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A decade of monitoring surveys for *Xylella fastidiosa* subsp. *pauca* in olive groves in Apulia (Italy) reveals a low incidence of the bacterium in the demarcated areas

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Abstract

In October 2013, *Xylella fastidiosa* subsp. *pauca* (Xfp) was reported as being associated with a decline of olive groves in Salento (Apulia, Italy) and named as "olive quick decline syndrome" (OQDS). Due to its quarantine status, a series of mandatory actions were established in the Apulia region to impede its further spread in the territory. Upon the demarcation of "containment" and "buffer" zones, monitoring surveys were carried out in these areas for sampling olive trees and other host plants that showed symptoms putatively attributable to OQDS. The eradication of plants that tested positive for Xfp, including also all the asymptomatic ones in a radius of 100 or 50 m, was performed over 10 years. An analysis, based on the official data of such surveillance activities over the period of a decade, was performed. The data show that the Xfp incidence in the "containment" and "buffer" zones is very low, especially during the last three campaigns from 2020–2021 to 2022–2023, when the bacterium was detected in a range of 0.06%–0.70% of the sampled plants. In most of the sampled trees that showed symptoms of OQDS, the bacterium was not detected. Based on these data and according to epidemiological models that verified the negligible role of asymptomatic olive trees in the spreading of OQDS, we propose to eliminate the rule requiring the uprooting of all host plants that surround one Xfp-positive tree in a radius of 50 m. Such an implementation could save many healthy centennial and monumental olive trees and the remarkable landscape that they contribute to.

KEYWORDS
centennial olive trees, epidemiology, olive quick decline syndrome, plant eradication

1 | INTRODUCTION

In Italy, the Apulia region is the major producer of olive oil, with a large surface area dedicated to olive orchards. The olive oil is also obtained from orchards containing centuries-old trees that, in many areas, characterize the landscape and the environment. Remarkably, only a fifth of the olive trees in the region are <50 years old (Ciervo, 2016). In 2010, olive growing contributed to 13% of the value of the agricultural production of the region, with an incidence of 26% on the value of Italian olive production. Apulian olive growing is characterized by the wide number of olive producers (Ciervo, 2020). Olive production in the Salento area dates back to the Bronze Age and, on account of its wide presence, has always contributed to the immeasurable heritage for the whole area, together with the many hypogea oil mills (Calabrese et al., 2012; Scortichini, 2020).

The main characteristics of this particular agro-ecosystem include the majestic beauty of the many centennial trees scattered throughout the whole area and the traditional way of cultivation of the olive orchards that respect the natural shape of the tree. The "Piana degli olivi monumentali" (a vast area located on the Adriatic coast between the province of Brindisi and Bari) has inestimable value, and it has been included in the National Register of Historical Rural Landscapes of the Italian Ministry of Agriculture, Food Sovereignty and Forests and has been proposed to be included in the UNESCO world heritage. In this area, more than 250,000 olive trees are present, of which the centennial ones are predominant, and the occurrence of many trees older than 1000 years should also be noted. This area portrays a relevant and peculiar landscape unique to the Mediterranean Basin (Calabrese et al., 2012; Scortichini, 2020).

These trees also represent a real ecological safeguard considering that, due to the extension of their canopy, which can sometimes reach 10 m in diameter, they protect the soil from water evaporation (triggered by high summer temperatures), thus ensuring an important hydrogeological and microclimatic balance and facilitating, for example, horticulture in the shade of their canopy even during the summer. The traditional olive groves constitute an agroecosystem that, as such, also constitutes a buffer system that protects the surrounding territory from the negative effects of intensive agriculture (Calabrese et al., 2012; Di Rienzo et al., 2018; Perrino & Calabrese, 2014).

The first *Xylella fastidiosa* outbreak was reported in the Gallipoli area in 2013 (Saponari et al., 2013) on about 8000–10,000 ha of olive groves. However, the olive tree dieback was noticed in the Gallipoli area 5–6 years before the official case record (Ciervo, 2020; Martelli, 2016; Scortichini, 2022). *X. fastidiosa* subspecies *pauca* (Xfp), subsequently identified among the causal agents of the "olive quick decline syndrome" (OQDS) in Salento (Cariddi et al., 2014), can survive on many wild and cultivated plant species (EFSA, 2016), and in Apulia, it is transmitted by a very prolific insect vector, namely *Philaenus spumarius* (Saponari et al., 2014). One year after the first detection, indeed, Xfp was reported on about 23,000 ha (Stokstad, 2015).

Xylella fastidiosa is included among the A2 list of quarantine phytopathogens for which the European Union, according to the European and Mediterranean Plant Protection Organization (EPPO), imposes severe rules for impeding their further spread in other areas of the country and outside the country.

With the aim of eradicating this alien pathogen, presumably introduced from abroad, the authorities referred to the rules established by the Council Directive of the European Union, 2000/29 and subsequent amendments and implementations that impose an eradication approach and concern "protective measures against the *introduction* into the Community of organisms harmful to plants or plant products and against their spread within the Community." However, according to Sosnowski et al. (2009), the successful eradication of quarantine phytopathogens requires various criteria to be fulfilled, namely (a) prompt identification of the causal agent found in a new area, (b) a restricted and limited infected area, (c) an effective local organization for crop uprooting and (d) biological features of the microorganism that guarantee its complete elimination. It should be said that at the time of the first record, none of these criteria were met for the Xfp outbreak in Salento.

However, the Apulia Region established its first eradication measures through Resolution 2023/2013, which included the uprooting of the infected olive trees that tested positive for Xfp and insecticide treatments against vectors in the outbreak area (Apulia Region, 2013). Subsequently, the European regulations have aggravated the provisions by also including the removal of non-infected plants in the 100 m radius of olive trees that tested positive for the bacterium. Moreover, the Apulia Region requested (dgR n. 1842 of 8 September 2014) and obtained (10 February 2015) from the national government the declaration of a state of emergency with the appointment of a special commissioner.

These implemented measures to control the spread of the bacterium assumed that "at the moment there are no certified methods of controlling *X. fastidiosa* except that of the eradication of infected plants" (Silletti, 2015). They were based on three principles: the uprooting of trees (infected, potentially infected and non-infected), the heavy use of pesticides to control the vector and the prohibition of planting host plants (including olive trees).

In May 2015, however, the European Food Safety Authority (EFSA) clearly stated "In the case (Apulia) of an infected area extending over tens of thousands of hectares, several more of these conditions are not fulfilled: (a) condition 1: early detection and rapid initiation of an eradication programme; (b) condition 2: there are many hosts and many potential vectors, often polyphagous; (c) condition 3. 'blind' molecular testing of many asymptomatic hosts will be necessary" (EFSA, 2015, p. 95). In regard to the Apulia scenario, it appeared quite evident that the eradication (i.e., the complete elimination of the pathogen from the infected area) was impossible (EFSA, 2015). Consequently, also based on previous failed attempts to eradicate *X. fastidiosa* in the USA (California), Taiwan and Brazil, the EFSA stated in May 2015 that the infected zone of Salento should be considered as established and eradication is no longer possible (EFSA, 2015).

The European Commission in May 2015 also pointed out that, in certain parts of the Lecce province, Xfp has been present for more than 2 years and it was no longer possible to eradicate it (Commission Implementing Decision UE 2015/789). Consequently, following this statement by the European Union, the Regional Government and the Italian Ministry of Agriculture, Food and Forestry have updated the former legislations. The new regulations described a series of mandatory technical and legislative measures aimed at preventing further Xfp spread in the territory of Apulia and established a demarcation of the Salento territory into "infected," "containment" and "buffer" areas, with the latter two located northwards of the "infected" area. Subsequently, these measures will be integrated by regulatory provision and implementation measures at European, national and regional level, and these areas have been moved several times northwards according to the detection of the pathogen in areas previously free from its occurrence (see Table 1 and Figures 1 and 2). The main rules, however, have remained substantially unchanged for 10 years, apart from the reduction from a 100 m radius to a 50 m radius for the uprooting of all host plants of the bacterium found

TABLE 1 Results of the monitoring surveys performed in Apulia from 2013 to 2023 to detect *Xylella fastidiosa* subsp. *pauca* in olive groves and other host plants in the whole demarcated areas.

Monitoring campaign	Territories in infected areas (including infected zone, containment zone and buffer zone) at the end of the monitoring campaign	Monitored surface (ha)	N. plants showing dieback symptoms	N. analysed plants	N. Xfp-positive plants	% n. Xfp-positive plants/n. analysed plants	% n. Xfp-positive plants/n. plants showing symptoms	N. plants felled
2013–2014 11/2013–10/2014 (Figure 1a)	Most of the province of Lecce	n.a.	n.a.	11,099 (7537 olive trees)	353 (291 olive trees)	3.18	n.a.	104
2014–2015 10/2014–12/2015 (Figure 1b)	Province of Lecce: all, Province of Brindisi: entire territory of 3 municipalities; outbreaks in 6 municipalities, Province of Taranto: outbreak in Avetrana	20,278	6025 (5981 olive trees)	51,409 (48,605 olive trees)	4191 (4169 olive trees)	8.15	69.56	1648
2016–2017 (2/2016–5/2017) (Figure 1c)	Province of Lecce: all, Province of Brindisi: entire territory of 16 municipalities; parts of 2 municipalities; outbreak in Ostuni Province of Taranto: entire territory of 15 municipalities; parts of 4 municipalities	156,887	7040 (6748 olive trees)	159,109 (141,749 olive trees)	1588 (1573 olive trees)	1.00	22.56	881
2017–2018 (7/2017–5/2018) (Figure 1d)	Province of Lecce: all, Province of Brindisi: all, Province of Taranto: entire territory of 22 municipalities, Province of Bari: entire territory of Locorotondo	164,591	18,630 (17,741 olive trees)	198,768 (170,704 olive trees)	3835 (3819 olive trees)	1.93	20.58	2662
2018–2019 (11/2018–7/2019) (Figure 2a)	Province of Lecce: all, Province of Brindisi: all, Province of Taranto: entire territory of 22 municipalities, Province of Bari: entire territory of Locorotondo	185,776	7004 (6776 olive trees)	68,500 (53,465 olive trees)	1074 (1072 olive trees)	1.57	15.33	974 (972 olive trees)
2019–2020 (8/2019–4/2020) (Figure 2b)	Province of Lecce: all, Province of Brindisi: all, Province of Taranto: entire territory of 22 municipalities, Province of Bari: entire territory of Locorotondo; 5 outbreaks in Monopoli	162,906	7346 (7088 olive trees)	50,967 (40,669 olive trees)	1396 (1364 olive trees)	2.74	19.00	1356 (1324 olive trees)
2020–2021 (4/2020–3/2021) (Figure 2c)	Province of Lecce: all, Province of Brindisi: all, Province of Taranto: entire territory of 22 municipalities, Province of Bari: entire territory of Locorotondo; 7 outbreaks in Monopoli and one outbreak in Polignano a Mare	161,417	10,005 (9765 olive trees)	172,006 (160,737 olive trees)	1205 (1191 olive trees)	0.70	12.04	1193 (1179 olive trees)
2021–2022 (5/2021–2/2022) (Figure 2d)	Province of Lecce: all, Province of Brindisi: all, Province of Taranto: entire territory of 22 municipalities, Province of Bari: entire territory of Locorotondo; 7 outbreaks in Monopoli e one outbreak in Polignano a Mare Province of BAT: one outbreak in Canosa di Puglia	23,602	4541 (4470 olive trees)	225,014 (188,009 olive trees)	146	0.06	3.21	1549
2022–2023 (6/2022–6/2023) (Figure 2e)	Province of Lecce: all, Province of Brindisi: all, Province of Taranto: entire territory of 22 municipalities, Province of Bari: entire territory of 4 municipalities; parts of Castellana Grotte	31,396	n.a.	266,366	366	0.14	n.a.	4733

Note: Data elaborated from the official reports of the phytosanitary service of Apulia Region.

Abbreviations: N, n: number; na, not assesse.

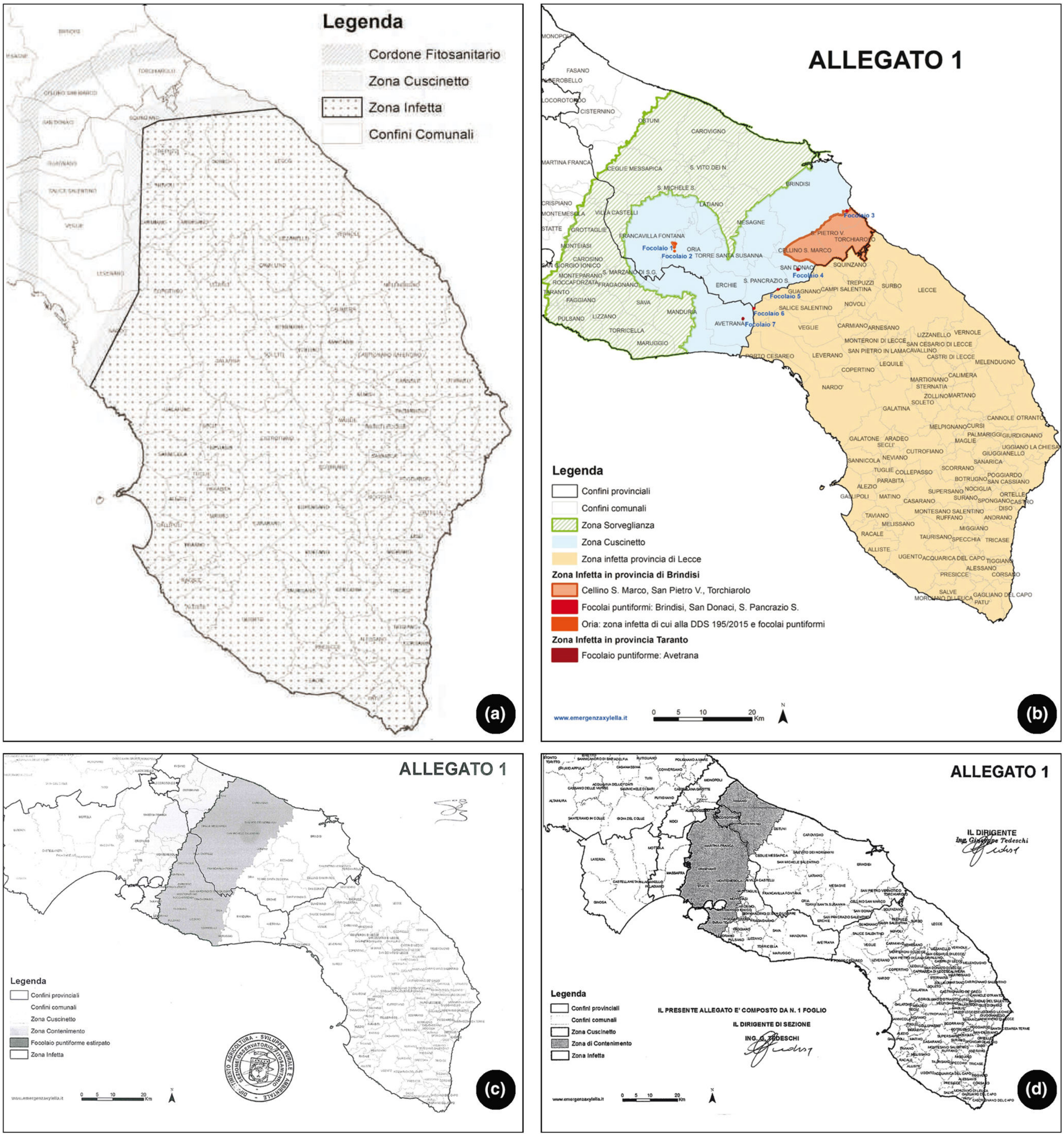

FIGURE 1 Geographical maps of the Apulia that indicate the displacement of the "containment" and "buffer" zones to be monitored for the occurrence of *Xylella fastidiosa* subsp. *pauca* from 2013–2014 to 2017–2018 (a–d) surveys. Based on data from the Apulia Region.

from olive trees that officially tested positive for Xfp (Commission Implementing Decision UE, 2020/1201).

In this paper, we show how the Xfp incidence in the olive groves of the demarcated areas of Apulia is very low, especially during the last 3 years of monitoring surveys. This finding would suggest removing the rule that imposes the eradication, in a radius of 50 m, of all asymptomatic plants that surround the Xfp-positive olive tree. Such a proposal is also corroborated by epidemiological models that verified the negligible role of the asymptomatic olive trees in the spreading of Xfp.

2 | MATERIALS AND METHODS

The official data obtained from the Apulia Region's phytosanitary service (2023a, 2023b) concern the following: the monitored

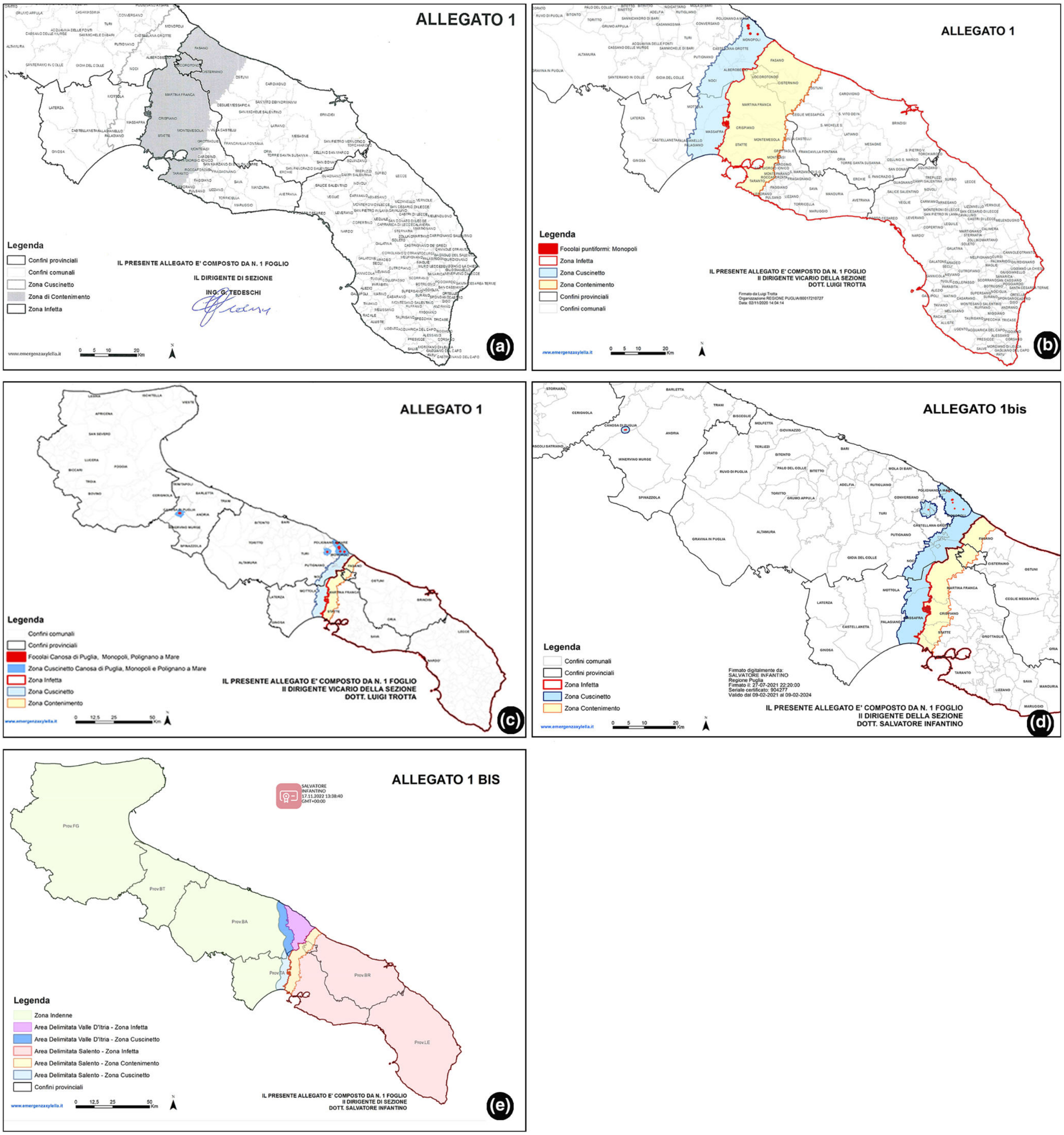

FIGURE 2 Geographical maps of the Apulia that indicate the displacement of the "containment" and "buffer" zones to be monitored for the occurrence of *Xylella fastidiosa* subsp. *pauca* from 2018–2019 to 2022–2023 (a–e) surveys. Based on data from the Apulia Region.

surface, the number of plants that showed dieback symptoms, the analysed plants, the number of Xfp-positive plants, and the number of uprooted plants, including olive trees within all demarcated areas of Apulia. They were collected by the phytosanitary service upon the monitoring surveys carried out from November 2013 to June 2023 in the olive groves of all demarcated areas by the Agenzia regionale per le attività irrigue e forestali (ARIF) and, upon subsequent analyses, for detecting the occurrence of the bacterium, which was performed by official laboratories all working in the Apulia (Figures 1 and 2; Table S1). Olive leaf samples collected from the olive groves and subsequently analysed in the laboratories were obtained by following the procedures described elsewhere (Regione Puglia, 2015; Scortichini & Cesari, 2019). After a presumptive serological analysis obtained through the ELISA test, a sample infected by Xfp was retained upon the confirmatory assay performed by using real-time PCR according to the procedures established by the EPPO PM7/24 standard diagnostic protocol for *X. fastidiosa* (EPPO, 2019).

The demarcation of the areas has changed over the years also from a nominal point of view. As a general rule, the territory is divided into two main demarcated areas: infected area including the "infected zone" and the "containment zone," and the free area containing the "buffer zone" and the "free zone." The infected area is where Xfp has been detected and is deemed to be established; it consists of the "infected zone," which is not monitored, and the "containment zone," which is a belt of 5 km at the border with the "buffer zone." The free area is considered free from Xfp; it consists of the "buffer zone," which is at the northern boundary of the "containment zone," and the "free zone," which is the furthest from the infected area. In recent campaigns (2021–2022 and 2022–2023) following the EU Regulation 2020/1201, new "delimited areas" where eradication measures are also applied (articles 7–11) have been introduced. In the "buffer zone," "free zone" and new "delimited areas," the positive plants and plants within a 100 m radius of the infected plants are uprooted (50 m radius from 2021). The "containment zone" is the unique zone where only the Xfp-positive plant is uprooted. The "infected area" is no longer monitored, and from 2019 to 2025, law 44/2019 made it possible for owners and holders of land to remove olive trees, also monumental, with a simple communication to the region, without having to prove that the olive trees are Xfp-positive or even show OQDS symptoms. This also applies to monumental olive trees.

3 | RESULTS

The results of the monitoring surveys performed in Apulia from 2013 to 2022 of the presence of Xfp in the olive groves are shown in Table 1. The hectares monitored during the surveys varied considerably during the years. It is worth noting that, apart from the second campaign that regarded 20,278 ha, from 2016 to 2015, the hectares monitored in each survey were always more than 150,000 and up to a maximum value of 185,776 in the fifth campaign (2018–2019). By contrast, the last two campaigns (from 2021 to 2023) have taken into consideration 23,602 and 31,396 ha, respectively. There are no data concerning the hectares monitored during the initial campaign of 2013–2014. The number of olive trees and other host plants sampled during the different surveys also varied considerably among the various campaigns. It, indeed, varied from a minimum of 11,099 (initial surveys from November 2013 to October 2014) to a maximum of 266,366 (survey from June 2022 to June 2023). A considerable number of trees sampled were also observed in another four campaigns when the sampled trees were higher in number than 150,000. Notably, despite a low surface monitored during the campaigns of 2021–2022 and 2022–2023 in comparison with previous surveys, the number of sampled trees is the highest of all the campaigns, specifically 225,014 from May 2021 to February 2022 and 266,366 from June 2022 to June 2023 (Figure 3). The number of positive trees seems to be absolutely independent of the number of plants analysed (Figure 4).

The Xfp incidence observed in the entire demarcated areas during the monitoring surveys varied, but, apart from the initial two surveys where the incidence reached 3.18% and 8.15% (surveys from 2013 to 2015), all other campaign incidences were always lower than 3.0%. It should be noted that during the last three campaigns (from 2020 to 2023) such incidences were very low and varied from 0.06% to 0.70%. Further notable data are that regarding the percentage of plants that tested positive for Xfp and that showed, at the time of sampling, the symptoms of OQDS. Apart from the high percentage observed during the campaign from October 2014 to December 2015, when 69.56% of olive trees that presented OQDS symptoms tested positive for Xfp, all other campaigns suggested that the relationships between the trees that clearly show symptoms of OQDS and the presence of Xfp in the analysed samples resulted were quite low, namely from a maximum of 22.56% (surveys from February 2016 to May 2017) to a minimum of 3.21% (campaign of May 2021 to February 2022) (Table 1). The number of plants eradicated upon the sampling and laboratory analyses varied throughout the 10-year duration. The highest number was during the fourth campaign (2017–2018) when 2662

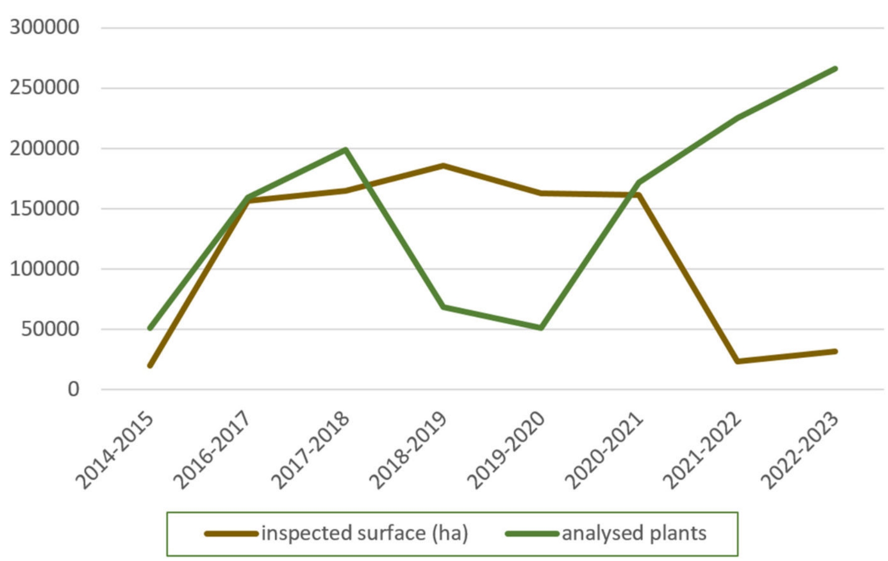

FIGURE 3 Inspected surface (hectares) and analysed plants from 2014–2015 to 2022–2023 of the entire demarcated areas of the Apulia. Based on data from the Apulia Region.

FIGURE 4 Analysed plants and *Xylella fastidiosa* subsp. *pauca*-positive plants from 2013–2014 to 2022–2023. Based on data from the Apulia Region.

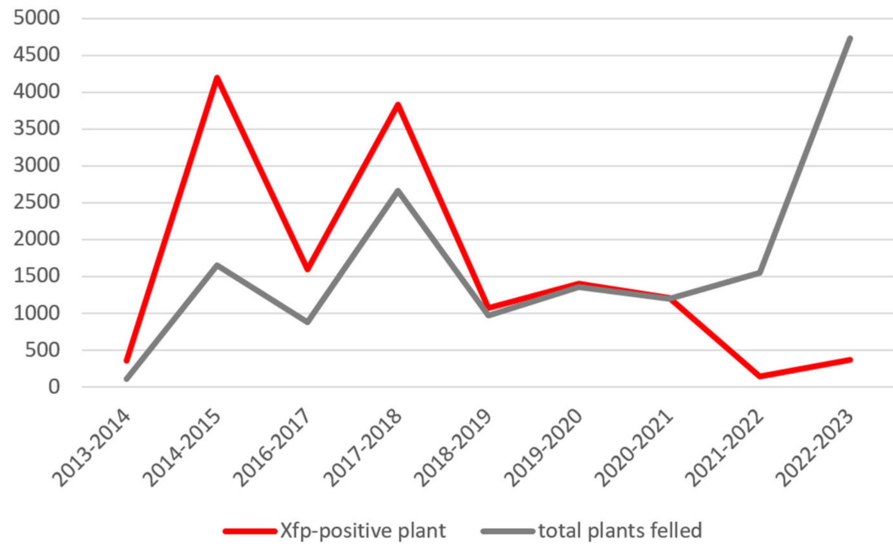

FIGURE 5 *Xylella fastidiosa* subsp. *pauca*-positive plants and total plants felled from 2013–2014 to 2022–2023. Based on data from the Apulia Region.

plants were uprooted and the last campaign (2022–2023) when 4733 plants were uprooted. In total, 15,100 plants have been cut down over 10 years as a result of monitoring campaigns, most of which were centuries-old olive trees, often monumental. In regard to the number of positive plants and the total number of uprooted plants (Figure 5), we can see three phases: (a) from 2013–2014 to 2017–2018, the uprooted plants are much fewer than the number of positive plants; (b) from 2018–2019 to 2020–2021, the number of plants felled is equivalent to about the number of positive plants; and (c) from 2021–2022 to 2022–2023, felling significantly exceeds the number of positive plants: over 1050% and almost 1300%, respectively.

A detailed report about the last two campaigns is shown in Table 2. Both campaigns reported a very low incidence of Xfp-infected olive trees and other host plants either in the "free" area or in the "containment," "buffer" and "delimited" areas. In any case, the incidence of the bacterium was lower than 0.23%, with a minimum incidence observed during 2021–2022 when Xfp was found present in 0.09% ("containment" zone), 0.11% ("delimited" area), 0.04% ("buffer" zone) and 0.0% ("not infected" area) of the analysed samples. Furthermore, in 2022–2023 the "buffer" zone has the same percentage as the previous campaign, while in the other three areas the percentage exceeded those of the previous year. The olive trees and other host plants that tested positive for Xfp numbered 146 and 366 during 2021–2022 and 2022–2023, respectively. However, by following the rule of 50 m, the number of uprooted trees that surrounded the olive tree or another host plant that tested positive upon sampling and laboratory analyses reached quite high in the last two campaigns. During 2021–2022, 1403 trees were eradicated, whereas during 2022–2023 4389 trees were eradicated. In both years, a relevant number of centennial and monumental olive trees were uprooted.

TABLE 2 Data referring to the last two monitoring surveys performed in the different demarcated zones of Apulia.

Demarcated zones		Monitoring campaign 2021–2022						Monitoring campaign 2022–2023					
		Monitored surface (ha)	N. analysed plants	N. Xfp-positive plants	% n. Xfp-positive plants/n. analysed plants	N. Xfp-positive plants felled	N. plants felled within a 50 m radius of the infected plant	Monitored surface (ha)	N. analysed plants	N. Xfp-positive plant	% n. Xfp-positive plants/n. analysed plants	N. Xfp-positive plants felled	N. plants felled within a 50 m radius of the infected plant
Free area	Not infected zones	6334	44,021	0	0	0	0	9051	65,317	101	0.15	100	1521
	Buffer zone	6315	43,810	17	0.04	17	1008	8116	56,659	22	0.04	22	1524
Infected area	Delimited areas where eradication measures are also applied (EU Reg. 2020/1201 art. 7–11)	2461	38,009	41	0.11	41	395	8830	78,341	95	0.12	85	1344
	Containment zone	8492	99,174	88	0.09	88	0	5399	66,049	148	0.22	137	0
	Total	23,602	225,014	146	0.06	146	1403	31,396	266,366	366	0.14	344	4389

Note: Data elaborated from the official reports of the phytosanitary service of Apulia Region.

Abbreviations: N, n: number.

4 | DISCUSSION

A number of studies, by applying a series of mathematical models based on the estimation of epidemiological parameters, vector feeding and the consequent spreading of the bacterium within and between the olive groves, described the shape of the invasion front and the rate of bacterium movement and calculated the potential spread of Xfp in the olive groves of Apulia (EFSA, 2019; Fierro et al., 2019; Jeger & Bragard, 2019; Kottelenberg et al., 2021; Strona et al., 2017; White et al., 2017). Such studies determined different average rates of bacterium spread per year among the olive groves, with calculations that ranged from 5 to 20 km per year (EFSA, 2019; Fierro et al., 2019; Kottelenberg et al., 2021). Such discrepancies are most probably related to a series of parameters related to both Xfp and its biological transmitting vector that are objectively difficult to precisely calculate as for any insect-transmitted plant disease (Jager and Bragard, 2019; White et al., 2020). However, by considering that the initial outbreak of OQDS approximately started during 2008 (Kottelenberg et al., 2021), the official front of Xfp movement during these 15 years (from Gallipoli area to Monopoli municipality) is about 150 km, corresponding to about 10 km per year. Within this scenario, our study ascertained that, apart from during the first two monitoring surveys performed in the municipalities where OQDS symptoms were first observed (i.e., the Gallipoli area) that showed an incidence of 3.18% and 8.15%, the incidence of Xfp throughout the entire demarcated areas was always low and never exceeded 2.73% and from 2020 onwards was always lower than 0.71%.

In addition, the trees that showed symptoms of OQDS and that were sampled and analysed for the occurrence of Xfp very often did not test positive for the bacterium. During the monitoring surveys of 2021, the percentage of olive trees with clear symptoms of OQDS that tested positive for Xfp was merely 3.21%. These findings confirm what already has been observed in a previous assessment of the campaigns determining the occurrence of Xfp when 3300 samples taken from symptomatic trees did not test positive for the bacterium (Scortichini & Cesari, 2019). This clearly shows that there is not always a clear relationship between symptoms of OQDS and the presence of Xfp.

These data reinforce the fact that other phytopathogens could be involved in OQDS and that they can induce very similar symptoms to those incited by Xfp. In fact, already from 2013 several studies have indicated various species of fungi associated with a decline of Apulian olive trees (Carlucci et al., 2013, 2015; Nigro et al., 2013). Additionally, it was recently observed that aggressive fungi such as *Neofusicoccum mediterraneum* and *N. stellenboschiana* have been very frequently isolated from olive trees that apparently show the typical symptoms of OQDS and in many cases they are also associated with Xfp (Brunetti et al., 2022; Manetti et al., 2023; Scortichini et al., 2023). The very low percentage of Xfp-infected trees that show decline symptoms associated with the occurrence of other phytopathogens frequently found in olive groves of Apulia would also suggest reconsidering the OQDS from a broader epidemiological point of view.

An important point to consider is the very low Xfp incidence and a very low rate of symptomatic trees observed during the last two campaigns throughout the demarcated areas. A total number of 512 Xfp-positive olive trees and other host plants, as revealed during 2021–2022 and 2022–2023, resulted in an additional 5792 trees that were uprooted according to the 50-m rule. Among these trees, a relevant number of asymptomatic centennial and monumental olive trees were included. It should be noted that, through epidemiological models, White et al. (2020) showed that asymptomatic olive trees have a low to negligible infectivity for Xfp and that they have little effect on the overall epidemiological dynamics of OQDS. To corroborate such finding, it should be added that some asymptomatic olive trees detected as Xfp-positive in 2015 that were not immediately uprooted due to pending legislative procedures and updating of demarcation areas are currently in an optimal vegetative and productive status many years after the detection of Xfp (Figures 6 and 7).

These facts, in addition to the relevant heritage represented by the olive groves of Apulia, allow proposing some modification of the current rules governing the management of Xfp throughout the demarcated areas. The epidemiological irrelevance of the asymptomatic trees for the further spreading of the bacterium

FIGURE 6 An olive tree located in the Lecce province that tested positive for *Xylella fastidiosa* subsp. *pauca* in 2015 as it appears on 14 October 2023. Upon detection, the tree was not uprooted due to a pending legal proceeding and the subsequent updating and shifting of demarcation areas.

(White et al., 2020) would suggest removing the 50-m rule in all the demarcated areas. The uprooting of the trees that surround the Xfp-positive olive tree in a radius of 50 m seems a detrimental cost, considering that the asymptomatic and Xfp-positive olive trees have a very low to negligible influence on avoiding the further spread of Xfp in the area.

Such a proposal is in accordance with observations made by the EFSA regarding the main source of uncertainty in the management of Xfp in Apulia (EFSA, 2019). In particular, when referring to the "option for cutting," they clearly state, "The adoption of a cutting strategy was the most effective option. An alternative option could consider the cutting of the infected plant only. This should provide that advantage of saving potentially healthy plants." The EFSA also stated the possibility that the detection techniques do not intercept the bacterium, which can occur at a low concentration within the tree (EFSA, 2019). It should be said that in recent years the techniques for detecting Xfp in olive trees have been greatly improved, now requiring only very few cells of the bacterium in the sample (Amoia et al., 2023; D'Onghia et al., 2022; Faino et al., 2021; Mastin et al., 2022; Passera et al., 2022). This proposal is in line with findings from other scientific studies with regard to measures taken to limit the spread of *X. fastidiosa*. For example, concerning the Balearic Island case, Olmo et al. (2021) state that "neither of those proposed measures was epidemiologically sound, since the pathogen was already widespread and established in the fields for several decades," and they also question the adequacy of eradication strategy without any prior investigation into the epidemiological history.

Moreover, the International Standard for Pathogen Measures (ISPM) adopted by the Food and Agriculture Organization (FAO) in agreement with the International Plant Protection Convention (IPPC) provide for the periodic review of the measures in order to assess whether the objectives were achieved or determine whether changes are necessary to modify the rules "as conditions change, and as new facts become available, phytosanitary measures shall be modified promptly, either by inclusion of prohibitions, restrictions or requirements necessary for their success, or by removal of those found to be unnecessary" (ISPM 4, IPPC Secretariat, 2017). In particular, it is stated that "throughout the eradication, the programme should be subject to periodic review to analyse and assess information gathered, to check that objectives are being achieved, and/or to determine if changes are required" (ISPM 9, IPPC Secretariat, 2016a). According to the ISPM 22, the current characteristics of the entire demarcated areas of the Apulia region, where Xfp is continuously monitored and its very low incidence was repeatedly found, would attain the status "areas of low pest prevalence" where "in a part of a country a specific pest occurs at low level and which is subject to effective surveillance, control or eradication measures" (ISPM 22, IPPC Secretariat, 2016b).

For these areas, modification of the current eradication rules would now seem appropriate. The elimination of the 50-m rule is proposed since it can represent a more rational approach that does not sacrifice the healthy trees, the landscape, the environment and the territorial economy, while concurrently allowing continuous monitoring of the occurrence of the bacterium in the areas.

FIGURE 7 Olive grove located in the Lecce province where, in 2015, the tree in the foreground was tested positive for *Xylella fastidiosa* subsp. pauca., as it appears on 14 October 2023. Upon detection, the tree was not uprooted due to a pending legal proceeding and the subsequent updating and shifting of demarcation areas. Also note the healthy vegetative status of the nearby olive trees.

Moreover, for another plant quarantine microorganism transmitted by an insect vector, namely "Flavescence doree phytoplasma of grapevine" spread by the leafhopper *Scaphoideus titanus*, the quarantine measures of uprooting concern solely the infected grapevine, and only when the incidence of the infection within the vineyard is about 20%–30% must the whole vineyard be uprooted (EFSA, 2016).

Concerning the OQDS epidemiology and its consequent management, it should also be added that it has been recently observed that the Xfp vector effectively spreads the bacterium mainly through the road system of Apulia, whereas the natural areas hamper such a spread (Bajocco et al., 2023). Due to the relevant number of roads that occur in Apulia, the very intensive traffic that characterizes this region especially during summer, this finding poses some uncertainty for an effective annulment of the Xfp occurrence in the region through the uprooting of olive trees, since it is not possible to eradicate the insect vectors in the infected area in the South of the region. *P. spumarius*-infected specimens, indeed, carried by vehicles starting from the infected area can reach the free areas within a few minutes and, subsequently, spread Xfp. Indeed, in 2015 the EFSA stated that "as observed in the Apulian area, insect vectors may hitch-hike for rather long distances on or in vehicles, even without plants [...] This means that infectious vectors may disseminate far from plots where the disease is present, which implies that eradication of indigenous insect vectors over a large area is not possible, as there are plenty of indigenous xylem sap feeder species associated with many kinds of plants" (EFSA, 2015, pp. 97–98). Finally, a working protocol for the effective management of Xfp in the olive groves of Apulia, based on the spray to the olive crown, once per month, from spring to autumn of a zinc–copper–citric acid biofertilizer that also has bactericidal properties, could also be applied in the various demarcated areas to reduce the bacterium cell density within the tree (Blonda et al., 2023; Scortichini et al., 2018; Tatulli et al., 2021).

In conclusion, this study shows, for the first time, an analysis based on the official 10-year data of monitoring surveys of olive groves in Apulia and clearly reveals (a) a very low incidence of Xfp-infected olive trees within the different demarcated areas especially during the last 3 years, even in the infected area; (b) a high number of uprooted healthy olive trees, in agreement with the "50-metre-radius" rule and despite the very low incidence rates of the bacterium; (c) a lack of correlation between the occurrence of Xfp in olive trees that show symptoms of OQDS; and (d) confirmation of the epidemiological irrelevance of the asymptomatic trees for the further spread of the bacterium, as shown by White et al. (2020).

Finally, this study presents a scientifically based proposal that could reduce the devastation of the environment, landscape, traditional agriculture and territorial economy and that could validly support the political decision-maker in his choices. Further developments of the research are necessary, and they could point in the direction of a more descriptive epidemiological investigation of OQDS by also including, other than Xfp, the aggressive fungi reported above.

AUTHOR CONTRIBUTIONS

Margherita Ciervo contributed to data acquisition and elaboration, methodology, preparation of the draft manuscript, review and editing. Marco Scortichini contributed to methodology, preparation of the draft manuscript, review and editing.

CONFLICT OF INTEREST STATEMENT

The authors declared no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

Amoia, S. S., Loconsole, G., Ligorio, A., Pantazis, A. K., Papadakis, G., Gizeli, E., & Minafra, A. (2023). A colorimetric LAMP detection of *Xylella fastidiosa* in crude alkaline sap of olive trees in Apulia as a field-based tool for disease containment. *Agriculture*, *13*, 448.

Apulia Region. (2013). *DgR n. 2023: Misure di emergenza per prevenzione, controllo ed eradicazione Xf associato al "CoDiRO"*. Bari.

Apulia Region. (2023a). Nota AOO_181/PROT/03/07/2023/0006075. Oggetto: monitoraggio ulivi positivi al batterio *Xylella fastidiosa*. Istanza di accesso agli atti ai sensi dell'art. 5 del d.lgs. 14/03/2013 n. 33 come sostituito dall'art. 6 comma 1 d.lgs. 97 del 2016. Riscontro (3 luglio 2023).

Apulia Region. (2023b). Nota AOO_181/PROT/28/08/2023/0007316. Oggetto: monitoraggio ulivi positivi al batterio *Xylella fastidiosa*. Istanza di accesso agli atti del 31/07/2023 (protocollo interno AOO_181/PROT/01/08/2023/0006891) ai sensi dell'art. 5 del d.lgs. 14/03/2013 n. 33 come sostituito dall'art. 6 comma 1 d.lgs. 97 del 2016. Riscontro (28 agosto 2023).

Bajocco, S., Raparelli, E., & Bregaglio, S. (2023). Assessing the driving role of the anthropogenic landscape on the distribution of the *Xylella fastidiosa*-driven "olive quick decline syndrome" in Apulia (Italy). *Science of the Total Environment*, *896*, 165231.

Blonda, P., Tarantino, C., Scortichini, M., Maggi, S., Tarantino, M., & Adamo, M. (2023). Satellite monitoring of a bio-fertilizer restoration in olive groves affected by *Xylella fastidiosa* subsp. *pauca*. *Scientific Reports*, *13*, 5695.

Brunetti, A., Matere, A., Lumia, V., Pasciuta, V., Fusco, V., Sansone, D., Marangi, P., Cristella, N., Faggioli, F., Scortichini, M., & Pilotti, M. (2022). *Neofusicoccum mediterraneum* is involved in twig and branch dieback of olive trees observed in Salento (Apulia, Italy). *Pathogens*, *11*, 53.

Calabrese, G., Ladisa, G., & Pettiti, L. (2012). Oliviculture and ancient olive orchards in the EU-Mediterranean area. In A. La Posta, C. Lacirignola, & G. Mimiola (Eds.), *A multi-scale and multi-level approach for conservation of ancient olive orchards in the Euro-Mediterranean region* (pp. 2–23). IAMB, CIHEAM - Mediterranean Agronomic Institute of Bari.

Cariddi, C., Saponari, M., Boscia, D., De Stradis, A., Loconsole, G., Nigro, F., Porcelli, F., Potere, O., & Martelli, G. P. (2014). Isolation of a *Xylella fastidiosa* strain infecting olive and oleander in Apulia, Italy. *Journal of Plant Pathology*, *96*, 425–429.

Carlucci, A., Lops, F., Cibelli, F., & Raimondo, M. L. (2015). *Phaeoacremonium* species associated with olive wilt and decline in Southern Italy. *European Journal of Plant Pathology*, *141*, 717–729.

Carlucci, A., Raimondo, M. L., Cibelli, F., Phillips, A. J., & Lops, F. (2013). *Pleurostomophora richardsiae*, *Neofusicoccum parvum* and *Phaeoacremonium aleophilum* associated with a decline of olives in Southern Italy. *Phytopathologia Mediterranea*, *52*, 517–527.

Ciervo, M. (2016). The olive quick decline syndrome (OQDS) diffusion in Apulia region: An apparent contradiction according to the agricultural model. *Belgeo*, *4*.

Ciervo, M. (2020). *Il disseccamento degli olivi in Puglia, evidenze, contraddizioni, anomalie, scenari* (p. 74). Un punto di vista geografico. Società Geografica Italiana.

Council Directive of European Union. (2000/29/EC). *Protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their within the Community*.

D'Onghia, A. M., Santoro, F., Minutillo, S. A., Frasheri, D., Gallo, M., Gualano, S., Cavallo, G., & Valentini, F. (2022). Optimisation of sampling and testing for asymptomatic olive trees infected by *Xylella fastidiosa* in Apulia region, Italy. *Phytopathologia Mediterranea*, *61*, 439–449.

Di Rienzo, V., Fanelli, V., Miazzi, M. M., Sabetta, W., & Montemurro, C. (2018). The preservation and characterization of Apulian olive germplasm biodiversity. *Acta Horticulturae*, *1199*, 1–6.

EFSA Panel on Plant Health (PLH). (2015). Scientific opinion on the risks to plant health posed by *Xylella fastidiosa* in the EU territory, with the identification and evaluation of risk reduction options. *EFSA Journal*, *13*, 3989.

EFSA Panel on Plant Health (PLH). (2016). Scientific opinion of the risk to plant health of Flavescence dorée for the EU territory. *EFSA Journal*, *14*, 4603.

EFSA Panel on plant health (PLH). (2019). Update of the scientific opinion on the risks to plant health posed by *Xylella fastidiosa* in the EU territory. *EFSA Journal*, *17*, 5665.

European Commission. (2015). *Commission Implementing Decision (EU) 2015/789 of 18 May 2015 as regards measures to prevent the spread within the Union of Xylella fastidiosa (Wells et al.)*. Official Journal of European Union.

European Commission. (2020). *Commission Implementing Regulation (EU) 2020/1201 of 14 August 2020 as regards measures to prevent the spread within the Union of Xylella fastidiosa (Wells et al.)*. Official Journal of European Union.

European Plant Protection Organization (EPPO). (2019). PM7/24 *Xylella fastidiosa*. *Bulletin OEPP/EPPO Bulletin*, *49*, 175–227.

Faino, L., Scala, V., Albanese, A., Modesti, V., Grottoli, A., Pucci, N., Doddi, A., L'Aurora, A., Tatulli, G., Reverberi, M., & Loreti, S. (2021). Nanopore sequencing for the detection and identification of *Xylella fastidiosa* subspecies and sequence types from naturally infected plant material. *Plant Pathology*, *70*, 1860–1870.

Fierro, A., Liccardo, A., & Porcelli, F. (2019). A lattice model to manage the vector and infection of the *Xylella fastidiosa* in olive trees. *Scientific Reports*, *9*, 8723.

IPPC Secretariat. (2016a). *International standards for pathogenic measures n° 9* (p. 10). Published by F.A.O. on behalf of the secretariat of the International Plant Protection Convention (IPPC).

IPPC Secretariat. (2016b). *International standards for pathogenic measures n° 22* (p. 10). Published by F.A.O. on behalf of the secretariat of the International Plant Protection Convention (IPPC).

IPPC Secretariat. (2017). *International standards for pathogenic measures n° 4* (p. 10). Published by F.A.O. on behalf of the secretariat of the International Plant Protection Convention (IPPC).

Jeger, M., & Bragard, C. (2019). The epidemiology of *Xylella fastidiosa*: A perspective on current knowledge and framework to investigate plant host-vector-pathogen interaction. *Phytopathology*, *109*, 200–209.

Kottelenberg, D., Hemerik, L., Saponari, M., & Van der Werf, W. (2021). Shape and rate of movement of the invasion front of *Xylella fastidiosa* spp. *pauca* in Puglia. *Scientific Reports*, *11*, 1061.

Manetti, G., Brunetti, A., Lumia, V., Sciarroni, L., Marangi, P., Cristella, N., Faggioli, F., Reverberi, M., Scortichini, M., & Pilotti, M. (2023). Identification and characterization of *Neofusicoccum stellenboschiana* in branch and twig dieback-affected olive trees in Italy and comparative pathogenicity with *N. mediterraneum*. *Journal of Fungi*, *9*, 292.

Martelli, G. P. (2016). The current status of the quick decline syndrome of olive in southern Italy. *Phytoparasitica*, *44*, 1–10.

Mastin, A. J., Van den Bosch, F., Bourhis, Y., & Parnell, S. (2022). Epidemiologically-based strategies for the detection of emerging pathogens. *Scientific Reports*, *12*, 10972.

Nigro, F., Boscia, D., Antelmi, I., & Ippolito, A. (2013). Fungal species associated with a severe decline of olive in southern Italy. *Journal of Plant Pathology*, *95*, 668.

Olmo, D., Nieto, A., Borràs, D., Montesinos, M., Adrover, F., Pascual, A., Gost, P. A., Quetglas, B., Urbano, A., De Dios Garcia, J., Velasco-Amo, M. P., Olivares-Garcia, C., Beidas, O., Juan, A., Marco-Noales, E., Gomila, M., Rita, J., Moralejo, E., & Landa, B. B. (2021). Landscape epidemiology of *Xylella fastidiosa* in the Balearic Islands. *Agronomy*, *11*, 473.

Passera, A., Grosso, V., Miotti, N., Rossato, M., Gaffuri, F., Casati, P., Delledonne, M., & Bianco, P. A. (2022). Nanoplate digital PCR assay for detection and quantification of *Xylella fastidiosa*. *Phytopathologia Mediterranea*, *61*, 489–503.

Perrino, E. V., & Calabrese, G. (2014). Vascular flora of the ancient olive groves of Apulia (southern Italy). *Natura Croatica*, *23*, 189–218.

Regione Puglia, Servizio Agricultura, & Ufficio Osservatorio Fitosanitario. (2015). Relazione su *Xylella fastidiosa*. 52.

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.

Saponari, M., Loconsole, G., Cornara, D., Yokomi, R. K., De Stradis, R. A., Boscia, D., Bosco, D., Martelli, G. P., Krugner, R., & Porcelli, F. (2014). Infectivity and transmission of *Xylella fastidiosa* by *Philaenus spumarius* (Hemiptera: Aphrophoridae) in Apulia, Italy. *Journal of Economic Entomology*, *107*, 1316–1319.

Scortichini, M. (2020). The multi-millennial olive agroecosystem of Salento (Apulia, Italy) threatened by *Xylella fastidiosa* subsp. *pauca*: A working possibility of restoration. *Sustainability*, *12*, 6700.

Scortichini, M. (2022). The epidemiology and control of "olive quick decline syndrome" in Salento (Apulia, Italy). *Agronomy*, *12*, 2475.

Scortichini, M., & Cesari, G. (2019). An evaluation of the monitoring surveys of the quarantine bacterium *Xylella fastidiosa* performed in containment and buffer areas of Apulia, southern Italy. *Applied Biosafety*, *24*, 96–99.

Scortichini, M., Chen, J., De Caroli, M., Dalessandro, G., Pucci, N., Modesti, V., L'Aurora, A., Petriccione, M., Zampella, L., Mastrobuoni, F., Migoni, D., Del Coco, L., Girelli, C. R., Piacente, F., Cristella, N., Marangi, P., Laddomada, F., Di Cesari, M., Cesari, G., … Loreti, S. (2018). A zinc, copper and citric acid complex shows promise for control of *Xylella fastidiosa* subsp. *pauca* in olive trees in Apulia region (southern Italy). *Phytopathologia Mediterranea*, *57*, 48–72.

Scortichini, M., Manetti, G., Brunetti, A., Lumia, V., Sciarroni, L., & Pilotti, M. (2023). *Xylella fastidiosa* subsp. *pauca*, *Neofusicoccum* spp. and the decline of olive trees in Salento (Apulia, Italy): Comparison of symptoms, possible interactions, certainties and doubts. *Plants*, *12*, 3593.

Silletti, G. (2015). *Piano degli interventi per fronteggiare il rischio fitosanitario connesso alla diffusione della Xylella fastidiosa (Well e Raju) nel territorio della regione Puglia, art. 1C.4 ordinanza del CDPC 225/2015*.

Sosnowski, M. R., Fletcher, J. D., Daly, A. M., Rodoni, B. C., & Viljanen-Robinson, S. L. H. (2009). Techniques for the treatment, removal and disposal of host material during programmes of plant pathogen eradication. *Plant Pathology*, *58*, 621–635.

Stokstad, E. (2015). Italy's olives under siege. *Science*, *348*, 620.

Strona, G., Carstens, C. J., & Beck, P. S. A. (2017). Network analysis reveal why *Xylella fastidiosa* will persist in Europe. *Scientific Reports*, *7*, 71.

Tatulli, G., Modesti, V., Pucci, N., Scala, V., L'Aurora, A., Lucchesi, S., Salustri, M., Scortichini, M., & Loreti, S. (2021). Further in vitro assessment and mid-term evaluation of control strategy of *Xylella fastidiosa* subsp. *pauca* in olive groves of Salento (Apulia, Italy). *Pathogens*, *10*, 85.

White, S. M., Bullock, J. M., Hooftman, D. A. P., & Chapman, D. S. (2017). Modelling the spread and control of *Xylella fastidiosa* in the early stages of invasion in Apulia, Italy. *Biological Invasions*, *19*, 1825–1837.

White, S. M., Navas-Cortes, J. A., Bullock, J. M., Boscia, D., & Chapman, D. S. (2020). Estimating the epidemiology of Xylella fastidiosa outbreaks in olives. *Plant Pathology*, *69*, 1403–1413.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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