

Bacterial leaf spot of *Hydrangea*: on a “new old” disease and the importance of getting it right in phytodiagnostics

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Introduction

The bacterial genus *Xanthomonas* mainly contains phytopathogenic members that cause devastating disease on over 400 plant species. Until recently *Xanthomonas hortorum* was mostly known to affect ornamental plants. In the last decade, *X. hortorum* was however reported on new hosts (e.g., lavender or chicory) and in new countries (e.g., Malaysia, Greece, Korea) with an increased frequency. This phytopathogen has been so far reported from 65 plant species in 20 botanical families (Dia *et al.*, 2022a). With this context, a «new old» disease of *Hydrangea* due to a bacterium related to *X. hortorum* has recently received an increased awareness.

Bacterial spot disease of *Hydrangea*

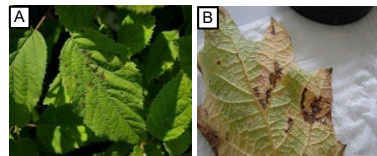


Figure 1. Natural leaf infection of (A) *Hydrangea arborescens* with typical symptoms of *Xanthomonas* leaf spot, and (B) of *Hydrangea quercifolia* showing ooze droplets on the dorsal leaf surface with typical leaf spot symptoms along the veins and margin.

Incidents of leaf spot were regularly observed on *Hydrangea arborescens* and *Hydrangea quercifolia* in retail nurseries in Flanders (BE) from 2011 to 2015 (Fig. 1). Based on partial *gyrB* sequencing, the causal agent was found to be related to *X. hortorum* (Cottyn *et al.*, 2021). Genomics was used to further characterize this phytopathogen.

The causal agent: *Xanthomonas hydrangeae*

Based on overall genome relatedness indices (Fig. 2), the bacterium responsible for leaf spot of *Hydrangea* was found to be phylogenetically close, yet distinct from, *X. hortorum*, and was named *X. hydrangeae* (Dia *et al.*, 2021).

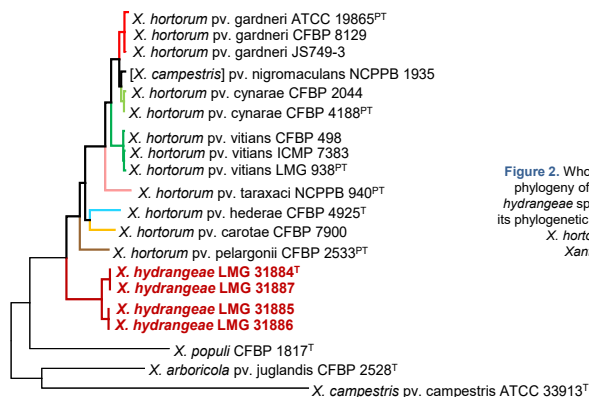


Figure 2. Whole-genome ML phylogeny of *Xanthomonas hydrangeae* sp. nov. showing its phylogenetic relationship to *X. hortorum* and other *Xanthomonas* spp.

History of bacterial spot on *Hydrangea*

The history of bacterial spot on *Hydrangea* is summarized in Table 2. *Xanthomonas* isolates from *Hydrangea* were first reported to affect ornamental plants in a commercial nursery in 1995 in Georgia (USA). In a disease note from 1996, the isolates, that are unfortunately not anymore available, were shown to be most similar to «*Xanthomonas campestris*» pv. carotae. Some communications continued to report the causal organism as *X. campestris* based on non-genetic methods, which could refer to the former name of *X. hortorum*. The New Disease Report and the subsequent description of *X. hydrangeae*, shed light on the occurrence of bacterial spot on *Hydrangea* caused by *X. hydrangeae*. It is likely that these occurrences would have gone unnoticed otherwise, highlighting the importance of publishing disease reports, even ten years after the initial interception of the disease in Belgium.

Table 2. Reporting of bacterial leaf spot on *Hydrangea* from the first documented case in 1995 to late 2021. Bold indicates reports that came to light after the New Disease Report by Cottyn *et al.* (2021). ND: not determined.

Identified causal organism	<i>Hydrangea</i> host(s)	Location (isolation year)	LAMP
<i>X. campestris</i> pv. carotae	<i>H. quercifolia</i>	Georgia, USA (1995)	ND
<i>X. hortorum</i>	<i>Hydrangea</i> sp.	Exact origin unknown but a customs reference indicates it might be from NL (2002)	+
<i>X. campestris</i>	<i>H. quercifolia</i> <i>H. macrophylla</i> <i>H. arborescens</i>	Michigan, USA (2010)	ND
<i>X. hortorum</i>	<i>Hydrangea</i> sp.	Netherlands (2011)	+
<i>X. campestris</i>	<i>H. quercifolia</i> cv. 'Little Honey'	Illinois, USA (2012)	ND
<i>X. campestris</i>	Wild <i>H. quercifolia</i>	Multiple states, USA (2018-2019)	ND
<i>X. hydrangeae</i>	<i>H. arborescens</i> (cvs. 'Invincibelle' and 'Bella Anna') and <i>H. quercifolia</i>	Belgium (2011, 2012, 2015)	ND
<i>X. hortorum</i>	<i>H. arborescens</i>, <i>H. quercifolia</i>	Netherlands (2020 – 2021)	+
<i>X. hortorum</i>	<i>H. quercifolia</i>	United Kingdom (2021)	+
<i>X. hortorum</i>	<i>H. quercifolia</i>	Italy (2021)	+
<i>X. hortorum</i>	<i>H. arborescens</i>	Germany (2021)	+

Plant trade as disease-spreading agent

From the four *Hydrangea* isolates that were the subject of the New Disease Report (Cottyn *et al.*, 2021), multiple potential introductions of *X. hydrangeae* to Belgium in 2011 and 2012 are hypothesized (Fig. 3). For LMG 31884^T for example, mother plants were supplied by a nursery in the USA to another nursery in Ethiopia which, in turn provided *Hydrangea* cuttings to a nursery in Belgium where the diseased plants were intercepted. The ever-increasing global plant material trade is known to lead to an increase in plant diseases, further highlighting the importance of quarantine measures and diagnostics.

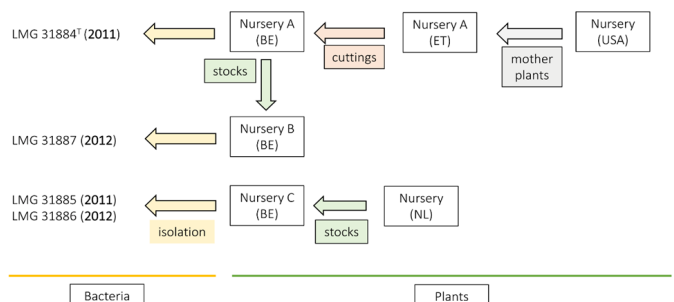


Figure 3. Retracing the potential introductions of *Xanthomonas hydrangeae* strains LMG 31884^T, LMG 31887, LMG 31885 and LMG 31886 to Belgium, where they were isolated in 2011 and 2012. The countries referred to in this figure are Belgium (BE), Ethiopia (ET), the Netherlands (NL) and United States of America (USA).

Host range of *X. hydrangeae*

X. hydrangeae is pathogenic on *H. arborescens*, mildly pathogenic on *H. quercifolia* and not pathogenic on *H. macrophylla* and *H. paniculate* (Table 1). Furthermore, two *X. hydrangeae* strains were differentially pathogenic on *Lycopersicon esculentum*, thus raising the question about the phytosanitary standing of this newly described bacterial phytopathogen.

Table 1. The pathogenicity of four *Xanthomonas hydrangeae* strains on four *Hydrangea* species and four of the known hosts of *Xanthomonas hortorum* (Cottyn *et al.*, 2021; Dia *et al.*, 2021; unpublished results). NT: not tested.

	<i>X. hydrangeae</i> LMG 31884 ^T	LMG 31885	LMG 31887	LMG 31886
<i>H. arborescens</i>	++	++	++	++
<i>H. quercifolia</i>	+	+	+	+
<i>H. macrophylla</i> and <i>H. paniculate</i>	-	-	-	-
<i>Lycopersicon esculentum</i>	+	NT*	NT	++
<i>Pelargonium zonale</i>	+	+	NT	NT
<i>Daucus carota</i>	NT	NT	NT	NT
<i>Hedera helix</i>	+	+	NT	NT

Conclusions

Given that the experimental host range informs about potential natural infections and host jumps, *X. hydrangeae* could affect tomato in natural settings. *X. hydrangeae* and *X. hortorum* pv. *gardenii* being genetically close, symptoms of bacterial spot on tomato caused by *X. hydrangeae* could have been falsely attributed to *X. hortorum* pv. *gardenii*. This hypothesis should be checked by screening strain collections of historical tomato isolates using for example the LAMP assays recently developed (Dia *et al.*, 2021; Dia *et al.*, 2022b). This cross-pathogenicity combined with an ever-increasing global plant trade also raises question about the phytosanitary standing of *X. hydrangeae* further highlighting the importance of quarantine measures and diagnostics. Additionally, our work illustrates the significance of publishing disease reports and of performing long term preservation of isolates for example by depositing them in a strain collection.

Further information and references available via tiny.one/poster-suppl-or:

