Willie Commelin Scholtendag 2004

Op donderdag 22 januari 2004 vond op de Uithof in Utrecht de Willie Commelin Scholtendag plaats. Deze jaarlijks terugkerende bijeenkomst wordt georganiseerd door de sectie voor de Fytopathologie van de Koninklijke Nederlandse Botanische Vereniging en heeft tot doel kennisuitwisseling tussen de fytopathologische onderzoeksgroepen op instituten, proefstations en universiteiten te bevorderen. De bijeenkomst werd bijgewoond door ongeveer tachtig personen. De samenvattingen van de presentaties staan hieronder weergegeven.

De datum voor de volgende WCS dag is vastgesteld op donderdag 20 januari 2005, wederom op de Uithof in Utrecht. U bent allen uitgenodigd om deel te nemen. Het bestuur van de sectie streeft naar een programma waarin alle actoren in het fytopathologisch onderzoek in Nederland vertegenwoordigd zijn en nodigt met name onderzoekers van instituten en proefstations uit een bijdrage te leveren. Voor nadere informatie over de KNBV sectie fytopathologie en de WCS dag kunt u zich wenden tot Guido Bloemberg, secretaris (bloemberg@rulbim.leidenuniv.nl / 071 527 5056) of Francine Govers, voorzitter (francine.govers@wur.nl / 0317 483 138).

Hosts, species and genotypes

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How we define and recognise species is a theme that is central to phytomycolo23gy. For the purpose of this talk, I will briefly discuss the various models currently employed for species recognition and point out the positive and negative aspects of each, using various examples of phytopathogenic fungi. To this end, the recognition of phylogenetic species by employing genealogical concordance appears to be the widely accepted, though the biological and morphological species concepts are still commonly used. In recent years the synergism between plant pathology and phytomycology has largely been lost and hence plant pathology as a science finds itself in a serious predicament. Most plant pathologists work with names that relate to outdated concepts. Few actually work with the organisms named in their grant proposals. In this talk I will present data to address various issues related to: (a) genomic data vs. the Saccardoan system and the anamorph names it gave rise to; (b) pathogen diagnostics and the value of epitypification; (c) genomic data that will indicate that many of the pathogen names we are currently using need to change; (d) the need of plant pathologists to ensure that they are represented in AToL initiatives; (e) the understanding that clonality, sex and variation mean we have to think about studying populations rather than random strains. Although the pros and cons of various proposed changes remain debatable, the mycological dogma we were taught is changing due to genomics. The biggest advantage to systematics is that these new approaches promise an eventual stability to a science that underpins plant pathology.

Downy mildew genomics: identification and functional analysis of genes encoding secreted proteins

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Downy mildews infect many important crops worldwide. To protect crops from downy mildew disease, natural resistance genes have been introduced into cultivars. However, resistance is usually rapidly overcome by the pathogen. The project 'Downy mildew genomics and plant disease resistance' aims to identify new resistance genes that mediate the recognition of important pathogen proteins and may therefore be more durable. A genomics approach is used to identify downy mildew genes that encode secreted proteins and that are specifically expressed during the infection process. Two downy mildew – plant interactions are studied: *Peronospora parasitica – Arabidopsis thaliana* and *Bremia lactucae* – lettuce. Over three thousand Expressed Sequence Tags (ESTs) have been collected from *B. lactucae* and *P. parasitica* conidiospore libraries. These ESTs have been screened for signal peptides and for similarity to genes or proteins in public databases. Microarray technology is being

used to study the expression of these genes during infection of the host. In addition, we are colecting a large number of ESTs from a subtracted library of the *P. parasitica – A. thaliana* interaction. Functional studies of selected *P. parasitica* secretory proteins will be carried out by (transient) expression in *A. thaliana* and *Nicotiana* sp.. *B. lactucae* genes encoding secreted proteins will be transiently expressed in lettuce to identify lines reacting with a hypersensitive response. These lines will be tested further for downy mildew resistance and can be used by lettuce breeders to obtain new resistance specificities to downy mildew disease.

This research is funded by the Dutch Technology Foundation (STW).

New bacterial strains for the control of tomato foot and root rot

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Tomato foot and root rot (TFRR) is an important tomato disease caused by the fungus *Fusarium oxysporum* f.sp. *redicis-lycopersici* (Forl). In our group we develop bacterial control agents to Forl and other fungal diseases in plants.

Pseudomonas strains were isolated from Spanish tomato plants and *Bacillus* strains were isolated from Mexican maize plants. In both cases plants were grown under conditions of sustainable agriculture. The isolates that appeared to be antagonistic towards Forl *in vitro*, were tested for control of TFRR under greenhouse conditions after applying them on tomato seeds or seedlings. *Pseudomonas chlororaphis* PCL 1391 and *Bacillus* sp. BS43 appeared to be very efficient in TFRR suppression. For these strains the presumed mechanism of biocontrol is antibiosis.

Since competitive root tip colonization can be an important trait in biocontrol, a number of Gram-negative bacteria with enhanced colonization properties was isolated. It was shown that some excellent colonizers could control TFRR under greenhouse conditions. Because these strains do not display antagonistic activity against Forl *in vitro*, we speculate that the mechanism(s) by which they control TFRR is/are induced systemic resistance and/or competition for nutrients and niches.

Boosting plant defense by beneficial microorganisms

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Plants have developed multiple strategies to protect themselves against pathogen attack, including preformed barriers and inducible defence mechanisms. Moreover, they interact with beneficial microorganisms able to reduce the effects of deleterious organisms. Some of these microorganisms, for example *Trichoderma* spp. fungi, can have a direct impact on the pathogen through antibiosis and parasitism. Others have a more indirect mode of action, as arbuscular mycorrhizal fungi. They colonise roots leading to an increased plant nutrition, competing with the pathogen for nutrients and colonisation sites and potentiating plant defence responses against a challenging pathogen. In fact, the most effective biocontrol agents combine different mechanisms. One of the most studied examples of combined strategies for

biocontrol are bacteria from the genus *Pseudomonas*. They can produce antibiotics and siderophores, weakening the pathogen in the soil. Root colonisation by selected strains result in induce systemic resistance (ISR) effective against a broad range of root and foliar pathogens. Interestingly, no major changes in gene expression have been related to the ISR state in the plant. Instead, induced plants show potentiated defence responses after infection with the challenging pathogen, a phenomenon called 'priming'. We hypothesise that priming of pathogen-induced genes allows the plant to react more effectively to the invader encountered, which might explain the broad-spectrum action of rhizobacteria-mediated ISR. The molecular mechanisms underlying priming are currently under study. Understanding the mechanisms by which beneficial microorganisms help the plant to defend themselves is key for developing safe, durable and environment friendly strategies in crop protection.

Suppression of take-all disease in soils from organic versus conventional farms in relation to native and introduced 2,4-diacetylphloroglucinol-producing Pseudomonas fluorescens

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In three sets of experiments with soils collected from organic and conventional farms, take-all disease on barley, wheat or triticale, caused by *Gaeumannomyces graminis*, was more suppressed in organically managed than in conventionally managed soils where crops had been grown in rotation. This was true for soils with naturally occurring *G. graminis* and for soils amended with inoculum of *G. graminis* var. *tritici* strain R3-111a-1. Suppression of *G. graminis* var. *tritici* was positively correlated with bacterial diversity in soil as determined by denaturing gradient gel electrophoresis (DGGE) analysis of 16S ribosomal DNA genes amplified from DNA directly extracted from soil. Disease severity in a take-all suppressive soil, where wheat had been grown in continuous monoculture, was intermediate between that in an organic and a conventional soil with crop rotation. Natural populations of 2,4-diacetylphloroglucinol-producing *Pseudomonas* species were abundant in soil from the monoculture wheat field, less abundant in conventional soil where triticale had been grown organically for two years, and almost absent in soil from an organic farm. Populations of a *Gfp*-tagged, 2,4-diacetylphloroglucinol-producing strain of *Pseudomonas fluorescens* introduced in soil declined faster in organically managed than in conventionally managed soils, and did not contribute as much to take-all suppression in the former than in the latter soils. Thus, the natural mechanism of take-all suppression in organically managed fields may be different from that in conventional fields with monoculture wheat or triticale, where 2,4-diacetylphloroglucinol-producing *Pseudomonas* species may be of importance.

Characterization of an MFS transporter from Mycosphaerella graminicola as a potent multidrug transporter

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The ascomycetous fungus *Mycosphaerella graminicola* is the causal agent of a severe disease on wheat called septoria tritici leaf blotch. Screening of *M. graminicola* EST libraries led to the identification of *MgMfs1*, a full length Major Facilitator Superfamily (MFS) gene with high homology to putative toxin transporters involved in virulence. Complementation of a *Saccharomyces cerevisiae* strain deficient in multiple drug transporter genes with *MgMfs1* resulted in an impressive decrease in sensitivity of *S. cerevisiae* to a broad range of synthetic and natural toxic compounds indicating that the encoded protein, MgMfs1, is involved in multidrug resistance. We propose that MgMfs1 can act as a virulence factor of *M. graminicola* and can be a determinant of the pathogen in sensitivity and resistance to fungicides.

Molecular characterization of MAP kinase signaling genes in Mycosphaerella graminicola and their role in pathogenicity

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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 regulation of other pathways/genes and in the establishment and development of *M. graminicola* on wheat. Through analyses of cDNA libraries of *M. graminicola*, several signal transduction genes have been identified. We optimized and exploited a method to disrupt the MAP kinase genes by using *in vitro* transposon mutagenesis system. We generate knockouts of these genes through homologous using *Agrobacterium*-mediated transformation recombination. The study of the role of these genes in virulence is in progression. As an example, a full-length cDNA clone that is highly homologous to a mitogen-activated protein, FUS3 in *Saccharomyces cerevisiae* was cloned. This MAP kinase possesses a 1068 bp open reading frame and encodes a 356aa sequence. 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 disruptant failed to cause any symptoms e.g. chlorosis, necrosis and pycnidia on wheat in either detached leaf or seedling bioassays. We measured the fungal biomass of this 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.

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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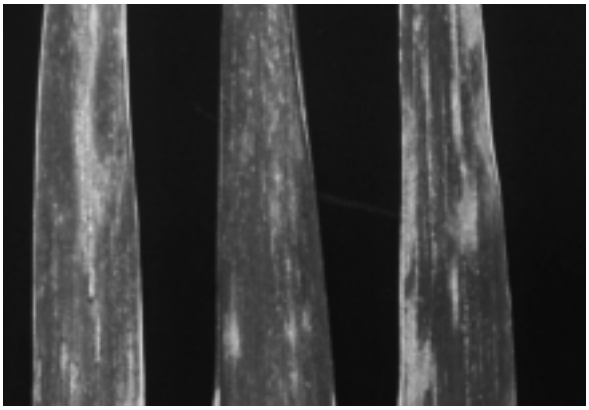
We report the identification of the first avirulence factor from a root-infecting pathogen. It is a cysteine-rich protein secreted by *Fusarium oxysporum* f.sp. *lycopersici* during colonization of tomato xylem vessels. The corresponding gene was identified with degenerated primers based on peptide sequences 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.

Molecular phylogeny of Phytophthora species; impact of reticulation and ecological parameters

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A molecular phylogenetic analysis of the genus *Phytophthora* was performed, based on both nuclear and mitochondrial DNA sequence data. Emphasis in our study was on species collected from the Toluca Valley in central Mexico, the presumed center of origin of *Phytophthora infestans* and other closely related species. A total of 113 isolates from 48 *Phytophthora* species and two *Pythium* species were used in this analysis. Phylogenetic analyses were performed for combined mitochondrial sequences, for combined nuclear sequences and for all sequences combined and between-data set congruence was tested. Results indicate that the classical taxonomic grouping as described by Waterhouse (1963) does not reflect true phylogenetic relations. *Phytophthora* species were redistributed into eight clades, providing a more accurate representation of phylogenetic relationships within the genus *Phytophthora*. The evolution and transition of morphological, pathogenic and reproductive traits was inferred from the cladogram generated in this study. Incongruence was found between phylogenies for nuclear and mitochondrial DNA, a possible indication for reticulate evolution in *Phytophthora* species.

Characterisation of the signal transduction pathway resulting in the hypersensitive response in planta

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The hypersensitive response (HR) is an efficient, active defence response in plants based on a resistance (*R*) gene in the plant that mediates resistance against a pathogen that contains the corresponding avirulence (*Avr*) gene. In tomato, the resistance gene *Cf-4* mediates specific recognition of the corresponding elicitor AVR4 produced by the pathogen *Cladosporium fulvum*. To study the signal transduction pathways resulting in HR, we have generated tomato seedlings that express both Cf-4 and AVR4. Since HR resulting from AVR4 recognition is suppressed at elevated temperatures (33°C), systemic HR in Cf-4/AVR4 tomato seedlings can be synchronised by a shift from high to low (20°C) temperature (De Jong *et al.*, 2002). This system will be further referred to as 'dying seedlings'.

In the past, several studies have been done to identify parts of the signal transduction pathway in cell suspensions. However, the dying seedlings give us a nice tool to study the signal transduction pathway in intact plants. The system allows studies on cell death, H_2O_2 production, callose formation, MAP kinase activity and alkalization of the leaves.

Furthermore, protein phosphorylation events that play a key role in signal transduction pathways can be studied in these dying seedlings. Several phosphorylation enzymes, such as Pto, Xa21, MAPKs and CDPKs are specifically activated during HR. To search for target proteins of phosphorylation enzymes in general, we aim to study changes in the phosphoproteome during HR in the dying seedlings. Differentially phosphorylated samples can be identified on Western blot by specific antibodies, whereas proteins can be isolated for further analysis by immunoprecipitations.