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Short Notes

Outbreak of *Xylella fastidiosa* subsp. *pauca* ST53 affecting wild and cultivated olive trees on the island of Mallorca, Spain

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Summary. The Balearic Islands have emerged as a hotspot for the invasive plant pathogen *Xylella fastidiosa* (*Xf*). Since 2016, the *Xf* subsp. *fastidiosa* and *multiplex* have been detected causing almond leaf scorch and Pierce's disease on the island of Mallorca, Spain, and a new sequence type (ST), ST80, of subsp. *pauca* is infecting wild and cultivated olive trees on the island of Ibiza. In addition, *Xf* subsp. *multiplex* ST81 is widespread in scrublands, and causes mild, sub-lethal dieback of wild olive trees in Menorca and Mallorca. A new outbreak is here reported of the *Xf* subsp. *pauca* in the municipality of Sencelles in the centre of Mallorca island. In early 2024, dying patches were observed in wild olive trees (*Olea europaea* var. *europaea* subsp. *sylvestris*). Samples from these trees were *Xf*-positive in different qPCR tests, and the pathogen was subsequently identified as belonging to ST53 of subsp. *pauca*, the same genetic variant responsible for olive quick decline syndrome in Apulia, Italy. More than 184 plants of eight hosts have tested positive for subsp. *pauca* within a demarcation zone of approx. 1 km radius. The identified host species include 124 wild olive trees, 40 cultivated olive trees, nine *Rhamnus alaternus*, six *Nerium oleander*, two *Lavandula angustifolia*, one *Laurus nobilis*, one *Lavandula dentata* and one *Polygala myrtifolia*. Of particular concern is detection of co-infections by *Xf* subsp. subsp. *pauca* and *multiplex* on plants from natural settings (wild olives, *L. dentata* and *R. alaternus*), posing potential risk of genetic recombinations. Intensive surveys are being carried out to contain the spread of ST53, and infected plants have been destroyed in the demarcated zone.

Keywords. Genetic diversity, olive quick decline syndrome, invasive pathogens, Multi-Locus Sequence Typing (MLST), disease outbreak.

INTRODUCTION

The vector-borne plant pathogenic bacterium *Xylella fastidiosa* (*Xf*) has recently emerged as a threat to agriculture in southern Europe. Following its first detection in Apulia (Italy) in 2013, associated with the rapid decline syndrome of olive trees (Saponari *et al.*, 2013), the European Union (EU) took decisive action with mandatory surveys to contain possible spread of the pathogen within the EU (Reg. EU 2016/2031). The three main subspecies of *Xf* have since then been detected, and their establishment confirmed, in several southern European countries, including the island of Corsica and the Balearic Islands (Saponari *et al.*, 2013; Denance *et al.*, 2017; Landa *et al.*, 2020; Olmo *et al.*, 2017; Carvalho-Luis *et al.*, 2022).

The Balearic Islands have become a hotspot for establishment of genetic diversity of this pathogen, which originated from North, Central and South America. Since 2016, the three main subspecies of *Xf*, *fastidiosa*, *multiplex*, and *pauca*, have been detected, along with unique genotypes such as the sequence type (ST) 81 (from subsp. *multiplex*) on the islands of Mallorca and Menorca, and the ST80 (from subsp. *pauca*) on the island of Ibiza (Olmo *et al.*, 2021). Subspecies *fastidiosa*, and in particular ST1, is only known on Mallorca island, where it caused severe outbreaks of Pierce's disease of grapevines and leaf scorch of almond (Moralejo *et al.*, 2019; Moralejo *et al.*, 2020). Some evidences suggest that the genotypes responsible for Pierce's disease (ST1) and almond leaf scorch (ST1 and ST81) in Mallorca were likely a single introduction via infected plant material from California, in approx. 1993 (Moralejo *et al.*, 2020; Velasco-Amo *et al.*, 2022).

METHODS

Early in 2024, plant health inspectors in the Balearic Islands noticed an unusually severe decline in a stand of wild olive trees (*Olea europaea* subsp. *europaea* var. *sylvestris*) (Figure 1). Samples from different tree branches were collected, were pooled, and then sent to the Balearic Islands Official Plant Health Laboratory (LOSVIB) for analyses. Sample extracts were prepared by homogenizing 0.5 g of leaf petioles in an extraction bag (BIOREBA®) and grinding them in 5 mL (1:10 weight:volume) of phosphate buffered saline (PBS) using a semi-automated homogenizer (Homex 7; BIOREBA®).

Total DNA extraction was carried out using 200 µL of each sample extract, and the EZNA HP Plant Mini kit (Omega-Biotek), which employs a CTAB-based method,



Figure 1. A group of wild olive trees with severe symptoms of quick decline syndrome. This photograph was taken at focus 0 near the municipality of Sencelles, Mallorca Island, Spain, where *Xylella fastidiosa* subsp. *pauca* ST53 was found for the first time in January 2024.

as described in the EPPO protocol (EPPO, 2023). The DNA extracts were subsequently tested for the presence of *Xf* by quantitative real-time PCR (qPCR), according to Harper *et al.* (2010). Aliquots of DNA from the *Xf* positive samples from the wild olive tree were sent to the Institute for Sustainable Agriculture (IAS-CSIC), Córdoba, Spain, for ST determination, which was carried out by conventional MultiLocus Sequence Typing (MLST) analysis (Yuan *et al.*, 2010). A nested-MLST (Cesbron *et al.*, 2020) analysis was used when not enough amplification product for direct sequencing was obtained when using the conventional MLST approach.

RESULTS AND DISCUSSION

MLST analyses identified the presence of alleles *leuA-7*, *petC-6*, *malF-16*, *cysG-24*, *holC-10*, *nuoL-16* and *glT-14* associated with *Xf* subsp. *pauca* ST53 in the affected wild olive tree. Additionally, the qPCR protocol of Dupas *et al.* (2019) validated the diagnosis as *Xf* subsp. *pauca*.

After confirming the diagnosis and recognizing the potential devastating impact if this subspecies were to spread, the phytosanitary authority of the Balearic Government implemented a targeted action plan. This included increasing surveys and intensive sampling in the area of the new outbreak. Additional measures were also established to those already in place from the current Regulation (EU) 2016/2031. These included analyses to determine the subspecies present in all the *Xf*-positive samples, in collaboration with the Valencian Institute of Agricultural Research (IVIA), using the qPCR protocols developed by Dupas *et al.* (2019) and Hodgetts *et al.* (2021).

Since the first detection in January 2024 and up to December 2024, a total of 1,328 samples from within the disease outbreak area have been analyzed. This has shown that 184 plants were infected by *Xf* subsp. *pauca*,

Table 1. Host plant species, numbers of plants analysed, and numbers infected by different subspecies *Xylella fastidiosa* (*Xf*), in single or in mixed infections, in the outbreak of subspecies *pauca* around the locality of Sencelles (Mallorca island, Spain)

Hosts	Total analyzed	<i>Xf</i> positive ^a	%	<i>Xylella fastidiosa</i> subspecies ^b					
				<i>pauca</i>	<i>mutlplex</i>	<i>fastidiosa</i>	<i>pauca/multiplex</i>	<i>pauca/fastidiosa</i>	<i>multiplex/fastidiosa</i>
<i>Laurus nobilis</i>	6	2	33	1	1				
<i>Lavandula angustifolia</i>	8	8	100	2	6				
<i>Lavandula dentata</i>	6	5	83		4		1		
<i>Nerium oleander</i>	22	6	27	6					
<i>Olea europaea</i> var. <i>europaea</i> subsp. <i>europaea</i>	57	41	72	37	1		3		
<i>Olea europaea</i> var. <i>europaea</i> subsp. <i>sylvestris</i>	929	155	17	106	29	2	18		
<i>Polygala myrtifolia</i>	1	1	100				1		
<i>Prunus dulcis</i>	8	1	13		1				
<i>Rhamnus alaternus</i>	190	26	14	5	8	6	3	1	2
<i>Salvia rosmarinus</i>	23	19	83		19				
Thirteen other plant species	78	-							
Totals	1328	264	19.88	157	69	8	26	1	2

^a Diagnoses performed using the qPCR test of Harper *et al.* (2010).

^b Subspecies assignment based on the qPCR tests as described by Dupas *et al.* (2019) and Hodgetts *et al.* (2021).

and eight different hosts tested positive for *Xf* subsp. *pauca*, from within a demarcated zone (approx. 1 km radius) around the first disease detection. The infected host species include 124 wild olive trees, 40 cultivated olive trees, nine *Rhamnus alaternus* plants, six of *Nerium oleander*, two of *Lavandula angustifolia*, and one each of *Laurus nobilis*, *Lavandula dentata* and *Polygala myrtifolia* (Table 1).

Despite the numerous cases of *Xf* subsp. *pauca* infections detected (Table 1), all cases analyzed by MLST, up to present, and within the initial focus of the outbreak belong to the ST53 (Figure 2). However, given that *Xf* subsp. *fastidiosa* and *Xf* subsp. *multiplex* were already established in the area, it was not surprising to find some mixed infections by these two subspecies in some individual plants. Eleven percent of *Xf*-positive samples were of mixed infections, with 26 cases of subspp. *pauca/multiplex*, one of subspp. *pauca/fastidiosa* and two of subspp. *multiplex/fastidiosa* (Table 1). The multiple infections probably may increase risk of genetic recombinations in the pathogen and the formation of new sequence types with different virulence and/or host ranges (Potnis *et al.*, 2019).

Although it is premature to draw definitive conclusions, the ST53 strain identified in Mallorca exhibited a level of virulence comparable to that associated with the olive quick decline syndrome observed in Apulia, based on the observed higher severity of symptoms in both wild and cultivated olive trees, compared to those induced by *Xf* subsp. *multiplex* ST81. Efforts are current-

ly underway (December 2024) to isolate the bacterium, to enable complete genome sequencing and carry out comparative analysis of the pathogen's compete genome with those of ST53 strains from Apulia.

A new procedure has been established based on targeted sequence capture enrichment of *Xf* in combination with high-throughput sequencing using the Illumina platform. This is being implemented to provide additional genomic information (Velasco-Amo *et al.*, 2021). Initial results based on approx. 126 genes have shown close genetic relatedness of the ST53 strain infecting the wild olive in Mallorca with all other ST53 strains isolated from Costa Rica and Italy (M.P. Velasco-Amo and B.B. Landa; *unpublished results*).

A complicating factor in containment of ST53 strains is the large population of wild olive trees throughout Mallorca, which may facilitate transmission of the pathogen to cultivated olive trees. Tight wild olive tree population networks can be dense reservoirs of *Xf*, and provide effective dispersal pathways to commercial olive plantations. It is also unclear whether previous infections of wild olive trees by ST81 of *Xf* subsp. *multiplex* will have influence subsequent infections by ST53. It is estimated that more than 50% of wild olive trees are infected with ST81 (Olmo *et al.*, 2021), so any interaction between ST53 and ST81 in co-infections, regardless of its intensity, could have important epidemiological implications (Jeger and Bragard, 2019).

Epidemiological models have shown that the most effective strategy for preventing spread of *Xf* is to decel-

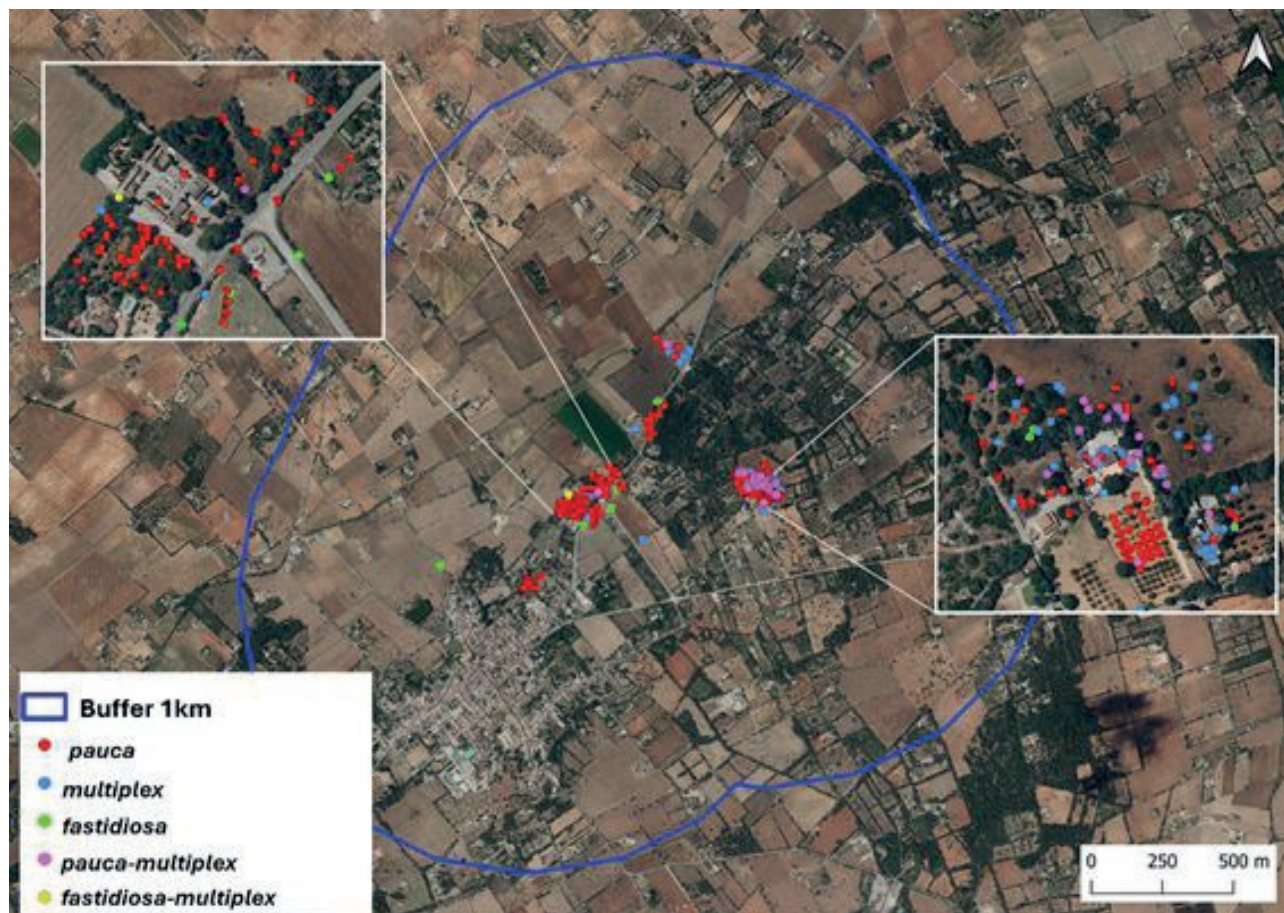


Figure 2. Map of the outbreak focus of *Xylella fastidiosa* subsp. *pauca* ST53 in the municipality of Sencelles, near the centre of Mallorca Island, Spain. The *Xf pauca*-positive plants were found within a radius of < 1 km. Both squares with amplified views show *Xf*-positive plants within the natural vegetation, formed mainly by wild olive trees near farms where other *Xf* subsp. *pauca* ST53 positive plants were found within olive orchards. The positive samples of *Xf* subsp. *pauca* ST53 plants were mixed with other infected with subsp. *multiplex*, and to a lesser extent with subsp. *fastidiosa*. In a few cases, co-infections by *Xf* subsp. *pauca* ST53 and subsp. *multiplex* were found in the same plant (Table 1).

erate transmission chains (White *et al.*, 2020; Giménez-Romero *et al.*, 2023). Accordingly, in Mallorca, the decision has been taken to eradicate infected plants wherever feasible. Although this is a difficult and arguably unattainable objective, the attempt can have significant long-term impacts. It is anticipated that populations of the principal insect vector of *Xf*, *Philaenus spumarius*, will progressively decline in Mallorca in response to rising temperatures associated with climate change (Giménez-Romero *et al.*, 2024). Unsuitable conditions for the vector may become significant in a period of approx. 15 to 20 years. Climate change could be beneficial for containing the outbreak, provided that exponential infection rates can be reduced. Appropriate implementation of these actions will alleviate the economic consequences of *Xf* for the Mallorcan olive production sector in the medium term.

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