



## NEW DISEASE REPORT

# First report of bacterial leaf spot of *Hydrangea* in retail nurseries in Belgium caused by strains assigned to a new *Xanthomonas hortorum* clade

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**FIGURE 1** Natural infection of *Hydrangea arborescens* with typical symptoms of *Xanthomonas* leaf spot.

The genus *Hydrangea* includes at least 23 species of ornamental plants that are highly valued for their large and long-lasting flowerheads. Incidents of leaf spot were regularly observed on *Hydrangea arborescens* and *H. quercifolia* in retail nurseries in Flanders (Belgium) from 2011 to 2015. Leaves showed brown to black irregular leaf spots (Fig. 1) which tended to blend into larger lesions (Fig. 2).



**FIGURE 2** Naturally infected leaf of *Hydrangea quercifolia* showing ooze droplets on the dorsal leaf surface with typical leaf spot symptoms along the veins and margin.

Leaf spots were aseptically excised and comminuted in sterile 10 mM phosphate buffer (PB, pH 7.0). Dilution plating was done on BD Difco™ Pseudomonas Agar F supplemented with sucrose (10 g/L). Large numbers of pale yellow round, convex *Xanthomonas*-like colonies had developed after four days incubation at 28°C. They induced a

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**TABLE 1** GenBank accession numbers matching the partial *gyrB* sequences of *Xanthomonas* isolates from *Hydrangea arborescens* and *Hydrangea quercifolia*

	Strain	GenBank Accession No.
gyrB Seq 1	GBBC2123	MW223005
gyrB Seq 2	GBBC2128	MW223006
gyrB Seq 3	GBBC2199	MW223007
gyrB Seq 4	GBBC2202	MW223008
gyrB Seq 5	GBBC3035	MW223009
gyrB Seq 6	GBBC3048	MW223010

hypersensitive reaction after infiltration in leaves of tomato and *Sedum*, but not in leaves of tobacco (cv. Xanthi NN).

The phylogenetic position in the genus *Xanthomonas* was determined with partial sequences of the DNA gyrase subunit B (*gyrB*) gene (Constantin et al., 2016), which were trimmed, aligned and compared with *gyrB* sequences of *Xanthomonas* benchmark strains in our Bionumerics database (Applied Maths, Bio-Mérieux) or retrieved from GenBank (NZ\_SMED00000000.1 from *Xanthomonas vitians* LMG 938<sup>neoPT</sup>). GenBank accession numbers of the partial *gyrB* sequences of *Xanthomonas* isolates obtained in this study are shown in Table 1. The *Hydrangea* leaf spot isolates were assigned to a new clade in the *gyrB*-based phylogeny (Fig. 3) of *X. hortorum* and clearly distinct from any of the existing pathovars in the species (Morinière et al., 2020). Further analysis using whole genome sequences and ANI values is ongoing to determine whether it should be considered a new *Xanthomonas* species.

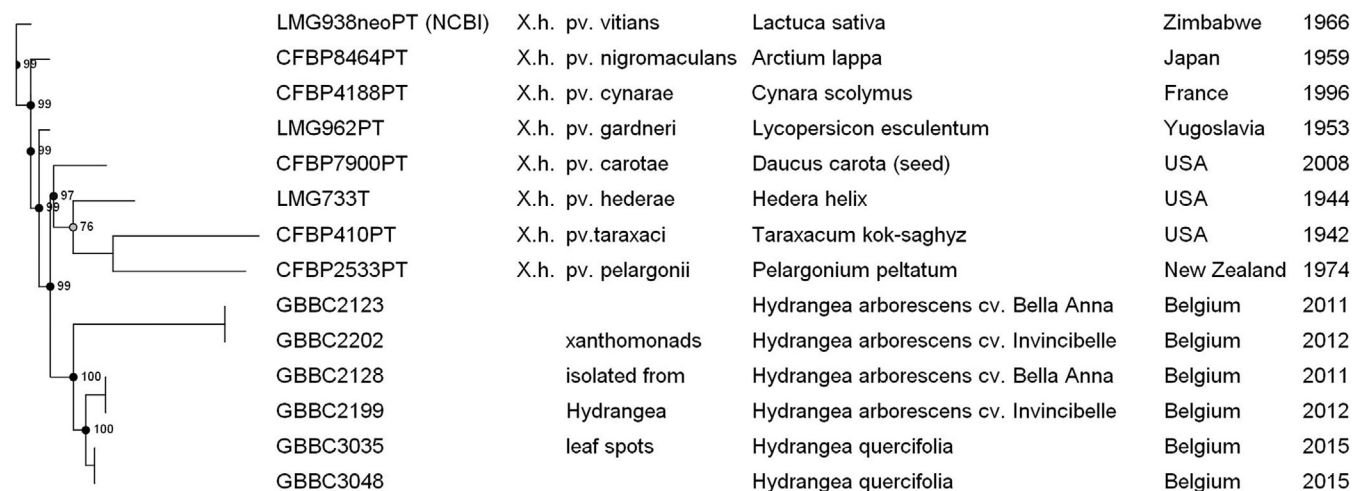
Pathogenicity was verified on *H. arborescens* and *H. quercifolia* by pressurised spray inoculation of the leaves with a suspension of 10<sup>8</sup> cfu/ml of each strain in PB in three replicates and with PB as negative control. Plants were covered for 48 hr with polythene sheet to sup-



**FIGURE 4** Leaf spot symptoms developing on *Hydrangea arborescens* four weeks after pressurised spray inoculation with *Xanthomonas* strain GBBC 2123 and kept in the greenhouse at 18/25°C.

port high humidity and facilitate infection. They were maintained in the greenhouse at 18/25°C (night/day). Leaf spots similar to those on the nursery plants developed on the inoculated leaves within four weeks. Koch's postulates were fulfilled by isolating from the leaf spots and by confirming the presence of identical *gyrB* sequences in re-isolated and inoculated strains.

This is the first record of a bacterial leaf spot of *Hydrangea* in Belgium. A leaf spot disease of oak leaf hydrangea caused by a pathovar of *Xanthomonas campestris* was reported in the past in Georgia, USA (Uddin et al., 1996). This may have been a similar pathogen, alas the



**FIGURE 3** Neighbour-joining phylogenetic tree based on 530 bp *gyrB* partial sequences of *Xanthomonas* strains. The Belgian strains isolated over different years from leaf spot symptoms of two *Hydrangea* species are indicated with a bracket. T = type strain; PT = pathotype strain; neoPT = neo pathotype strain. Assignment to clusters is underpinned by the cophenetic correlation coefficient.



strain is not obtainable (R. Gitaitis, pers. comm.). The isolates were obtained from deciduous *Hydrangea* species. Infected plants that overwintered at the ILVO premises developed symptoms the following year but eventually the disease was no longer observed. Also, in the nurseries the disease has not been visible over the past few years. Stocks of *Hydrangea* plants are regularly renewed and summers have been hot and dry. The environmental conditions have certainly not been favourable for infection. The relevance of this pathogen is also underpinned by the presumed introduction in cuttings from the USA. It suggests that it is still present there after its first report in 1996. So, it may be re-introduced and re-emerge when conditions are more favourable.

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