Siebentes Nachwuchswissenschaftlerforum 2014

26. - 28. November
in Quedlinburg

- Abstracts -

Berichte aus dem Julius Kühn-Institut

177

Julius Kühn-Institut
Bundesforschungsinstitut für Kulturpflanzen

Kontaktadresse
Heike Riegler
Julius Kühn-Institut (JKI)
Bundesforschungsinstitut für Kulturpflanzen
Informationszentrum und Bibliothek
Erwin-Baur-Straße 27
06484 Quedlinburg

Telefon +49 (0)3946 47-123
Telefax +49 (0)3946 47-300

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

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

ISSN 1866-590X
DOI 10.5073/berjki.2014.177.000

Greetings from the President

Dear Young Scientists,

Welcome to the 7th Young Scientists Meeting of the Julius Kühn-Institut at the JKI head quarters in Quedlinburg.

The “Wissenschaftsrat” being the independent evaluation board of the German Government for federal research institutions visited the JKI this spring. The JKI has been positively evaluated. In the evaluation report the commission states that ‘the JKI is very committed to mentoring young scientists’ and that ‘due to the attractive working environment, there are no problems recruiting new PhD students.’ You, as young scientists, have contributed to this success by organizing and attending the Young Scientists Meeting. I would like to express my gratitude to the members of the organizing board for their efforts and for the time they invested so that the tradition of the Young Scientists Meeting is continued and the concept of the event is carried into the future.

Prompted by the feedback from last year’s attendees, the classic program of talks and poster sessions has been modified. Each institute will be introduced briefly before its scientists present their research projects. Also the poster presenters are facing a new challenge this year, having to perform a series of so called “elevator pitches”. In concise two minutes each topic has to be presented without showing numbers, graphs or results. In times of “science slams” and “pecha kucha nights” audiences favor more popular science presentations. All contributions will be in English, as a practical training for the international conferences, you will be attending in the future.

Two keynote lectures complete the program. Friedrich Fauser from the Karlsruhe Institute of Technology talks about techniques in plant breeding, a central topic at the JKI. Steffen Kecke, head of the JKI data processing department, gives an overview of electronic resources and data collections, valuable tools for your research.

I am convinced that all participants will personally benefit from the NWF by expanding their knowledge and by increasing their presentation skills. In addition, you get the chance to get to know each other during two joint evening events and expand your personal network, an important base for your future professional life.

I hope you all enjoy your stay at Quedlinburg and return home enriched by the experience and inspired both scientifically and socially.

Quedlinburg, November 2014

Dr. Georg F. Backhaus
President of the JKI

Content

Session 4: Institute for Plant Protection in Fruit Crops and Viticulture

Session 5: Institute for Epidemiology and Pathogen Diagnostics

Session 6: Institute for Biological Control

Postersession

Keynote 2

Keynote 1

CRISPR/Cas-based genome engineering

Friedrich Fauser[1], Simon Schiml[1], Jeannette Steinert[1], Gabriele Ilg[1] and Holger Puchta[1]
[1] Karlsruhe Institute of Technology (KIT), Botany II
Email of corresponding author: friedrich.fauser@kit.edu

The CRISPR/Cas system is becoming the major tool for targeted mutagenesis in eukaryotes to induce either double-strand breaks (DSBs) or single-strand breaks at preselected genomic sites. Thus, homologous recombination (HR) can be enhanced and targeted mutagenesis can be achieved by error-prone non-homologous end joining (NHEJ). Recently, we were able to demonstrate heritable targeted mutagenesis in *Arabidopsis thaliana* as well as the first application of a Cas9 nickase in plants. Using a natural nuclease and marker genes, we also developed an *in planta* gene targeting (GT) strategy in which both the GT vector and the target locus are activated simultaneously via DSB induction during plant development.

We demonstrate that the *in planta* GT strategy can be used for natural genes by Cas9-mediated DSB induction. We were able to integrate a resistance cassette into the *ADH1* locus of *A. thaliana* via HR. Heritable events were identified using PCR-based genotyping, characterized by Southern blotting and confirmed on the sequence level. Moreover, a major concern is the specificity of the CRISPR/Cas system. Off-target effects might be avoided using two adjacent sgRNA target sequences to guide the Cas9 nickase to each of the two DNA strands, resulting in the formation of a DSB. By amplicon deep sequencing, we demonstrate that this Cas9 paired nickase strategy has a mutagenic potential comparable to that of the nuclease. We also demonstrate the stable inheritance of such mutations in *A. thaliana*.

Taken together, we provide the plant community with a highly efficient CRISPR/Cas system. Most notably, the *in planta* GT strategy does not rely on efficient transformation and regeneration procedures, indicating the benefit for application in crop plants to improve elite cultivars.

This work is funded by the European Research Council (Advanced Grand 'COMREC') and by the Federal Ministry of Education and Research (PLANT 2030, Grand 'TAMOCRO').

Session 1

Institute for Grapevine Breeding Geilweilerhof

Berry color mutants of traditional grapevine cultivars

Franco Röckel[1], Ludger Hausmann[1], Erika Maul[1], Reinhard Töpfer[1]
[1] Julius Kühn-Institut, Institute for Grapevine Breeding Geilweilerhof, Siebeldingen
Email of corresponding author: franco.roeckel@jki.bund.de

The color of the berries is one of the most important fruit traits in grape and can vary from green/yellow to blue/black. Responsible for the coloration is mainly the anthocyanin composition and concentration in the berry skin, whereas the berry flesh mostly remains colorless. It has been shown that anthocyanin biosynthesis is controlled by two adjacent MYB-related transcription factor genes, *VvmybA1* and *VvmybA2*, located on chromosome 2. Loss-of-function mutations in both genes, an insertion of a Ty3-gypsy-type retrotransposon (*Gret1*) in the promoter region of *VvmybA1* and two amino acid-changing mutations in the coding sequence of *VvmybA2*, were identified leading in combination to a non-functional allele. Independent genetic studies revealed that white-skinned cultivars are homozygous for the non-functional allele, whereas colored-skinned cultivars possess at least one functional allele.

Because berry color mutations are a relatively frequent event, a lot of different color mutants have been selected since the rise of viticulture. Color recoveries, from white to red berries, are the most observed mutations, all of which may have a different molecular reason. Large scale parentage analysis identified the white-skinned cultivar 'Heunisch Weiss', with at least 120 offsprings in the European wine growing countries, as the most prolific grape since its rise in the first millennium A.D. in Western Europe. For example, it could be confirmed that 'Heunisch Weiss' is a parent of the famous cultivars 'Riesling', 'Chardonnay', 'Elbling', 'Gamay' and 'Blaufränkisch'.

This study focuses on the analysis of the molecular basis of color recovery in the bud sports 'Heunisch Dreifarbig', 'Heunisch Rotgestreift', 'Riesling Rot' and 'Elbling Rot' and its underlying mechanisms.

Quality determining loci in grapevine

Franziska Huber[1], Rudolf Eibach[1], Florian Schwander[1], Reinhard Töpfer[1]
[1] Julius Kühn-Institut, Institute for Grapevine Breeding, Siebeldingen
Email of corresponding author: franziska.huber@jki.bund.de

Nowadays, wild vine accessions are valuable sources for all kinds of pathogen resistances and therefore are important resistance donors in grapevine breeding. With the aim of reducing chemical plant protection products, they are used to generate new resistant cultivars. Usually, several pseudo backcrossings with *V. vinifera* cultivars are necessary to re-establish a wine quality comparable to the traditional cultivar's while introgressing the pathogen resistances. All in all decades are required to complete backcross and selection for wine quality.

In this study, a population of the hybrid `Catawba´ and the elite cultivar `Lemberger´ is investigated concerning the Labrusca-typical ("foxy") flavors which are frequently appreciated in table grapes but undesired in wines. This study aims at developing quality-correlating genetic markers that are applicable for marker-assisted selection.

For almost 200 years there were several assumptions about the origin of `Catawba´. Among these, `Catawba´ was supposed to be the result of a cross between a high-quality European cultivar (*Vitis vinifera*) and a native North American wild grape (*Vitis labrusca*).

The *Vitis vinifera* parent could be identified by genetic fingerprinting. This old hybrid is very popular in North America and is still used for juice and table grape production in the region of New York State. Besides this, the parent-child relationship between `Catawba´ and `Concord´ could be confirmed by SSR marker techniques.

This knowledge promotes the search for genomic areas responsible for the biosynthesis of Labrusca-typical aromas such as Furaneol ("strawberry") and methyl anthranilate ("mothball") in the offspring of the cross `Lemberger´ x `Catawba´. Anyway, this finding is a gain in the field of genetic resources in viticulture and to implement new traits into the breeding program.

Sequence analysis of loci *Rpv10* and *Rpv3* for resistance against grapevine downy mildew (*Plasmopara viticola*)

Jens Dudenhöffer[1], Florian Schwander[1], Reinhard Töpfer[1], Eva Zyprian[1]
[1] Julius Kühn-Institut, Institute for Grapevine Breeding Geilweilerhof, Siebeldingen
Email of corresponding author: eva.zyprian@jki.bund.de

Among several other partners the Julius Kühn-Institut at Siebeldingen is involved in a cooperative project called “Bacchus” funded by the 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).

The oomycete *Plasmopara viticola* causing downy mildew 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 an extensive amount of fungicide applications are necessary to enable the cultivation of healthy grapes. For that reason newly bred mildew resistant varieties are a major contribution to reduce the amount of fungicides for sustainable 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 e. g. cv. 'Regent') and *Rpv10* (Asian origin e. g. cv. 'Solaris') are known.

Up to now we could assemble most of the region of *Rpv10* via amplicon sequencing. *Rpv3*-linked molecular SSR-markers were used to screen for contigs from the *Rpv3* locus in two different Next Generation Sequencing datasets of resistant cultivars. Three possible candidate genes for each downy mildew resistance locus could be found so far. They possess protein domains showing similarity to resistance genes found in other plant species. In comparison to the grapevine reference genome of 'Pinot noir' (PN40024) - which is susceptible for *Plasmopara viticola* - some genes show major differences in essential protein domains.

RNASeq analysis was done of plants carrying *Rpv10*, *Rpv3* or none of these loci. The samples were collected in the very early hours after inoculation with the pathogen to investigate the expression level of the genes possibly conferring resistance against *Plasmopara viticola*. The analysis of the RNASeq data is in progress.

First candidate genes in the resistance locus *Ren3* against grapevine powdery mildew (*Erysiphe necator*)

Daniel Zendler[1], Pierre Schneider[1], Reinhard Töpfer[1], Eva Zyprian[1]
[1] Julius Kühn-Institut, Institute for Grapevine Breeding Geilweilerhof, Siebeldingen
Email of corresponding author: eva.zyprian@jki.bund.de

One of the most devastating fungal diseases in European grapevine *Vitis vinifera* L., is powdery mildew. This disease which is caused by the pathogen *Erysiphe necator* leads to enourmous annual losses of yield if no counteractions such as the excessive use of fungicides are taken. The pathogen was introduced to Europe around 1845 from America and has spread over nearly all grapevine growing regions worldwide.

American wild *Vitis* species have obtained a natural resistance to *E. necator* due to co-evolution and are preferred resources for grapevine resistance breeding. Up to now there are several loci known originating from these wild *Vitis* species which confer resistance to powdery mildew. One of these is *Ren3* which was first detected in the cultivar 'Regent' by FISCHER et al. (2004).

The project analyzing this resistance locus includes the creation of a physical map of *Ren3* by assembling BAC clone sequences together with next generation sequencing data obtained from cultivars carrying *Ren3* This physical map will be the basis for the identification of candidate genes which confer resistance to powdery mildew.

To elucidate the function of candidate genes found in *Ren3*, differential gene expression has to be evaluated by performing qRT-PCR. This could confirm a possible interaction in the assumed resistance mechanisms.

Candidate genes which show differential expression upon infection with *E. necator* have to be transferred into susceptible grapevine cultivars to further confirm their involvement in the resistance to powdery mildew.

In order to get a better understanding of the attack of *E. necator* and the defense of grapevine microscopic studies have to be performed comparatively with resistant and susceptible grapevine cultivars. This will shed light on the different plant reactions and hopefully help to understand the mechanisms of resistance.

The project is funded by DFG (Deutsche Forschungsgemeinschaft) Zy 11/9-1.

Session 2

Institute for Strategies and Technology Assessment and Institute for Biosafety in Plant Biotechnology

Relevance of extreme weather events to specialty crops

Sandra Krengel[1], Friedrich Louis[1], Hermann-Josef Krauthausen[1], Bernd Freier[2]
[1] State Education and Research Center of Viticulture and Horticulture and Rural Development, Department Phytomedicine, Neustadt/Weinstraße
[2] Julius Kühn-Institut, Institute for Strategies and Technology Assessment, Kleinmachnow
Email of corresponding author: sandra.krengel@dlr.rlp.de

Extreme weather events and their enormous potential to cause yield and quality losses in agricultural and horticultural crops are well known and deeply dreaded. However, detailed investigations and descriptive data are currently sparsely or even not available. The joint project „Agrarrelevante Extremwetterlagen“ aims to investigate if and how the relevance of extreme weather events will increase or if new extremes will occur in the course of climate change. The sub-project on specialty crops generates findings for wine, vegetable (asparagus, onion, carrot and cabbage), fruit (apple) and hop production using literature research, expert interviews and data analyses.

Experts (advisors and farmers) scored the severity of extreme weather events during the year with 0 (minor), 1 (medium) and 2 (high) and drew up a ranking within the extremes. The scores were used to calculate severity marks. The gained results were matched with findings from literature research on consequences, injury thresholds and currently available management methods. Additional exemplary data analyses serve to quantify damage potential. Injury thresholds are used to request the future likelihood of the relevant extreme weather events and to derive regional impacts.

In grapes hail, late frost and winter frost are classified to be most relevant. The investigated vegetables are mostly threatened by dryness, waterlogging and hail. Apple producers in Northern Germany (Altes Land) are afraid of hail, late frost and waterlogging, while the experts in Southern Germany (Lake Constance) ranked hail, dryness and late frost for apple production. Hop in nearby production area “Hallertau” in Southern Germany is mostly affected by drought and heat, hail and dryness. First analyses of yield data confirm statements from literature and expert interviews that extreme weather events are able to cause enormous damages up to total loss. For instance due to a late frost event in May 2011, yield in frost sensitive vineyards in the cropping area “Pfalz” was reduced by 27 to 44% compared to years without frost after budding.

The present study illustrates exemplary relationships and confirms that the intensity of impacts of extreme weather events strongly depends on species, varieties and site. Consequently, functional management strategies have to be developed applying a situation-related approach.

Initiation of meiotic double strand breaks in Arabidopsis depends on two different SPO11 proteins

Thorben Sprink[1] and Frank Hartung[1]
[1] Julius Kühn-Institut, Institute for Biosafety in Plant Biotechnology
Email of corresponding author: thorben.sprink@jki.bund.de

Pairing and balanced distribution of allelic chromosomes during meiosis depends in most organisms on the initiation of double strand breaks (DSBs) by the protein SPO11. SPO11 is an evolutionary conserved meiotic transesterase, which introduces DSBs to the DNA during early prophase I of meiosis. Whereas in animals and fungi only one single SPO11 is present, plants have at least two meiotic active SPO11 proteins (SPO11-1 and SPO11-2) which are essential for proper chromosome distribution and recombination processes. Single knock out mutants of SPO11-1 as well as SPO11-2 are nearly sterile due to random chromosome segregation during meiosis.

In all so far sequenced land plants orthologous genes to *Arabidopsis* SPO11-1 and SPO11-2 can be found. Our aim is to investigate whether the function of SPO11-1 and -2 is species specific or interchangeable between different near or far related species.
We were able to show that it is, in some cases, possible to interchange SPO11-1 as well as SPO11-2 between *Arabidopsis* and a different species.

Furthermore we were able to identify a species specific splicing pattern of SPO11-1 and SPO11-2. We will present results on heterologous complementation approach as well as splicing patterns of different plants SPO11-1 and -2 genes, as well as patterns from *Arabidopsis* plants carrying SPO11 from a different species. Additionally we investigate if the function of SPO11-1 and -2 is sequence specific and if they work together. For this purpose we interchanged regions between SPO11-1 and -2. We will present results on these swapped gene approach.

Additionally we designed and produced antibodies against *Arabidopsis* SPO11-1 and -2 for use in immunofluorescence-microscopy to get a closer look on the behavior and the distribution of SPO11-1 and -2 during meiosis. We will show images using these and other antibodies on different *Arabidopsis* mutant lines from the complementation approaches.

By analyzing the results of these experiments we should be able to answer the question if there is an sequence and/or species specific function of SPO11-1 and -2 and if the specific splicing pattern has influence on the function if SPO11. With the new antibodies we should also be able to get a closer look on the behavior of SPO11-1 and -2 during meiosis.

Session 3

Institute for Plant Protection in Field Crops and Grassland

Control of pyrethroid resistant pollen beetles

Meike Brandes[1,2], Anna Schmitz[2], Udo Heimbach[1], Bernd Ulber[2]
[1] Julius Kühn-Institut, Institute for Plant Protection in Field Crops and Grassland, Braunschweig
[2] Georg-August-University Göttingen, Department of Crop Sciences, Division of Plant Pathology and Crop Protection, Section Agricultural Entomology, Göttingen
Email of corresponding author: meike.brandes@jki.bund.de

In the last years control of pollen beetle (*Meligethes aeneus* F.) in Germany was mainly based on the application of synthetic pyrethroids. The extensive and indiscriminate use of this insecticide class resulted in a high selection pressure on the beetles, ensuing in the formation of resistance, which has spread over many European countries.

Insecticide applications should reduce yield loss by bud feeding and the reproduction of the beetles to minimize the infestation pressure in following cultures, e.g. vegetable crops and winter oilseed rape in the following year.

To test the effect of insecticides on the reproduction of pollen beetles a field trial was carried out near Braunschweig in 2013. Egg laying was significantly reduced by application of the neonicotinoid Biscaya (active ingredient thiacloprid) in BBCH 60 or 65. Consequently a reduced number of larvae and new pollen beetles hatched. Application of the pyrethroid insecticide Karate Zeon (active ingredient lambda-cyhalothrin) resulted in an increase of the next generation. In 2014 the effect of Biscaya and Mavrik (active ingredient tau-fluvalinat) on population dynamic was investigated. The insecticides were applied in different growth stages (BBCH 55 or 60).

Shortly before and after application the number of beetles was counted in the different plots. Additionally the number of eggs per bud and the number of larvae dropping to the ground for pupation was recorded. The larvae were investigated for parasitization with the key larval parasitoids *Tersilochus heterocerus* and *Phradis* spp.
Furthermore the new pollen beetle generation emerging from treated and untreated plots was trapped in photoeclectors. In the field trial 2014 the application of the insecticides Biscaya and Mavrik had an effect on the population dynamic whereby Biscaya was more effective.

Neonicotinoids and bees: Effects on honeybees, bumblebees and solitary bees in oilseed rape grown from Clothianidin-treated seed

Nadine Kunz[1], Anke Dietzsch[1], Malte Frommberger[1], Ina Wirtz[1], Matthias Stähler[2], Eva Frey[3], Ingrid Illies[4], Winfried Dyrba[5], Abdulrahim Alkassab[6], Jens Pistorius[1]
[1] Julius Kühn-Institut, Institute for Plant Protection in Field Crops and Grassland, Braunschweig
[2] Julius Kühn-Institut, Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection, Berlin, [3] Apicultural State Institute, University Hohenheim, Stuttgart, [4] Bavarian State Institute for Viniculture and Horticulture, Department of Honey Bee Research & Beekeeping, Veitshöchsheim
[5] Beekeeping State Association Mecklenburg-Vorpommern, Beekeeping Centre, Bantin,
[6] Ruhr-Universität Bochum, Department for Biology and Biotechnology, Bochum
Email of corresponding author: nadine.kunz@jki.bund.de

To date, the potential side effects of oilseed rape treated with neonicotinoids on the mortality, development and reproduction have been mainly investigated for honeybees. However, for solitary bees and bumblebees hardly any higher tier studies in semi-field or field conditions are available and validated methods to evaluate potential risks of pesticides are still lacking. Thus, field trials and semi-field trials were conducted in five federal states in spring 2014 using the Western honeybee (*Apis mellifera* L.), the buff-tailed bumblebee (*Bombus terrestris* L.) and the red mason bee (*Osmia bicornis* L.) as model organisms. Small colonies (*A. mellifera, B. terrestris*) and artificial trap nests with cocoons (*O. bicornis*, each 33 male and female cocoons) were placed right next to flowering oilseed rape (*Brassica napus* variety SHERPA® or AVATAR®). There were five treated and five control fields, and four colonies and three trap nests per field, resulting in 40 colonies of *A. mellifera* and *B. terrestris* and 30 trap nests with *O. bicornis* per treatment. Additionally 48 tents were set up before flowering of oilseed rape, each tent containing one small honeybee colony, two small bumblebee colonies and three trap nests with solitary bees. Before, during and after exposure mortality and brood development were regularly assessed. Samples of nectar or honey and pollen were regularly collected and the samples were analysed for residues. Overwintering success of honeybee colonies, fertility of bumblebee queens and hatching rate of solitary bee cocoons are going to be assessed in spring 2015.

These long-term investigations are part of the “ABO 2014” project which is coordinated by the Julius Kühn-Institut in Braunschweig. This study aims to evaluate potential risks of neonicotinoids for honeybees and other pollinators and to develop new evaluation methods. Partly funded by BMEL, BVL and JKI.

Session 4

Institute for Plant Protection in Fruit Crops and Viticulture

Development of antagonistic bacteria for field control of fire blight

Christine Hübert[1], Helmut Junge[2], Kristin Dietel[2], Helmut Junge[2], Annette Wensing[1], Wilhelm Jelkmann[1]

[1] Julius Kühn-Institut, Institute for Plant Protection in Fruit Crops and Viticulture, Dossenheim

[2] ABiTEP GmbH, Berlin

Email of corresponding author: christine.huebert@jki.bund.de

Application of antagonists is considered a possible alternative towards use of antibiotics like streptomycin in fire blight control. Mechanisms of biological control by bacterial antagonists are diverse and can base on competition or include more direct means like toxin production.

The Gram-negative bacterium *Erwinia tasmaniensis* is closely related to the fire blight pathogen *Erwinia amylovora*. It shares not only many physiological traits, but is also well adapted to the fire blight habitat. Whereas *E. tasmaniensis* does not produce any toxins against *E. amylovora*, an inhibition due to competition for nutrients and/or living space between both bacteria is conceivable.

The Gram-positive bacterium *Bacillus amyloliquefaciens* is considered a classical soil habitant. While it is not well adapted to survival on aerial plant surfaces, it possesses interesting antagonistic features due to the production of a broad spectrum of secondary metabolites which are partly toxic for *E. amylovora*.

In this project we compare performance of both antagonists in a number of laboratory setups and in field trials.

Co-cultivation of *E. tasmaniensis* and a luminescent reporter strain of *E. amylovora* as an indicator strain revealed reduced pathogen growth, but no inhibition after application of supernatants. We also investigated various *Bacillus* isolates for their effect on *E. amylovora*. Application of supernatants revealed promising results in growth inhibition of the pathogen. However, efficiency of secondary metabolites depends on medium and growth phase. The effects were visible in dual-culture assays as well as in agar diffusion analysis. In detached-flower assays reduction in symptom development could be observed after application of antagonists, but the results still revealed considerable fluctuations. We also compared population development on inoculated flowers.

Nevertheless, for field operation any antagonist has to be conserved for long term storage and formulations have to become active within a short time frame after application. A combination of both metabolite formulations and antagonists might help to cover part of this problem and should be tested in further examinations.

Drosophila suzukii – quo vadis?

Felix Briem[1], Heidrun Vogt[1]
[1] Julius Kühn-Institut, Institute for Plant Protection in Fruit Crops and Viticulture, Dossenheim
Email of corresponding author: felix.briem@jki.bund.de

Since its first record in Germany in 2011 the Spotted Wing Drosophila (SWD), *Drosophila suzukii*, spread rapidly through the country. Currently it occurs almost all over Germany. Native to South East Asia, SWD was able to overcome its natural barriers by import/export of fruits. In 2008 first individuals occurred in the USA and Southern Europe. During the following years it spread through Europe and reached Southern Germany in 2011.

Since SWD is very polyphagous it infests a wide range of fruits. In addition, its high reproduction rate and short generation cycle of 10 to 14 days turns SWD into an extraordinary harmful pest for fruit production and viticulture. Contrary to our native Drosophilidae SWD, equipped with a serrated ovipositor, lays eggs into healthy, ripening and ripe fruits, usually with several eggs per fruit. The larvae hatch within 24 hours after oviposition. Their feeding activity in the pulp results in a rapid collapse of infested fruits. Without countermeasures, this leads to a complete crop loss.

In 2013, there was a large increase in population as indicated by our monitoring traps. At the same time, the number of locations where SWD was found increased, too. The highest trap captures were recorded from September 2013 onward. This correlates with the availability of fruits as food resource and for oviposition (e.g. blackberry and raspberry).

The application of mass trapping or bait sprays in berries showed no success in pest control. The increase in fly captures was particularly noticeable in forests and forest-edges in November, while captures in orchards decreased. This might reflect the migration behavior of adult SWD searching suitable overwintering sites. At these hotspots, we carried out further studies in order to identify preferred overwintering niches. The extraordinary mild winter 2013/2014 together with the increased number of surviving individuals resulted in a very early repopulation of cropland in spring 2014. Consequently, at the beginning of May we recorded infestation of early cherry varieties (Earlise and Burlat) for the first time in Germany. Delayed application of insecticides (Mospilan SG, SpinTor), resulted in massive crop losses.

By early July, the number of individuals grew rapidly and blackberries and raspberries were infested (up to 40 eggs per fruit). This extreme scenario led to a high infestation in late summer and autumn crops, as well as to a significant increase in individual numbers.

With the ambition of developing alternative control measures, we will continue to study biology and ecology of the pest, in particular by searching for attractants and repellents.

Session 5

Institute for Epidemiology and Pathogen Diagnostics

Characterisation of microbial communities and detection of the putative causal agents in lesions of sugar beet with symptoms of girth scab

Katja Fröhlich[1], Kornelia Smalla[1]
[1] Julius Kühn-Institut, Institute for Epidemiology and Pathogen Diagnostics, Braunschweig
Email of corresponding author: katja.froehlich@jki.bund.de

To date the pathogen(s) causing girth scab of sugar beet is unknown. Symptoms of girth scab on sugar beet resemble common scab of potato thus in this study it was hypothesized that the same pathogens cause both diseases or at least are part of the disease development. Potato scab is induced by pathogenic *Streptomyces* spp. *A. cochlioides* is a long known pathogen of sugar beet causing root rot (black root) in older roots. Both girth scab and root rot show symptomatic lesions that appear on the surface of the beet, but beets show a different appearance above the ground.

While previous studies concentrated on the isolation of pathogens from sugar beet lesions with girth scab symptoms in the present study aimed to analyse the microbial communities and to detect the putative causal agents in total DNA from lesions of sugar beet derived from different geographic locations.

DGGE was used to analyse 16S rRNA gene fragments (bacteria, *Streptomycetaceae)* and ITS (fungi) fragments amplified from total community DNA (TC-DNA) to analyse the composition of dominant bacteria, *Streptomycetaceae* and fungi. In addition, the presence of pathogenic *Streptomyces* spp. in the microbial community was detected by PCR-Southern blot hybridisation of *Streptomyces* ITS and the pathogenicity determinants *txtAB*, *nec1*, *tomA.* For the detection of *A. cochlioides* the specific primers *ef1α* and Southern blot hybridisation with a digoxigenin-labeled probe were used to detect the oomycete in TC-DNA in order to detect the putative causal agents of girth scab of sugar beet lesions. 16S and ITS fingerprints of samples originating from the same region clustered together but DNA extraction technique influenced the clustering. The PCR Southern blot hybridisation revealed the presence of *Streptomyces* spp. in microbial communities of all samples but not in all of them pathogenicity determinants were detected.

A. cochlioides could not be detected in the TC-DNA from lesions of sugar beets analysed here. These results eliminated *A. cochlioides* as the causal agent of girth scab.

Identification and efficacy of naturally occurring fungi associated with cereal cyst nematode *Heterodera filipjevi* and wheat

Samad Ashrafi[1,2,3], Amer Dababat[2], Maria Finckh[3], Wolfgang Maier[1]
[1] Julius Kühn-Institut, Institute for Epidemiology and Pathogen Diagnostics, Braunschweig
[2] CIMMYT-Turkey, Ankara, Turkey
[3] University of Kassel, Faculty of Organic Agricultural Sciences, Ecological Plant Protection, Witzenhausen
Email of corresponding author: samad.ashrafi@jki.bund.de

Cereal cyst nematodes (CCNs, *Heterodera avenae* group) are a group of plant parasitic nematodes, which comprise about 12 species, occurring worldwide. Within this group *H. avenae*, *H. filipjevi* and *H. latipons* are the most damaging species causing significant yield losses and economic damage. Despite the application of several management strategies such as the use of resistant cultivars and cultural methods (e.g. crop rotation) yield losses still persist. Therefore, it is important to integrate biological control method with the aforementioned approaches to reduce the damage of nematodes more effectively.

Preliminary outcomes of a field trial in experimental fields of CIMMYT in Turkey revealed a strong reduction of CCN populations suggesting soil suppressive activities. It was hypothesized that these were caused by nematode-antagonistic fungi or bacteria Therefore, the present study is pursuing the following aims: i) isolation and identification of naturally occurring fungi associated with *H. filipjevi* (cysts and eggs*)* and wheat; ii) evaluation of antagonistic effects of the isolates on *H. filipjevi* iii) investigation of the modes of action of fungal isolates towards *H. filipjevi*.

To achieve the above mentioned goals *in vivo* screening of a total of 100 fungi isolated from either cyst or wheat root samples was carried out to evaluate their biocontrol potential against *H. filipjevi*. Of these, ten isolates showed promising results to be used in detailed studies. All fungal isolates were then molecularly identified. The 10 isolates obtained from the *in vivo* study were identified as *Pochonia chlamydosporia*, *Acremonium persicinum*, *Paecilomyces fumosoroseus* and *Fusarium acuminatum*. The first three belong to the Clavicipateles and are known as nematode parasites. In addition to the *in vivo* tested isolates molecular analyses of nearly fifty additional fungal isolates of field-collected cyst samples indicated a variety of fungal species belonging to the genera of *Embellisia*, *Ophiosphaerella*, *Periconia*, and *Ilyonectria* spp. Also, while some of these fungi have previously been reported as plant endophytes, they have here been isolated from eggs and cysts of *H. filipjevi* for the first time. This newly found association might be exploited for the biological control of the nematode in future.

Factors influencing the fate of human pathogens in the plant environment

Eva Fornefeld[1], Ute Zimmerling[1], Kornelia Smalla[1]
[1] Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, Institute for Epidemiology and Pathogen Diagnostics, Braunschweig
Email of corresponding author: eva.fornefeld@jki.bund.de

Fresh fruits and vegetables contaminated with human pathogenic bacteria can cause illnesses. As foodborne outbreaks associated with fresh produce are a growing concern, there is a need to further analyse the dissemination of human pathogens to plants and their persistence in the plant environment. The factors influencing survival need to be better understood aiming at optimisation of agricultural practices and possibly reducing outbreaks of produce associated illnesses.

Here, we analysed the influence of the factors preadaptation and the presence of sludge as a fertilizer on the survival of *Salmonella* in soil. In a greenhouse experiment *Salmonella enterica* LT2, was applied to soil and its survival was monitored. The experiment included six different treatments: soil amended with sludge or not and each inoculated with (i) *Salmonella*, (ii) preadapted *Salmonella* or (iii) no inoculums. Soil was sampled regularly and numbers of *Salmonella* were monitored using culture-dependent and -independent methods.
The *Salmonella*-CFU decreased from about 10^6 to 10^3 per g dry soil within five weeks. Direct plating showed significantly higher numbers of *Salmonella* in the treatment with preadapted Salmonella without sludge compared to the other treatments from 10 days after inoculation (dpi).
The significant differences were confirmed using qPCR for 14 and 21 dpi. *Salmonella* was detected in soils 98 dpi using PCR-Southern blot hybridization and after 119 days using enrichment culture and plating.
Furthermore the effect of sludge on the soil microbiome was analysed. Bacterial communities were compared by denaturing gradient gel electrophoresis (DGGE). 16S fingerprints showed a strong influence of sludge on the bacterial community of soil. Inoculated samples showed distinct *Salmonella*-bands at 0 dpi which were weaker at 21 dpi. Soil samples were also analysed with regard to abundance of mobile genetic elements like class 1 integrons and genes conferring resistances to antibiotics or disinfectants which were detected at similar levels in all soil samples indicating that sludge did not have an effect on their abundance.

The results indicate that the factor preadaptation promoted survival of *Salmonella* in soil and show a lower survival of *Salmonella* in the presence of sludge. Despite of a rapid decline of Salmonella in soil our data showed a long term survival at low abundance.

Session 6

Institute for Biological Control

The entomopathogenic fungus *Isaria* sp. for insect pest control in vegetables

Katharina Saar[1], Andreas Leclerque[2], Dietrich Stephan[1]
[1] Julius Kühn-Institut, Institute for Biological Control, Darmstadt
[2] Geisenheim University, Institute for Microbiology and Biochemistry, Geisenheim
Email of corresponding author: katharina.saar@jki.bund.de

The growing area of vegetables in the EU covers more than 3.000.000 ha. BIOCOMES is an EU funded project to provide fundamental information for the development of plant protection products, based on biocontrol agents (BCA). Currently, the common control of various insect pests is managed mainly by synthetic insecticides. Nevertheless, several pest insects cause considerable damage in agriculture due to resistance to pesticides.

The aim of the BIOCOMES work package is to develop a new fungal BCA for pest insect control in open field crops and in greenhouses. Presently, we investigate the integration of entomopathogenic fungi into a control strategy.
Within different treatments and pre- and post-harvest applications in protected and non-protected cropping systems, we compare the efficacy of at least ten *Isaria* spp. strains under different laboratory conditions.
Moreover, the host range of these strains was screened against white flies and the moth *Spodoptora exigua*, in order to determine the relationship of clade specific differences between virulence and pathogenity factors.
Additionally, the effect on beneficial insects like the predatory mite *Typhlodromus pyri* and the seven-spot ladybird, *Coccinella septempunctata*, will be evaluated to assess the possibility for implementation of entomopathogenic fungi in an integrated pest management strategy.
As entomopathogenic fungi are known to produce a wide range of secondary metabolites e.g. antibiotics or repellents, selected strains will be screened for secondary metabolites and enzyme activities.
Moreover, different molecular biological studies have been evaluated.
Currently, first results of the phylogenetic relationship between the different isolates, as well as different specifications will be shown.

PhopGV baculoviruses for control of *T. absoluta* in tomato and *P. operculella* and *T. solanivora* in potato

Andreas Larem[1], Eva Fritsch[1], Karin Undorf-Spahn[1], Johannes A. Jehle[1]
[1] Julius Kühn-Institut, Institute for Biological Control, Darmstadt
Email of corresponding author: andreas.larem@jki.bund.de

A promising method for the biological control of insect caterpillars is the usage of baculoviruses. Several different baculoviruses have already been commercialized as highly selective biocontrol agents for insect pest control. The tomato leaf miner *Tuta absoluta* has shown resistance to chemical insecticides, therefore biological alternatives are searched to control this pest insect. Previous studies have shown that there may be the opportunity to use a single baculovirus isolate to control three different but close related insect species, i.e. *Phthorimea operculella* (potato tuber moth), *Tecia solanivora* (Guatemalan potato moth) and *T. absoluta* (tomato leaf miner). Isolates of *Pthorimea operculella* granulovirus (PhopGV) were found to infect all of these three pests. To find a highly virulent isolate to control these three pests it is necessary to characterize different isolates by biological and molecular means. As an outcome of this research the development of a combined control of different pests by highly selective baculoviruses is aimed at.

Detection and quantitation of mixed infected samples with *Agrotis* specific baculoviruses

Gianpiero Gueli Alletti[1], Jörg T. Wennmann[1], Johannes A. Jehle[1]
[1] Julius Kühn-Institut, Institute for Biological Control, Darmstadt
Email of corresponding author: gianpiero.gueli.alletti@jki.bund.de

Four baculoviruses, namely *Agrotis segetum* nucleopolyhedrovirus A (AgseNPV-A), *A. segetum* (Agse) NPV-B, *A.ipsilon* (Agip) NPV and *A. segetum* granulovirus (AgseGV) from the genera *Alpha*- and *Betabaculovirus*, respectively, are known to infect larvae of the lepidopteran pests *A. segetum* and *A. ipsilon*. The potential as biocontrol agents against *Agrotis* species benefits of two abilities, namely to crossinfect both pests *A. segetum* and *A. ipsilon* and to coinfect single larvae in mixed portions. In order to obtain a detailed understanding of mixed infections, especially between *Agrotis spp*. NPV and GV, and the amount of virus progeny produced, reproducible quantitative methods are necessary. This has been already set up with a SybrGreen-based RT-qPCR assay with specific primers binding in the core gene *polyhedrin*, or *granulin* respectively. By this method, mixed infections of AgseNPV-B and AgseGV in portions of their corresponding median lethal concentrations have been examined in experiments with neonate larvae of *Agrotis segetum*.

These findings revealed first hints to viral interactions between AgseNPV-B and AgseGV, as given by their potential virus progeny per larva. Ongoing experiments will consider investigations of mixed infections in cell culture studies with varieties of *Agrotis* specific baculoviruses and will be used for viral quantitation and microscopic picturing of mixed infections.

The SybrGreen-based RT-qPCR assay, however, requires a single RT-qPCR detection for each single virus and is thusly time- and sample-consuming. This lacks the ability for being used in experiments with more than two viruses, and/or in experiments with cell cultures of *Agrotis ipsilon*. An alternative assay with highly-specific *TaqMan* probes improves the quantification by a simultaneous RT-qPCR of up to four viruses in one reaction.

A novel mode of resistance of codling moth against *Cydia pomonella granulovirus*

Annette J. Sauer[1], Eva Fritsch[1], Karin Undorf-Spahn[1], Johannes A. Jehle[1]
[1] Julius Kühn-Institut, Institute for Biological Control, Darmstadt,
Email of corresponding author: annette.sauer@jki.bund.de

The codling moth (CM, *Cydia pomonella*) is one of the most devastating pests in nearly all pome fruit growing regions. An alternative to the application of chemical insecticides is the application of *Cydia pomonella granulovirus* (CpGV) (family *Baculoviridae*), which is registered as biological control agents in 34 countries worldwide. Since 2005, CM populations with a reduced susceptibility to CpGV products have been reported from about 40 plantations in seven European countries. For many of these CM populations, the resistance could be traced back to a single, dominant allele that is linked to the sex chromosome Z. CpGV-M, the so-called Mexican isolate, was the common agent used in all commercial CpGV products registered in Europe. Currently, resistance management strategies are based on the application of improved CpGV products, containing resistance-overcoming isolates. However, a CM field population, termed NRW-WE showed even resistance to most resistance overcoming CpGV isolates, suggesting a second mode of CpGV resistance.

In order to elucidate the inheritance of this type of resistance and after failure of single crossing experiments, successive mass crossings under virus pressure were carried out to establish a genetically homogenous resistant strain of the CM population NRW-WE. Subsequent reciprocal crossing experiments with the resulting CM strain and a susceptible laboratory CM strain (CpS) followed by bioassays fitted to a dominant but autosomal inheritance model. Further analyses of the mode of resistance are under way.

Poster Session

The use of semiochemicals for stored product protection

Tina Gasch[1]
[1] Julius Kühn-Institut, Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection, Berlin-Dahlem
Email of corresponding author: tina.gasch@jki.bund.de

Post-harvest, plant products are stored for a certain time period to ensure a continuous and season-independent food supply. During storage, the goods are highly vulnerable to infestation by microorganisms and insects. In case of a mass propagation of a stored product pest, the means of choice are inorganic insecticidal gases and contact insecticides. Due to the cumulated occurrence of resistances against commonly used pesticides as well as a growing awareness on the environmental risks and consequences of intensive pesticide use, biological and biotechnical approaches to counter stored product pests experience an increasing demand.

Semiochemicals, substances that mediate the communication of an organism with its environment, can be exploited to monitor, decimate and combat insect pests in an environmentally sustainable way. Using different methods of natural product analytics like Gas Chromatography coupled to Flame Ionization Detection (GC-FID), Mass Spectrometry (GC-MS), or Electroantennographic Detection (GC-EAD), our group at the JKI Berlin aims to identify new food attractants, insect pheromones, and plant-derived insect repellents that can be utilized in stored product protection.
Several headspace methods, like Needle-Trap Device (NTD), Solid-Phase

Microextraction (SPME) and Closed-Loop Striping Analysis (CLSA) are implemented to trap and enrich the respective volatiles *in situ*. Behavioral assays, e. g. in flight chambers, y-olfactometers and 4-field walking arenas, complement the analytical findings and verify the biological activity of the isolated compounds. The poster presents the application potential of the different insect semiochemicals as well as the experimental methods used in our group.

M-OVICARD: Analyzing physical cues for grapevine moth oviposition for the development of a Decision Support System

Anna Greif[1], Margit Rid[2], Jürgen Gross[2], Christoph Hoffmann[1]
[1] Julius Kühn-Institut, Institute for Plant Protection in Fruit Crops and Viticulture, Siebeldingen
[2] Julius Kühn-Institut, Institute for Plant Protection in Fruit Crops and Viticulture, Dossenheim
Email of the corresponding author: anna.greif@jki.bund.de

The European grapevine moths (GVM) *Lobesia botrana* (Denis & Schiffermüller) and *Eupoecilia ambiguella* (Hubner) (Lepidoptera: Tortricidae) are polyphagous insects, which can cause severe economic losses in European viticulture.

Female grapevine moths are attracted by the host plant to lay eggs on flower buds and grapes at different phenological stages. Beside the direct damage on grapes by feeding, the hatched larvae injure fruits and thus promote infections of pathogenic fungi such as *Botrytis cinerea*, which can lead to a decrease of wine quality.

Besides mating disruption, the only control opportunity consists of insecticide treatments. Most of the modern insecticides against GVM have to be applied around larvae hatching from the eggs.

For effective applications, it would be necessary to conduct egg monitoring in vineyards. Usually this is a laborious and time consuming task for winegrowers. As a result, the application occurs prophylactically which is not in compliance to integrated pest management. Hence the aim of this project is to develop a monitoring tool, which offers the opportunity to determine the timing and necessity of grape berry moth control. Ideally, the tool should be as attractive or even more than the grape itself and deliver, in reference to the number of eggs, a correlation with pest infestation.

To create a “Moth Oviposition Card” (M-OVICARD), it is necessary to identify the parameter influencing the egg laying process. Basically, olfactory cues, released from host plants, guide the females to oviposition substrates from the distance. Once they reach the plant, volatiles in combination with contact and/or visual stimuli lead to induction of the egg laying process.

The construction of the “M-OVICARD” is made possible by a cumulative work of two facilities of the JKI-Institute for Fruit Crops and Viticulture. The location in Dossenheim consecrates on the identification of the attractive substances whereas in Siebeldingen the corresponding bioassays are carried out.

Primarily, the bioassays are structured according to dual-choice tests.
An analysis on volatiles acting over distance is conducted in small tents in the greenhouse. Visual, olfactory and contact-chemosensory plant cues of grapes and suitable surfaces as well are analyzed in small cages under controlled conditions in the climatic chamber. Quantification is primarily based on the number of eggs laid.

Correlation of grape leaf metabolites with *Plasmopara viticola* resistance traits

Maike Gruenwald[1], Detlef Ulrich[1]
[1] Julius Kühn-Institut, Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection, Berlin and Quedlinburg
Email of corresponding author: maike.gruenwald@jki.bund.de

Plasmopara viticola is an obligate biotrophic oomycete which infects all cultivars of the European grapevine (*Vitis vinifera*). In contrast to *V. vinifera* the native American species such as *V. labrusca* or *V. riparia* are resistant to the pathogen. In the 19th century *P. viticola* was unintendedly imported from Northern America to Europe, where it encountered with the highly susceptible European grapevines. The oomycete infiltrates the host tissue through the stomata of leaves, flowers and small berries. Though infection can happen throughout the whole vegetation phase, it bears the highest potential of severe yield losses at time of flowering. Once stomata penetration occurs, the susceptible European vines fail to stop the proceeding infection. However, American vine species have gained the necessary traits to stop or at least slow down host internal growth of *P. viticola*. Though resistance mechanisms are not yet fully understood, it is assumed that secondary metabolites are involved as active components or signaling compounds. Thus, we screened leaf metabolites of eleven grapevine genotypes with different *P. viticola* resistance traits. Leaf homogenates were analyzed by GC-MS and LC-MS to cover the patterns of volatile as well as non-volatile metabolites. Non-targeted chemometrical data processing was used to obtain metabolite fingerprints of grapevine leaves at the plant developmental stage of flowering. Leaf metabolites of the eleven tested genotypes were analyzed in two subsequent years.

Principal component analyses (PCA) of the metabolite fingerprints arrange all susceptible *V. vinifera* cultivars close to each other. Interspecific genotypes and the resistant *V. labrusca* samples are positioned in close proximity, and the *V. riparia* samples segregate from both groups. These observations apply to the fingerprints of volatiles as well as of non-volatiles. PCA generates groups which divide the grapevine genotypes into their biological filiation which is at the same time a separation into susceptible and resistant genotypes. Resistance traits of the genotypes were correlated with their metabolite profiles. Spearman rank correlation isolated all together four different metabolites with high correlation coefficient moduli in both of the two subsequent years. These were methyl salicylate, (*E*)-beta-ocimene and two known-unknown compounds. Further identification with approved mass spectrometry tools are in progress. Whereas only methyl salicylate could be found in intense correlation with high resistance traits, the other three where strongly correlated with susceptibility.

Phaeomoniella chlamydospora as causal agent of Esca: occurrence and detection in grapevine nurseries

Nicolai Haag[1], Michael Fischer [1]
[1] Julius Kühn-Institut, Institute for Plant Protection in Fruit Crops and Viticulture, Siebeldingen
Email of corresponding author: nicolai.haag@jki.bund.de

Esca is a grapevine trunk disease that can be found worldwide. In Europe two mitosporic fungi, *Phaeomoniella chlamydospora* (*Pch*) and *Phaeoacremonium aleophilum* (*Pal*), as well as the basidiomycete *Fomitiporia mediterranea* (*Fmed*) are considered to be the main causal agents of the disease. Esca not only occurs in older vineyards, but also young vineyards and even planting material may be affected by the associated fungi. In the last years this situation led to considerable economic losses which are steadily increasing. Due to the lack of sufficient information on biology, occurrence, paths of infection and spreading behavior of the pathogens, a direct control measure is not available to date. As basis for development of effective control strategies the acquisition of information on the occurrence and epidemiological aspects of *Pch,* which is supposed to be the most relevant causal agent in plant material production, is of great importance.

In the present study, the occurrence of *Pch* is to be investigated over a period of three years. Various substrates, such as grapevine wood, callusing media and water in three different grapevine nurseries in Rhineland-Palatinate and Baden-Wuerttemberg are considered. The acquired information is expected to give some indication of potential inoculum sources, infection pathways and spreading behavior.

At the beginning of 2014, various wood samples of different rootstock and scion cultivars and water samples from hydration tanks were collected and investigated with respect to the presence of *Pch*. Furthermore wood samples of rooted grafts and samples from callusing media were taken prior to planting in the nursery. In the first place, wood samples were investigated visually regarding *Pch*-characteristic wood symptoms. Suspicious wood samples were additionally checked for the presence of *Pch* by cultivation on potato dextrose agar (PDA) medium and through nested polymerase chain reaction (nested PCR). Likewise water samples and callusing media were investigated by cultivation measures and nested PCR.

For the most part, wood samples of rootstock and scion cuttings as well as rooted grafts were inconspicuous in terms of *Pch*-associated symptoms or showed diffuse browning.

To date, *Pch* could be identified sporadically in wood samples from rootstock cuttings, water samples from hydration tanks as well as callusing medium. In addition, several fungi isolated from rootstock cuttings and plant residues on grafting tools could be assigned to the genus *Cadophora*, which contains fungal species suspected to play a role in trunk disease development.

Setup of a phenotyping pipeline in grapevine breeding

Anna Kicherer[1], Katja Herzog[1], Markus Wieland[2], Christian Müller[3], Michael Pflanz[4], Steffen Kecke[3], Heiner Kuhlmann[2], Reinhard Töpfer[1]
[1] Julius Kühn-Institut, Institute for Grapevine Breeding Siebeldingen [2]University of Bonn, Institute for Geodesy and Geoinformation, Bonn. [3]Julius Kühn-Institut, Department of Data Processing, Quedlinburg. [4]Leibniz Institute for Agricultural Engineering Potsdam-Bornim, Potsdam.
Email of corresponding author: anna.kicherer@jki.bund.de

The acquisition of phenotypic data in grapevine breeding is usually done directly in the field by visual estimations. In general OIV descriptors or the BBCH scale are applied to assess the phenotypes into classes. The phenotyping is strongly limited by time, costs and the subjectivity of records. Due to that limitation, objectivity, automation and precision of phenotypic data evaluation is crucial in order to 1) reduce the consisting phenotyping bottleneck, 2) increase the efficiency of grapevine breeding, 3) enable further important genetic research and 4) assure improved vineyard management.

For these purposes a phenotyping pipeline was setup and tested in a plot of genetic resources. It ranges from the automated image acquisition directly in the field using the PHENObot, to data management, data analysis and the interpretation of gained phenotypic data for grapevine breeding aims. The PHENObot, consists of an automated guided vehicle system, a calibrated camera system, a Real-Time-Kinematic GPS system and a computer for image acquisition and image storage.

Specifically developed software was applied in order to capture geo referenced images directly in the vineyard. The geo reference is afterwards used for the post-processing data management in a database. As phenotypic traits are to be analyzed within the phenotyping pipeline the detection of berries and the determination of the berry size and color were considered.

The application of the phenotyping pipeline enables the fast acquisition of image data from at least 250 individual grapevines per hour directly in the field, using the PHENObot, and represents the basis for high-throughput, automated and non-invasive data sampling in the field. The following automated analysis of these images using Matlab® permits the generation of objective and precise phenotypic data.

Improving weed control for the promising future crop *Taraxacum koksaghyz* as an alternative source for natural rubber

Regina Kölzsch[1], Katja Thiele[1], Frank Hartung[1], Joachim Schiemann[1]
[1] Julius Kühn Institut, Institute for Biosafety in Plant Biotechnology, Quedlinburg
Email of corresponding author: regina.koelzsch@jki.bund.de

The rubber tree *Hevea brasiliensis* is currently the only commercial source of natural rubber, whose worth at the world market for rubber consumer products was $200 billion in 2011. Besides the production of goods like medical equipment, textiles and household items, 70 % of the yearly production is expended by the tire industry. Whilst this market is predicted to grow further but the rubber yield of *H. brasiliensis* has reached its growth limit (500-1500 kg ha^{-1} a^{-1}), searches for alternative sources for natural rubber are in the current focus of the industry. Those should unify several important characteristics like fast growth and annual harvest and allow production of high quality rubber that's easy to extract.

A very promising candidate is *Taraxacum koksaghyz* - a dandelion originating from Kazakhstan. However, it's able to grow also under central European climate conditions, which would facilitate cultivation in Europe to e.g. reduce transportation costs. Besides being a more economically alternative to *H. brasiliensis*, the *T. koksaghyz* rubber is not causing any allergic reactions like the rubber from *H. brasiliensis*. Nevertheless, the rubber types are biochemically very similar. Advantageously, the dandelion is annual and the whole plant can be harvested to gain the rubber which is stored in the roots.

T. koksaghyz has already been cultivated in the Soviet Union from the 1930ies till the 1950ies and was also used as an emergency source during WWII in many countries. Several research projects are presently working on improving and optimizing *T. koksaghyz* to make it an additional and especially economic source of natural rubber. As a result of the BMBF-funded project TARULIN one of the partners - Continental Germany - already started with producing prototype tires consisting of *T. koksaghyz* rubber (recently presented at the IAA 2014). This underlines the practicability of this enterprise but there is an urgent need for further investigations on *T. koksaghyz* cultivation and the rubber processing to reach a commercial level of productivity.

The EVITA project is a consortium of Russian and German partners from university, national research institutes and rubber industry. The focus of our task lies on enhancing the productivity of the crop by introducing an herbicide resistance to *T. koksaghyz* as it is known that especially early overgrowing by weeds is a major reason for low crop yield. This aim will be reached by the new CRISPR/Cas-method for introducing specific DNA mutations into the genomic sequence coding for the enzyme acetohydroxyacid synthase (AHAS) which is a well described target for imidazolinone herbicides.

Morphological, physiological and proteomic responses of potato cultivars to nitrogen deficiency in an *in vitro* system

Philipp Meise[1], Annegret Schum[1], Sylvia Seddig[1], Frank Ordon[1]
[1] Julius Kühn-Institut, Institute for Resistance Research and Stress Tolerance, Groß Lüsewitz
Email of corresponding author: philipp.meise@jki.bund.de

Nitrogen is an essential nutrient which directly influences crop yield and quality. Potatoes require high levels of nitrogen up to 200 kg per hectare, especially in early developmental stages for canopy development. The above-ground biomass is generally related to the photosynthetic capacity and therefore contributes essentially to tuber yields. Nitrogen fertilizers have been used intensively with negative side effects on the environment, e.g. nitrate leaching to the groundwater. This is a special problem in potato production due to the shallow root system of potato and the practice of total nitrogen input in a single treatment at the beginning of growing. Identification of traits contributing to improved nitrogen uptake and utilization may help in breeding of potato cultivars with optimized nitrogen efficiency.

Previous studies on *in vitro* grown table potatoes indicated that responses to nitrogen deficiency varied considerably in a set of table potato cultivars. Especially differences in nitrogen uptake capacity and root development were observed. This suggested cultivar-depend differences in response to nitrogen deficiency and nitrogen use efficiency. In the present study the developed method was used to screen a set of starch potato cultivars for their response to nitrogen deficiency under highly controlled *in vitro* conditions. Liquid MS media with four nitrogen levels (60, 30, 15 and 7.5 mMol/l) were used and plant development and nitrogen uptake evaluated after 7, 11, 14 and 18 days of incubation.

Here we present results obtained on two table cultivars (Lambada and Topas) and seven starch cultivars. Lambada produces high biomass under high nitrogen availability and reacts with a strong decrease under nitrogen deficiency. In contrast Topas produces less biomass under high nitrogen availability, but does not react with a strong decrease under limited conditions. The nitrogen uptake and root development on Lambada is faster than on Topas. We use these differently reacting cultivars to classify the starch cultivars for their response to nitrogen deficiency. To achieve this we determine the biomass production of roots and shoots (fresh and dry matter), the chlorophyll- and crude protein content. Furthermore, differences in the nitrogen uptake during the cultivation period will be demonstrated.

In addition, the *in vitro* system is used to study early proteomic changes in the shoot proteome on the cultivars Topas and Lambada. Using 2D IEF/SDS PAGE method to separate and MALDI-TOF MS to indentify the proteins. First results obtained revealed more differently regulated proteins in Topas than in Lambada.

Comparative pathogenicity of *Meloidogyne hapla* populations on *Rosa corymbifera* 'Laxa'

Abdalmenem Hawamda[1], Beira-Hailu Meressa[1] and Johannes Hallmann[1]
[1] Julius Kühn-Institut, Federal Research Center for Cultivated Plants, Institute for Epidemiology and Pathogen Diagnostics, Münster
Email to corresponding author: beira-hailu.meressa@jki.bund.de

Among plant-parasitic nematode, the root-knot nematodes *Meloidogyne* hapla is the most damaging species on cut roses throughout the world. In this study, the effects of two *Meloidogyne hapla* populations from Ethiopia and Germany on *Rosa corymbifera* Laxa growth and status of nematode reproduction were investigated. One month old seedlings transplanted into a 2 l capacity pots, were infected separately with either of the two populations at initial population densities of 0, 3.1, 6.3, 9.4, 12.5, 15.6, 18.8, and 21.9 second-stage infective juveniles (J2) per gram of dry soils and allowed to grow for ten months under greenhouse conditions. Both nematode populations significantly reduced the relative root fresh weight at all *M. hapla* initial densities. Comparatively, the population from Ethiopia affected growth of the plant root than the German population and was more sever with increasing nematode density. On the other hand, root gall severity increased until an initial density of 15.6 J2 per g dry soil was reached in both populations. Higher nematode reproduction was obtained at the lowest initial densities of the Germany population (69.3 J2/g soil) than the Ethiopian (38.4 J2/g soil). Regardless of the higher final nematode population of the German population, the Ethiopian population demonstrated to be more damaging to *R. corymbifera*.

Vinedress: A wound closure for vines made of polymer fibers

Melanie Molnar[1], Michael Fischer[1]
[1] Julius Kühn-Institut, Institute for Plant protection in Fruit Crops and Viticulture, Siebeldingen
Email of corresponding author: melanie.molnar@jki.bund.de

During the last decades Esca has become one of the main diseases in vineyards. Esca, a complex comprising the three wood living fungi Phaeomoniella chlamydospora (Pch), Phaeoacremonium aleophilum (Pal) and Fomitiporia mediterranea, causes high yield losses every year and weakens the plants resulting in low quality vines. For all of these fungi, wounds in the bark of the vines are considered as the main entrance for spores. Therefore particular attention has to be paid to the winter pruning of vines where spores can easily invade the plant through numerous pruning wounds close to the stem head. For that reason the wounds have to be protected.

Conventional ways of protection like resins, waxes or the application of fungicides have not led to an improvement of the situation so far. As a result a new wound closure based on polymer fibers is currently tested. The wound closure will be applied directly after the pruning providing a physical barrier for the pathogens of the Esca disease, especially Pch and Pal as they infect the plant in an early age.

The new closure consists of electrospun fibers. The process of electrospinning provides a physically stable and elastic mat of fibers. The pore size of the fiber mats can be modified during the spinning process, defining not only the physical barrier for spores, but also properties like air- and water permeability. This permeability should promote the healing process of the plant and prevent rotting. Through the selection of the used polymers the fibers also should be biodegradable.

In this approach the tightness of different polymers is tested against spores and germination tubes of the fungi. First results show that not only pore size has an influence on the result but also the hydrophobicity of the polymers. The biodegradability of the different polymers is tested in soil and in the vineyard. Furthermore different methods of applications are evaluated as the fibers can be applied on the canopy or the electrospinning process can take place directly in the vineyard.

Transformation Associated Recombination of an AgseNPV-B bacmid

Laurin R. Monnheimer[1] ,Gianpiero Gueli Alletti[1], Johannes A. Jehle[1]
[1] Julius Kühn-Institut, Institute for Biological Control, Darmstadt
Email of corresponding author: gianpiero.gueli.alletti@jki.bund.de

Four baculoviruses, namely *Agrotis segetum* nucleopolyhedrovirus A (AgseNPV-A), *A. segetum* (Agse) NPV-B, *A.ipsilon* (Agip) NPV and *A. segetum* granulovirus (AgseGV) from the genera *Alpha-* and *Betabaculovirus*, respectively, are known to infect larvae of the lepidopteran pests *A. segetum* and *A. ipsilon*. All four baculoviruses have a potential for being used as biological control agent against *Agrotis* pests. Despite genomic sequence information, studies about the infection cycle on a molecular and cellular level are still in favor. These studies require bacmids, recombinant baculoviruses, for their genomic manipulation and successive functional studies of genes in larvae and cell culture assays. In the past Bacmid construction was performed either in insect cell culture or in recombination's with *E. coli*. These methods were either time-consuming or lacked in the recombination quality of large and highly repetitive genomes like for *E. coli*.

This study presents a novel method for the construction of bacmids; a yeast transformation associated recombination (TAR) cloning. TAR cloning in yeast simplifies the recombination of large (semi-) synthetic-DNA constructs, e.g. as experiments of C. Venter have shown for *Mycoplasma genitalae*, or modification of entire pathways. Furthermore it allows the recombination of numerous different DNA fragments, also from different species background, in one single step. The AgseNPV-B genome was therefore decomposed in thirty fragments with overlapping ends. In addition to that the shuttle vector pYES-1L with homologues overlaps was amplified. This shuttle vector allows a replication of the Bacmid in *S. cerevisae* and *E. coli*. This study investigated the possibilities of facilitating the fragment construction via long range PCRs and a comparison of the transformation efficacy between chemically– and electro-competent yeast cultures

Genetic fingerprinting of sugar beet cyst nematodes based on pathogenicity gene *vap1* for epidemiological studies

Rasha Nuaima[1], Andreas Westphal[1], Holger Heuer[1]
[1] Julius Kühn-Institut, Institute for Plant Protection in Field Crops and Grassland and Institute for Epidemiology and Pathogen Diagnostics, Braunschweig
Email of corresponding author: rasha.haj-nuaima@jki.bund.de

Heterodera schachtii, the sugar beet cyst nematode (SBCN), is a crucial pest in sugar beet production (Schmidt, 1992). Nematode management involves combinations of crop rotations, host plant resistance, cropping practices, chemical and biological control, all of which may have specific genotype-level interaction with the plant parasitic nematodes (Castagnone-Sereno, 2002; Blok, 2005). Knowledge on the epidemiology and genetic variability of *Heterodera schachtii* populations is important to preserve the durability of resistant sugar beet varieties (Plantard and Porte, 2004). The use of resistant cultivars may change gene frequencies, and consequently reduce the efficacy of the resistance.

The objective of our study was to develop a genetic fingerprinting technique based on variation of the pathogenicity gene *vap1* to investigate the genetic variability among nematode populations of different regional origin. Soil samples were collected from four sugar beet fields in each of four regions in Germany (Peine/Hildesheim, Söllingen, Göttingen, Rheineland). Cyst nematodes were extracted from the soil and reared on *Brassica napus* cv. NK-fair under controlled condition. The propagated cysts were extracted from the soil and ten cysts from every population were taken for DNA extraction by nematode lysis buffer (Holterman et al, 2009). The *vap1* genes were amplified by PCR using a novel primer set. Gene variants occurring in each population were separated by denaturing gradient gel electrophoresis (DGGE). The resulting patterns of bands were compared by using the GelCompar II 6.5 software.

Substantial variation among the samples was detected. However, populations from the same region did not show a consistent pattern, so that significant differences were not observed among regions.
Our plan is to investigate the genetic variability among individual juveniles and cysts of each population to compare the variation within and between populations from distant fields. Moreover, in greenhouse experiments the virulence /aggressiveness of *Heterodera schachtii* populations for different host plants will be determined and related to the observed genetic differences.

M-OVICARD: Analyzing chemical cues for grapevine moth oviposition for the development of a Decision Support System

Margit Rid[1], Anna Greif[2], Christoph Hoffmann[2], Jürgen Gross[1]
[1] Julius Kühn-Institut, Institute for Plant Protection in Fruit Crops and Viticulture, Dossenheim
[2] Julius Kühn-Institut, Institute for Plant Protection in Fruit Crops and Viticulture, Siebeldingen
Email of corresponding author: margit.rid@jki.bund.de

The European grape berry moth, *Eupoecilia ambiguella* and the grapevine moth *Lobesia botrana* are the most serious pests in European vineyards. They can develop up to four generations in one growing season, depending on weather conditions. While the first generation prefers the flower buds for oviposition, the second generation lays its eggs on green berries.

Larvae developed from the second generation eggs damage the grapes and may transmit bacteria and fungi, especially grey mould *Botrytis cinerea*, which are able to develop rapidly on injured berries.

For insects it is of vital importance to perceive and find suitable cues for locating food, finding a mate and oviposition sides. Olfaction plays a central role in inter- and intra-specific chemical communication. Additionally, contact chemoreception, tactile and visual cues contribute to host finding and acceptance.

For oviposition site acceptance several sequences have to be fulfilled in turn or at the same time. Firstly, the gravid females get guided to the suitable environment, such as the vineyard, mostly through olfactory and visual cues. For the location of its host plant grape in the vineyard olfactory signals are very important. Once the moth has landed on the plant, olfactory as well as tactile, visual and contact-chemosensory cues are contributing to the decision to oviposit. *L. botrana* is capable of perceiving signals via receptors on antennae, proboscis, tarsi as well as on the ovipositor.

A successful control method consists on the treatment of the pest at its most vulnerable life stage. The prediction of the moths oviposition would therefore help in designing an optimal treatment schedule. This would help to reduce sprayed pesticide amounts to acceptable levels.

Traps equipped with female sex pheromone are used currently for determining the activity of male moths, but they do not provide reliable information for timing female oviposition behavior, for which no measuring method exists.
The poster summarizes the known sequences for host location and acceptance *in Lobesia botrana* and *Eupoecilia ambiguella* and gives a short outline on our current attempts for filling the gaps.
Beside analytical analysis, behavioral and electrophysiological experiments will be carried out for developing an innovative oviposition monitoring tool.

Determining herbicide resistance by molecular means

Dagmar Rissel[1], Lena Ulber[1]
[1] Julius Kühn-Institut, Institute for Plant Protection in Field Crops and Grassland, Braunschweig
Email of corresponding author: dagmar.rissel@jki.bund.de

Herbicide resistance is a steadily increasing problem for farming worldwide. Resistance to specific active ingredients or herbicide mode of action is a consequence of frequent and biased herbicide use. But not all herbicide modes of action are affected similarly. At the moment, most weed species exhibit resistance to herbicide actives belonging to the groups of ACCase inhibitors and ALS inhibitors. Additionally, weed species do not have the same inherent risk to develop herbicide resistance. In Germany, the most critical weeds are the grasses Alopecurus myosuroides (ALOMY) and Apera spica-venti (APESV). Another weed species that is also gaining attention for its potential to develop resistance is the scentless mayweed Tripleurospermum perforatum (MATIN). To manage existing herbicide resistance on fields and to prevent further development and spreading, a deep understanding how herbicide resistance works is required. Two major mechanisms are known: target-site resistance and non-target-site resistance. Target-site resistance is characterized by the alteration of the target protein structure so that the herbicide cannot bind to its target anymore. This alteration is due to an amino acid substitution in the amino acid sequence of the target protein. Several of these substitutions can occur in one protein leading to resistance against different active ingredients. Examples for this are the ALS protein of APESV where 7 different amino acid substitutions were observed to evoke resistance to different ALS inhibitors or the ACCase protein of ALOMY with 5 different amino acid substitutions involved in resistance to ACCase inhibitors. All amino acid substitutions result from single nucleotide polymorphisms (SNPs) in the coding DNA sequence. Different molecular methods, such as PCR Amplification of Specific Alleles (PASA), derived Cleaved Amplified Polymorphic Sequences (dCAPs) or pyrosequencing were described to determine SNPs in literature. In the herbology lab at the JKI in Braunschweig, these methods were adapted for specific SNPs in the ACCase gene of ALOMY and in the ALS gene from ALOMY and MATIN. So, target-site resistance was successfully determined in field samples of ALOMY and in MATIN plants grown in the greenhouse.

Non-target site resistance describes a collection of plant protective measures against herbicide action, such as an enhanced metabolism of the herbicide in the weed or a spatial separation of the herbicide and its target. In contrast to target-site resistance, the understanding and diagnosis of non-target-site resistance is still in its beginnings. Only a few actors were identified by now. Additionally, some genes were shown to be up-regulated in non-target-site herbicide resistant plants. They provide interesting candidates for the future research.

Steps to unravel the sequence of the resistance locus *Ren3* against powdery mildew (*Erysiphe necator*) originally identified in the grapevine cultivar 'Regent'

Pierre Schneider[1], Daniel Zendler[1], Reinhard Töpfer[1], Eva Zyprian[1]
[1] Julius Kühn-Institut, Institute for Grapevine Breeding Geilweilerhof, Siebeldingen
Email of corresponding author: eva.zyprian@jki.bund.de

Powdery mildew is one of the most devastating diseases of grapevine (*Vitis vinifera* L). The disease is caused by *Erysiphe necator* Schw. (syn. *Uncinula necator* (Schw.) Burr, anamorph *Oidium tuckeri* Berk.), an ascomycete fungus, which was introduced from North America to Europe in 1845. The traditional European cultivars are highly susceptible to the fungus and still today huge amounts of fungicides are necessary to defeat the pathogen.

Many North American *Vitis* species developed resistance against *Erysiphe necator* due to co-evolution of host and pathogen. This process promoted the development of a genetic locus called *Ren3*, which was characterized by Fischer et al. (2004) in the cultivar 'Regent'. Later Dudenhöffer and Zyprian (2012) showed that several genes are located within this region, which show great similarity to genes known to mediate resistances in other plants.

Recent work in this project focuses on unraveling the complete sequence of the *Ren3* locus via screening of 22.000 BAC clones. In a 4D approach a `Regent´ BAC library was screened for BACs located within the locus. The gaps left in the sequence will be closed by re-sequencing of previously identified BAC clones and amplicon sequencing.

The obtained sequences permit searching for open reading frames which contain functional domain structures already known from identified resistance genes. Subsequently an expression analysis will be performed.

Some of these genes shall be cloned into a binary expression vector and checked for functionality upon transformation of susceptible grapevine cultivars with *Agrobacterium tumefaciens*.

The project is funded by DFG (Deutsche Forschungsgemeinschaft) Zy 11/9-1.

Breeding Late-Blight resistant potatoes for organic farming

Michael Sprengel[1], Thilo Hammann[1]
[1] Julius Kühn-Institut, Institute for Breeding Research on Agricultural Crops, Groß Lüsewitz
Email of corresponding author: michael.sprengel@jki.bund.de

Late blight in potato, caused by fungus-like oomycete *Phytophthora infestans*, is one of the most disastrous diseases worldwide. Due to constraints in the use of fertilizers and pesticides, organic farming is faced with an even higher challenge as compared to conventional farming. Pre-breeding for quantitative, race non-specific late-blight resistance may serve an option for enabling sustainable potato growing in organic farming.

To take advantage of this potential a program focused on breeding of potato varieties for organic farming in Germany was initiated by a network of organic farmers, potato breeders and research institutes. The project aims at combining low susceptibility to late blight, resistance to other diseases, and quality traits in pre-breeding materials.

To achieve this goal, a total of 158 varieties and breeding clones were evaluated for their susceptibility against late blight on foliage and tubers. Four experiments with different test methods, i.e. a field trial, a detached-leaf assay, a tuber-slice assay, and a whole-tuber test were carried out over three years. Infestation of potato tops with late blight in the field was assessed via Delta-rAUDPC values which had been corrected for maturity. Maturity and quality scores of these clones were determined in an additional field trial under fungicide application.

Maturity is an essential trait in the context of assessing late-blight resistance. Most of the tested JKI breeding clones showed very low Delta-rAUDPC values, which illustrates the enhancement achieved in breeding for late-blight resistance within this material. A large part of these clones are early maturing, thus exemplifying that the correlation of late-blight resistance and late maturity can be broken up. As expected, infestation of the tubers was only loosely correlated with the infestation of the foliage as assessed in the detached-leaf assay or in the field. Some pre-breeding clones exhibited low infestation of the tubers in tuber-slice and whole-tuber tests. Correlations of Delta-rAUDPC values and results of the laboratory assays were in a medium range, which indicate a non–substitutability of the field experiment in predicting quantitative late blight resistance.

The project is expected to provide information on the potential of late-blight resistant potato breeding clones in contributing to sustainable potato growing via reduced use of copper-based fungicides in organic farming systems.
The project belongs to the governmental program „Bundesprogram Ökologischer Landbau und andere Formen nachhaltiger Landwirtschaft“ (BÖLN) and is funded by the BMEL.

DURESTrit - mapping NHR-genes from barley secondary gene pool *Hordeum bulbosum*

Behrend v. Hülsen[1], Brigitte Ruge-Wehling[1]
[1] Julius Kühn-Institut, Institute for Breeding Research on Agricultural Crops, Groß Lüsewitz
Email of corresponding author: behrend.huelsen@jki.bund.de

As part of the ERA-CAPS program the DURESTrit consortium is an international collaboration between the Julius Kühn-Institut, the Leibniz Institute of Plant Genetics and Crop Plant Research and several national and international partners. Aim is the localization and functional characterization of nonhost resistances from the primary and secondary gene pool in barley.

Nonhost Resistance (NHR) is the resistance plants possess against the majority of pathogens in the environment. Therefore transferring components of NHR to crop plants from related species via introgression lines will give us new tools to protect our crops against pathogens.

Our part of the project is to map new powdery mildew resistance loci for Barley, derived from the secondary gene pool *Hordeum bulbosum*. We use several 2HS and 2HL introgression lines differing in their *H. bulbosum* and *Hordeum vulgare* backround. All introgressions bear at least two different resistances against *Blumeria graminis f. sp. hordei.*

For molecular characterization a mapping population will be developed. Phenotyping will be carried out by detached leaf assays with different powdery mildew isolates, checking the spectrum of resistance.

Fine Mapping of the introgressions will be performed by novel marker techniques, e.g. Exome Capture and GBS. Based on the resulting sequence information CAPS, InDel, as well as SNP marker will be constructed for marker assisted selection of the novel resistance loci. Additional transcript information can be used as candidate genes getting closer to resistance loci.

Recombinant plants with reduced introgression sizes will be selected with minimized introgression size and linkage drag to increase the value for breeding purposes.

Identification of genomic regions involved in drought stress induced leaf senescence and drought stress tolerance in juvenile barley

Gwendolin Wehner[1], Christiane Balko[1], Eva Zyprian[2], Frank Ordon[1]
[1] Julius Kühn-Institut, Institute for Resistance Research and Stress Tolerance, Quedlinburg/ Groß Lüsewitz [2] Julius Kühn-Institut, Institute for Grapevine Breeding Siebeldingen
Email of corresponding author: gwendolin.wehner@jki.bund.de

Premature leaf senescence induced by environmental stress conditions, e.g. drought stress, is an important factor for growth limitation and yield losses. Drought tolerance is a complex quantitative trait that is controlled by various genes. With genome wide association mapping (GWAS) we got a powerful tool to itemize such complex pathways by the detection of quantitative trait loci (QTL) suited to be used in future plant breeding programs.

In greenhouse pot experiments 113 German barley (*Hordeum vulgare* L.) cultivars and 43 accessions of the Spanish Barley Core Collection were tested for their response to drought stress. At the end of a four weeks stress period (BBCH 33) chlorophyll content which is an indicator of leaf senescence, as well as the chlorophyll fluorescence, content of free proline, content of soluble sugars, osmotic adjustment and the aboveground biomass production indicative for drought stress response were determined in the control and stress variant. The panel showed obvious phenotypic variation for all traits, significantly correlated to drought stress induced leaf senescence.

Association mapping was performed using the genotypic data generated with the Illumina 9k iSelect SNP Chip and the phenotypic data out of the pot experiments. One major QTL for drought stress induced leaf senescence was found on barley chromosome 5H, whereas another strong QTL was found on chromosome 2H. NCBI Blast search of the significant marker sequences pointed out that respective SNPs are in some cases located in genes coding for proteins related to drought stress or leaf senescence eg. serine/ threonin protein kinase (SAPK9), or nucleotide pyrophosphatase (AVP1).
Tolerance ranking of phenotypic data (drought susceptibility index DSI) with the leaf senescence parameter chlorophyll content and the drought stress parameter biomass yield revealed some tolerant and sensitive genotypes reacting in the same way for both traits. Furthermore, tolerance ranking with gene expression data (fold change) with four genes out of the GWAS showed identical sensitive and tolerant genotypes for these genes. By comparing the DSI and fold change ranking two tolerant and one sensitive genotype was found out of the 156 analysed barley genotypes.

Construction of full-length cDNA clones of *Apple chlorotic leaf spot virus* in two different methods

Lei Zhang[1], Wilhelm Jelkmann[1]
[1] Julius Kühn-Institut, Institute for Plant Protection in Fruit Crops and Viticulture, Dossenheim
Email of corresponding author: lei.zhang@jki.bund.de

Apple chlorotic leaf spot virus is a flexuous filamentous particle of approximately 680 to 780 nm in length and 12 nm in width, and is the type species of genus *Trichovirus* within the family *Betaflexiviridae*. The genome size of *Apple chlorotic leaf spot virus* is about 7.5 kb, of which the 3'- and 5'- ends are well conserved, and till now there are 14 complete genome sequences of different strains of the virus are available in the nucleotide database of National Center for Biotechnology Information.

For constructing a clone of a certain gene or DNA fragment, there are several different methods, generally including TA cloning, cloning using restriction enzymes and ligation-independent cloning. The TA cloning should be the simplest one, but for constructing infectious clones of plant viruses, the latter two are always preferred. According to publications, by the way of cloning using restriction enzymes, the infectious cDNA clones of *Apple chlorotic leaf spot virus* and *Apple stem grooving virus* have been constructed, while ligation-independent cloning methods were successfully used for constructing the cDNA clones of *Apple stem pitting virus* and *Apple stem grooving virus.* In our study, a commercial product of In-Fusin HD Cloning Kit (Takara) and a ligation-independent method of circular polymerase extension cloning (CPEC) are employed.

An isolate of ACLSV from a peach tree was chosen for generating the cDNA clone. Total RNA of 100 mg leaf tissue from an infected peach tree was extracted using RNeasy Plant Mini Kit (Qiagen). The cDNA was generated by RT-PCR with RevertAid Premium Reverse Transcriptase (Thermal Scientific). Full-length fragments of ACLSV (inserts) and linear pV297 (a pBin vector, E. Maiss, Hannover) were produced from PCRs using Precisor High-Fidelity DNA Polymerase (BioCat). Assembly reactions were performed with gel purified inserts and vectors using In-Fusion HD Cloning Kit (Takara) according to the manufacturer's instruction and by a circular polymerase extension cloning (CPEC) method, respectively, followed by transforming NEB 10β competent cells. Possibly successful full-length cDNA clones of the *Apple chlorotic leaf spot virus* were identified with PCRs using three different primer pairs and sequent XbaI digestion with isolated plasmids (Qiangen Miniprep Kit). Finally, *Agrobacterium* strain ATHV was transformed and used for infecting tobacco plants (*N. occidentails* 37b).

Keynote 2

Vorstellung der zentralen Datenverarbeitung des JKI

Steffen Kecke[1]
[1] Julius Kühn-Institut, Zentrale Datenverarbeitung, Quedlinburg
Email of corresponding author: steffen.kecke@jki.bund.de

Hand-out

„Presentation of the data processing group of the Julius Kühn-Institut"

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

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

Heft 153, 2010: NEPTUN 2009 – Gemüsebau. Dietmar Roßberg, 72 S.

Heft 154, 2010: Bewertung der Resistenz von Getreidesortimenten: Planung und Auswertung der Versuche mit Hilfe der SAS-Anwendung RESI 2. Eckard Moll, Kerstin Flath, Ines Tessenow, 109 S.

Heft 155, 2010: Biofumigation als Pflanzenschutzverfahren: Chancen und Grenzen. Beiträge des Fachgespräches vom 5. Mai 2010 in Bonn-Roleber. Bearbeitet von: Johannes Hallmann, Johannes Keßler, Rita Grosch, Michaela Schlathölter, Florian Rau, Wolfgang Schütze, Matthias Daub, 102 S.

Heft 156, 2010: Netz Vergleichsbetriebe Pflanzenschutz - Jahresbericht 2009. Bearbeitet von: Bernd Freier, Jörg Sellmann, Jürgen Schwarz, Marga Jahn, Eckard Moll, Volkmar Gutsche, Wolfgang Zornbach. Unter Mitwirkung von: Anita Herzer, Merle Sellenriek, Rene Brand, Benita Burghardt, Christiane Seidel, Florian Kluge, Ute Müller, Christina Wagner, Christoph Hoffmann und den Pflanzenschutzdiensten der Länder, 83 S.

Heft 157, 2010: Drittes Nachwuchswissenschaftlerforum 2010; 23. - 25. November in Quedlinburg - Abstracts , 47 S.

Heft 158, 2010: 14. Fachgespräch: „Pflanzenschutz im Ökologischen Landbau – Probleme und Lösungsansätze“. Phosphonate. Bearbeitet von Stefan Kühne, Britta Friedrich, 34 S.

Heft 159, 2011: Handbuch. Berechnung der Stickstoff-Bilanz für die Landwirtschaft in Deutschland, Jahre 1990 – 2008. Martin Bach, Frauke Godlinski, Jörg-Michael Greef, 28 S.

Heft 160, 2011: Die Version 2 von FELD_VA II und Bemerkungen zur Serienanalyse. Eckard Moll, 34 S.

Heft 161, 2011: Netz Vergleichsbetriebe Pflanzenschutz - Jahresbericht 2010 - Analyse der Ergebnisse der Jahre 2007 bis 2010. Bearbeitet von Bernd Freier, Jörg Sellmann, Jürgen Schwarz, Marga Jahn, Eckard Moll, Volkmar Gutsche, Wolfgang Zornbach, 86 S.

Heft 162, 2011: Viertes Nachwuchswissenschaftlerforum 2011 - Abstracts - , 62 S.

Heft 163, 2012: Bewertung und Verbesserung der Biodiversität leistungsfähiger Nutzungssysteme in Ackerbaugebieten unter Nutzung von Indikatorvogelarten. Jörg Hoffmann, Gert Berger, Ina Wiegand, Udo Wittchen, Holger Pfeffer, Joachim Kiesel, Franco Ehlert, 215 S. , Ill., zahlr. graph. Darst.

Heft 164, 2012: Fachgespräch: „Kupfer als Pflanzenschutzmittel“ Berlin-Dahlem, 1. Dezember 2011. Bearbeitet von Stefan Kühne, Britta Friedrich, Peter Röhrig, 102 S.

Heft 165, 2012: Nationaler Aktionsplan zur nachhaltigen Anwendung von Pflanzenschutzmitteln – Bericht 2008 bis 2011. Bernd Hommel, 162 S.

Heft 166, 2012: Netz Vergleichsbetriebe Pflanzenschutz - Jahresbericht 2011 - Analyse der Ergebnisse der Jahre 2007 bis 2011. Bearbeitet von Bernd Freier, Jörg Sellmann, Jürgen Schwarz, Bettina Klocke, Eckard Moll, Volkmar Gutsche, Wolfgang Zornbach, 104 S.

Heft 167, 2012: Fünftes Nachwuchswissenschaftlerforum 2012, 4. - 6. Dezember in Quedlinburg, 50 S.

Heft 168, 2013: Untersuchungen zur Bildung von Furocumarinen in Knollensellerie in Abhängigkeit von Pathogenbefall und Pflanzenschutz. Andy Hintenaus, 92 S.

Heft 169, 2013: Pine Wilt Disease, Conference 2013, 15th to 18th Oct. 2013, Braunschweig / Germany, Scientific Conference, IUFRO unit 7.02.10 and FP7 EU-Research Project REPHRAME - Abstracts -. Thomas Schröder, 141 S.

Heft 170, 2013: Fachgespräch: „Kupfer als Pflanzenschutzmittel“, Berlin-Dahlem, 7. Dezember 2012. Bearbeitet von Stefan Kühne, Britta Friedrich, Peter Röhrig, 89 S.

Heft 171, 2013: Sechstes Nachwuchswissenschaftlerforum 2013, 27. - 29. November in Quedlinburg - Abstracts - , 52 S.

Heft 172, 2013: Netz Vergleichsbetriebe Pflanzenschutz, Jahresbericht 2012, Analyse der Ergebnisse der Jahre 2007 bis 2012. Bearbeitet von Bernd Freier, Jörg Sellmann, Jörn Strassemeyer, Jürgen Schwarz, Bettina Klocke, Hella Kehlenbeck, Wolfgang Zornbach, 111 S.

Heft 173, 2014 Statusbericht Biologischer Pflanzenschutz 2013. Johannes A. Jehle, Annette Herz, Brigitte Keller, Regina G. Kleespies, Eckhard Koch, Andreas Larem, Annegret Schmitt, Dietrich Stephan, 117 S.

Heft 174, 2014 47th ANNUAL MEETING of the SOCIETY FOR INVERTEBRATE PATHOLOGY and INTERNATIONAL CONGRESS ON INVERTEBRATE PATHOLOGY AND MICROBIAL CONTROL, 176 S.

Heft 175, 2014 NEPTUN-Gemüsebau 2013. Dietmar Roßberg, Martin Hommes, 44 S.

Heft 176, 2014 Rodentizidresistenz. Dr. Alexandra Esther, Karl-Heinz Berendes, Dr. Jona F. Freise, 52 S.


JKI
Julius Kühn-Institut
Bundesforschungsinstitut für Kulturpflanzen