specialized hyphae inside the plant cell, so-called haustoria. These structures represent the most intimate contact zone between pathogen and host and play a vital role in the uptake of nutrients and the exchange of information. In order to suppress plant defense mechanisms, rust fungi produce specialized proteins, so-called effectors. These proteins are secreted into the extrahaustorial matrix or even into the plant cell, where they are expected to interact with plant proteins. Knowledge about these mechanisms might provide valuable clues for plant protection in the future, especially with respect to RNAi based approaches. Our work is focused on the identification and analysis of novel effector proteins from different rust species. To select for putative secreted proteins, we are working with a method called „Signal Sequence Trap" (Jacobs et al., 1999). This method has already been used for *Uromyces fabae* (Link and Voegele, 2008) and *Uromyces appendiculatus*. We are using cDNA libraries of *Phakopsora pachyrhizi* generated from infected soybean leaves (*Glycine max*). To enrich the cDNA for fungal cDNA we employ Subtractive Hybridization. Comparing the different rust secretomes across species might lead to the identification of novel effector proteins, essential for virulence.

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(DPG AK Wirt-Parasit-Beziehungen)

3) Haustorial transciptomes of *Uromyces appendiculatus* and *Phakopsora pachyrhizi* – identification of families of candidate effectors

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Rust fungi are biotrophic pathogens, which mean that they do not kill their respective host plants but are dependent on living tissue for propagation. Among them are species with major economic impact like *Phakopsora pachyrhizi* and *Uromyces appendiculatus*, infecting soybean and common bean respectively. A hallmark feature of biotrophic fungi are haustoria, which were shown to be the interface for nutrient uptake in rust fungi and probably are the place where effector proteins are transferred to the plant. Effector proteins can suppress the host resistance response and may have other functions in influencing the plant host, making establishment and maintenance of the biotrophic interaction possible. To identify effector candidates we did transcriptome sequencing using the next generation sequencing technology 454 pyrosequencing. For this cDNA was prepared from isolated haustoria of both U. appendiculatus and *P. pachyrhizi*. Comparing our annotation results with those for prebiotrophic structures we could corroborate findings that haustoria have indeed important functions in energy and amino acid metabolism. Blasting our sequences against other rust and basidiomycete genome sequences, predicting secreted proteins and building gene families through a clustering analysis, we could identify genes and gene families that are secreted and that are specific to rust fungi or subclades to the rust fungi. In addition to this, interesting motifs and expression patterns make these genes and gene families good candidate effectors.

(DPG AK Wirt-Parasit-Beziehungen)

Abstracts Arbeitskreis Mykololgie 2012

1) Ash dieback in southwest Germany and genetic investigations on the causal agent *Hymenoscyphus pseudoalbidus*

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Ash dieback is observed in southwest Germany since 2009 and causes increasingly damage. At present, planting of ashes is not recommended. The question arises in which extent *Fraxinus excelsior* will be suitable for forestry purposes in future. Characteristic symptoms of this new disease include wilting of leaves, premature leave fall, shoot dieback and bark necroses. In young trees, the disease leads to bushy or dwarf growth, whereas the wood quality of older trees is endangered by formation of secondary shoots at the stems. Data concerning the occurrence of the disease in Baden-Württemberg was collected every year since 2009. Ash dieback occurred on 2505 ha in autumn 2009, 4106 ha in autumn 2010 and 8526 ha in autumn 2011. On 3133 ha the disease was rated as threatening whole stands. Particularly affected are stands in the Rhine valley. The genetic variation of 64 isolates of the causal agent *Hymenoscyphus pseudoalbidus* from southwest Germany was analysed by RAMS-fingerprinting (random amplified microsatellites) using the primer $VDV(CT)_7C$. A considerable amount of genetic variation among these strains was detected. That implies that the fungus has a heterothallic life cycle. Furthermore, the findings suggest a fast increase in genetic variation, although more research is needed to confirm this result. Nevertheless, it is a cause of concern, keeping in mind that the pathogen may be able to overcome the reported individual resistance of *F. excelsior* in future. Also, this could explain the contradiction between the indications of *H. pseudoalbidus* as an invasive species on the one hand and its high genetic variation on the other hand. There was no evidence for a connection between the geographic origin and the genetic distance of the strains. This is an indication that the dispersal of the pathogen's ascospores by the wind takes place over large distances. But also trading of nursery material may have contributed to this phenomenon.

(DPG AK Mykologie)

2) Grey mould isolates from German strawberry fields show multiple fungicide resistance and represent a novel clade between *B. cinerea* and *B. fabae*

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Grey mold is a major problem of fruit and vegetable production worldwide. For control of Botrytis, strawberries receive multiple fungicide treatments each year. Grey mold populations from different German strawberry growing regions were tested for their sensitivity against Botrytis fungicides and for their genetic variability. Fungicide resistance was observed, including many isolates with multiple resistance against the majority of fungicides tested. A novel, stronger variant of the previously described multidrug resistance phenotype MDR1 was observed, called MDR1 h, conferring threefold higher, partial resistance to cyprodinil and fludioxonil. The majority of strawberry iso-

lates, in particular those with MDR1 h and multi-resistant phenotypes, were genetically distinct from *B. cinerea* populations that occur in vineyards. DNA sequencing indicated that the strawberry isolates indeed represented a separate clade, similar to *B. cinerea* and *B. fabae*. A PCR assay was developed that allowed reliable discrimination of 'strawberry type' Botrytis genotypes from *B. cinerea sensu stricto*. 'Strawberry type' genotypes were found to be dominating in German strawberry growing regions, but almost absent from vineyards. We raise the hypothesis that these genotypes have been selected in German strawberry fields partly because of their ability to accumulate more fungicide resistance mutations than *B. cinerea sensu stricto*.

(DPG AK Mykologie)

3) Molecular methods for fungicide resistance detection

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Fungicides resistance is a growing problem since the introduction of target specific fungicides. Determination of the baseline sensitivity of a fungal population and detection of sensitivity changes after market introduction is required for a sustainable use of fungicides. Different monitoring methods are available for detection of fungicide sensitivity of fungal isolates or population including *in vivo*, *in vitro* and molecular genetic techniques. Molecular genetic techniques show with respect to throughput, duration time and amount of pathogen material advantages over *in vivo* and *in vitro* methods. Also additional information on pathogen populations can be obtained by using quantitative methods. Before the usage of a molecular genetic assay for fungicide resistance detection the exact resistance mechanism must be determined. Verification of DNA sequences, specificity and validation with an *in vivo* or *in vitro* assay is also essential to avoid wrong results. CAPS-PCR (Cleaved Amplified Polymorphic Sequences-PCR) is a simple combination of target gene amplification and additional cleavage of the polymorphic site with a restriction enzyme. This method shows limitations with respect to quantification, detection limit and restriction enzyme limitation. From the molecular genetic assays real-time PCR show the greatest sensitivity. With the coupling of allele-specific primers with real-time PCR, a detection limit lower than 1% mutation in a wildtype population (e.g. S524T in the *cyp51*), can easily established with an additional quantification option. Different codons for the same amino acid, e.g. F129L in the cytochrome *b* of *Pyrenophora teres*, or different mutations at the same codon, e.g. P225F/L or H272Y/R in the SdhB gene of *Botryotinia fuckeliana*, would require a complex real-time PCR assay. Newer real-time PCR instruments offer the option of High-Resolution melting. Depending on sequence and instrument the application is limited to genotyping. The limitations of the allele-specific real-time PCR can be solved by pyrosequencing, which is a sequencing-by-synthesis method based on the online detection of pyrophosphate which is released during the elongation of a sequencing primer by DNA polymerase. Besides the quantification option and detection limit between 5–10%, the sequencing of short DNA fragments provides additional information on nearly related pathogen species, for example *M. nivale* and *M. majus*.

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4) *Verticillium*-monitoring of former agriculturally used land for the risk minimization of following planted woods

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On a field in Brandenburg, which was formerly used as agricultural land, there have been planted different species and subspecies of trees in the year 2010. 34 soil assays were taken from the rhizosphere in November 2011 of trees with wilt symptoms, e.g. *Acer*, *Quercus*, *Platanus*. The soilsuspension was examined in the laboratory for the existence of microsclerotia of *Verticillium dahliae*. The objective of this present thesis is to give suggestions for the selection of urban trees concerning their sensitivity to *Verticillium dahliae* and the possibilities for minimizing and fighting against the contamination.

(DPG AK Mykologie)

5) Sweets for my sweet: Carbohydrate metabolism in the parasitic and non-parasitic stage of *U. fabae*

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The mobilization, uptake and metabolism of carbohydrates from host tissue is of crucial importance for obligate biotrophic pathogens like the broad bean rust *Uromyces fabae*. In the course of our studies different genes involved in carbohydrate metabolism during the parasitic stage of *U. fabae* were identified, including an invertase, a haustorium-specific hexose transporter with high affinity for D-Glucose and D-Fructose and different enzymes catalyzing subsequent metabolic conversions of acquired carbohydrates. In contrast, only little is known about the molecular mechanisms of carbohydrate metabolism during the non-parasitic stage. Therefore our research also focused on the identification of enzymatic pathways, providing physiological energy during the germination of spores and subsequent colonization of the host plant. To identify genes involved in sugar metabolism, we use different PCR-approaches based on sequence information from other rust fungi like *Puccinia*- or *Melampsora*-species. The use of real-time PCR also enabled us to quantify the activity of different genes, involved in the metabolism of sugars at different stages of fungal growth. Our research shall provide new insights in the carbohydrate metabolism of obligate biotrophic organisms and help to understand the complex mechanisms and relationships making rust fungi such a successful group of parasites.

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