IIp-1

A SMALL, CYSTEINE-RICH PROTEIN SECRETED BY FUSARIUM OXYSPORUM DURING COLONIZATION OF XYLEM VESSELS IS REQUIRED FOR I-3-MEDIATED RESISTANCE IN TOMATO

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We report the identification if the first avirulence factor from a root-infecting pathogen. It is a cysteine-rich protein secreted by Fusarium oxysporum f.sp. lycopersici during colonization of tomato xylem vessels. The corresponding gene was identified with degenerated primers based on peptide sequences and encodes a 30 kD protein, designated Six1 for Secreted in xylem 1. The central part of Six1 corresponds to the 12 kD protein found in xylem sap of infected plants. Disruption of the SIX1 gene in a wild-type strain results in breaking of I-3-mediated resistance, suggesting that I-3-mediated resistance requires secretion of Six1 in xylem vessels. On susceptible plants, SIX1-deleted strains are less virulent than wild-type. In forma specialis lycopersici, SIX1 lies on a chromosomal region with a high density of transposons. SIX1 is absent in isolates belonging to other formae speciales, suggesting that it may be associated with host-specificity. We are now investigating if variation in virulence on I-3 plants amongst natural isolates is associated with variation in the SIX1 sequence.

IIp-2

THE ROLE OF REACTIVE OXYGEN SPECIES IN THE CLAVICEPS PURPUREA/RYE INTERACTION.

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The ascomycete C. purpurea is an ubiquitious phytopathogen with a broad host range including many grasses and cereals. It infects only grass florets and causes the ergot disease.
Until now no clear evidence for incompatibility has been found in the interaction C. purpurea/rye. Nevertheless H_2O_2 generation could be shown in rye ovaries colonized by the fungus. Because of C. purpurea's biotrophic lifestyle one would expect a set of ROS scavenging enzymes preventing oxidative damage from both the fungus itself and the host tissue. Investigation of the function of such enyzmes by targeted inactivation of genes encoding the most abundant enzymes, catalase C/D and superoxide dismutase A, revealed that none of them alone is required for pathogenicity.
Interestingly, probably all catalase isoforms of C. purpurea are under control of a bZIP transcription factor (CPTF1) which we recently identified during an in planta EST-analysis. It activates transcription of a catalase gene, cpcat1, after treatment with 0.5 mM H_2O_2 in axenic culture.
CPTF1 is essential for full virulence of C. purpurea on rye. Cytological investigation of rye ovaries colonized by a cptf1 deletion mutant lead to first time observation of an oxidative burst-like accumulation of H_2O_2 around the infecting hyphae but also in distant tissue areas. Since the mutant seems to secrete even more H_2O_2 in axenic culture (due to downregulation of catalases), this observation could be explained by an induction of the host defense reaction by the fungal ROS.
Additionally, two NADPH oxidases have been identified which could be involved in superoxide anion generation, the substrate of SOD.
We now focuse on signal chain components involved in oxidative stress signalling. The CPTF1 sequence contains potential MAP kinase phosphorylation sites. Two MAP kinases of C. purpurea, CPMK1 and CPMK2 are obviously not involved in the CPTF1 mediated cpcat1 induction. A recently identified HOG-homologous MAP kinase could be a candidate for the oxidative stress signal transmitter and is currently characterized. Furthermore functional analysis of histidine kinases is in progress.

IIp-3

THE GPMK1 MAP-KINASE OF FUSARIUM GRAMINEARUM REGULATES THE PRODUCTION OF CELL WALL DEGRADING ENZYMES

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Fusarium graminearum secretes cell wall degrading enzymes during colonization of its host. Recently, we reported that Δgpmk1 mitogen-activated protein-kinase mutants are apathogenic and cannot invade wheat spikes. Now, we show the regulation of various cell wall degrading enzymes via this map-kinase pathway. In a quantitative assay a F. graminearum wild type strain and Δgpmk1 mutants were analysed concerning their ability to produce various cell wall degrading enzymes. The gpmk1 disruption had no effect on the production of polygalacturonases or amylases. However, it could be shown that Gpmk1 regulates the early induction of endoglucanase, xylanase and protease activity. Since the disruption of the Gpmk1 MAP kinase leads to an apathogenic phenotype, these results suggest the infection process of F. graminearum to depend on the secretion of cell wall degrading enzymes particularly during the early infection stages.

IIp-4

TRANSCRIPTOME ANALYSES DURING THE ECTOMYCORRHIZAL DEVELOPMENT BETWEEN PAXILLUS INVOLUTUS AND BIRCH (BETULA PENDULA)

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Certain fungi maintain a genetic constitution that enables them to form functional symbiosis with plants in one type of interaction we know as ectomycorrhiza. In this association between plant roots and fungal hyphae, the fungi derive photosynthetic sugars from the plant, which in turn benefit from the fungus-mediated assimilation of nutrients. This phenomenon is a classic example of mutualism, and is of fundamental ecological importance. We are interested in the molecular and genetic background to the development of this tissue and the signals that govern and maintain a compatible symbiotic interaction. For this purpose we are analyzing the gene expression in the symbiotic tissue. As a model we use the common ectomycorrhizal interaction between the fungus Paxillus involutus and the plant Betula pendula (birch). A previous investigation using microarray analyses on fully developed mycorrhiza have shown significant differences between fungal and plant transcription during mycorrhizal conditions vis-à-vis free-living conditions (Johansson et al. 2004 MPMI 17:202). In this study we have been using microarrays to sample the mycorrhizal interaction at five different time points during the first 21 days of development. Analyses of 2400 cDNA probes of either fungal or plant origin show significant regulation for 251 fungal and 138 plant genes as compared to the control. Groups of fungal and plant genes are identified that show co-regulation at different stages of the mycorrhizal development.

THE ROLE OF DOTHISTROMIN TOXIN IN PINE NEEDLE BLIGHT

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Dothistroma pini Hulbary is a filamentous fungus that causes needle blight in conifers throughout the world. The main hosts for D. pini are Pinus spp.. D. pini produces the difuroanthraquinone mycotoxin dothistromin (DOTH). DOTH is structurally similar to sterigmatocystin and aflatoxin produced by some Aspergillus species and there is evidence that DOTH shares steps in its biosynthesis with aflatoxin. Using aflatoxin biosynthesis genes as probes dothistromin (dot) genes have been found[1]. The relationship between the expression of dot genes and the production of DOTH has been determined. Although it was shown that purified DOTH reproduces the symptoms of disease when injected into pine needles[1], the role of DOTH in pine needle blight has not been confirmed. It is necessary to understand the role of DOTH in the infection and disease process to find new possible targets for disease control. To specify the role of DOTH in the infection process D. pini mutants have been made. Pathogenicity tests are in progress and infection processes with wild type and DOTH deficient mutants will be investigated. This will show if DOTH is necessary for pine needle infection and disease symptoms. GFP expressing D. pini strains are being constructed to observe the infection process in detail.

1 Bradshaw et al., 2002: Applied and Environmental Microbiology 68(6) 2885-2892

2 Shain and Franich, 1981; Physiological Plant Pathology 19: 49-55

MOLECULAR DISSECTION OF STAGONOSPORA NODORUM – WHEAT INTERACTION

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The Australian Centre for Necrotrophic Fungal Pathogens (ACNFP) was created to develop an understanding of necrotrophic fungal pathogen/host interactions at the molecular level, particularly those affecting Australian crops. One such project within the centre is focused on the interaction between the fungus Stagonospora nodorum and wheat. Stagonospora nodorum is the causal agent of leaf and glume blotch on wheat and is responsible for $60M (AUD) of crop loss in Australia each year. Whilst also appearing to be an economically important pathogen throughout the world, very little is known at a molecular level about how the fungus infects wheat. Using a range of classical microscopic techniques supplemented by the use of GFP-expressing strains, we have characterised the infection biology of the fungus. The fungus appears to penetrate the leaf both via stomata and directly through the cuticle. Small penetration structures, which we term hyphopodia, are normally found at penetration sites. Within 2-3 days the fungus ramifies through the depth of the leaf. This precedes obvious cell death. Pycnidia develop within 5-8 days or as soon as the leaf senesces.
We have begun dissecting this interaction using a variety of molecular techniques. We have developed a small collection of ESTs and developed moderate throughput methods to generate knock-out strains. Transformation and homologous recombination are efficient.
We have investigated genes previously associated with pathogenicity and some novel genes. Amongst the known genes are an alpha subunit of a heterotrimeric g protein encoding gene. The mutants have multiple phenotypes including lack of sporulation, lack of pigmentation, reduced osmo-tolerance and reduced secretion. Analysis of this gene has differentiated stomatal and direct penetration and has identified DOPA as the likely precursor of the melanin. Amongst the genes not previously associated with pathogenicity is a mannitol-1-phosphate dehydrogenase. Knock-outs of this gene lack all detectable M1PDH activity and have radically altered neutral sugar contents. Gross symptom production was not changed but the time taken to sporulate was increased markedly. This mutant has focused our attention on the conditions required for sporulation on the host.

IIp-7

CHARACTERIZATION OF ARABIDOPSIS THALIANA UDP-GLUCOSYL-TRANSFERASES INACTIVATING THE FUSARIUM MYCOTOXIN DEOXYNIVALENOL

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Plant pathogenic fungi of the genus Fusarium cause agriculturally important diseases of small grain cereals and maize. The trichothecene mycotoxins produced by Fusarium species inhibit eukaryotic protein biosynthesis and presumably interfere with the expression of genes induced during the plants defense response. The F. graminearum mycotoxin deoxynivalenol (DON) is a virulence factor and frequently accumulates in grain to levels posing a threat to human and animal health.

We have used heterologous expression in Saccharomyces cerevisiae to clone and characterize a gene from Arabidopsis thaliana, encoding a UDP-glycosyltransferase, that is able to confer resistance to deoxynivalenol. This enzyme catalyzes the transfer of glucose from UDP-glucose to the hydroxyl group at carbon 3 of deoxynivalenol, as shown by LC-MS/MS. Experiments with a wheat germ extract coupled transcription /translation system demonstrated that the DON-glucoside has highly reduced ability to inhibit protein synthesis in vitro. Expression of the glucosyltransferase in Arabidopsis is developmentally regulated and induced by deoxynivalenol as well as the plant defense signalling compounds salicylic acid, ethylene and jasmonic acid. Constitutive overexpression in Arabidopsis leads to enhanced tolerance of seedlings against deoxynivalenol.

The identified deoxynivalenol-glucosyltransferase is a member of a huge gene family (more than 100 genes in Arabidopsis!) and is located in a gene cluster consisting of six members of the UDP-glycosyltransferase subfamily UGT73C, all of which are highly similar in their amino acid sequences, but have different substrate specificity. Using domain shuffling the N-terminal part of UGT73C5 could be identified to be essential for deoxynivalenol inactivation. The relevance of detoxification reactions and structural diversity of trichothecenes in plant-pathogen co-evolution are discussed.

(Funded by the Austrian genome programme GEN-AU)

IIp-8

THE DATABASE OF PCR PRIMERS FOR PHYTOPATHOGENIC FUNGI

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Among the modern nucleic acid-based techniques, those involving the polymerase chain reaction are most suited for early detection of phytopathogenic fungi, due to their high sensitivity and the automating potential. Primer sets can be designed to get specificity at the genus, species, or physiological race level, in order to distinguish a given pathogen even from closely related entities.
So far, very few are the examples of specific primer set collections reported in the literature while no on-line repository is available. Here we present the first on-line searchable database of primer sets useful for the detection of plant pathogenic fungi. This web resource is totally implemented with open source software (PHP, MySQL). Primer set details can be retrieved by organism's name, primer's name, nucleotide sequence comparison, target DNA, PCR technique, authors' name, journal and year of publication. Each record is directly linked to other reference databases to allow easy access to the correct nomenclature, taxonomical position and anamorph/teleomorph connections of the pathogen, GenBank deposited source sequences of the primer sets and reference contents.
The database is open to data additions after free registration of the submitter and following system administrator review of submitted data.

SCLEROTINIA SCLEROTIORUM STEM ROT OF CANOLA IN AUSTRALIA

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Stem rot caused by the ascomycete *Sclerotinia sclerotiorum* is now a significant cause of yield loss in Australian Brassica crops. The broad host range (over 400 species, including crops and weeds) of this fungus enables populations to persist and spread easily, and effective and economically feasible disease control measures are lacking. In 1999, yield losses ranged from 5-20% affecting up to 180,000 ha in New South Wales. Currently sown canola lines have no resistance although some genotypes with resistance have been identified in China. Knowledge of the mechanisms employed by the fungus during infection of canola and the underlying genetics of the host-pathogen interaction is imited.

We have collected more than 220 isolates of *S. sclerotiorum* from canola petals at mid-flowering in different regions in eastern Australia. Very high levels of inoculum were present - over 90 % of petals collected in New South Wales and one site in Victoria contained *Sclerotinia sclerotiorum,* whilst there was lower inoculum at a second Victorian site (35%). DNA fingerprints from 80 of these isolates are currently being analysed for genetic diversity. The fingerprints, created using a repetitive element of nuclear DNA provided by Prof. Linda Kohn, Canada, will be compared with a database of 3000 North American isolates.

A second aim is to generate mutant strains of *S. sclerotiorum* that have reduced pathogenicity on canola, and to use these to identify genes important for infection. To date, Poly Ethylene Glycol (PEG) -mediated transformation is the only method of transforming *Sclerotinia* that has been reported. We have produced a Green Fluorescent Protein (GFP)-expressing strain by this method, and fluorescence is observed in both hyphae and sclerotia. These transformants are being used for cytological studies of the infection process on canola and on other Brassica species.

IDENTIFICATION OF THE ROLE OF CHITINASES FROM THE ANTAGONISTIC *CLONOSTACHYS ROSEA* STRAIN IK726 IN BIOCONTROL.

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The fungus *Clonostachys rosea* (syn. *Gliocladium roseum*) is a common saprophyte worldwide. *C. rosea* strains have antagonistic features against a wide variety of phytopathogenic fungi. A strain of *C. rosea,* IK726, was isolated from barley roots infected with *Fusarium culmorum* and evaluated in an *in vivo* screening program. The strain has proven highly efficient against seed-borne diseases of cereals under field conditions. Furthermore, a formulation of the strain with long shelf-life has recently been developed. The modes of action of *C. rosea* as a biological control agent are not well understood but enzymatic activity, mycoparasitism, substrate competition, induced resistance and production of secondary metabolites all have been suggested to play a role. A few recent studies have shown that *C. rosea* produces cell wall degrading enzymes (CWDEs) including chitin, glucan and cellulose degrading enzymes. Thus, enzymatic activity, including activity of chitinases, likely plays a significant role for successful biocontrol of *C. rosea* but has not been studied earlier. The main aim with the Ph.D. project is to identify chitinases and their genes expressed during the antagonistic interactions of *C. rosea* strain IK726 and plant pathogens. The project involves identification of which type(s) of chitinases is expressed, cloning of chitinase genes using PCR strategies and studies on their expression.

IIp-11

GENE EXPRESSION PROFILES IN BLUMERIA GRAMINIS

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Blumeria graminis f. sp. *hordei* (*Bgh*) is the causal agent of barley powdery mildew, an economically significant disease that can cause yield losses of up to 40%. *Bgh* is an obligate biotrophic plant pathogen; it can only complete its life cycle on a living host. The asexual life cycle of *Bgh* on the host proceeds in a highly ordered fashion. The conidium produces a primary germ tube and subsequently an appressorial germ tube, which forms the appressorium. By a combination of physical force and enzymatic action, the appressorium sends a penetration peg into the plant cell, where a haustorium starts to develop. Once the nutrient supply is established, the fungus grows by spreading mycelium over the plant leaf surface.

We have developed microarrays to study global gene expression in *Bgh*. The arrays are about 3500 sequenced and annotated cDNAs derived from *Bgh* libraries. We probe the arrays with fluorescent cDNA from RNA extracted from the fungus at 8 different stages of development, from the ungerminated conidium to a fully sporulating, mature colony.

Expression analysis is examined with Genedata Expressionist™ software and reveals patterns of expression that identify some gene clusters that are likely to play key roles at various developmental stages. This data allows us to propose some models for metabolic and signalling pathways that regulate and control development of infection in this obligate biotrophic pathogen.

IIp-12

EFFECTS OF SEVERAL FUNGICIDES ON THE EXPRESION OF THE FUMONISIN BIOSYNTHETIC GENE, FUM-5, AND THE MFS EFFLUX PUMPS OF Fusarium verticillioides BY REAL TIME PCR.

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Fumonisins are toxic secondary metabolites produced by the Gibberella fujikuroi species complex, particularly F. verticillioides, F. proliferatum and F. nygamai. The genes involved in the fumonisin biosynthesis are clustered in the genome of the fumonisin-producing strains of F. verticillioides. One of these genes, fum5 (=fum1) encodes a polyketide synthase, which is present in the fumonisin-producing F. verticillioides strain FV2, but not in the fumonisin non-producing strain FV16. In a previous work, a MFS (Major Facilitator Superfamily) efflux pump encoding gene had been identified in both strains of *F. verticillioides* and showed an enhanced expression in the fumonisin-producing strain FV2 in comparison with the fumonisin non-producing isolate FV16, suggesting a role of these efflux pump in the export of the toxin. In this work, we report the comparison of this gene in both strains and the results of the analysis of the effect of several antifungal compounds, including fungicides (carbendazime, propiconazol, benomile), antibiotics (cicloheximide, higromicine B) and food preservatives (propionic acid) in the expression of fum-5 gene and MFS coding gene by real time PCR in both isolates. The results of fum5 expression are discussed in relation to the growth and concentration of the antifungal compounds.

The comparison of the effects of the antifungal compounds on the expression of MFS coding gene in both strains indicated a higher level of expression of the MFS coding gene in the strain FV2 than in the strain FV16 in most of the cases, suggesting a role of fumonisins in the activation of the expression of MFS, and possibly other efflux pump genes. Furthermore, we described the efficiency of the real time PCR assay to be used in future assays on the control of fumonisin production.

Supported by the CAM07G/0007/20031 and the AGLZ001-2927-C05-05 projects.

NOVEL FACTORS INVOLVED IN THE INTERACTION MECHANISMS OF TRICHODERMA, A MYCOPARASITE AND AN AVIRULENT OPPORTUNISTIC PLANT SYMBIONT.

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Trichoderma-based biofungicides are a reality in commercial agriculture, with more than 50 formulations registered worldwide as biopesticides or biofertilizers. Several research strategies have been applied to identify the main genes and compounds involved in the complex, three-way interactions between fungal antagonists, plants and microbial pathogens. Proteome and genome analyses have greatly enhanced our ability to conduct targeted and genome-based functional studies. We have obtained reproducible 2-D maps of the entire fungal proteome in various conditions of interaction, which permitted the isolation of many proteins related to specific functions. Many differential proteins from several biocontrol strains of Trichoderma spp. during the in vivo interaction with different plants and/or several phytopathogenic fungi have been isolated and analyzed by MALDI-TOF. Relevant genes have been cloned and specifically inactivatedn, to demonstrate their function in biocontrol and induction of disease resistance. GFP-based reporter systems with interaction-inducible promoters allowed the characterization of regulatory sequences activated by the presence of the pathogen or the plant. From extensive cDNA and EST libraries of genes expressed during Trichoderma-pathogen-plant interactions, we have identified and determined the role of a variety of novel genes and gene-products, including ABC transporters specifically induced during antagonism with other microbes; enzymes and other proteins that produce or act as novel elicitors of Induced Resistance in plant and promote root growth and crop yield; proteins possibly responsible of a gene-for-gene avirulent interaction between Trichoderma and plants; mycoparasitism-related inducers released from fungal pathogens and that activate biocontrol in Trichoderma; fungal promoters specifically induced during mycoparasitism and plant colonization; plant proteins and a novel phytoalexin induced by the presence of the fungal antagonist; ecc. We have also transgenically demonstrated the ability of Trichoderma to transfer heterologous proteins into plants during root colonization. Finally we have used GFP and other markers to monitor the interaction in vivo and in situ between Trichoderma and its host (the fungal pathogen or the plant).

***FUSARIUM OXYSPORUM GAS1* ENCODES A 1,3-β-GLUCANOSYL-TRANSFERASE REQUIRED FOR VIRULENCE ON PLANTS**

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The family of 1,3-β-glucanosyltransferases includes Gas1p from *Saccharomyces cerevisiae*, Phr1p and Phr2p from *Candida albicans* and Gel1p from *Aspergillus fumigatus* which are implicated in fungal cell wall biosynthesis and morphogenesis. Deletion of *PHR1* genes in *C. albicans* results in pH-conditional defects in growth, morphogenesis and virulence on mice. The role of 1,3-β-glucanosyltransferases in fungal pathogenesis to plants has not been explored. We have cloned the *gas1* gene encoding a putative1,3-β-glucanosyltransferase from the vascular wilt pathogen *Fusarium oxysporum*. In contrast to *C. albicans,* expression of *F. oxysporum gas1* was independent of ambient pH and of the pH response transcription factor PacC. *F. oxysporum* mutants lacking a functional *gas1* allele showed a similar growth phenotype as the wild type strain in submerged culture but exhibited an abnormally restricted colony morphology on solid media. This restricted growth phenotype was partially relieved in the presence of the osmotic stabilizer sorbitol, suggesting that it is caused by alterations in the fungal cell wall. Northern analysis showed an increase in the *Δgas1* mutants, of transcript levels of genes implicated in cell wall biogenesis such as *chsV* encoding a class V chitin synthase or *rho1* encoding a small G protein, suggesting the presence of a compensatory mechanism for cell wall integrity. The *Δgas1* mutants were strongly impaired in virulence on tomato plants, both in a root infection assay and in a fruit invasion model. Our results suggest that 1,3-β-glucanosyltransferases are required for plant infection by fungal pathogens.

IIp-15

THE ROLE OF SECRETED PROTEINS IN THE VIRULENCE OF AN APOPLAST-COLONIZING PLANT PATHOGENIC FUNGUS

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Septoria lycopersici is a hemibiotrophic fungal plant pathogen which can infect a variety of solanaceous hosts. S. lycopersici colonizes the plant leaf by growing between plant cells in the apoplastic space. The fungal hyphae never penetrate the host cell or form specialized feeding structures. Thus, the principal way in which the fungus can interact with the host to promote disease relies upon secretion of molecules into the apoplastic space. In order to better understand pathogenesis, we are studying the proteins which are secreted into the apoplastic space by S. lycopersici.
Currently, two S. lycopersici secreted proteins have been identified. The first protein is the enzyme tomatinase which catalyzes the breakdown of alpha-Tomatine, a tomato compound with anti-fungal activity. This protein has been the focus of other researchers in the lab. The second protein is SEP (Septoria extra-cellular protein). SEP shows limited sequence similarity with the PhiA protein of Emericella nidulans. Mutants which lack PhiA show altered phialide and conidial development. The crystal structure of the Sep protein has been solved, and the structure is that of a beta-trefoil fold protein. S. lycopersici mutants which lack SEP are being generated and the phenotype will be presented as well as molecular characterization of the SEP-encoding gene.
Further work is also planned to identify and characterize more S. lycopersici secreted proteins with the goal of determining their role in pathogenesis. Secreted proteins will be identified via analysis of apoplastic wash fluid followed by MALDI-TOF MS/MS or database mining for ESTs which contain a signal peptide for secretion. The biological activity of these proteins will be assessed in planta. S. lycopersici mutants will be generated which lack the putative secreted proteins. The mutants will be assessed for changes in host range and/or virulence. This study will provide important information about the mechanism of pathogenesis for S. lycopersici and possibly other plant pathogenic fungi which colonize the apoplastic space.

IIp-16

The multigenic endoPG gene family from the necrotrophic fungus *Sclerotinia sclerotiorum* : characterization and expression during plant pathogenesis

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The fungus *Sclerotinia sclerotiorum* is an ubiquitous and polyphage pathogen, agent of soft-rot diseases. *S. sclerotiorum* invades its host and rapidly macerates the plant tissues. Among the numerous cell wall-degrading enzymes secreted by the fungus during pathogenesis, endopolygalacturonases (endoPGs) play a major role as they can alone cause tissue maceration. *S. sclerotiorum* endoPGs isozymes are encoded by a large family of genes. A subfamily constituted by the genes *pg1*, *pg2* and *pg3* (*pg1-3*) encodes neutral pI isoforms and has been previously characterized. To improve our understanding of this pectinolytic system, we have isolated and characterized three additional distinct endoPG genes, named *pg5*, *pg6* and *pg7.* The analysis of the amino acid sequences of PG5; PG6 and PG7 revealed the presence of highly conserved domains considered to be the signature of this family of enzymes. The predicted unprocessed endoPGs proteins range in length from 370 to 392 amino acids and contain a signal peptide. The predicted isoelectric points of PG5, PG6, and PG7 are 4.7, 5.5, and 6.9 respectively. The amino acid sequences of PG5, PG6, and PG7 show significant homology to other fungal endoPGs. As expected, the highest amino acid identity was found with those of the related necrotroph *B. cinerea* (79% to 89%). The relationship between the sequences from *S. sclerotiorum* and *B. cinerea* was studied by the prediction of a phylogenetic tree. It appeared that genomic organization of the *S. sclerotiorum* and *B. cinerea* endoPG families are similar. Expression of the *S. sclerotiorum* endoPG gene family was analyzed during pathogenesis of carrot roots. Whereas transcripts of the *pg1-3* genes were detected by Northern blot, transcripts of *pg5, pg6*, and *pg7* could only be detected by RT-PCR. *pg1-3* genes were expressed during the phase of colonization of the healthy tissues, whereas *pg5* was transcribed during the final phase of maceration, and *pg6* and *pg7* exhibited a more constant expression pattern. In the course of infection, acidification of the ambient medium by oxalic acid secretion creates a sequence of environemental conditions that necesseraly activates or repressess the transcription of the endoPG genes. Even if the signification of large endoPG gene families in necrotrophic fungi is not clearly explained, their presence provides an efficient and flexible pectinolytic system that may contribute to the success of *S. sclerotiorum* as a polyphage fungus.

GENETIC AND PATHOGENIC VARIABILITY IN RHIZOPYCNIS VAGUM D.F. FARR, A ROOT ROT AND ENDOPHYTIC FUNGUS

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The coelomycete Rhizopycnis vagum is known to belong to the complex of root rot pathogens contributing to “vine decline” of cucurbits, a disease responsible for considerable economic losses in several parts of the world. Although primarily reported from cucurbit roots, the fungus has also been recently reported as associated with other, unrelated hosts, such as tomato plants showing typical corky root symptoms (Porta-Puglia et al., 2001). A different, endophytic association was described with wild, asymptomatic Pinus halepensis and Rosmarinus officinalis plants in Italy (Girlanda et al., 2002). To assess host-specific and geographical differentiation within R. vagum, pathogenic and endophytic isolates from cucurbit, tomato, P. halepensis and R. officinalis plants in Italy and Northern and Central America were analysed for polymorphisms in single and multilocus markers; pathogenicity assays on melon were also performed under semicontrolled conditions. Phylogenetic analyses of separated and combined ITS (ITS1-5.8S-ITS2) and Gpd (glyceraldehyde 3-phosphate dehydrogenase) sequences and UPGMA analysis of 42 microsatellite loci indicated lower diversity within the American than the Italian isolates, some of the latter clustering with the formers; Fst and Gst indices based on ITS and Gpd haplotype and microsatellite marker frequences revealed high genetic differentiation on both a geographical and a host basis. These results indicate that selection may be important in the association of both pathogenic and nonpathogenic R. vagum with specific hosts. Inoculation experiments established that endophytic isolates may exhibit pathogenic potential on a cucurbit host.

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FUNCTIONAL ANALYSIS OF THE FUNGAL TETRASPANIN PLS1 DURING RICE INFECTION BY MAGNAPORTHE GRISEA.

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The non-pathogenic mutant punchless was obtained by insertional mutagenesis in the rice blast fungus Magnaporthe grisea. This mutant produces melanised appressoria with normal turgor that fail to penetrate into host leaves. The gene inactivated in this mutant encodes a putative integral membrane protein of 225 aa (Pls1). This protein is structurally related to the tetraspanin family(1) and could control an appressorial function essential for the penetration of the fungus into host leaves(2). Genes homologous to PLS1 were identified in other phytopathogenic fungi such as Botrytis cinerea and Colletotrichum lindemuthianum. The B. cinerea ortholog of PLS1 is required for fungal penetration into host plant(3). Identifying the appressorial functions controlled by Pls1 should help understanding fungal appressorium-mediated penetration. This approach requires the identification of proteins upstream or downstream of Pls1. Fungal tetraspanins exhibit conserved domains such as the small intracellular loop (ECL1), motifs of the large extracellular loop (ECL 2) and the C-terminal cytoplasmic tail. These domains of Pls1 were modified by site-directed mutagenesis and their functionality was assessed by complementation of the apathogenic pls1Δhph mutant. The cysteine motifs of ECL2 and the C-terminal tail are required for Pls1 function. Moreover, the C-terminal tail is necessary for the vacuolar localisation Pls1. Proteins interacting with Pls1 will be investigated using TAP-tag purification of Pls1 complexes and yeast two-hybrid method. In order to identify functions controlled by Pls1, appressorial gene expression profiles were compared between wild-type and pls1Δhph mutant. Most of the genes repressed in pls1Δhph are involved in protein secretion, suggesting a role for Pls1 in vacuolar sorting or protein trafficking.

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IIp-19

CHITINASE GENE FROM TRICHODERMA ATROVIRIDE CONFERS FUSARIUM RESISTANCE TO GM-BARLEY

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The plant pathogenic fungi *Fusarium* spp. are known to produce a range of mycotoxins that cause diseases in humans such as cancer, hormonal disturbances and allergy. As many of the mycotoxins are heat stable, these compounds cannot be removed through the chain of processing, and once present in the grains at harvest, they will also be present in the final product. The reduction of the original infection of *Fusarium* will thus be the only way to reduce the amount of *Fusarium*-produced mycotoxins in the final food products. Cereal genes conferring resistance to *Fusarium* infection have not yet been identified, but in some wheat cultivars, resistance loci have been mapped. *Trichoderma* genes encoding chitinolytic enzymes involved in biocontrol have been introduced into several plant species and have been shown to increase the plants' resistance against fungal pathogens.
At the Norwegian Crop Research Institute we have produced GM-barley where a fungal endochitinase gene, *ech42* from *T. atroviride* regulated by the barley promoter Ltp2, has been inserted resulting in increased resistance towards *Fusarium* infection. The advantage of the *Ltp*2 promoter is that it permits a gene to be expressed only in the aleurone layer of developing seeds. Interestingly, this point of time is exactly the time where *Fusarium* infects the spikes of barley.
One of the resulting transformed plant lines, PL9, seemed to be especially promising. The copy number was estimated by the real-time PCR method to be low. Study on the inheritance of the transgenes in T_1 progeny revealed a Mendelian 3:1 segregation pattern.
The expression of the chitinase gene, *ech42,* was studied in the T_1 generation using quantitative real-time RT-PCR assay. Some T_1 progenies showed very high *ech42* expression while others had either very low or no detectable expression at all. After inoculation with *Fusarium culmorum,* all *ech42* containing T_1 progenies coming from PL9 showed high resistance. The amount of *F. culmorum* present after point inoculation of the spikes was quantified by real-time PCR analysis. Extremely low amounts or no *F. culmorum* could be detected in seeds located at the same spike close to the point inoculated grains compared to the huge amounts found in wild type control plants.

IIp-20

LACCASE PRODUCTION IN *FUSARIUM OXYSPORUM*

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Laccases are blue copper phenol oxidases, which catalyse the oxidation of molecular oxygen into water. Many plant pathogenic fungi produce different laccase isoenzymes encoded by multiple genes. For example, *Rhizoctonia solani* has four laccase genes, *Gaeumennomyces graminis var. tritici* has three and *Botrytis cinerea* has two. Nevertheless, the biological role of the majority of fungal laccases remaining uncertain. Grapewine produce the secondary metabolite resveratrol, considered as a phytoalexin which protects the plant against *B.cinerea* infection, laccases produced by this pathogenic fungus are assumed to detoxify resveratrol facilitating the colonization of the host plant (Schouten, A. et al, 2002). The cereal root pathogen *G. graminis var tritici* penetrates the lignitubers by producing lignin degrading enzymes, such as laccases among others (William A.E., 1999). The tomato pathogen *Fusarium oxysporum* f.sp. *lycopersici* penetrates the roots and invades the vascular system by producing different hydrolytic enzymes: polygalacturonases, pectinases, xylanases and proteases (Roncero et. al., 2003).
The presence of multiple laccase genes has been evidenced by bioinformatic analysis in the closely related species *F. graminearum* (http://www.broad.mit.edu/annotation/fungi/fusarium/), using the *lac1* and *lac2* sequences previously isolated (Córdoba et al, ECFG6 2002). Recently four new laccase encoding gene fragments have been isolated, from *F. oxysporum* genomic DNA by PCR amplification, and named *lac3, lac4, lac5* and *lac9*. The role of laccases in pathogenicity is being studied by targeted replacement of the encoding genes with each inactive allele. The pathotype of disrupted lac1 mutants was indistinguishable from the wild type strain, indicating that the gene is not essential for virulence. Further characterization of the laccase system is being accomplished by construction of knockout mutants. In addition, time-course production of total laccase activity has been determined in the *Δlac1* mutant, in a *ΔpacC* mutant (affected in a pH-dependent transcription activating factor; Caracuel et.al., 2003), in comparison with the wild type strain 42.87 grown on minimal medium containing sucrose as sole carbon source during 5 days. Extracellular activity increases along growth time in both strains the *Δlac1* mutant and wild type, in contrast *ΔpacC* mutant does not secrete any detectable activity under the conditions studied. Expression analysis of the six *F. oxysporum* laccase genes is being carried out, by northern hybridization or RT-PCR under induction conditions for laccase (presence of copper, tannic acid, resveratrol) and at different ambient pHs.

PATHOGENIC AND NON-PATHOGENIC MODES OF CONIDIAL GERMINATION

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Colletotrichum gloeosporioides f. sp. aeschynomene (C.g.a) is a hemibiotrophic fungus that is specifically pathogenic on the weed Aeschynomene virginica. C.g.a. conidia do not germinate readily in water due to self-inhibiting materials. We previously found that pea extract can overcome this inhibition: when submerged in pea extract conidia divide within less that an hour and one of the resulting cells differentiates a germ tube. Further studies revealed that in rich medium without pea extract conidia first swell and then form two germ tubes on opposite sides of the conidium. Addition of cAMP to rich medium resembles the major events that occur during Aspergillus nidulans conidial germination. On a solid surface as on plants, conidia germinate only from one side even in the absence of pea extract, but form appressoria only on hydrophobic surfaces or on a hydrophilic surface in the presence of pea extract. The two modes of conidial germination suggested that they may have different roles and might be important under different conditions. To test the effect of germination conditions on pathogenicity, conidia were germinated in pea extract and in rich medium, and then inoculated on plants. Only the conidia that were germinated in pea extract were fully pathogenic on A. virginica plants, whereas conidia that were incubated in rich medium caused only minor disease symptoms. These results suggest that C.g.a., and possibly other plant pathogenic fungi retain two separate germination pathways one of which is pathogenic specific.

INDUCTION OF INFECTION PLAQUES IN OCULIMACULA SPP. THE CEREAL EYESPOT FUNGI

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Cereal eyespot, caused by the closely-related fungi Oculimacula yallundae and O. acuformis (formerly Tapesia yallundae and T. acuformis, Crous et al., 2003), is one of the most important diseases of wheat, barley, and rye in temperate regions. These pathogens infect the stem base, predisposing the plant to lodging; if vascular tissues are penetrated the ears become white and fail to mature. This results in poor grain quality and lower yields. Oculimacula infects the plant by two different mechanisms. Initially, conidia germinate and form simple infection hyphae that penetrate the coleoptile. A mechano-stimulus, created by contact between the coleoptile and the first leaf sheath, is then sensed by Oculimacula and triggers the development of an infection structure or plaque. Plaques are complex multicellular mats with multiple infection hyphae required for penetration of the next leaf sheath. Mechano-sensing is therefore key to the infection process. The object of this project is to examine the mechanisms involved in mechano-sensing. An in vitro technique has been developed to test mechano-sensing and plaque formation. Both O. yallundae and O. acuformis respond to the in vitro mechano-stimulus but the architecture of the plaque differs between the two species; O. yallundae forms dense plaques whereas O. acuformis forms digitate plaques. Mutants of O. yallundae, created using the chemical mutagen N'-methyl-N'-nitro N-nitrosoguanidine (NTG), have been screened to identify those unable to sense pressure or to form functional plaques. To date, mutants unable to form plaques have not been found, but interestingly a significant number that form more plaques than the wild type have been isolated. This result suggests the plaque induction process is negatively regulated.

Mechano-sensing in Oculimacula underlies plaque formation. Initial pharmacological studies in our group suggest that a stretch-activated channel may be involved in the signal pathway. Experiments are in progress to isolate the receptor for this stimulus and to investigate the role G proteins have on plaque formation. This research will provide information on how the mechano-stimulus is sensed and on the signal transduction pathway that leads to infection structure formation.

Ref. Crous et al 2003 European Journal of Plant Pathology 109:841-850

IIp-23

TOXIN PRODUCTION BY FINNISH *FUSARIUM* STRAINS IN ARTIFICIALLY INOCULATED WHEAT AND BARLEY

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The aim of the work was to study the capability of Finnish *F. avenaceum, F. artrhosporioides, F. tricinctum, F. culmorum. F. graminearum . F. sprotrichioides* and *F. poae* strains to produce mycotoxins on spring barley cultivar Scarlett and spring wheat cultivar Mahti on field conditions. Artificial inoculation (three strains per species/inoculum) increased *F. culmorum* and *F. sporotrichioides* contamination levels in both wheat and barley in 2002. *F. avenaceum, F. arthrosporioides, F. tricinctum* and *F. graminearum* inoculation on barley had no clear effect on the contamination level, while both wheat and barley were slightly sensitive to *F. poae* and wheat clearly sensitive to *F. avenaceum*. Spraying by the spores of other *Fusarium* species reduced *F. culmorum* contamination in wheat, while spraying by the spores of any species strongly increased *F. tricinctum* and less strongly *F. poae* contamination in barley, as compared to the unsprayed control.

In inoculated barley *F. culmorum* treatment induced deoxynivalenol (DON, 3 mg/kg) and zearalanone (ZEN, 0.096 mg/kg) production and *F. sporotrichioides* HT-2 production. Nivalenol (NIV), moniliformin (MON), enniatins (ENN) and beauvericin (BEA) were incuded in all inoculated barley samples, except for the one inoculated by *F. culmorum*. The high DON levels in control wheat (1 mg/kg) and barley (0.9 mg/kg) were inhibited in inoculated wheat and barley, except for the plots inoculated with *F. culmorum*. The DON level of the plots of wheat and barley inoculated by *F. culmorum* were 22-fold and 3-fold, respectively, compared to the non-inoculated plots. The highest NIV levels (ca. 0.3-0.6 mg/kg = 6-8-fold compared to the non-inoculated sample) were found in the plots with the highest *F. poae* contamination, while the highest HT-2 (0.466 mg/kg) and T-2 (0.062 mg/kg) levels were found in the wheat plot inoculated with *F. sporotrichioides*. The ZEN concentration (0.045 mg/kg) was highest in the wheat plot inoculated with *F. culmorum*, while no ZEN was detected in other inoculated wheat plots or in the neighbouring wheat field. In wheat the highest MON, BEA and ENN levels were induced by *F. avenaceum/ F. tricinctum. F.avenaceum/F. sporotrichioides* and *F. poae/F.avenaceum/F.arthrosporioides/F.tricinctum*, respectively.

Based on our results it seems that both cultivars of the present work are clearly sensitive to *F. culmorum* and *F. sporotrichioides*, while inoculation with other *Fusarium* species inhibited the natural infection by *F. culmorum* and DON production. It seems that the spraying treatment by any species, except for *F. culmorum,* incuced the natural infection of *F. tricinctum* and *F. poae* in barley, which caused higher MON, ENN, NIV and BEA production.

IIp-24

TOXIN PRODUCTION BY FINNISH *FUSARIUM* STRAINS IN AUTOCLAVED RICE

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The aim of the work was to study the capability of Finnish *F. avenaceum, F. artrhosporioides, F. tricinctum, F. culmorum. F. graminearum, F. sprotrichioides* and *F. poae* strains to produce mycotoxins in pure cultures grown on autoclaved rice.

All three *F. poae* strains produced different amounts of beauvericin (BEA), enniatins (ENN), fusarenon-X (FX), diacetoxyscirpenol (DAS) and nivalenol (NIV), while no deoxynivalenol (DON) and 3-acetyldeoxynivalenol (3AcDON) were produced. Only small amounts of zearalenone (ZEN) and moniliformin (MON) were produced by *F. poae*. All three *F. culmorum* and *F. graminearum* strains had similar toxin profiles and they produced DON, 3AcDON (except for one *F. culmorum* strain) and ZEN and smaller amounts of BEA, ENN, and NIV. The greatest production of DON and ZEN was found in a *F. graminearum* strain. Both *F. sporotrichioides* strains produced BEA, DAS, HT-2 and T-2 and smaller amounts of ENN and ZEN. *F. avenaceum, F. arthrosporioides* and *F. tricinctum* strains had similar toxin profiles and all strains of these species produced ENN and MON and smaller amounts of BEA and ZEN. In one *F. tricinctum* strain a small amount of DON was also produced. According to our results *F. avenaceum, F. arthrosporioides* and *F. tricinctum* were not able to produce great amounts of trichothecenes, but they could produce different amounts of ENN and MON. *F. arthrosporioides* strains were most effective in producing ENN and MON. *F. poae* strains were most effective in producing NIV, F-X and BEA, while *F. sporotrichioides* strains were most effective in producing HT-2, T-2 and BEA. There were also a great intraspecific variation in the amounts of different toxins produced. We are going to continue studying toxin production of the same strains in different temperature and humidity conditions.

DEVELOPMENT OF SPECIFIC PRIMERS FOR A PHYTOPATHOGENIC STRAIN OF *FUSARIUM OXYSPORUM*

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The aim of the study was to be able to monitor the presence of a *F.oxysporum* strain in order to study the mechanism of action of biological agents.
The randomly amplified polymorphic DNA (RAPD) and universally amplified PCR (UP-PCR) techniques were used for screening 21 RAPD and two UP-PCR primers in order to develop strain-specific primers for *Fusarium oxysporum* strain 246 VIZR, the casual agent of wilt in tomato. The RAPD and UP-PCR products were very similar in all tested strains, although variation between the isolates was obtained by both methods. Primer OPA6 proved to be strain specific. Strain 246 VIZR gave amplification products of 400 and 1970 bp which were not revealed among the products of 28 other *F.oxysporum* strains. The specific products were cloned and sequenced and primer pairs designed according to the sequences of these fragments were tested on specificity. The designed primer pairs detected not only strain 246 VIZR but also about 40-50% of other *F.oxysporum* strains used in work.
DNA samples isolated from tomato plants treated or untreated with a suspension of *F.oxysporum* conidia and fungi grown in peat were also used in order to test the specific primers. The new primer pairs did not detect the strain 246 VIZR from infected tomatos. The *F. oxysporum* specific primer pair FO1f/r designed on the sequence divergence within the internal transcribed spacer region of nuclear ribosomal DNA detected *F. oxysporum* on one of the infected tomatos, while in uninfected plants no PCR products were obtained.
The specific PCR products of strain 246 VIZR and two closely related strains were sequenced and compared with each other in order to design more specific primers to separate strain 246 VIZR from other *F. oxysporum* strains. Differences in the sequences of the three strains were obtained and new more specific primers were designed for the strain 246 VIZR. We are now testing the specificity of the new primers both in pure culters and .infected plants.

FOPTA1a AND FOPTA1b: TWO TRANSCRIPTION FACTORS DIFFERENTIALLY INVOLVED IN PATHOGENICITY OF FUSARIUM OXYSPORUM F.SP. PHASEOLI

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One of the severe diseases of common bean (Phaseolus vulgaris) is the Fusarium wilt, produced by Fusarium oxysporum f.sp. phaseoli J.B. Kendrick & W.C. Snyder. The plant pathogen Fusarium oxysporum is a common soilborne fungus with a worlwide distribution. It can grow as a saprophitic organism but many isolates have also the capabilities to infect and cause wilt disease in different plant hosts. We are interested in the molecular basis of the differences that allow the pathogenic isolates to penetrate the host and cause the disease.

We have previously isolated and characterized the gene Fopta1 (Fusarium oxysporum phaseoli transcription activator 1), that belongs to the Gal4 family, which is specific of highly virulent strains that infect common bean plants. We have analysed its expression“in vitro” (by means of RT-PCR) and “in vivo” by Real Time PCR, detecting a dramatic expression peak in infected plant stems 24-48 hours after plant inoculation. To determine the role of Fopta1 in pathogenicity and/or virulence, we also inactivated the native copy by gene disruption, using a cloned copy of the gene with the hygromycin resistance gene inserted in the coding region. Analysis of the knock-out mutants obtained by Agrobacterium-mediated transformation of a highly virulent strain, showed the presence of a second copy of Fopta1 that we have named Fopta1b. Fopta1b is 80% homologous at the nucleotide level with Fopta1a, the main differences being located in the promoter region. Further analyses have shown that Fopta1b is also present both in weakly virulent and nonpathogenic strains (which lack Fopta1a).

These facts suggest that Fopta1a and Fopta1b may have the same origin but have acquired different functions along the evolution. We are currently characterizing mutants with both genes inactivated, and also double mutants, in an effort to elucidate their role in the pathogenicity process.

IIp-27

A NEW GENE IN AN ARBUSCULAR MYCORRHIZAL FUNGUS MODULATED BY NITROGEN: AN AMINO ACID PERMEASE?

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Arbuscular mycorrhizas (AMs) are widespread mutualistic symbioses, involving over 80% of land plants and a limited number of fungi, belonging to Glomeromycota. They were overall considered for their active role in the contribution to plant phosporus nutrition. Recent data obtained in microcosm experiments suggest the possibility of an important role in the uptake of organic nitrogen: AM symbiosis enhance decomposition and increase nitrogen capture, but there is no direct evidence of amino acid transport from extraradical hyphae to the host plant (Hawkins et al. 2000, Hodge et al. 2001).
The aim of the research is to investigate whether an AM fungus, *Glomus mosseae*, particularly present in agricultural systems, has the molecular bases to improve the uptake of organic nitrogen. A DNA sequence (*GmosAAP1*) with high similarity with other amino acid transporters was identified. Expression analyses performed on various steps of the fungal life cycle indicate that *GmosAAP1* is expressed in the external mycelium but not in the asymbiotic phase. In order to investigate whether gene expression is regulated by different nitrogen sources, *Cucumis sativus* mycorrhizal roots were treated for 3 days with 2 micromolar or 2 millimolar nitrate, ammonium or an aminoacid pool containing leucine, alanine, asparagine, lysine and tyrosine. Preliminary results, obtained by Real-Time RT-PCR assays on external mycelium from the different treatments, suggest that *GmosAAP1* transcription responds to available N compounds.

This work was supported by the European Union GENOMYCA project (Project no. QLK5-CT-2000-01319)(www.dijon.inra.fr/bbceipm/genomyca/)

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IIp-28

IDENTIFICATION OF FUNGAL GENES OVER-EXPRESSED DURING INFECTION OF ARABIDOPSIS BY MACROARRAY ANALYSIS.

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Our group is studying the ascomycete Botrytis cinerea, a broad-spectrum pathogen of plants. In order to investigate the molecular mechanisms of pathogenicity of Botrytis without a priori, a genomic approach was adopted: 6.559 fungal sequences obtained from a cDNA library were analyzed by bioinformatic resources. 3032 unigenes (approximately one third of the estimated number of genes) were thus obtained and spotted onto nylon filters for macroarray expression studies.
Here we present a study of the expression profiling of these unigenes during the interaction of Botrytis with one of its hosts, Arabidopsis thaliana. The infected leaves of the plant, harvested at three different times during the period of infection, served as the material for the extraction of RNAs subsequently used as the “interaction” probes. The macroarray filters were hybridized in parallel with the “reference” probes, that is, the RNAs of Botrytis grown in vitro in two different culture media (reference: “Botrytis without plant”), as well as the RNAs of the uninfected plant.

The resulting data has been treated statistically using GeneAnova software (based on the analysis of the variance of expression) and clustered through Genesis. We could then search for genes potentially implicated in the Botrytis-Arabidopsis pathogenic interaction among the unigenes that were systematically over-expressed during the infection (compared to in vitro growth). The list of unigenes coming out of this analysis was furthermore compared with two types of existing data:

1) Two subtractive libraries representing the interaction of Botrytis with Arabidopsis thaliana and Vitis vinifera
2) A previous study made on the transcriptome of a mutant with an altered pathogenicity phenotype (Viaud et al, Mol. Microbiol, 2003, 50, 1451-1465).
A certain number of unigenes have shown a good agreement between their expression profiles for these conditions and the results of the present study. We consider these unigenes as good candidates for a reverse genetics analysis aimed at verifying their potential role in the “pathogenicity arsenal “ of Botrytis.

THE CELLULOSE-BINDING ELICITOR LECTIN (CBEL) FROM PHYTOPHTHORA PARASITICA IS A NEW PATHOGEN-ASSOCIATED MOLECULAR PATTERN

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The oomycete pathogen Phytophthora parasitica produces a cell wall glycoprotein which binds to fibrous cellulose, agglutinates erythrocytes and elicits defence responses and necrosis in the host plant tobacco (1). It consists in a repetition of two domains containing each a Cellulose-Binding Domain (CBD) of the fungal type and an Apple/PAN domain found in animal proteins. CBEL has been shown to be involved in recognition of cellulose, adhesion to this substrate, and cell wall deposition in Phytophthora parasitica (2).
The distribution, activity range and structure-activity relationships of CBEL were further investigated. Homologues of CBEL were found in all Phytophthora strains tested. Infiltration of CBEL into the non-host plants French Bean, Zinnia and Arabidopsis thaliana induced defence reactions in these species. The use of A. thaliana plants mutated in perception of either jasmonic acid (coi1) or ethylene (ein2), or expressing the salicylate hydrolase gene (nahG), showed that defence induction by CBEL involves at least three different signalling pathways in Arabidopsis (3).
Structural requirements for elicitor activity in tobacco were studied by leaf infiltration of recombinant proteins produced in E. coli, or by transient expression using a Potato Virus X vector. The deletion/mutation analysis showed that the integrity of the whole sequence, as well as specific amino acid residues of the CBDs, are involved in elicitor activity.
Taken together, the results shown by this and previous studies suggest that CBEL is a new Pathogen-Associated Molecular Pattern (PAMP) from Phytophthora.

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A FUNGAL MITE IS TRANSACTIVATED BY IMPALA, A MEMBER OF THE TC1-MARINER SUPERFAMILY

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The mimp1 element, previously identified in the fungus Fusarium oxsporum has structural features of miniature inverted-repeat transposable elements (MITEs) : short size (approximately 220 bp), TIRs of 27 bp, a TA target site, no coding capacity, a stable secondary structure. The mimp TIRs are very similar to those of impala, a Tc1-like family known to contain autonomous elements. However the central region is not homologous to any part of the full-length element. Analysis of the distribution and nucleotide divergence of mimp1 elements in a wide range of strains belonging to different Fusarium species showed that they are generally present in 5-20 copies and very homogeneous both in sequence and size. In addition, a strong correlation between the presence of mimp1 and impala was established. To better understand this relationship, we investigated transactivation of mimp1 by an impala element using a phenoytypic assay for excision.

Here we show that a mimp1 element, inserted in the first intron of the niaD gene encoding nitrate reductase, can excise leaving short footprints when an impala source of transposase is provided. Reinsertion occurs in about 90% of all excision events. These results clearly demonstrate that this fungal MITE is actively transposing by a cut-and-paste mechanism through the action of a transposase provided in trans by a Tc1-mariner member.

The mobility of the transposon mimp1 represents an interesting alternative tool for gene tagging in filamentous fungi. Because several MITE families seem to be associated with genes in plants, we started a comparative analysis of mimp1 insertion sites in different genetic contexts. The position of resident versus newly transposed mimp1 elements relative to ORFs should reveal their insertion site specificity as well as the effect of selection and the impact of mimp1 insertions.

IIp-31

FUNCTIONAL ANALYSIS OF *CLSTE12*, A STE12-LIKE GENE FROM THE BEAN PATHOGEN *COLLETOTRICHUM LINDEMUTHIANUM*

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Colletotrichum lindemuthianum is the causal agent of anthracnose of bean. This fungus produces a cell wall-associated polygalacturonase, CLPG2, during the early stages of pathogenesis (1). Recently, the regulatory sequences involved in *CLPG2* transcriptional induction during pathogenesis were identified (2). These elements showed significant sequence homologies with the yeast filamentation and invasion response element (FRE). In yeast, this element controls the expression of genes induced during the invasive development or pseudohyphal growth. It binds two different transcriptional factors named Ste12 and Tec1 which form a heterodimer. A gene encoding a STE12-like transcription factor was isolated from C. lindemuthianum and named *CLSTE12*. This gene codes for a 705 amino acid protein which contained two putative DNA-binding motifs, a homeodomain at the N-terminal region and two Cys2His2 zinc finger at the C-terminal region. The homeodomain is present in all Ste12-family proteins ever identified. The second DNA binding motif, the Cys2His2 zinc fingers is absent from Saccharomyces cerevisiae and Candida albicans Ste12 proteins but present in Magnaporthe grisea and Colletotrichum lagenarium proteins. The protein was produced in E. coli and purified. Gel shift experiments revealed that the recombinant CLSTE12 was able to cooperate with the yeast TEC1 protein to bind a sequence containing a FRE.
To define the function of CLSTE12, *clste12* mutants were created by targeted gene replacement. Saprophytic growth of the mutants was indistinguishable from those of the wild type strain. However, these mutants did not produce anthracnose lesions in bean tissues, indicating that CLSTE12 is essential for pathogenicity of C. lindemuthianum.

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IIp-32

ROLE OF CUTINASES IN THE SOILBORNE PATHOGEN *FUSARIUM OXYSPORUM*

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Cutin is an insoluble lipid polyester covering the surface of all aerial parts of the plant. This polymer is thought to play a key role in protection against the invasion by pathogenic microorganisms. Fungal leaf pathogens secrete cutinolytic enzymes able to depolymerize the plant cutin. In *Fusarium solani* three highly homologous cutinase genes, *cut1, cut2* and *cut3* have been described. While expression of *cut2* and *cut3* is constitutive at basal levels, expression of *cut1* is induced to high levels in the presence of cutin monomers and this induction is mediated by the transcription factor Ctf1α (Li and Kolattukudy, 1997, JBC 272:12462).
F. oxysporum is a soilborne pathogen that infects its host plants strictly through the roots. To study the role of cutinases in root infection, we have isolated *ctf1,* the *F. oxysporum* orthologue of *F. solani ctf1*α . The deduced protein of 902 amino acids shares 91% identity with Ctf1α and contains a Cys_6Zn_2 binuclear cluster motif, three signals for nuclear localisation and numerous consensus sites for protein phosphorylation. Gene replacement was used to create *F. oxysporum* mutants harbouring an interrupted copy of *ctf1*. We also produced strains expressing the *ctf1* gene under the control of the constitutive *Aspergillus nidulans gpdA* promoter. Mycelial growth and conidiation of the two classes of mutants were similar to that of the wild type strain. Northern analysis of genes encoding different plant cell-wall degrading enzymes (*pg1, pgx4, pg5, prt1, xyl4*) as well as *cut1* encoding a cutinase with high identity to the three *F. solani* cutinases, showed no significant differences between the wild type strain and *ctf1* mutants. Experiments are currently underway to identify the gene functions and enzymatic activities regulated by Ctf1. In contrast to *F. solani* (Li *et al*., 2002, JBC 277:7905) knockout of the *ctf1* gene did not significantly affect virulence of *F. oxysporum* on tomato plants, suggesting that cutin-activated transcriptional regulation plays differential roles in soilborne and foliar plant pathogens.

SECONDARY METABOLISM AND AVIRULENCE IN *MAGNAPORTHE GRISEA*

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Isolates of the rice blast fungus *Magnaporthe grisea* that carry the avirulence gene *ACE1* are specifically recognized by rice varieties carrying the resistance gene *Pi33*. *ACE1* was isolated by map based cloning and encodes a natural hybrid between a polyketide synthase (PKS) and a nonribosomal peptide synthetase (NRPS). Together with the lovastatin biosynthetic enzyme LNKS from *A. terreus*, Ace1 defines a novel family of eukaryotic PKS/NRPS that appears to be widespread in ascomycetes. Since Ace1 is not a secreted enzyme and its biosynthetic activity is required for avirulence, we believe that the fungal signal recognized by resistant rice plants is the secondary metabolite whose synthesis depends on Ace1. Construction of an *ACE1* overexpressing strain is underway to facilitate the purification and characterization of the polyketide produced by Ace1.
Expression of *ACE1* is detected exclusively in penetrating appressoria. The same penetration-specific expression was also observed for ten other genes that are located adjacent to *ACE1* in the genome (the *ACE1* gene cluster). Based on homology, these genes may be involved in secondary metabolite production and export. Individual gene disruption experiments should reveal whether these functions are required for the biosynthesis of the avirulence signal. Microbial gene clusters often include regulatory genes involved in the transcriptional control of the cluster. We identified a Gal4-like transcriptional activator in the *ACE1* gene cluster. The presence of a corresponding binding site in the *ACE1* promoter further strengthens the idea that this putative transcription factor may be involved in the regulation of *ACE1* expression. In two convergent approaches, overexpression and disruption of this transcriptional activator, and site directed mutagenesis of its putative binding site in the *ACE1* promoter, we are aiming to elucidate its role in the regulation of expression of the *ACE1* gene cluster. This may pave the way to the constitutive expression of the entire *ACE1* cluster, facilitating the purification of the final metabolite and characterization of its role in avirulence signaling.

A MAP KINASE GENE, *MgMK1*, IS ESSENTIAL FOR PATHOGENICITY IN *MYCOSPHAERELLA GRAMINICOLA*

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The infection of *Mycosphaerella graminicola,* the causal agent of septoria tritici leaf blotch of wheat, is initiated by germination of conidia and entry of the germ tubes through the stomates. Subsequent intercellular growth in close contact with mesophyll cells and colonization of the tissue leads to chlorosis, necrosis and pycnidia formation. So far the molecular mechanisms involved in pathogenesis and infection process are poorly understood in this pathogen. Infection is triggered by perception of the host by the fungal pathogen through physical and/or chemical signals leading to cascades of biological processes needed for establishment and successful colonization. We are particularly interested in understanding the role of the signal transduction pathways/genes in the establishment and development of *M. graminicola* on wheat. Through analyses of cDNA libraries of *M. graminicola,* we identified a full-length cDNA clone containing a ~2.2kb insert sequence that is highly homologous to a mitogen-activated protein (MAP kinase), FUS3 in *Saccharomyces cerevisiae*. In other fungi this gene has been identified as a crucial factor for pathogenicity. This MAP kinase, designated *MgMK1,* possesses a 1068 bp open reading frame and encodes a 356aa sequence. We generated a knockout of this gene by *in vitro* transposon mutagenesis, followed by *Agrobacterium*-mediated transformation through homologous recombination. The disruptant showed no differences in germination, sporulation and growth rate *in vitro* as compared to the wild type isolate IPO323 or transformants with an ectopic integration of the construct. However, the *MgMK1* disruptant failed to cause any symptoms e.g. chlorosis, necrosis and pycnidia on wheat in either detached leaf or seedling bioassays. We monitored the fungal biomass of *MgMK1* disruptant in the absence of visual symptoms and determined only a slight increase of fungal biomass over time using Real Time PCR (TaqMan). However, this increase was tremendously lower than the increase of biomass of the wild type isolate IPO323. Our results indicate that non-pathogenic transformants/isolates can survive in or on hosts without causing symptoms.

IIp-35

PATHOGENICITY GENES IN THE MYCOPARASITE CONIOTHYRIUM MINITANS

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Coniothyrium minitans is an aggressive mycoparasite of sclerotia (resting bodies) of its host Sclerotinia sclerotiorum, an economically important plant pathogen. Although there has been much research on the use of C. minitans as a biocontrol agent, little is known at the molecular level of the mechanisms by which it infects its host. We are attempting to identify, clone and characterise C. minitans genes required for pathogenicity against S. sclerotiorum.
Transformation systems have been developed using both Agrobacterium tumefaciens and Restriction Enzyme Mediated DNA Integration (REMI). Both techniques gave comparable numbers of transformants, with 37 transformants/5x10^5 germlings and 32 transformants/mg of DNA respectively. Southern analysis has shown single-copy integration in 17% of REMI and 37% of Agrobacterium transformants.
From a collection of 3000 REMI-HindIII transformants and 1000 Agrobacterium-derived transformants, nine pathogenicity mutants, two auxotrophic mutants and 39 secondary metabolite mutants were identified. DNA flanking the sites of insertion in four REMI-derived pathogenicity mutants was recovered using plasmid rescue. Sequencing of one of these revealed two putative ORFs with homology to (i) Schizosaccharomyces pombe RRM3/PIF1 helicase involved in mtDNA repair and recombination and (ii) a pisatin demethylase gene (PDA) from Nectria haematococca.
To recover wild-type copies of the putative pathogenicity genes, a C. minitans cosmid genomic library has been prepared and is being screened via macro-arrays. Progress of this screening and approaches for insertional inactivation of pathogenicity genes will be discussed.

IIp-36

REGULATION OF XYLANOLYTIC GENES IN FUSARIUM OXYSPORUM

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Fusarium oxysporum is an economically important plant pathogen that enters the host plant through the roots and colonises them by spreading upwards through the vascular system. During infection, F. oxysporum encounters a number of polymers that constitute the structural components of the plant cell wall, of which xylan represents the major hemicellullose fraction. We have previously identified four structural xylanase genes of F. oxysporum, xyl2-xyl5. Here we present the characterization of the F. oxysporum orthologue of the Aspergillus nidulans xlnR gene, encoding a transcription factor required for expression of xylanolytic genes. Electrophoretic mobility shift experiments showed specific binding of a recombinant FoXlnR-6His-tag protein to the sequence GGCTAA present in the promoter of the xyl4 gene, which was abolished by site-directed mutagenesis of the G located at the second position. At present we are carrying out experiments to establish the possible effects of this mutation on xylanase gene expression in vivo. To study the role of XlnR in F. oxysporum, we have created a ΔxlnR loss-of-function mutant and a $xlnR^C$ strain carrying the gene under the control of the AngpdA promoter, resulting in strongly increased xlnR transcript levels. Extracellular xylanase activity and xyl4 transcripts were significantly increased in the $xlnR^C$ strain as compared to the wild type, and reduced in the ΔxlnR mutant. Root infection assays on tomato plants showed that neither knockout nor overexpression of the xlnR gene produced significant alterations in virulence of F. oxysporum. At present, we are studying the mechanisms of transcriptional regulation of the xlnR gene.

SAPONIN GLYCOSYL HYDROLASES AS VIRULENCE DETERMINANTS IN "Septoria lycopersici"

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A number of plant pathogens are known to degrade the antimicrobial secondary metabolites of their hosts and in some cases this phenomenon has been shown to be associated with virulence. The fungus "Septoria lycopersici" (a foliar pathogen of various Solanaceous species) produces the extracellular enzyme tomatinase, which hydrolyses the tomato steroidal glycoalkaloid saponin alpha-tomatine to the less toxic product, beta-$_2$-tomatine. Tomatinase-minus mutants of "S. lycopersici" are unable to degrade alpha-tomatine and display enhanced sensitivity to this compound. These mutants are still able to cause disease on tomato leaves, but trigger enhanced cell death and elevated expression of plant defence genes during the early stages of infection. A more striking effect of loss of tomatinase is seen on an alternative Solanaceous host, "Nicotiana benthamiana". Tomatinase-minus mutants of "S. lycopersici" are unable to cause disease on this host and elicit a hypersensitive-like response. Our results indicate that tomatinase is involved in a novel two-component process in which host saponins are hydrolysed and the resulting hydrolysis products then suppress induced defence responses via interference with fundamental disease resistance signal transduction processes. Further characterisation of the role of tomatinase in interactions between "S. lycopersici" and Solanaceous hosts is in progress.

ROLE OF CUTINASES IN THE SOILBORNE PATHOGEN *FUSARIUM OXYSPORUM*

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Session 2. Fungal-Plant Interactions.

Cutin is an insoluble lipid polyester covering the surface of all aerial parts of the plant. This polymer is thought to play a key role in protection against the invasion by pathogenic microorganisms. Fungal leaf pathogens secrete cutinolytic enzymes able to depolymerize the plant cutin. In *Fusarium solani* three highly homologous cutinase genes*, cut1, cut2* and *cut3* have been described. While expression of *cut2* and *cut3* is constitutive at basal levels, expression of *cut1* is induced to high levels in the presence of cutin monomers and this induction is mediated by the transcription factor Ctf1α (Li and Kolattukudy, 1997, JBC 272:12462).
F. oxysporum is a soilborne pathogen that infects its host plants strictly through the roots. To study the role of cutinases in root infection, we have isolated *ctf1,* the *F. oxysporum* orthologue of *F. solani ctf1*α . The deduced protein of 902 amino acids shares 91% identity with Ctf1α and contains a Cys_6Zn_2 binuclear cluster motif, three signals for nuclear localisation and numerous consensus sites for protein phosphorylation. Gene replacement was used to create *F. oxysporum* mutants harbouring an interrupted copy of *ctf1*. We also produced strains expressing the *ctf1* gene under the control of the constitutive *Aspergillus nidulans gpdA* promoter. Mycelial growth and conidiation of the two classes of mutants were similar to that of the wild type strain. Northern analysis of genes encoding different plant cell-wall degrading enzymes (*pg1, pgx4, pg5, prt1, xyl4*) as well as *cut1* encoding a cutinase with high identity to the three *F. solani* cutinases, showed no significant differences between the wild type strain and *ctf1* mutants. Experiments are currently underway to identify the gene functions and enzymatic activities regulated by Ctf1. In contrast to *F. solani* (Li *et al*., 2002, JBC 277:7905) knockout of the *ctf1* gene did not significantly affect virulence of *F. oxysporum* on tomato plants, suggesting that cutin-activated transcriptional regulation plays differential roles in soilborne and foliar plant pathogens.

IIp-39

INVOLVEMENT OF PHOSPHOLIPASE D IN THE REGULATION OF AN ACID PROTEASE PRODUCTION IN BOTRYTIS CINEREA.

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Upon contact with plant tissues, the necrotrophic fungus B. cinerea secretes numerous lytic enzymes that participate in the host colonisation by reducing the host defences and by degrading the host cellular components to provide nutrients. The production of these enzymes is regulated by a complex array of signals that transduce information on the extracellular pH, the available nutrients in the medium or the presence of a potential host. In order to understand the molecular basis of these signals transduction pathways, the regulated production of the acid protease ACP1 has been used as a model. ACP1 is encoded by a single gene whose expression is induced upon contact with a host plant and repressed by neutral or alkaline pHs.
We identified phospholipase D as a signalling component involved in the regulation of the *acp1* gene. The expression of this gene decreased when the fungus was incubated in the presence of phospholipase D inhibitors whereas that of other lytic enzymes encoding genes was not affected. Incubation of the fungus under different growth conditions and direct measurements of the lipase activity showed variations that correlated with *acp1* expression and with the status of available sulphur and nitrogen sources in the growth medium. On the other hand, modification of phospholipase D activity did not interfere with the perception of pH by the fungus. Phospholipase D therefore appears to play an important role in the regulation of one set of fungal lytic enzymes and molecular analyses are underway to fully characterize it.

IIp-40

A SECRETED LIPASE OF FUSARIUM GRAMINEARUM IS A MAJOR VIRULENCE FACTOR TO WHEAT

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Fusarium head blight (FHB) caused by the fungus Fusarium graminearum Schwabe (Gibberella zeae (Schwein.) Petch) is one of the most destructive diseases of cereals in humid-temperate climates. Apart from reduced grain quality and yield, FHB is also responsible for the contamination of grain with toxins which reveals a serious threat to the health of humans and animals. Fungal pathogens have evolved a number of different strategies to infect and colonize host plants. Therefore the identification of pathogenicity factors is of great importance for the understanding infection processes. Like other fungal pathogens, F. graminearum secretes various extracellular enzymes which are supposed to be involved in host infection.
We could detect, clone and characterize for the first time a secreted lipase (FGL1) of F. graminearum. The 1056 bp-ORF of the FGL1 is interrupted by two introns, and the encoded lipase consists of 337 amino acids with a calculated molecular weight of 35.7 kDa (after cleavage of the signal peptide).This lipase shows a high homology to the known lipase NHL1 from Nectria haematococca. Expression analysis of FGL1 indicated that the gene can be induced by suitable substrates and is repressed by catabolites. In planta, FGL1 transcripts were already detected one day after inoculation of wheat spikes.
To evaluate the role of FGL1 during the infection process we created lipase deficient mutants (Δfgl1) by gene disruption and compared them with wild type strains. Gene disruption of FGL1 resulted in a significantly reduced extracellular lipolytic activity of the yielded strains. After infection of wheat spikes, the Δfgl1 strains shows a drastically reduced virulence. The infected region was limited only to directly inoculated spikelets.
In summary our results show for the first time on a molecular level the direct evidence that a secreted lipase is a major fungal pathogenicity factor.

MOLECULAR, CHEMICAL, AND IN PLANTA ANALYSES OF TRICHOTHECENE MUTANTS OF THREE FUSARIUM GRAMINEARUM FIELD ISOLATES WITH DIFFERENT PHYTOPATHOGENIC PROPERTIES

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Head blight caused by the ascomycetic fungus Gibberella zeae (Fusarium graminearum) is one of the most destructive diseases of cereals. It causes yield reductions and contaminates grain with mycotoxins, which constitutes a potential risk for human and animal nutrition. One important class of mycotoxins produced by several Fusarium species are the trichothecene derivatives (e.g. nivalenol, deoxynivalenol). Trichothecenes accumulate in Fusarium-infested food and non-specifically affect most eukaryotes. We want to investigate whether virulence is only determined by the presence of the trichothecenes or is a quantitative character that is heterogeneously determined by several factors differing from one isolate to the other. Three isolates of F. graminearum, well characterized in field experiments, were selected:
a medium aggressive isolate that produces nivalenol,
a medium aggressive isolate which forms deoxynivalenol,
a highly virulent producing high levels of deoxynivalenol.

The Tri5 genes of these three isolates were cloned, sequenced, and disrupted by transformation mediated homologous recombination. The molecular analysis, the trichothecene quantification, and the phytopathogenic properties of the mutants will be presented.

GENETIC TRASFORMATION OF *PYRENOCHAETA LYCOPERSICI* OF TOMATO

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Poster presentation, Session 2: Fungal-Plant Interactions

Corky root, caused by P*yrenochaeta lycopersici*, is an important soil-borne disease of tomato in Italy. The study of the fungal infection process of tomato roots and the monitoring of the propagation of *P. lycopersici* in the soil are hampered by the slow growth of the fungus and the difficulties in the isolation procedures. A transformation protocol using the *Escherichia coli gusA* gene coding for β-glucuronidase was choosen to obtain genetically engineered fungal strains for histochemically detecting the presence of hyphae into the roots. GUS assay has become a widely used reporter system in the study of filamentous fungi, mainly because of enzyme stability and the high sensitivity and amenability of the assay by fluorometric or histochemical techniques. The highly virulent *P. lycopersici* isolate ISPaVe ER-1211 was stably co-transformed with plasmids pNOM102 containing the β-glucuronidase gene and pAN7-1 for hygromycin resistance. Protoplasts obtained from mycelium were transformed in the presence of PEG giving an efficiency of 0.1-0.2 transfomants/µg of DNA/10^7 protoplasts. Among the transformants obtained, two of them revealed stable after several replicates on non-selective medium. No difference in morphology and virulence between transformants and the wild type were observed. DNA analyses of transformants were carried out. This system provides a practical mean of screening of tomato varieties with different resistance at different time after inoculation. It will also facilitate the observation and detection of the fungus in soil.

IIp-43

A SYSTEM FOR DIRECTED INTEGRATION IN THE py*r*G LOCUS OF Aspergillus awamori GENOME.

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The BiP protein is a molecular chaperone member of the heat shock 70 protein family (HSP70) also known as glucose-regulated protein (GRP78). It is found in the lumen of the endoplasmic reticulum (ER) and participates in the translocation of newly synthesized proteins to avoid their aggregation before the correct folding. The expression level of the BiP-enconding gene (bipA) is strongly increased under situations of cellular stress.
To study the role *of* bip*A* promoter and the UPR elements in the gene expression we have designed a system of directed integration in a specific locus of A. awamori (pyrG locus). We have obtained an A.awamori pyrG mutant strain (56pyrG-) by UV radiation. Transformation of this strain with a plasmid that contains 1000pb of the bipA promoter fused to a reporter gene (lac*Z* gene of E.coli) and a mutant copy of the pyrG gene of A.niger resulted in the integration by homologous recombination of a single copy of the bipA promoter fused to the lacZ gene in the pyrG locus of A.awamori. By this method we could also integrate into the A. awamori genome, in a single copy, several fragments of the promoter obtained by the action of S1 nuclease on the 5´end. This strategy is being used to analyse the role of different boxes (heat stress elements and UPR elements) on bipA gene expression.

IIp-44

CHARACTERISATION OF ISOLATES OF *PHOMA TRACHEIPHILA* BY RAPD-PCR, MICROSATELLITE-PRIMED PCR AND rDNA ITS1/ITS2 SEQUENCING AND DEVELOPMENT OF SPECIFIC PRIMERS FOR *IN PLANTA* PCR-DETECTION.

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The mitosporic fungus *Phoma tracheiphila* is the incitant of the “mal secco” disease of lemon and other *Citrus* species. The pathogen invades the xylem and causes impairment of the water transport system of the plant, leading to wilting of branches and eventually to plant death. When grown in liquid substrate, the fungus is able to produce a complex of phytotoxic glycoproteins, among which a 60 kDa toxin coded Pt60 was shown to reproduce the disease symptoms when injected into plant leaves. Moreover, pectolytic enzymes are produced by *P. tracheiphila* and their role as pathogenicity or virulence factors is being currently evaluated. With the aim of seeing whether the variability in virulence observed among *P. tracheiphila* isolates may be related to differences at the genome level, a collection of 35 isolates representing Italian populations of this pathogen was characterised by different molecular tools and compared to representatives of other phytopathogenic *Phoma* species. These included analysis of the distribution of RAPD and microsatellite markers and sequencing of the internal transcribed spacer region of the nuclear rDNA genes. The result obtained with 10 RAPD primers and 8 microsatellite primers indicates that this pathogen presents a high level of genetic homogeneity, leading to overlapping patterns upon amplification with most of the tested primers. Accordingly, ITS1/2 sequences were highly conserved among all the tested isolates of *P. tracheiphila*. The ITS1/2 of *P. tracheiphila* were compared with those of species commonly found on Citrus as pathogen and those of other *Phoma* species (namely *P. betae*, *P. cava*, *P. exigua*, *P. fimeti*, *P. glomerata*, *P. lingam*, and *P. medicaginis*) and *P. tracheiphila*-specific primers were designed matching diverging ITS1/2 regions. A PCR assay coupled to electrophoretic separation of PCR products made it possible to detect *P. tracheiphila* in naturally infected *Citrus* wood tissue. The limit of detection was 50 pg of genomic DNA. The PCR assay is being applied to study the colonisation pattern by *P. tracheiphila* in artificially inoculated lemon plants.

MOLECULAR BASIS OF MITOCHONDRION-ENCODED RESISTANCE TO Q_0 INHIBITOR FUNGICIDES IN PODOSPHAERA FUSCA

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Strobilurins are inhibitors of respiration and constitute a major class of modern agricultural fungicides. Strobilurins have a single-site mode of action. They inhibit mitochondrial respiration by binding to the Q_0 site of the cytochomre bc1 enzyme complex, thus blocking electron transfer in the respiration pathway and leading to energy deficiency due a lack of ATP, and are also known as Q_0 inhibitors (Q_0Is). It is well known that single-site inhibitors generally posses a high risk of resistance development if resistant mutants are not impaired in their ability to survive and reproduce in the agricultural environment. Following the commercial introduction of Q_0Is in 1996, resistant isolates were detected in field populations of several phytopathogenic fungi and soon after, the mechanisms underlying resistance to Q_0I fungicides in those pathogens were investigated. Thus, it is assumed that the main mechanism conferring Q_0I resistance is a point mutation in the mitochondrial cytochrome b gene (cytb) leading to an amino acid change from glycine (G) to alanine (A) at position 143 (G143A) in the Q_0 centre.

Powdery mildew is one of the most important diseases of cucurbits worldwide. Disease induces as the most characteristic visual symptom a whitish, talcum-like, powdery growth developing on both leaf surfaces, petioles and stems. The main causal agent of cucurbit powdery mildew in Spain is Podosphaera fusca, an obligate biotrophic ectoparasite which is responsible for important yield losses in cucurbit crops under field and greenhouse conditions. Fungicide application is currently the principal control practice in most cucurbit crops for managing powdery mildew. As part of a research program on cucurbit powdery mildew management in Spain, we are carrying out a detailed analysis on fungicide resistance in populations of P. fusca from different cucurbit production areas. To date, we have detected a frequency of resistance to Q_0I fungicides (azoxystrobin, kresoxim-methyl and trifloxystrobin) up to 17%, with minimal inhibitory concentration values >500 μg/ml for resistant isolates. In addition, we are characterizing cytb alleles from Q_0I resistant and sensitive P. fusca isolates by mapping the G143A substitution.

VIRULENCE AND THE PRODUCTION OF ENDO-1,4-B-GLUCANASE BY ISOLATES OF ALTERNARIA ALTERNATA INVOLVED IN THE MOLDY-CORE DISEASE OF APPLES

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Alternaria alternata, is the predominant fungal pathogen responsible for moldy-core in apple cvs. of the Red Delicious group. Here we report on the association between virulence of natural isolates of A. alternata, their production of endo-1,4-β-glucanase (EG) and moldy-core development in apple fruits. Based on decay development following wound-inoculation of mature fruits, three of 150 isolates, collected in three orchards in northern Israel and representing low, moderate and high virulence, were selected and designated Rm44, Er30 and Sh42, respectively. All three isolates secreted EG when grown on enzyme inducing medium (EIM) containing commercial cellulose or apple cell walls and this production was related with their degree of virulence. Polyacrylamide gel electrophoresis (PAGE) revealed small quantitative differences between the three isolates, relative to their virulence, but no qualitative differences. A single band with a molecular mass of 23 kDa showing EG activity was produced by isolates Er30 and Sh42 but not by Rm44 grown on EIM containing fruit cell walls. A commercial cellulase preparation (containing endo and exo-1,4-β-glucanase) placed on pricked fruit mimicked the formation of symptoms that developed on A. alternata-inoculated fruits within 2-4 days. Inoculation of bloom clusters at full bloom with A. alternata resulted in significantly higher infection in fruits inoculated with isolate Sh42 (58%) than in fruits inoculated with isolate Rm44 (30%). Our results suggest that the moldy-core symptoms caused by A. alternata, in apple, could be related to the ability of the fungus to produce EG in developing lesions.

IIp-47

QUANTIFICATION OF FUSARIUM GRAMINEARUM IN INFECTED PLANT MATERIAL BY REAL-TIME PCR APPLYING A SPECIES SPECIFIC TAQMAN PROBE

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Fusarium graminearum (teleomorph: Gibberella zeae) is a major cause for cereal scab. We developed a Real-Time PCR assay to selectively quantify F. graminearum in infected wheat. The assay is based on the detection of a 111-bp part of the ß-tubulin gene of F. graminearum using a pair of newly developed primers and a TaqMan probe with specificity on the species level. As a reference we constructed a plasmid standard for ß-tubulin quantification. We showed that the developed assay was able to specifically detect all 9 Austrian F. graminearum isolates tested in our evaluation whereas applying the method on the most closely related Fusarium species no product formation could be observed (e.g. F. sporotrichoides, F. poae, F. culmorum, F. cerealis). The limit of quantification in a background of wheat DNA was determined to be 10 copies of the ß-tubulin gene while the detection limit was even lower than that. The method led to reproducible results, which were linear (R^2 >0,97) over five orders of magnitude (from 10 to 10^5 templates).
This assay was then employed in planta using wheat samples inoculated with F. graminearum in a field experiment. During all phases of infection of the plant (day 1 to day 16) samples were taken, nucleic acids were extracted and then subjected to further analysis. The developed method allowed the detection of F. graminearum in early stages of infection before the appearance of any visible symptom of the disease. In addition we assessed the expression of tri5 (trichodien synthase encoding) following a RT-PCR approach combined with Real-Time quantitation using SYBRgreen I as fluorescent dye. The method developed in this study allows a fast identification and quantitation of plant-infections by F. graminearum at very early stages where classical microbiological and toxin analysis methods fail to detect the pathogen.

IIp-48

FOPTA1, DETECTED IN FORMAE SPECIALES OF FUSARIUM OXYSPORUM

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The plant pathogen Fusarium oxysporum is a common soilborne fungus with a worlwide distribution. Within the species there is a high level of host specificity with over 120 described formae speciales and races capable of causing vascular wilt diseases of many agricultural crops. This combination of wide range of infection as species and host specificity as formae speciales makes F. oxysporum an atractive model for the study of the molecular interactions involved in pathogenicity and/or virulence.

We have previously isolated and characterized the gene Fopta1 (Fusarium oxysporum f.sp. phaseoli transcription activator 1), that belongs to the Gal4 family, which is specific of highly virulent strains that infect common bean plants. In order to determine whether this gene is also present in other pathogenic strains of F. oxysporum we screened representants of 15 different formae speciales. We designed primers for PCR analyses based on the nucleotide sequence of Fopta1 from a highly virulent strain of F. oxysporum f.sp. phaseoli. By using different combinations of primers aimed to obtain the promoter region and the coding region close to the promoter, we have obtained homologous fragments from formae speciales pisi, lycopersici, betae, vasinfectum, lupini and raphani. Comparison of the cloned sequences shows a very high identity among the copies of this gene. Southern analyses have shown that, besides the presence of the Fopta1a copy of the gene, there is also a second copy named Fopta1b, as it is the case of the highly virulent strains of F. oxysporum f.sp. phaseoli (see abstract by B. Ramos et al.).

These results suggest that Fopta1a and Fopta1b are conserved among the pathogenic isolates of F. oxysporum, and that Fopta1a is required for pathogenicity in a wide range or formae speciales.

USE OF FORWARD AND REVERSE GENETICS AND COMPARATIVE GENOMICS TO DISSECT THE Fusarium EAR BLIGHT – WHEAT INTERACTION

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This Fusarium ear blight project is part of the new Wheat Pathogenesis programme at Rothamsted Research which aims to identify the common molecular themes underlying disease formation on cereal hosts caused by non-biotrophic fungi. The two other species investigated are the leaf blotch pathogen Mycosphaerella graminicola (Septoria tritici) and the eyespot pathogen Oculimacula yallundae, formerly named *Tapesia yallundae.*

F. graminearum and *F. culmorum* cause ear blight disease on small grain cereals. Infections at anthesis, not only lower grain quality but also lower grain safety because both fungal species produce a range of trichothecene mycotoxins post-infection[1].

Our research aims to identify Fusarium genes and gene networks of functional importance to the outcome of the cereal interaction. By a forward genetics approach, involving random plasmid insertion and ear pathogenicity tests, nine disease attenuated Fusarium (daf) mutants have been recovered. Each daf mutant differed in its ability to (a) cause disease symptoms on the ear, leaf and roots of wheat plant, (b) affect grain fill and (c) synthesise trichothecene mycotoxins. By a reverse genetics approach, our laboratory and others have demonstrated that two distinct Mitogen Activated Protein Kinases (MAPKs) Map1 and Mgv1, are independently required for infection and subsequent spread within the wheat ear[2]. We have now selected, via comparative genomics, other candidate fungal pathogenicity genes for targeted gene disruption and biological evaluation.

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Rothamsted Research receives grant-aided support from the Biotechnology and Biological Sciences Research Council (BBSRC) of the UK. This work is supported in part by Monsanto and Syngenta.

SEXUAL DEVELOPMENT AND FUNCTION IN GIBBERELLA ZEAE.

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Gibberella zeae (anamorph Fusarium graminearum) produces its sexual spores (ascospores) in sacs called asci. The asci are produced in ephemeral perithecia which are produced on the surface of field debris and which, when mature, fire their spores into the air. We have been investigating formation of perithecia and the mechanism of forcible ascospore discharge through a variety of molecular, histological and physiological techniques. We will present the results of these studies. Among the findings will be results of microarray analyses to identify genes expressed during perithecium maturation, and evidence that accumulation of osmolytes is important to generation of the turgor pressure for discharge of these spores. The recent availability of a genomic sequence for *F. graminearum* has greatly facilitated the study of perithecium development and function, and host colonization.

IIp-51

IDENTIFICATION AND ANALYSIS OF THE AUROFUSARIN GENE CLUSTER IN SPECIES OF THE FUSARIUM GRAMINEARUM COMPLEX

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The filamentous ascomycetes of the genus Fusarium are living partly as saprophytes in the soil, partly as necrotrophic plant pathogens. Fusarium culmorum is a major pathogen of the cereals in Northern Europe. Like the closely related Fusarium graminearum, a major pathogen in North America, it is causing head blight and foot and root rot. Due their ability to produce a range of different toxins these fungi are of concern in feed and food production. Recently, the first release of the complete genome of F. graminearum was made publicly available at the Whitehead Institute.

We are currently working on the identification of genes and molecular factors involved in pathogenesis of F. culmorum. One strategy involves random DNA mutagenesis of species in the complex using Agrobacterium tumefaciens mediated transformation. We have presently produced about 2000 transformants. To identify potential pathogenicity genes, we are planning two ways of screening the transformants. The first involves a screen for secondary metabolism mutants, followed by pathogenicity testing of each identified mutant. The second involves a direct pathogenicity screen. The first target chosen of the former screen was the toxic red pigment aurofusarin, due the expected easily detectable phenotype of mutants: a culture of a mutant should appear white or pale, as opposed to the red wild type. To this date 3 white, 1 yellow and 6 pink mutants have been produced. Until now, three of the mutants, two white and one pink, have been localised to the same gene cluster. The organisation of this gene cluster in the species of the F. graminearum complex is under investigation.
We are currently working on implementing an HPLC screen to identify mutants in other secondary metabolite pathways.

IIp-52

FUSARIUMSCREEN™, A SENSITIVE, REALTIME AND NON-DESTRUCTIVE MONITORING TOOL FOR FUSARIUM INFECTION IN CEREALS

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A complex of *Fusarium* spp. causes head blight on wheat. Unlike the advances in the area of Fusarium genomics, progress in the understanding of the infection process and the possible resistance mechanisms of the host is slow. In wheat several different resistance mechanisms to *Fusarium* are currently recognised, including resistance to infection, resistance to colonisation and resistance to kernel infection. To exploit these natural types of resistance in wheat breeding programs it is important to monitor the infection process to identify and characterise the type and the level of resistance in wheat genotypes. We developed FusariumScreen™ to quickly identify different levels and mechanisms of resistance in wheat. FusariumScreen™ is based on high throughput fluorescence imaging (HTFI) and enables fast, detailed, non-destructive studies of the *Fusarium*-cereal interactions. We inoculated wheat heads with a *Fusarium*::GFP transformant and utilised FusariumScreen™ to identify the stress response of the plant and the colonisation process of the *Fusarium*::GFP strain. All images are quantified in an automated image analysis pipeline. The integration of the plant stress response and the increase of fungal biomass enables efficient screening of wheat lines and will generate invaluable information about the infection process and the genetic variation for resistance mechanisms in cereals to *Fusarium* species.

IN VITRO AND IN PLANTA EXPRESSIÓN OF THE BOTRYTIS CINEREA ALTERNATIVE OXIDASE CODING GENE.

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Getting energy during the infection and colonization process is a primary problem any plant pathogen must face. Mitochondria are the energy producing engines in eukaryotic cells. Most fungi retain a core mitochondrial electron transfer chain similar to that utilised by mammalian mitochondria involving the participation of three large protein complexes (I, III and IV). In each complex electron transport is coupled to proton tanslocation. The resultant proton motive force is used for ATP synthesis. In addition to this cytochrome-involved respiratory pathway, higher plants, protists and fungi possess an alternative cyanide-resistant pathway, based on the activity of an altenative oxidase enzyme which accepts electrons from the ubiquinol pool and catalyses the reduction of dioxygen to water.
Botrytis cinerea is an important plant pathogenic fungus with a wide host range. Previous work carried out in our laboratory suggested a causal link between altered mitocondrial metabolism and pathogenicity of B. cinerea. In order to evaluate in this necrotroph the role of fungal respiration through the alternative oxidase pathway during the interaction of the pathogen with its host, we decided to clone the B. cinerea alternative oxidase gene, analise its expresión pattern during the interaction and characterise it functionally by generating mutants deficent in the alternative oxidase activity which will be tested for pathogeniciy. Northern analisis during saprophytic growth indicated that expression of the alternative oxidase gene is developmentally regulated, increassing significantly once gemination of conidia in liquid medium took place. In planta, its level of expression is below the limit of detection by Northern analysis, even at late stages of the infection process, during maceration of the infected tissue, indicating that the expression of the gene is down-regulated in planta. Transformation experiments are currently being performed to obtain mutants deficient in this enzimatic activity by gene replacement. Their possible alterations, both during saprophytic growth and during the interaction of the pathogen with its host, will be investigated.

This work was supported by grant AGL2001-2052 (Ministerio de Ciencia y Tecnología, Spain).

A MULTIDISCIPLINARY APPROACH TO DISSECT THE MOLECULAR BASIS OF THE NEOTYPHODIUM LOLII / RYEGRASS SYMBIOSIS

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Neotyphodium lolii is a fungal endophyte that lives entirely within the intercellular spaces of its grass host, perennial ryegrass. Infection is symptomless and the endophyte relies on the host plant for dissemination via the seed. The association is mutually beneficial since the endophyte confers a number of biotic and abiotic advantages to the host, including enhanced plant growth, protection from certain mammalian and insect herbivores, enhanced resistance to nematodes and some fungal pathogens and in some associations enhanced drought tolerance.

We are using a multidisciplinary approach to dissect the molecular basis of this important symbiosis and intend to link the knowledge gained from basic biology and cytology with the fields of Genomics, Transcriptomics, Proteomics and Metabolomics. To achieve this we have used isogenic ryegrass lines infected or uninfected with endophyte and a combination of Suppression Subtractive Hybridisation (to enrich for plant and fungal transcripts that are differentially expressed during the symbiosis), Microarray analysis, 2D-gel electrophoresis (to identify novel proteins associated with symbiosis), and metabolic profiling (also see abstract by L. J .Johnson et al.). To aid our proteomics approach we have also generated both fungal genomic and EST databases.

By linking these approaches we hope to identify genes which are important in both the establishment and maintenance of symbiosis, some of which could be fundamental to understanding how plant-fungal interactions, particularly evasion of host defences, are regulated. In addition, by combining transcriptomics with metabolomics, we intend to elucidate how endophyte infection influences host secondary metabolism, which we hypothesise is correlated with many of the endophyte conferred enhancements mentioned above. In accordance with this we have so far identified a significant number of up and down-regulated genes involved in secondary metabolism as well as genes involved in signalling, defence/stress responses and protein turnover.

IIp-55

ELUCIDATING THE ROLE OF THE F-BOX PROTEIN FRP-1 IN PATHOGENESIS OF FUSARIUM OXYSPORUM.

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During evolution, plants have developed effective ways to defend themselves against microbial invasion. A microbial pathogen has to break through these defences in order to colonize the plant. To investigate the genetic basis of this ability of pathogens, we use the interaction between the soil-borne fungus Fusarium oxysporum f.sp. lycopersici and its host tomato as a model system. Using insertional mutagenesis of F. oxysporum, an F-box protein called Frp1 was found to be required for pathogenicity. It was shown that this protein, like other F-box proteins, binds to Skp1, a subunit of the E3 complex. This complex is involved in the ubiquitinylation of proteins recruited by F-box proteins, marking them for degradation by the proteasome. To unravel the function of Frp1, other interacting proteins will be searched for, in particular ones that are recruited for degradation. To do so, a yeast two-hybrid screening using only the C-terminal part of Frp1 will be carried out. Another approach is the identification of interacting proteins by tandem affinity purification (TAP) and mass-spectrometry. TAP-tagged Frp1 will be generated and used to fish interacting proteins from an extract of Fusarium oxysporum. Interacting proteins will then be analysed for their role in pathogenicity.