmids of different incompatibility groups (IncN, IncQ, IncP-1, IncW, IncU, Low GC), integrons containing gene cassettes of different sizes and several ARGs (*sul1*, *sul2*, *sul3*, *tetA*, *aadA1*) in many of the samples. Our data indicate that not only piggery manure but also the fermentation residues from biogas plants used for fertilization of field soils might contribute to spreading antibiotic resistance genes and mobile genetic elements.

Does field application of manure from sulfadiazine treated pigs affect the abundance and transferability of resistance genes?

Jechalke, S.[1], Kopmann, C.[1], Rosendahl, I.[2], Groeneweg, J.[3], Weichelt, V.[1], Krögerrecklenfort, E.[1], Brandes, N.[1], Nordwig, M.[1], Ding, G-C.[1], Siemens, J.[2], Heuer, H.[1], Smalla, K.[1]

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The agricultural use of manure containing antibiotics for fertilization is assumed to promote the formation and spreading of resistance genes by mobile genetic elements such as plasmids which poses a risk for human health, e.g. by increasing resistance problems in human antibiotic therapy.

The DFG research unit FOR566 aims at identifying key processes that control the fate and effects of veterinary medicines in soil. Sulfadiazine (SDZ), used as a model compound in this project and belonging to the class of sulfonamides, is among the most widely used veterinary antibiotics in the EU. It is excreted largely unchanged by the animals and enters agricultural soils through the use of manure and slurry as fertilizer.

In the field study presented here, the effect of manure from pigs treated with sulfadiazine (SDZ-manure) on abundance and transferability of sulfonamide resistance genes *sul1* and *sul2* in the rhizosphere of maize and grass were compared to the effects in bulk soil. A significantly higher abundance of both *sul* genes was detected in the plots after application of SDZ-manure compared to manure from untreated pigs. The abundance of *sul* genes relative to bacterial ribosomal genes was significantly lower in the rhizosphere than in bulk soil. However, only in SDZ-manure treated rhizosphere the *sul* gene abundance constantly deviated from control treatments with time after manuring, suggesting an ongoing antibiotic selection. Transferability of sulfonamide resistance was analysed by capturing resistance plasmids from soil communities into *E. coli*. Increased rates of plasmid capture were observed in samples from SDZ-manure treated bulk soil and rhizosphere of maize and grass. More than 97% of the captured plasmids belonged to the LowGC-type, giving further evidence for their important contribution to the environmental spread of antibiotic resistance.

In summary, SDZ-manure applied to the field significantly increased the abundance and transferability of antibiotic resistance. However, the effect in bulk soil and rhizosphere differed which needs to be considered in further risk assessments.

Population dynamics of bank voles and human Puumala virus infections in Germany

Reil, D.[1,3], Rosenfeld, U.M.[2], Imholt, C.[1], Schmidt, S.[2], Kratzmann, N.[2], Eccard, J.[3], Ulrich, R.G.[2], Jacob, J.[1]

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The rodent-associated Puumala virus (PUUV), a hantavirus species, is widely spread in Europe. It is part of a series of zoonotic human pathogens and is transmitted by forest-living bank voles (*Myodes glareolus*). Infections with PUUV can cause *Nephropathia epidemica* (NE), a mild form of the hemorrhagic fever with renal syndrome (HFRS). Its reservoir species, the bank vole, shows seasonal variations and multi-annual fluctuations in population size, which is partially related to climatic conditions. The number of human PUUV infections also fluctuates. Hence, years with high bank vole abundances might be related to a large number of notified PUUV infections in humans.

The aim of our study is to closely investigate the potential correlations between climate, bank vole population dynamics and PUUV infections in humans and to use these correlations for predictive models.

Since 2010, changes in population dynamics of forest rodents, especially bank voles, are monitored in several regions of Germany using Ugglan live traps. Each trapping site is sampled three times a year. Blood samples of each trapped individual are taken to serologically detect PUUV infections and to estimate the rate of PUUV seroprevalence in bank vole populations. We were able to show a positive correlation between high numbers of human PUUV infections and high population densities of PUUV infected bank voles. Furthermore, we are analysing data on climate and bank vole abundance from several states of Germany by performing CART (c̲lassification a̲nd r̲egression t̲ree)-analyses to identify climate parameters that are clearly linked to changes in the population density of bank voles. On the basis of predicted climate data (Climate Service Center - Hamburg) we aim to make predictions about the population development of bank voles and finally about the risk of infection for humans with PUUV.

This study is commissioned and funded by the Federal Environment Agency (UBA) within the Environment Research Plan of the German Federal Ministry for the Environment, Nature Conservation and Nuclear Safety (BMU) (grant number 3709 41 401) and the Robert Koch-Institute (grant number 1362/1-924).

Screening repellents for the management of rodent damage to subsurface drip irrigation systems

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Worldwide the use of subsurface drip irrigation systems (SDI) in agriculture increases to boost production in semi-arid and arid areas. Because of low water use these subsurface pipe systems ensure effective soil watering in the fields. However, there are increasingly reports from SDI users of problems with rodent damage. Rodents can cause extensive damage to the pipes and therefore be responsible for a considerable water loss and extensive repair. So far, it is not known why the animals gnaw on the pipes. It may be because they search for water resources, they grind their teeth on the pipes or the pipes simply block the animals' movements in subsurface tunnels. On the one hand we would like to find an answer to this question, on the other hand we aim to identify a substance that is repellent to the rodents. We will present a short overview of the project including preliminary results of choice experiments with common voles. Common voles are the major vertebrate pest species in agriculture in Europe and occur in dry regions of southern and western Europe. Choice trials were conducted with common voles singly held in standard cages. Voles were supplied with different secondary plant compounds and other active ingredients attached to granules that were mixed in a food matrix. First results indicate that some compounds efficiently repel voles from feeding treated material. This raises hope to identify a repellent that can be tested in subsequent enclosure and field trials with common voles and other relevant rodent species. An effective repellent applied to piping material or in the vicinity of pipes will help to minimize damage to SDI systems and consequently conserve water resources.

Section 2

Biological pest control

Natural products against bird damage

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In agriculture bird feeding of seeds and seedlings causes substantial harvest and income losses. Furthermore, there are unintentional intoxications of birds if they consume toxic baits, which have been intended to be used for managing other species. Therefore, in this project a repellent shall be developed for the treatment of seeds and baits to avoid bird feeding.

In conventional agriculture chemically synthesized methiocarb is available as an effective seed treatment as protection against bird damage. However methiocarb is toxic and its use is not allowed in organic farming. In this project a repellent based on toxicologically harmless substances extracted from plants shall be found. Substances of plants will be fractioned until their repellent compounds can be identified by HPLC.

Repellent effects will be tested by food choice experiments with corn seeds, which are treated with the plant substances and with untreated corn seeds with pigeons and pheasants in aviaries. The same method will be applied for seedlings. Best substances in the aviaries trials will be tested furthermore in field experiments.

At the end a biological repellent should be developed, which could be applied for management of bird feeding.

The project is founded by the Federal Ministry of Food, Agriculture and Consumer Protection decided by the German Bundestag.

Eco-friendly regulation of the box tree pyralid, especially with entomopathogenic nematodes

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The box tree pyralid *Cydalima perspectalis* (Walker 1859) is an invasive alien moth from East Asia which occurs in Central Europe since 2007. It is an insect pest on plants of the genus Buxus, causing serious damage. Because of the rapid spread in Germany and nearby countries like Switzerland, one part of this study was testing different ways of eco-friendly regulation with commercially available beneficials and biological control agents.

In addition to host acceptance and search performance tests with *Trichogramma* wasps in the laboratory, the susceptibility of *C. perspectalis* larvae to three entomopathogenic nematode (EPN) species was investigated by using a bioassay system. *Steinernema carpocapsae* was the most effective nematode which produced mortalities ranging from 80 to 100% at four concentrations (25, 50, 100, 200 EPN/larvae). Also *Steinernema feltiae* produced high mortalities, whereas the treatment with *Heterorhabditis bacteriophora* only low mortality rates brought forth. The investigation of the infectivity of various larval instars (2nd and 4th) by *S. carpocapsae* demonstrated a mortality of 100% at 50, 100 and 200 EPN/larvae for both larval instars. On the other hand entomopathogenic nematodes are not effective against pupae of *C. perspectalis*. The susceptibility of *C. perspectalis* larvae under natural conditions in the laboratory was carried out on box trees. After application of *S. carpocapsae* with a pressure sprayer could be shown a mortality of 95%, despite the formation of type-specific webs. One experiment was applied to determine the persistence of *S. carpocapsae* on the foliage of box trees. After the application in the field, treated branches where sampled and fed to *C. perspectalis* larvae, followed by incubation in the laboratory. Even after 16 h of exposure, the resulting rate of insect mortality was 95%. First field experiments on infested box hedges were carried out with *S. feltiae.* The experiments point out free-eating larvae generally can be infected and above all, larvae in winter cocoon are not susceptible to infection.

This study has shown that under laboratory conditions *S. carpocapsae* is a suitable biological control agent for *C. perspectalis,* but the experimental conditions in field such as the time of application and the application rates used to be optimized.

Evaluation of the efficacy of a diatomaceous earth (SilicoSec) against *Callosobruchus maculatus* F. (Coleoptera: Chrysomelidae) on three cowpea varieties

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In search for alternative methods to residual synthetic pesticides in protection of cowpea seeds *Vigna unguiculata* (L.) Walp. in storage, the incidence of three varieties of cowpea (CRSP, LORI and TN5 - 78) on the insecticidal effectiveness of SilicoSec (diatomaceous earth) and on an insecticide of reference (Malagrain: Malathion 5%) was evaluated against *Callosobruchus maculatus* Fab. (Coleoptera: Bruchidae) under laboratory conditions of (t ≈ 21.5 - 26.5°C; r.h. ≈ 71.0 - 84.5 %). The grains of the three varieties of cowpea were mixed with the diatomaceous earth at four rates (0.5, 1, 1.5 and 2 g/kg) and with Malagrain® with the recommended amount of 0.5 g/kg. The susceptibility of the varieties of cowpea against the attack of *C. maculatus*, the mortality of the adults, the production of progeny of *C. maculatus* and the damage on the grains treated with diatomaceous earth were evaluated. The mortality of the adult beetles was recorded after 1, 2, 4 and 6 days post-exposure. Variety LORI with an index of susceptibility of 7.87 were weakly susceptible to *C. maculatus*. SilicoSec caused a significant and increasing mortality in beetles according to the rates and periods of exposure. One day after exposure, Malagrain caused 100% of mortality in beetles. SilicoSec was most effective on the variety TN-78. The greatest rates of SilicoSec (2 g/kg) caused 100% of mortality for the three varieties after 4 days of exposure. The LC_{50} values at 4 days were of 0.20 g/kg for varieties TN5-78 and LORI and 0.54 g/kg for variety CRSP. As in the case of Malagrain, SilicoSec reduced the emergence of the progeny on average, the percentage of grain damaged and the mass losses for the three varieties of cowpea by more than 80%. These results suggest that varietal resistance and the use of diatomaceous earth against the devastating insects in storage of cowpea could represent an alternative for the integrated management against *C. maculatus*. SilicoSec could profitably replace Malagrain for the protection of the grains of cowpea against cowpea weevil attacks.

Section 3

Population genetics and resistance research

Analysis of *Plasmopara viticola* resistance locus *Rpv10* and comparison to *Rpv3*

Dudenhöffer, J.[1], Zyprian, E.[1], Töpfer, R.[1]

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The oomycete *Plasmopara viticola* is one of the most important pathogens of grapevine (*Vitis vinifera* L.). Especially all traditional European cultivars used for wine production are highly susceptible. As a result a great quantity of fungicides is necessary to enable a rewarding cultivation of grapes. For that reason newly bred resistant varieties are a major contribution to reduce the fungicidal applications in viticulture.

Many North American Vitis species possess resistance genes due to co-evolution with *Plasmopara viticola*. Beyond that Asian Vitis species also exhibit such resistances although their evolutionary origin is not understood yet. Among others, the two loci *Rpv3* (American origin) and *Rpv10* (Asian origin) have been identified.

In this study on the one hand we try to compare *Rpv3* and *Rpv10* carriers for example by performing leaf disc assays of *Plasmopara viticola* infections and comparative RNA sequence analysis to identify differentially expressed genes. On the other hand we will analyze the genomic DNA sequence of 'Solaris' (*Rpv10*) to find possible candidate genes which are responsible for the expression of resistance against *Plasmopara viticola*. Furthermore the project is aimed at developing new markers closely linked to *Rpv10* to improve the marker-assisted breeding of new resistant grapevine cultivars.

Among several other partners the Julius Kühn-Institute in Siebeldingen is involved in the project which is called “Bacchus” and is funded by the interregional program “Interreg IV Upper Rhine” of the objective “European territorial cooperation”. The grant is provided by the “European Regional Development Fund” (ERDF) allocated by the European Union (EU).

Pathogen defense in plants – transcriptional regulation of the promoter from *Vitis vinifera* PR10

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Erysiphe necator (synonym Uncinula necator [Schw.] Burr) causes Powdery Mildew of grapes and is one of the major pathogens in viticulture. It was introduced to Europe in the 19th century from North America. The European grape *Vitis vinifera ssp. vinifera* is highly susceptible to this ascomycete fungus. Since then it is unavoidable to spray the plants with high amounts of fungicides. These treatments can be reduced by breeding of resistant cultivars. It has become increasingly common to use molecular markers correlated with traits of interest, like pathogen resistance for efficient marker-assisted breeding. However, a deeper understanding of the knowledge about defense mechanisms in the plants would highly improve the development and efficiency of the usage of resistance-correlated markers.

To unravel the molecular mechanisms of plant defense, differential gene expression studies were carried out previously using a resistant and susceptible genotype, respectively, inoculated with *Erysiphe necator*. It could be shown that the candidate gene pathogenic-related protein 10 (PR10) is 50-fold up regulated in the resistant cultivar and only weakly in the susceptible (< 3-fold). In this work the promoter of PR10 was cloned from both a resistant and susceptible genotype to get an idea about transcription factors that regulate the expression of PR10. The fragments were sequenced, analysed for *cis*-regulatory motifs *in silico* and linked to a luciferase reporter gene. The constructs were then tested in a transient grapevine transformation assay in combination with different transcription factors which had also been shown to be differentially regulated in the resistant cultivar after inoculation with *Erysiphe necator*. In these studies the promoter of PR10 appears to be activated by WRKY33, ethylene-responsive transcription factor 5 (ERF5) and CZF1/ZFAR1.

Non-targeted analysis of the grapevine leaf metabolome

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Powdery mildew of grapevine (*Plasmopara viticola*) is a commonly occurring disease in the temperate climate of northern Europe. It infests leaves as well as grapes. Powdery mildew was brought to Europe in the 19th century when American grapevine species were imported to control the grapevine phylloxera in France. These species are partially tolerant to or even resistant against powdery mildew. In contrast, cultivars of the European grapevine *Vitis vinifera* are strongly affected by *P. viticola*. If untreated, it can cause severe decrease in harvest yield.

Thus, comparing the leaf metabolome of different *Vitis spp.* may give better understanding about the resistance mechanisms and may lead to new resistance biomarkers. The focus of the analysis is laid on detecting volatile organic compounds (VOCs), since interactions between plants, fungi, and animals often proceed via volatile signaling and / or defense compounds.

The determination of volatile patterns of grapevine leaves was carried out via a non-targeted analysis approach. The grapevine leaf VOCs were extracted by headspace-SPME and detected by quadrupol MS after gas chromatographic separation. The non-targeted approach ensures that all of the detected metabolites are included in the data processing. Non-targeted methods enable an unbiased processing of metabolic data and prevent inadvertent neglects of unknown or new compounds.

A total of 238 volatile compounds were detected in analyses of the metabolite profile of seven different *Vitis vinifera* varieties and interspecific hybrids at three sampling dates. More than half of these (137) are putatively annotated or identified by now. Interestingly, only a few metabolites like linalool or methyl salicylate occur in all analyzed probes with high concentration. However, most of the metabolites are specific for a certain developmental stage. They are either detected solely in one stage or show higher concentration in a certain developmental stage. For example, the concentration of beta-cyclocitral is high in the first sampling date and is decreased in the subsequent sampling dates.

Linkage Disequilibrium and Population Genetics in Spring Barley

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Analysing Linkage Disequilibrium (LD) and population structure is crucial for conducting genome wide association studies to lower the outcome of false positive marker-trait associations and to provide insight into the genomic constitution of the population analysed. Therefore, we genotyped 716 spring barley (*Hordeum vulgare*) lines containing high yielding accessions of actual breeding programs from two German breeding companies and a set of landraces from the fertile crescent, Africa and the Far East by iSelect SNP array technology enveloping 7800 markers. Results from LD analysis on the germplasm described above including an explanation of the methodology applied using a yet unpublished high density genetic map of the barley genome are presented. Furthermore detection of population structure has been performed by several approaches. A very common method is Bayesian clustering with the program *STRUCTURE*. There, the usage of linkage and allele frequency models shows enhanced estimation of population structure resulting in three clusters, whereas simple models can produce overestimated outputs up to 17 clusters. Besides multivariate analysis like principle component analysis and multidimensional scaling (MDS) reveals genetic diversity and is much faster, which is of special importance concerning the increasing amount of genetic information available. Two major findings from MDS are that on the one hand landraces clearly distinguish from breeders lines. On the other genetic diversity detected within the breeder's lines is much higher than within the landraces arising from the fact that the marker set was developed using breeders genotypes and therefore miss to detect diversity in wild relatives alleles.

High resolution mapping of the BaYMV/BaMMV resistance gene *rym*13

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Barley yellow mosaic virus (BaYMV) and *Barley mild mosaic virus* (BaMMV) transmitted by the soil-borne plasmodiophorid *Polymyxa graminis* cause severe yield losses up to 50% in barley (*Hordeum vulgare* L.).

Because chemical measures are neither effective nor ecologically sound, the only way of preventing these yield losses is breeding for resistance.

The aim of this project is, therefore to isolate the resistance gene *rym13* located on chromosome 4HL via a map based cloning approach. This gene originates from the Taiwanese cultivar ´Taihoku A´, which turned out to be resistant against BaMMV, BaYMV, BaYMV-2, BaMMV-Teik and BaMMV-Sil.

For fine mapping a high-resolution mapping population comprising 5,181 F_2-plants of the cross ´Taihoku A` x ´Plaisant` corresponding to a resolution of 0.0096 % recombination was constructed. F_2-plants were analyzed with flanking markers WMS06 and HVM67 and the target interval between these flanking markers turned out to be 12.84 cM. Plants carrying a heterozygous recombination event within this interval were selfed in order to identify homozygous recombinants. For this purpose twelve plants per F_2-plant were analysed in F_3 with respective markers.

Using this approach, a set of 475 homozygous segmental recombinant inbreed lines (RILs) has been obtained and was used for marker saturation and phenotyping. For this purpose, plants were mechanical inoculated with BaMMV–ASL 1 in the climatic chamber, and were sown in BaYMV/BaMMV infested fields. The virus titer was estimated by DAS-ELISA. In addition, marker saturation was conducted in a first step by mapping flanking markers in published high density maps of barley. Using this approach the marker interval carrying *rym*13 was shortened to 1.42 % recombination. Further marker saturation will be conducted by exploiting the barley/rice/ Brachypodium/sorghum syteny and using NGS-data available in barley.

Section 4

Plant biology and development

Apple and strawberry MADS-box genes and their function in plant developmental pathways

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MADS-box transcription factors play a crucial role in several plant developmental processes and have been studied in details in the model plant *Arabidopsis thaliana*. However, little is known about their function in crops of important agricultural and commercial value. Our study aims to investigate their role in two representative Rosaceae crops: apple and strawberry. A phylogenetic analysis of apple MADS-box genes indicated the presence of a subclade containing genes involved in important developmental pathways such as flowering induction and dormancy. We investigated the function of these genes in the sequenced apple cultivar 'Golden Delicious'. The open reading frames (ORFs) of these genes were validated experimentally from cDNA libraries of different apple tissues. Expression studies using qPCR seems to indicate that the apple MADS-box genes analyzed are not involved in flower development but are more likely involved in response to winter chill. Real Time gene expression and epigenetic studies of dormant buds collected during the chilling period suggested that the activity of the genes is regulated by long exposure to cold. A potential candidate gene for manipulating the switch to flowering in apple crops was identified.

We also performed a phylogenetic analysis of MADS-box genes in another Rosaceae spp, *Fragaria vesca* (wild strawberry) and selected three MADS-box genes to perform gene expression analyses. These genes were knocked down using RNA interference approach and some regenerants were analyzed at the molecular level. Further information about gene function will be available after the phenotypic analysis of the transgenic lines.

Progress in molecular characterization of members of the apple lipoxygenase (LOX) gene family involved in volatile metabolism

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Lipoxygenases (LOXs) are nonheme iron-containing enzymes that catalyze the dioxygenation of fatty acids and are ubiquitous among eucaryotes. LOX catalyzes the addition of molecular oxygen to polyunsaturated fatty acids to produce respective hydroperoxides and have many different putative physiological functions in higher plants. Plant LOXs have been proposed to form biologically active compounds both during normal developmental stages such as germination or growth as well as during responses to environmental stress such as wounding or pathogen attack. LOXs also play a decisive role in the production of volatiles that influence the flavour and aroma of fruits and vegetables.

By a bioinformatic LOX gene mining about 40 different apple sequences were identified as putative members of the LOX family. After a second round of screening for putative functional genes, a phylogenetic tree of the LOX gene family in *Malus*, including totally 23 genes, was calculated. Two sub-trees were found which differentiate the LOX sequences according to their positional specificity of linoleic acid oxygenation (9-LOX or 13-LOX). The positional information of these sequences published in the frame of the '*Malus* x *domestica* Whole Genome Assembly and Annotation' project was used to create a map of the LOX genes with their positions on the apple chromosomes. Some LOX genes are located on apple chromosomes that show strong collinearity, as for instance the chromosomes 2 and 7 or 4 and 12, respectively. It is suggested by several researchers that the apple genome was duplicated during a genome-wide duplication event. Therefore, some of the LOX genes located on these chromosomes might be of the same evolutionary origin.

Cloning of full-length LOX genes was performed by a PCR-based strategy. Based on the sequences of 16 LOX genes, gene-specific PCR primers have been developed and used for semi-quantitative RT-PCR to determine LOX gene expression patterns in apple tissues during fruit ripening and pathogen attack. A variety of SNPs was detected by direct sequencing of PCR fragments amplified with the gene-specific primers in apple cultivars. Presently, SNPs of some candidate genes are analyzed by the TASSEL software package for their association with quantitatively and qualitatively assessed apple volatiles putatively involved in fruit aroma.

SPO11 dependent initiation of meiotic double strand breaks in *Arabidopsis thaliana*

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Meiosis plays a key role in sexual reproduction in plants as well as in animals and fungi, by dividing the chromosome set in half and forming gametes. One of the major steps in meiosis is the prophase, during this stage meiotic recombination occurs leading to the physical connection of homologous chromosomes and exchange of genetic material between them.

This process eventually leads to faithful segregation of the allelic chromosomes and depends in nearly all analyzed eukaryotic organisms on the initiation of double strand breaks (DSBs). SPO11, a meiosis specific transesterase, is one of the main enzymes introducing DSBs during prophase. Whereas in animals and fungi only a single SPO11 for the initiation of DSBs is present, plants have at least two meiotic active SPO11 proteins (SPO11-1 and SPO11-2). Both are essential for the initiation of meiotic DSBs in *Arabidopsis thaliana* (Ath). Single knockout mutants of Ath_SPO11-1 as well as Ath_SPO11-2 are nearly sterile because random chromosome segregation during meiosis occurs.

In all so far sequenced plants genes homologous to Arabidopsis SPO11-1 and -2 exist. Our aim is to investigate whether the function of these two SPO11 proteins is species and/or sequence specific. To analyze this we use orthologous genes from different land plants (*Carica papaya*, *Oryza sativa* and *Physcomitrella patens*) as well as cDNAs from lesser related organisms such as *Chlamydomonas reinhardtii* and *Mus musculus* for heterologous complementation of SPO11 mutants from Arabidopsis.

We will also swap gene regions between the two SPO11 in Arabidopsis to figure out by homologous complementation if the function is sequence specific.

With this information we should be able to answer the questions if there are sequence and/or species specific functions of each SPO11 and which region(s) of the proteins are essential for the initiation of meiotic double strand breaks.

Section 5

Environmental impacts of agriculture II

Transport of pesticides in branched rivernetworks of small waterstreams

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In Germany the dominant surface water types near agricultural fields are small streams and ditches. These water bodies are especially vulnerable to pesticides.

In this work we present a prediction model based on transport equations for the pesticide exposure in networks of small surface water bodies.

For the computational analysis we established a discrete and topographic node network with an resolution of 25 meter per segment. The modelled nodes are derived from the natural river flow via spatial data bases. Each node has a degree of ingoing and outgoing arcs which route the incoming and outgoing water stream from and to the next node.

Further characteristics for river sections like width and depth of a stream and average flow velocity are calculated and can be configured in the network data model.

Every single node can be assigned with load information which consists of loading time, the specific substance and the loaded mass. The initial load information is assessed by a probabilistic approach for drift and a run-off model which was linked to a Geospatial Information System (GIS) evaluation on entrance points and run-off pathways from critical source areas. The load is routed through the modelled network in dependence of the average flow velocity.

For each segment in the network the concentration level will decrease and the length of the pesticide wave in flow direction will increase. The resulting concentration of a substance is summarized over all passing concentration levels.

Based on such results it is possible to analyse and compare different scenarios of risk management.

Rodenticide residues in non-target small mammal species and their occurrence in owl pellets

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Worldwide, the biocidal use of anticoagulant rodenticides (AR) is common, caused by hygienic as well as stored product protection aspects. Beside target rodents like Norway rats and house mice, non-target rodents and other small mammals are able to consume bait (primary poisoning). Secondary poisoning happens when raptors or scavengers consume small mammals containing AR residues. Data on AR residues in non-target species are missing in Germany. The aim of our study is to screen for residues in non-target small mammal species.

Because of higher abundance of rats and house mice on farms in cold months, we designed field experiments in autumn and late winter. Before, three days after start and after a systematic rodent control campaign we trapped rodents on transects beginning at bait boxes and ending about 100 meter away from the farm. Brodifacoum rolled oats bait was used. To detect the distribution of poison liver samples of caught mammals were analyzed for all eight registered anticoagulant rodenticides using a high performance liquid chromatography (HPLC) method.

Pellets from barn owls (*Tyto alba*) were collected from nest and resting sites on the same farms to investigate owl food composition, which enables calculating the potential risk of secondary poisoning in combination with known residues in prey species. Pellet samples were taken monthly throughout the year.

First results show that brodifacoum residues mainly occurred in the direct surrounding of a farm. In this area *Apodemus*-species more frequently contained residues than *Microtus* species. The pattern of residues in bank voles varied between autumn and winter session. Unexpectedly we found, that even shrews showed partly high concentrations of brodifacoum, documenting them to consume quite significant amounts of bait. Voles were always preyed upon most frequently by barn owls. *Apodemus*-species were found in pellets from all investigated farms as well, and in every season. More results of further experiments on these farms are needed to calculate the risk of owls through secondary poisoning.

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Effects of thermical weed control methods in apple orchards on population dynamics of the common vole (*Microtus arvalis*)

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The common vole (*Microtus arvalis*) is the most important vertebrate pest species in European agriculture because of its damage potential during outbreaks. Management usually relies on the use of rodenticides. However, common voles are important components in agro-ecosystems. In risk assessment prior to authorization of a plant protection product in the EU, the common vole is used as a model species for herbivorous small mammals.The attempt to protect the common vole as a non-target species from unwanted effects of plant protection products needs to be balanced with fighting it as a major pest species. To base risk assessment models on relevant and realistic data, extensive and detailed information about common vole ecology and behavior in different habitats and crops is vital. In the past, many studies have been conducted dealing with population development and population dynamics of common voles in different habitats and under the effects of various landscape modifications. And yet, there still are questions left to be answered. Weed control methods aim to reduce weeds in crops. For voles, weeds represent food and shelter. By reducing or destroying this source, it might be possible to observe changes in population development and dynamics of common voles and use this knowledge for risk assessment and for pest management methods.This project deals with two different thermical weed control methods in an apple orchard in Thuringia. Each of three replicates is treated with an open flame or hot steam system for weed control. Herbicide treated parts serve as an experimental control. We use capture-mark-recapture to assess the impact of these methods on changes in population dynamics and demography of common voles.

Improving nitrogen use efficiency in rice-wheat rotations in southeastern china

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Excessive use of mineral nitrogen (N) fertiliser is a common practice in rice-wheat rotations in southeastern China. However, at the same time the N use efficiency (NUE) in this rice-based cropping system is very low. The consequences are high N losses to water bodies (surface and ground water) and to the atmosphere. These losses from arable land can easily be reduced by applying 20-30% less mineral N fertilizer compared to the farmers practice without any reduction in grain yield and with a clear increase in NUE. To demonstrate this, field experiments on farmers` field sites were conducted from 2008 to 2011 for three consecutive rice-wheat double crop rotations in the two pilot counties Yixing and Huai'an in Jiangsu Province. The experimental design followed the so-called "3+x" approach with three different N fertilization treatments (conventional, reduced and zero-N application) and two agronomical ("x") treatments within each N fertilization level. Effects on crop growth, N nutrient status, mineral N in the soil (N_{min}) and grain yields were determined and nitrogen balance sheets were calculated. In spite of a much lower N fertilization rate, no significant change on crop growth, N nutrient status and grain yield were observed in the reduced N fertilization treatments in any year and crop. However, a significant increase of NUE could be achieved and the calculated nitrogen balances showed a clear decrease in nitrogen unaccounted for in the reduced N fertilization treatments compared to the farmers practice. Therefore, we can estimate that the N losses to the environment can be efficiently decreased by reducing the overall nitrogen fertilization rate without any decline in grain yield for rice and wheat.

Soil type affects rhizosphere microbial community and biocontrol of *Rhizoctonia solani*

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The soil borne pathogen *Rhizoctonia solani* is hard to control with fungicides. The use of antagonistically active bacteria could be an environmental friendly alternative. Two promising biocontrol strains could be identified under laboratory and greenhouse conditions. However, the biocontrol activity under field conditions is often inconsistent. The reason for this variability is until now unknown. An important factor influencing the crop yield, plant health and disease symptoms is the soil type. Soil types differ in chemical and physical conditions which may lead to different microbial communities. To understand the complex interactions between the soil type, plant and microbial community a field experiment with a unique plot system containing three soil types was performed. The dry weight and disease severity of lettuce was analyzed as well as the rhizosphere colonization of the two biocontrol strains. The dry weight in the three soil types is similar, but there are differences in the establishment of the pathogen. Surprisingly, the colonization density of the biocontrol strains was independent from the soil type. PCR-DGGE analysis showed that the rhizosphere microbial communities of lettuce in the three soil types differed significantly. The biocontrol strains had a negligible effect on the indigenous microbial community. Amplicon pyrosequencing of 16S rRNA genes from bulk soil and rhizosphere samples revealed taxonomic groups which were enriched in the rhizosphere of lettuce, or which differed between soil types. The dominant phyla in bulk soil and rhizosphere of all three soil types were *Proteobacteria, Actinobacteria, Fimicutes, Acidobacteria* and *Bacteroidetes.* The fast growing *Proteobacteria* were enriched in the rhizosphere compared to the bulk soil whereas the relative abundance of the other dominant groups decreases. The *Firmicutes* were enriched in one of the loamy soils, but decreased in the other two soils. A detailed analysis of the OTU report showed that many OTUs are enriched in the rhizosphere but also a noticeable number of OTUs are not influenced by the rhizosphere environment. Also there are significant differences between the soil types; especially the two loamy soils seem to enrich other OTUs than the sandy soil.

Poster

Microbial communities associated with juveniles of *Meloidogyne* spp. in soil

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Root-knot nematodes (*Meloidogyne* spp.) are one of the most damaging crop pathogens in the world. The newly-hatched juveniles of this obligate parasite have a free-living stage in the rhizosphere soil of the host plant where a high diversity of microbial species is present. Specific attachment of microbes to the cuticle of juveniles may play an ecological role, e. g. in transport of microbes through soil, microbial endophytic colonization or co-infection of roots, induction of plant defence systems, or microbial infection of the nematode. A better insight into nematode-microbe interactions might result in the discovery or improved application of biocontrol agents. The aim of this study was to assess by DNA-based techniques the species of bacteria and fungi that specifically attach to *Meloidogyne incognita* juveniles in soil. Bacterial and fungal communities of arable soil and that of juveniles extracted from this soil were compared by PCR-DGGE fingerprints of 16S rRNA gene or ITS fragments, respectively. Numerous nematode-specific microbial ribotypes were identified which were abundant on the nematode surface but not in the surrounding soil. Some bands from Fungi, Bacillus, Alphaproteobacteria and Betaproteobacteria were consistently enriched on most replicate samples of juveniles. This was observed for four different races of *M. incognita*. In contrast, Pseudomonas, Actinobacteria, and Enterobacteria showed high variability among replicate juveniles and races, suggesting that species of these taxa were not specifically attaching to the nematode cuticle.

Phenotyping of *Brassica napus* genotypes for resistance to drought

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Rapeseed (*Brassica napus* L.) is one of the most recently domesticated major crop species, and due to intensive breeding has become the most important oilseed crop in Europe concerning production. However, modern varieties are based on a relative small subset of the available genetic diversity. Therefore, the project of the Pre-Breed Yield consortium aims at estimating the genetic diversity present in oilseed rape on the phenotypic and genotypic level as the base for a directed improvement of yield and yield stability.

In this respect drought as one of the most serious production constraints worldwide came into focus in rape seed breeding. In the subproject at JKI respective rapeseed genotypes are evaluated for drought tolerance in rain-out shelter trials and in parallel a high throughput screening system is established. The project contributes to the characterization of genetic resources and their use in breeding programs as well as to adapting crops to the changing climate.

In 2011, the first rain-out shelter trial was conducted and growth chamber experiments for the establishment of a high throughput screening system were started.

53 genotypes were analysed for drought stress reaction by examining six indicator traits. In growth-chamber trials induced osmotic stress conditions were applied with PEG6000 at a concentration of 40% and wilting stress on leaf discs and whole leaves. First results for *relative water content* (RWC) and *chlorophyll content* (CC) point out significant effects ($P \geq 0{,}001$) of induced stress relative to the control treatment. RWC performance decreases significantly under wilting conditions for 24h and differences between genotypes were observed. The analysis of physiological traits like *membrane stability* (MSI) and the accumulation of *proline* and s*oluble sugars* as factors contributing for *osmotic adjustment* under drought stress are in progress. Results of a pre-trial indicate also striking alterations of these traits under induced drought conditions. Based on this, correlations of these traits to the results on agronomic performance obtained in the rain-out shelter trials will be calculated.

Influence of minerals, charcoal and litter on microbial response to phenanthrene in artificial soils

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Polycyclic aromatic hydrocarbons (PAHs) are important environmental pollutants which often persist in soil. The biochemistry of microbe-driven degradation of PAHs is well-studied though less is known about abiotic soil environmental factors influencing the microbe-phenanthrene interaction. Since minerals and charcoal are major soil components we matured different microbial communities in artificial soils based only on variation in the mineral content (montmorillonite, illite, ferrihydrite) and presence of charcoal for 2 years after addition of Luvisol microorganisms and sterile manure. Established microbial communities in soils were exposed to PAHs' model compound phenanthrene (2 mg/g) to study microbial functionality depending on soil composition. Furthermore, litter which was previously shown to enhance microbial activity was added to the soils (1 wt%). Both treatments with corresponding controls were further incubated for 63 days with sampling on day 0, 7, 21 and 63. A natural Luvisol soil was treated similarly for control. Fingerprints by denaturing gradient gel electrophoresis (DGGE) based on 16S rRNA or ITS gene fragments, respectively, amplified from extracted total community DNA were generated. The presence of phenanthrene-degradative genes was screened by PCR-Southern Blot detection and soil treatments were subjected to a chemical phenanthrene analysis. DGGE fingerprints revealed that the addition of phenanthrene and litter caused a shift in microbial community composition. Differences in bacterial response to phenanthrene were seen depending on litter addition and soil composition. The incubation time was determined as additional influencing factor. Furthermore, a dominance of a specific genotype for phenanthrene degradation is assumed based on PCR-Southern Blot analysis.

In conclusion, microbial response to phenanthrene was shown to be soil composition-, litter- and time-dependent. Hence, this study provided new insights into the complex soil interaction network.

Semi-field experiments with the entomopathogenic fungus *Isaria fumosorosea* (Isolate Pfr4) for control of various fruit moth species

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Several fruit moth species cause serious damage in fruit plantations. Especially in organic agriculture only limited control strategies are available. Within a national funded project on biological control of the plum fruit moth in organic agriculture we investigated the integration of entomopathogenic fungi in a biocontrol strategy.

The efficacy of the entomopathogenic fungus *Isaria fumosorosea*, isolate Pfr4, was tested in various semi-field experiments in a plum orchard of the JKI, Institute for Plant Protection in Fruit Crops and Viticulture (Dossenheim). Therefore, submerged spores of Pfr4 were produced in liquid culture by using a modified medium described by Samsinakova (1966). After filtration and centrifugation, the spore suspension was sprayed with a backpack sprayer on the stem and on the soil or on bark mulch as artificial hideout under the plum fruit trees. For the evaluation of the natural population of the plum fruit moth *Cydia funebrana* trapeze tents and for artificially released vine moth *Eupoecilia ambiguella* photoeclectors were used for collecting emerged moths. In both experiments it was not possible to catch the moths back.

Pfr4 can be produced in different production systems by formation of different types of spores. To select the most suitable production system experiments on the persistence and storability were carried out with aerial conidia produced on solid substrates and submerged spores produced in liquid culture. To get aerial conidia we float them off agar plates. Experiments on the persistence on bark mulch were evaluated over three months under semi-field conditions. Every second week bark mulch samples were taken and L_5-larvae of fruit moths were added to test whether the fungus is still able to infest them. Despite intensive UV radiation and heavy rainfall a long persistence over the whole experimental time of both aerial conidia and submerged spores was noticed. For comparison of the storability aerial conidia and submerged spores of Pfr4 were freeze-dried and samples were incubated at temperatures between 5° and 80°C for 6 days. After that the germination rate was investigated. The results will be presented.

Whether the isolate Pfr4 could be evidenced by using PCR will be discussed.

Use of nectar providing plants by the parasitoid wasp *Ascogaster quadridentata* WESMAEL (Hymenoptera: Braconidae)

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The codling moth *Cydia pomonella* is the main pest in apple production. Oviposition takes place on the fruit and subsequent feeding of larvae cause direct fruit damage. Infested apples can only be used for the production of apple juice, thus lowering yield and income for the farmer. The codling moth has many specific natural enemies. One of the most important ones is the braconid wasp *Ascogaster quadridentata.* This wasp places its eggs inside the eggs of the codling moth. The wasp's larva develops inside the codling moth larva. When the wasp's larva is fully developed it leaves the codling moth larva which dies afterwards. Although the apple is still damaged by the parasitized codling moth larva, the population density of the pest will be reduced in the next generation. The adult braconid wasp lives on nectar, thus requiring the presence of blooming plants. In many plantations however, soil treatment removes any flowering vegetation. The aim of the present work is to elaborate recommendations to the farmer which nectar producing plants should be planted or conserved in the field to allure braconid wasps. Therefore, the effects of different flowering plant species on the longevity and parasitism capacity of *A. quadridentata* were evaluated. In the field, plastic cylinders were installed on specimen of a particular plant species. The following plants where used during the study: Alyssum, buckwheat, carrot, *Echium sp.*, flax, parsnip, phacelia, white mustard and a control with grassy, non flowering vegetation. Five wasp couples were given in each cylinder. Furthermore, coding moth eggs, laid on plastic sheets from a laboratory rearing, were added and replaced on Mondays, Wednesdays and Fridays with new ones. After two weeks yellow sticky papers were placed in each cylinder to catch the surviving wasps. The eggs were kept until the eclosion of the larvae. Then parasitism of the larvae was determined by dissection. Therefore larvae were frozen, then dissected and examined for the presence of wasp larva under the binocular. Results showed that nectar of parsnip supported both survival of wasps and parasitism of codling moth eggs best from all tested plants.

Sensitive and specific detection method for *Pseudomonas savastanoi* isolates from *Mandevilla sanderi*

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The ornamental plant *Mandevilla sanderi* originating from Middle and South America has become increasingly popular over the last decade mainly because of its copiously formed red flowers. In 2008 breeders of *Mandevilla sanderi* observed for the first time large necrotic lesions with chlorotic rings on leaves and tumor formation on stems. The potential causal agents isolated from the lesions of leaves of diseased plant material were identified initially by metabolic profiling (BIOLOG) as *Pseudomonas savastanoi* pv. glycinea or pv. nerii. Several pathovars of *P. savastanoi* infect woody plants, e.g., *P. savastanoi pv.* savastanoi is known as an important pathogen of olive trees (*Olea europaea)* in the Mediterranean area. The BOX fingerprints were similar for *P. savastanoi* isolates from different host plants, plasmid restriction patterns and sequencing of plasmid-located pathogenicity determinants revealed that *Mandevilla* isolates contained similar plasmids distinct from those of other isolates. A *repA*-based detection method was established. The present study was carried out to do molecular characterization of *Pseudomonas savastanoi* isolates from *Mandevilla sanderi* in comparison to isolates originating from olive trees, oleander, jasmine and privet. This information can be used as a basis for the development of a sensitive and specific detection method for the pathogen from total community DNA.

Identification of genomic regions involved in drought stress tolerance in barley by association genetics

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In the face of global warming drought stress tolerance is becoming an important goal in barley breeding. So far there have been no comprehensive results on genotypic differences in drought stress tolerance in German winter barley cultivars. Therefore, the project aims at determining diversity on the phenotypic and genotypic level in order to identify genomic regions involved in drought stress tolerance by genome wide association studies (GWAS).

For this purpose 128 genotypes were analyzed in rain-out shelter trails for drought stress tolerance at flowering and in parallel were genotyped using the newly developed 9k iSelect chip. First results obtained in rain-out shelter trials revealed significant differences between stressed and non stressed plants and also between genotypes in response to drought stress concerning the content of proline and soluble sugars as well as senescence and yield parameters.

Based on the analysis of 6073 polymorphic SNPs a large genetic variation could be detected within the set of genotypes. Based on these phenotypic and genotypic results association genetics studies will be conducted.

SNPs being significantly associated to drought stress tolerance will be converted into easy to or Pyrosequencing marker. These will allow the effective marker-assisted selection for drought stress tolerance in barley.

Non-vector spread of the pine wood nematode, *Bursaphelenchus xylophilus*, with wood chips to non-infected trees

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The quarantine pest pine wood nematode (PWN) *Bursaphelenchus xylophilus*, native in North America, can be found in wood and root parts of infected conifer trees with focus on *Pinus species*. At the beginning of the 20th century this species was introduced into Japan supposedly with timber imports where it induced pine wilt expression on susceptible native pine species. Further outbreaks outside Europe are reported from China, Korea, Taiwan and Mexico. In 1999 PWN was found in Portugal, which today is declared as complete infested, since followed by first single tree outbreaks in Spain. The European Union (EU) member states have to undertake measures to prevent further spread of this nematode according to the emergency measures of the EU Commission.

Within the framework of the EU project REPHRAME "Analysis of the PWN to spread, survive and cause pine wilt in European coniferous forests in support of EU plant health policy" the risk of non-vector spread (without *Monochamus galloprovincialis*) of PWN through various pathways to healthy forests has to be determined. One example of such non-vector spread is the transmission with wood chips to non-infected trees which will be examined in the present study.

Pinus sylvestris logs were inoculated with a fresh *B. xylophilus* isolate from Portugal, sealed in plastic bags and incubated for 24 days at 25 °C in a climate chamber. Approximately 80 kg fresh wood chips were produced with a wood mill. In August 2012 the wood chips were placed at 3-4 years old *Pinus sylvestris* in two greenhouses at 15 °C and 25 °C. At the end of the test period the nematodes will be extracted from the trees and the wood chips to determine their density per gram dry matter.

Phytosanitary measures concerning wood chips from PWN infected areas are already included in the existing trade regulations. This study quantifies the risk of non-vector spread with wood chips and helps to evaluate the already existing measures.

HT-Phenotyping methods for yield parameters in grapevine

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Marker-assisted-selection (MAS) is one of the most important analysis methods to speed up grapevine breeding. Complementary to genotyping techniques the quality, objectivity and precision of phenotyping methods are determining. Non-invasive, high-throughput techniques based on images and software applications for image analysis are becoming state-of–the-art in plant science.

Building on the scientific methods developed in CROP.SENSe.net, such as acquisition of georeferenced images and image-based detection methods, new phenotypic tools for field acquisition are implemented. This is done in cooperation with working groups from Bonn University.

A large set of reference data taken in the field are used for testing and evaluation of the image based detection methods. Focus is laid on growing stages, diseases symptoms and in particular yield aspects, such as berry size, berry number, clusters per shoot and vine yield.

As a first step a high-throughput image interpretation tool for the lab was developed at Bonn University. It makes it possible to acquire the number and size of grape berries from RGB images in a much shorter period of time than manual measurements.

Development of a high-throughput phenotyping screening system for drought tolerance in barley

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Barley is the second most important crop species next to wheat in Europe. Barley yields have substantially increased during the last decades, but in recent years the linear trend decreased which is believed to be associated with drought periods in spring and summer. Climate models predict for Germany an increase in drought periods, therefore drought tolerance will be an important goal in barley breeding in the future. Because of its importance drought tolerance is included in the project BARSELECT that deals with the implementation of genomic selection in barley breeding. As a prerequisite to include this trait in the genomic selection procedure a reliable high-throughput screening system for drought tolerance has to be developed.

To achieve this, in a first step 63 six-rowed winter barley cultivars, which have been analysed in association genetics studies (GABI-GENOBAR), already, are grown under controlled conditions in a growth chamber up to the four leaf stage. Stress is applied by wilting single leaves or leaf disks or by putting leaf disks on a PEG medium for 48h. On wilting leaves the relative water content as well as the chlorophyll fluorescence is estimated. On leaf disks the osmotic adjustment, the content of soluble sugars and free proline is analysed as well as the cell membrane stability which is determined by measuring the electrical conductivity of ions leaching in double distilled water.

For these six physiological parameters significant differences between the stressed and non stressed plants were detected indicating a successful stress application. The results also revealed significant differences between the GABI-GENOBAR genotypes after stress application which is a prerequisite to score the traits as indicator for drought tolerance.

Results now will be correlated to data obtained on these genotypes in two years rain-out shelter trials for drought tolerance and in two years pot experiments for early leaf senescence that are received in two additional projects funded by the BMELV and the IZN. Based on the correlations, three out of these six traits will be selected, and subsequently 750 six-rowed winter barley breeding lines and varieties and 750 DH lines derived from crossings between selected genotypes will be analysed for these traits.

Methods of crop physiology to determine drought tolerance of winter rye (*Secale cereale L.*)

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Rye will probably be greatly affected by drought stress induced yield losses due to climate change, because it is typically grown on sandy soils with low water holding capacity. The aim of this study is to make statements about the drought tolerance mechanisms of rye and their use in plant breeding.

In the year 2011, 16 winter rye genotypes were examined in a drought environment under a foil tunnel without rainfall and irrigation from shooting stage until harvest, and a control environment with optimum irrigation in Braunschweig, Germany. Different phenological, physiological, and agronomic determinations were carried out. Among others, canopy temperature, carbon isotope discrimination, leaf area index, stomatal conductance, chlorophyll content, yield, and yield components were measured. Under the influence of drought stress, maturity was reached 12 days earlier and the total above-ground biomass yield decreased by 47 %. All investigated traits showed significant differences between environments and in some cases also between the genotypes. Canopy temperature, stomatal conductance, and carbon isotope discrimination were significantly correlated with grain and total above-ground biomass yield under drought stress. Canopy temperature showed the highest correlations (max. r = 0.76***; $p < 0.05$) of the investigated traits, but it is very dependent on climate conditions, especially in the temperate climate zone.

Impact of a vesicular arbuscular mycorrhiza symbiosis on biotic and abiotic stress tolerance of wheat

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The expected increase of drought and heat periods, in particular early summer drought, will result in reduction of yield and quality of the wheat grain (*Triticum aestivum*) harvested. The identification of stress tolerant wheat genotypes is one of the most promising approaches to reduce the negative impact of abiotic stress. Root endophytic mycorrhizal fungi are beneficial to many plant species by increasing water and nutrient uptake leading to increased yield under stress conditions. Therefore, the identification of wheat genotypes showing a better colonization with respective mycorrhiza fungi may be an opportunity to reduce the impact of abiotic stress on yield and quality. To achieve this, a set of 100 wheat genotypes are tested in order to detect genetic differences related to their ability to generate a symbiosis with arbuscular mycorrhizal fungi and to get information on the impact of this symbiosis on agronomic traits under stress conditions.

Genotypes are grown in pot trials in a drought stressed (25% maximal water capacity) and a control variant (75% maximal water capacity) with and without mycorrhization in three replications. Traits of agronomic performance, e.g. flowering time, plant height, yield and yield compounds were assessed. The analyzation of root colonization by mycorrhizal fungi was performed by PCR analysis (Janoušková et al., 2009) and an ink vinegar stain of root segments (Vierheilig et al., 1998).

The successful inoculation with a mixture of *Glomus intraradices, Glomus etunicatum* and *Glomus claroideum* could be confirmed by the root stain and PCR analyses. Mycorrhizal specific primer pairs showed that the majority of wheat genotypes were colonized by one or more mycorrhizal species of which G. *intraradices* was predominant. Typical mycorrhizal structures like intraradical hyphae, spores and vesicles become visible using light microscopical techniques. Furthermore, significant differences in plant height were observed. However other traits showed no difference between inoculated plants and the non-inoculated control. Respective trials will be repeated and additional trials to get information on the benefit of mycorrhizal symbiosis under conditions like phosphorus deficiency and biotic stress will be conducted. In parallel these genotyping using the 90k iSelect chip available for wheat will be conducted in order to identity QTL involved in mycorrhization and tolerance to abiotic stress via genome wide association genetics studies.

Fine mapping of 5 resistance genes on introgressions of *Hordeum bulbosum* in barley with SNP markers

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Barley growing is threatened by plant diseases such as scald (*Rhynchosporium secalis*), leaf rust (*Puccinia hordei*) or the soil-borne virus complex of BaMMV, BaYMV-1 and -2, the latter of which is transmitted by the fungus *Polymyxa graminis*. Breeding of resistant varieties can minimize the yield losses. The wild *Hordeum* species *H. bulbosum*, which makes up the secondary gene pool of barley, is a source of resistance genes against these fungi and viruses. Interspecific crosses of *H. vulgare* x *H. bulbosum* resulted in different recombinant diploid barley progeny with dominantly inherited immunity against the two fungi and the virus complex, respectively. Two virus resistance genes, *Rym14*^{Hb} and *Rym16*^{Hb}, have been introgressed to the short arm of chromosome 6 (6HS) and the long arm of chromosome 2 (2HL), respectively. The gene *Rrs16*^{Hb}, which confers immunity to *R. secalis,* was introgressed to the short arm of barley chromosome 4 (4HS). Two introgressions containing genes for resistance to *P. hordei* are present on chromosomes 2HL and 5HL, respectively. Mapping populations for the 5 resistance genes segregated in a 3:1 ratio of resistant to susceptible plants, suggesting a dominant monogenic inheritance of the different resistances. For the fine mapping of these genes SNP markers will be developed on the basis of sequence information generated by exome capture and the mapping populations genotyped with the Illumina VeraCode GoldenGate Genotyping Assay, Genotyping By Sequencing and Pyrosequencing. Finally, recombinant plants with reduced introgression sizes containing the resistance genes will be identified by marker-assisted selection for barley breeding.

Identification of candidate genes for a BaYMV/BaYMV-2 resistance gene located on chromosome 5H of barley

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Barley yellow mosaic virus diseases caused by different strains of soil-borne *Barley yellow mosaic virus* (BaYMV) and *Barley mild mosaic virus* (BaMMV) is a serious threat for winter barley production in Europe and Asia due to yield losses up to 50 percent. Chemical measures to prevent these high yield losses are neither effective nor acceptable for ecological reasons. Thus, the only way to control barley yellow mosaic virus disease is to grow resistant cultivars. There are several loci known conferring resistance to the different strains of BaMMV and BaYMV. A new resistance gene being only effective against BaYMV and BaYMV-2 was located on chromosome 5H.

In order to identify and isolate candidate genes for this locus a map based cloning approach was started. Using co-dominant flanking markers a high resolution mapping population was constructed based on 5085 F_2-plants (0.0098 cM resolution) derived from the cross ‘HHOR4224’ x ‘Igri’. The interval carrying the resistance gene was estimated at 12.09% recombination according to these analyses.

Currently the marker saturation of the target interval is in progress using all available marker and sequence information in barley, and employing synteny to rice, Brachypodium and sorghum. Up to now 11 markers have been located in the interval exercising this approach. Overall 730 segmental recombinant inbreed lines were identified and phenotypic analysis of 22 RILs gives first hint that the BaYMV/BaYMV-2 gene is located between markers k0xx1 and GBSxx1 comprising an interval of 2.8% recombination.

In order to integrate the BaYMV/BaYMV-2 resistance gene properly into the high resolution map all available segmental RILs are to be tested in field trials at two locations in the growing season 2012/2013.

Penetration behavior of different aphid species on *Lupinus angustifolius* L. genotypes

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Due to the high level of protein content in the seeds of up to 40%, the ability to fix nitrogen by symbiosis with rhizobia and the deep root system improving soil structure and quality, lupin cultivation is of growing interest. Wild types of lupins accumulate alkaloids which are toxic metabolites working as deterrents for several pests and diseases. A high content of alkaloids prohibits the use of lupins in human and animal diets.

Breeding of cultivars with a low content of alkaloids (“sweet lupins”) in the 1930’s enhanced the use of lupin seeds for human and animal nutrition, but led to a higher susceptibility to e.g. different aphids. Aphids harm plants by penetrating the cells, feeding on the phloem sap, leading to an inhibition of plant growth and by serving as vectors of different viruses.

Three genotypes of blue lupin (*Lupinus angustifolius* L.) with different levels of alkaloid concentrations - the sweet genotypes “Boregine” and “Bo083521AR” with a low content of alkaloids, and the bitter, alkaloid rich genotype “Azuro” - were evaluated with regard to the penetration behavior of the lupin aphid (*Macrosiphum albifrons),* which is well adapted to alkaloids in *Lupinus angustifolius* L., and the polyphagous aphid species green peach aphid (*Myzus persicae)* and black bean aphid (*Aphis fabae)*.

A method to record the penetration behavior of aphids is the Electrical Penetration Graph (EPG). Aphids are fixed on a thin gold wire by a droplet of silver glue. The conductivity of this attachment allows carrying low current through the aphid when stylet penetration occurs. The wired aphids produce an electrical signal during the penetration progress. Depending on the penetrated plant tissue and the different parts of penetration procedure, e.g. salivation or phloem sap ingestion, the recorded waveforms of wired aphids show different patterns.

The aim of the test is to analyze, how far the penetration behavior of the well adapted lupin aphid is different from that of the polyphagous green peach and black bean aphid on bitter and sweet lupin genotypes. Results obtained up to now give hint that the lupin aphid is unaffected by the alkaloid level while the green peach and the black bean aphid show an alkaloid-level depending penetration behavior.

Monitoring, analysis and modeling of yield and quality dynamics of *Lolium perenne* varieties for biogas production

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The overall aim of this project is to increase the biogas production of perennial ryegrass (*Lolium perenne*) by model-based optimization of the harvesting date. In a preliminary step it was analyzed statistically, whether the harvesting date influences yield and quality of *L. perenne* varieties. In a second step a model for yield and quality development of the varieties was set up and parameterized.

In the first step 20 varieties of *L. perenne* differing in maturing times were cultivated at five sites in Germany and the Netherlands. Two harvesting treatments were applied: consistent harvest (H_c), where all varieties were harvested at the same time, as it is common practice, and differentiated harvest (H_d), where each variety was harvested at growth stage of ear emergence. Yield was characterized as dry matter yield, and quality parameters, such as crude protein and crude fiber, were determined by Near Infrared Spectroscopy (NIRS). Yield and quality data were analyzed descriptively and by ANOVA. It was shown that yield and grass quality varied mainly between site and year. A significant interaction between variety and harvesting treatment indicated, that yield and quality of both harvesting treatments differed particularly between the early and late varieties: Yield of early varieties was greater in H_c, while yield of late varieties was lower in H_c than in H_d. On the contrary, quality of early varieties was lower in H_c, while quality of late varieties was greater in H_c than in H_d.

In the second step, a logistic growth model for yield and quality development was set up, which also includes the most important environmental conditions, such as temperature and soil moisture. For model parameterization, eight *L. perenne* varieties with different maturing times were cultivated at four locations in Germany and harvested in time series. Yield and quality of *L. perenne* and environmental conditions were monitored. The model fitted well to both, yield and quality data of *L. perenne* varieties at all sites. The differences in yield and quality development were reflected by the model through differences in rates of growth and temperature coefficients: Early varieties had lower rates of growth and temperature coefficients than late varieties.

It can be concluded, that this model can help to characterize *L. perenne* varieties and determine the optimal date of harvest for an increased biogas production.

Identification of QTLs for drought stress induced leaf senescence in barley using genome wide association studies

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Due to climate change tolerance to drought stress has become an important goal in plant breeding. Therefore, the aim of this project is to identify genomic regions involved in drought stress induced leaf senescence in early developmental stages in barley by applying genome wide association studies.

In pot experiments (three replications each) 156 barley genotypes including 64 six-rowed, 49 two-rowed German cultivars and 43 accessions of the Spanish Barley Core Collection (SBCC) are tested for their response to early drought stress and induction of leaf senescence under control (70% water capacity) and stress conditions (20% water capacity).

During the stress period physiological parameters, e.g. the photosystem II efficiency, the chlorophyll content, the content of proline and soluble sugar and the aboveground biomass production were determined.

First results revealed significant differences between the treatments and between genotypes concerning the parameters mentioned above.

In parallel this set of genotypes was analysed with the 9k iSelect chip available for barley. In summary 6807 SNPs turned out to be polymorphic. Those being mapped and showing a minor allele frequency >5% were used for whole genome association studies taking into account population structure and kinship. Based on this procedure significant associations were observed for all traits analysed.

Study of the transmission efficiency of *Wheat dwarf virus* with different geographic origins of the leafhopper *Psammotettix alienus*

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Due to the climate changes insect-transmitted viruses become more important. The leafhopper species *Psammotettix alienus* is the vector of the *Wheat dwarf virus* (WDV). Higher temperatures in autumn advantage the infection of cereals with WDV. For resistance tests it is important to know the transmission efficiency of WDV with *Psammotettix alienus*, which are living in the experimental rearing. Three different geographic origins of the vector (Quedlinburg, Beijing and Prague) were used for the transmission tests. To make sure that the origins belong to the species *Psammotettix alienus*, the *aedeagus* must be dissected. Two viruses are distinguished: the Wheat dwarf virus (WDV) and the Barley dwarf virus (BDV). One isolate of each virus was taken for transmission test. ELISA was used to detect the virus in the plants. The leafhoppers were divided in body and head. The heads were tested by quantitative real-time PCR, the bodies by qualitative PCR with subsequent gel electrophoresis. The results show that the transmission efficiency is different between the three origins. The origin Beijing has the highest transmission efficiency (WDV 75%, BDV 77%); the transmission efficiency of the origin Quedlinburg (WDV 58%, BDV 63%) is nearly as good as the origin Beijing; the origin Prague showed heterogeneous test results between both viruses (WDV 76%, BDV 23%). In most of the leafhopper bodies WDV/BDV were detected. Also some heads were positive tested with WDV/BDV, although the DNA isolation from the heads was difficult. There is more investigation necessary to secure the present results.

Heat shock induced flowering of PtFT apple plants

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Apple plants have a long vegetative phase of 7 to 10 years involving a time consuming, non-economical process of breeding apple resistant to fire blight, scab and powdery mildew. An early onset of flowers would be a great advantage to release disease resistant pre-breeding material. Overexpression of the flower promoting genes *LEAFY* and *FLOWERING LOCUS T* from *Arabidopsis thaliana* in poplar (*Populus trichocarpa*) and *BpMADS4* from *Betula pendula* in apple (*Malus* × *domestica*) led to an accelerated development of flowers. Overexpression of the *BpMADS4* gene in apple resulted in constitutive flowering from in vitro culture to greenhouse. A continuous development of fruits was achieved based on the constitutive expression of the 35S promoter in these plants. This extensive fruit production resulted in an abnormal vegetative plant development, in small-sized fruits and in high fruit drop due to a lack in nutrient supply. As an improved approach a heat-induced flowering was established based on the heat-shock promoter *Gmhsp 17.5-E* (HSP) from soybean (*Glycine max*). In poplar a heat-regulated expression of the poplar *FLOWERING LOCUS T* (*PtFT*) gene resulted in flowering after certain heat-treatments. We introduced the *PtFT1* and *PtFT2* gene from poplar under the HSP-promoter into apple. The conditions for heat-induced regulation of these genes in apple were studied.

Molecular characterization of BYDV resistance gene *Ryd4*^{Hb} introgressed from *Hordeum bulbosum* into barley

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Barley yellow dwarf virus (BYDV) causes high yield losses worldwide. Thereby aphids play an important role as virus vectors. Such yield losses caused by BYDV may be reduced using virus-tolerance genes from the primary genepool of barley. Additionally, complete resistance to the virus has been introgressed *via* interspecific crosses from the secondary genepool within the tetraploid wild species *Hordeum bulbosum* to barley chromosome 3HL. Previous studies indicate that this resistance is governed by a dominant gene, *Ryd4*^{Hb}. In the present study, a BC_2F_4 mapping population consisting of 134 individuals was used to establish a molecular-marker map for *Ryd4*^{Hb}. Using the model genome of *Oryza sativa*, the Massive Analysis of cDNA Ends (MACE) approach was applied to develop novel markers. One of these markers cosegregated with *Ryd4*^{Hb}. For a fine-mapping of *Ryd4*^{Hb} a BC_2F_6 family of 454 individuals will be used.

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