134-Taubenrauch, K.; Kühne, T.

Julius Kühn-Institut, Bundesforschungsinstitut für Kulturpflanzen

Ermittlung der Direktwirkung von Fungiziden auf *Mycosphaerella anethi* im Agarplattentest

Evaluation of direct fungicide impact on Mycosphaerella anethi in an agar plate test

In den letzten Jahren hat sich das Befallsniveau von *M. anethi* an Fenchel zunehmend erhöht; in allen Anbaugebieten kommt es regelmäßig zu hohen Ertragsausfällen bis hin zum Totalausfall. Der Pilz ist nachweislich samenübertragbar, Fungizidanwendungen zeigten in der Praxis keine ausreichende Wirkung zur Schadensreduzierung. Bisher existierte kein praktikables Verfahren zur Befallseinschätzung von latent infizierten Fenchelfrüchten. Zur tatsächlichen Fungizidwirkung auf das Mycel von *M. anethi* lagen keine Untersuchungen vor, da der Erreger bisher als nicht kultivierbar auf Agar galt und daher als Reinkulturen nicht verfügbar waren. Innerhalb der Projektarbeit ist es gelungen eine Methode zur Inkulturnahme des Pilzes zu entwickeln und das Mycel zu vermehren, sodass erstmals Laborversuche zur Direktwirkung von Fungiziden auf das Pilzwachstum durchgeführt werden konnten. Die überwiegende Anzahl der getesteten Fungizide ist bisher nicht zur Bekämpfung des Erregers im Fenchelanbau zugelassen. Es sollen die ersten Ergebnisse zur Beeinflussung des Pilzwachstums dargestellt werden.

135-Niepold, F.

Julius Kühn-Institut, Bundesforschungsinstitut für Kulturpflanzen

Methylierungen der Tri5 und Tri14 Mykotoxin-Gene sind bei *Fusarium sporotrichioides* mit der Endonuklease MspJI nachweisbar

Methylated mycotoxin-genes Tri5 and Tri14 in Fusarium sporotrichioides are detectable by applying the endonuclease MspJI

Bei *Fusarium sporotrichioides* wurde die Methylierung von DNA der beiden Mykotoxin-Gene Tri5 und Tri14 unter verschiedenen Infektionsbedingungen untersucht. Für diese epigenetischen Untersuchungen wurde das Restriktionsenzym MspJI verwendet, das DNA immer nur dann schneidet, wenn diese auch bei der Nukleotid-Folge Guanosin/Cytosin (G/C) methyliert ist. Unmethylierte G/C-haltige DNA wird von diesem Enzym nicht geschnitten, weshalb hier in einfacher Weise eine Unterscheidung in der Methylierung möglich ist. Eine Restriktion oder Nichtrestriktion wurde mit PCR-Primern nachgewiesen, die die Sequenzen beider Gene abdeckten. So war eine Methylierung der für die Mykotoxin-Gene Tri5 und Tri14 codierende DNA mit MspJI nur nachweisbar, wenn *F. sporotrichioides* seine Wirtspflanzen Gerste und Mais erfolgreich infizierte. Alle anderen Bedingungen hatten keinen Einfluss auf die Methylierung der DNA beider Gene. Ein endgültiger Nachweis einer Methylierung der Tri-Gene ist die Behandlung mit Bisulfit, das nur die unmethylierten Cytosine verändert, nicht aber die methylierten, was dann durch eine DNA-Sequenzierung gezeigt werden kann.

137-Ha, X.; Wei, T.; von Tiedemann, A.

Georg-August-Universität Göttingen

Epidemiological and phytopathological studies on wheat blast (*Magnaporthe grisea*) – characterisation of pathotypes, host specificity and resistance in wheat

Magnaporthe grisea is the causal pathogen of wheat blast, which can cause significant yield losses in subtropical wheat production. We tested the optimal conditions for the development of wheat blast. The effects of temperature (20, 23, 26, 29 and 32 °C) and spike wetness duration (24 h, 48 h, 72 h and 96 h) at the flowering stage were studied in climate chambers with the susceptible wheat cultivar BR18. The results showed that temperatures > 26 °C are conducive for infection and growth of *M. grisea*, which is capable to induce high disease severity even at 29 °C and 32 °C. A minimum spike wetness time of 24 h was required for infection; wetting periods above 24 h had little additional effect on wheat blast development. Host specificity of wheat and rice strains was analysed on leaves of wheat and rice plants inoculated with Magnaporthe strains at the 3- or 4-leaf stage. At 6 dpi, host compatible strains triggered large necrotic spots and could be reisolated from infected lesions. In contrast, strains on a non-homologous host showed few white or necrotic spots and no expanding lesions. Phylogenetic relationships among *M. grisea* isolates from wheat, rice, finger millet and ryegrass, were tested by Amplified Fragment Length Polymorphisms (AFLP). A clear differentiation between wheat and rice strains was observed.

Isolates from wheat predominantly clustered with isolates from finger millet and ryegrass in genetic distance analysis suggesting evolutionary relationship.

Twenty-seven wheat lines were assessed for resistance to wheat blast in a standardized screening assay in the greenhouse. Inoculations were performed on the leaves and ears in separate experiments to test the organ-specific responses. Leaf infection was not correlated with ear symptoms. Upon ear inoculation at flowering stages, cultivar MILAN showed the highest resistance to *M. grisea*, but this was associated with a relatively high susceptibility to *Fusarium* head blight (FHB, *Fusarium* graminearum). Conversely, SUMAI 3 and GONDO-CBRD were susceptible for *M. grisea*, but relatively more resistant to *F. graminearum*. Differential interactions of *M. grisea* and *F. graminearum* with these three wheat cultivars were studied on ears by confocal laser scanning microscopy (CLSM) at different time points of disease development. At 24 h post-inoculation (hpi), hyphae of both pathogens were observed in anthers, some following the filament towards the ovary. At 48 hpi, the tips of palea and stigma were uniformly invaded, while only *F. graminearum* showed initial infection of the rachilla. Colonization with both pathogens on anthers, filament, stigma and palea was similar on the three wheat cultivars. A hypothesis is that differences in resistance of the three cultivars are expressed in more interior tissues in the spikelets. In the resistant MILAN/M. grisea interaction a strong accumulation of H2O2 within 48 h post-inoculation (hpi) was detected in the palea, which was not found in the two susceptible cultivars. The histochemical localization of H2O2 in the palea tissue indicates the involvement of active oxygen species (AOS) in the resistance response of wheat plants against *M. grisea*. This study indicates the existence of resistance in wheat lines specific for wheat blast caused by *M. grisea*, with no cross-reactivity to another important ear pathogen, *F. graminearum* causing *fusarium* head blight.

138-Kamble, A.[1)]; Koopmann, B.[2)]; von Tiedemann, A.[2)]

[1)] University of Pune, India
[2)] Georg-August-Universität Göttingen

Comparative analysis of defense responses in *B. napus* against *V. longisporum* during host and chemically induced resistance

Verticillium longisporum is a soilborne vascular fungal pathogen of oilseed rape and poses a major threat to its cultivation. Lignification of cell walls is a common defense response and has been shown to also enhance resistance against vascular pathogens. The incorporation of phenylpropanoid derived monolignols in lignin and lignin like polymers following infection results in strengthening of cell walls and improves structural rigidity thus limiting the degradation of cell walls by exogenous enzymes and also limits diffusion of enzymes and toxins from the fungus to the host. In the present investigation, we compared the expression of resistance in respect to the phenylpropanoid metabolism in a susceptible ('Falcon') and a resistant genotype (SEM 05-500256) of *Brassica napus*.

Our earlier work on β aminobutyric acid (BABA) induced resistance in *B. napus* against *V. longisporum* demonstrated early and significant increase in phenylalanine ammonia lyase (PAL) activity in hypocotyls suggesting higher synthesis and accumulation of phenylpropanoids. This increase in PAL activity was found to correlate with large numbers of phenol storing cells surrounding the vessels. Similar kind of defense responses were also reported in case of genotypic resistance in *B. napus* against *V. longisporum* wherein resistance was correlated with higher levels of soluble and cell wall bound phenolics, phenol storing cells and lignin formation in hypocotyls. We also observed a strong and significant increase in salicylic acid (SA) during the susceptible interaction of *B. napus* against *V. longisporum* which correlated with a higher amount of pathogen DNA found in the hypocotyls. These results suggested the possibility of pathogen mediated diversion of the cinnamic acid pool (a common precursor for both SA and lignin biosynthesis) towards SA, to the expense of a rapid and effective lignin biosynthesis hence weakening the plant defense response against fungal invasion. Our present study is based on this hypothesis and we are investigating the role of SA as a negative regulator of resistance in *B. napus* response to *V. longisporum*. Work is in progress to examine the level of salicylic acid, qualitative and quantitative profiling of soluble and wall bound phenolic compounds using HPLC, activities of enzymes of the phenylpropanoid pathway, such as PAL, peroxidase, cinnamate 4-hydroxylase and a histochemical analysis to get more insight into the phenolic profiling, alterations in the chemical composition and regulation of lignin biosynthesis.