Phytopathologia Mediterranea
The international journal of the
Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/pm

Short Notes

Isolation and molecular characterization of a *Xylella fastidiosa* subsp. *multiplex* strain from almond (*Prunus dulcis*) in Apulia, Southern Italy

Vito MONTILON[1], Oriana POTERE[1], Leonardo SUSCA[1], Francesco MANNERUCCI[1], Franco NIGRO[1], Stefania POLLASTRO[1,*], Giuliana LOCONSOLE[2], Francesco PALMISANO[3], Claudio ZAZA[3], Marco CANTATORE[3], Francesco FARETRA[1]

[1] *University of Bari Aldo Moro, Department of Soil, Plant and Food Sciences (DiSSPA), Via Amendola 165/A, 70126 Bari, Italy*
[2] *National Research Council, Institute for Sustainable Plant Protection, Via Amendola, 122/D, 70126 Bari, Italy*
[3] *Regional Plant Protection Service-Apulia Region, Lungomare Nazario Sauro, 51, 70100 Bari, Italy*
*Corresponding author. E-mail: stefania.pollastro@uniba.it

Summary. *Xylella fastidiosa* is a xylem-limited phytopathogenic bacterium under regulation in the European Union as a priority pest. Given the potential risk posed by this pathogen to cultivated and ornamental plants, mandatory annual surveys and laboratory testing are required in Member States to early detect outbreaks. In the course of surveys carried out during early spring 2024 in the Apulia region (Southern Italy), *X. fastidiosa* subsp. *multiplex* was identified using quantitative real-time Polymerase Chain Reaction (qPCR), in a non-symptomatic sample from an almond tree (*Prunus dulcis*) in an orchard located in Santeramo in Colle, in Bari province. Multilocus sequence typing (MLST) was used to identify the subspecies and sequence type (ST) of the bacterium using the genomic DNAs extracted from the infected sample. Comparative sequence analysis of the seven MLST allele genes indicated that the obtained nucleotide sequences completely matched allele sequences of *X. fastidiosa* in PubMLST database corresponding to the allelic profile (Sequence Type) ST26 related to subsp. *multiplex*. Bacterial colonies consistent in morphology with *X. fastidiosa* were isolated from asymptomatic host samples and identity was confirmed by real-time PCR analysis. This is the first report of detection of *X. fastidiosa* subsp. *multiplex* ST26 in the EU.

Keywords. *Xanthomonadaceae*, MLST, priority pest, sequence type, xylem-limited bacterium.

OPEN ACCESS

Citation: Montilon, V., Potere, O., Susca, L., Mannerucci, F., Nigro, F., Pollastro, S., Loconsole, G., Palmisano, F., Zaza, C., Cantatore, M., & Faretra, F. (2024). Isolation and molecular characterization of a *Xylella fastidiosa* subsp. *multiplex* strain from almond (*Prunus dulcis*) in Apulia, Southern Italy. *Phytopathologia Mediterranea* 63(3): 423-429. doi: 10.36253/phyto-15810

Accepted: December 9, 2024

Published: December 30, 2024

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Editor: Anna Maria D'Onghia, CIHEAM/Mediterranean Agronomic Institute of Bari, Italy.

ORCID:
VM: 0000-0001-8565-4166
OP: 0009-0003-7182-3143
FN: 0000-0002-8273-0052
SP: 0000-0001-8806-1905
GL: 0000-0002-5154-7829
FF: 0000-0003-1063-0607

INTRODUCTION

Xylella fastidiosa (*Xanthomonadaceae*) (Wells *et al*., 1987) is a gram-negative plant pathogenic bacterium comprising several subspecies, which are pathogenic to a broad spectrum of host plants including agricultural crops

of economic importance, ornamentals and natural vegetation (EFSA, 2023). The pathogen is limited to host plant xylem tissues (Purcell and Hopkins, 1996), leading to symptoms generally related to xylem vessel occlusion, which include scorching of leaves and dieback that vary in severity depending on the host susceptibility. The pathogen is naturally transmitted by xylem sap-feeding leafhoppers (*Cicadellidae*) and spittlebugs (*Cercopidae*) (Hopkins, 1989), and is spread over long distances through movement of infected plant material or infectious insect vectors (Purcell and Hopkins, 1996; Loureiro *et al*., 2024).

Following the first confirmed report of *X. fastidiosa* in the European Union (EU) in 2013, in Salento, Apulia Region, Southern Italy (Saponari *et al*., 2013), where a strain of *X. fastidiosa* subsp. *pauca* Sequence Type (ST) 53 (Giampetruzzi *et al*., 2015; Giampetruzzi *et al*., 2017) was found to cause the Olive Quick Decline Syndrome (OQDS) (Martelli, 2016; Saponari *et al*., 2017), EU emergency measures against plant pests were updated with the new plant health Regulation (EU) 2016/2031 (European Commission, 2016). Under this regulation, *X. fastidiosa* became a priority pest (European Commission, 2019), and has been since subjected to mandatory annual surveys by Member States to prevent its entrance and spread within the EU (European Commission, 2020; European Commission, 2024). As a result of extensive survey activities, *X. fastidiosa* has also been detected in France, Spain, and Portugal (Denancé *et al*., 2017; Olmo *et al*., 2017; Marco-Noales *et al*., 2021; Carvalho-Luis *et al*., 2022; EFSA, 2023), in which several sequence types (STs) of the bacterium belonging to different subspecies were identified on various plant species. More recently, *X. fastidiosa* subsp. *multiplex* ST87 has been found in the Tuscany region of Italy (Saponari *et al*., 2019), and a new outbreak of the subspecies *fastidiosa* ST1 emerged in a location in the province of Bari in Apulia (Cornara *et al*., 2024).

Official inspections performed for the detection and identification of the bacterium and its subspecies are regulated by Commission Implementing Regulation (EU) 2020/1201, amended and corrected by Commission Implementing Regulation (EU) 2024/2507), that specify which molecular tests must be used for the identification of *X. fastidiosa* and its subspecies.

Inspections are based on visual surveys, and collection of representative plant samples for pathogen diagnosis to species level by real-time Harper PCR (Harper *et al*., 2010, erratum 2013). Following the diagnostic confirmation of the positive detection of *X. fastidiosa* in previously free areas or in new plant host species, multilocus sequence typing (MLST) analysis (Yuan *et al*., 2010) is the most common test used for the assignment of positive samples to subspecies and Sequence Type (ST). Real-time PCR methods based on Dupas *et al*. (2019) and Hodgetts *et al*. (2021) can also be used for subspecies assignment (CI Regulation (EU) 2024/2507).

This paper reports identification of *X. fastidiosa* subsp. *multiplex* ST26 in a non-symptomatic sample from an almond tree (*Prunus dulcis*) in an orchard in Apulia, in the province of Bari (Southern Italy) (Europhyt outbreak notification n. 2549). This discovery occurred in the context of the regional surveillance program for *X. fastidiosa* associated with OQDS, enforced by the Plant Health Service of the Apulia Region, and carried out by the Regional Agency for Irrigation and Forestry Activities (ARIF).

MATERIALS, METHODS, AND RESULTS

Samples were randomly collected in March 2024, from a site that included orchards in the municipal territory of Santeramo in Colle, a few tens of kilometres from the west of the *X. fastidiosa* subsp. *pauca* ST53 demarcated area (DA). The plant material delivered to the laboratory of the Department of Soil, Plant and Food Sciences, University of Bari (Italy) consisted of mature lignified branches from non-symptomatic almond trees, which were refrigerated until testing.

Samples were prepared by debarking the hardwood cuttings and scraping the exposed surfaces of the wooden tissues with a sterile razor blade. From each sample, 0.5 g of wood shavings were placed in extraction bags (BIOREBA®) and then ground in 5 mL (1:10 weight:volume) of CTAB extraction buffer using a semi-automated homogenizer (Homex 7, BIOREBA®). Total nucleic acids were extracted using a cetyltrimethylammonium bromide (CTAB) based method (Loconsole *et al*., 2014; EPPO, 2023). Samples from an OQDS-infected and a non-infected plant were included in the DNA extraction as positive (PIC) and negative (NIC) isolation controls (EPPO, 2023).

DNA extracts were analysed by quantitative polymerase chain reaction (qPCR) assays carried out according to Appendix 5 of the EPPO Diagnostic Standard for *X. fastidiosa* PM 7/24 (5) based on the protocol of Harper *et al*. (2010, erratum 2013). Total nucleic acids of PIC and NIC were run alongside the samples, and a negative amplification control (NAC) was included. A positive amplification control (PAC) consisting of a suspension at a known concentration of *X. fastidios*a subsp. *pauca* ST53 cells was also included in the same plate of the qPCR assay. According to the guidelines issued by the Plant Health Service of the Apulia Region (DDS no. 31 of 13 May 2022) for the

monitoring and eradication of the pathogenic bacterium at regional level, samples that produced by qPCR (Harper *et al*., 2010) a quantification cycle (Cq) ≤32 were considered positive, while samples that did not exhibit exponential amplification were considered negative. If the Cq was greater than 32 the result was considered undetermined, and the sample was re-tested.

The qPCR assays for individual trees revealed presence of *X. fastidiosa* in one almond tree identified with the code ID 19107. The Cq value produced for this sample by qPCR was 28,54. Both the negative (NIC and NAC) and positive (PIC and PAC) controls produced the respective expected results.

Consequently, an aliquot of DNA of this sample was used for multilocus sequence typing (MLST) analysis (Yuan *et al*., 2010), according to the Appendix 16 of the EPPO Diagnostic Standard PM 7/24 (5) (EPPO, 2023), to further characterize the *X. fastidiosa* genotype detected outside the *X. fastidiosa* DA. Amplicons with the expected size were sequenced by Macrogen Inc., Seoul, South Korea. Allele sequences were then assembled by BioEdit Sequence Alignment Editor version 7.2.5 software and analyzed using the PubMLST database (http://pubmlst.org/xfastidiosa/) to identify allele types. Allele sequences amplified from the infected almond tree had 100% nucleotide identity with those of alleles *leuA*_5, *petC*_3, *malF*_3, *cysG*_3, *holC*_6, *nuoL*_3, and *gltT*_5 corresponding to the ST26 genotype belonging to *X. fastidiosa* subsp. *multiplex* (EPPO, 2023). The sequences obtained for the MLST alleles were deposited in GenBank under accession numbers: *leuA* allele 5, PQ535574; *petC* allele 3, PQ535575; *malF* allele 3, PQ535576; *cysG* allele 3, PQ535577; *holC* allele 6, PQ515132; *nuoL* allele 3, PQ535578; and *gltT* allele 5, PQ535579. A phylogenetic network, inferred through the concatenation of the MLST sequences of all *X. fastidiosa* (STs) retrieved from the PubMLST database (Jolley *et al*., 2018), and conducted using the Neighbor-Net method implemented in Splits Tree4 (version 4.12.2) (Huson and Bryant, 2006), indicated that the genotype ST26 shared close similarity to a complex of strains of subsp. *multiplex* (Figure 1).

An aliquot of the DNA sample extracted from the infected almond tree was also analyzed by real-time tetraplex PCR assay (Dupas *et al*., 2019) to further confirm the isolated bacterium subspecies. This test was carried out using the reaction volumes and amplification conditions validated in the test performance study for the *X. fastidiosa* subspecies identification previously con-

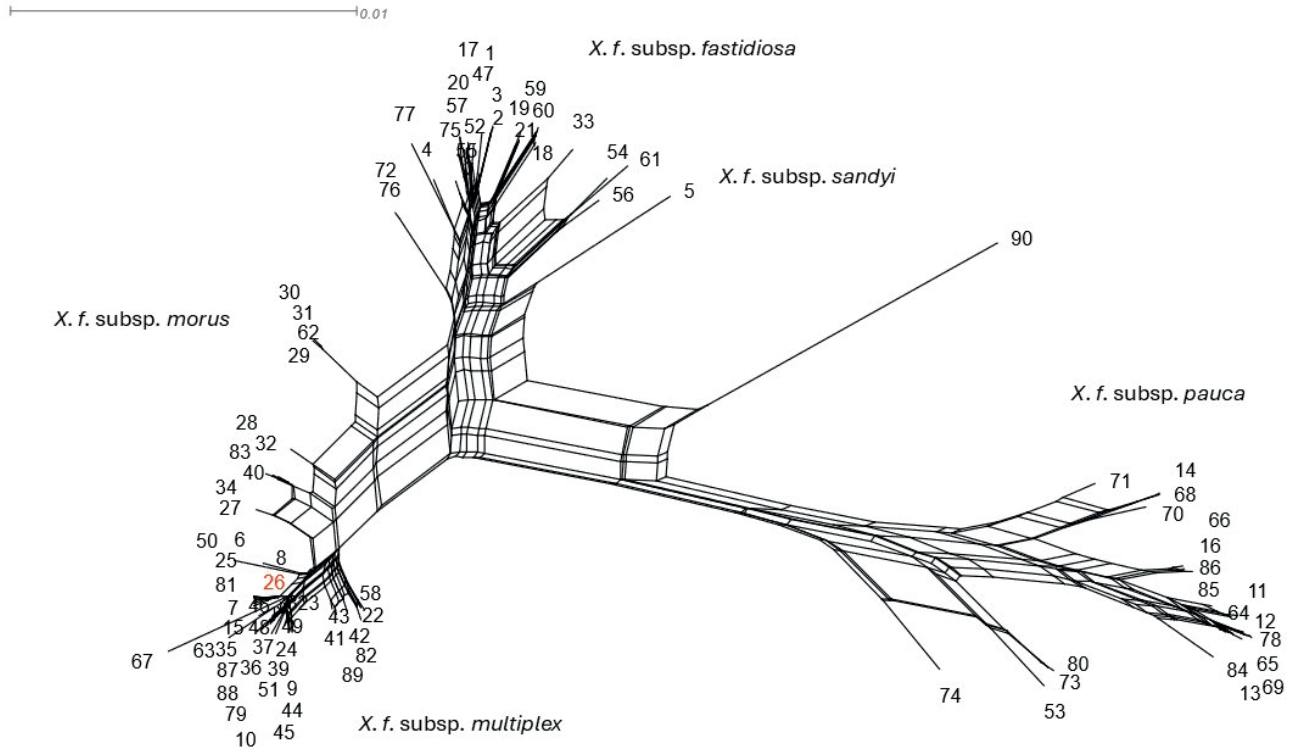

Figure 1. Neighbor net network analysis based on concatenated sequence alignments of the seven MLST genes representing distribution and phylogenetic relationships of the 90 sequence types (STs) of *X. fastidiosa* belonging to the subspp. *multiplex*, *morus*, *fastidiosa*, *sandyi* or *pauca* identified to date. The nextwork tree includes the ST26 (red font) identified in the present study.

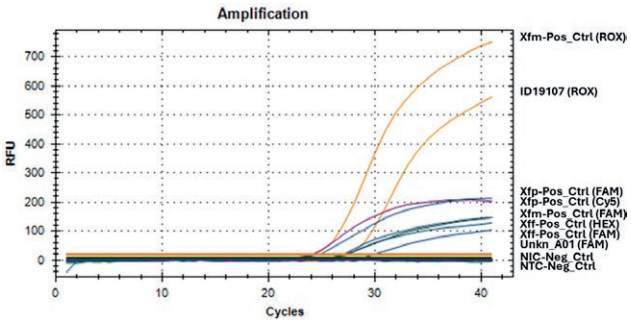

Figure 2. Results from multiplex real-time PCR based on primer sets reported in Dupas *et al.* (2019). The blue exponential curves were generated by the primer-probe set, labelled with fluorophore FAM, which detected bacteria at species levels in the positive controls, and in the sample ID 19107. The green exponential curve was generated by the primer-probe set, labelled with HEX, which specifically detected subsp. *fastidiosa* in the positive control (Xff-Pos_Ctrl). The orange exponential curves were generated by the primer-probe set, labelled ROX, which detected the subsp. *multiplex* in the positive control Xfm-Pos, and in the sample ID 19107. The violet exponential curve was generated by the primer-probe set, labelled Cy5, which detected subsp. *pauca* in the positive control Xfp-Pos. Negative amplification controls (NIC-Neg_Ctrl and NTC-Neg_Ctrl) are also indicated. Quantification cycles are indicated on the x axis, and relative fluorescence units (RFUs) are indicated on the y axis.

ducted by the Official Laboratories of the Italian National Plant Protection Organization (NPPO), with coordination of the National Reference Laboratory, constituted by the Council for Agricultural Research and Economics, Research Centre for Plant Protection and Certification (CREA-DC) (Pucci *et al.*, 2023). For the assay, PACs were provided by CREA-DC and consisted of DNA extracted from plant samples infected by isolates of *X. fastidiosa* subsp. *fastidiosa*, subsp. *multiplex* or subsp. *pauca*. The tetraplex real-time PCR assay detected and identified subsp. *multiplex* in the sample ID 19107, producing a Cq value of 27.47 (Figure 2). All DNAs extracted from the PAC tested positive for the corresponding target subspecies of *X. fastidiosa* when using the appropriate subspecies-specific primers and probes, while no amplification reaction occurred for the other subspecies. No signal amplification was observed for the NAC.

Attempts were made to isolate the bacterium. Lignified portions (length 4 to 8 cm) recovered from cuttings of non-symptomatic host plant branches that had tested positive by qPCR were surface sterilized in a laminar flow hood by soaking for 2 min in 2% (v/v) sodium hypochlorite solution and 2 min in 70% ethanol, and then rinsed three times each for 2 min in sterile distilled water. The tissue portions were then each cut in half, squeezed with sterile pliers by pressing the external ends, and the freshly cut faces were blotted onto buffered cysteine-yeast extract

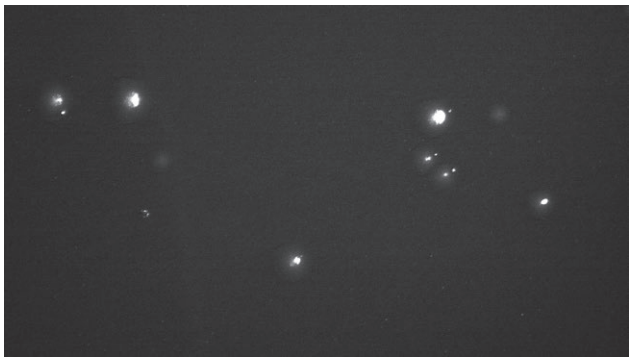

Figure 3. Bacterial colonies of *X. fastidiosa* subsp. *multiplex* ST26 isolated from almond tree branches on buffered cysteine yeast extract (BCYE) agar 30 d after isolation.

(BCYE) medium (Wells *et al.*, 1981). The inoculated plates were then incubated at 28°C in the dark for at least 30 d and were periodically observed using a light microscope for appearance of colonies with morphological characteristics typical of *X. fastidiosa* (Wells *et al.*, 1981; 1987) (Figure 3). Typical colonies were re-isolated and their identity as *X. fastidiosa* was confirmed using the qPCR assay of Harper *et al.* (2010, erratum 2013).

DISCUSSION

The subsp. *multiplex* of *X. fastidiosa* is native to North America (Nunney *et al.*, 2019), and is known to have a wide plant species host range. This includes peach (*P. persica*), plum (*P. domestica*), almond (*P. dulcis*), and several forest and shade trees (Schaad *et al.*, 2004; Nunney *et al.*, 2013; Nunney *et al.*, 2019). Strains of *X. fastidiosa* subsp. *multiplex* have also been reported in associations with olive (Krugner *et al.*, 2014), and with grapevine (Almeida and Purcell, 2003). To date, in the European Union, strains belonging to the ST6, ST7, ST81 and ST87 of *X. fastidiosa* subsp. *multiplex* have been reported on almond and other hosts, including cultivated and ornamental species in Corsica, mainland France, the Balearic Islands, in Spain, and in Italy in Tuscany and Lazio (EFSA, 2022; Trkulja *et al.*, 2022). The strain ST26 identified in Apulia is distinct from those found in other Italian and European regions, indicating that ST26 has been introduced from a different and unknown location.

Xylella fastidiosa subsp. *multiplex* ST26 has been previously detected only on *P. domestica* in Brazil, where it was thought to have been introduced from the North America (Coletta-Filho *et al.*, 2017). ST26 is reported to mainly affect stone fruit trees (*Prunus* spp.), particularly plum (Coletta-Filho *et al.*, 2017; Nunney *et al.*, 2019).

After the notification of detection of *X. fastidiosa* multiplex ST26 to the competent Plant Health Service of the Apulia Region, a DA was established, and emergency control measures have been carried out to limit the spread of the bacterium to surrounding areas, in accordance with the legislative provisions under Regulation (EU) 2020/1201 (European Commission, 2020) amended by Regulation (EU) 2024/2507 (European Commission, 2024). A monitoring campaign is currently underway (in 2024) to determine the extent of the epidemic outbreak. Further research is required to consider the host range of ST26, seasonal development of the leaf scorch symptoms this strain causes, and the presence of infective vectors.

FUNDING

Funding for the plant diagnostic and testing service was received from Regione Puglia, in the framework of the Agreement with the Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, "Servizio di analisi di laboratorio ufficiali per rilevare la presenza di *Xylella fastidiosa* sul territorio della Regione Puglia". Research on characterization of the pathogen strain was supported by Agritech National Research Center, funded by European Union Next Generation EU (Piano Nazionale Di Ripresa E Resilienza (PNRR) – Missione 4 Componente 2, Investimento 1.4 – D.D. 1032 17/06/2022, CN00000022).

ACKNOWLEDGEMENTS

The authors thank Dr Giuseppe Incampo, Daniele Cornacchia, Adriano Pacifico and Antonio Ceglie (University of Bari, Italy) for conducting some of the analyses described in this paper. Support of the Regional Agency for Irrigation and Forestry Activities (ARIF) is also acknowledged.

LITERATURE CITED

Almeida R.P.P., Purcell A.H., 2003. Biological Traits of *Xylella fastidiosa* Strains from Grapes and Almonds. *Applied and Environmental Microbiology* 69(12): 7447–7452. https://doi.org/10.1128/AEM.69.12.7447-7452.2003

Carvalho-Luis C., Rodrigues J.M., Martins L.M., 2022. Dispersion of the Bacterium *Xylella Fastidiosa* in Portugal. *Journal of Agricultural Science and Technology A* 12(1). https://doi.org/10.17265/2161-6256/2022.01.005

Coletta-Filho H.D., Francisco C.S., Lopes J.R., Muller C., Almeida R.P., 2017. Homologous Recombination and *Xylella Fastidiosa* Host–Pathogen Associations in South America. *Phytopathology* 107(3): 305–312. https://doi.org/10.1094/PHYTO-09-16-0321-R

Cornara D., Boscia D., D'Attoma G., Digiaro M., Ligorio A., … Saponari M., 2024. An Integrated Strategy for Pathogen Surveillance Unveiled *Xylella Fastidiosa* ST1 Outbreak in Hidden Agricultural Compartments in the Apulia Region (Southern Italy). *European Journal of Plant Pathology*. https://doi.org/10.1007/s10658-024-02945-7

Denancé N., Legendre B., Briand M., Olivier V., de Boisseson C., … Jacques M.A., 2017. Several Subspecies and Sequence Types Are Associated with the Emergence of *Xylella Fastidiosa* in Natural Settings in France. *Plant Pathology* 66(7): 1054–1064. https://doi.org/10.1111/ppa.12695

Dupas E., Briand M., Jacques M.A., Cesbron S., 2019. Novel Tetraplex Quantitative PCR Assays for Simultaneous Detection and Identification of *Xylella Fastidiosa* Subspecies in Plant Tissues. *Frontiers in Plant Science* 10: 1732. https://doi.org/10.3389/fpls.2019.01732

European Commission, 2016. Regulation (EU) 2016/2031 of the European Parliament of the Council of 26 October 2016 on protective measures against pests of plants, amending Regulations (EU) No 228/2013, (EU) No 652/2014 and (EU) No 1143/2014 of the European Parliament and of the Council and repealing Council Directives 69/464/EEC, 74/647/EEC, 93/85/EEC, 98/57/EC, 2000/29/EC, 2006/91/EC and 2007/33/EC. *Official Journal of the European Union L* 317: 4–104. http://data.europa.eu/eli/reg/2016/2031/oj/eng

European Commission, 2019. Commission Delegated Regulation (EU) 2019/1702 of 1 August 2019 supplementing Regulation (EU) 2016/2031 of the European Parliament and of the Council by establishing the list of priority pests. *Official Journal of the European Union L* 260: 8–10. http://data.europa.eu/eli/reg_del/2019/1702/oj/eng

European Commission, 2020. Commission Implementing Regulation (EU) 2020/1201 of 14 August 2020 as regards measures to prevent the introduction into and the spread within the Union of *Xylella fastidiosa* (Wells *et al.*). *Official Journal of the European Union L* 269: 2–39. http://data.europa.eu/eli/reg_impl/2020/1201/oj/eng

European Commission, 2024. Commission Implementing Regulation (EU) 2024/2507 of 26 September 2024 amending and correcting Implementing Regulation (EU) 2020/1201 as regards measures to prevent the

introduction into and spread within the Union of *Xylella fastidiosa* (Wells *et al*.) and amending Implementing Regulation (EU) 2020/1770 as regards the list of plant species not exempted from the traceability code requirement for plant passports. *Official Journal of the European Union L*. http://data.europa.eu/eli/reg_impl/2024/2507/oj/eng

EFSA (European Food Safety Authority), Delbianco A., Gibin D., Pasinato L., Morelli M., 2022. Update of the *Xylella* spp. host plant database: systematic literature search up to 30 June 2021. *EFSA Journal* 20(1): 7039. https://doi.org/10.2903/j.efsa.2022.7039

EFSA (European Food Safety Authority), Gibin D., Pasinato L., Delbianco A., 2023. Update of the *Xylella* Spp. Host Plant Database – Systematic Literature Search up to 31 December 2022. *EFSA Journal* 21(6): 8061. https://doi.org/10.2903/j.efsa.2023.8061

EPPO (European Plant Protection Organization), 2023. PM 7/24 (5) *Xylella Fastidiosa*. *EPPO/OEPP Bulletin* 53(2): 205-276. https://doi.org/10.1111/epp.12923

Giampetruzzi A., Chiumenti M., Saponari M., Donvito G., Italiano A., … Saldarelli P., 2015. Draft Genome Sequence of the *Xylella fastidiosa* CoDiRO Strain. *Genome Announcements* 3(1): e01538-14. https://doi.org/10.1128/genomeA.01538-14

Giampetruzzi A., Saponari M., Almeida R.P.P., Essakhi S., Boscia D., ... Saldarelli P., 2017. Complete Genome Sequence of the Olive-Infecting Strain *Xylella fastidiosa* subsp. *pauca* De Donno. *Genome Announcements* 5(27): 10.1128/genomea.00569-17. https://doi.org/10.1128/genomea.00569-17

Harper S.J., Ward L.I., Clover G.R.G., 2010. Development of LAMP and Real-Time PCR Methods for the Rapid Detection of *Xylella fastidiosa* for Quarantine and Field Applications. *Phytopathology*® 100(12): 1282–1288. (erratum 2013). https://doi.org/10.1094/PHYTO-06-10-0168

Hodgetts J., Glover R., Cole J., Hall J., Boonham N., 2021. Genomics informed design of a suite of real-time PCR assays for the specific detection of each *Xylella fastidiosa* subspecies. *Journal of Applied Microbiology* 131(2): 855–872. https://doi.org/10.1111/jam.14903

Hopkins D.L., 1989. *Xylella Fastidiosa*: Xylem-Limited Bacterial Pathogen of Plants. *Annual Review of Phytopathology* 27(1): 271–290. https://doi.org/10.1146/annurev.py.27.090189.001415

Huson D.H., Bryant D., 2006. Application of Phylogenetic Networks in Evolutionary Studies. *Molecular Biology and Evolution* 23(2): 254–267. https://doi.org/10.1093/molbev/msj030

Jolley K.A., Bray J.E., Maiden M.C.J., 2018. Open-Access Bacterial Population Genomics: BIGSdb Software, the PubMLST.Org Website and Their Applications. *Wellcome Open Research* 3: 124. https://doi.org/10.12688/wellcomeopenres.14826.1

Krugner R., Sisterson M.S., Chen J., Stenger D.C., Johnson M.W., 2014. Evaluation of Olive as a Host of *Xylella fastidiosa* and Associated Sharpshooter Vectors. *Plant Disease* 98(9): 1186–1193. https://doi.org/10.1094/PDIS-01-14-0014-RE

Loconsole G., Potere O., Boscia D., Altamura G., Djelouah K., … Saponari M., 2014. Detection of *Xylella fastidiosa* in olive trees by molecular and serological methods. *Journal of Plant Pathology* 96(1): 7–14. https://doi.org/10.4454/JPP.V96I1.041

Loureiro T., Serra L., Martins A., Cortez I., Poeta P., 2024. *Xylella Fastidiosa* Dispersion on Vegetal Hosts in Demarcated Zones in the North Region of Portugal. *Microbiology Research* 15(3): 1050–1072. https://doi.org/10.3390/microbiolres15030069

Marco-Noales E., Barbé S., Monterde A., Navarro-Herrero I., Ferrer A., … Roselló M., 2021. Evidence that *Xylella fastidiosa* is the Causal Agent of Almond Leaf Scorch Disease in Alicante, Mainland Spain (Iberian Peninsula). *Plant Disease* 105(11): 3349–3352. https://doi.org/10.1094/PDIS-03-21-0625-SC

Martelli G.P., Boscia D., Porcelli F., Saponari M., 2016. The Olive Quick Decline Syndrome in South-East Italy: A Threatening Phytosanitary Emergency. *European Journal of Plant Pathology* 144(2): 235–243. https://doi.org/10.1007/s10658-015-0784-7

Nunney L., Vickerman D.B., Bromley R.E., Russell S.A., Hartman J.R., … Stouthamer R., 2013. Recent Evolutionary Radiation and Host Plant Specialization in the *Xylella Fastidiosa* Subspecies Native to the United States. *Applied and Environmental Microbiology* 79(7): 2189–2200. https://doi.org/10.1128/AEM.03208-12

Nunney L., Azad H., Stouthamer R., 2019. An Experimental Test of the Host-Plant Range of Nonrecombinant Strains of North American *Xylella Fastidiosa* subsp. *multiplex*. *Phytopathology*® 109(2): 294–300. https://doi.org/10.1094/PHYTO-07-18-0252-FI

Olmo D., Nieto A., Adrover F., Urbano A., Beidas O., ... Landa B.B., 2017. First Detection of *Xylella fastidiosa* Infecting Cherry (*Prunus avium*) and *Polygala myrtifolia* Plants, in Mallorca Island, Spain. *Plant Disease* 101(10): 1820–1820. https://doi.org/10.1094/PDIS-04-17-0590-PDN

Pucci N., Scala V., Cesari E., Crosara V., Fiorani R., … Loreti S., 2023. An Inter-Laboratory Comparative Study on the Influence of Reagents to Perform the Identification of the *Xylella Fastidiosa* Subspecies Using Tetraplex Real Time PCR. *Horticulturae* 9(9): 1053. https://doi.org/10.3390/horticulturae9091053

Purcell A.H., Hopkins D.L., 1996. Fastidious xylem-limited bacterial plant pathogens. *Annual Review of Phytopathology* 34: 131–151. https://doi.org/10.1146/annurev.phyto.34.1.131

Saponari M., Boscia D., Nigro F., Martelli G.P., 2013. Identification of Dna Sequences Related to *Xylella fastidiosa* in Oleander, Almond and Olive Trees Exhibiting Leaf Scorch Symptoms in Apulia (southern Italy). *Journal of Plant Pathology* 95(3): 668. https://doi.org/10.4454/JPP.V95I3.035

Saponari M., Boscia D., Altamura G., Loconsole G., Zicca S., ... Martelli G.P., 2017. Isolation and Pathogenicity of *Xylella Fastidiosa* Associated to the Olive Quick Decline Syndrome in Southern Italy. *Scientific Reports* 7(1): 17723. https://doi.org/10.1038/s41598-017-17957-z

Saponari M., D'Attoma G., Kubaa R.A., Loconsole G., Altamura G., … Boscia D., 2019. A new variant of *Xylella fastidiosa* subspecies *multiplex* detected in different host plants in the recently emerged outbreak in the region of Tuscany, Italy. *European Journal of Plant Pathology* 154(4): 1195–1200. https://doi.org/10.1007/s10658-019-01736-9

Schaad N.W., Postnikova E., Lacy G., Chang C.J., 2004. *Xylella fastidiosa* subspecies: *X. fastidiosa* subsp *piercei*, subsp. nov., *X. fastidiosa* subsp. *multiplex* subsp. nov., and *X. fastidiosa* subsp. *pauca* subsp. nov. *Systematic and Applied Microbiology* 27(3): 290-300.

Trkulja V., Tomić A., Iličić R., Nožinić M., Milovanović T.P., 2022. *Xylella Fastidiosa* in Europe: From the Introduction to the Current Status. *The Plant Pathology Journal* 38(6): 551–571. https://doi.org/10.5423/PPJ.RW.09.2022.0127.

Wells J.M., Raju B.C., Nyland G., Lowe S.K., 1981. Medium for Isolation and Growth of Bacteria Associated with Plum Leaf Scald and Phony Peach Diseases. *Applied and Environmental Microbiology* 42(2): 357–363. https://doi.org/10.1128/aem.42.2.357-363.1981

Wells J.M., Raju B.C., Hung H.Y., Weisburg W.G., Mandelco-Paul L., Brenner D.J., 1987. *Xylella fastidiosa* gen. nov., sp. nov: Gram-Negative, Xylem-Limited, Fastidious Plant Bacteria Related to *Xanthomonas* spp. *International Journal of Systematic and Evolutionary Microbiology* 37(2): 136–143. https://doi.org/10.1099/00207713-37-2-136

Yuan X., Morano L., Bromley R., Spring-Pearson S., Stouthamer R., Nunney L., 2010. Multilocus Sequence Typing of *Xylella fastidiosa* Causing Pierce's Disease and Oleander Leaf Scorch in the United States. *Phytopathology* 100(6): 601–611. https://doi.org/10.1094/PHYTO-100-6-0601