Identification and characterization of *Cercospora beticola* necrosis-inducing effector CbNip1

Malaika K. Ebert[1,2,3], Lorena I. Rangel[1], Rebecca E. Spanner[1,2], Demetris Taliadoros[4,5], Xiaoyun Wang[1], Timothy L. Friesen[1,2], Ronnie de Jonge[6,7,8], Jonathan D. Neubauer[1], Gary A. Secor[2], Bart P. H. J. Thomma[3,9], Eva H. Stukenbrock[4,5], Melvin D. Bolton[1,2]. [1]Edward T. Schafer Agricultural Research Center, USDA Agricultural Research Service, Fargo, North Dakota, USA. [2]Department of Plant Pathology, North Dakota State University, Fargo, North Dakota, USA. [3]Laboratory of Phytopathology, Wageningen University, Wageningen, Netherlands. [4]Environmental Genomics Group, Max Planck Institute for Evolutionary Biology, Plön, Germany. [5]Christian-Albrechts University of Kiel, Kiel, Germany. [6]Plant-Microbe Interactions, Department of Biology, Utrecht University, Utrecht, Netherlands. [7]Department of Plant Systems Biology, VIB, Ghent, Belgium. [8]Department of Plant Biotechnology and Bioinformatics, Ghent University, Ghent, Belgium. [9]University of Cologne, Institute for Plant Sciences, Cluster of Excellence on Plant Sciences (CEPLAS), Cologne, Germany.
Corresponding Author(s): Melvin Bolton (melvin.bolton@usda.gov)
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ABSTRACT
Cercospora beticola is a hemibiotrophic fungus that causes cercospora leaf spot disease of sugar beet (*Beta vulgaris*). After an initial symptomless biotrophic phase of colonization, necrotic lesions appear on host leaves as the fungus switches to a necrotrophic lifestyle. The phytotoxic secondary metabolite cercosporin has been shown to facilitate fungal virulence for several *Cercospora* spp. However, because cercosporin production and subsequent cercosporin-initiated formation of reactive oxygen species is light-dependent, cell death evocation by this toxin is only fully ensured during a period of light. Here, we report the discovery of the effector protein CbNip1 secreted by *C. beticola* that causes enhanced necrosis in the absence of light and, therefore, may complement light-dependent necrosis formation by cercosporin. Infiltration of CbNip1 protein into sugar beet leaves revealed that darkness is essential for full CbNip1-triggered necrosis, as light exposure delayed CbNip1-triggered host cell death. Gene expression analysis during host infection shows that *CbNip1* expression is correlated with symptom development in planta. Targeted gene replacement of *CbNip1* leads to a significant reduction in virulence, indicating the importance of CbNip1 during colonization. Analysis of 89 *C. beticola* genomes revealed that *CbNip1* resides in a region that recently underwent a selective sweep, suggesting selection pressure exists to maintain a beneficial variant of the gene. Taken together, CbNip1 is a crucial effector during the *C. beticola*–sugar beet disease process.

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