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Phylodynamic Insights into Global Emergence and Diversification of the Tomato Pathogen *Xanthomonas hortorum* pv. *gardneri*

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Data availability: The read files and genome assemblies for all strains sequenced for this study are available under NCBI bioproject PRJNA940592. Further information for previously sequenced strains is available in Supplementary Table S1.

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The emergence of plant pathogens is often associated with waves of unique evolutionary and epidemiological events. *Xanthomonas hortorum* pv. *gardneri* is one of the major pathogens causing bacterial spot disease of tomatoes. After its first report in the 1950s, there were no formal reports on this pathogen until the 1990s, despite active global research on the pathogens that cause tomato and pepper bacterial spot disease. Given the recently documented global distribution of *X. hortorum* pv. *gardneri*, our objective was to examine genomic diversification associated with its emergence. We sequenced the genomes of *X. hortorum* pv. *gardneri* strains collected in eight countries to examine global population structure and pathways of emergence using phylodynamic analysis. We found that strains isolated post-1990 group by region of collection and show minimal impact of recombination on genetic variation. A period of rapid geographic expansion in *X. hortorum* pv. *gardneri* is associated with acquisition of a large plasmid conferring copper tolerance by horizontal transfer and coincides with the burgeoning hybrid tomato seed industry through the 1980s. The ancestry of *X. hortorum* pv. *gardneri* is consistent with introduction to hybrid tomato seed production and dissemination during the rapid increase in trade of hybrid seeds.

Keywords: bacterial plant pathogen, genome evolution, phylodynamics

Global estimates of crop yield losses due to plant diseases are in excess of 10% annually (Savary et al. 2019; Strange and Scott 2005). In the monoculture landscape of modern agriculture, plant pathogens have evolved a wide array of adaptation mechanisms to the diverse natural and anthropogenic forces imposed in crop production systems (Dutta et al. 2021; Hessenauer et al. 2021; Singh et al. 2023). Plant disease outbreaks, whether due to emergence of a new pathogen or new strains of a known pathogen, continue to pose major risks to food security globally (McCann 2020; McDonald and Stukenbrock 2016). Understanding the evolutionary factors that underpin pathogen emergence is therefore central to sustainable disease mitigation and management. Implementing crop protection through quarantine and surveillance, for example, requires knowledge of the origin and dissemination of target plant pathogens (Carvajal-Yepes et al. 2019). However, attempts to understand the origin and long-distance movement of plant pathogens are constrained by lack of information on source populations, strength of population bottlenecks, and environmental conditions that aid emergence and local adaptation (Gladieux et al. 2015; McCann et al. 2013). Nevertheless, increasing access to genetic and genomic data from diverse geographic locations and precise dates of isolation have allowed at least partial reconstruction of the origin and dissemination of some historical and recently emerged plant pathogens or strains (Gladieux et al. 2015; Goss et al. 2014; McCann et al. 2013; Patané et al. 2019).

Tomato (*Solanum lycopersicum* L.) is cultivated globally (FAO 2022), with about 5.1 million ha dedicated to tomato cultivation worldwide, yielding over 187 million metric tons (FAO 2022). Tomato originated in the Andean region, with initial domestication occurring in Mexico (Bai and Lindhout 2007; Blanca et al. 2022; Denham 2014; Peralta and Spooner 2007; Razifard et al. 2020; Robertson and Labate 2007). Intensive domestication outside of the Americas began in Europe in the late 17th century and became the epicenter of intensive domestication and cultivation in the late 1800s when Europeans began to widely accept tomato as an edible crop (Denham 2014; Peralta and Spooner 2007). Disease has been a constant constraint in tomato production (Gleason and Edmunds 2005; Jones et al. 2016; Linn and Luckmann 1965). The distribution of some tomato pathogens can only be explained by global movement on seed (Ansari et al. 2019; Davino et al. 2020; Jones 2021; Potnis et al. 2015).

Bacterial spot of tomato is a disease with global distribution (Goode and Sasser 1980; Osdaghi et al. 2021). *Xanthomonas* spp. can be seedborne, and bacterial spot of tomato is primarily spread through movement of seeds and seedlings (Abrahamian et al. 2019, 2021; Dutta et al. 2014, 2016; Giovanardi et al. 2018; Ignjatov et al. 2017). Here, we focus on the intercontinental diversification of one of four pathogens responsible for this disease, *X. hortorum* pv. *gardneri* (Timilsina et al. 2015). *X. hortorum* pv. *gardneri* (synonym *X. gardneri* and *X. cynarae* pv. *gardneri*) was originally isolated in the early 1950s in former Yugoslavia (Šutic 1957) and was not reported elsewhere for more than three decades, despite active global research on bacterial spot disease (Bouzar et al. 1994; Jones et al. 2000). In recent years, *X. hortorum* pv. *gardneri* has emerged as an aggressive pathogen in tomato-production regions on four continents (Cuppels et al. 2006; Hamza et al. 2010; Kebede et al. 2014; Ma et al. 2011; Quezado-Duval et al. 2004; Rashid et al. 2016). The change in causal agent forced new resistance breeding strategies to be developed for targeting *X. hortorum* pv. *gardneri* and production systems to continue their reliance on copper-based bactericides for disease control (Potnis 2021). The species *X. hortorum* contains multiple pathovars that cause bacterial spot and/or blight on more than 15 plant families (Dia et al. 2022). These other pathovars include *X. hortorum* pv. *cynarae*, *X. hortorum* pv. *vitians*, *X. hortorum* pv. *pelargonii*, *X. hortorum* pv. *carotae*, *X. hortorum* pv. *hederae*, and *X. hortorum* pv. *taraxaci* (Morinière et al. 2020). All pathovars of *X. hortorum* share an average nucleotide identity above 95 but differ in host range and specificity (Dia et al. 2022; Morinière et al. 2020; Timilsina et al. 2019a).

Multilocus sequence analysis of 28 *X. hortorum* pv. *gardneri* strains from five continents failed to uncover genetic variation (Timilsina et al. 2015), and whole-genome sequencing confirmed that there was little variation in the core genome among *X. hortorum* pv. *gardneri* strains from the United States (Schwartz et al. 2015). The objectives of this study were to use whole genomes of a collection of *X. hortorum* pv. *gardneri* strains from countries on three continents to investigate genomic diversity and evolution of the pathogen across tomato-production regions and estimate the timing of global emergence of *X. hortorum* pv. *gardneri*.

Results

Core genome and recombination

X. hortorum pv. *gardneri* genome size ranged from 5.16 to 5.44 Mbp and carried between 4,324 and 4,617 coding sequences (Supplementary Table S3). High degrees of similarity in the pairwise whole-genome comparisons verified that all strains were *X. hortorum* pv. *gardneri*; in all comparisons, the average nucleotide identity (ANI) values were higher than 99.9% at over 95% genome coverage (Supplementary Fig. S1).

The pangenome analysis of 62 *X. hortorum* pv. *gardneri* strains determined that 4,038 out of 5,103 annotated genes were core genes (Supplementary Fig. S2). A core genome phylogeny using concatenation of core gene alignments (approximately 4.12 Mbp) demonstrated that the strain ATCC 19865, which was isolated in the early 1950s and deposited in the NCPPB culture collection in 1953, is distinct from the more recently isolated strains (Supplementary Fig. S3). Core genome phylogeny of representative *X. hortorum* pv. *gardneri* strains, together with *X. hortorum* genomes from NCBI, showed that these strains are monophyletic and distinct from other *X. hortorum* pathovars (Supplementary Fig. S4).

Core genes were used to infer the prevalence of homologous recombination among *X. hortorum* pv. *gardneri* strains. Overall, the *X. hortorum* pv. *gardneri* strains showed an estimated rate of recombination to mutation (R/θ) of 0.132 (95% highest posterior density [HPD]: 0.104, 0.160) and probability of recombination per site (ν) of 0.174 (0.160, 0.189) across all branches. Recombination and mutation had a near-equal role in *X. hortorum* pv. *gardneri* nucleotide variation ($R/\theta \times \delta \times \nu = 1.116$ [0.844, 1.446]). Notably, there was little recombination in the internal branches of the phylogeny; 78 of 90 inferred recombination affected individual strains only, with some strains showing no recombination (Supplementary Fig. S5).

Accessory genome and plasmid typing

Pangenome analysis identified a total of 1,065 accessory genes; however, individual strains carried only 315 to 607 accessory genes. Clustering of *X. hortorum* pv. *gardneri* strains based on the presence or absence of accessory genes revealed three major clusters (Fig. 1; Supplementary Fig. S6). Notably, there was a clear distinction in the number of accessory genes between the clusters, with cluster A carrying fewer than 400 and cluster B and C carrying more than 500 accessory genes. To determine if the observed difference in accessory gene content is associated with the presence of plasmids, we analyzed plasmid content in the whole-genome sequences. At least two different sizes of plasmids were identified among the strains from plasmid typing; the smaller plasmid (S-plasmid) was approximately 45 kbp in size, and the larger plasmid (L-plasmid) was approximately 211 Kbp (Supplementary Table S4). The S-plasmid was present in all strains except one from the United States, whereas the L-plasmid was present in all cluster B and C strains but was missing in cluster A strains (Supplementary Table S4; Supplementary Fig. S6). The presence/absence of plasmids was also verified by electrophoresis of purified plasmids to obtain the plasmid profile of several strains (Supplementary Fig. S7). An approximately 26-Kbp plasmid similar to *X. hortorum* pv. *gardneri* strain CFBP8129 plasmid p26 (Dia et al. 2020) may also be present in some strains (data not shown).

Both S- and L-plasmids are closely related to previously sequenced plasmids in other xanthomonads, including those pathogenic on tomato. The L-plasmid from *X. hortorum* pv. *gardneri* strains in cluster B (hereby, L1-plasmid) showed 99.9% identity to plasmid pLH276.1 from *X. citri* subsp. *citri* (Richard et al. 2017b) and was previously reported in copper-resistant *X. hortorum* pv. *gardneri* and *X. euvesicatoria* pv. *perforans* (Richard et al. 2017a). The L-plasmid in cluster C strains (hereby, L2-plasmid) was more similar (>99% identity) to pLMG911.1 from *X. vesicatoria* (Richard et al. 2017a; Supplementary Table S4; Supplementary Fig. S6). There was >99.85% sequence identity among L1- or L2-plasmids in the same cluster; the sequence identity was <98.25% for all pairwise cross-cluster comparisons between an L1- and an L2-plasmid (coverage >84% for all; Supplementary Fig. S8). The S-plasmid had 99.9% identity to plasmid pLH3.3 (Richard et al. 2017a). The S-plasmid is predicted to be conjugative, and both L-plasmids are predicted to be mobilizable (Supplementary Table S5). These plasmids carry genes that impact bacterial fitness and virulence, such as the transcriptional activator-like (TAL) effector *avrHah1* (Schornack et al. 2008; Schwartz et al. 2017) in the S-plasmid and copper and arsenic resistance gene clusters *copLAB*, *cusAB-smmD/czcABCD*, and *arsBHCR* in the L-plasmid (Richard et al. 2017a).

Type III effectors

Type III effector content of strains in this study, based on tBLASTn analysis, is presented in Supplementary Table S6. All previously reported effectors of *X. hortorum* pv. *gardneri* (Schwartz et al. 2015) were present across our collection of strains. Additionally, XopAU and XopAZ, two recently characterized effectors (Kara 2016; Teper et al. 2018), were also present in all strains in this study, including the reference strain. Most effectors were identical in sequence among strains (Supplementary Table S6). Relatively higher polymorphism was found in sequences of some effectors such as AvrBs1, XopD, XopAD, and XopE2 (Supplementary Table S6).

Copper resistance and *cop* cluster

Both L-plasmids carry the copper resistance gene cluster, *copLAB* (Richard et al. 2017a). To verify that the strains carrying the *cop* clusters annotated in L-plasmids are copper resistant, we tested copper sensitivity of selected strains from each cluster. Strains carrying either of two L-plasmids grew on solid agar media amended with 200 ppm copper sulfate pentahydrate,

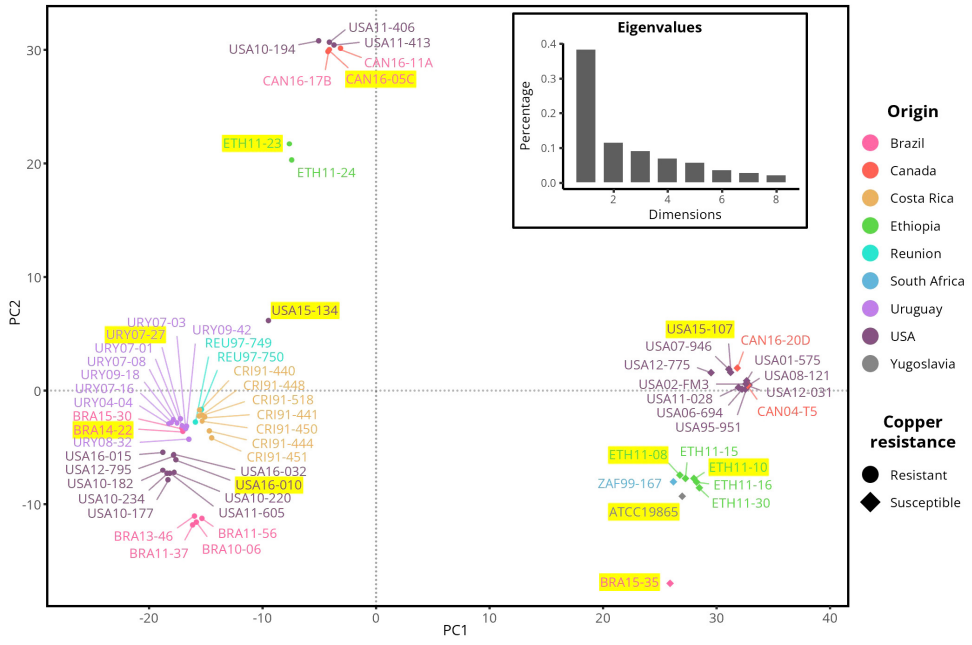

Fig. 1. Principal component analysis of *Xanthomonas hortorum* pv. *gardneri* strains based on the presence/absence of accessory genes. PC1 contained more than three times the variation of PC2. PC1 separates strains containing the L-plasmid (PC1 coordinates < 0) from strains lacking the L-plasmid (PC1 coordinates > 0). Strains are colored by country, and the labels of strains that were experimentally tested for copper tolerance are highlighted in yellow (Supplementary Table S6). JS749-3 and strains from Uruguay were also reported as copper tolerant (Montelongo García 2012; Richard et al. 2017a).

while the strains lacking the L-plasmid did not (Supplementary Table S7).

The arrangement of genes in the copper clusters of both L-plasmids was identical to the copper cluster (*copLABMGCDF*) in *X. citri* subsp. *citri* strain A44 (XccA44) (Behlau et al. 2011); however, there was only 95 to 96% nucleotide identity between *copLAB* clusters in the two L-plasmids. Interestingly, two strains from Brazil and all from Uruguay also carried a second *cop* cluster in their L-plasmid (Supplementary Table S4); the arrangement of open reading frames (ORFs) (*copLABMGF*) in the second cluster was different from XccA44 and similar to *X. alfalfae* subsp. *citrumelonis* (synonym *X. euvesicatoria* pv. *citrumelonis*) strain 1381 (Behlau et al. 2011).

X. hortorum pv. *gardneri* evolution timescale

To place the evolution of *X. hortorum* pv. *gardneri* in a temporal context, we inferred an *X. hortorum* pv. *gardneri* phylogeny based on 1,009 nonrecombinant, core chromosomal single nucleotide polymorphisms (SNPs) identified from variant calling. Statistically significant temporal signals and positive slopes were identified on 29 nodes of the core chromosomal SNP phylogeny (Supplementary Fig. S9). The most recent common ancestor (MRCA) of strains isolated after 1990 was dated to 1945 (1922, 1966) (Fig. 2). All but one of these strains were categorized into three clades. Clade I consisted of strains from the United States, Costa Rica, Réunion, Uruguay, Brazil, and South Africa, and the MCRA was dated to 1963 (1950, 1975). Near-simultaneous branching within clade I was associated with spread to different continents through 1971 (1961, 1981). All but two strains in clade I contained the L1-plasmid, so acquisition of the L1-plasmid was inferred to be prior to dissemination of these strains to different continents. Clade II split from clade I around 1960 (1943, 1975), just before the period of rapid branching within clade I, and contained strains from Ethiopia whose MRCA was dated to 1983 (1968, 1996). Clade III split early in the ancestry of *X. hortorum* pv. *gardneri* and contained strains isolated from the United States and Canada, with an MRCA dated to 1991 (1986, 1994). Clades II and III contained far fewer strains with the L-plasmid. Multiple occasions of acquisition of the L2-plasmid were inferred in clades II and III, while one strain in clade III had the L1-plasmid.

Most strains clustered by location in the Bayesian phylogeny. Strains from the United States clustered in clade I and clade III. The United States strains in clade I diverged more recently (since 2006) than those within clade IV (since 1991), suggesting a more recent introduction of the clade I lineage into Ohio, Michigan, and Indiana, U.S.A. Clade III contained two subclades that each diversified starting in the late 1990s, in which strains from Pennsylvania clustered with strains from either Ohio, U.S.A., or Canada. In clade I, strains from Brazil were found in two clusters, one of which was nested within strains from Uruguay.

Discussion

The first *X. hortorum* pv. *gardneri* strain was first isolated in the early 1950s by Šutic (1957) in former Yugoslavia. All other strains are genetically distinct from that strain, suggesting a different source for the Yugoslavia outbreak. It was approximately 40 years before *X. hortorum* pv. *gardneri* strains were isolated from tomato leaflets in Costa Rica in 1991 (Bouzar et al. 1999) and from a 1995 outbreak in a processing tomato field in Pennsylvania, U.S.A. (Kim et al. 2010), with strains from each of these outbreaks representing distinct clades. Strains collected from Pennsylvania and Ohio, U.S.A., and Ontario, Canada (Fig. 2; clade III), are descendants of the same source as the 1995 Pennsylvania outbreaks. Strains collected in Ohio, Indiana, and Michigan, U.S.A. (Fig. 2; clade I), resulted from a second, later introduction to the United States and share ancestry with the strains found decades earlier in Costa Rica. Brazil was the only country other than the United States that showed two possible introductions based on our strain collection. Note that other studies have confirmed the presence of *X. hortorum* pv. *gardneri* in other regions of the world not included in this study (Dehghan-Niri and Rahimian 2016; Rashid et al. 2016).

X. hortorum pv. *gardneri* emergence following a surge in trade in hybrid tomato seeds

Our results demonstrate that the global emergence of *X. hortorum* pv. *gardneri*, long after the first report in the 1950s, followed a global surge in international trade of hybrid tomato seed. This may not be surprising, as commercial success of hybrid corn in the first half of the 20th century encouraged hybridization of other crops, and the first hybrid tomato cultivars were released by 1940 (Dorst 1946; Emsweller 1961). However, it was not until between 1962 and 1977 that the tomato hybrid seed industry witnessed similar success, with a 300-fold increase in F1 hybrid tomato seeds exported globally during that 15-year period (Known-You Nursery and Seed Production Company Ltd. 1978). *X. hortorum* pv. *gardneri* diversification coincides with this early period of hybrid tomato seed production. Specifically, strains collected from multiple countries in the Americas and Africa are descended from major nodes in our phylogeny dated to the period of 1960 through 1970, at which time F_1 hybrids began changing the dynamics of tomato production due to their early and high yields, large fruit sizes, and long shelf-life (Mutschler et al. 1988; Scott et al. 2013; Tigchelaar et al. 1978). In the period of rapid growth in tomato production in the late 1970s through 1980s, we see evidence of regional diversification of *X. hortorum* pv. *gardneri*, likely resulting from other anthropogenic factors such as seedling production and distribution systems and the use of copper for disease management.

Selection for acquisition of copper tolerance

The population structure of *X. hortorum* pv. *gardneri* is largely clonal, with homologous recombination in the core genome having occurred largely at the single strain level. The relative clonality of *X. hortorum* pv. *gardneri* contrasts with extensive recombination in the core genome of the bacterial spot pathogen *X. euvesicatoria* pv. *perforans* (Jibrin et al. 2018; Newberry et al. 2019; Timilsina et al. 2015, 2019b). However, we observed that *X. hortorum* pv. *gardneri* strains varied in the presence/absence of a large plasmid that carries one or more copper resistance gene clusters (Richard et al. 2017a). Copper resistance was likely acquired in response to selection from extensive use of copper bactericides, which were recommended for control of bacterial spot pathogens as early as the 1930s (Bender and Cooksey 1986; Miller and Crosier 1936; Stall et al. 1986). Copper resistance was previously reported for strains collected from Costa Rica (Behlau et al. 2013; Bouzar et al. 1999) and Uruguay (Montelongo García 2012; Maria Ines Siri, *personal communication*). *Xanthomonas* species are known to acquire copper resistance via transmissible plasmids from local sources (Behlau et al. 2012). Our analysis suggests the plasmid was acquired early in the ancestry of clade I strains, which may have contributed to their ability to establish and persist in tomato-production regions on multiple continents. In contrast, fewer strains in clades II and III contained the large plasmid, and these strains appear to have largely acquired it from a different source than clade I (likely the bacterial spot pathogen *X. vesicatoria* during co-infection on tomato). These results suggest that there may have been different dissemination pathways for clade I versus clades II and III. Copper resistance was likely acquired in response to selection from extensive use of copper

bactericides, which were recommended for control of bacterial spot pathogens as early as the 1930s.

Updates on the type III effectors profile of *X. hortorum* pv. *gardneri*

Results from this study build upon previous reports of type III effectors in *X. hortorum* pv. *gardneri* (Potnis et al. 2011; Schwartz et al. 2015). The putative identification of all previously described 11 common effectors in all sequenced strains suggests that these effectors are important in maintaining the core function of this species in its interaction with tomato host. Our re-sequencing of some of the strains examined in previous studies provided added insights into the distribution of effectors. For example, AvrBs7 was only reported in two strains previously

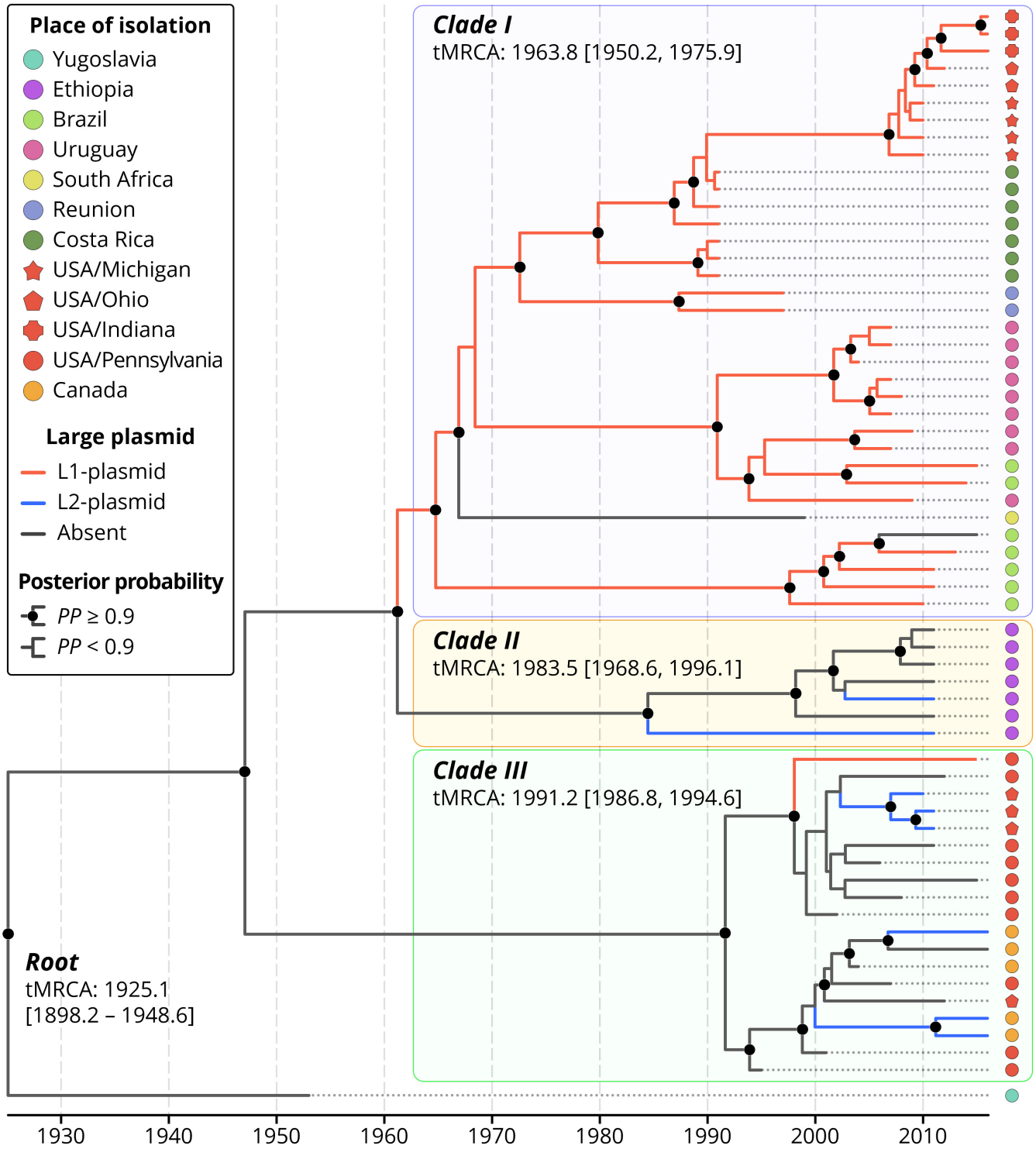

Fig. 2. Calibrated phylogeny of *Xanthomonas hortorum* pv. *gardneri* strains showing the global population expansion of the species. The phylogeny is based on chromosomal core nonrecombinant single nucleotide polymorphisms. The tree was generated using BEAST v1.10.4 using the HKY substitution model, the asymmetric substitution model for the presence/type of large plasmid, and the coalescent Skygrid model under an uncorrelated relaxed clock. The overall evolutionary rate was 2.81×10^{-7} (95% highest posterior density [HPD]: 2.11×10^{-7}; 3.49×10^{-7}). Branch coloring shows inferred ancestral presence of the large plasmid (L-plasmid) in its two observed forms or absence. tMRCA = dating of most recent common ancestor.

(Schwartz et al. 2015). Our results showed that this may have been due to either sequencing or assembly error, as AvrBs7 is present in all strains. Future studies on the observed allelic diversity in some of the putatively identified effectors such as AvrBs1, XopAD, XopAS, XopD, XopE2, and XopJ1 will provide improved understanding of their impact on pathogen evolution and host-pathogen interactions.

Conclusions

Global trade has provided a mechanism for disseminating seedborne pathogens intercontinentally. The ancestry of *X. hortorum* pv. *gardneri* is consistent with the burgeoning hybrid tomato seed industry and dissemination during the rapid increase in the trade of hybrid seeds. However, the apparently small number of genetically distinct lineages of *X. hortorum* pv. *gardneri* in any one country suggests that there has not been pervasive movement of novel *X. hortorum* pv. *gardneri* strains into the global seed-to-field tomato-production systems. Disease management with the chronic use of copper-based products selected for rapid acquisition of copper tolerance by horizontal gene transfer. Previous authors have hypothesized a link between the seed trade and dissemination of plant pathogens (McDonald and Stukenbrock 2016; Sikes et al. 2018). Hybrid seeds of many crops are important in local, regional, and global trade (Almekinders and Louwaars 2002; Schreinemachers et al. 2017). Diversification could impact genes such as effectors like *avrHah1* that may impact host-pathogen interaction. Continuous global monitoring of the pathogen population is required to draw conclusions on the impact of effectors in pathogen diversification. The complex production chains for vegetable crops and various sources of seeds provide opportunities for continued pathogen dissemination, leading to the potential for geographically widespread emergence or re-emergence of disease.

Materials and Methods

Genome sequencing, assembly, and annotation

A collection of 62 strains from three continents was used to study the phylogeographic structure of *X. hortorum* pv. *gardneri* (Supplementary Table S1). Strains were collected from Africa (Ethiopia, Réunion, and South Africa), North America (Canada, Costa Rica, and the United States), and South America (Brazil and Uruguay). Altogether, 55 *X. hortorum* pv. *gardneri* strains were sequenced for this study and analyzed together with whole-genome sequences of seven previously sequenced genomes (Potnis et al. 2011; Schwartz et al. 2015). Genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega Corp., Madison, WI, U.S.A.), and libraries were prepared using the Illumina DNA Prep Kit (Illumina Inc., San Diego, CA, U.S.A.). The whole genomes of all strains were paired-end sequenced with either Illumina MiSeq or NextSeq 2000 to generate 2- × 150-bp reads. Raw reads were trimmed for adapters using Cutadapt v2.8 (Martin 2011) and were assembled into contigs with SPAdes v3.15.3 (Prjibelski et al. 2020) using the '–careful' switch. Contigs with fewer than 500 bp and a k-mer coverage of 2.0 were filtered out. Pilon v1.24 (Walker et al. 2014) was used to polish draft assemblies, coupled with BWA v0.7.17 (Li and Durbin 2009) for alignment of reads and SAMtools v1.15 (Danecek et al. 2021) for sorting of alignment file. Prokka v1.14.6 (Seemann 2014) was used for gene annotation. Pairwise whole-genome ANI was calculated by Pyani v0.2.10 (Pritchard et al. 2016) using Nucmer v3.1 (Kurtz et al. 2004) for genome alignment.

Pangenome analyses

The pangenome of the *X. hortorum* pv. *gardneri* strains (Supplementary Table S2) was analyzed with Roary v3.12.0 (Page et al. 2015) with default parameters, except the core gene presence cutoff was set at 100% of strains (-cd 100), and the identity threshold was set to 90% (-i 90). To obtain a core gene alignment for *X. hortorum* strains, a separate pangenome analysis was conducted using the same parameters. IQ-TREE v2.1.3 (Nguyen et al. 2015) was used to infer a maximum likelihood-based phylogeny using a concatenated core gene alignment produced by Roary and 1,000 bootstraps using the ultrafast method (Minh et al. 2013). FigTree v1.4.4 (tree.bio.ed.ac.uk/software/figtree) was used to visualize the tree. Based on the presence/absence of accessory genes in Roary pangenome analysis of *X. hortorum* pv. *gardneri* strains, the accessory genome phylogeny was developed using FastTree v2.1.10 (Price et al. 2010), and the matrix of gene content was visualized with roary2svg.pl (github.com/tseemann/nullarbor).

Estimating rates and impacts of homologous recombination in *X. hortorum* pv. *gardneri*

Signals of recombination among the core genome of *X. hortorum* pv. *gardneri* strains were identified by ClonalFrameML v1.11 (Didelot and Wilson 2015) using the core gene alignment from Roary and the core gene phylogeny from IQ-TREE. Confidence intervals for rates of recombination to mutation (R/θ), recombination tract length (δ), and probability of recombination per site (ν) were calculated from 1,000 bootstraps.

Plasmid typing

Various tools within MOB-suite v3.1.0 (Robertson and Nash 2018) were used to screen the *X. hortorum* pv. *gardneri* whole-genome assemblies for plasmids; MOB-cluster identified and clustered plasmids, and MOB-recon reconstructed the plasmids from contigs. The transferability of identified plasmids (conjugative versus mobilizable) as well as the type of relaxase and mate-pair formation was identified by MOB-typer. Pairwise ANI, which was used to infer similarity between plasmids, was calculated using Pyani v0.2.10 (Pritchard et al. 2016).

Plasmid profiles

Plasmids were purified from suspensions of each *X. hortorum* pv. *gardneri* strain tested using a modified alkaline lysis method (Kado and Liu 1981). Bacterial cells were suspended in a solution of 50 mM Tris containing 0.57 M sodium chloride, 0.04 M sodium hydroxide, and 3% w/v sodium dodecyl sulfate and were lysed by incubation at 28°C for 15 min. DNA was extracted from cell lysates with two volumes of phenol-chloroform-isoamyl alcohol (25:24:1). Plasmid profiles for strains were determined by agarose gel electrophoresis of DNA on a 0.5% agarose gel in Tris-acetate buffer (40 mM Tris-acetate and 1 mM EDTA, with a pH of 8.2) at 5 V/cm (Maniatis et al. 1982).

Copper cluster analysis

The positions of the *cop* resistance cluster were identified using BLAST+ v2.10.1 (Camacho et al. 2009). The individual nucleotide coding sequences of the *cop* cluster of *X. citri subsp. citri* strain A44 were used as queries for BLASTn of whole genomes of *X. hortorum* pv. *gardneri* strains (similarity ≥70%; query coverage ≥50%) (Behlau et al. 2011). The copper clusters were classified based on percentage identity (threshold: 98%) and the presence or absence of individual *cop* ORFs.

Copper resistance test

Copper resistance of strains was tested by spotting on nutrient agar (NA) medium (BBL; Becton Dickinson and Co, Franklin Lakes, NJ, U.S.A.) amended with 100 or 200 ppm copper sulfate (Stall et al. 1986). Petri plates were incubated at 28°C and examined for colony growth after 1 and 3 days. Copper-sensitive *X. euvesicatoria* pv. *perforans* strain 91-118 and copper-resistant

X. euvesicatoria pv. *perforans* strain 2010 were used as controls (Basim et al. 2005).

Type III effector content

Amino acid sequences for all *Xanthomonas* type III effectors were acquired from the INRA *Xanthomonas* Portal (biopred.net/xanthomonas/t3e.html). An in-house script (github.com/rknx/eff-search) was used to perform the homology search and identify alleles. Briefly, the nucleotide BLAST database was generated from bacterial genomes using the BLAST+ tool (Camacho et al. 2009). The tBLASTn tool was used for identifying the effector sequences in the genomes. Hits exceeding 40% query coverage and 70% identity were extracted for cross comparison among the strains and allele number assignment. Different copies of XopE2 were distinguished, with 95% identity as the cut-off. Truncated hits were identified by examining the presence of the first and the last codon in the BLAST alignment.

Variant calling

A multifasta alignment of core chromosomal SNPs between the *X. hortorum* pv. *gardneri* strains was generated using the ProkSNPTree pipeline (Sharma 2022). Briefly, the trimmed FASTQ reads were aligned to reference genome *X. hortorum* pv. *gardneri* strain JS749-3 (Richard et al. 2017a) using BWA v0.7.17 (Li and Durbin 2009), and the alignment file was sorted and converted to binary format with SAMtools v1.15 (Danecek et al. 2021). Variations from reference were identified by the HaplotypeCaller tool in GATK v4.1.9.0 (DePristo et al. 2011). All nonSNP and nonchromosomal variations were ignored; the remaining SNPs were then filtered as follows: depth $\geq$ 10, quality-normalized depth $\geq$ 10, mapping quality $\geq$ 50, and reference allele depth $\leq 0.1 \times$ alternate allele depth. Chromosome-level sequence alignment of all strains was generated from the reference chromosome and high-quality SNPs using GATK FastaAlternateReferenceMaker. Gubbins v3.3.0 (Croucher et al. 2015) was used to identify potentially recombinant regions in the alignment, and SNPs in these regions were filtered out before subsequent analyses. The nucleotide at the nonrecombinant core SNP positions for each strain was concatenated together to produce core SNP alignment.

Bayesian phylogeographic analyses

The presence of temporal signals for each clade was inferred for the core SNP alignment using Phylostems (Doizy et al. 2023). The discrete phylogeographic analysis was performed using the core SNP alignment in Bayesian Evolutionary Analyses of Sampled Trees (BEAST) v1.10.4 (Drummond et al. 2012) using the following as priors: HKY nucleotide substitution model with empirical base frequencies and gamma distribution of site-specific rate heterogeneity, an asymmetric substitution model for the presence or absence of an L-plasmid with Bayesian stochastic search variable selection (BSSVS), an uncorrelated relaxed clock, and Bayesian Skyline (Hall et al. 2016). Ancestral states were reconstructed at all ancestors, utilizing the L-plasmid state as the trait. Markov Chain Monte Carlo samplers were run for 200 to 250 million generations. Adequate mixing was assessed by calculating the effective sampling size (ESS) of parameter estimates (cutoff ESS $\geq$ 200). The maximum clade credibility (MCC) trees were inferred from the posterior distribution of trees using TreeAnnotator v1.10.4 (Drummond et al. 2002), specifying a burn-in of 10% and median node heights, and were visualized in FigTree v1.4.4 (tree.bio.ed.ac.uk/software/figtree).

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