**PS 11-526**

**PROTEOMIC ANALYSIS OF THE APOPLAST OF CLADOSPORIUM FULVUM-INFECTED TOMATO REVEALS NOVEL VIRULENCE FACTORS**

Melvin D. BOLTON1,2, Jack H. VOSSEN1, Iris J.E. STULEMEIJER1, Grady VAN DEN BERG1, Henk L. DEKKER1, Chris G. DE KOSTER1, Pierre J.G.M. DE WIT1, Matthieu H.A.J. JOOSTEN1, and Bart P.H.J. THOMMA1.

1Laboratory of Phytopathology, Wageningen University, Binnenhaven 5, 6709 PD Wageningen, The Netherlands; 2Department of Plant Pathology, North Dakota State University, Fargo, ND 58105-5012, USA. "Swammerdam Institute for Life Sciences, Mass Spectrometry, Nieuwe Achtergracht 166, 1018 WV Amsterdam, The Netherlands. bart.thomma@wur.nl

During growth on its host tomato, the biotrophic fungal pathogen Cladosporium fulvum secretes several effector proteins into the apoplast (Thomma et al., 2005). Eight of these C. fulvum effectors have previously been characterized. To discover novel C. fulvum proteins that play a role in virulence, we utilized a two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) approach to visualize proteins secreted during compatible and incompatible C. fulvum-tomato interactions. Several proteins that accumulated during infection were identified by mass spectrometry (MS) and were shown to be previously described. For proteins that were not identified by MS, N-terminal sequencing and peptide fragment spectra obtained with liquid chromatography (LC)--MS/MS provided peptide sequence information. PCR with degenerate primers based on peptide sequences yielded coding sequence for three novel C. fulvum proteins; PhIC, Ecp6, and Ecp7. PhIC shows homology to fungal phialides, proteins that occur in sporogenous cells that release conidia. Ecp6 contains LysM domains that may be involved in chitin binding, similar to the function of C. fulvum Avr4 (van Esse et al., 2007). Ecp7 codes for a small, cysteine-rich protein with no homology to any known proteins. We demonstrate that Ecp6 and Ecp7 are crucial for C. fulvum virulence by exploiting RNAi-mediated gene silencing.


**PS 11-527**

**THE CHITIN-BINDING CLADOSPORIUM FULVUM EFFECCTOR PROTEIN AVR4 IS A GENUINE CYSTEINE PROTEASE INHIBITOR.**

John W. van ‘t KLooSTER1, Marc W. van der KAMP1,2, Jacques VERVOORT, Jules BEEKWILDER1, Bart P.H.J. THOMMA1, Matthieu H.A.J. Joosten1 and Pierre J.G.M. DE WIT1.

1Laboratory of Phytopathology, Wageningen University, Wageningen, The Netherlands; 2Laboratory of Biochemistry, Wageningen University, Wageningen, The Netherlands; 3Plant Research International, Wageningen, The Netherlands. John.vantklooster@wur.nl

Cladosporium fulvum (syn. Passalora fulva) is an extracellularly growing biotrophic fungal pathogen that causes tomato leaf mold. The Cf2-dependent resistance towards C. fulvum is based on the indirect recognition of the fungal elicitor protein Avr2. Our previous studies have demonstrated that the interaction of Avr2 with the tomato cysteine protease Rcr3 triggers a Cf2-mediated hypersensitive response (HR), which complies to the so-called guard hypothesis. Although the Avr2 protein acts as a cysteine protease inhibitor, its amino acid sequence does not show significant homology with other sequences present in public databases. Since disulphide bridge formation is important for the 3-dimensional structure of proteins and may support the identification of functional homologues, we elucidated the disulphide bridging pattern of the Avr2 protein. Subsequent bio-informatic analysis has led to the identification of a number of amino acids that could be important for the interaction of Avr2 with Rcr3. PCR-based site-directed mutagenesis was applied to obtain mutant Avr2 proteins upon heterologous expression in the yeast Pi- chia pastoris. All proteins were tested for HR-inducing activity in Cf2 tomato plants. A selection of the Avr2 mutant proteins were used in Rcr3-inhibition assays to determine their binding affinity when compared to the native Avr2 protein. This study indicates that the C-terminal part of Avr2 is crucial for the HR inducing activity as well for the inhibition of Rcr3. Since the Avr2 protein only shows very limited homology to known protease inhibitors, it can be concluded that Avr2 is a novel type of cysteine protease inhibitor.