DOI: 10.1111/epp.13023

ORIGINAL ARTICLE

The first surveillance report for *Xylella fastidiosa* in olive and stone fruit orchards in Palestine

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Abstract

Xylella fastidiosa has been identified as the causal agent of several horticultural plant diseases that have resulted in major economic and cultural heritage losses. In the last decade, *X. fastidiosa* emerged as a destructive phytopathogen on olive trees in the Apulia region, Italy, prompting widespread surveillance throughout the Mediterranean basin. The present paper reports monitoring efforts for *X. fastidiosa* in Palestine on olive for 5 years (2017–2022) and 1 year (2022) on stone fruit trees, as a result of international collaboration projects. No signs of olive quick decline syndrome were observed on olive trees in all the Palestinian olive-growing lands. This observation was confirmed by molecular tests using LAMP technology and PCR. In addition, 500 leaf samples from stone fruit trees (almond, apricot, peach, nectarine and plum) were tested using LAMP and PCR. All of these samples were negative for *X. fastidiosa*, even though few of the samples from almond trees in Idna (Hebron governorate) and apricot trees in Bal'a (Tulkarm governorate) showed leaf scorch-like symptoms. This study provides confirmation that these important horticultural crops in Palestine (olive and stone fruits) are still free of *X. fastidiosa*. Preventive measures and surveillance of these and other horticultural crops such as grapevine and citrus trees are strongly recommended.

KEYWORDS
almond leaf scorch disease, LAMP, olive quick decline syndrome, *Xylella fastidiosa*

Premier rapport de surveillance de *Xylella fastidiosa* dans des vergers d'oliviers et d'arbres fruitiers en Palestine

Xylella fastidiosa a été identifié comme l'agent causal de plusieurs maladies de plantes horticoles qui ont entraîné des pertes majeures du patrimoine économique et culturel. Au cours de la dernière décennie, *X. fastidiosa* est apparu comme un agent phytopathogène destructeur des oliviers dans la région des Pouilles (Italie), provoquant une surveillance généralisée dans tout le bassin méditerranéen. Cet article rend compte des efforts de suivi de de *X. fastidiosa* en Palestine pendant 5 ans (2017–2022) sur les oliviers, et de 1 an (2022) sur les arbres fruitiers à noyaux, grâce à des projets de collaboration internationale. Aucun signe de syndrome de déclin rapide des oliviers n'a été observé dans toutes les terres oléicoles palestiniennes. Cette observation a été confirmée par des tests moléculaires utilisant la technologie LAMP et la PCR. 500 échantillons de feuilles de Prunus (amande, abricot, pêche, nectarine et prune) ont également été testés à l'aide de ces technologies. Tous les échantillons étaient négatifs pour *X. fastidiosa*, même si certains de ces échantillons provenant d'amandiers d'Idna (gouvernorat d'Hébron) et d'abricotiers de Bal'a (gouvernorat de Tulkarem) présentaient des symptômes de brûlure des feuilles. Cette étude confirme que ces cultures horticoles importantes

en Palestine (olives et fruits à noyau) sont toujours exemptes de *X. fastidiosa*. Des mesures préventives et une surveillance de ces cultures horticoles, ainsi que d'autres cultures comme la vigne et les agrumes sont fortement recommandées.

Первое сообщение о надзоре за *Xylella fastidiosa* в садах маслины и косточковых культур в Палестине

Xylella fastidiosa была идентифицирована как возбудитель нескольких болезней садовых растений, которые привели к крупным потерям для экономики и культурного наследия. В последнее десятилетие *X. fastidiosa* стала разрушительным фитопатогеном для оливковых деревьев в регионе Апулия (Италия), что стало поводом для начала широкомасштабного надзора во всем Средиземноморском бассейне. В настоящей статье сообщается о 5-летнем мониторинге (2017–2022 гг.) *X. fastidiosa* на оливковых деревьях и 1-летнем мониторинге (2022 г.) на косточковых фруктовых деревьях в результате реализации проектов международного сотрудничества в Палестине. Никаких признаков синдрома быстрого увядания оливок (olive quick decline syndrome; OQDS) не наблюдалось на оливковых деревьях на всех палестинских землях, где выращиваются оливки. Это наблюдение было подтверждено молекулярными тестами с использованием технологии LAMP и ПЦР (PCR). Кроме того, с помощью LAMP и ПЦР было протестировано 500 образцов косточковых фруктов (миндаль, абрикос, персик, нектарин и слива). Все они были отрицательными относительно *X. fastidiosa*, хотя некоторые образцы с миндальных деревьев в г. Идна (Idna; провинция Хеврон) и абрикосовых деревьев в г. Бала (Bal'a; провинция Тулькарм) показали симптомы, похожие на ожог листьев. Проведенное исследование подтверждает, что такие важные садовые культуры в Палестине, как маслина и косточковые, по-прежнему свободны от *X. fastidiosa*. Настоятельно рекомендуются профилактические меры и наблюдение за этими и другими садовыми культурами (виноград и цитрусовые).

1 | INTRODUCTION

Xylella fastidiosa was recognized in recent decades as the causal agent of Pierce's disease in grapevines, and as an emerging phytopathogen on olive trees in Italy in 2013 (Almeida, 2016; EPPO, 2023; Martelli et al., 2016; Rapicavoli et al., 2018; Saponari et al., 2013). It was also associated with several plant diseases worldwide, causing major economic and cultural heritage losses. *X. fastidiosa*, a gram-negative bacterium within the family Lysobacteraceae (Gammaproteobacteria), is an obligatory phytopathogen and colonises plant xylem (Wells et al., 1987). It has a wide range of host plants. It was reported as a highly destructive pathogen that can cause plant wilting, decline and death. In general, leaf scorch can be observed in water-stressed plants (Daugherty et al., 2011; Marucci et al., 2005; McElrone et al., 2001).

So far, four subspecies of *Xylella fastidiosa* have been reported in different hosts: *Xylella fastidiosa* subsp. *fastidiosa*, associated with disease of almond, citrus and grapevines; *Xylella fastidiosa* subsp. *pauca*, associated with disease of citrus, coffee and olive; *Xylella fastidiosa* subsp. *multiplex*, which causes leaf scorch diseases in a wide range of trees and perennial plants; and *Xylella fastidiosa* subsp. *sandyi*, associated with oleander leaf scorch. Recently, a fifth putative subspecies (*X. fastidiosa* subsp. *morus*) has been proposed, the same as a newly described *Xylella* species denoted *Xylella taiwanensis* (Su et al., 2016). *Xylella fastidiosa* was reported to cause phoney peach disease in the Southern United States, and bacterial leaf scorch, citrus variegated chlorosis disease, oleander leaf scorch, and Pierce's disease in grapevines in Brazil. It was found to cause olive quick decline syndrome in olive trees in the Salento area of Southern Italy (Saponari et al., 2013).

Unlike plant diseases, *X. fastidiosa*'s connection with hemipteran vectors (the sole mechanism for natural dissemination) is not determined by particular plant–pathogen combinations. Research has shown that insect vectors may disseminate all genotypes of *X. fastidiosa* (Almeida & Nunney, 2015). *X. fastidiosa* vectors include sharpshooter leafhoppers (Cicadellidae subfamily Cicadellinae) and spittlebugs (Cercopoidea, families Aphrophoridae, Cercopidae, and Clastopteridae) (Sicard et al., 2018). Other xylem-feeding insects might also be regarded as potential vectors (Purcell & Hopkins, 1996). Leafhoppers (Cicadellidae) of the subfamily Cicadellinae (sharpshooters) and spittle bugs or froghoppers (Cercopidae) are by far the most frequent vector species found in *X. fastidiosa*'s native area in North America. Meanwhile *Cicadella viridis* (Cicadellinae) and the meadow spittle bug, *Philaenus spumarius* (Cercopidae), are common and widespread in Central and Southern Europe (Godefroid et al., 2022).

TABLE 1 Number of samples collected from olive trees and stone fruit orchards in the West Bank during the field surveys.

Governorate	Olive growing area (hectare)	Number of samples from olive trees (2017–2022)	Selected orchards (for stone fruit)	Number of stone fruit samples (2022)
Jenin	15 000	20	4	78
Nablus	13 000	20	–	–
Tulkarm	12 000	20	3	60
Qalqilia	5500	20	–	–
Tubas	1800	5	4	100
Sulfite	5800	20	–	–
Ramallah	10 000	20	3	62
Hebron	6300	20	5	100
Jerusalem	1200	10	–	–
Bethlehem	2900	5	6	100
Total	73 500	160	25	500

One of the main factors influencing *X. fastidiosa* is the climate. It affects pathogen transmission, plant-pathogen infection dynamics, host plant growth conditions and distribution, as well as the dynamics of vector populations. Higher temperatures are generally associated with higher feeding rates (Son et al., 2010), sharpshooter vector survival (Son et al., 2009), transmission efficiency (Daugherty et al., 2009), *X. fastidiosa* multiplication rate (Feil & Purcell, 2001), shorter plant incubation and latency periods (Daugherty et al., 2017), and greater persistence of infections in plants (Lieth et al., 2011). Likewise, greater temperatures with higher precipitation are linked to increased densities of vector populations in the field, and precipitation is positively correlated with the establishment or spread of *X. fastidiosa* (Bosso et al., 2016a, 2016b).

Reliable detection techniques are needed for detection of the pest during monitoring. PCR-based approaches (conventional PCR, real-time quantitative PCR) and LAMP tests are often more sensitive than serological methods, with high specificity and strong selective capacities for detecting tiny amounts of bacteria in plants and insect vectors (Guan et al., 2013; Huang, 2009; Huang et al., 2006; Minsavage et al., 1994; Oliveira & de Lencastre, 2002; Rodrigues et al., 2003). Several PCR tests can detect *X. fastidiosa* DNA after purification. However, contamination with PCR inhibitors is a common issue in PCR tests of environmental tissue samples (Bextine et al., 2004; Chen et al., 2008; Fatmi et al., 2005).

In 2017, the Food and Agriculture Organization and the governments of Palestine, Algeria, Egypt, Lebanon, Libya, Morocco and Tunisia launched project TCP/RAB/3601, entitled "Strengthening capacities to prevent the introduction and spread of *X. fastidiosa* – Olive Quick Decline Syndrome in NENA countries" to enhance phytosanitary measures and increase awareness of *X. fastidiosa* diseases. Furthermore, an agreement was reached to carry out applied agricultural research with the goal of providing all parties involved in the stone fruit industry (almond, apricot, peach, nectarine and plum) with accurate, dependable and efficient means of detecting *X. fastidiosa*.

In this paper, we report the result of 5 years of efforts (2017–2022) to monitor *X. fastidiosa* in Palestine in several governorates of the West Bank that was carried out on olive and stone fruits,[1] highly important horticultural crops in Palestine, as a result of such collaboration.

2 | MATERIALS AND METHODS

2.1 | Field inspection and sample collection

Field surveys were conducted in orchards of stone fruits (almond, apricot, peach, nectarine and plum) and for olive trees located in the West Bank during the years 2017–2022. These orchards were distributed over the governorates of West Bank as seen in Table 1. A total of 670 samples were collected from petioles and leaves for laboratory tests. The samples were collected from trees that appeared to have nonspecific scorch-like symptoms (which could have been indicative of *X. fastidiosa*), as well as those that did not.

2.2 | Molecular detection and analysis

2.2.1 | DNA extraction and Conventional PCR

DNA was extracted from the collected leaf tissues (petioles, midribs and basal leaf portions) following the cetyltrimethyl ammonium bromide (CTAB)-based

[1]For the purpose of this paper the categories for stone fruit used are according to the EPPO non-taxonomic Codes for stone fruit crops and therefore olive is not considered a stone fruit.

extraction. Briefly, 500 mg of leaf tissues was crushed in liquid nitrogen then extracted with 5 mL of CTAB extraction buffer in accordance with the EPPO diagnostic Standard (EPPO, 2023). One millilitre of extract was transferred into a 1.5-mL micro-centrifuge tube and the sample was heated at 65°C for 30 min before centrifugation at 16 000*g* for 5 min. One millilitre of the supernatant was mixed with the same volume of chloroform:isoamyl alcohol (24:1) in a new 2-mL micro-centrifuge tube, mixed well by shaking and centrifuged at 16 000*g* for 10 min. Then 700 µL of the supernatant was mixed with approximately 0.7 volume of cold 2-propanol before being incubated at −20°C for 20 min. Centrifugation of the samples was performed at 16 000*g* for 20 min and the pellets were collected and washed with 1 mL of 70% ethanol. An additional centrifugation at 16 000*g* for 10 min and decantation in 70% ethanol could be performed. Then the air-dried pellet was re-suspended in 100–150 µL of TE buffer or nuclease-free water.

The PCR test was carried out according to the EPPO Diagnostic Standard for *X. fastidiosa* based on Minsavage et al. (1994). The forward primer RST31 (GCGTTAATTTTCGAAGTGATTCGATTGC) and the reverse primer RST33 (CACCATTTCGTATCCCGGTG) were used, which code for a 733-bp sequence of the bacterial *rpoD* gene located at the 30 gene end coding for an RNA polymerase sigma-70 factor. Then, 10 ng of purified DNA template was mixed with the GoTaq® DNA Polymerase (Promega) PCR reaction kit with the above-mentioned primers. Positive amplification control (PAC) was provided courtesy from the Istituto Agronomico Mediterraneo di Bari Italy, and water was used as negative amplification control (NAC). PCR parameters were in 40 cycles of DNA amplification previously heated at 95°C for 1 min. Each cycle consisted of 95°C for 30 s, followed by 55°C for 30 s and 72°C for 45 s extension. The final extension step was subjected to 72°C for 5 min. The PCR products were visualized in 2% agarose gel under UV light after GelRed® Nucleic Acid Gel (Biotium) staining.

2.2.2 | Real-time LAMP test

The real-time LAMP test was performed using the ICGENE small system, following the EPPO diagnostic Standard (EPPO, 2023). An Enbiotech Rapid DNA extraction kit and a LAMP mix kit were used. The kits are designed to extract DNA from plant material (petioles and twigs) and amplify the *X. fastidiosa* target sequence.

Briefly, 0.5 g of plant materials (petioles and main veins) was DNA extracted in 5 mL of extraction buffer (1% Triton x-100, 20 mM Tris–HCl, 20 mM EDTA), then placed into an extraction bag (Bioreba), homogenized or crushed with a hammer and incubated at room temperature for 15 min before 1 mL of the extracts was collected in a sterile tube. Next, 5 µL of the extract was diluted in 200 µL of extraction buffer, vortex mixed and incubated in a LAMP device at 65°C for 10 min.

The following step involved 2.5 µL of extracted DNA for each tube for the amplification process. The tubes were directly placed into the ICGENE mini device and started directly in the pre-set DNA extraction program. The LAMP reaction was prepared in a 0.2-mL safe-lock tube and used the same extraction procedure, adding 5 µL of primer mix (1 µM of each internal primer [FIP and BIP], 0.1 µM of each external primer [F3 and B3] and 0.5 µM of each loop primer [LF and LB]). The primers mix was provided by Enbiotech Xylella Screen Glow as a separate strip and ready to use (Yaseen et al., 2017). For each tube, 22.5 µL of LAMP master mix, 30 µL of mineral oil and 2.5 µL of extracted DNA were added. PAC and NAC controls were included in the analysis. The tubes were directly placed into the LAMP device, and the number of each sample was associated with the tablet real-time-LAMP software. Results were automatically viewed on the screen at the end of amplification.

3 | RESULTS

3.1 | Olive

Field surveys over 73 500 hectares of olives orchards distributed over 10 governorates of West bank were carried out during 2017–2021 and showed no remarkable symptoms of olive quick decline syndrome. The only (non-specific) symptoms observed were dried branches and leaves on some trees. Laboratory testing was therefore used to test for the bacterium's presence. LAMP as well as conventional PCR detection methods (Figure 1) were applied to 160 samples gathered from olive orchards and confirmed negative results for *X. fastidiosa* in tested olives trees growing in Palestinian lands.

3.1.1 | Stone fruits

Surveys of stone fruit orchards were carried out in the West Bank, covering more than 100 stone fruit orchards in the main governorates where stone fruits flourish. Leaf scorch, a *Xylella fastidiosa* disease-like symptom, was observed on some almond trees in Idna in the Hebron governorate and on apricot trees in Bal'a at Tulkarm orchards (Figure 2). This symptoms involved browning of the leaf tissues, including leaf margins and tips, and occasional yellowing.

Conventional PCR and LAMP tests were also used. None of the (500) samples from stone fruit tested positive for *X. fastidiosa*. This study confirmed that stone fruit orchards in Palestinian lands are free from *X. fastidiosa*.

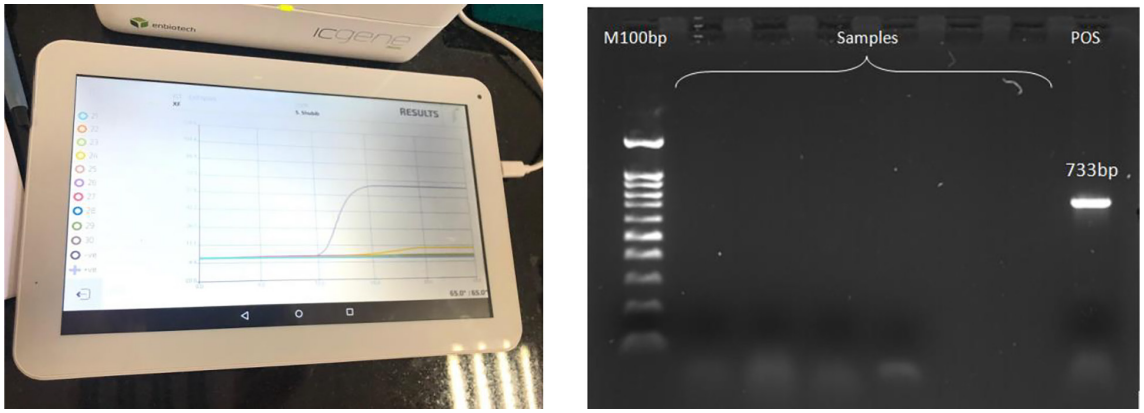

FIGURE 1 Sample processing for real-time LAMP detection (left) and conventional PCR using the forward primer RST31 and the reverse primer RST33 with band size 733 bp (right). Negative results were seen for all the samples. POS, positive amplification control.

FIGURE 2 Leaf scorch was seen on surveyed almond trees in Idna, Hebron governorate and on apricot trees in orchards in Bal'a, Tulkarm.

4 | DISCUSSION

Field surveys in Palestinian orchards exhibited non-specific disease symptoms of *X. fastidiosa* on olives, almond and apricots, and these samples all tested negative for the bacterium using molecular tests. *X. fastidiosa* is an important plant pathogen infecting several economic important crops (Bosso et al., 2016a, 2016b; Loconsole et al., 2014; Martelli et al., 2016; Purcell & Hopkins, 1996; Saponari et al., 2013, 2014). There has been great concern about its spread in the Mediterranean basin region, therefore monitoring its spread is considered to be of the utmost importance (White et al., 2017). *X. fastidiosa* has spread to several other European regions (southern France, the Balearic Islands (Spain) and Germany) (EPPO Global Database, 2024). In the Middle East, it was reported in Iran (Amanifar et al., 2014), Northern Israel (Zecharia et al., 2022) and recently in Lebanon (Choueiri et al., 2023). This study provided research-based evidence that *X. fastidiosa* was absent in the two tested crops (stone fruits and olives) in Palestine. This information is critical for regional biosecurity and can guide surveillance efforts. Knowing that the bacteria has a wide host range, continuous monitoring on those crops and other fruit trees crops, such as grapevine and citrus, is highly recommended. Phytosanitary measurements, including *X. fastidiosa* surveillance, are advisable as the sole effective preventive measures.

ACKNOWLEDGEMENTS

The authors would like to thank both An-Najah National University and National Agricultural Research Centers in Palestine for the use of their laboratories.

FUNDING INFORMATION

This research was funded by Food and Agriculture Organization projects in collaboration agreements with Ministry of Agriculture and An-Najah National University.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

Adequate and clear descriptions of the applied materials and tools are provided in the materials and method section of manuscript. In addition, the obtained data is clearly justified from the figures and tables in the manuscript.

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How to cite this article: Alabdallah, O., Alkowni, R., Radwan, J., Ghzayal, S., Jaradat, S., Shubib, S. et al. (2024) The first surveillance report for *Xylella fastidiosa* in olive and stone fruit orchards in Palestine. *EPPO Bulletin*, 54, 236–242. Available from: https://doi.org/10.1111/epp.13023