PhytoFrontiers™
An Open Access Journal from The American Phytopathological Society

Mini-Review

Bacterial Leaf Spot of *Hydrangea*: On a "New Old" Disease and the Importance of Diagnostic Specificity to Reduce Cross-Reactivity and Improve Surveillance

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Accepted for publication 7 February 2025.

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Funding: N. C. Dia was supported by the Swiss National Science Foundation (SNSF; grant IZCOZ0_177064). This article is based upon work from COST Action CA16107 EuroXanth, supported by COST (European Cooperation in Science and Technology). T. H. M. Smits and J. F. Pothier were supported by the Department of Life Sciences and Facility Management of the Zurich University of Applied Sciences (ZHAW) in Wädenswil. The open access article processing charges of this publication were funded by the SNSF.

The author(s) declare no conflict of interest.

Abstract

The causal organism of bacterial leaf spot of *Hydrangea* L., *Xanthomonas hydrangeae*, was the subject of a disease report and a new bacterial species description in 2021. These publications, along with the development of a specific isothermal diagnostic assay for *X. hydrangeae* and the recent awareness of this pathogenic bacterium, combined with the wet weather conditions during the summer of 2021 in Europe, have revealed a broader historical and current prevalence of this disease. Global trade of plants appeared to play an important role in the dissemination of this pathogen. Furthermore, scouring the literature revealed multiple instances, mainly in the United States, of bacterial leaf spot of *Hydrangea*, previously attributed to other *Xanthomonas* species (e.g., *X. campestris*, *X. hortorum*). The first known indication of bacterial leaf spot of *Hydrangea* dates to 1995 in Georgia (U.S.A.). However, these 1995 isolates are no longer available, and their identity as *X. hydrangeae* cannot be confirmed. This mini-review outlines the challenges encountered when studying *X. hydrangeae*, as related to (i) the development of a diagnostics assay targeting *X. hydrangeae*, especially given its phylogenetic similarity to *X. hortorum*, and (ii) the information discontinuity regarding historical and current incidence of bacterial leaf spot of *Hydrangea*.

Keywords: diagnostics, LAMP assays, *Xanthomonas hydrangeae*

Diagnosis of *Xanthomonas* phytopathogens faces multiple challenges, which have been previously documented (Catara et al. 2021). The 16S rRNA gene within the *Xanthomonas* genus is not discriminative enough to reliably separate between *Xanthomonas* species (Catara et al. 2021; Hauben et al. 1997). Numerous *Xanthomonas* species are divided into pathovars: pathogenic varieties on specific plant hosts and usually named after their hosts (Young et al. 1978, 1992, 2001). Even though the pathovar concept is very useful in a phytosanitary context, it has no taxonomic standing and can further complicate the development of long-lasting phytodiagnostic methods. This is relevant because of the evolving taxonomic classification of *Xanthomonas* species (e.g., within the *Xanthomonas* genus, but also from *Xanthomonas* to other bacterial genera and vice

versa) and the wide host range of the species and pathovars of the genus (Vauterin et al. 1995).

Xanthomonas pathogens are responsible for many plant diseases on as many as 400 different plant species (Leyns et al. 1984; Saddler and Bradbury 2015). These phytopathogens cause bacterial streak (e.g., of rice, wheat, barley), bacterial blight (e.g., of carrot, hazelnut, ornamental plants), bacterial canker (e.g., of citrus), and bacterial spot (e.g., of pepper, tomato, strawberry, stone fruit trees, and ornamental plants). The ornamental hosts of *Xanthomonas* include, among other genera, *Begonia*, *Pelargonium*, *Zinnia*, *Poinsettia*, *Tagetes*, *Syngonium*, *Hedera*, and *Ficus* (Balaž et al. 2016; Benedict et al. 1990; Dickey and Zumoff 1987; Duff 1991; Dye 1967; Rockey et al. 2015; Sahin et al. 2003).

Some of the *Xanthomonas* species pathogenic to ornamental plants are well studied. For example, *X. axonopodis* pv. *begoniae* and *X. hortorum* pv. *pelargonii*, causing bacterial blight on *Begonia* and *Pelargonium*, respectively, were extensively characterized (Balaž et al. 2016; Barel et al. 2015; Demirci et al. 2019; Vauterin et al. 1990). *Xanthomonas* diseases of other ornamental plants are not studied in detail due to their decreased economic importance.

The purpose of this mini-review is to report on the development of a specific diagnostic molecular assay for surveillance, detection, and discrimination between *Xanthomonas* species causing leaf spots on *Hydrangea* ornamentals. *Hydrangea* plants are popular ornamentals in many geographical regions (Fulcher et al. 2016; Rinehart et al. 2018; Sherwood et al. 2022). There are 70 *Hydrangea* species (BOLD 2024; Ratnasingham et al. 2024), native to the Americas and to Asia (McClintock 1957). International *Hydrangea* trade, more specifically from nurseries (e.g., in the United States) to Europe or Africa, is common (Fulcher et al. 2016; Wu et al. 2021).

Xanthomonas strains on diseased oakleaf hydrangeas (*Hydrangea quercifolia*) were first reported from a commercial ornamental nursery in Georgia (U.S.A.) in the summer of 1995 (Uddin et al. 1996). Similar bacterial leaf spots on *Hydrangea* were observed from 2011 to 2015 in Belgium. The diseased *Hydrangea* plants, grown in Belgian commercial nurseries, were sent to the Plant Diagnostic Center of the Flanders Research Institute for Agriculture, Fisheries, and Food (ILVO, Belgium) for further examination. There, xanthomonads were isolated from *H. arborescens* (cultivars Invincibelle and Bella Anna) and from *H. quercifolia* (Cottyn et al. 2021). A later comprehensive taxonomic study demonstrated that these isolates represented a previously undescribed *Xanthomonas* species close to *X. hortorum*, named after its host *X. hydrangeae* (Dia et al. 2021). As evidence of the different host range of this species, strains have been tested on hosts that are common to *X. hortorum*. Preliminarily spray inoculation experiments showed that *X. hydrangeae* LMG 31884^T and LMG 31886 cause moderate and severe bacterial leaf spot symptoms, respectively, on tomato plants (*Solanum lycopersicum*) (N. C. Dia, B. Cottyn, and J. F. Pothier *unpublished data*) but not on other *X. hortorum* host plants (*Hedera hibernica*, *Pelargonium zonale*, *Taraxacum kok-saghyz*, and *Daucus carota* cultivar Nerac; Dia et al. 2021). Otherwise, this phenotype could not be observed for *X. hortorum* pv. *carotae* CFBP 7900, which did not develop spots on *H. arborescens* (Dia et al. 2021).

Based on genetic and genomic evidence (Dia et al. 2021), it was hypothesized that at least two distinct but concurrent introductions of *X. hydrangeae* occurred in Belgium between 2011 and 2012 (Fig. 1). The first introduction was retraced to mother plants, supplied by a nursery in the United States to another nursery in Ethiopia, which, in turn, provided *H. arborescens* cuttings to its subsidiary nursery in Belgium. A second introduction originated from *H. arborescens* cuttings supplied by a nursery in the Netherlands to a nursery in Belgium, where the diseased plants were intercepted by ILVO. The mother plants likely originated from the United States for both introductions of *X. hydrangeae* to Belgium. However, it remains unclear at which stage of the itinerary of plant trade the infection(s) with *X. hydrangeae* occurred. Another strain (LMG 31938) was isolated in 2015 from *H. quercifolia* in the same Belgian nursery, but the origin of this infection could not be traced.

Due to its high phylogenetic similarity to *X. hortorum* and the demonstrated cross-pathogenicity for some strains within the *X. hortorum–X. hydrangeae* species complex (Dia et al. 2022a, b), it appeared crucial to develop a highly specific and fast diagnostic method that could distinguish the *X. hydrangeae* strains (clade

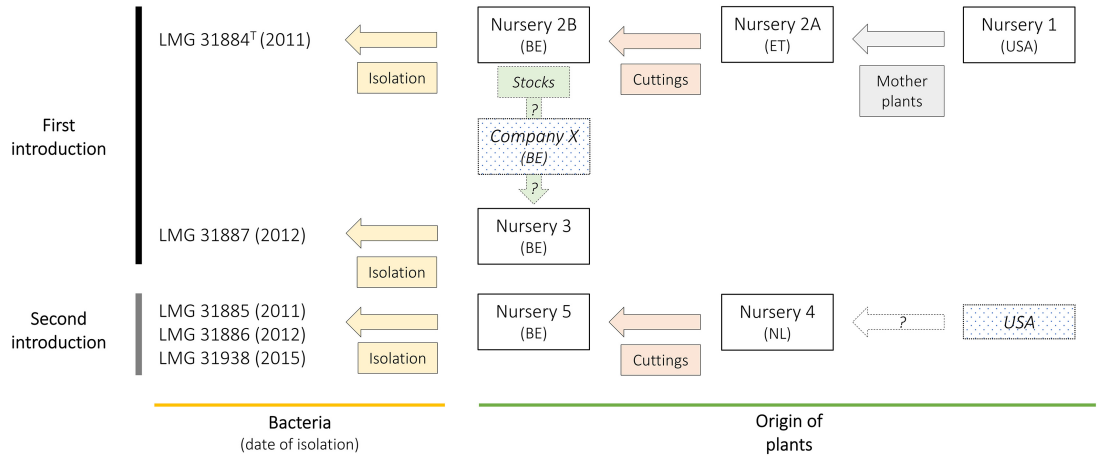

FIGURE 1

Retracing the two 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 from *Hydrangea arborescens* (cultivars Invincibelle and Bella Anna). Although no direct exchange between Nursery 2B and Nursery 3 could be traced, the two strains (LMG 31884^T and LMG 31887) from the first introduction are so closely related phylogenetically (Dia et al. 2021) that it is plausible, though unconfirmed, that stock plants were exchanged via an intermediary, Company X. Strain LMG 31938 was isolated from *Hydrangea quercifolia* in Nursery 5 in 2015, but the origin of this infection could not be traced. The countries referred to in this figure are Belgium (BE), Ethiopia (ET), the Netherlands (NL), and the United States of America (USA). Nursery 2B in Belgium is the subsidiary of Nursery 2A in Ethiopia.

C; Fig. 2) from the five other clades of this species complex (A1, A2, B1, B2, and B3; Fig. 2) (Dia et al. 2022a). Thus, in addition to the formal description of the new species, a genome-informed isothermal diagnostic assay based on loop-mediated isothermal amplification (LAMP) was developed for diagnostic purposes to identify this organism in the laboratory or even in the field (Dia et al. 2021, 2022a), allowing for accurate detection and targeted strategies for the effective management of this phytopathogen. This *X. hydrangeae*-specific LAMP assay was validated using a reference set of 62 reference strains, including a limited set of four *X. hydrangeae* strains, and a validation set of 60 xanthomonad strains from culture collections or diagnostic laboratories, classified or inconclusive (Dia et al. 2022a). The assay demonstrated 100% specificity, sensitivity, and efficiency, with a consistent detection limit of 250 fg and 2.5 pg of genomic DNA (equivalent to 42.1 and 421 genome copies) without and with spiking of plant cell extracts, respectively.

The knowledge on the historical and current incidence of bacterial leaf spot of *Hydrangea* is summarized in Table 1. Sources include disease reports, one research paper, one species description, personal communications, blog posts on extension websites, and noncompliance reports from the European and Mediterranean Pest Protection Organization (EPPO). *Xanthomonas* species were reported on *Hydrangea* starting in 1995, with rare incidences afterward, but every year between 2018 and 2022 in various countries in Europe (the Netherlands, Belgium, the United Kingdom, Italy, and Germany) and North America (multiple states in the United States).

After the first mention of an unusual bacterial leaf spot on oakleaf *Hydrangea* (Mullen 1995), a report describing the symptoms and the various phenotypic traits of the isolates on *H. quercifolia* was published one year later (Uddin et al. 1996). Here, the isolates were identified as "*Xanthomonas campestris*" based on carbon utilization and fatty acid analysis. More specifically, the *Hydrangea* isolates were described as similar to "*X. campestris*" pv. *carotae*, and, to a lesser degree, to "*X. campestris*" pv. *vitians* (Uddin et al. 1996). Since then, the taxonomic revision of the genus *Xanthomonas* has caused the reclassification of these pathovars of *X. campestris* into *X. hortorum* (Vauterin et al. 1995).

Less than a month after the publication of the New Disease Report (Cottyn et al. 2021), the Plant Clinic of Fera Science Ltd. (York, United Kingdom) initiated contact with ILVO and Zurich University of Applied Sciences. They had a series of strains isolated from *Hydrangea* plants of diverse geographic origins with symptoms of bacterial leaf spot that were intercepted by the Plant Clinic of Fera in 2002 (origin unknown, probably the Netherlands), in 2011 and 2020 (the Netherlands), in 2021 (Germany, the Netherlands, the United Kingdom, and Italy), in 2022 (the Netherlands, Italy, Belgium, and unknown origin), and in 2023 (the Netherlands, France, and unknown origin). Some of the findings from 2021 were also published through the EPPO's reports, as a result of notifications received from the United Kingdom (EPPO 2021a, 2022). Based on partial sequence analysis of the housekeeping gene *gyrB*, it was shown that the sequences of *Hydrangea* isolates of Fera clustered with those of *X. hydrangeae* strains from Belgium (A. Aspin, *personal communication*, June 2021 and October 2024). Furthermore, the identity of a subset of these *Hydrangea* sp. isolates was confirmed by using the *X. hydrangeae*-specific LAMP assay (Dia et al. 2022a). It was therefore confirmed that the causal agent of the plants intercepted by Fera was this pathogen.

More recently, bacterial leaf spot was reported on *H. quercifolia* in Tuscany (Italy) in 2022, and the causal organism was identified as *X. hydrangeae* (Campigli and Rizzo 2023), using, among other methods, the *X. hydrangeae*-specific LAMP (Dia et al. 2022a). This paper confirmed the occurrence of *X. hydrangeae* on symptomatic plants independent of our efforts and constitutes an independent assay verification confirming the transferability of the *X. hydrangeae*-specific LAMP diagnostic assay to another laboratory (Groth-Helms et al. 2023).

The publication of the disease report (Cottyn et al. 2021) and the new species description (Dia et al. 2021) shed light on the occurrence of bacterial leaf spot on *Hydrangea* caused by *X. hydrangeae*. In fact, most reports of bacterial leaf spot on *Hydrangea* were made in 2021, after the new disease report was published. We speculate that this is due to increased awareness about *X. hydrangeae*. Additionally, climatic factors, such as the wet summer in the European regions, may have provided more favorable conditions for the disease to thrive. In fact, *X. hortorum* pathovars have been reported to thrive in warm, humid, and wet environments in greenhouses, nurseries, and fields (Dia et al. 2022b; Uddin et al. 1996).

The development of a diagnostic tool (i.e., LAMP; Dia et al. 2021), together with the novel description of a plant pathogen, thus led quickly after its publication to an improvement of the diagnostic process. It is likely that these occurrences would have gone unnoticed without the publication of the 2021 disease report, highlighting the importance of publishing disease reports, even 10 years after the initial interception of the disease in Belgium.

Historically, the causal organism of bacterial leaf spot on *Hydrangea* was reported as either *X. campestris* (Byrne 2010; Cleveland 2012; Mullen 1995; Sherwood et al. 2021; Uddin et al. 1996) or *X. hortorum* (A. Aspin, *personal communication*; EPPO 2021a, 2022) (Table 1). We have shown that the retrospective

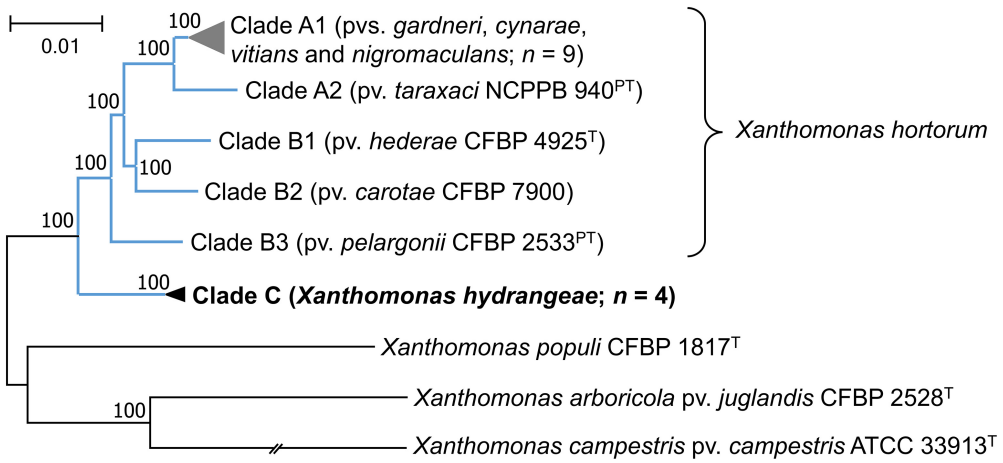

FIGURE 2
Whole-genome maximum-likelihood core phylogeny of the *Xanthomonas hortorum–Xanthomonas hydrangeae* species complex. The tree branches in blue indicates the various clades within this species complex. The clade C corresponding to *X. hydrangeae* is indicated in bold. Three outgroup type strains of *Xanthomonas* are included in the analysis.

identification of the pathogen as *X. hydrangeae* is possible. However, most of the historical incidences of disease were characterized using nongenetic methods or using identification with partial sequencing of the *gyrB* gene, which is usually the first step when an infection caused by *Xanthomonas* is suspected. Even though the *gyrB* gene resolution is better than 16S rRNA gene identification at the species level (Catara et al. 2021; Hauben et al. 1997), its discriminative power depends on the parameters chosen and on the other strains used to reconstruct the phylogeny (Parkinson et al. 2007, 2009).

When describing *X. hydrangeae* as a novel species, the strains were also tested for cross-pathogenicity on host plants of the *X. hortorum* pathovars, the closest phylogenic neighbors of *X. hydrangeae*. This led to the observation that the tested *X. hydrangeae* strains also caused bacterial spot symptoms on tomato, which is more known as a host for *X. hortorum* pv. *gardneri* (Dia et al. 2022b; Larrahondo-Rodríguez et al. 2022). This pathovar is classified as an EPPO A2 organism (EPPO 2021b) and is described as a regulated nonquarantine pest by the European Union Plant Health Law (European Union 2016, 2019, 2020; Picard et al. 2018) on pepper (*Capsicum annuum*) and tomato (*Solanum lycopersicum*) seeds, propagating and planting material. Given that the experimental host range informs about potential natural infections and host jumps (Jacques et al. 2016), it could be possible that *X. hydrangeae* affects tomato plants in natural settings as well. This would mean that symptoms of bacterial leaf spot

TABLE 1

Historical and current instances of bacterial leaf spot on *Hydrangea* spp.[a]

Host(s)	Location (isolation year)	Initial characterization (number of recent samples)	Identified causal organism[b]	Reference(s)	*Xanthomonas hydrangeae* LAMP result[c]
Hydrangea quercifolia	Georgia, U.S.A. (1995)	Carbon source utilization and fatty acid analysis	*X. campestris* pv. *carotae*	Mullen 1995; Uddin et al. 1996	Strains not available
**Hydrangea* sp.	The Netherlands[d] (2002)	*gyrB* identification	*X. hortorum* (*X. hydrangeae*)	A. Aspin, *personal communication* 2020–2021	+ (Dia et al. 2022a)
H. quercifolia, *Hydrangea macrophylla*, and *Hydrangea arborescens*	Michigan, U.S.A. (2010)	Unclear	*X. campestris*	Byrne 2010	Not tested
**Hydrangea* sp.	The Netherlands (2011)	*gyrB* identification	*X. hortorum* (*X. hydrangeae*)	A. Aspin, *personal communication* 2021	+ (Dia et al. 2022a)
H. quercifolia cv. Little Honey	Illinois, U.S.A. (2012)	Microscopy[e]	*X. campestris*	Cleveland 2012	Not tested
**H. arborescens* (cvs. Invincibelle and Bella Anna) and *H. quercifolia*	Belgium (2011, 2012, 2015)	*gyrB* identification	*X. hydrangeae*	Cottyn et al. 2021; Dia et al. 2021	+ (Dia et al. 2022a)
Wild *H. quercifolia*	Florida, Tennessee and Mississippi, U.S.A. (2018–2019)	Tolerance of *H. quercifolia* to bacterial leaf spot	*X. campestris*	Sherwood et al. 2021	Not tested
**H. arborescens* and *H. quercifolia*	The Netherlands (2020–2021)	*gyrB* identification, $n = 1$ (2020), $n = 10$ (2021)	*X. hortorum* (*X. hydrangeae*)	A. Aspin, *personal communication* 2021; EPPO 2021a, 2022	+ for 12 from 12 tested strains (Dia et al. 2022a)
**H. quercifolia*	United Kingdom (2021)	*gyrB* identification, $n = 2$			
**H. quercifolia*	Italy (2021)	*gyrB* identification, $n = 1$			
**H. arborescens*	Germany (2021)	*gyrB* identification, $n = 3$			
**Lactuca sativa* (romaine, green leaf, and red leaf varieties)	Pennsylvania, U.S.A. (2022)	16S rRNA identification, $n = 2$	*X. hydrangeae*	Martinez 2023	Not tested
**H. quercifolia*	Italy (2022)	Multilocus sequence analysis (*gyrB*, *rpoD*, *dnaK*, and *fyuA*) and LAMP	*X. hydrangeae*	Campigli and Rizzo 2023	+ (Campigli and Rizzo 2023)
**H. arborescens*	Italy (2022), the Netherlands (2023), France (2023)	*gyrB* identification, $n = 1$ (2022), $n = 4$ (2023)	*X. hydrangeae*	A. Aspin, *personal communication* 2024	Not tested
**H. macrophylla*	The Netherlands (2023)	*gyrB* identification, $n = 1$			
**H. quercifolia*	The Netherlands (2022), Italy (2022), Belgium (2022), NA[f] (2022–2023)	*gyrB* identification, $n = 7$ (2022), $n = 1$ (2023)			

[a] The instances that came to light after the publication in *New Disease Reports* (Cottyn et al. 2021) are indicated by an asterisk before the host name. The reports are listed by chronological year of isolation. The identity of a subset of these *Hydrangea* isolates is tested with the *Xanthomonas hydrangeae*-specific loop-mediated isothermal amplification (LAMP) assay.
[b] Information in parentheses is the new correct identity.
[c] As described in Dia et al. (2022a). Information in parentheses indicates who performed the analysis.
[d] The exact origin of the strains is unknown, but custom reference indicates that the country of origin could be the Netherlands.
[e] Personal communication with Travis Cleveland (February 2022) revealed yearly observation of the disease to varying degrees of severity.
[f] NA, not available.

on tomato caused by *X. hydrangeae* may have been mistakenly attributed to *X. hortorum* pv. *gardneri* in the past. Testing this hypothesis would require a retrospective screening of historical strain collections of tomato isolates assigned to the latter species using the LAMP assay (Dia et al. 2022a) to ensure the correct assignment of the pathogens to the right species. This may then even lead to an inclusion of *X. hydrangeae* on the EPPO A2 list, just like *X. hortorum* pv. *gardneri*.

Depositing strains as live cultures in two public culture collections located in two different countries is a requirement for novel taxa descriptions (Chun et al. 2018; Lapage et al. 1992). Because the description of novel species requires time-consuming work (Moore et al. 2010) and strain deposition is not required for reporting new diseases in short communications, many novel taxa might not be formally described at all.

The first *Hydrangea* isolates (1995 to 1996) were not available, as they were neither placed in the authors' private strain collection nor deposited in a public culture collection (R. D. Gitiatis, *personal communication*, June 2020). Therefore, their identity cannot be confirmed, even though they are most likely *X. hydrangeae* strains based on the limited information available. Their availability would have allowed for consolidation of knowledge regarding bacterial leaf spot on *Hydrangea* and, eventually, the description of *X. hydrangeae* earlier. The benefits of microbial resource centers such as culture collections to scientific advancement and reproducibility are well documented (Broders et al. 2022; Overmann 2015), and requiring strain deposition also for short communications might prove useful. This mini-review reinforces the importance of obtaining sufficient strains to develop assays that detect and identify genetically distinct pathogens that cause similar diseases (also in the same host, e.g., *Xanthomonas* spp. causing tomato bacterial spot), thus determining the specificity of a diagnostic assay. This is crucial for designing assays for validation success (Groth-Helms et al. 2023).

Conclusions

New knowledge and the subsequent awareness raised about bacterial leaf spot of *Hydrangea* revealed that *X. hydrangeae* may be commonly prevalent within Europe and that global trade of plants plays an important role in its dissemination. This mini-review supports the importance of continuity within the field of phytodiagnostics by studying the case of bacterial leaf spot on *Hydrangea*, outlining the challenges faced in developing a diagnostic tool for its detection. The availability of such a tool is now expected to reveal the presence of the bacterium in other countries and eventually even on other plants.

Acknowledgments

The authors thank Nicola Rhyner for his support in the laboratory experiments that preceded this mini-review, Marilena Palmisano for her technical expertise in designing diagnostics assays, and Ronald D. Gitiatis and Travis Cleveland for providing valuable information through personal correspondence.

Literature Cited

Balaž, J., Ivanović, Ž., Davidović, A., Iličić, R., Janse, J., and Popović, T. 2016. Characterization of *Xanthomonas hortorum* pv. *pelargonii* isolated from geranium in Serbia. Plant Dis. 100:164-170.

Barel, V., Chalupowicz, L., Barash, I., Sharabani, G., Reuven, M., Dror, O., Burdman, S., and Manulis-Sasson, S. 2015. Virulence and *in planta* movement of *Xanthomonas hortorum* pv. *pelargonii* are affected by the diffusible signal factor (DSF)-dependent quorum sensing system. Mol. Plant Pathol. 16:710-723.

Benedict, A. A., Alvarez, A. M., and Pollard, L. W. 1990. Pathovar-specific antigens of *Xanthomonas campestris* pv. *begoniae* and *X. campestris* pv. *pelargonii* detected with monoclonal antibodies. Appl. Environ. Microbiol. 56:572-574.

BOLD. 2024. BOLD Systems - Taxonomy Browser: *Hydrangea*. https://bench.boldsystems.org/index.php/Taxbrowser_Taxonpage?taxid=179659 (accessed 23 October 2024).

Broders, K., Aspin, A., Bailey, J., Chapman, T., Portier, P., and Weir, B. S. 2022. Building more resilient culture collections: A call for increased deposits of plant-associated bacteria. Microorganisms 10:741.

Byrne, J. 2010. Bacterial leaf spot on hydrangea. https://www.canr.msu.edu/news/bacterial_leaf_spot_on_hydrangea (accessed 28 January 2022).

Campigli, S., and Rizzo, D. 2023. First report of *Xanthomonas hydrangeae* causing leaf spot on oakleaf hydrangea (*Hydrangea quercifolia*) in Tuscany, Italy. Plant Dis. 107:2514.

Catara, V., Cubero, J., Pothier, J. F., Bosis, E., Bragard, C., Đermić, E., Holeva, M. C., Jacques, M.-A., Petter, F., Pruvost, O., Robène, I., Studholme, D. J., Tavares, F., Vicente, J. G., Koebnik, R., and Costa, J. 2021. Trends in molecular diagnosis and diversity studies for phytosanitary regulated *Xanthomonas*. Microorganisms 9:862.

Chun, J., Oren, A., Ventosa, A., Christensen, H., Arahal, D. R., da Costa, M. S., Rooney, A. P., Yi, H., Xu, X.-W., De Meyer, S., and Trujillo, M. E. 2018. Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. Int. J. Syst. Evol. Microbiol. 68:461-466.

Cleveland, T. 2012. Bacterial leaf spot on oakleaf hydrangea. Home, Yard & Garden Pest 5. http://hyg.ipm.illinois.edu/article.php?id=367 (accessed 17 December 2021).

Cottyn, B., De Paepe, B., Dia, N. C., Van Malderghem, C., Pothier, J. F., and Van Vaerenbergh, J. 2021. First report of bacterial leaf spot of *Hydrangea* in retail nurseries in Belgium caused by strains assigned to a new *Xanthomonas hortorum* clade. New Dis. Rep. 43:e12008.

Demirci, A. Ş., Palabiyik, İ., Apaydin, D., Gümüş, T., Mirik, M., and Arici, M. 2019. Rheological behaviour and yield characterization of gum from local isolates: *Xanthomonas hortorum* pv. *pelargonii* and *Xanthomonas axonopodis* pv. *begonia*. Int. J. Agric. Environ. Food Sci. 3: 226-232.

Dia, N. C., Cottyn, B., Blom, J., Smits, T. H. M., and Pothier, J. F. 2022a. Differentiation of the *Xanthomonas hortorum – Xanthomonas hydrangeae* species complex using sensitive and selective LAMP assays. Front. Agron. 4:898778.

Dia, N. C., Morinière, L., Cottyn, B., Bernal, E., Jacobs, J. M., Koebnik, R., Osdaghi, E., Potnis, N., and Pothier, J. F. 2022b. *Xanthomonas hortorum* – beyond gardens: Current taxonomy, genomics, and virulence repertoires. Mol. Plant Pathol. 23:597-621.

Dia, N. C., Van Vaerenbergh, J., Van Malderghem, C., Blom, J., Smits, T. H. M., Cottyn, B., and Pothier, J. F. 2021. *Xanthomonas hydrangeae* sp. nov., a novel plant pathogen isolated from *Hydrangea arborescens*. Int. J. Syst. Evol. Microbiol. 71:005163.

Dickey, R. S., and Zumoff, C. H. 1987. Bacterial leaf blight of *Syngonium* caused by a pathovar of *Xanthomonas campestris*. Phytopathology 77:1257-1262.

Duff, J. 1991. Angular leaf spot of ornamental *Ficus* spp. due to *Xanthomonas campestris* pv. *fici*. Australas. Plant Pathol. 20:1-2.

Dye, D. W. 1967. Bacterial spot of ivy caused by *Xanthomonas hederae* (Arnaud, 1920) Dowson 1939, in New Zealand. N. Z. J. Sci. 10:481-485.

EPPO. 2021a. EPPO report on notifications of non-compliance from the United Kingdom (January 2021 - October 2021). https://gd.eppo.int/reporting/article-7209 (accessed 2 January 2022).

EPPO. 2021b. EPPO global database. https://gd.eppo.int/ (accessed 25 April 2021).

EPPO. 2022. EPPO report on notifications of non-compliance from the United Kingdom (November - December 2021). https://gd.eppo.int/reporting/article-7236 (accessed 2 January 2022).

European Union. 2016. Regulation (EU) 2016/2031 of the European Parliament of the Council of 26 October 2016 on protective measures against pests of plants, amending Regulations (EU) No 228/2013, (EU) No 652/2014 and (EU) No 1143/2014 of the European Parliament and of the Council and repealing Council Directives 69/464/EEC, 74/647/EEC, 93/85/EEC, 98/57/EC, 2000/29/EC, 2006/91/EC and 2007/33/EC. Official J. Eur. Union L317:4-104.

European Union. 2019. Commission implementing directive (EU) 2019/2072 of 28 November 2019 establishing uniform conditions for the implementation of regulation (EU) 2016/2031 of the European Parliament and the Council, as regards protective measures against pests of plants, and repealing commission regulation (EC) no 690/2008 and amending commission implementing regulation (EU) 2018/2019. Official J. Eur. Union L319: 1-278.

European Union. 2020. Commission implementing directive (EU) 2020/177 of 11 February 2020 amending council directives 66/401/EEC,

66/402/EEC, 68/193/EEC, 2002/55/EC, 2002/56/EC and 2002/57/EC, commission directives 93/49/EEC and 93/61/EEC and implementing directives 2014/21/EU and 2014/98/EU as regards pests of plants on seeds and other plant reproductive material. Official J. Eur. Union L41: 1-77.

Fulcher, A., Owen, J. S., and LeBude, A. 2016. Hydrangea production: Species-specific production guide. Report No. PB 1840-B. Pages 1-12. University of Tennessee Institute of Agriculture and University of Tennessee Extension.

Groth-Helms, D., Rivera, Y., Martin, F. N., Arif, M., Sharma, P., and Castlebury, L. A. 2023. Terminology and guidelines for diagnostic assay development and validation: Best practices for molecular tests. PhytoFrontiers 3:23-35.

Hauben, L., Vauterin, L., Swings, J., and Moore, E. R. B. 1997. Comparison of 16S ribosomal DNA sequences of all *Xanthomonas* species. Int. J. Syst. Bacteriol. 47:328-335.

Jacques, M.-A., Arlat, M., Boulanger, A., Boureau, T., Carrère, S., Cesbron, S., Chen, N. W. G., Cociancich, S., Darrasse, A., Denancé, N., Fischer-Le Saux, M., Gagnevin, L., Koebnik, R., Lauber, E., Noël, L. D., Pieretti, I., Portier, P., Pruvost, O., Rieux, A., Robène, I., Royer, M., Szurek, B., Verdier, V., and Vernière, C. 2016. Using ecology, physiology, and genomics to understand host specificity in *Xanthomonas*. Annu. Rev. Phytopathol. 54:163-187.

Lapage, S. P., Sneath, P. H. A., Lessel, E. F., Skerman, V. B. D., Seeliger, H. P. R., and Clark, W. A. 1992. Principles. International Code of Nomenclature of Bacteria: Bacteriological Code, 1990 Revision. https://www.ncbi.nlm.nih.gov/books/NBK8807/ (accessed 4 February 2022).

Larrahondo-Rodríguez, E., Liao, Y.-Y., and Huerta, A. I. 2022. Diagnostic guide for bacterial spot of tomato and pepper. Plant Health Prog. 23:355-361.

Leyns, F., De Cleene, M., Swings, J.-G., and De Ley, J. 1984. The host range of the genus *Xanthomonas*. Bot. Rev. 50:308-356.

Martinez, E. R. 2023. Phenotypic and genetic analyses of the lettuce bacterial leaf spot pathogen, *Xanthomonas hortorum* pv. *vitians*, and the development of a rapid detection technique. PhD thesis. Pennsylvania State University, University Park, PA. https://etda.libraries.psu.edu/files/final_submissions/29279

McClintock, E. 1957. A monograph of the genus *Hydrangea*. Pages 147-256 in: Proceedings of the California Academy of Sciences, 4th series. California Academy of Sciences, San Francisco, CA.

Moore, E. R. B., Mihaylova, S. A., Vandamme, P., Krichevsky, M. I., and Dijkshoorn, L. 2010. Microbial systematics and taxonomy: Relevance for a microbial commons. Res. Microbiol. 161:430-438.

Mullen, J. 1995. Southeast region report. Pages 97-102 in: Plant Diagnostics Quarterly, vol. 16. G. W. Simone and G. Ruhl, eds. Purdue University Plant and Pest Diagnostic Lab, West Lafayette, IN, U.S.A.

Overmann, J. 2015. Significance and future role of microbial resource centers. Syst. Appl. Microbiol. 38:258-265.

Parkinson, N., Aritua, V., Heeney, J., Cowie, C., Bew, J., and Stead, D. 2007. Phylogenetic analysis of *Xanthomonas* species by comparison of partial gyrase B gene sequences. Int. J. Syst. Evol. Microbiol. 57: 2881-2887.

Parkinson, N., Cowie, C., Heeney, J., and Stead, D. 2009. Phylogenetic structure of *Xanthomonas* determined by comparison of *gyrB* sequences. Int. J. Syst. Evol. Microbiol. 59:264-274.

Picard, C., Afonso, T., Benko-Beloglavec, A., Karadjova, O., Matthews-Berry, S., Paunovic, S. A., Pietsch, M., Reed, P., van der Gaag, D. J., and Ward, M. 2018. Recommended regulated non-quarantine pests (RNQPs), associated thresholds and risk management measures in the European and Mediterranean region. EPPO Bull. 48:552-568.

Ratnasingham, S., Wei, C., Chan, D., Agda, J., Agda, J., Ballesteros-Mejia, L., Ait Boutou, H., El Bastami, Z. M., Ma, E., Manjunath, R., Rea, D., Ho, C., Telfer, A., McKeowan, J., Rahulan, M., Steinke, C., Dorsheimer, J., Milton, M., and Hebert, P. D. N. 2024. BOLD v4: A centralized bioinformatics platform for DNA-based biodiversity data. Pages 403-441 in: DNA Barcoding: Methods and Protocols. Springer US, New York, NY, U.S.A.

Rinehart, T. A., Wadl, P. A., and Staton, M. E. 2018. An update on *Hydrangea macrophylla* breeding targets and genomics. Acta Hortic. 1191:217-224.

Rockey, W., Potnis, N., Timilsina, S., Hong, J. C., Vallad, G. E., Jones, J. B., and Norman, D. J. 2015. Multilocus sequence analysis reveals genetic diversity in xanthomonads associated with poinsettia production. Plant Dis. 99:874-882.

Saddler, G. S., and Bradbury, J. F. 2015. *Xanthomonas*. Pages 1-53 in: Bergey's Manual of Systematics of Archaea and Bacteria. W. B. Whitman, ed. John Wiley & Sons, Chichester, U.K.

Sahin, F., Abbasi, P. A., Ivey, M. L. L., Zhang, J., and Miller, S. A. 2003. Diversity among strains of *Xanthomonas campestris* pv. *vitians* from lettuce. Phytopathology 93:64-70.

Sherwood, A., Alexander, L. W., Clark, M. D., McNamara, S., and Hokanson, S. C. 2021. Horticultural characterization of wild *Hydrangea quercifolia* seedlings collected throughout the species native range. HortScience 56:1023-1033.

Sherwood, A., Clark, M. D., and Hokanson, S. C. 2022. Oakleaf hydrangea (*Hydrangea quercifolia* Bartr.): Horticulture, genetics, breeding, and conservation. Pages 1-33 in: Horticultural Reviews. I. Warrington, ed. John Wiley & Sons, Chichester, U.K.

Uddin, W., McCarter, S. M., and Gitaitis, R. D. 1996. First report of oakleaf hydrangea bacterial leaf spot caused by a pathovar of *Xanthomonas campestris*. Plant Dis. 80:599.

Vauterin, L., Hoste, B., Kersters, K., and Swings, J. 1995. Reclassification of *Xanthomonas*. Int. J. Syst. Bacteriol. 45:472-489.

Vauterin, L., Vantomme, R., Pot, B., Hoste, B., Swings, J., and Kersters, K. 1990. Taxonomic analysis of *Xanthomonas campestris* pv. *begoniae* and *X. campestris* pv. *pelargonii* by means of phytopathological, phenotypic, protein electrophoretic and DNA hybridization methods. Syst. Appl. Microbiol. 13:166-176.

Wu, X., Hulse-Kemp, A. M., Wadl, P. A., Smith, Z., Mockaitis, K., Staton, M. E., Rinehart, T. A., and Alexander, L. W. 2021. Genomic resource development for hydrangea (*Hydrangea macrophylla* (Thunb.) Ser.)—A transcriptome assembly and a high-density genetic linkage map. Horticulturae 7:25.

Young, J. M., Bull, C. T., De Boer, S. H., Firrao, G., Gardan, L., Saddler, G. E., Stead, D. E., and Takikawa, Y. 2001. Classification, nomenclature, and plant pathogenic bacteria - A clarification. Phytopathology 91:617-620.

Young, J. M., Dye, D. W., Bradbury, J. F., Panagopoulos, C. G., and Robbs, C. F. 1978. A proposed nomenclature and classification for plant pathogenic bacteria. N. Z. J. Agric. Res. 21:153-177.

Young, J. M., Takikawa, Y., Gardan, L., and Stead, D. E. 1992. Changing concepts in the taxonomy of plant pathogenic bacteria. Annu. Rev. Phytopathol. 30:67-105.