New Disease Reports

Natural infection of *Clavibacter michiganensis* subsp. *sepedonicus* in tomato (*Solanum tuberosum*)

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Five tomato plants cv. 'Merlice' on Maxifort rootstock were received late April 2014 from a substrate crop in Flanders, Belgium. They exhibited yellowing and necrosis of the leaf mesophyll, withering of leaflets and wilting of whole leaves. Vascular tissues were probed at various nodes of the stem because explicit discolouration of the xylem was not observed. The tissues were fragmented in sterile 10 mM phosphate buffer (PB) and the dilution was plated on Pseudomonas Agar F containing 5g/l of sucrose. Creamy white colonies developed from the diluted extracts after seven days incubation at room temperature (Fig. 1). A pure culture from each diseased tomato plant (designated GBBC 1958 to GBBC 1962) was identified as *Clavibacter michiganensis* subsp. *sepedonicus* (Cms) in taxon-specific conventional PCR (Pastrik & Rainey, 1999) and TaqMan real-time PCR (Schaad *et al*., 1999). They were also assigned to the *gyr*B sequence cluster of Cms (Fig. 2) with unique subspecies signatures and displayed specific biomarker proteins of Cms in MALDI-TOF (Fig. 3) (Zaluga *et al*., 2011). The vascular tissue extracts tested positive for Cms in an immunofluorescence test with polyclonal antiserum (Prime Diagnostics, The Netherlands) and with the monoclonal antibody 9A1 (Agdia Biofords, France). They tested negative for *C. m.* subsp. *michiganensis* (Cmm) in an immunofluorescence test with polyclonal antiserum (Prime Diagnostics, The Netherlands).

Pathogenicity of the five isolates was tested in tomato and potato using suspensions of about 10^8 cells/ml in PB. Tomato plantlets with two fully developed leaves were inoculated by stem infiltration (Zaluga *et al*., 2013) and placed in a growth chamber at 20-25°C. High-grade seed tubers cv. 'Fontane' were inoculated by dipping a potato knife in the cell suspension and making a longitudinal cut from the heel end over about two-thirds of the tuber. After overnight retention at 16°C, they were hand planted in preformed ridges in a contained field plot at ILVO. Buffer-inoculated controls separated each test object. The type strains of Cmm and Cms were used as pathogenic controls. In tomato plantlets, the five Cms isolates displayed flaccidity and chlorosis of leaf margins, wilting or necrosis of individual leaf parts and finally wilting of whole leaves (Fig. 4). The first symptoms appeared 10-12 days after inoculation and progressed slowly. In potato plants, the five Cms isolates caused a general burned appearance with rolling and necrosis of leaf margins, mottling and yellowing between veins (Fig. 5). Ring rot symptoms were identified in the progeny tubers and confirmed by TaqMan PCR. Each isolate was recovered from infected test plants and re-identified as Cms by the *gyr*B barcode.

Although tomato is considered a host upon artificial inoculation, this is to our knowledge the first report of a natural infection of *C. m.* subsp. *sepedonicus* in tomato plants. The incidence in the crop was limited to ten successively arranged plants in one row, suggesting transmission from a primary infected plant but at a low rate. The origin of the infection is unknown. Inspection of tomato crops issued from the same seed lot did not result in additional findings. The official status of the pathogen in Belgium is reported as under eradication on tomato and absent on potato (EPPO, 2014). The five isolates are deposited at the LMG collection (strain numbers 28446-28450). The corresponding *gyr*B sequences are available at NCBI (GenBank Accession Nos. KP899559-KP899663).

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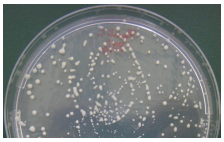

Figure 1

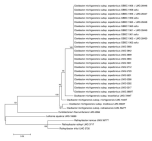

Figure 2

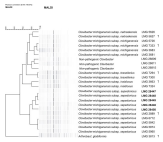

Figure 3

Figure 4

Figure 5

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