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Xanthomonas arboricola pv. *juglandis* and pv. *corylina*: Brothers or distant relatives? Genetic clues, epidemiology, and insights for disease management

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Abstract

Background: The species *Xanthomonas arboricola* comprises up to nine pathovars, two of which affect nut crops: pv. *juglandis*, the causal agent of walnut bacterial blight, brown apical necrosis, and the vertical oozing canker of Persian (English) walnut; and pv. *corylina*, the causal agent of the bacterial blight of hazelnut. Both pathovars share a complex population structure, represented by different clusters and several clades. Here we describe our current understanding of symptomatology, population dynamics, epidemiology, and disease control.

Taxonomic status: Bacteria; Phylum *Proteobacteria*; Class *Gammaproteobacteria*; Order *Lysobacterales* (earlier synonym of *Xanthomonadales*); Family *Lysobacteraceae* (earlier synonym of *Xanthomonadaceae*); Genus *Xanthomonas*; Species *X. arboricola*; Pathovars: pv. *juglandis* and pv. *corylina*.

Host range and symptoms: The host range of each pathovar is not limited to a single species, but each infects mainly one plant species: *Juglans regia* (*X. arboricola* pv. *juglandis*) and *Corylus avellana* (*X. arboricola*. pv. *corylina*). Walnut bacterial blight is characterized by lesions on leaves and fruits, and cankers on twigs, branches, and trunks; brown apical necrosis symptoms consist of apical necrosis originating at the stigmatic end of the fruit. A peculiar symptom, the vertical oozing canker developing along the trunk, is elicited by a particular genetic lineage of the bacterium. Symptoms of hazelnut bacterial blight are visible on leaves and fruits as necrotic lesions, and on woody parts as cankers. A remarkable difference is that affected walnuts drop abundantly, whereas hazelnuts with symptoms do not.

Distribution: Bacterial blight of walnut has a worldwide distribution, wherever Persian (English) walnut is cultivated; the bacterial blight of hazelnut has a more limited distribution, although disease outbreaks are currently more frequently reported. *X. arboricola* pv. *juglandis* is regulated almost nowhere, whereas *X. arboricola* pv. *corylina* is regulated in most European and Mediterranean Plant Protection Organization (EPPO) countries.

Epidemiology and control: For both pathogens infected nursery material is the main pathway for their introduction and spread into newly cultivated areas; additionally, infected nursery material is the source of primary inoculum. *X. arboricola* pv. *juglandis* is also disseminated through pollen. Disease control is achieved through the phytosanitary certification of nursery material (hazelnut), although approved certification schemes are not currently available. Once the disease is present in walnut/hazelnut groves, copper compounds are widely used, mostly in association with dithiocarbamates; where allowed, antibiotics (preferably kasugamycin) are sprayed. The emergence of strains highly resistant to copper currently represents the major threat for effective management of the bacterial blight of walnut.

Useful websites: https://gd.eppo.int/taxon/XANTJU, https://gd.eppo.int/taxon/XANTCY, https://www.euroxanth.eu, http://www.xanthomonas.org

KEYWORDS

bacterial blight, hazelnut, walnut, *Xanthomonas arboricola*

1 | INTRODUCTION

Global demand for nuts is currently strong and rapidly increasing, and among in-shell and table nuts almonds, walnuts, and hazels represent the three major crops of such horticultural species (Shahbandeh, 2020). The growing interest in hazel and walnut production worldwide, together with the increasing concerns raised by producers, extension services, and phytosanitary authorities focusing on acceptable and effective management strategies, make bacterial diseases, mainly those caused by *X. arboricola*, of relevant interest (COST, 2011).

X. arboricola pathovars are known to be the most important phytopathogenic bacteria of stone fruits and nuts (Lamichhane, 2014). Nine pathovars have been proposed so far: pv. *pruni* causing disease on stone fruits; pv. *corylina* pathogenic to hazelnut; pv. *juglandis* pathogenic to Persian (English) walnut; pv. *fragariae* the causal agent of strawberry bacterial leaf blight; pv. *populi* causing disease on poplar and pv. *celebensis* pathogenic to banana (Janse et al., 2001; Vauterin et al., 1995); pv. *arracaciae*, causing disease on *Arracacia xanthorrhiza*; pv. *guizotiae* pathogenic to *Guizotia abyssinica*; and pv. *zantedeschiae* the causal agent of blight symptoms on *Zantedeschia aethiopica* (also former pvs of *X. campestris*) (Fischer-Le Saux et al., 2015; Joubert & Truter, 1972; Pereira et al., 1971; Yirgou, 1964). According to the host range, three of them, pv. *pruni* (Xap), pv. *juglandis* (Xaj), and pv. *corylina* (Xac), represent highly phylogenetically related strains that cluster in three distinct clonal complexes (Fischer Le-Saux et al., 2015). Additionally, the species *X. arboricola* holds many unclassified plant-associated bacteria besides the described pathovars. The pathovar *juglandis*, although clustering in different clades, does not appear to be a homogeneous group of strains (Giovanardi et al., 2016; Scortichini et al., 2001), whereas pv. *corylina* is divided into two main clusters (Fischer Le-Saux et al., 2015). Due to repeated outbreaks and significant crop losses, these two pathovars of *X. arboricola* have gained attention in recent decades. Such interest is even greater due to the progressive spread of pv. *corylina* into new geographic areas, and the increasing challenge posed by the management of the latter. Nowadays, bacterial blight is still considered a major disease and a limiting factor in the production of walnuts, as reported 20 years ago (Teviotdale & Schroth, 1998).

2 | DISTRIBUTION AND IMPORTANCE/ ECONOMIC IMPACT

Bacterial blight of walnut (WBB), caused by Xaj, occurs worldwide in almost all areas where the Persian walnut (*Juglans regia*) is grown. It has also been reported that Xaj might be associated with some fungal pathogens, resulting in a specific disease called brown apical necrosis (BAN) (Belisario et al., 2002; Moragrega & Özaktan, 2010; Moragrega et al., 2011). Xaj is also related to a more recently reported disease, so-called vertical oozing canker (VOC) (Hajri et al., 2010). These diseases are the most serious among the biotic stresses affecting Persian walnut trees (Frutos & López, 2012; Lamichhane, 2014; Leslie et al., 2006). The presence of Xaj has already been confirmed in many geographical regions on all continents: (EPPO, 2020; Figure 1a). It is a pity that no updates have been published in some areas for over 30 years, meaning that the problem with Xaj probably (a) does not pose specific concerns to orchardists any more, (b) occurs only as local and sporadic outbreaks, or (c) has not been described in a scientific report. The distribution of complex walnut diseases is not uniform in space and time, as they may not occur regularly and with similar severity in the same area in different years.

Due to the possibility of infection of all the above-ground organs of the tree, WBB, BAN, and VOC have a great economic impact (Fu et al., 2018). The diseases may decrease the effectiveness of nursery production and reduce crop quality and yield due to premature fruit

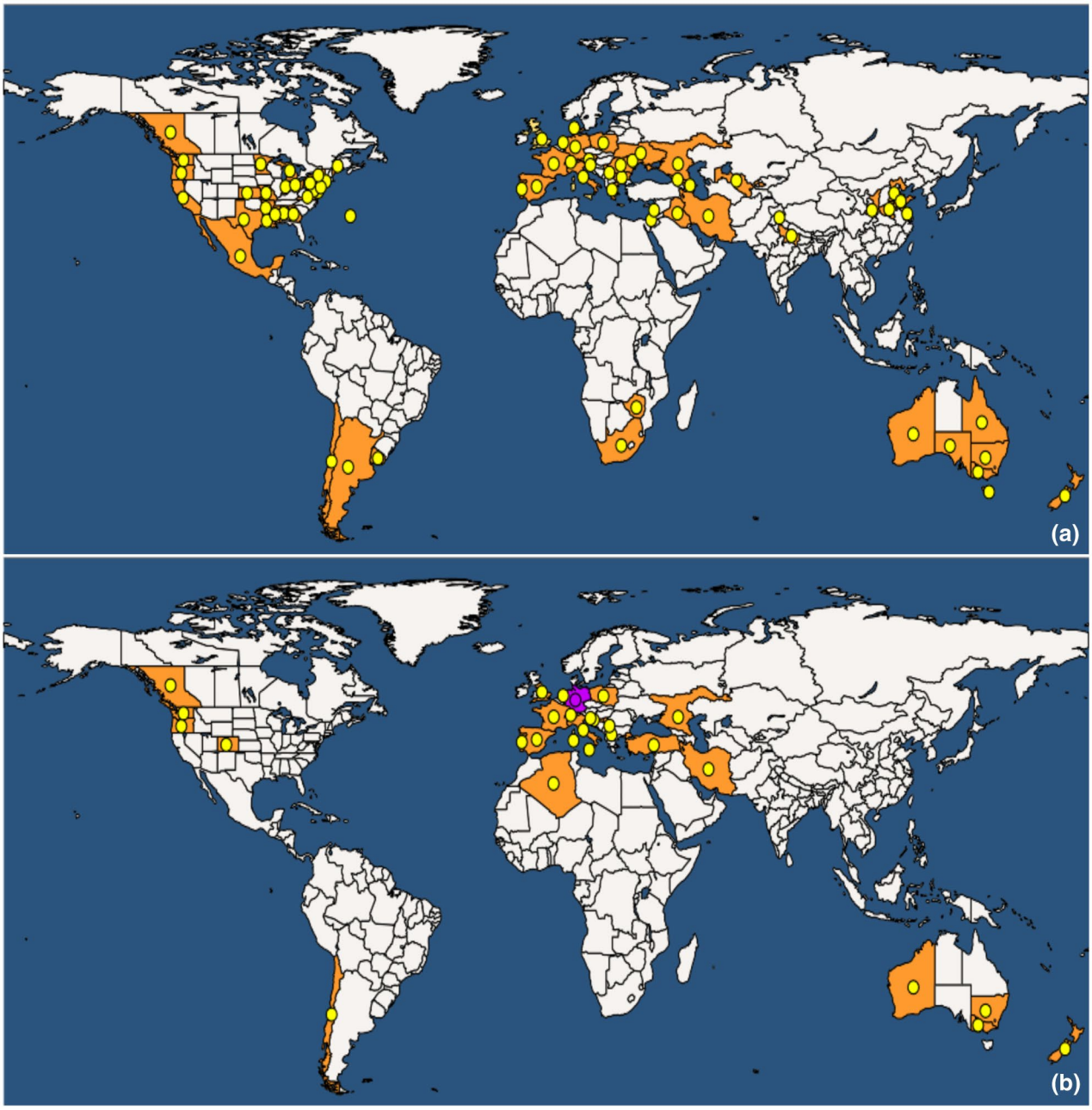


FIGURE 1 Worldwide distribution of *Xanthomonas arboricola* pv. *juglandis* (a) and pv. *corylina* (b) based on EPPO Global Database EPPO (2021) EPPO Global Database https://gd.eppo.int.; yellow, present; purple, transient

drop, as well as causing shell staining and kernel browning of the nuts still hanging on the tree (Belisario et al., 2001, 2002; Bouvet, 2005; Hajri et al., 2010; Lang & Evans, 2010; Lindow et al., 2014; Özaktan et al., 2009; Rudolph, 1933). It is expected that, due to global warming and the popularity of walnut nuts, the harmfulness of these complex diseases will increase.

According to the information given on the CABI website, bacterial blight of hazelnut (HBB) has already been reported in some countries of Asia, Africa, Europe, North and South America, and Oceania (CABI, 2019; Figure 1b). There is still a risk of it being introduced into other countries. The lack of confirmed reports on the occurrence of the disease may be related to the lack of monitoring in a given area or incorrect diagnostics. It is also expected that the situation may change significantly in the coming years due to the increase in the global trade of nursery material, resulting in the establishment of new orchards in some countries (Bayramoglu et al., 2010; authors' unpublished data). The economic impact of HBB is related primarily to planting material, which may be rejected due to the presence of a regulated organism, but the dieback of buds and new shoots can also cause great damage in orchards. It should be pointed out that although many reports have been published on the occurrence of the disease (Ćalić et al., 2009; Cirvilleri et al., 2006; EPPO, 2004; Guerrero & Lobos, 1987; Kazempour et al., 2006; Lamichhane et al., 2012; Luisetti et al., 1975; Puławska et al., 2010; Webber et al.,

2020; Wimalajeewa & Washington, 1980), very little or no information is available on the economic losses it has caused.

3 | DISEASE SYMPTOMS/HOST RANGE

The Persian (English) walnut (*J. regia*) is the major host of Xaj, although other plants belonging to the same genus might be occasionally found infected as well, for example Eastern black walnut (*J. nigra*), Southern California black walnut (*J. californica*), Northern California black walnut (*J. hindsii*), butternut (*J. cinerea*), Japanese walnut (*J. ailantifolia*, *J. ailantifolia* var. *cordiformis*), and hybrids *J. hindsii* × *J. regia* 'Paradox' and *J. nigra* × *J. regia* 'Royal' (Bradbury, 1967; Miller & Bollen, 1946; Smith, 1914; Smith et al., 1912).

The symptoms of WBB can be observed on all above-ground organs (Figure 2a–g). On the leaves, small water-soaked spots appear in the parenchymatic tissue in late spring. They enlarge, can coalesce, and turn into brown necrotic lesions with a blackish central area. They are often surrounded by a greenish or yellowish glow or a "chlorotic halo". On twigs necrotic lesions can develop, which become black and dry, and the twigs subsequently die. The pollen produced in catkins may also be colonized with Xaj, thus serving as an efficient dissemination pathway for the pathogen (Ark, 1944). On the fruits, initially small, round, water-soaked, dark lesions, which rapidly turn necrotic, deepen, and collapse, can be present. At high humidity and warm temperatures, droplets of bacterial slime may ooze from the lesions. The affected fruits shrink, and in most cases drop off prematurely. Late infections, during shell hardening, are usually limited to the epicarp of the fruit, with the infected nuts showing a necrotized husk. It is worth emphasizing that the symptoms on the leaves and fruits, especially in their early stage, may easily be confused with those caused by the fungi *Marssonina* spp. (or *Colletotrichum* spp.), both of which are the causal agents of walnut anthracnose. However, in the case of anthracnose, dry brown to grey spots with acervuli are observed. On twigs and shoots, necrotic cankers may occur (Janse, 2006; Lang & Evans, 2010; Miller & Bollen, 1946; Scortichini, 2010; Stapp, 1961). The characteristic symptoms of BAN manifest themselves as apical necrosis originating at the stigmatic end of the fruit (Figure 2h,i). On fallen fruit, a brown patch appearing exclusively at the blossom end can occur, as well as blackening and rotting of inner tissues. The symptoms observed differ from those of WBB, where blackish greasy spots, with or without a yellow halo, not restricted to the stigmatic end of the fruit are present (Belisario et al., 2002; Moragrega & Özaktan, 2010). The symptoms of VOC develop in woody tissues. Initially, they include longitudinal deformations of the affected trunks, followed by

FIGURE 2 Symptoms induced by *Xanthomonas arboricola* pv. *juglandis* on walnut. Symptoms of walnut bacterial blight (WBB): necrotic lesions on fruitlets and fruits (a–c); necrotic spots on leaves, sometimes surrounded by a chlorotic halo (d, e); necrotic spots on twigs where walnut catkins are developing (f); necrosis/cankers on woody tissue (g). (h, i) Symptoms of brown apical necrosis (BAN) on fruits. (j–l) Symptoms of vertical oozing canker (VOC): longitudinal deformations and vertical cankers, oozing in spring, on trunks of *Juglans regia*

vertical cankers developing on both the trunks and branches, with brown to black exudates, observed mainly in summer. In the final stage, severe distortion and cracking of the affected trunks become evident (Figure 2j–l; Hajri et al., 2010).

The most important host of Xac is *Corylus avellana* (hazel). Other plants species, for example *C. pontica*, *C. maxima*, and *C. colurna*, have been found to be susceptible as well, but are considered minor hosts (Anonymous, 1986; EPPO, 2004a). HBB symptoms

FIGURE 3 Hazelnut bacterial blight caused by *Xanthomonas arboricola* pv. *corylina* (a) Symptoms on leaves: spots (in the corner) and characteristic V-shaped lesions, (b) fruit shell elongated brown to black necrotic lesions, (c) longitudinal shoot necrosis, (d) shoot dieback and leaf blight, (e) canopy leaf blight, spotting of husk and fruit

occur on all of the above-ground organs of the infected trees, but in contrast to WBB, the nuts are rarely affected. The disease is considered to be the main limiting factor in nursery production. The long and densely growing shoots on mother plants are very susceptible during high-humidity or rainy periods, when the disease can spread very quickly. In the nurseries and orchards, dying of both leaf and flower buds, surrounded by necrotic damage, is observed. Additionally, small, slightly convex brown spots, most often in the shape of an ellipse, appear along the shoots. With time, they expand to form longitudinal cankers sometimes covering the entire shoot circumference (Figure 3c, d). In the spring and summer, partial or total dieback of new lateral shoots and twigs is observed. The most dangerous are cankers on the stems of young trees, which may cause the death of the entire tree. On the leaves initially single, yellow-green, water-soaked, small angular lesions are formed, which may subsequently turn necrotic and coalesce. Necrosis can also start from the margin of the leaf blade. The necrotic leaf tissue is often surrounded by light green or chlorotic discoloration (in a characteristic 'V' shape) (Figure 3a). On the shell of the fruit, round or elongated brown to black necrotic lesions are present, but the involucre of the shell shows oily or necrotic round spots. (Figure 3b,e; Anonymous, 1986; Lamichhane et al., 2013; Miller et al., 1949). In favourable conditions, bacterial exudate may ooze from the necrotic lesions.

4 | TAXONOMIC HISTORY

The pathogens of both diseases were first isolated on the Pacific coast of the USA in the late nineteenth–early twentieth century. WBB was first observed in southern California. The causal agent was named *Pseudomonas juglandis* by Pierce (1901), who had already mentioned the great amylolytic properties of the bacteria and its aggressiveness on *J. regia*, describing it as one of the most pathogenic species of the genus known to date. It was reclassified as *Xanthomonas juglandis* on the creation of this genus (Dowson, 1939). At that time, the classification of plant pathogens at the generic level was controversial and confusing, with the coexistence of several classification systems based on different classification criteria: indeed, the bacterium was also named *Phytomonas juglandis* (Bergey et al., 1939) or *Bacterium juglandis*. Although filbert blight was also first observed in the early twentieth century (Barss, 1913) in Oregon, it took 25 years before the first detailed description of the causal agent was published. Miller et al. (1940) were puzzled by the close similarities between the two pathogens and questioned their relationships. Indeed, the filbert blight strains could only be distinguished from the walnut strains by their differential pathogenic behaviour on their hosts and none of the biochemical and physiological tests were discriminative. While Miller et al. (1940) hesitated to classify the filbert strains as a variety of *Phytomonas juglandis*, they finally named them *Phytomonas corylina* for convenient reasons. The name *X. corylina* was preferred by other authors (Star & Burkholder, 1942). Both pathogens were reclassified as pathovars of *X. campestris* (Dye, 1978) in anticipation of the purge of bacterial species names linked to the creation of the Approved Lists of Bacterial Names (Skerman et al., 1980). When Vauterin et al. (1995) redefined *Xanthomonas* species based on DNA–DNA hybridizations, *X. campestris* pv. *juglandis* and *X. campestris* pv. *corylina* were reclassified in the newly proposed species *X. arboricola* along with pv. *pruni*, pv. *populi*, pv. *celebensis*, and type C strains of *X. campestris* pv. *poinsettiicola*. The pathotype strain of *X. arboricola* pv. *juglandis* CFBP 2528^{T} = NCPPB 411^{T} = LMG 747^{T} = ATCC 49083^{T} = ICMP 35^{T} was chosen as the type strain of the species. The pathotype strain of *X. arboricola* pv. *corylina* is CFBP 1159^{PT} = NCPPB 935^{PT} = LMG 689^{PT} = ATCC 19313^{PT}. According to present taxonomic status Xac and Xaj belong to the *Gammaproteobacteria* class, the *Lysobacterales* order (earlier synonym of *Xanthomonadales*), and the *Lysobacteraceae* family (earlier synonym of *Xanthomonadaceae*).

5 | MICROBIOLOGICAL PROPERTIES/ PHENOTYPIC CHARACTERS

Xaj and Xac share the common microbial properties of *Xanthomonas* genus. They are gram-negative rods (1.1–3.8 × 0.3–0.7 μm) usually motile thanks to a single polar flagellum. They are strictly aerobic. Colonies appear as yellowish, glistening, and mucoid colonies. Xaj and Xac also share the common bacteriological features of *X. arboricola* (Vauterin et al., 1995). Among them, the ability to metabolize quinate is a major discriminative character of *X. arboricola* strains that is unique to this species. This character has been proven stable among Xaj populations and is revealed on succinate-quinate medium, on which a greenish halo develops around a streak of *X. arboricola* strains (Lee et al., 1992). It should be noted that, although they metabolize quinate, the strains are not able to use it as sole source of carbon and thus this characteristic cannot be tested on Biolog plates. Xaj and Xac strains produce a set of specific exoenzymes and can hydrolyse starch, gelatin, esculin, and Tween 80. They share highly similar biochemical properties and cannot be differentiated only on this basis. Molecular diagnostics should be preferred for accurate identification.

6 | DETECTION AND IDENTIFICATION

The diagnostic protocol for HBB was originally prepared by EPPO and consists of the description of disease symptoms, isolation of the pathogen using complex media, for example glucose-yeast extract-calcium carbonate agar (GYCA), yeast extract-peptone-glucose agar (YPGA), or yeast extract-dextrose-calcium carbonate (YDC), tests for pathogenicity, and phenotypic characters (EPPO, 2004b; Schaad et al., 2001). However, it is known that these tests are not suitable for all strains of Xac. Some discrepancies have been noted in phenotypic descriptions, such as utilization of L-arabinose, maltose, glycerol, D-xylose, lactose, and raffinose (Puławska et al.,

2010). The polyclonal antibody and commercial kits for immunofluorescence (IF) and/or double-antibody sandwich ELISA (from Loewe Biochemica) can be useful for screening and early pathogen detection; however, they do not have sufficient specificity and/or sensitivity and can give an ambiguous response, including false-positive/-negative results (Prokić et al., 2012). Hitherto, several DNA-based molecular assays useful for the identification and detection of Xac have been developed. For its preliminary identification, primers X1/X2, specific for the *Xanthomonas* genus (Maes, 1993), are routinely used. The species-level primers XarbQ-F/XarbQ-R, based on regions of the quinate metabolic gene *qumA*, can also be applied as the first identification test for Xac (Pothier et al., 2011). In addition, primers XapY17-F/XapY17-R (Pagani, 2004), included together with XarbQ in the duplex-PCR assay, for identification and detection of Xap, also cross-react with Xac strains (Pothier et al., 2011; Webber et al., 2020). Recently, analysis of partial sequences of selected housekeeping genes has been widely used for identification and the determination of the taxonomic position of Xac (MLSA; Webber et al., 2020; Young et al., 2008). For discrimination of pathovars within *X. arboricola* and differentiation of the Xac strains, the rep-PCR (using the ERIC-, rep-, and BOX-PCR primers sets) was found to be very useful (Puławska et al., 2010; Scortichini et al., 2002). More recently, based on the comparison of available genomes of *X. arboricola* pathovars, the specific sequence fragments from the genome were selected for Xac and used for designing specific markers. Studies have shown that the developed systems are reliable in the detection of Xac directly in plant material, and are characterized by high sensitivity and specificity (authors' unpublished data).

Regarding the detection of Xaj, it should be noted that the WBB symptomatology, detailed in section 3, provides to trained phytopathologists an immediate perception that the aetiological agent is most probably Xaj. However, this phytopathometric assessment of symptoms does not replace the need for an accurate diagnosis of the disease through detection of the bacteria in plant samples and their identification. Gironde et al. (2009) reported a PCR-based detection of Xaj that targets a genomic marker using a primer pair (XajF and XajR), which unfortunately was not provided and therefore has limited use for the community. Later, based on comparative genomics, a set of nine genomic markers (XAJ1 to XAJ9) were identified to discriminate Xaj from other pathovars and closely related *Lysobacteraceae* (Fernandes et al., 2017). While four out of the nine markers were broad-range, that is, present in most of the Xaj strains assayed regardless of their genetic diversity, five markers were narrow-range and were only detected in a subset of the Xaj strains analysed. The authors used these differences to define hybridization patterns capable of discriminating between different Xaj strains (Fernandes et al., 2017). To meet the need to have a reliable and fast, culture-independent, detection method of Xaj directly in walnut leaves and fruits with symptoms, a multiplex PCR using three broad-range markers (XAJ1, XAJ6, and XAJ8) was proposed in the same study (Fernandes et al., 2017). Recently, a quantitative PCR (qPCR) using markers XAJ1 and XAJ6 was described to estimate the load of Xaj cells in infected fruits as a measure of its virulence, that is, the pathogen fitness to colonize the host (Martins et al., 2019).

7 | XAJ AND XAC WITHIN *X. ARBORICOLA* POPULATION STRUCTURE

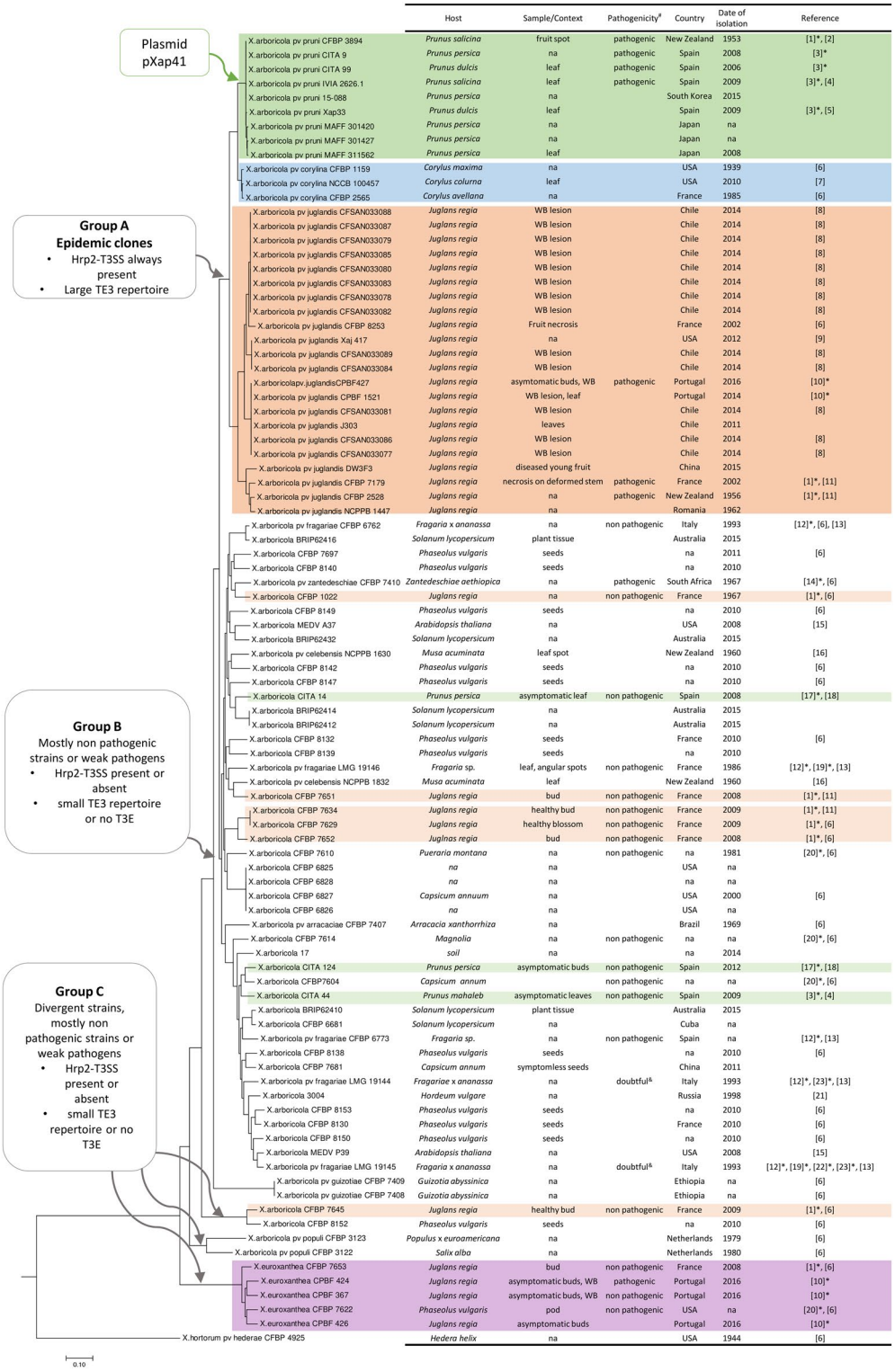
The genetic cohesion of *X. arboricola* species has been confirmed by partial sequencing of housekeeping genes and later by phylogenomic analyses. Indeed, within the genus diversity, *X. arboricola* strains (including Xaj and Xac) form a distinct cluster, clearly separated from other described species on phylogenetic trees based on four concatenated genes (Young et al., 2008) or *gyrB* alone (Parkinson et al., 2009), or on 993 concatenated proteins from the core proteome (Merda et al., 2017). Since its description, additional strains and pathovars have been reclassified within *X. arboricola*, and non- or low pathogenic strains not classified in pathovars (Essakhi et al., 2015; Fischer-Le Saux et al., 2015; Parkinson et al., 2009).

Within the diversity of the species, Xaj and Xac correspond to cohesive genetic clusters. Strains from pv. *juglandis* and pv. *corylina* split into two separate monophyletic groups as soon as a sufficient number of genes (i.e., seven) is used in multilocus sequence analysis (MLSA) to provide a robust phylogenetic signal (Fischer-Le Saux et al., 2015). However, if fewer genes are used, the robustness on the branches decreases and Xaj or Xac strains do not longer cluster into unique groups. For instance, using partial *gyrB* alone cannot discriminate Xaj and Xac from Xap, as some strains from these three pathovars share the same *gyrB* allele (Fischer-Le Saux et al., 2015; Kałużna et al., 2014; Webber et al., 2020). Genetic clustering of Xaj and Xac according to pathovar classification has been confirmed by phylogenomic studies (Figure 4) (Garita-Cambronero et al., 2016c; Merda et al., 2017).

Population genetics and comparative genomic studies showed that the three pathovars attacking stone and nut fruits trees (Xaj, Xac, and Xap) correspond to three epidemic clones that share a common ancestor (Merda et al., 2016, 2017). Therefore, their close phylogenetic relatedness is supported by highly similar accessory genomes with, for instance, 10 type III effector (T3E) genes in common, not retrieved in non- or low virulence strains (Garita-Cambronero et al., 2018; Merda et al., 2017). These genes are not grouped on a plasmid or on a pathogenicity island but are scattered in the genomes with conserved flanking regions and thus may have been gradually acquired by their common ancestor through a long-term evolution process (Merda et al., 2017). This ancient accumulation of a large set of shared T3E genes, among which several are known to suppress the pathogen-associated molecular pattern-triggered immunity, may contribute to the actual epidemic success of the three major pathovars. From this common ancestor, host-driven divergence has occurred. Further acquisitions of differential T3E genes may account for host specialization as hypothesized by Hajri et al. (2009, 2012). Thus, contrary to other pathogens that emerge following a single acquisition event (Barash & Manulis-Sasson, 2009), it seems that Xaj

and Xac emergence is the result of a long evolutionary history with gradual accumulation of virulence determinants. Additional studies are needed to further decipher the evolutionary history of nut and stone fruit tree pathogens, and the potential role of host domestication and host jumps in the patho-adaptative process (Jacques et al., 2016).

At the species level, *X. arboricola* fits into the epidemic population structure described by Maynard-Smith et al. (1993), within which one can distinguish epidemic clones composed of a limited number of highly frequent haplotypes (group A composed of the successful pathovars Xaj, Xac, and Xap) and a network of highly diverse strains with a high recombination rate (group B including non- or low pathogenic strains and unsuccessful pathovars) (Figure 4) (Merda et al., 2016). Multilocus sequence typing (MLST) showed that Xac, Xaj, and Xap form three clonal complexes of host specialized strains on their respective host, with the same sequence type that can be retrieved



	Host	Sample/Context	Pathogenicity[#]	Country	Date of isolation	Reference
X.arboricola pv pruni CFBP 3894	*Prunus salicina*	fruit spot	pathogenic	New Zealand	1953	[1]*, [2]
X.arboricola pv pruni CITA 9	*Prunus persica*	na	pathogenic	Spain	2008	[3]*
X.arboricola pv pruni CITA 99	*Prunus dulcis*	leaf	pathogenic	Spain	2006	[3]*
X.arboricola pv pruni IVIA 2626.1	*Prunus salicina*	leaf	pathogenic	Spain	2009	[3]*, [4]
X.arboricola pv pruni 15-088	*Prunus persica*	na		South Korea	2015	
X.arboricola pv pruni Xap33	*Prunus dulcis*	leaf		Spain	2009	[3]*, [5]
X.arboricola pv pruni MAFF 301420	*Prunus persica*	na		Japan	na	
X.arboricola pv pruni MAFF 301427	*Prunus persica*	na		Japan	na	
X.arboricola pv pruni MAFF 311562	*Prunus persica*	leaf		Japan	2008	
X.arboricola pv corylina CFBP 1159	*Corylus maxima*	na		USA	1939	[6]
X.arboricola pv corylina NCCB 100457	*Corylus colurna*	leaf		USA	2010	[7]
X.arboricola pv corylina CFBP 2565	*Corylus avellana*	na		France	1985	[6]
X.arboricola pv juglandis CFSAN033088	*Juglans regia*	WB lesion		Chile	2014	[8]
X.arboricola pv juglandis CFSAN033087	*Juglans regia*	WB lesion		Chile	2014	[8]
X.arboricola pv juglandis CFSAN033079	*Juglans regia*	WB lesion		Chile	2014	[8]
X.arboricola pv juglandis CFSAN033085	*Juglans regia*	WB lesion		Chile	2014	[8]
X.arboricola pv juglandis CFSAN033080	*Juglans regia*	WB lesion		Chile	2014	[8]
X.arboricola pv juglandis CFSAN033083	*Juglans regia*	WB lesion		Chile	2014	[8]
X.arboricola pv juglandis CFSAN033078	*Juglans regia*	WB lesion		Chile	2014	[8]
X.arboricola pv juglandis CFSAN033082	*Juglans regia*	WB lesion		Chile	2014	[8]
X.arboricola pv juglandis CFBP 8253	*Juglans regia*	Fruit necrosis		France	2002	[6]
X.arboricola pv juglandis Xaj 417	*Juglans regia*	na		USA	2012	[9]
X.arboricola pv juglandis CFSAN033089	*Juglans regia*	WB lesion		Chile	2014	[8]
X.arboricola pv juglandis CFSAN033084	*Juglans regia*	WB lesion		Chile	2014	[8]
X.arboricolapv.juglandisCPBF427	*Juglans regia*	asymtomatic buds, WB	pathogenic	Portugal	2016	[10]*
X.arboricola pv juglandis CPBF 1521	*Juglans regia*	WB lesion, leaf		Portugal	2014	[10]*
X.arboricola pv juglandis CFSAN033081	*Juglans regia*	WB lesion		Chile	2014	[8]
X.arboricola pv juglandis J303	*Juglans regia*	leaves		Chile	2011	
X.arboricola pv juglandis CFSAN033086	*Juglans regia*	WB lesion		Chile	2014	[8]
X.arboricola pv juglandis CFSAN033077	*Juglans regia*	WB lesion		Chile	2014	[8]
X.arboricola pv juglandis DW3F3	*Juglans regia*	diseased young fruit		China	2015	
X.arboricola pv juglandis CFBP 7179	*Juglans regia*	necrosis on deformed stem	pathogenic	France	2002	[1]*, [11]
X.arboricola pv juglandis CFBP 2528	*Juglans regia*	na	pathogenic	New Zealand	1956	[1]*, [11]
X.arboricola pv juglandis NCPPB 1447	*Juglans regia*	na		Romania	1962	
X.arboricola pv fragariae CFBP 6762	*Fragaria x ananassa*	na	non pathogenic	Italy	1993	[12]*, [6], [13]
X.arboricola BRIP62416	*Solanum lycopersicum*	plant tissue		Australia	2015	
X.arboricola CFBP 7697	*Phaseolus vulgaris*	seeds		na	2011	[6]
X.arboricola CFBP 8140	*Phaseolus vulgaris*	seeds		na	2010	
X.arboricola pv zantedeschiae CFBP 7410	*Zantedeschiae aethiopica*	na	pathogenic	South Africa	1967	[14]*, [6]
X.arboricola CFBP 1022	*Juglans regia*	na	non pathogenic	France	1967	[1]*, [6]
X.arboricola CFBP 8149	*Phaseolus vulgaris*	seeds		na	2010	[6]
X.arboricola MEDV A37	*Arabidopsis thaliana*	na		USA	2008	[15]
X.arboricola BRIP62432	*Solanum lycopersicum*	na		Australia	2015	
X.arboricola pv celebensis NCPPB 1630	*Musa acuminata*	leaf spot		New Zealand	1960	[16]
X.arboricola CFBP 8142	*Phaseolus vulgaris*	seeds		na	2010	[6]
X.arboricola CFBP 8147	*Phaseolus vulgaris*	seeds		na	2010	[6]
X.arboricola CITA 14	*Prunus persica*	asymptomatic leaf	non pathogenic	Spain	2008	[17]*, [18]
X.arboricola BRIP62414	*Solanum lycopersicum*	na		Australia	2015	
X.arboricola BRIP62412	*Solanum lycopersicum*	na		Australia	2015	
X.arboricola CFBP 8132	*Phaseolus vulgaris*	seeds		France	2010	[6]
X.arboricola CFBP 8139	*Phaseolus vulgaris*	seeds		na	2010	
X.arboricola pv fragariae LMG 19146	*Fragaria sp.*	leaf, angular spots	non pathogenic	France	1986	[12]*, [19]*, [13]
X.arboricola pv celebensis NCPPB 1832	*Musa acuminata*	leaf		New Zealand	1960	[16]
X.arboricola CFBP 7651	*Juglans regia*	bud	non pathogenic	France	2008	[1]*, [11]
X.arboricola CFBP 7634	*Juglans regia*	healthy bud	non pathogenic	France	2009	[1]*, [11]
X.arboricola CFBP 7629	*Juglans regia*	healthy blossom	non pathogenic	France	2009	[1]*, [6]
X.arboricola CFBP 7652	*Juglnas regia*	bud	non pathogenic	France	2008	[1]*, [6]
X.arboricola CFBP 7610	*Pueraria montana*	na	non pathogenic	na	1981	[20]*, [6]
X.arboricola CFBP 6825	*na*	na		USA	na	
X.arboricola CFBP 6828	*na*	na		na	na	
X.arboricola CFBP 6827	*Capsicum annuum*	na		USA	2000	[6]
X.arboricola CFBP 6826	*na*	na		USA	na	
X.arboricola pv arracaciae CFBP 7407	*Arracacia xanthorrhiza*	na		Brazil	1969	[6]
X.arboricola CFBP 7614	*Magnolia*	na	non pathogenic	na	na	[20]*, [6]
X.arboricola 17	*soil*	na		na	2014	
X.arboricola CITA 124	*Prunus persica*	asymptomatic buds	non pathogenic	Spain	2012	[17]*, [18]
X.arboricola CFBP7604	*Capsicum annum*		non pathogenic	na	na	[20]*, [6]
X.arboricola CITA 44	*Prunus mahaleb*	asymptomatic leaves	non pathogenic	Spain	2009	[3]*, [4]
X.arboricola BRIP62410	*Solanum lycopersicum*	plant tissue		Australia	2015	
X.arboricola CFBP 6681	*Solanum lycopersicum*	na		Cuba	na	
X.arboricola pv fragariae CFBP 6773	*Fragaria sp.*	na	non pathogenic	Spain	na	[12]*, [13]
X.arboricola CFBP 8138	*Phaseolus vulgaris*	seeds		na	2010	[6]
X.arboricola CFBP 7681	*Capsicum annum*	symptomless seeds		China	2011	
X.arboricola pv fragariae LMG 19144	*Fragariae x ananassa*	na	doubtful[&]	Italy	1993	[12]*, [23]*, [13]
X.arboricola 3004	*Hordeum vulgare*	na		Russia	1998	[21]
X.arboricola CFBP 8153	*Phaseolus vulgaris*	seeds		na	2010	[6]
X.arboricola CFBP 8130	*Phaseolus vulgaris*	seeds		France	2010	[6]
X.arboricola CFBP 8150	*Phaseolus vulgaris*	seeds		na	2010	[6]
X.arboricola MEDV P39	*Arabidopsis thaliana*	na		USA	2008	[15]
X.arboricola pv fragariae LMG 19145	*Fragaria x ananassa*	na	doubtful[&]	Italy	1993	[12]*, [19]*, [22]*, [23]*, [13]
X.arboricola pv guizotiae CFBP 7409	*Guizotia abyssinica*	na		Ethiopia	na	[6]
X.arboricola pv guizotiae CFBP 7408	*Guizotia abyssinica*	na		Ethiopia	na	[6]
X.arboricola CFBP 7645	*Juglans regia*	healthy bud	non pathogenic	France	2009	[1]*, [6]
X.arboricola CFBP 8152	*Phaseolus vulgaris*	seeds		na	2010	[6]
X.arboricola pv populi CFBP 3123	*Populus x euroamericana*	na		Netherlands	1979	[6]
X.arboricola pv populi CFBP 3122	*Salix alba*	na		Netherlands	1980	[6]
X.euroxanthea CFBP 7653	*Juglans regia*	bud	non pathogenic	France	2008	[1]*, [6]
X.euroxanthea CPBF 424	*Juglans regia*	asymptomatic buds, WB	pathogenic	Portugal	2016	[10]*
X.euroxanthea CPBF 367	*Juglans regia*	asymptomatic buds, WB	non pathogenic	Portugal	2016	[10]*
X.euroxanthea CFBP 7622	*Phaseolus vulgaris*	pod	non pathogenic	USA	na	[20]*, [6]
X.euroxanthea CPBF 426	*Juglans regia*	asymptomatic buds		Portugal	2016	[10]*
X.hortorum pv hederae CFBP 4925	*Hedera helix*	na		USA	1944	[6]

FIGURE 4 Phylogenetic tree showing *Xanthomonas arboricola* pv. *juglandis* (Xaj) and *X. arboricola* pv. *corylina* (Xac) lineages within the genetic diversity of *X. arboricola*. The nearest recently described species *X. euroxanthea*, which encompasses pathogenic and nonpathogenic strains from walnut, is also represented and highlighted in purple. *X. hortorum* is used to root the tree. Whole-genome sequences from *X. arboricola* available at the National Center for Biotechnology Information (NCBI) were collected and grouped according to their percentage of shared *k*-mers at a threshold of 50% (Briand et al., 2020). Only strains clustering with the type strains of *X. arboricola* and *X. euroxanthea*, respectively, were retained. Duplicated genomes from the same strain and genomes with no metadata were discarded. Genomes were annotated with Prokka (Seemann, 2014) and the tree constructed on 259,046 single-nucleotide polymorphisms (SNPs) from the core alignment with panX software (Ding et al., 2018). *X. arboricola* pv. *pruni* (Xap), Xac, and Xaj strains are highlighted in green, blue, and orange, respectively. *X. arboricola* strains from *Juglans regia* and *Prunus* spp. outside Xaj and Xap lineages are highlighted in light orange and light green, respectively. [#]Pathogenicity towards host of isolation according to published inoculation tests (see reference column). *Reference relative to pathogenicity testing otherwise relative to genome publication: 1, Essakhi et al. (2015); 2, López-Soriano et al. (2016); 3, Garita-Cambronero et al. (2016c); 4, Garita-Cambronero et al. (2016a); 5, Garita-Cambronero et al. (2014); 6, Merda et al., 2017; 7, Ibarra Caballero et al. (2013); 8, Higuera et al. (2015); 9, Pereira et al. (2015); 10, Martins et al. (2020); 11, Cesbron et al. (2015); 12, Vandroemme et al. (2013); 13, Gétaz et al. (2018); 14, Fischer-Le Saux et al. (2015); 15, Wang et al. (2018); 16, Harrison et al. (2016); 17, Garita-Cambronero et al. (2017); 18, Garita-Cambronero et al. (2016b); 19, Gétaz et al. (2020); 20, Vauterin et al. (1996); 21, Ignatov et al. (2015); 22, Ferrante and Scortichini (2018); 23, Janse et al. (2001) contrary to Vandroemme et al. (2013) and Gétaz et al. (2020), Janse et al. (2001) and Ferrante and Scortichini (2018) found the strain to be pathogenic

on different continents decades apart, a feature of pandemic pathogens (Boudon et al., 2005; Fischer-Le Saux et al., 2015; Marcelletti et al., 2010; Webber et al., 2020). By contrast, most strains from the recombinant network (Group B) have been shown to be nonpathogenic (Essakhi et al., 2015; Garita-Cambronero et al., 2016a, 2016c) or to exhibit a doubtful virulence, like the ones of pv. *fragariae* (Ferrante & Scortichini, 2018; Gétaz et al., 2020; Vandroemme et al., 2013). They do not cluster according to the host of isolation (Figure 4) (Merda et al., 2016). These observations suggest that they may be generalists.

It is noteworthy that *X. arboricola* Group B and divergent lineages (designated Group C in Merda et al., 2016) include look-alike strains isolated from the same host as Xaj and Xap (Figure 4) (Essakhi et al., 2015; Garita-Cambronero et al., 2016a, 2016b, 2016c). Recently, pathogenic and nonpathogenic strains isolated from *J. regia* in Portugal have been classified as a new species of *Xanthomonas euroxanthea* (Martins et al., 2020). This novel species corresponds to one of the divergent lineages from Group C and also encompasses nonpathogenic strains isolated from *J. regia* and *Phaseolus vulgaris* in France and the USA, respectively (Figure 3). Even though in planta inoculation of these look-alike strains on their host of isolation often does not produce symptoms, some necroses are sometimes observed (Garita-Cambronero et al., 2017; Martins et al., 2020). However, when measured, the bacterial population size after 21 days of incubation was shown to be limited with these strains compared to Xaj or Xap (Essakhi et al., 2015; Garita-Cambronero et al., 2017). Those strains able to cause necrotic symptoms on the same hosts as the major pathovars can be misidentified as Xaj, Xac, or Xap. Accurate identification of epidemic clones requires careful molecular tests with appropriate markers and in-depth pathogenicity tests, including evaluation of in planta pathogen multiplication.

The pathovar definition is based on distinctive pathogenicity, which refers to host range and symptomatology. The above observations challenge this pathovar concept and question the need to include a genetic dimension to the pathovar definition.

8 | GENETIC DIVERSITY WITHIN XAJ AND XAC

Of the three major pathovars attacking stone fruits and nuts, Xaj is the most polymorphic, contrasting with Xap, which is the most monomorphic (Figure 4) (Boudon et al., 2005; Fischer-Le Saux et al., 2015; Marcelletti et al., 2010). The relevant level of genetic polymorphism and different genetic lineages were revealed in Xaj by molecular fingerprinting methods (amplified fragment length polymorphism [AFLP], PCR melting profile [PCR MP], repetitive-PCRs [rep-PCRs]) and MLST/MLSA applied to extensive collections, with representative strains from different countries and continents, or to collections from epidemiological surveys in more restricted regions (Giovanardi et al., 2016; Kałużna et al., 2014; Loreti et al., 2001; Marcelletti et al., 2010; Scortichini et al., 2001). However, no consensus clustering of Xaj in a determined number of lineages emerged from these studies. No clear relation between these genetic lineages and geographic origins could be evidenced (Fernandes et al., 2018; Kałużna et al., 2014; Marcelletti et al., 2010). Strains sharing the same genetic profile could be retrieved on different continents decades apart (Loreti et al., 2001; Marcelletti et al., 2010), while Xaj strains isolated from the same restricted geographical origin (Italian Romagna region for instance) over a short period show the same level of genetic diversity as a worldwide collection (Fernandes et al., 2018; Giovanardi et al., 2016). It was even mentioned that a single leaf can host diverse Xaj strains (Fernandes et al., 2018; Scortichini et al., 2001). Extensive exchanges of propagation material over the world might contribute to worldwide dispersal of different sequence types with a high fitness.

Recombination events within the Xaj pathovar revealed by MLST analyses may also contribute to its diversification (Fischer-Le Saux et al., 2015; Kałużna et al., 2014; Marcelletti et al., 2010). The observed predominance of recombination over mutation as the driving force within Xaj can explain its greater diversity compared to Xap, whose main evolutionary force is

mutation (Fischer-Le Saux et al., 2015). Indeed, a single event of homologous recombination can bring numerous polymorphic sites, compared to a mutation event leading to a single polymorphic nucleotide. Coexistence of diverse Xaj isolates in the same plant, as seen by Scortichini et al. (2001) or Fernandes et al. (2018), could favour genetic exchanges between them. Despite its high genetic diversity, Xaj was found to be clonal, with most sequence types clustered in a single clonal complex (Fischer-Le Saux et al., 2015; Marcelletti et al., 2010).

In the early 2000s the VOC symptoms appeared in French walnut orchards. A specific f-AFLP lineage within Xaj was shown to be responsible for this new disease (Hajri et al., 2010). MLST and multilocus variable-number tandem repeat analysis (MLVA) later confirmed that the strains responsible for VOC symptoms were highly genetically related within Xaj diversity (Cesbron et al., 2014; Fischer-Le Saux et al., 2015). In contrast, strains isolated from BAN symptoms did not cluster in a single phylogenetic lineage; they were under diversifying selection (Marcelletti et al., 2010).

Strains of Xac show an intermediate level of genetic diversity when compared to Xaj and Xap. An extensive collection with representative strains from diverse European countries and from Oregon, USA, was studied using rep-PCRs and profiles of whole proteins: such studies evidenced five and three groups, respectively, with no relation to geographic origins (Scortichini et al., 2002). Slightly distinct profiles were also produced by rep-PCRs on strains isolated in Poland (Puławska et al., 2010). These strains could not be differentiated with *gyrB* and *rpoD* partial sequencing (Fischer-Le Saux et al., 2015; Puławska et al., 2010). Indeed, MLSA reveals less polymorphism than rep-PCRs, with only two major groups identified so far (Fischer-Le Saux et al., 2015; Webber et al., 2020). Xac strains cluster in a clonal complex within which mutation was found to be four times more frequent than recombination (Fischer-Le Saux et al., 2015). An MLVA scheme based on 16 VNTRs was proposed as a promising method for epidemiological surveys (Cesbron et al., 2014). An extensive survey, including ancient and new strains from worldwide origins, is needed to get further insights about Xac routes of invasion (Webber et al., 2020).

There is an ongoing debate as to whether the Xac pathotype strain is representative of the pathovar based on genotypic, phenotypic, and pathogenic profiles. This strain was found to be divergent based on rep-PCRs and slightly differed from other Xac strains as presented by biochemical and pathogenicity tests (Puławska et al., 2010; Scortichini et al., 2002;). However, an atypical genetic profile was not retrieved, either by MLST or by MLVA (Cesbron et al., 2014; Fischer-Le Saux et al., 2015). The pathotype strain isolated in Oregon (USA) from *Corylus maxima* in 1939 shares the same MLST sequence type and highly similar MLVA pattern as French isolates from the first epidemics of hazelnut blight reported in France. Interestingly, one of the first orchards where symptoms were detected was planted with imported material from Oregon (Luisetti et al., 1975). This specific culture of the pathotype strain used by Scortichini et al. (2002) might be impaired in its growth ability, which could explain both faint biochemical reactions and low virulence.

9 | INSIGHTS ON VIRULENCE FACTORS FROM COMPARATIVE GENOMICS

Over 100 whole-genome sequences of *X. arboricola* are currently available at the NCBI Genome Resources genome databases (https://www.ncbi.nlm.nih.gov/assembly/organism/56448/all/, accessed 25 September 2020), providing a valuable pangenome patrimony for comparative genomics studies capable of scrutinizing putative pathovar-specific pathoadaptations.

The first genome sequences for Xac and Xaj were obtained from strain NCCB100457 (Ibarra Caballero et al., 2013) isolated from the ornamental *Corylus colurna* in Colorado (USA) in 2010 (Ibarra et al., 2012) and from strain NCPPB1447 (Noh & Cha, 2012) isolated from *J. regia* in Romania in 1963. Up to now, a total of three Xac and 11 Xaj genome assemblies have been deposited in the NCBI Genome Resources (https://www.ncbi.nlm.nih.gov/assembly/organism/487821/all/ and https://www.ncbi.nlm.nih.gov/assembly/organism/195709/all/, respectively, accessed 28 August 2020). These include the genome sequences of the type strains for both pathovars, namely Xac CFBP 1159^{PT} and Xaj CFBP 2528^{T}, with their main features displayed in Table 1. It is important to notice that for Xaj, additional genomes are also available, but reported as *X. arboricola* (e.g., Higuera et al., 2015). Furthermore, among all these genomes, only Xaj CPBF 427, isolated in Portugal in 2016, resulted from a hybrid assembly of Illumina short-reads and Oxford Nanopore Technology long-reads, which allowed the circular genome sequence to be completed in a single scaffold (Teixeira et al., 2020). The remaining genome sequences are morcellated in variable numbers of contigs or scaffolds. However, several complete genome sequences obtained with the hybrid assembly of short- and long-read technologies are expected to be released soon for these two pathovars (authors' unpublished data).

TABLE 1 Summary of *Xanthomonas arboricola* pv. *corylina* CFBP 1159^{PT} and pv. *juglandis* CFBP 2528^{T} genome sequences.

	***X. arboricola* strain**	
	CFBP 1159^{PT}	**CFBP 2528^{T}**
Pathovar name	*corylina*	*juglandis*
Host of origin	*Corylus maxima*	*Juglans regia*
Place of collection, year	Oregon, USA, 1939	New Zealand, 1956
Assembly accession	GCA002939845.1	GCA_001013475.1
Genome size (bp)	5,105,973	5,084,477
G + C content (%)	65.50	65.60
No. of scaffolds	124	8
No. of CDS	4,104	4,132

CDS, coding DNA sequence.

The genomic data available for these two *X. arboricola* pathovars have been mainly used for the precise classification of these bacterial pathogens, for comparative genomics studies addressing primarily virulence and pathogenicity-related genes, and to open the way for genomic epidemiology (Cesbron et al., 2015; Ibarra Caballero et al., 2013; Martins et al., 2020; Merda et al., 2017). Although no proper comparative genomics analysis has been conducted with the three Xac genome sequences available, this has been performed in the case of Xaj to link their genomic landscape with phenotypic traits mainly related to pathoadaptations (Cesbron et al., 2015; Merda et al., 2017), or more recently to propose *X. euroxanthea* as a new walnut-infective species (Martins et al., 2020). Remarkably, the screening of putative virulence/pathogenicity genes in two nonpathogenic *X. arboricola* strains (CFBP 7634 and CFBP 7651) and in a nonpathogenic strain of *X. euroxanthea* (CPBF 367), all isolated from symptomless walnut buds, showed that these strains were deficient for the type III secretion system (T3SS) (Cesbron et al., 2015; Martins et al., 2020).

Despite these seminal contributions, the low number of genomes available at the moment still does not enable a population-based characterization of genetic determinants of virulence and pathogenicity, with particular emphasis on the highly conserved T3SS of the Hrp2 family and T3E genes that have been previously characterized by PCR amplification in numerous *Xanthomonas* pathovars, including Xac and Xaj (Essakhi et al., 2015; Hajri et al., 2012; Merda et al., 2016).

The distribution based on PCR analysis of 53 T3E and 11 T3SS genes in 10 Xac and 20 Xaj strains showed that all tested strains harboured the Hrp2-T3SS genes, but that the T3E repertoires varied between these two *X. arboricola* pathovars (Hajri et al., 2012). Using this approach, a total of 21 T3E genes could be detected in Xac, the only exception being the pathotype strain CFBP 1159^{PT} isolated from the ornamental species *C. maxima* (Scortichini et al., 2002) and lacking *xopH* (Hajri et al., 2012). A similar observation was made using the first Xac available genome sequence, as strain NCCB100457 was also isolated from an ornamental *Corylus* species (Ibarra Caballero et al., 2013; Ibarra et al., 2012). This first Xac genome sequence did question the presence of the transcription activator-like (TAL) effector AvrBs3-encoding gene, as evidenced by PCR analysis (Hajri et al., 2012). Although short-read sequencing technology is not adapted for sequencing this repeated region, the authors performed a PCR with *avrBs3*-specific primers to confirm the absence of this gene in NBBC100457 (Ibarra Caballero et al., 2013). However, the presence of homologous sequences was evidenced in the other two available genomes for Xac, namely CBFP 1159^{PT} and CFBP 2565 (Merda et al., 2016, 2017).

In Xaj, PCR analyses distinguished two different repertoires corresponding to the two lineages described in this pathovar (Essakhi et al., 2015; Hajri et al., 2010, 2012). Indeed, 16 and 17 T3E genes were detected in Xaj and in VOC strains, respectively. The differences consisted of VOC strains harbouring *xopAI* and *xopB* whereas *xopAH* was only reported in non-VOC strains (Essakhi et al., 2015; Hajri et al., 2010, 2012). This result was later confirmed by comparative genomics analysis of these two lineages (Cesbron et al., 2015) but also extended these T3E repertoires as seven additional T3E genes, namely *xopAL1*, *xopG*, *xopAA*, *xopAB*, *awr4*, *sfrJ*, and *xopAR*, could be predicted (Cesbron et al., 2015).

Hitherto, differences in genomic content in Xaj, Xac, and Xap have been shown, with distinct profiles of virulence determinants such as secretion systems, chemotaxis, adhesion, and cell-wall degrading enzymes (Garita-Cambronero et al., 2018). Among the most striking differences is the high number of T3E or secreted (T3SP) proteins in the pathovars, compared to their absence or limited number in Group B of strains described above (Garita-Cambronero et al., 2017; Hajri et al., 2012; Merda et al., 2016, 2017). Notably, the *Hrp2* cluster is lacking in some Group B strains (Essakhi et al., 2015; Garita-Cambronero et al., 2016c, 2017; Ignatov et al., 2015; Merda et al., 2016, 2017), most probably as a consequence of its loss (Merda et al., 2017).

Moreover, in contrast to other Xaj strains, the presence of an integrative and conjugative element (ICE) including a *copABCDFGK* gene cluster, conferring copper resistance in VOC strains, was evidenced (Cesbron et al., 2015). The acquisition of copper resistance, thanks to this mobile element, may represent a founder event that has contributed to the emergence of this aggressive clone. Moreover, it is worth mentioning here the genes existing in Xac and Xaj strains, that is, copper tolerance genes located on plasmids (Behlau et al., 2011; Richard et al., 2017). With reference to previous studies on *Xanthomonas* plasmids, Stall et al. (1986) and Bender et al. (1990) suggested that such plasmids containing *cop* genes are ubiquitous and readily transferred. Later, Gardan et al. (1993) associated the copper resistance observed in a large collection of French isolates with the presence of a conjugative plasmid. A recent study on a large collection of Xaj isolates showed that most of them were copper tolerant or copper resistant and molecular characterization of those isolates revealed the presence of the *copLAB* gene cluster, typically present in xanthomonads and conferring on them copper resistance (Giovanardi et al., 2016).

10 | EPIDEMIOLOGY

Propagation material latently harbouring Xaj is the main pathway of pathogen introduction into new areas. Due to the wide geographical distribution of Xaj, cuttings and scions taken from mother trees are frequently already latently contaminated by the pathogen. Traditionally, grafted rootstocks are kept for rooting in supervised and controlled nursery fields: there, they may become infected through bacterial dissemination from nearby infected plants or groves via wind-driven rain or pollen. A study conducted in Italy highlighted that micropropagated plants might be infected as well: indeed, walnut plants raised in screen houses and used to obtain meristematic tissue from buds revealed symptomless infections (authors' unpublished data). Polito et al. (2005) showed a high level of pollen parentage originating from pollen sources outside the orchard using simple-sequence repeat (SSR)-based paternity analyses: this

confirms the importance of pollen in the short and medium distance dissemination of Xaj. Viable pathogenic bacteria were repeatedly isolated from pollen (Giovanardi et al., 2016) and infected pollen also represents a possible pathway of introduction into healthy walnut groves, in case of mechanical pollination. Pinillos and Cuevas (2008) highlighted the importance of artificial tree crop pollination to increase production, therefore to anticipate walnut production and/or increase fruit set, artificial pollination was proposed as a possible strategy (Atefi & Khoshnevis, 1990). More recently, with the use of specific drones as pollen carriers and pollinating devices, artificial pollination of walnut has become a practicable method in large commercial groves (Cozzolino et al., 2017).

From season to season, Xaj survives in buds, small or large cankers on trunks, branches and twigs, and diseased fruits that remain in the walnut groves. Recovery of Xaj from fruit mummies left in walnut groves is possible up to 8 months from infection (Miller & Boller, 1946). The role of herbaceous plants and grasses in walnut orchards as a possible reservoir of Xaj has been investigated: Esterio and Latorre (1982) consistently isolated Xaj from several spontaneous species in all four seasons and proved that those isolates were pathogenic to walnut. Nevertheless, the epidemiological role of such a possible source of primary inoculum remains obscure.

While buds are the major overwintering sites for Xaj populations (Mulrean & Schroth, 1982), there is a high degree of spatial segregation of the walnut blight pathogen within buds (Lindow et al., 2014). The colonization and overwintering of Xaj in walnut buds, later developing in female and male flowers, can occur both epiphytically and internally (between the scales and the apex of the bud): in a study on the ecology of Xaj on walnuts in California, 90% of colonized buds and 45% of colonized catkins had both epiphytic and internal populations of the pathogen (Mulrean & Schroth, 1982).

Xaj activity in walnut groves, and consequently bacterial blight incidence and severity, strongly depends on environmental conditions, climatic events, and the amount of primary inoculum available in orchards. A pattern of colonization of embryonic and developing leaves by Xaj suggests that they become inoculated shortly after emergence from the bud and that moisture was the mechanism for moving the inoculum (Lindow et al., 2014). Although xanthomonads generally prefer high temperature and humidity, Xaj increases its populations in buds and cankers in early spring, colonizing the developing catkins, sprouts, and female flowers (Lindow et al., 2014; Mulrean & Schroth, 1982). Temperature in the range 4–30 °C, high humidity, and leaf wetness are necessary for pathogen multiplication and penetration into the host tissue through lenticels, stomata, leaf scars, wounds, and stigmas: it has been calculated that as little as 5 min of wetness is sufficient to allow Xaj penetration into fruitlets (Miller & Bollen, 1946). The infection process can occur as soon as buds break and growth begins, therefore in early spring; different to other xanthomonads, Xaj seems to be not much affected by relatively low temperatures. Penetration of Xaj occurs primarily through stomata (Garcin et al., 2001). Once penetrated, Xaj rapidly colonizes the walnut tissue surrounding the entry point, but without becoming systemic. Necrotic lesions readily appear on fruits and leaves then, later in summer, on twigs as small cankers.

From lesions, secondary inoculum may evade and disseminate in the grove during rains, therefore wind-driven rain splashes (or water splash of sprinkler irrigation) are important in bacterial dispersal (Adaskaveg et al., 2000; Stall et al., 1993). Xaj has a long epiphytic phase and may easily survive on any plant surface and on pruning tools, tractors, and other machinery used in the orchard. Nonetheless, pruning and drip irrigation do not appear to be efficient means of pathogen dissemination in orchards. Conversely, mechanical harvesters, including shaking, sweeping, and picking machines, produce thick dust during harvesting: in affected walnut groves, Xaj is abundant in such dust clouds and can easily disseminate far away from diseased trees (Giovanardi et al., 2016). Although Xaj is easily detectable from spring to autumn, secondary inoculum can cause symptoms on fruits and leaves until early summer; the small cankers that may develop in late summer are possibly the result of lenticel colonization during the previous months.

In the first description of the disease (Barss,), although without solid evidence from that time, it was assumed that infected planting material was the main pathway of the pathogen spread from the place of origin over long distances over the world. This assumption was later confirmed by monitoring hazelnut planting material in international trade. Large-scale dissemination could occur when apparently healthy, but latently infected propagation materials are introduced (Alvarez, 2004; Janse & Wenneker, 2002).

The restricted presence of Xac within the EPPO region suggests rather limited natural spread of the bacterium, even though the inoculum is present in spontaneous *Corylus* populations (Scortichini, 2002). Favourable temperature and humidity facilitate epiphytic survival of the bacterium, which could be further spread short distances by wind-driven rain and splashing. Pruvost and Gardan (1988) confirmed that Xac can maintain high populations (10^6–10^7 cfu/ml) on the leaf surface. The bacterium could survive on the fallen leaves for several months, but not in the soil (Gardan & Deveaux, 1987).

When established in one area, Xac population mainly overwinters in the infected buds and cankers on hazelnut twigs and branches. The buds are colonized by the epiphytic population before closing, especially during heavy rainfalls in autumn. Leaf scars and other wounds may serve as the entry for the rain-driven inoculum as well. The pathogen remains latent during the winter. Early next spring bacteria continue to multiply and colonize the plant tissue, causing bud and twig necrosis. The buds are susceptible from their initial development until bud-break in spring and may be completely killed or only partially affected. Leaf infections occur when the tissue is young, water-congested, and the stomata open (Miller et al., 1949).

Shoots emerging from the buds generally become infected from infected bud scales. The intensity of secondary infections depends on the host plant age, weather conditions, and management practices. Plants up to 5 years old are the most susceptible. Humid weather and moderate temperatures (around 20 °C) favour infection during the season. However, the incidence is reduced in dry and

hot weather. Bacterial blight incidence is highly correlated to stress caused by spring frost, drought, and winter pruning (Moore et al., 1974). Pruning and wounding may contribute to the infection spread (Miller et al., 1949). So far there have been no indications that insects and mites may have an important role in HBB spread. Lamichhane et al. (2013) demonstrated a positive correlation of climatic (high rainfall and spring frost) and soil (high nitrogen and low magnesium levels) factors in the occurrence and spread of HBB.

11 | DISEASE CONTROL/INTEGRATED MANAGEMENT

Chemical control of WBB is essentially based on repeated use of copper compounds together with dithiocarbamates. Copper mixed with mancozeb proved to be the most effective bactericidal mixture against Xaj (Adaskaveg, 2009, 2015). The addition of dithiocarbamates to copper compounds is suggested to control Xaj populations that developed copper resistance. The main issue concerning the use of copper-based products is the ability of Xaj and Xac to detoxify copper, a property linked to the *cop* gene cluster (see above) that strongly limits its efficacy. Where allowed, antibiotics are used as well. Kasugamycin, an aminoglycoside from *Streptomyces kasugaensis*, is the most effective, with an activity comparable to copper (Adaskaveg et al., 2010). Kasugamycin is used to minimize resistance development against copper-based bactericides. Kasugamycin may also be added with dithiocarbamates, although its antibacterial efficacy does not improve significantly (Adaskaveg, 2015). Because Xaj activity in walnut groves strongly depends on environmental conditions and the amount of inoculum, a disease forecast model was developed. Therefore, the application of protective sprays is based on a spray forecast software, XanthoCast, a walnut blight model (Adaskaveg et al., 2000). XanthoCast calculates a 7-day cumulative index based on temperature and leaf wetness: in conducive conditions, during prolonged wet springs and rains, sprays should be done at 7- to 10-day intervals to obtain adequate disease control.

Early attempts to specifically control xanthomonads by using bacteriophages were done in the early 1970s, but they did not raise particular interest (Rao, 1970). More recent research confirmed the presence of several bacteriophages that are lytic to Xaj and Xap may be used singularly or in cocktails (Civerolo & Keil, 1969; Gašić et al., 2019; Retamales et al., 2016; Saccardi et al., 1993).

Several studies showed the antibacterial activity on nanoparticles, in particular silver nanoparticles, with possible positive implications in agriculture (Singh et al., 2018). Nanoparticles are promising to overcome copper tolerance in xanthomonads as well, as highlighted by Carvalho et al. (2019). So far, no attempts to control Xaj or Xac in the field using nanoparticles has been done, but a report by Ghadamgahi et al. (2014) indicated that both silver nanoparticles and zinc nanoparticles were able to inhibit the growth in vitro of Xaj. The use of nanotechnologies in plant protection is an emerging field that needs further study to evaluate their efficiency, but also to investigate the fate of nanoparticles and their safety for public health and the environment.

In the past, preliminary studies were done to understand the susceptibility of *Juglans* species to Xaj: *J. mandshurica* and *J. regia* were the most susceptible, whereas *J. nigra* was found to be resistant (Belisario et al., 1999). To date, no Xaj-resistant genotypes of *J. regia* are widely available, although differences among walnut cultivars in their susceptibility to the bacterium are reported (Frutos & López, 2012). In Europe and Asia, local selections from wild populations indicated that a certain degree of resistance might be found, but this has not been associated with particular markers (Frutos & López, 2012; Jiang et al., 2020). The accumulation of specific phenolic compounds and the activity of peroxidases, phenylalanine ammonia-lyase, and polyphenol oxidases were associated with a relative tolerance of walnut to Xaj infections, with superoxide dismutase and catalase activity as defence regulators (Jiang et al., 2020; Solar et al., 2012). Martínez-García et al. (2016) described a high-quality draft genome sequence of *J. regia* 'Chandler': they identified a second polyphenoloxidase gene (*JrPPO2*) homolog to *JrPPO1* and, in addition, about 130 genes of the GGT superfamily, where genes *JrGGT1* and *JrGGT2* appear to have the most significant role in the phenolics pathway. Therefore, investigations of the phenolics biosynthesis pathways in *J. regia* may contribute to breeding tolerant walnut cultivars and phenolic compounds may be regarded as potential markers for walnut blight resistance.

Hazelnut protection from HBB is mainly based on the prevention and integration of various treatments and practices. The use of disease-free planting material is a primary condition for HBB prevention and control. Nursery material should be produced in pathogen-free areas. In addition, nurseries should be distant from areas where hazelnut commercial orchards are grown (Lamichhane & Varvaro, 2014). Pisetta et al. (2016) significantly reduced the population of Xac in hazelnut suckers by treatment of the planting material with hot water. The authors concluded that after exposure to 42 °C for 45 min, the hazelnut propagative material could be safe enough for further trade and planting. However, due to the latent nature of the pathogen, the plants for planting should be tested prior to exportation to other countries, thus complying with existing phytosanitary legislation. Infection of young plants is considered a high risk due to their high susceptibility and lack of efficient postinfection treatment.

Because HBB can be sometimes confused with abiotic stress, such as sunscald and winter damage, the bacterial aetiology of the disease should be confirmed by laboratory testing of symptomless propagative material or new reservoirs. The most suitable time to collect and test samples for the Xac is during spring.

Genetic resistance is apparently not a measure of choice for Xac control because most of the hazelnut cultivars are susceptible (https://pnwhandbooks.org/node/3758). However, proper plant management and cultivation practices could contribute to lower susceptibility. Keeping nitrogen content in the soil at the optimal rate seems to be critical. Excessive nitrogen may stimulate intensive growth and prolonged formation of susceptible young tissue

(Lamichhane et al., 2013; Miller et al., 1949). Additionally, nitrogen excess can extend the season, postponing the leaf fall and delaying the overwintering phase. All this creates multiple chances for the bacterium to penetrate and invade the tissue.

Pruning out of the infected twigs and branches could reduce the sources of inoculum but cannot eradicate the disease. Due to the pathogen presence in symptomless tissue, it is advised to make cuts 30–50 cm below apparently affected tissue toward the healthy parts. Between cuts, the pruning tools should be soaked in disinfectant, such as 70% ethanol or sodium hypochlorite solution (may be corrosive). It is recommended that the two pruners method is used: have one soaking in the disinfectant while using the other, then switch pruners. To ensure effectiveness the pruners should be cleaned while exposed to the disinfectant.

Moisture stress should be controlled by irrigation, especially during the first three seasons after planting. However, to avoid continuous leaf moisture and wetting irrigation should be localized, such as drip irrigation, instead of overhead irrigation. Installation of the shading or anti-hail nets could prevent canopy sunburns. Field exposure, planting density, and row direction should facilitate good aeration and fast evaporation of the leaf surface moisture after rainfall or humid weather.

Chemical treatments are almost exclusively based on the application of copper compounds. However, the effectiveness of these treatments is rather limited because copper-based bactericides work on contact and thus do not reach bacterial populations inside dormant buds and cankers, typical of Xac. Therefore, the strategy should be to act preventively by targeting the Xac epiphytic population. The first treatment should be scheduled in late August or early September, before the first heavy rains. Sprays should be repeated when 75% of the leaves have dropped (Miller et al., 1949). The choice of the copper product should be based on the antiresistance strategy principles and spraying frequency should minimize the chances for pathogen resistance development and increase in copper soil residues. Prokić et al. (2018) reported an increased tolerance of some Serbian Xac strains to copper sulphate.

Despite several attempts to control bacterial diseases of fruit and nut trees by innovative approaches, such as the use of antagonist bacteria, glucohumates, and bacteriophages (Biondi et al., 2009; Gašić et al., 2019; Obradović et al., 2020), no such studies are available for Xac.

12 | FUTURE PERSPECTIVES

Recently, published advances in our understanding of Xaj and Xac have improved knowledge about the pathogens, but have also revealed knowledge gaps and some questions remain unanswered.

Phylogenetic studies pointed out the need to further decipher the evolutionary history of nut and stone fruit tree pathogens, and the potential role of host domestication and host jumps in the patho-adaptative process. In addition, the role of a new species, *X. euroxanthea*, in this story should be considered. Insights from comparative genomics indicate the need to conduct such analysis for Xac, but should also pay attention to *X. arboricola*-related strains, that is, non-pathogenic strains, and determine the role of strains from multiple lineages of bacteria in the same host plant, for example, *X. euroxanthea*. It is worth mentioning that hitherto comparative transcriptome analysis is missing for Xac and Xaj.

As it happens two other phytopathogenic bacteria having a long epiphytic phase, Xaj and Xac, are strongly influenced by agroclimatic conditions. Therefore, it appears fundamental in designing future disease management strategies for walnut blight that specific disease forecast models are implemented in those growing regions where they are not used. In case of HBB, no specific disease forecast model has ever been developed: in such case, farmers lack a fundamental tool for disease management. This is a gap that should be urgently filled.

Phytosanitary controls of the propagation material to detect pathogens in their possible latent phase, as well as before the planting of new orchards, are key to ensuring that growers may initiate the cropping of hazel and walnut by using safe plant material.

Copper resistance is a typical feature of most Xaj isolates while in the case of Xac such a feature appears to be a concrete risk. As described, the high recombination rate of Xaj and similar behaviour shown by Xac might be responsible for an efficient gene flow among microbial communities in the orchards, including genetic elements responsible for copper resistance. This fact questions the use of copper sprays: indeed, copper treatments are currently reported to be not sufficient for optimal disease control and, additionally, increase the risk of environmental pollution and challenge food safety. As a possible sustainable solution in the near future, attempts should be focused on the search for beneficial microbes to be used in implementing biological control methods, asis currently done with bacteriophages.

Finally, we are confident that plant geneticists will consider the need to exploit possible resistance features reported in tolerant *Juglans* or *Corylus* species and direct plant breeding towards commercial genotypes or cultivars that can show a high degree of tolerance towards both bacteria. Therefore, a holistic approach is recommended and required to identify the best solutions to overcome the challenges posed by both phytopathogenic bacteria.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed in this study.

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