

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.

JACOBS, K.A., et al., 1999: A genetic selection for isolating cDNA clones that encode signal peptides. *Methods Enzymol.* **303**, 468-479.

LINK, T.I., R.T. VOEGELE, 2008: Secreted proteins of *Uromyces fabae*: similarities and stage specificity. *Mol. Plant Pathol.* **9**, 59-66.

(DPG AK Wirt-Parasit-Beziehungen)

### 3) Haustorial transcriptomes 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.

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## Abstracts Arbeitskreis Mykologie 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 leaf 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)<sub>7</sub>C. 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.

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### 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-