EBERT, MALAIKA K.[1,2]*, BART P. H. J. THOMMA[2] and MELVIN D. BOLTON[1], [1]USDA-ARS, Northern Crop Science Laboratory, Fargo, North Dakota, USA and [2]Laboratory of Phytopathology, Wageningen University, The Netherlands. **Identification of *Cercospora beticola* effectors.**

ABSTRACT

Cercospora Leaf Spot (CLS), caused by the hemibiotrophic fungus *Cercospora beticola*, is the most destructive foliar disease of sugar beet worldwide. Plant pathogens, including *C. beticola*, secrete effector proteins into the apoplast to promote their virulence during infection. To combat the effects of pathogen effectors, plants have developed resistance (R) proteins that are able to recognize specific effectors and trigger defense responses. Although many effectors have been characterized in several pathosystems, no *C. beticola* effector proteins have been reported to date. Here, we show two different approaches that led to the identification of two *C. beticola* effectors.

We have shown recently that that the tomato R protein Ve1 provides resistance against *Verticillium dahliae* race 1 strains that secrete the effector protein VdAve1 (Avirulence on Ve1 tomato). Comparative genomics revealed the presence of a VdAve1 ortholog, *CbAve1* in the *C. beticola* genome. We have shown previously using agroinfiltration in *Nicotiana benthamiana* that tomato Ve1 recognizes CbAve1, resulting in a hypersensitive response. To determine whether CbAve1 is a virulence factor during the infection of sugar beet plants, Δ*CbAve1* mutant strains were developed. Sugar beet variety 86RR66 was inoculated with either a *C. beticola* wild type or Δ*CbAve1* mutant strain. At 16 dpi, the leaves of the wild type infected plants displayed more symptoms compared to the Δ*CbAve1* mutant infected plants. Furthermore, significantly less fungal biomass was found in the leaf material inoculated with the Δ*CbAve1* mutant strains than in the leaf material infected with the wild type. These results show that CbAve1 is a virulence factor for *C. beticola* and deletion of *CbAve1* compromises *C. beticola* virulence.

Besides comparative genomics, we also developed a phenotype based approach for effector identification. To identify *C. beticola* proteins involved with virulence, we grew *C. beticola in vitro* under specific conditions and tested culture filtrate for necrosis-inducing activity by infiltration into sugar beet leaves. Culture filtrate from one growth condition reliably caused necrosis in sugar beet leaves within 24 h. Treatment of culture filtrate with a mixture of proteases abolished necrosis-inducing activity, confirming that the *C. beticola* effector(s) responsible for necrosis was proteinaceous in nature. Active culture filtrates were partially purified using liquid chromatography (LC). A single (LC) fraction was repeatedly identified that caused necrosis upon infiltration into host tissue. MS/MS analysis of this fraction identified three *C. beticola* proteins. Each protein exhibited classic effector characteristics, including secretion signal, high cysteine content and low molecular weight (6 to 11 kDa). All candidate effector proteins were produced in *Pichia pastoris*. Infiltration of the candidate effector CbNip10 caused necrosis in sugar beet leaves, while the other two effector candidates did not.