Nachwuchswissenschaftlerforum
Young Scientits Meeting

Viertes Nachwuchswissenschaftlerforum 2011

29. November - 1. Dezember
in Quedlinburg

- Abstracts -

Berichte aus dem Julius Kühn-Institut

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Julius Kühn-Institut
Bundesforschungsinstitut für Kulturpflanzen

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Der Forschungsbereich des Bundesministeriums für Ernährung, Landwirtschaft und Verbraucherschutz (BMELV) hat seit dem 1. Januar 2008 eine neue Struktur.
Die Biologische Bundesanstalt für Land- und Forstwirtschaft (BBA), die Bundesanstalt für Züchtungsforschung an Kulturpflanzen (BAZ) sowie zwei Institute der Bundesforschungsanstalt für Landwirtschaft (FAL) wurden zum Julius Kühn-Institut - Bundesforschungsinstitut für Kulturpflanzen zusammengeschlossen. Das Johann Heinrich von Thünen-Institut (vTI) wurde aus der Bundesforschungsanstalt für Fischerei, der Bundesforschungsanstalt für Forst- und Holzwirtschaft und aus Teilen der Bundesforschungsanstalt für Landwirtschaft errichtet.

The research branch of the Federal Ministry of Food, Agriculture and Consumer Protection (BMELV) has been reorganized. The former Biological Research Centre for Agriculture and Forestry (BBA) has been merged with other institutions. The newly established Julius Kühn Institute (JKI), Federal Research Centre for Cultivated Plants, is working on plant protection, plant breeding, crop and soil science. The Johann Heinrich von Thünen Institute (vTI) was created from the German Federal Research Centre for Fisheries, the German Federal Research Centre for Forestry and Forest Products and part of the German Federal Agricultural Research Centre.

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Dr. Georg F. Backhaus

Grußwort des Präsidenten

Liebe Nachwuchswissenschaftlerinnen, liebe Nachwuchswissenschaftler,

wenn eine wissenschaftliche Tagung zum vierten Mal in Folge mit hoher Beteiligung stattfindet, kann man sicherlich bereits von einem Erfolgskonzept sprechen. Das Nachwuchswissenschaftlerforum des Julius Kühn-Instituts (NWF) ist eine solche Veranstaltung. Über 50 Studierende, Doktoranden und PostDocs aus 14 Instituten des JKI präsentieren auch in diesem Jahr wieder Ergebnisse aus ihren wissenschaftlichen Arbeiten. Es macht mich stolz miterleben zu dürfen, wie eine so große Zahl junger Forscher aus den verschiedensten Fach- und Wissenschaftsgebieten gemeinsam eine Konferenz eigenständig plant und durchführt. In Umfang und Gestaltung kann das NWF tatsächlich ein Vorbild für andere Tagungen am JKI und nicht nur dort sein. Hervorheben möchte ich die in diesem Jahr erstmalig durchgeführten Poster-Präsentationen. Sie erlauben jedem Teilnehmer, auch dieses Mittel der Vorstellung und Diskussion von Forschungsergebnissen unabhängig von einem strengen Vortragsplan zu nutzen. Ganz besonders freue ich mich darüber, dass unser Kooperationspartner am Hauptstandort, das GutsMuths-Gymnasium in Quedlinburg, einen Beitrag zum kulturellen Rahmenprogramm des NWFs leistet. Ich wünsche mir, dass hieraus weitere Impulse für die Zusammenarbeit beider Einrichtungen erwachsen. Neue Impulse für Ihre wissenschaftlichen Arbeiten wünsche ich auch Ihnen. Tauschen Sie sich aus, diskutieren Sie fleißig und vor allem: Lassen Sie das 4. NWF wieder zu einem vollen Erfolg werden!

Greetings from the president

Dear Young Scientists,

The Young Scientists Meeting of the Julius Kühn-Institute takes place in the fourth consecutive year and can therefore surely be characterized as a concept for success. This year again, more than 50 graduates, PhD students and PostDocs from 14 institutes of the JKI present their scientific results. It makes me proud to witness the realization of a conference which was independently planned and accomplished by the young scientists themselves. In scope and configuration the Young Scientists Meeting has the property to act as a pattern for further conferences at the JKI and not only there. In particular, I emphasize the new poster sessions which are part of the meeting for the first time. Participants thereby have the opportunity to choose between different ways of presenting scientific work. Furthermore, I am indeed very pleased that our cooperation partner, the GutsMuths-Gymnasium, Quedlinburg, contributes a cultural event. By these means, I would really appreciate to see further impetus arising for the cooperation of both institutions. This gives me the cue for wishing you, the young scientists, every success for your future scientific work and career. Have a mutual exchange, discuss sedulously and, what's more, let the 4th Young Scientists Meeting again become a complete success!

Dr. Georg F. Backhaus

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Sixth Session: Mixed Presentations

Posters

First Section:
Biological Control

Molecular characterization of different isolates of CpGV and their activity against *Cydia molesta*

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The codling moth granulovirus (*Cydia pomonella* granulovirus, CpGV) is an efficient biological agent of the codling moth which is of great importance in organic fruit growing. Currently there are CpGV preparations from different manufactures available in Europe and about 100-150.000 ha per year are used. Although CpGV has a very narrow host range, in addition to *C. pomonella* also *C. molesta* can be infected with lower efficiency. So far there is no effective control agent in organic farming for *C. molesta*, therefore, the direct control approach using CpGV is tested. Four different CpGV isolates were tested in bioassay against a laboratory strain of *C. molesta* and the LC_{50}s (median lethal concentration) of these isolates were determined. The rating was carried out after 7 and 14 days. In addition to the biological activity of isolates against *C. molesta* the CpGV isolates were characterized by restriction analysis. The restriction profiles were compared to the known profiles of CpGV-M. A virus isolate (CpGV-V22, Andermatt Biocontrol), which has great potential as bioinsecticide, is a mixture of different genotypes. Therefore an *in vivo* cloning procedure was performed. Thereby, a pure genotype "V22P" could be isolated from this mixture of different genotypic variants. In view of using the cloned *in vivo* CpGV-V22P in pest control it is interesting to determine to what extent the individual genotype shows a higher infectivity for *C. molesta* than the corresponding CpGV-V22 isolate. The differences in biological activity between mixture and pure genotype are determined in a bioassay.

Identification of physiologically active volatile compounds in dried apple, dried apricot and dried almonds on *Plodia interpunctella* (HÜBNER) (Lepidoptera: Pyralidae)

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Monitoring of insects by the use of attractant semiochemicals is becoming more and more interesting since semiochemicals can be used against many different insect species. *Plodia interpunctella*, the major pest of packaged food in transit and storage, infests goods such as grain and grain products, nuts, seeds, chocolate, dry pet food and dried fruits. Dried apricot, dried apple and dried almonds were used in the present study in order to identify attractant odorants which could be used as lure in traps for the monitoring of this moth. Firstly, volatile compounds of these three products were collected by headspace-solid phase microextraction and identified by gas chromatography/mass spectrometry (GC-MS). Secondly, some volatiles among those obtained from chemical analysis were chosen based on literature observations. The electrophysiological activity was tested by electroantennographic measurements (EAG) at a standard concentration of 1µg/µl. Thirdly, to evaluate the sensitivity of *P. interpunctella* antenna to the compounds, a dose-response test was carried out. Of the test compounds which, at the standard concentration (1µg/µl) elicited significant EAG responses, different concentrations ranging from 10^{-3} to 100 µg/µl were puffed to the antenna in further EAG tests. The results revealed that, benzaldehyde, (*E*)-2-octenal and (*Z*)-2-heptenal consistently elicited EAG responses in male antennae. The same observation was made with (*E*)-2-octenal and 1-heptanol in female antennae. Limonene, however, elicited a rather low EAG response in both sexes at all tested concentrations. In general, the response increased with increasing concentration of the volatile. The highest increase in response occurred in both sexes between 1 and 10 µg/µl for 4 compounds tested. These findings indicate the evidence of a behavioral activity, attractive or repellent, induced by each tested compound on *P. interpuctella*. The respective activity will be determined through behavioral bioassays of each compound.

Turnip rape as a trap crop and natural pesticides – solutions for the pest disease in organic rape seed cropping?

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In Germany cultivated area with organic oilseed rape is very small between 2300 ha and 4000 ha (Ami, 2011). Important reasons for the small area are different insect pests which often cause important yield losses up to total loss of yield. A mixed cropping system of rapeseed and 10 % turnip rape as trap crop was compared with oilseed rape in pure stand to demonstrate the reduction of infestation by insect pests. Furthermore the application of biopesticides like pyrethrum / rape oil (Spruzit® Neu), spinosad (SpinTor), diatomeen earth (SiO2) / sunflower oil and rock powder / water was tested. Oilseed rape showed a higher infestation by stem weevils (*Ceutorhynchus* spp.) in the mixed cropping system compared to rapeseed in pure stand. The reduction of the pollen beetle (*Meligethes aeneus*) on the rapeseed buds depends on higher attractiveness of turnip rape as a consequence of advanced growth and a sufficient amount of turnip rape plants. The application of pyrethrum and spinosad against stem weevils had no effect. Spinosad was the only agent that caused a satisfying reduction of the pollen beetle. Certainly neither the mixed cropping system nor the bio pesticides causes economocally growth of yield.

AMI, 2011. AMI-Marktbilanz Getreide Ölsaaten Futtermittel 2011.

Competition and Synergy Related Baculoviruses

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The application of baculoviruses has a large impact in biological pest control. By now vast numbers of viruses are known that infect numerous hosts in crop cultures. Larvae of the genus *Agrotis* (Lepidoptera) are widespread worldwide and harm a lot of crop cultures. The research-focus of the diploma-thesis "Development of Molecular-Biological Detection Methods for *Agrotis* Specific Baculoviruses" was the three baculoviruses, who are isolated from *Agrotis* genera: *Agrotis ipsilon nucleopolyhedovirus* (AgipNPV), *Agrotis* se*getum* nucleopolyhedovirus English strain (AgseNPV-B) and *Agrotis* se*getum* granulovirus (AgseGV). The applicability of these baculoviruses against *Agrotis* species is explored by analyzing the virulence parameters.

A sub goal was to prove synergic and competitive effects of a mix-infection with AgseNPV-B and AgseGV by qualitative and quantitative PCR and to draw conclusions from that for the evolution of these three viruses.

Second Section:
Breeding Research - Horticultural Crops

Marker-Assisted Selection (MAS) for *Vf*-scab resistance in apple (*Malus domestica*)

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Apple scab caused by the fungal pathogen *Venturia inaequalis* is one of the most important diseases in all apple growing areas around the world. The infection can cause severe damage to leaves and fruits making them unmarketable. Therefore extensive fungicide applications up to 15 times or more per year with different fungicides are required to prevent economic losses. Due to the demand for more sustainable production fungicide treatments could be reduced by planting scab resistant cultivars. The most commonly used apple scab resistance gene in breeding programs is *Rvi6* (the new denomination of *Vf*), derived from *Malus floribunda* 821. Until now more than 70 scab resistant cultivars are reported carrying the *Rvi6* gene. Planting these *Rvi6* cultivars allows the reduction of fungicide treatment.

The Züchtungsintiative Niederelbe (ZIN) is an union of apple growers in the North-Western part of Germany focusing on breeding new cultivars. ZIN, together with the FH Osnabrück and the JKI are partners participating in a network called WeGa. The topic of the three partners is to identify *Rvi6* scab resistant apple cultivars with high fruit quality. This project includes the genotyping of apple selections concerning *Vf* resistance, the analysis and identification of aroma compounds by gas-chromatography and the evaluation of inner and outer fruit quality. Here we will present first results of the Workpackage 1.2: "Marker-Assisted Selection (MAS) for *Vf*-scab resistance in apple (*Malus domestica*)" which is part of the cluster "Product Safety by Sustainable Plant Protection" of the WeGa network project.

An evaluation approach of salt stress tolerance in carrots

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Salinisation is a major problem in crop and vegetable production. Globally the soil salinity increases as a result of extensive irrigation of crops and vegetables. Many activities are carried out to develop salt stress tolerant cultivars in many crop species worldwide. Carrot has been described as sensitive to salt stress by several authors, but there are no statements to salt stress tolerance in broad carrot collections e.g. gene bank material, landraces and modern cultivars.

To create new salt stress tolerant carrot genotypes, a suitable evaluation approach was necessary. Results of the methodological pre-experiments, the impact of salt solution to the germination of carrot seeds and the influence of different salt concentrations on agronomical and morphological characters as well as sugar compounds in roots were presented.

Finally, a method was established and more than 120 carrot genotypes from different geographic origin were evaluated for their ability to tolerate salt stress. A new carrot cultivar with an enhanced salt stress tolerance may lead to a sustained increase to the global carrot production.

Usage of intraspecific variability of lemon balm, *Melissa officinalis*, for the generation of new gene pools for plant breeding

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Lemon balm is a spice- and teadrug, which is used for over 2000 years, because of its lemon aroma. The main components are essential oil and rosmarinic acid. The pharmaceutical use as watery or alcoholic extract is due to its sedative, spasmolytic and antiviral effectiveness.

In the framework of the “Demonstrationsprojekt" “Verbesserung der internationalen Wettbewerbsposition des deutschen Arznei- und Gewürzpflanzenanbaus am Beispiel der züchterischen und anbautechnologischen Optimierung von Kamille, Baldrian und Zitronenmelisse" (KAMEL), lemon balm stands as a example for leafy drugs. This conglomerate of different projects tries to optimise the whole process of generating quality drugs and concerns plant breeding, plant growing, harvesting and drying. In 2010 and 2011 92 accessions of lemon balm from the collections of the federal state institute for agriculture of Bavaria and the *ex situ* gene bank of the Leibniz-Institute of Plant Genetics and Crop Plant research were tested in field trials. Special attention was given to agronomical qualities like winter hardiness, yield and essential oil content.

On the basis of these trials we could measure the potential of single accessions and evaluate the relation between morphological properties and qualitative characteristics. With the help of molecular markers (AFLP), we were able to generate the first phylogenetic tree of *Melissa officinalis*, which shows the degree of relation between the accessions and the collections. This information was of considerable importance for choosing partners for crossing.

Breeding strategies for the development of resistant strawberry cultivars against the pests *Botrytis cinerea* and *Xanthomonas fragariae*

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Organic strawberry production is mostly hampered by fungal and bacterial plant diseases. Especially the grey mould disease caused by the fungus *Botrytis cinerea* Pers. (teleomorph *Botrytinia fuckeliana*) and the bacterial angular leaf spot disease caused by *Xanthomonas fragariae* (Kennedy & King) are two of the most important diseases in organic strawberry production. Pesticides are not permitted in organic farming and indirect plant protecting measures such as cultivation methods and application of plant strengthening products are only less effective. Planting of resistant cultivars seems to be the most promising strategy to improve the productivity in organic strawberry production. Although commercially cultivated strawberry cultivars differ in their susceptibility to these diseases no resistant cultivars are available on the marketplace. On this account we started a program on evaluation of strawberry genetic resources for their resistance/susceptibility to *B. cinerea* and *X. fragariae*, respectively. We collected different strains of each pathogen and tested their virulence to a defined set of strawberry genotypes. Subsequently, we established methods for artificial fruit/leaf inoculation in the greenhouse for both pathogens and started the evaluation of strawberry cultivars. Furthermore, breeding clones and wild species of the Fruit Gene Bank Dresden-Pillnitz will be tested. Based on the results resistant/tolerant genotypes will be selected and used in test crosses to determine the general and specific combining ability. The results of the test crosses will lead to the identification of genotypes that are suitable for a targeted resistance breeding against *B. cinerea* and *X. fragariae*, respectively.

Selected apple and strawberry transcription factors and their relevance to fruit development

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MADS-box transcription factors and their role in flower and fruit development have been widely studied in the model plant *Arabidopsis thaliana*. However, they are poorly studied in major crop plants and fruit trees. Our study aims to extend the knowledge on MADS-box genes of the *Rosaceae* family that includes crops of important agricultural and commercial value. We selected individual genes of this gene family involved in developmental processes and studied their function at the molecular level in two *Rosaceae* representative species. A phylogenetic analysis of apple MADS-box genes suggests the presence of a subclade containing genes that are close to the *Arabidopsis AGL24* gene. Fifteen of these *AGL24*-like genes were investigated with regard to their function in the apple cultivar 'Golden Delicious'. The ORFs of these genes were validated experimentally from cDNA libraries of different apple tissues. Expression studies using qPCR indicate that the apple MADS-box genes belonging to this subclade do not play any role in flower development, but they are more likely involved in response to vernalization (dormancy-associated MADS-box, DAM). Furthermore, we studied also the putative *F. vesca* homologues, but they did not show any expression in the respective tissues of strawberry. Therefore, we focused our study on MADS-box genes with homologues of known function in *Arabidopsis* to perform gene expression and functional analysis. After phylogenetic analysis, three genes that are most likely the strawberry homologues of *Arabidopsis PISTILLATA* and *AGAMOUS* were chosen as candidate genes for post-transcriptional gene silencing using a RNA interference approach. The molecular and phenotypic analysis of the transformants will help to explain how the ABCE model genes act in *Rosaceae*.

Generation of cisgenic apple (*Malus x domestica* BORKH.) with a biotic resistance to apple scab caused by *Venturia inaequalis*

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The cultivated apple (*Malus* x *domestica* Borkh.) is one of the economically most important fruit crop worldwide. Among hundreds of excising apple cultivars only a handful of them are favoured by consumers due to their appearance, quality, flavour and storability. These cultivars are all susceptible to different plant diseases like apple scab caused by *Venturia inaequalis*. The introduction of the natural occurring scab resistance gene *HcrVf2* from *Malus floribunda 821* by classical breeding is time consuming and expensive, because of self-incompatibility, heterozygosity and a long juvenile period of 5 to 12 years. Genetic engineering offers the opportunity to overcome these limitations. The introduction of *HcrVf2* into the genome of existing cultivars via *Agrobacterium*-mediated plant transformation requires an efficient selection as realized by the *neomycin phosphotransferase II* marker gene in apple. Such marker genes conferring resistances to antibiotics are not accepted by the consumer and need to be eliminated. A vector was developed allowing the site-specific excision of all unwanted DNA sequences after selection, mediated by a heat-shock inducible expression of the FLP recombinase. The vector contains beside *HcrVf2* under its own regulatory elements a recombination cassette flanked by direct repeated *flp* recognition target sites. The recombination cassette comprises of two marker genes *nptII* and *dao1*, both driven by a *CaMV 35S* promoter, as well as the *flp* recombinase gene under control of the heat-inducible *Gmhsp17.5-E* promoter. The second marker gene *dao1* codes for the DAAO protein, which converts the non-toxic amino acids D-valin and D-isoleucin to plant toxic products. A further selection of gene modified cells in which the FRT-flanked box was removed by recombination is possible. Using this vector gene modified lines were produced and investigated by PCR, RT-PCR. First results will be presented.

Coparative QTL mapping for fire blight resistance

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Fire blight is one of the most necrotic diseases affecting pome fruits like pear and apple or other members of the *Rosaceae* family. It is caused by the gram negative bacteria *Erwinia amylovora* (Burrill) Winslow *et al*.. The massive economical losses of the pome fruit producing industry in the last decade as well as the lack of efficient control strategies and the susceptibility of common apple cultivars justify an interest in fire blight resistance breeding. An effective solution would be planting of resistant cultivars with high fruit quality which are comparable to market leading cultivars. In the past many efforts have been made to study the genetics of fire blight resistance. A first mapping for fire blight resistance in the cross population 'Idared' x *Malus* × *robusta* 5 (Mr5) using the *E. amylovora* strain Ea222 resulted in the detection of a major QTL on LG 3 of Mr5, assuming a major gene responsible for the resistance. In the present study we inoculate the cross population with a deletion mutant strain (pZYRKD3-1) of the $avrRpt2_{EA}$ avirulence gene of *E. amylovora*. To compare the results, we additionally inoculate the progenies of 'Idared' x *Malus* × *robusta* 5 with the wild type strain of the deletion mutant (Ea1189). After inoculation with the wild type strain Ea1189 the average necrosis shoot length of the progenies was 40 %. In contrast to the wild type, the deletion mutant strain caused a 37 % higher average necrosis length (total shoot necrosis of 77 %). In comparison to Ea222, we were able to confirm the QTL on LG 3 after inoculation with the wild type strain Ea1189. The deletion mutant strain (pZYRKD3-1) of *E. amylovora* caused a breakdown of the QTL. The results imply that the knock out of the avirulence gene $avrRpt2_{EA}$ causes a higher virulence of the mutant strain and an overcoming of resistance of Mr5. The different host-pathogen interactions are a first evidence for a gene for gene relationship between *Malus × robusta 5* and *E. amylovora* (Burrill) Winslow *et al*..

Promoter analysis of pathogen inducible genes of grapevine

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Vitis vinifera ssp. vinifera is an important fruit species in Europe but it is susceptible to Powdery and Downy Mildew caused by *Erysiphe necator* and *Plasmopara viticola*. Both Pathogens were introduced from North America in the 19th century. Since then it is necessary to treat the plants with high amounts of fungicides which causes high costs and is environmentally unfriendly. These measures can be reduced by breeding and use of resistant grapevines. Modern breeding is supported by the use of molecular markers. The development of resistance-correlated markers could be strongly improved by the understanding of the mechanism of pathogen defense. To get an idea which genes are involved in the mechanism of pathogen defense differential gene expression studies (qRT-PCR) were done in previous work. Some candidate genes like specific transcription factors, PR5 and PR10 were found to be differentially upregulated in resistant plants after the attack of *Erysiphe necator*. In the actual project the promoters of the genes were cloned from a resistant and a susceptible grapevine. They were sequenced and analyzed *in silico*. In further work the transcriptional regulation will be analyzed in transient expression systems and heterologous systems with the help of reporter genes.

Induction of early flowering in poplar and in apple for a speed-up in disease resistance breeding

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Breeding of apple is a long lasting process. Apple plants go through a juvenile phase-without the possibility of sexual reproduction and realization of crossing. The introgression of relevant traits i.e. resistance against diseases like fire blight, powdery mildew and apple scab, from wild species into cultivars with a marketable fruit quality by classical breeding is a time consuming task. Based on a shortened juvenile phase such important breeding processes could be realized within a more economical time frame. The introgression and overexpression of the *BpMADS4*-gene from silver birch (*Betula pendula* Roth.) into apple (*Malus × domestica* Borkh.) resulted in a shortened juvenile phase. However, the expression of the gene driven by the constitutive 35S promoter from the *Cauli flower mosaik virus* led to an extensive production of flowers, which only could be counteracted by permanent elimination of dispensable buds by hand.

This project focuses on an induced and controlled early flowering by introgression of the *BpMADS4*-gene driven by the heat-inducible promoter *Gmhsp 17.5-E* from soybean (*Glycine max* (L.) Merr.) into apple (*Malus × domestica* Borkh.).

Localisation and fine-mapping of the downy mildew resistance locus *Rpv10* in grapevine

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The pathogen *Plasmopara viticola* causing downy mildew is an oomycete native to North America. It was distributed throughout almost all wine growing regions around the world. Interestingly several accessions of the Asian wild grape *Vitis amurensis* possess resistance to *P. viticola*. Introgression of this resistance source into the gene pool of the European high quality grapevine *V. vinifera* resulted in new cultivars, such as 'Solaris', resistant to downy mildew. QTL analysis of a cross population derived from a cross between the breeding strain Gf.Ga-52-42 and the cultivar 'Solaris' revealed the resistance locus Rpv10 on chromosome 09. A subsequent fine mapping resulted in confidence intervals of less than one cM corresponding to 79 kb between the flanking markers on the grapevine reference genome. Four potential resistance candidate genes were identified in this section of the sequence. SSR markers flanking the locus can be used in marker assisted breeding (MAS) for screening genetic resources and breeding material. A set of 94 *V. amurensis* descendants was screened and 22 genotypes carrying the Rpv10 locus were identified. These genotypes are potential sources to introduce the Rpv10 locus into the breeding process for pyramiding resistance loci by combining different resistance donors. This is expected to result in plants which possess high and durable resistance properties. MAS offers the possibility to select these promising genotypes. By taking advantage of newly developed markers for Rpv10 the locus can be used immediately in resistance breeding to achieve high level of downy mildew resistance by pyramiding with further resistance loci.

Third Section:
Breeding Research – Agricultural Crops

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 one of the most important diseases of winter barley in Europe and Asia and can cause yield losses up to 50 percent. In barley 9 different loci conferring resistance to the different strains of these viruses are known. One of these loci being effective only against BaYMV and BaYMV-2 is located on chromosome 5H. Therefore, the aim of this project is to identify and isolate candidate genes for this recessive resistance gene applying a map based cloning approach. In a first step a high resolution mapping population is constructed on 5000 F2 progeny (0.01 cM resolution) derived from the cross 'HHOR4224' x 'Igri' using co-dominant flanking markers. Up to now 5085 plants have been analysed and the interval estimated at 12.95 cM. At present marker saturation of the target region is conducted using all sequence and marker information available for this interval in barley and employing synteny to rice, Brachypodium and sorghum. First polymorphic markers located in this interval have already been identified and mapped. Respective closely linked or co-segregating markers will facilitate the identification of candidate genes by analyzing BAC contigs. In the end this project will lead to a better understanding of the molecular basis of the resistance against *Barley yellow mosaic virus*.

PlantsProFood – New Varieties of Narrow-Leafed Lupin for Food Industry – Genetic and Molecular Analysis of New Variability

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Sweet narrow-leafed lupins shall be developed as novel protein resource for food production. The project aims at improving the competitiveness of narrow-leafed lupins in Germany's agriculture *via* generation of breeding progress. It is meant as a contribution to the political efforts to promote the use of homegrown legumes as an alternative protein resource. A network of four research groups and ten regional companies will process the entire value chain from new varieties to food production.

Based on EMS mutation novel phenotypes were selected from cultivar 'Boruta'. For phenotyping and genotyping F_2 populations have been developed. The genetic analysis revealed a 1:3 ratio, which is expected for the inheritance of recessive mutations. Molecular markers have been established from different legume genomes and will be useful for marker assisted selection. In cooperation with a lupin breeder stable and high yielded cultivars based on the novel phenotypes will be available for the food industry.

Phenotypic and molecular characterisation of new clubroot resistance genes derived from the primary gene pool of *Brassica napus*

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Clubroot is a serious soil-borne disease of cruciferous crops caused by the obligate biotrophic protist *Plasmodiophora brassicae* which causes galls on roots leading to premature death of the plant. Most problematic is the longevity of the resting spores in the soil up to 20 years and the fact that there are no economically reasonable control measures once a field has been infested. Currently, due to the raising density of oilseed rape cultivation within the last decades the number of contaminated fields detected in many European regions is constantly increasing. Therefore, as up to now only one race specific resistance is incorporated in adapted winter oilseed rape cultivars, it is essential to broaden the genetic base of resistance.

Potential resistance donors out of forage rape *(Brassica napus) and* rutabaga *(B. napus var. napobrassica)* cultivars have been selected by phenotypic resistance against several clubroot isolates in order to generate segregating RIL and DH populations. By phenotyping with clearly differentiating *P. brassicae* isolates under greenhouse conditions, genotyping with a SNP marker set and subsequent QTL mapping several genomic regions which presumably are involved in clubroot resistance have been detected in 2 RIL populations.

Identification of molecular markers associated with QTLs in rye

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Winter rye (*Secale cereale* L.) is a traditional cereal in Middle and Eastern Europe with versatile uses. Rye bread is particularly appreciated due to the high nutritional value of the rye grain. Rye is also used for feeding purposes and as a source for renewable energy to supplement and mix up biomass crop rotations.

Highly productive hybrid varieties keep rye growing competitive in modern agricultural production systems. Because of the complexity of hybrid performance, breeders take much effort to identify best performing cross progenies between inbred lines originating from heterotic pools. Up to now, no markers for quantitative inherited trait loci (QTLs) governing grain yield, thousand-grain weight, plant height or days to heading have been described for rye. Markers associated with QTLs could be used to improve these complex inherited traits more efficiently in practical rye breeding programs.

Recent progress in marker technology renders association mapping in rye on a genome-wide scale a feasible task. We have fingerprinted elite lines representing a successful hybrid rye breeding program with SSR as well as high-throughput, microarray-based DNA markers. Furthermore, candidate gene sequences for selected traits were included. Marker analysis enabled us to assess the genetic diversity in elite inbred lines of rye and to clearly distinguish both heterotic groups ‘Petkus’ and ‘Carsten’ at the molecular level. Correlation analysis identified markers with significant effects on the analysed traits. Sequence information on associated markers allowed for comparative QTL mapping between rye and rice. This approach revealed that orthologs of some of these markers coincide with QTLs that have been reported in rice. Results obtained in this study contribute to elucidate the molecular basics of agronomic important, complex inherited traits in rye.

Fourth section:
Diagnostic and Detection Methods

Resistance and tolerance of potato varieties to *Ditylenchus destructor*

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The potato tuber rot nematode *Ditylenchus destructor* is a nematode pest of potato and listed as a quarantine nematode in many countries. *Ditylenchus destructor* damages tubers and cause tuber rot under storage. Information on resistance and tolerance of potato varieties to *D. destructor* is limited. Experiments were conducted to determine multiplication and tuber damage caused by *D. destructor* on different potato varieties. In Experiment 1, resistance and tolerance of 21 potato varieties were evaluated in a greenhouse at a temperature of 20 +/- 3°C. Potato plants were challenged with 2000 nematodes (female, males and juveniles) upon germination. Pots were replicated five times per variety and laid out in a completely randomized design for twelve weeks. In Experiment 2, multiplication and damage caused by *D. destructor* at different initial population (Pi) densities (0, 100, 500, 1000 and 2000 nematodes) on the potato variety 'Désirée' were evaluated. In both experiments, nematodes were isolated and counted from 5g of tuber tissue and percentage internal and external tuber damage scored. Nematode numbers isolated from tubers varied significantly ($p<0.05$) among potato varieties. Juveniles were the dominant nematode stage isolated. Results from Experiment 1 indicated that inoculation of potato varieties with *D. destructor* leads to significant differences ($p<0.05$) in external and internal potato tuber damage among the 21 potato varieties. Percentage external and internal damage ranged between 7.2 to 44.5 % and 0 to 22 %, respectively. As *D. destructor* initial population densities increased so did the tuber damage in Experiment 2. Percentage external and internal damage ranged between 0 to 68 % and 0 to 56 %, respectively. High nematode densities caused severe potato tuber damage on the variety 'Désirée'. Knowledge on resistance and tolerance of potato varieties is important in the management of *D. destructor.*

Resistance against powdery and downy mildew of grapes - Searching for biomarkers in the grape leaf metabolome

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Powdery and downy mildew of grapevine are widespread diseases, which may cause severe harvest losses. In organic farming only copper containing fungicides are permitted. Since copper accumulates in soil and can be toxic to diverse organisms, the European Commission considers whether or not copper containing fungicides should be restricted. Thus, finding potent naturally occurring copper substitutes is an important research field. Although induced phytoalexines in grape leaves of European cultivares have long been discussed in the literature (LANGCAKE und PRYCE, 1977), wild type grape species and interspecific grape varieties show more efficient resistance mechanisms against mildew. Therefore, comparing the metabolic fingerprints of these resistant grape species with representatives of the more susceptible European grape varieties by non-targeted analysis assays might give some hints to additional resistance biomarkers. Our analyses consider the impact on the metabolome caused by grape species and leaf development stadium. Furthermore, the leaves of different plants of the same species (resp. varieties) were analyzed to examine the particular impact on the metabolite composition. In a first step of the research project we focused on headspace-SPME-GC-MS to analyze the different volatile organic compounds (VOCs) of the leaf metabolome. We could already identify about 70 out of 110 volatiles. Though the chromatographic fingerprints of the volatile patterns of individual species and varieties consist of mainly the same components, there are detectable differences in peak quality and quantity. Multivariate statistical data processing (PCA) showed that both the leaf development stadium and the grape species (resp. variety) have a great impact on the volatile composition. In contrast, the distinctions in the metabolomes of different plants which belong to the same variety are less obvious. Regarding the early stage of investigation clear statements are not yet possible and additional studies are required.

LANGCAKE, P. and R. J. PRYCE, 1977: New Class of Phytoalexins from Grapevines. Experientia **33** (2), 151-152.

Development of a rapid immuno-based method for simultaneous detection of potato viruses with SPR (Surface Plasmon Resonance)

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Phytopathogenic viruses cause substantial losses in yield and may reduce the quality of potatoes in all potato-growing areas. Aside from the control of insect pests as vectors, the use of healthy, virus-free propagated material is the only way to face this problem. In the European Union, ELISA (Enzyme-linked immunosorbent assay) tests for several viruses are required for certified seed potatoes. Because ELISA can detect just one single pathogen per assay, it is relatively time consuming. Additionally, viruses cannot be detected in the potato tuber itself, but in seedlings derived from the germinated tuber. The production of seedlings only for virus test purposes is time consuming and expensive. SPR (Surface Plasmon Resonance) technology is a spectroscopic method to measure the layer thickness on a surface on the nanometer scale. Mostly the SPR-systems are based on SPR-chips coated with a thin metal surface, mainly gold. The binding of virus particles to a specific antibody, which is immobilized on a gold surface, causes a detectable increase in layer thickness. This change can be monitored in real-time, without any modifications of the antibody, which enables a label-free serological detection method. The goal of this project is to establish a Lab-on-a-chip immunoassay, at least as sensitive as conventional ELISA assays, allowing the simultaneous detection of the six most important potato viruses PVA, PVM, PVS, PVX, PVY and PLRV by means of SPR.

Fifth Section:
Epidemiology and Population Dynamics

Analysis of the formation of submersspores and molecular characterization of different isolates of the entomopathogenic fungi *Metarhizium anisopliae*

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The genus *Metarhizium* is one of the best characterized entomopathogenic fungi and the pathogenicity is known against 200 different insect species. To increase the fields of application and reduce the production costs of these fungi, it is essential to maximize the rate of spores obtained during liquid fermentation. Under this aspect, the work concentrates on testing of fermentation parameters in shake-flask cultures, like media composition, incubation-temperature and agitation of the flasks. During these tests, a media composition was found where the fungi produced spores, whereas in another setup no sporeformation could be detected. The observation of this different growth led to another approach in which the differential gene expression was analyzed for *M. anisopliae* strain 43 cultivated in two different liquid media. Therefore, RNA was isolated from the cultures and reverse transcribed into cDNA. Subsequently, the method of suppression subtractive hybridization PCR (SSH-PCR) was used to amplify differentially expressed genes. Due to optimization of the SSH-PCR, a necessary combination between amplification and suppression of the PCR was achieved. After verifying that these genes are indeed differentially expressed, the results should enable the identification of sporulation related genes.

Parallel to this work DNA was extracted from some isolates of *Metarhizium* to amplify a partial sequence of the β-Tubulin gene (*tubB*) and the ITS-region of the ribosomal RNA operon. With these results, phylograms were created with the methods of Neighbor-Joining (NJ), Minimum Evolution (ME) and Maximum-Likelihood (ML) to determine the phylogenetic relationships of the isolates.

Development of the population model of the Western corn rootworm

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The Western corn rootworm (*Diabrotica virgifera virgifera*) is an agricultural pest, which was caught in a pheromone trap in Germany first time in 2007. According to the first estimates Germany has a loss given default of 25 million € (BAUFELD et al., 2006) caused by the high damage potential of these pest per year. A simulation model, which can be used by the internet, was developed to predict, to identify, and to schedule monitoring dates and pest control dates. For a realistic description of the abundance it was necessary to identify and to estimate all important influencing factors affecting the occurrences of this pest. The relationship between these factors and the most important population dynamics processes was mapped by mathematical and deterministic rules into the simulation model. A first version of this simulation model can be used on the internet portal http://diabrotica.jki.bund.de. The user interface allows a single field simulation supported by GIS-elements like a map window and zoom functions to get optimized pest control dates. Also a daily updated risk map is shown for the federal state Bavaria and Baden - Wurttemberg. In the project progression the model needs to be validated and if necessary modified.

BAUFELD P., J.-G. UNGER, U. HEIMBACH (2006): Ein bedeutender Quarantäneschädling im Mais: Westlicher Maiswurzelbohrer *Diabrotica virgifera virgifera* LeConte. Informationsblatt der BBA.

Approaches to model the dispersal of the Western corn rootworm

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The Western corn rootworm (*Diabrotica virgifera virgifera*) is one of the most important pests on maize in the world and endemic to North America. In 1992, *Diabrotica virgifera virgifera* was first detected in Europe close to the Airport of Belgrade. Since then the Western corn rootworm has actively spread through Europe. In 2007, first beetles were caught in pheromone traps in Germany. The previous spread of *Diabrotica* in Europe as well as in North America varied from year to year and from region to region. This shows that dispersal is effected by regional conditions. Hence, a dispersal model should be developed which integrates all relevant regional conditions. The model will consist of the following four components: situation of *Diabrotica*, regional spread, long distance flights and global spread. The component "situation of *Diabrotica*" will include the population development of the Western corn rootworm under regional conditions. The second component of the model, "regional spread", will contain all flights over short distances. The direction of the flights depends on corn growing because the Western corn rootworm follows maize over short distances. Additionally, barrier cells, like cities, are integrated in the component. The Western corn rootworm, however, does not follow maize directly when the beetle flies over long distances and no barriers exist. This behavior is regarded in the component "long distance flights". Furthermore, the Western corn rootworm was detected far away behind the established spread line because of hitchhiking on various means of transport. This fact will be taken into account in the component "global spread".

Sixth Session:
Mixed Presentations

Analysis of costs for the use of pesticides in winter wheat and winter oil seed rape based on Network of Reference Farms Plant Protection 2007-2010

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Since 2007, the Network of Reference Farms Plant Protection has been in operation. Its aim is to gather information about the intensity of pesticide use in main crops and regions every year. Minimum need assessments are also carried out by experts from the plant protection services. Based on these findings, economic analyses regarding the pesticide use in the farms were conducted on winter wheat and winter oil seed rape. Altogether, 13.264 single measures on 1.457 fields were considered during the period 2007-2010. The data were entered into an Oracle-database and evaluated according to regions and pesticide groups. Additionally, for every plant protection measure, both the product costs and operating costs were determined. Based on the intensity of the pesticide treatment measured as Treatment Frequency Index (TFI), the average costs of one plant protection measure and the costs of a TFI at 1.0 were determined. Furthermore, the total costs for plant protection measures were calculated. On average for four years, they amounted to 214.40 € for winter wheat and 247.00 € for winter oil seed rape per hectare per year. Finally, the data were evaluated in correlation with several influences. In winter wheat, there were positive correlations between total costs and yield as well as total costs and soil value and a negative correlation between total costs and farm area. In contrast, winter oil seed rape showed positive correlations between total costs and farm area as well as between total costs and field size. In conclusion, the results showed that the costs for plant protection measures in winter oil seed rape were clearly higher than the costs in winter wheat. This resulted mainly from the overall higher TFI and the higher expenditures for herbicides and insecticides in winter oil seed rape.

Dispersal dynamics of common voles (*Microtus arvalis*) at field-refuge-boundaries

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Common voles (*Microtus arvalis*) can disperse from refuges (e.g. field edges) to arable land and, at high abundances, cause significant losses in agriculture and forestry. To minimise damage it is useful to prevent voles from dispersing to the fields. To apply timely and spatially targeted management methods, sound knowledge about the distribution patterns of voles at field refuge boundaries is required. This study, funded by the German Federal Environmental Foundation (DBU), aims to investigate population dynamics and dispersal patterns of common voles as a basis for a sustainable vole management.

Field sites are located in Saxony-Anhalt, Germany. Grassland areas below wind energy plants, from which common voles invade fields, are used as replicated experimental refuges. To measure dispersal pressure, barrier fences that allow immigration but prevent emigration were installed at some refuges. Since October 2009, population dynamics and dispersal rate from refuges to fields were surveyed monthly by using capture-mark-release. Recapture probability within each trapping session was at least 50%. In general, extrapolated vole density per refuge averaged 150-300 ind./ha. Mowing in June and September 2010 reduced vole abundance clearly. A population increase in August 2010 as well as in August 2011 led to extrapolated abundances of 850 and 1,300 ind./ha, respectively. During this process, numbers rose higher in refuges without barrier fence than in fenced refuges. Telemetry studies and aerial pictures were used to detect vole dispersal dynamics onto the fields for individuals and on population level. Although there was a low vole density, no establishment of refuge-born individuals could be detected by means of live-trapping. Therefore, it can be assumed that the maximum population density in the refuges is not yet reached. Subsequent DNA analyses on collected tissue samples will allow drawing conclusions about possible migration movements between refuges and fields.

Economic analysis of plant protection strategies in winter rye based on long-term field trials in Dahnsdorf

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Plant protection strategies are often evaluated with respect to their efficacy to reduce pest incidence and to improve yield loss relationships. But, the decision on their practical application is based on the economic advantage. Such economic analyses require comprehensive data like those provided by long-term field trials conducted by JKI in Dahnsdorf on "Comparing strategies for environmentally sound plant protection". These experiments compare two different plant protection intensities over a period of 11 years since 1997: "normal"pesticide application according to the observed pest situation (100% strategy) and a reduced dosage with only half of the "normal" pesticide dosage (50% strategy). Both intensities are applied to herbicides (H), fungicides (F) and a combination of herbicides and fungicides (HF). Additionally, two farming systems BS1 and BS2 with slight differences in pesticide application are studied. To identify the economically most efficient plant protection strategy additional costs and revenues caused by the use of pesticides are determined. The treatments H, F and HF for both the 100% and 50% strategies are compared to the untreated control. The additional costs comprise expenses for pesticides and their application. Extra earnings are calculated and presented as net present value (NPV). Analysing the NPVs show that only the HF treatment leads to considerable positive NPVs throughout both strategies and farming systems. Sole herbicide application (H) yields to positive NPVs within the 50% intensity. The positive effect of higher yields of the 100% strategy is outweighed by higher costs for pesticide usage. HF BS2 turned out to be less efficient than BS1. In H no considerable difference between the faming systems can be observed. F BS1 leads to positive NPVs with the 100% strategy being more efficient. Further analyses of climate and pest occurence data is required to explain some the observed differences.

Exposure of non-target species to anticoagulant rodenticides

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Anticoagulant rodenticides (AR) are often used to control rodent pests. The delayed effect of these agents causes two ways of potential poisoning of non-target animals. On the one hand there is a risk to ingest bait from bait stations (primary poisoning); on the other hand the poison can be transferred from poisoned rodents to their predators (secondary poisoning). Until now systematic investigations of these risks are missing in Germany. Hence our aim is to analyze, if residues of ARs can be detected within the food chain (bait, prey, and predator). The focus concentrates on non-target rodents and owls as predators on and around farms. In a field experiment non-target rodents are trapped along different distances to the farm before and after a standardized rodent control. Additionally we trap target animals and collect fresh barn owl (*Tyto alba*) pellets from known nesting or resting sites. We use Brodifacoum for pest control, because it is the most commonly used AR following our farmer within the investigation area (Muenster, North-Rhine Westphalia, Germany). Liver samples of all trapped animals and pellets are analyzed for the eight agents (Brodifacoum, Bromadiolone, Chlorophacinone, Coumatetralyl, Difenacoum, Difethialone, Floucomafen and Warfarin), licensed as biocides to control rats and house mouse. Additionally we monthly collect pellets to analyze the food composition of the owls. These data, in combination with the results of the residue analysis, will give us a background to calculate a potential risk of secondary poisoning of barn owls. Because of seasonal variation of the owl's food composition and variation of species within the trapping different seasons of the year will be under investigation. Preliminary results of the field work and the food composition of barn owls will be presented as well as methods used in this project.

Posters

Predation efficiency of ladybird beetles *Harmonia axyridis* and *Coccinella septempunctata* on *Daktulosphaira vitifoliae*

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The ladybird beetles *Harmonia axyridis* and *Coccinella septempunctata* are used for biological control in cultures like hop or field crops. *H. axyridis* was therefore introduced in several countries. In viticulture, both species are not always welcome due to their potential to influence the taste of wine. Their potential in reducing the grapevine pest *Dactulosphaira vitifoliae* (grape phylloxera) has not been investigated so far. *D. vitifoliae* is an important root damaging, leaf feeding and gall-forming insect for grapevines of *Vitis vinifera*. In Europe there is a lack of efficient natural enemies to control grape phylloxera. In our trials, *H. axyridis* adults consumed appr. 90% of grape phylloxera eggs (500 offered simultaneously to each beetle) within 24 h. When fed exclusively with these eggs, the average developmental period (from 1st instar to adult stage) was 18.40 +/- 7.73 days, the mortality 33%, and the average adult weight 18.20 +/- 4.12 mg. In contrast, *C. septempunctata* consumed less than 20% of offered phylloxera eggs in 24 h and could not complete its larval development. Thus, the mortality rate was 100%. Field observations on grapevines with and without leaf galls showed that *H. axyridis* was significantly more abundant on grapevines than *C. septempunctata*, and also more abundant on grapevines infested by leaf galls of grape phylloxera. Its abundance was significantly lower on leaves infested by a few leaf galls, than on higher infested leaves ($p<0.05$). Thus, we conclude that *H. axyridis* can use D*. vitifoliae* as prey and track grapevines with high infestation of leaf galls under field conditions. In conclusion, *H. axyridis* represents an efficient predator for grape phylloxera in vineyards. In contrast, *C. septempunctata* plays no role for the biological control of *D. vitifoliae.*

Development of a sensor to monitor the thickness of grapevine berry skin

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The necrotrophic fungus *Botrytis cinerea* Pers. which is the anamorph of the ascomycete *Botryotinia fuckeliana* Whetzel affects more than 200 plant species. On grapevine the fungus grows either as grey mold (bunch rot) or as noble rot. Grey mould of grapevines causes huge harvest losses in vineyards. One important objective of the present study is the development of a sensor to determine the thickness of the grapevine berry skin and of the wax layer. A prototype sensor was constructed measuring a specific physical property of berry skin as a potential indicator for *Botrytis* susceptibility. We analyzed seven cultivars during the grape berry ripening and a F1-breeding population at a defined time of ripening. In parallel the *Botrytis* infection and grape cluster architecture was evaluated in the vineyard. Preliminary results indicate a correlation between the measured physical value and thickness of cuticle as well as *Botrytis* susceptibility.

A new experimental system to study the virus entry into plant growing points

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Understanding the mechanisms of plant-virus interaction is an essential prerequisite to develop appropriate defence approaches against pathogens. Plant growing points containing the shoot apical meristem have been thought to be protected from virus infection, particularly PVX. We developed a new experimental system, consisting of PVX and recombinant PVX-Cre (P1 recombinase), to study virus entry into growing points. In comparison to PVX infected plants, PVX-Cre infected plants were more severely diseased, stunted and later displayed a "recovery" phenotype. It is known that the recovery-inducing RNA viruses have the unusual ability to infect meristems (RATCLIFF *et al.*, 1999). Based on phenotypic observations of PVX and PVX-Cre infected *N. benthamiana* plants we proposed a more efficient apical colonization by PVX-Cre. To test this hypothesis, Cre-transgenic *N. benthamiana* plants were inoculated with PVX-GFP. Most of the apical shoots investigated exhibited GFP fluorescence. Results reported from studies with other RNA viruses indicated that RNA silencing is implicated in virus meristem exclusion (QU *et al.*, 2005; SCHWACH *et al.*, 2005). To investigate if PVX-Cre is able to suppress RNA silencing, we performed a transient expression assay using *N. benthamiana* plants and a *gfp*-expressing construct. In the absence of PVX-Cre the *gfp* expression was weak most probably due to silencing. Co-expression of PVX-Cre with the *gfp*-containing construct leads to enhanced *gfp* expression. These findings indicate that PVX-Cre might provide a silencing suppressor activity, which allows overcoming the meristem exclusion. Detailed analysis of this phenomenon is in progress.

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The role of the *avrRpt2EA* gene in the host-pathogen interaction *Malus × robusta* 5 - *Erwinia amylovora*

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The enterobacterium *Erwinia amylovora* is a quarantine pathogen, which causes the necrotrophic fire blight, a disease of plants of the rosaceous family (pear, apple, quince etc.). The relevance of this disease, especially in the apple production, is immense and the only effective control by antibiotics restricted. Therefore, resistant wild species like *Malus x robusta* 5 (Mr5) are important for both breeding and genetic studies on the resistance mechanism. Until now, only a few genes are known to be involved in the resistance mechanism. One of these genes encodes the effector protein AvrRpt2$_{EA}$ from *E. amylovora*, which is homologue to the initial found effector protein *avrRpt2* of *Pseudomonas syringae*. Surprisingly, an *avrRpt2*$_{EA}$ mutant strain, in which the gene was disrupted and replaced with an antibiotic resistance cassette, could break the resistance of *Malus × robusta* 5. This result suggests that the *avrRpt2*$_{EA}$ gene plays an important role in the resistance. To get a better understanding of the mechanism, the first step was to compare the nucleotide sequence and the resulting amino acid sequence of the *avrRpt2*$_{EA}$ gene of different *E. amylovora* strains. Various genes were then used to complement the *avrRpt2*$_{EA}$ mutant strain and several wild type strains. For the evaluation of the virulence, Mr5 shoots were inoculated and the susceptibility to the bacterium was evaluated by determining the necrotic shoot length. Furthermore, the interacting partner of the AvrRpt2$_{EA}$ protein in *Malus × robusta* 5 was studied. Therefore, the Yeast Two-Hybrid System, a method to determine protein-protein interactions, was used. In this system the *avrRpt2*$_{EA}$ gene acts as "bait" and was screened against putative interacting partners in Mr5.

Key factors influencing fate and activity of bacterial inoculants and their effect on the indigenous microbial community in the rhizosphere

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Rhizoctonia solani is a soil-borne plant pathogen which causes bottom rot disease and leads to a massive loss of lettuce and potato every year. The lack of effective fungicides makes it difficult to control this and other plant pathogens (WELLER *et al.*, 2002). So it is necessary to find alternative strategies. A promising approach is the use of natural antagonists of the plant pathogen. Under laboratory and greenhouse conditions two isolates, *Pseudomonas jessenii* RU47 and *Serratia plymuthica* 3Re4-18, showed the ability to reduce disease symptoms (ADESINA *et al.*, 2007). But the efficiency of biocontrol agents was reported as very variable and the reason for this variability is largely unknown (ROBINSON-BOYER *et al.*, 2009). Therefore, a better understanding of the interaction of the microbial community, the plant rhizosphere and the soil is required for a successful exploitation of this antagonistic potential. Within the frame of a DFG-Project a field experiment has been set up with a unique experimental plot system in Großbeeren comparing three different soil types. This made it possible to analyze the influence of the soil type independently from other factors such as climate and cropping history. Each soil type came under six different treatments with four replicates each: 1. water control, 2. pathogen control, 3. inoculation with *P. jessenii* RU47, 4. inoculation with *S. plymuthica* 3Re4-18, 5. inoculation with *P. jessenii* RU47 and *R. solani*, and 6. inoculation with *S. plymuthica* 3Re4-18 and *R. solani*. From the extracted total community-DNA 16S rRNA gene fragments were amplified and run in a DGGE-Gel to give insights into the structural diversity of microbes in the rhizosphere (SMALLA *et al.*, 2001). This molecular fingerprint technique and the following statistical analysis showed a clear difference between the three different soil types. These results revealed that the soil type is the driving factor for the composition of the microbial community while the treatment with the biocontrol agents was of minor influence.

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Towards functional markers for apple aroma - Assessment of allelic diversity in members of the lipoxygenase (LOX) gene family

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At the NWF 2010 the background and the major aims of the project were presented together with first results on bioinformatic LOX (lipoxygenase) gene mining. In the forthcoming presentation, a comprehensive phylogenetic tree of the apple LOX gene family will be displayed, that proves the determination of sequences as genes and indicates different alleles. Furthermore, an overview about the so far from *Malus* cloned series of LOX genes is given, and the strategy for direct sequencing and evaluation of the allelic state of selected LOX genes is demonstrated.

In the past, it was a laborious and expensive task to sequence large sets of gene-specific amplicons produced by specific primers in different cultivars and to obtain sufficient information concerning the allelic diversity of a putative candidate gene. Thus, fragment length analyses of simple sequence repeat (SSR) arrays were used for association analyses, but because of much lower frequency of loci they have lower information content, compared with single nucleotide polymorphisms (SNPs), which are an abundant source of molecular variation in a plant genome. Nowadays, it is possible to use sequence data from databanks, to create gene specific primers instead of degenerate primers. Especially for larger gene families, such as LOX, highly specific PCR primers are crucial for achieving accurate sequencing results from a heterozygous plant like apple. After sequencing the PCR fragments of chosen LOX members in 29 apple cultivars with available aroma profiles, several putative SNPs have been identified. These allelic data will further be used for association analyses of LOX genes and their alleles which might be involved in volatile organic compound (VOC) production in apple. The final goal of the reported investigation is to develop SNP-based functional marker assays that can be used for marker assisted selection of fruit quality traits in young apple seedlings.

Fate and effect of sulfadiazine in bulk soil and in the rhizosphere of maize: a mesocosm study

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Spread and evolution of antibiotic resistance genes through agriculture pose a possible risk for human health, e.g. by increasing resistance problems in human antibiotic therapy. The "DFG Forschergruppe 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 (KOOLS *et al.*, 2008). It is excreted largely unchanged by the animals and enters agricultural soils through the use of manure and slurry as fertilizer. Thereby, it can have effects on the functional and structural composition of the soil microbial community and its activity and may promote the formation and spreading of resistance genes by mobile genetic elements such as plasmids (HEUER *et al.*, 2011). Recently it was shown by BRANDT *et al*. that the addition of artificial root exudates increased the bacterial community tolerance towards SDZ, indicating that the rhizosphere might be a hotspot of resistant bacteria (BRANDT *et al.*, 2009). On the other hand, the dissipation of bioaccessible SDZ-concentrations was accelerated in rhizosphere soil (ROSENDAHL *et al.*, 2011). However, knowledge of the abundance and dynamics of sulfonamide resistance genes in the rhizosphere is scarce. We therefore will present results on the fate and effect of SDZ in bulk soil and rhizosphere of maize plants which were studied in a mesocosm experiment. The abundance and dynamics of sulfonamide resistance genes (*sul1*, *sul2*) and major plasmid vectors were assessed by cultivation-independent approaches (qPCR; exogenous plasmid isolation). The main findings were (I) the significantly increased abundance of *sul* genes when the soil was treated with manure containing SDZ, (II) the majority of the plasmids captured belonged to the novel LowGC-type family, and (III) unexpectedly the relative abundance of *sul* genes was lower in the rhizosphere compared to bulk soil.

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Bacterial community in artificial soils structured by mineral composition and charcoal responded to phenanthrene

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In soil, different organic, inorganic and biological constituents are contacting each other and forming large biogeochemical interfaces upon which important processes for the ecosystem act. As it is still not known how these different components interact, this study focuses on the interplay of soil minerals and charcoal with microbial communities. Since the comparison of microbial communities from natural soils is very problematic, seven different artificial soils were used. They are characterized by a known mineral composition consisting of illite, montmorillonite, ferrihydrite, boehmite, charcoal and quartz sand. After adding autoclaved manure as organic matter and the same microbial community extracted from a natural Cambisol to each artificial soil mixture, they were incubated under constant environmental conditions up to 18 months. Furthermore, the response of microbial communities at biogeochemical interfaces to persistent organic pollutants using phenanthrene as example was explored. Therefore, one-year old artificial soils were spiked with phenanthrene (2 g/kg) and incubated for another 70 days. By a cultivation-dependent approach it was shown that the bacterial density in soil treated with phenanthrene is higher. For *Fungi,* higher colony forming units (CFUs) in the soil with charcoal were monitored whereas the soil with illite showed a higher diversity. Cultivation-independently, total community DNA was extracted and the 16S rRNA gene and ITS amplicons for *Bacteria* or for *Fungi*, respectively, were used in denaturing gradient gel electrophoresis (DGGE) to generate molecular fingerprints. DGGE analysis showed that the mineral composition and charcoal influence the establishment of microbial communities in artificial soils, even after a long incubation time. Especially the charcoal soil showed a distinctly different pattern compared to other artificial soils without charcoal. Additionally, the DGGE data revealed a bacterial response to phenanthrene spiking in the long term.

To conclude, mineral composition, charcoal and persistent organic pollutants using phenanthrene as model compound are important factors that shape the composition of the microbial communities established in artificial soils.

Suppression of *Globodera pallida* in monoculture cropping of potato

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Potato cyst nematodes*, Globodera pallida* and *G. rostochiensis*, can cause serious yield losses in potato. Plant-parasitic nematodes inhabit the soil matrix that supports microbial communities. In some soils, microbes confer soil suppressiveness. In these, plant-parasitic nematodes cannot develop to high population densities, despite the presence of a susceptible host and favorable environmental conditions. Specific suppressiveness against soil-borne diseases is transferable with small portions of soil to non-suppressive soils. Potentially, suppressive soils are sources of highly effective biological control organisms. For example in previously published studies, *Dactylella oviparasitica* isolated from suppressive soil in California reduced population densities of the sugarbeet cyst nematode (*Heterodera schachtii*) as well as root-knot nematodes (*Meloidogyne* spp.). The objectives of this study were to determine whether a potato monoculture soil had become specifically suppressive to *G. pallida*, and if *D. oviparasitica* can suppress the population density of *Globodera* spp. In the first experiment, soil taken from microplots infested with *Globodera pallida*, and untreated or treated with *D. oviparasitica*, were tested for specific soil suppressiveness against *G. pallida*. In two consecutive cropping cycles of potato in these plots, nematode eggs had increased in 2009 but decreased in 2010. In greenhouse pot tests, population density development of added *G. pallida* was compared after one potato crop in sandy soil amended with 10% untreated or pasteurized portions of soil from these microplots. In contrast to the hypothesis, population densities did not decline in the untreated soil containing treatment. In a second greenhouse experiment with potato, suppressive potential of *D. oviparasitica* to different pathotypes of PCN (*G. pallida* Pa2 and Pa3, and *G. rostochiensis* Ro 1 and Ro2) was evaluated. Soil amendment with *D. oviparasitica* did not impact the number of cysts, eggs and juvenile of these nematode species. Further studies to elucidate the soil suppressiveness to nematodes are ongoing.

Occurrence of plant-parasitic nematodes in cut-flowers of Ethiopia

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In Ethiopia, the floriculture sector is booming that makes it the 2nd and 6th largest rose exporter in Africa and in the world, respectively, due to its favorable diverse agroclimate. Similar to other crops, cut flower production also faces problems that reduces both qualitative and quantitative yield. Usually pests are common problems of which nematodes are among the prevalent pests attacking floricultural crops. They infect most living plant parts including flowers, buds, leaves, stems and roots. However, information on the occurrence, biodiversity and damage potential of plant-parasitic nematodes on cut flowers are almost non-existent, although damage caused by nematodes is repeatedly mentioned by the growers. Therefore this survey was initiated to monitor the occurrence, distribution, and abundance of plant-parasitic nematodes associated with cut-flowers in Ethiopia. Accordingly, the survey was carried out from July to September 2011 covering 14 flower farms representing different regions, agroclimate and cut flower species. Per farm, 10 to 14 soil samples composed of 40 soil cores from the top 20 cm were collected randomly for rose, freesia, carnation, gypsophila, and statice making a total of 152 samples. Then aliquots of 200 ml soil were used to extract nematodes using the modified Baermann technique and heat killed and fixed in TAF before they were brought to JKI for morphological analysis. The preliminary observation indicates that cut flowers are a host to one or more nematode genera. At least the following nematode genera are identified as being detected: *Helicotylenchus*, *Criconemella*, *Aphel-encoide, Meloidogyne*, *Pratylenchus* and *Xyphinema*. Indeed, the detection level appears variable among sampling sites that might be as a result of the pesticide and fungicides applied. Nevertheless, this survey shows the presence of potential plant-parasitic nematodes in the cut flower farms of Ethiopia which is a start for future nematode management strategies.

Approaching the dominant dwarfing gene Ddw1 in rye by comparative genetics

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Winter rye (*Secale cereale* L.) is a traditional cereal in Germany with versatile uses for human and animal nutrition as well as a substrate for bioenergy production. Rye displays higher stability in yield compared to wheat and barley on marginal-gain soils due to its pronounced abiotic stress tolerance. Rye breeding aims to develop new high yielding varieties which are, in view of the global climate change, more robust and less demanding in water and fertilizer. These efforts are directed to enhance the sustainability of rye production and to keep rye competitive in modern agricultural production systems.

Lodging resistance ranks among the major breeding goals in rye. Improved lodging resistance will reduce the application of growth regulators and, thus, increase the effiency of rye production. The main approach to overcome lodging is a reduction of plant height by exploiting dwarfing mutants. In wheat and rice the alteration of plant height by recessive dwarfing genes resulted in a dramatically increase of crop yield. In rye, particularly the dominant dwarfing gene *Ddw1* has been used in Eastern European and Finnish breeding programs to improve lodging in population varieties. The potential of *Ddw1* for breeding highly productive hybrid rye varieties has yet not been elucidated. The implementation of *Ddw1* in the development of homozygous dwarf inbred lines is hampered, as an efficient and reliable method to distinguish homo- and heterozygous dwarf genotypes is currently not available. In this study, we are approaching *Ddw1* located on the long arm of rye chromosome 5R by comparative genetics. We have identified the *Ddw1*-orthologous regions in rice, *Brachypodium*, and *Sorghum*, and were able to use the gene models of these grasses to develop conserved orthologous sequence (COS) markers for *Ddw1*. Results will be presented on mapping the novel COS markers relative to *Ddw1*.

Reproductive potential of *Heterodera schachtii* on various weeds in fallow periods before sugar beet cultivation

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The sugar beet cyst nematode *Heterodera schachtii* is one of the most important pests in sugar beet production and causes severe yield losses. In Europe, typical prevention methods are the use of wide crop rotations, resistant cover crops, and resistant and tolerant cultivars of sugar beet. It is not fully understood to what extent weeds can support reproduction of *H. schachtii* in fallow periods prior to sugar beet. Objectives of this study were (A) to determine the weeds present in otherwise fallow fields during late summer, and (B) to analyze their reproductive potential. In these experiments over three years, the most important weeds were: white goose foot, black nightshade, annual mercury, chickweed, field penny-cress, ivy-leaved speedwell, red-root amaranth, field bindweed, red deadnettle, earth, smoke black bindweed, redshank, small nettle, corn chamomile, chamomile, field mustard and gallant soldier. For greenhouse experiments, additional weed species were selected primarily some that belong to the families of Brassicaceae, Amaranthaceae, Caryophyllaceae, Asteraceae and Fabaceae. In the greenhouse experiments with susceptible and resistant sugar beet cultivars as controls, mustard, pepperweed, field penny-cress, cowherb, chickweed, tall mustard, shepherd's-purse and black mustard supported high levels of reproduction of *H. schachtii*, similar to those of susceptible standards. Red sorrel, hare's ear mustard, hairy tare, night-flowering catchfly, chamomile, yellow pea, corn cockle, black nightshade, cleavers and common vetch allowed low levels of cyst nematode reproduction comparable to the resistant standards. Additional field experimentation with a smaller range of weed species is ongoing to test how the results from the greenhouse relate to nematode reproduction under field conditions.

The overall aim of this project is to determine the relative reproductive potential of weeds to generate management recommendations for weed control before sugar beet cultivation in *H. schachtii*-infested fields.

There's something in the water - Guttation and the risk for honey bee colonies (*Apis mellifera* L.): a worst case semi-field scenario in maize

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The study was performed under semi-field conditions in Lucklum (Braunschweig, North Germany), in seed treated (Clothianidin) and untreated maize. The tents (4 treatment and 2 control) had an area of 96m^2 (16 x 6m) each and were covered with a gauze. The study was repeated twice, in the first run (BBCH 13-15) with two variants, one with and one without artificial water source containing uncontaminated tap water. In the second run (BBCH 15-19) all variants had an artificial water source. Bee colonies used were of similar size with approximately 10.000 bees. The adult mortality of bees was assessed in dead bee traps and on linen sheets in the crop. The flight activity and behavior of bees at the entrance of the hives and in three flight squares in the crop were determined once daily. The observation time in the tents were 10 days, the observation of brood development for 100 brood cells per hive was conducted nearly for four weeks. During the whole observation period the occurrence of guttation was documented. In the first run of the study, in the artificial extreme situation without any additional water supplies a high impact on mortality and also on the brood development was observed, indicating the sensitivity of the test system but representing an unrealistic worst case scenario. In variants with treated maize and additional water supply, no effects on adult mortality and brood were observed. The results of the second run are also with no effects on adult mortality or brood development.

Influence of temperature on hatch of beet cyst nematodes (*Heterodera schachtii* and *Heterodera betae*)

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Temperature influences the life cycle biology of nematodes. The active part of the life cycle of cyst nematodes starts when the second stage juvenile hatches from the egg and leaves the cyst. Cyst nematodes exhibit considerable variation in temperature preferences for hatching. The aim of this study was to investigate the differences in minimal and optimal hatch temperatures of the beet cyst nematode species *Heterodera schachtii* and *H. betae*. Hatching dynamics were observed at 6 temperatures (5, 10, 15, 20, 25 and 30 °C) during a period of 6 weeks. Results showed that *H. schachtii* hatched between 10 and 30 °C with the highest hatching rates at 25 and 30 °C. Hatching of *H. betae* only started from 15 °C onwards and highest hatching rates were attained at 25 °C. *H. betae* still hatched at 30 °C, but hatching rates were lower than for *H. schachtii*. The results suggest different temperature preferences for hatch for both beet cyst nematode species.

The *Agrotis* baculovirus complex: multiple viruses for multiple pests

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Larvae of the genus *Agrotis* (Lepidoptera: Noctuidae) are known to be severe soil pests on a wide range of field crops and vegetables in Europe, Asia and Africa. *Agrotis spec*. are highly susceptible for a broad number of baculoviruses and in the past, two Alphabaculoviruses (AgseNPV-A and AgseNPV-B) and one Betabaculovirus (AgseGV) were isolated from the common cutworm *A. segetum*. From larvae of the black cutworm *A. ipsilon* another Alphabaculovirus, *Agrotis ipsilon nucleopolyhedrovirus* (AgipNPV), was isolated. Bioassay analysis demonstrated the cross-infectivity of all four baculoviruses for both hosts, which made them potential biocontrol agents for the control of cutworms. Especially in terms of resistance management the usage of a combination of different baculoviruses is regarded to be useful. In order to develop methods for identification of the different viruses we developed a multiplex polymerase chain reaction (PCR) and quantitative PCR (qPCR) based method. The genome of AgseNPV-B was completely sequenced and a comparative genome analysis of AgseNPV-B, AgseNPV-A and AgipNPV was conducted. Phylogenetic analysis confirmed the close relationship of AgseNPV-B and AgipNPV by a high sequence similarity, although the genome length and number of open reading frames (ORF) of AgseNPV-B and AgseNPV-A were more alike.

For biological characterization bioassays and the determination of the median lethal dose (LC50) of AgipNPV and AgseNPV for their common host *A. segetum*, were performed. This work is the basis to analyze the molecular and cellular interaction of these viruses in mixed infections and to optimize the application of these viruses for *Agrotis* control.

Variation in virus content among individual leaves of susceptible barley infected with *Barley dwarf virus*

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Wheat dwarf virus (WDV) and *Barley dwarf virus* (BDV) are circular single-stranded DNA-viruses belonging to the family *Geminiviridae*. Infected cereals are dwarfed, stunted, show yellow streaks and have a drastically reduced yield or die off early. WDV was first described in 1961 by VACKE in the Czech Republic. Since then it was found throughout Europe. In 2007 a strain infecting mostly barley was described as *Barley dwarf virus* (SCHUBERT *et al*.). It shows about 83-84% nucleotide identity with WDV. Both viruses are transmitted by the leafhopper *Psammotettix alienus* in a persistent non-propagative manner.

To study virus infection a very sensitive qPCR assay was developed. It allows detection and quantification of both viruses due to primers in conserved regions of the coat protein gene. The highly susceptible winter barley 'Rubina' was inoculated with 3 viruliferous leafhoppers under controlled greenhouse conditions. Samples of all leave tips were taken 7, 14, 21 and 28 days after inoculation (dpi) and analyzed with qPCR. Viral copies were detectable in all leaf tips, but virus concentrations varied between leaves. The eldest leaf, which was the inoculated one, had a virus content in the range of 10^3 copies/30 ng plant DNA 7 dpi increasing to 10^5 copies at 28 dpi. The younger leaves had a higher virus DNA content than the eldest one at every sampling date, the youngest reaching viral loads in the range of 10^8 copies 28 dpi. No significant difference could be found between the virus content of the tip and the base of the same leaf.

In conclusion it is advisable to harvest the youngest leaf tip to test plants for virus infection. For experiments where virus contents of plants are compared the leaf tip of the same level should be sampled.

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