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Management of the olive decline disease complex caused by *Xylella fastidiosa* subsp. *pauca* and *Neofusicoccum* spp. in Apulia, Italy

Marco Scortichini[a,*], Stefania Loreti[b], Valeria Scala[b], Nicoletta Pucci[b], Massimo Pilotti[b], Giuseppe Tatulli[b], Erica Cesari[b], Alessia L'Aurora[b], Massimo Reverberi[c], Nicola Cristella[d], Paolo Marangi[d], Palma Blonda[e], Cristina Tarantino[e], Maria Adamo[e], Sabino Maggi[e], Gianluigi Cesari[f], Chiara Roberta Girelli[g], Federica Angilè[g], Mudassar Hussain[g], Danilo Migoni[g], Francesco Paolo Fanizzi[g]

[a] *Council for Agricultural Research and Economics (CREA), Research Centre for Olive, Fruit and Citrus Crops, Via di Fioranello, 52, I-00134, Roma, Italy*
[b] *Council for Agricultural Research and Economics (CREA), Research Centre for Plant Protection and Certification, Via C.G. Bertero, 22, I-00156, Roma, Italy*
[c] *Department of Environmental Biology, University Sapienza, Piazzale Aldo Moro, 5, I-00165, Roma, Italy*
[d] *Terranostra S.r.l.s., Via Monte Grappa, 48, I-72021, Francavilla Fontana, (BR), Italy*
[e] *Institute of Atmospheric Pollution Research, National Research Council of Italy, c/o Interateneo Physics Department, Via Amendola 173, I-70126, Bari, Italy*
[f] *Agrifound Italia, Borgo Santa Croce, 6, I-50122, Firenze, Italy*
[g] *Department of Biological and Environmental Sciences and Technologies (Di.S.Te.B.A), University of Salento, Campus Ecoteckne, Via Prov.le Lecce-Monteroni, I-73100, Lecce, Italy*

A R T I C L E I N F O

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A B S T R A C T

Xylella fastidiosa subsp. *pauca* (*Xfp*) is associated with olive quick decline syndrome (OQDS), a severe disease affecting the olive groves of Salento (Apulia, Italy). Through a series of interdisciplinary studies, an effective management strategy aimed at maintaining the traditional olive germplasm has been developed and evaluated. Specifically, a systemic biocomplex formulation containing zinc (4%), copper (2%) and citric acid, is sprayed on the tree canopy once per month from spring to early autumn. The strategy also includes sustainable vector control through agronomical techniques as well as regular tree pruning and soil fertilization. Quantitative real-time PCR assessments performed in a mid- and long-term studies showed a significant reduction in the *Xfp* concentration in the leaf xylem tissue upon treatment, improving olive tree yield. Both ^{1}H-NMR metabolomic and mass spectrometric lipidomic analyses of leaf extracts revealed the occurrence of biomarkers linked to disease or tree restoration. The effects of mannitol and oleuropein derivatives, 13-oxylipins/DOX-oxylipins and 9-oxylipins appear to be related to the attenuation of disease symptoms. Both techniques indicate rapid reprogramming of metabolic tree activity upon spray treatment to regain tree health. Multiscale satellite imagery monitoring through high-resolution Sentinel-2, very high-resolution Pleiades and vegetation indices confirmed the robustness of the strategy over several years in both experimental and productive olive groves. Currently, this strategy is applied in many infected olive groves in Salento. Notably, some aggressive fungal species belonging to the *Neofusicoccum* genus have been recently found to be associated with olive trees that show symptoms similar to those induced by *Xfp*. Coinfections between this bacterium and fungi have also been frequently observed, suggesting the need for a more in-depth assessment of the epidemiology and management of OQDS.

1. *Xylella fastidiosa*: an old but emerging quarantine phytopathogen

Xylella fastidiosa is a long-known pathogenic bacterium that represents a longstanding problem in the Americas, despite being recently introduced to Europe. The first report of a disease caused by *X. fastidiosa* dates to the end of 1800, when this species was detected on grapevines in California and denoted Pierce's disease, and this disease significantly impacted the viability of growing grapes. Viticulture was largely abandoned in the area (Hopkins and Purcell, 2002), and this disease became

* Corresponding author.
E-mail address: marco.scortichini@crea.gov.it (M. Scortichini).

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a limiting factor for grape cultivation (Myers et al., 2007). By using a combination of population and evolutionary genomics approaches, it has been estimated that this pathogen was introduced into Apulia (southern Italy) in approximately 2008 (Sicard et al., 2021). Following the occurrence of this pathogen in the European territory, the European Union (EU) included it in the list of Union quarantine (EU Regulation, 2019/2079, Annex II, part B), and the European and Mediterranean Plant Protection Organization (EPPO) in the A2 quarantine list of bacterial pathogens.

Following Apulia infection, new recoveries of *X. fastidiosa* occurred in Italy (i.e., the Tuscany and Latium regions), France, Spain, Portugal, Switzerland, Germany, and other countries such as Israel and Iran, thus affecting several host species (Trkulja et al., 2022; EPPO, 2023). Recently, out of the delimitated area of *X. fastidiosa* subsp. *pauca* (*Xfp*), new recoveries of *X. fastidiosa* subsp. *fastidiosa* and of *X. fastidiosa* subsp. *multiplex* occurred in Apulia region. *X. fastidiosa* is highly polyphagous and capable of surviving on 696 host plants; in many cases, it is symptomless (i.e., latent) (European Food Safety Authority EFSA, 2023) and is divided into subspecies based on host range and genetic relationships (Nunney et al., 2014). Recently, the subspecies *pauca, multiplex* and *fastidiosa*, the latter including the 'sandyi' and 'morus' populations, have been reclassified based on comparative genomic analyses (Marcelletti and Scortichini, 2016a; Denancé et al., 2019) (Table 1).

In addition to subspecies categorization, *X. fastidiosa* can also be subdivided into sequence types (STs) using a multilocus sequence typing (MLST) approach based on the sequencing of seven housekeeping genes, namely, *holC*, *nuoL*, *gltT*, *cysG*, *petC*, *leuA* and *malF* (Yuan et al., 2010). The introduction and spread of the different strains occurred through mechanisms of recombination and translocation between plant hosts. MLST demonstrated that two of the three subspecies of *X. fastidiosa* found in the United States (i.e., the *X. f.* subsp. *fastidiosa* and 'sandyi' populations) are nonnative and were presumably introduced in 1880 (i. e., the population 'sandyi') and 1890 (i.e., subsp. *fastidiosa*) after the first outbreaks occurred in the United States (Nunney et al., 2010; Yuan et al., 2010). Previous MLST analyses suggested that there is only one form of *X. fastidiosa* native to the United States, namely, *X. f.* subsp. *multiplex* (Nunney et al., 2010). *X. f.* subsp. *pauca* became pathogenic only recently on citrus and coffee (crops cultivated in Brazil for several hundred years) via intersubspecific recombination; a candidate donor is the subspecies infecting plum in the region since 1935 (possibly *X. f.* subsp. *multiplex*) (Nunney et al., 2012). Another divergent strain from subsp. *pauca* responsible for Citrus variegated chlorosis and coffee leaf scorching can cause OQDS in the Apulia region, namely, the "De Donno" strain. This strain is closely related to a strain of *Xfp* from Costa Rica, which infects oleander and coffee plants. It is suspected that the introduction of *Xfp* to Salento (Apulia, Italy) resulted from the importation of ornamental plants, and this event seems to be relatively recent (Marcelletti and Scortichini, 2016b; Giampetruzzi et al., 2017). *X. f.* subsp. *multiplex* originated from the southeastern United States and was more recently spread in California, Brazil, and Europe. Whole-genome sequence analyses of *X. f.* subsp. *multiplex* from America in comparison with strains associated with recent outbreaks in southern Europe indicated multiple introductions of *X. f.* subspecies *multiplex* into Italy, Spain, and France, with a likely origin in California, USA (Landa et al., 2020).

Table 1
Xylella fastidiosa subspecies and populations based on comparative genomic analyses (Marcelletti and Scortichini, 2016a; Denancé et al., 2019). The main host plants infected by *X. fastidiosa* strains belonging to different subspecies and populations have also been reported (European Food Safety Authority EFSA, 2023).

Xylella fastidiosa	subspecies	population	Main host plants
	fastidiosa		*Vitis* spp., Peach, Plum, Almond, Sweet cherry, Oaks, Maples
		sandyi	Oleander, *Hemerocallis* spp., *Magnolia grandiflora*
		morus	*Morus alba*
	multiplex		Almond, Peach, Olive, Blueberry, Polygala, *Cystus* spp., *Hebe* sp., *Viburnum tinus*
	pauca		*Citrus* spp., *Coffea* spp., Olive, Almond, Polygala, Oleander, Elm, Sycamore, Oak

If prompt detection of the pathogen is essential for monitoring its introduction and/or diffusion in the territory, the identification of subspecies and STs is crucial in the case of a new outbreak of *X. fastidiosa* in a pest-free area or in association with new plant hosts. The diagnostic tests for the detection/identification of *X. fastidiosa* are widely described in the diagnostic protocol of the EPPO PM7/24 (5), and Annex 4 of the EU regulation 2020/1201 lists the methods allowed for official diagnoses. Briefly, plant samples collected in areas bordering infected areas must first be assessed through molecular (i.e., real-time PCR) assays. In the case of positive results, the MLST analysis has to be performed from the isolated strain or directly from the plant material to define its ST as well as the presence of possible recombination among different strains that drove the evolution and adaptation of *X. fastidiosa* to new plant hosts, thus providing useful information on its possible origin (Landa et al., 2020).

Crop substitutions and altered land use patterns in response to *X. fastidiosa* pressure can have a large socioeconomic impact, particularly in areas where agriculture is directly linked to cultural identity, which occurred in Salento (Apulia region, southern Italy) with the olive quick decline syndrome (OQDS) (Scortichini, 2020a).

The progression of the *X. fastidiosa* epidemic in Salento impacted not only agricultural production and the landscape but also cultural heritage and social dynamics (Colella, 2023). The replacement of infected and dead olive trees with fruit tree species considered immune to the bacterium has also been proposed as a new cultivation option for this region (Alhajj Ali et al., 2023).

2. Olive decline in salento: a complex disease

The epidemic caused by *Xfp* in the Salento area represents the most serious phytosanitary event that has occurred in recent years in Italy, with very serious economic, landscape and social consequences. The olive groves of Salento are a notable agricultural crop that is strictly linked to a territory through a plurimillennial history (Primavera et al., 2017) along which all the human populations that inhabited such an area benefited from the tree yield and the consequent trade (Calabrese et al., 2012; Lanfranchi and Giannetto, 2012; Scortichini, 2020a). Public concern was further created in 2020 due to danger related to these high-value millennial trees, which, in addition to being protected by regional law, have been proposed for UNESCO World Heritage status (Sportelli, 2020). It should also be noted that local cultivars that have traditionally been trained in Salento since ancient times, namely, Cellina di Nardò and Ogliarola salentina, are characterized by high nutritional and human health value (i.e., polyphenols) (Del Coco et al., 2014; Negro et al., 2019). In October 2013, *X. fastidiosa* was shown to be associated with declining olive trees (Saponari et al., 2013) in a restricted area of the Apulian region (i.e., Salento, province of Lecce, southeastern Italy) and characterized as subspecies *pauca* in 2014 (Cariddi et al., 2014). This bacterium was proposed to be the causal agent of OQDS. In Salento, *Xfp* is characterized by wide polyphagia, with a range of 56 host/plant species (European Food Safety Authority EFSA, 2023), including ornamental species, wild plants typical of the Mediterranean flora, and crop species such as olive, sweet cherry and almond. This bacterium is naturally transmitted by insect vectors such as *Philaenus spumarius*, which feeds on the xylem sap of host plants (Cornara et al., 2017). In olive, symptoms of OQDS include leaf scorching, scattered wilting of twigs and branches starting from the top of the tree canopy and expanding to the remaining crown, delayed growth, desiccation, and plant death. In several species, the pathogen multiplies and colonizes the vascular system, inducing alterations via the occlusion of xylem vessels by bacterial aggregates embedded in an exopolysaccharide matrix and tyloses and gums produced by the plant in response to infection (De La Fuente et al., 2008; Rapicavoli et al., 2018).

Symptoms resembling OQDS were already reported by olive growers some years before bacterial identification. Genomic studies suggest that the genome of the strain identified in the Apulia region (namely, sequence type ST53) is closely related to that of Costa Rica (Marcelletti and Scortichini 2016b; Giampetruzzi et al., 2017). However, concerning its introduction to Apulia, 2008 may not be strictly related to when the pathogen was first introduced in Italy, when it started to be adapted to olive trees as a host plant or when the epidemic started (Sicard et al., 2022). By following the monitoring reports of the Phytosanitary Service of the Apulia region, meteorological data on the increase in temperature of the Earth's surface, logistic functions coupled with fitting models (Kottelenberg et al., 2021) and in-depth analysis of the latency period of the disease, it can be postulated that the diffusion of OQDS started in 2002–2003 (Scortichini, 2022) At the time of its first report in 2013, approximately 10,000 ha were already affected, corresponding to approximately 1 million olive trees (Martelli, 2016). Twelve months later, the number of compromised hectares was more than 20,000 ha, and a few years later, the disease was defined as endemic (Strona et al., 2017) and considered no longer eradicable. To apply measures for containing pathogens on the quarantine list, the National and European Phytosanitary Authorities proposed moving from an "eradication" strategy to a "containment" strategy (European Commission, Commission Implementing Decision, 2015/789).

The difficulties and consequent delays in the correct identification of the causal agent of OQDS of olive trees have allowed the rapid spread of the disease in the Salento territory, where the olive grove is present in extensive monocultures of two autochthonous susceptible cultivars for many kilometres (Scortichini, 2022). Another significant predisposing factor may have been favourable conditions for the vector *P. spumarius*, the predominant xylem-sap feeder in that area, which plays a crucial role in spreading the pathogen and is able to cause repeated inoculations in the olive tree canopy (Cornara et al., 2017). The widespread presence of spontaneous host plants and ornamental shrubs, nonoptimal agronomic practices (i.e., hard pruning), adverse climatic conditions (drought, frost, and extreme rainy events) (Scortichini et al., 2018) and alterations in the equilibrium of microelements in the soil (Del Coco et al., 2020) have further favoured the rapid spread of the bacterium in this region (Scortichini, 2022).

Phytosanitary legislation has demarcated three main areas related to *Xfp* outbreaks in Apulia that can be modified northwards according to disease progression: a) the "infected" area, which includes a vast portion of the territory further south of Salento (i.e., the whole Lecce and Brindisi provinces and part of Taranto province) and b) the "containment" and "buffer" areas subject to monitoring and laboratory analyses for identifying new disease foci. Currently, the bacterium is consolidating in the "containment" zone, breaking through the "buffer" zone, and emerging into the safe zone close to Bari Province. The pathogen is estimated to move with an advance front of approximately 20 km per year (Kottelenberg et al., 2021). Remarkably, during recent field surveys to perform epidemiological studies on olive decline, leaf wilting and twig and branch diebacks apparently resembled those incited by *Xfp* (Fig. 1). However, by carefully observing the different facets of the disease, some consistent differences in OQDS were revealed. Among these, foliar and wood infection allowed us to differentiate OQDS from the branch and twig dieback caused by some *Botryosphaeriaceae* fungi, namely, *Neofusicoccum* spp.; the name "branch and twig dieback" (BTD) was given to the disease (Brunetti et al., 2022; Manetti et al., 2023). The most typical symptom caused by *Neofusicoccum* spp. consists of leaves that show a reddish-brown leaf blade discolouration, followed by complete leaf wilting. In contrast, *Xfp* causes leaf tip wilting without reddish discolouration (Fig. 2).

Transverse sections of branches infected by such fungi showed typical "V"-shaped necrotic lesions caused by these fungi (Fig. 3); such

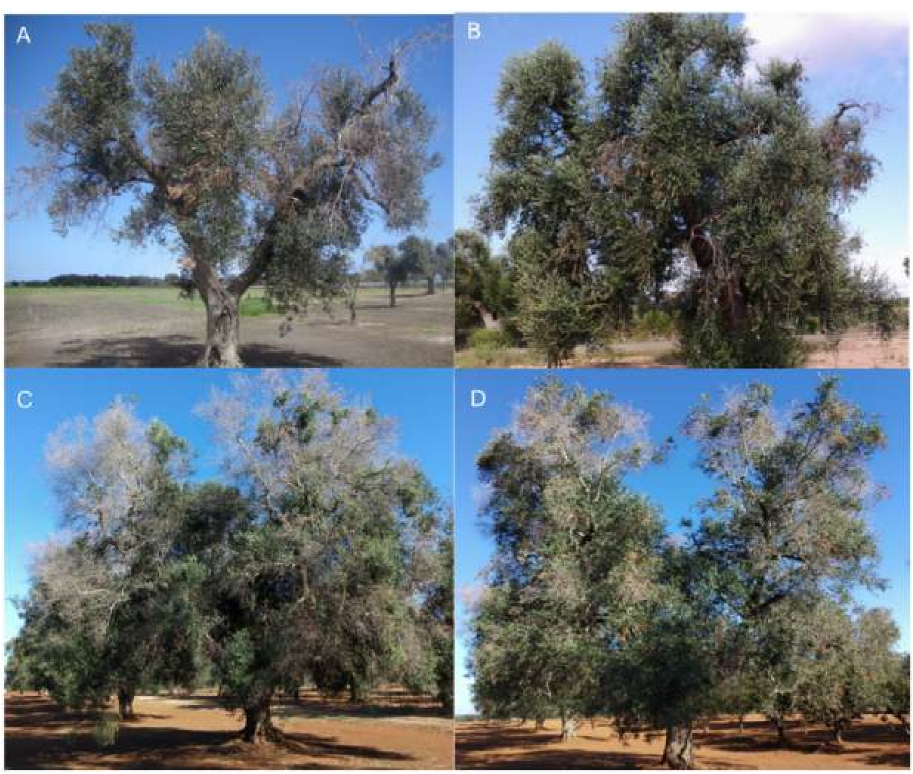

Fig. 1. Symptoms of twig and branch diebacks caused by *Xylella fastidiosa* subsp. *pauca* (*Xfp)* (A and B) and *Neofusicoccum* spp. (C and D) to olive trees in Salento (Apulia, Italy). Such symptoms can be easily confounded during field surveys for sampling leaves to detect the bacterium.

Fig. 2. Healthy olive leaves (A, D) and leaf tip wilting induced by *Xylella fastidiosa* subsp. *pauca* (B and C), and leaf reddening caused by *Neofusicoccum* spp. (E and F) in olive trees in Salento (Apulia, Italy). The two phytopathogens were detected (*Xfp*) or isolated (*Neofusicoccum*) from infected trees.

lesions were never observed in branches solely infected by *Xfp*. These fungi were found to be significantly pathogenic to olive trees in pathogenicity tests, and *N. mediterraneum* showed aggressiveness in terms of its bark cankering and wilting capacity (Brunetti et al., 2022; Manetti et al., 2023; Scortichini et al., 2023). These fungi should be considered quite dangerous since in pathogenicity tests, they exhibit virulence similar to that already reported for the same fungi that cause severe olive dieback in Spain, California, and other regions of Italy (Moral et al., 2010, 2017; Úrbez-Torres et al., 2013; Linaldeddu et al., 2023). Notably, species belonging to *Botryosphaeriaceae* also cause relevant damage to olive cultivation in other countries, such as Australia, South Africa, Uruguay, Croatia, and Tunisia (Sergeeva et al., 2009; Kaliterna et al., 2012; Triki et al., 2015; Spies et al., 2020; Hernandez-Rodriguez et al., 2022), as well as to the wild oleaster in Sardinia (Italy) (Manca et al, 2010). A recent survey performed in several regions of Italy revealed that such aggressive fungi are consistently found in the olive groves of Veneto, Lombardy, and Sardinia (Linaldeddu et al., 2023) as well as in Latium and Tuscany (Manetti et al., 2023) (Fig. 4). Interestingly, in our ongoing surveys in Salento, we ascertained that *Xfp*, *Botryosphaeriaceae* and even other fungal families can consistently coinfect the same olive trees that show significant wood discolouration, among other symptoms (Manetti et al., 2023; Scortichini et al., 2023).

This would suggest that a complex disease is causing the decline in olive grove productivity in Salento. We also stress that such olive diebacks can be easily confounded during monitoring surveys for assessing *Xfp* occurrence in the demarcated areas of Salento (Scortichini and Cesari, 2019; Ciervo and Scortichini, 2024)

Molecular analyses revealed that many trees showing OQDS symptoms did not host the bacterium, and in one of the most recent extensive surveys (i.e., 2022), only 3.21% of the olive trees that showed dieback symptoms hosted *Xfp* (Ciervo and Scortichini, 2024). Within this context, it should be noted that some years ago, *Phaoeoacremonium* spp., *Neofusicoccum parvum*, *Diplodia seriata*, and *Pleurostomophora richardsiae* were found to be associated with declines in olive groves in Apulia (Carlucci et al., 2013, 2015). Such fungi were consistently found either in northern or southern Apulia, even though they were not found together with *Xfp* in the same tree (Carlucci et al., 2020). More recently, *Apiospora marii* (synonym: *Arthrinium marii*) was found to be the causal agent of twig dieback and wood discolouration in very young olive trees in Fasano (Brindisi Province) and in Andria (north of Bari) (Gerin et al., 2020). The occurrence of fungi related to olive tree decline in Apulia and their relationships with *Xfp* are consistent phenomena that deserve additional in-depth studies.

3. Management of plant diseases: basic principles and common applications for quarantine bacterial pathogens

According to the principles of plant disease control illustrated by the American Phytopathological Society, in most cases, when a phytopathogen is established in an area, it is not possible to eliminate (i.e., eradicate at zero level) it from a crop, but it is possible to reduce its severity and maintain disease progression below thresholds that allow the crop to produce yearly (i.e., the field of applying a "cure" or "therapy" strategy) (https://www.apsnet.org/edcenter/disimpactmngmnt/topc/EpidemiologyTemporal/Pages/ManagementStrategies.aspx). This principle is even more stringent for woody or perennial crops since the uprooting of the trees in the attempt to eliminate the pathogen is not economically viable and is sometimes doubted due to the economic and/or cultural relevance represented by the crop for a certain territory. For quarantine pathogens that are ruled by supranational phytosanitary laws aimed at eliminating a pathogen introduced for the first time from abroad in a specific territory, it is technically and biologically difficult to eradicate, especially when the quarantine microorganism is not detected or isolated soon after its introduction (European Food Safety Authority PLH Panel, 2015), and long-term attempts to eradicate *X. fastidiosa* from vineyards in the U.S.A. have failed (European Food Safety Authority PLH Panel, 2015). Additionally, *Xfp* in the olive groves of Apulia represents an example where the slow detection of a quarantine bacterium

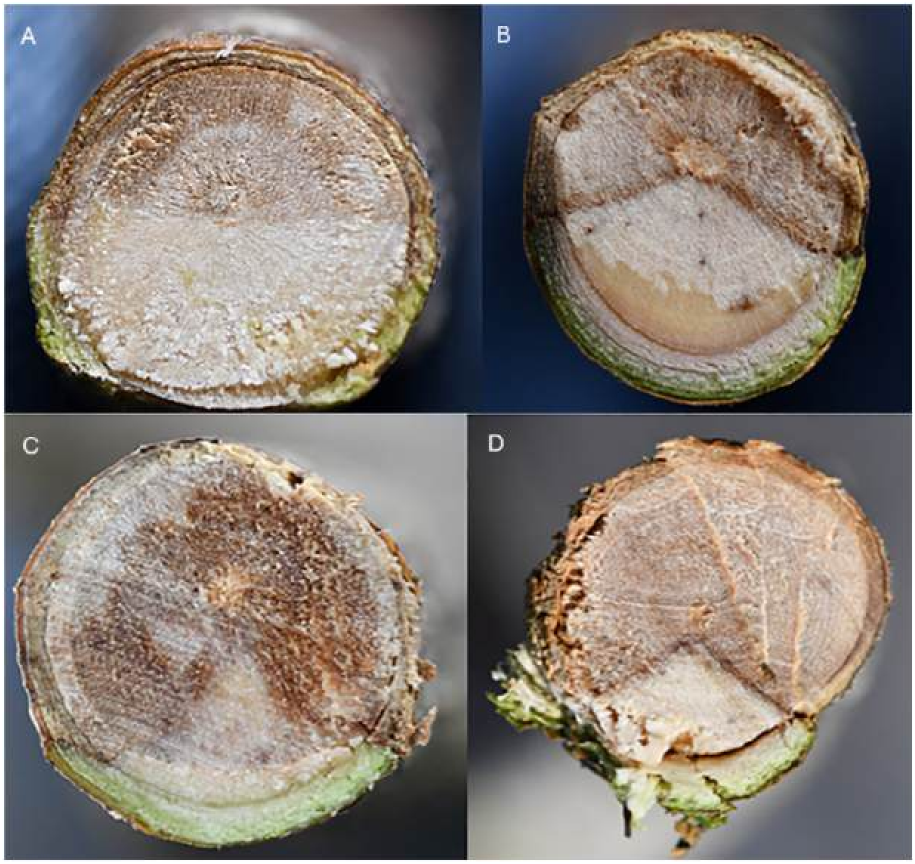

Fig. 3. Typical "V"-shaped necrotic lesions caused by *Neofusicoccum* spp. on the olive branch, as observed in olive groves of Salento, that show symptoms of branch and twig dieback. A: initial wood discoloration; B: "V"-shaped necrotic lesion on half of the branch; C, D: the "V"-shaped lesion has reached almost the whole branch diameter. Olive trees infected only by *Xylella fastidiosa* subsp. *pauca* did not show such signs of disease (Manetti et al., 2013).

and the large occurrence of diseased trees made the eradication of the pathogen in the infected area no longer feasible (Burbank, 2022). Moreover, for a polyphagous bacterium largely spread in the area by a very prolific insect vector such as *P. spumarius*, eradication in the area would be a failure (European Food Safety Authority PLH Panel, 2015).

Within quarantine and emerging bacteria, effective field control has also been achieved in areas where the pathogen is common, such as *Erwinia amylovora*, the causal bacterium of fire blight of pome fruits, and *Pseudomonas syringae* pv. *actinidiae*, the causal bacterium of kiwifruit bacterial canker. In both cases, an integrated approach based on the knowledge of the epidemiological cycle of the pathogen and on the timely application of different types of effective compounds allows for the cultivation of the crop in an infected area (Stockwell et al., 2010; Farkas et al., 2012; De Jong et al., 2019). Keeping this in mind, we tried to develop a disease management strategy that also includes the control of the vector through a sustainable approach that enables olive trees infected by *Xfp* to produce regularly in the presence of the bacterium within the tree.

4. Studies to reduce the impact of *Xylella fastidiosa* on crops

Notably, until 1987, the most common and well-known disease caused by *X. fastidiosa*, known as Pierce's disease in the U.S.A., was caused by an unknown 'viral agent' (Wells et al., 1987). Consequently, the control measures for mitigating the severity of the disease were, until that period, mainly based on the control of the insect vectors and the removal and substitution of the symptomatic plants. However, such methods have very rarely resulted in the successful elimination of the pathogen from the field (Hopkins and Purcell, 2002). Later, the discovery of other novel emerging diseases caused by the bacterium further promoted the assessment of additional control strategies to mitigate the damage caused by *X. fastidiosa* to cultivated and ornamental plant species (Hopkins and Purcell, 2002). Currently, both preventive and curative strategies aimed at preventing and/or reducing the incidence and severity of diseases caused by *X. fastidiosa* are being studied in the field (Kirkou et al., 2018; Burbank, 2022). Among the curative strategies under study by different research groups aimed at lowering the concentration of the pathogen within the xylem tissue and managing the disease in the presence of the pathogen are the use of bacteriophages, antagonistic bacteria, antibacterial microelements, natural compounds, nanoparticles, and synthetic peptides (Table 2). Antibiotics are also studied and employed in some circumstances, but due to their restriction in Europe to reduce the.

risk posed to humans in terms of antibiotic resistance development, antibiotics are not a feasible option. Cold or environmental therapy, based on the reduction in the bacterial concentration in the xylem vessels upon the occurrence of air temperatures close to or below 0 °C, has received some attention in the past (Purcell, 1977, 1980), but it has not been studied in detail. The identification of tolerant or possibly resistant cultivars is another important goal in identifying diseases caused by *X. fastidiosa* (Kirkou et al., 2018; Della Coletta-Filho et al., 2020; Morelli et al., 2021), and currently, several studies have aimed to determine the source of resistance within grapevine, citrus and olive germplasms (Della Coletta-Filho et al., 2020; Pavan et al., 2021; Aguero et al., 2022). It should be noted that the control of insect vectors through the timely distribution of insecticides, the possible application of species-specific vector parasitoids or the mechanical elimination of eggs from the ground and weeds is another pillar for field control of the bacterium (Kirkou et al., 2018; Della Coletta-Filho et al., 2020). To date, most of the studies carried out with potential curative strategies include *in vitro*

Fig. 4. Map showing the current occurrence of *Neofusicoccum* spp. in olive groves in different regions of Italy (i.e., Lombardy, Veneto, Tuscany, Latium, Sardinia, and Apulia), indicated by the typical hairy conidiomata of *N. mediterraneum*. In Apulia, the occurrence of *Xylella fastidiosa* subsp. *pauca*, indicated by the bacterium cells, has also been reported.

tests, greenhouse assays performed with potted plants, or a few plants trained in open fields (Kirkou et al., 2018), and apart from *Xfp* on olive trees in Italy (Scortichini et al., 2018; Tatulli et al., 2021), there is no strategy that has been verified in open fields for a long time period. Some promising lines of research are briefly reported herein.

Among bacteriophages, some lytic broad host-range strains have been shown to reduce Pierce's disease symptoms in preliminary glasshouse inoculations of potted plants carried out with a cocktail of four phages (Ahern et al., 2014; Das et al., 2015). Additional phages with the potential to reduce *X. fastidiosa* activity have been recently found in Mediterranean areas (Clavijo-Coppens et al., 2021).

Endophytic antagonistic bacteria also represent a potential valid strategy to reduce the activity of *X. fastidiosa* within the xylem tissue. Nonvirulent *X. fastidiosa* strains were first used to verify this approach, yielding promising results (Hopkins, 2005). In addition, other endophytic species have shown some interesting activity in mitigating *X. fastidiosa* symptoms. When sprayed on foliage, a strain of *Parabulkholderia phytofirmans*, namely, PsJN, significantly reduced Pierce's disease severity by possibly priming the expression of innate disease resistance pathways (Baccari et al., 2019). However, its activity was not detected in olive trees infected with *Xfp* (Morelli et al., 2019). Antagonistic activity towards *X. fastidiosa* was also found for *Pseudomonas fluorescens*, obtained from grapevine, and for *Curtobacterium flaccumfaciens*, isolated from citrus (Araújo et al., 2002; Deyett et al., 2017). Culture filtrates of epiphytic and endophytic bacterial species have also shown interesting antibiofilm activity towards *Xfp* (Mourou et al., 2022). Additionally, some endophytic fungi, namely, *Aureobasidium* sp. And *Cladosporium* sp., have shown antagonist activity *in vitro* against *X. f.* subsp. *fastidiosa* (Rolshausen and Loper, 2010).

Table 2
Main options under study to reduce the impact of *Xylella fastidiosa* on crops.

	Tool proven effective in *in vitro* or *in planta* assays	References
Bacteriophages	Lytic broad host range phages	Ahern et al. (2014); Das et al. (2015); Clavijo-Coppens et al. (2021)
Antagonistic bacteria	Non virulent *X. fastidiosa* strains	Hopkins (2005)
	Curtobacterium flaccumfaciens	Araújo et al., 2002
	Pseudomonas fluorescens	Deyett et al. (2017)
	Paraburkholderia phytofirmans	Baccari et al. (2019)
Antagonistic fungi	*Aureobasidium, Cladosporium*	Rolshausen and Roper (2010)
Microelements	Zinc	Kirkpatrick et al., 2003; 2004; Cobine et al. (2013); Navarrete and De La Fuente (2015)
Natural compounds	Cathecol, caffeic acid, resveratrol	Maddox et al. (2010)
	Radicinin	Aldrich et al. (2015)
	Oleuropein, veratric acid	Bleve et al. (2018)
	Phenolic olive leaf extracts	Vizzarri et al. (2023)
Nanoparticles	Chitosan-coated Fosetil-Al nanocristals	Baldassarre et al., 2020
	Thymol nanoparticles	Baldassarre et al., 2023
	Silver nanoclusters	Orfei et al. (2023)
Synthetic peptides	Peptides 1036, RIJK2	Moll et al. (2021)
	Peptides Ascaphin-8, DASamP1, DASamP2	El Handi et al. (2022)
Resistant cultivars	Grapevine, Citrus, Olive	Kirkou et al. (2018); Della Coletta-Filho et al. (2020); Morelli et al. (2021)

Among the antibacterial microelements, zinc was among the first to be investigated for possible activity towards *X. fastidiosa* and was the first to be evaluated as having a promising curative effect. This microelement was applied to vineyards exposed to Pierce's disease as zinc sulfate through foliar spray, trunk endotherapy or application to the soil and showed partial efficacy in reducing the incidence and severity of the disease in different grapevine cultivars (Kirkpatrick et al., 2003, 2004). The strict link between zinc and *X. fastidiosa* virulence was ascertained through a series of basic studies that revealed that at a certain dose (i.e., 0.25 mM), zinc inhibited bacterial biofilm formation (Cobine et al., 2013). In addition, zinc detoxification *in planta* is required to incite full *X. fastidiosa* virulence, suggesting that removal of such microelements from host plant tissue is necessary before colonization can begin (Navarrete and De La Fuente, 2015). An important corollary of this feature is that zinc represents a preformed defence for the plant that limits the growth of the bacterium, and, consequently, a manipulation of the zinc content in plants could represent a disease management strategy (Navarrete and De La Fuente, 2015). Recently, the possibility of applying zinc-based compounds to control *X. fastidiosa* has gained particular attention. The nanoformulation of zinc oxide, namely, Zinkicide®, distributed to the soil significantly reduced the *in planta* multiplication of both *X. f.* spp. *fastidiosa* and *multiplex* strains in tobacco and blueberry (*Vaccinium* sp.) plants grown in greenhouse conditions without inciting any phytotoxicity (Shantharaj et al., 2023). In addition, zinc-based formulations have shown antibacterial activity against *Xfp* both in *in* *vitro* tests and with potted olive plants (Del Grosso et al., 2021, 2022). The possible utilization of copper as an ion with bactericidal activity against *X. fastidiosa* has also been considered (Cobine et al., 2013). However, its specific ability as a unique microelement to counteract bacterial multiplication *in planta* has not been shown to be significant (Kirkpatrick et al., 2003, 2004; Ge et al., 2020).

In vitro tests revealed the antibacterial activity of several natural compounds towards *X. fastidiosa*. Radicinin, a phytotoxin obtained from the grapevine endophytic fungus *Cochliobolus* sp., was shown to reduce the growth of *X. f.* subsp. *fastidiosa* (Aldrich et al., 2015). Several phenolic compounds have antibacterial effects on *X. f.* subsp. *fastidiosa* and *pauca*. Flavonoids, stilbenes and coumarins showed antibacterial activity against *X. f.* subsp. *fastidiosa*, with catechol, caffeic acid and resveratrol being the most effective (Maddox et al., 2010), whereas 4-methylcathecol, catechol, veratric acid, caffeic acid, and oleuropein showed bacteriostatic activity towards *Xfp* (Bleve et al., 2018). Phenolic extracts from olive leaves have shown bacteriostatic activity against *Xfp* (Vizzarri et al., 2023). The possible utilization of synthetic peptides obtained through standardized techniques is another strategy currently under study. In preliminary studies, some synthetic peptides have shown either bactericidal and antibiofilm activity towards all *X. fastidiosa* subspecies (Moll et al., 2021; El Handi et al., 2022) or to promote a reduction in the *X. f.* subsp. *fastidiosa* population in potted almond plants when injected into the trunk through endotherapy (Moll et al., 2022). Nanomaterial compounds (i.e., chitosan-coated fosetyl-Al nanocrystals and thymol nanoparticles) showed interesting bactericidal effects on *X. fastidiosa* subspecies (Baldassarre et al., 2020, 2023). Similalrly, silver nanoclusters also showed a relevant *in vitro* efficacy (Orfei et al., 2023). A mixture of plant extracts also reduced field symptoms caused by *Xfp* (Bruno et al., 2020).

5. Management of *X. fastidiosa* subsp. *pauca* in olive groves of salento

The uniqueness of the olive agroecosystem, combined with the impossibility of achieving effective eradication of the bacterium, which was already present in over approximately 10,000 ha at the time of the first report, prompted us to study the possibility of coexisting with the pathogen through the application of a field management strategy. The overall sustainability of the strategy was also considered. We proposed a low-cost strategy through the utilization of a compound that could also be utilized for organic farming and that does not perturb the soil ecological equilibrium. Herein, we summarize the main results concerning either the principles that have led us to investigate the possibility of mitigating *Xfp* outbreaks or the field efficacy and the basic knowledge that has been achieved by the studies that have been performed within this framework.

5.1. Preliminary assessments

The ability of a compound to reach the xylem tissue of leaves and, possibly, of twigs and achieve a significant reduction in the long-term *Xfp* concentration in olive trees without causing any phytotoxic effects on the tree without altering the environment implies the contemporary occurrence of several characteristics, the most important of which are i) having bactericidal activity against the pathogen, ii) being highly systemic in the xylem tissue, iii) being able to release the active elements in the xylem tissue, iv) not being present in the oil, and v) being effective over a long-term period. According to the studies of Cobine et al. (2013) and Navarrete and De la Fuente (2015), a biocomplex prototype formulation, namely, PRC2022 (Pireco Products B.V., The Netherlands), which contains zinc (4% w/w) and copper (2% w/w) salts complexed with the hydracid citric acid, was chosen for the studies and applied. Other points that are important for choosing this biocomplex include its ease of use, its absence of phytotoxicity, and its affordability.

The possible bactericidal activity of PRC2022 was ascertained through a series of *in vitro* assays (Tatulli et al., 2021). Such tests showed relevant bactericidal activity towards all the *X. f.* subsp. *fastidiosa*, *multiplex* and *pauca* strains tested, including the "De Donno" strain isolated from olive trees in Salento that showed OQDS. The antibacterial activity was ascertained both in broth tubes and on a bacterial culture substrate. The absence/reduction of growth was also ascertained through quantitative real-time PCR over 30 days after the inoculation of the tubes, indicating a long-lasting effect of the antibacterial activity. Notably, antibacterial activity towards *X. fastidiosa* was also observed at a biofertilizer dilution of up to 1:100, and its minimum bactericidal concentration (MBC) was 400 mg L^{-1} or 200 mg l^{-1} when combined with zinc or copper, respectively. PRC2022 also significantly reduced biofilm formation in all the tested *X. fastidiosa* subspecies (Fig. 5). These data indicated that the biocomplex can potentially reduce either pathogen vessel colonization (planktonic phase) or the biofilm phase, thus justifying further testing in the field (Tatulli et al., 2021).

To assess the ability of biocomplex to effectively reach the xylem tissue of olive trees, a series of *ad hoc* studies were performed. Through confocal laser scanning microscopy and fluorescence quantification, it has been ascertained that PRC2022 reached the olive xylem tissue both after the spraying of the canopy or through endotherapy, thus demonstrating its effective systemicity. Fluorescence staining of leaves, leaf petioles and two- and five-year-old twigs revealed that the biocomplex clearly migrated through the xylem tissue from the entry points up to 80 cm of trunk height. The release of zinc and copper in the xylem of the samples was assessed through coupled plasma atomic emission spectroscopy. The analysis revealed that these ions were effectively released in the xylem. These data clearly indicated that the biocomplex reaches the xylem tissue and corroborated its potential use in the field (Scortichini et al., 2018).

5.2. Field trials and quantitative real-time PCR for testing biocomplex efficacy in the medium- and long-term

In parallel with the *in vitro* and *in planta* studies described above, we verified the effectiveness of the biocomplex on *Xfp* directly under open-field conditions. Thus, we selected traditional olive groves located in the infected area of Salento (i.e., Lecce Province) and trained them on local susceptible cultivars, namely, Ogliarola salentina and Cellina di Nardò, whose ages varied from 25 to more than 70 years. The first preliminary study was carried out at Veglie and, subsequently, at Galatone and Cannole to verify the effectiveness of the strategy in the medium- and long-term (Scortichini et al., 2018; Tatulli et al., 2021). In the first study (Scortichini et al., 2018), spray treatments on the olive crowns, performed during spring and autumn, induced a significant reduction in both field symptoms (i.e., twig wilting) and pathogen concentration within the leaves. No zinc or copper residues were found within the oil obtained from trees that received the biocomplex for more than three years.

In the medium-term evaluation (i.e., farms that applied the control strategy for three or four consecutive years), Leccino trees were also present on the Galatone farm. Generally, a trend that indicated a reduction in field symptoms during the year on both farms and for all cultivars was observed, with the number of wilted twigs increasing in March and decreasing in July and October. The Ogliarola salentina and Cellina di Nardò cultivars were confirmed to be more sensitive to *Xfp* than the Leccino cultivar. At the end of the season, just before harvest, only a few new wilted twigs per tree were recorded for all cultivars on both farms (Tatulli et al., 2021). Foliar treatments induced a significant reduction in the *Xfp* concentration in all cultivars, as observed by quantitative real-time PCR analyses. The first score of the mean bacterial concentration was obtained in March. At Galatone, Leccino trees had a lower bacterial concentration (i.e., mean of 9.0 10^2 CFU g^{-1}) than did Cellina di Nardò (1.7 10^4 CFU g^{-1}) and Ogliarola salentina (8.7 10^3 CFU g^{-1}).

A similar trend was also observed in the Cannole grove, which

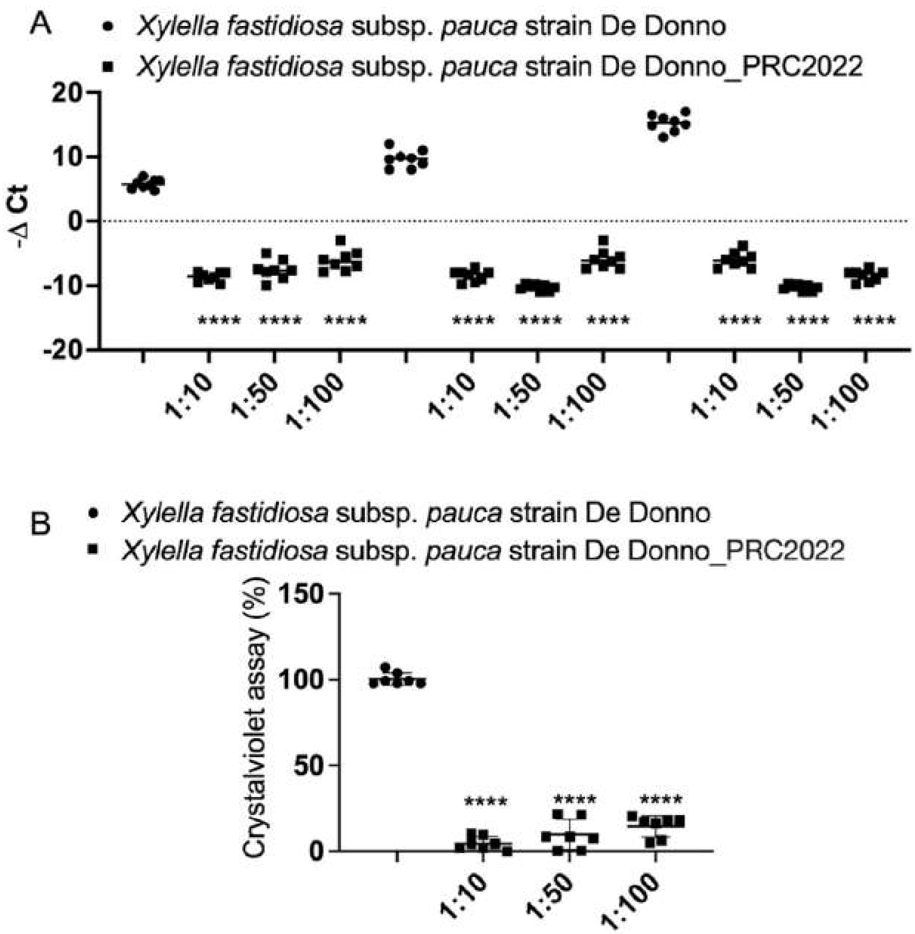

Fig. 5. A) *In vitro* significant efficacy of biocomplex PRC2022 dilutions (i.e., 1:10, 1:50, 1:100) on the planktonic growth of the *Xylella fastidiosa* subsp. *pauca* strain "De Donno" (control), as assessed by real-time PCR at 6-, 15- and 30-days post inoculation (from left to right). B) *In vitro* biofilm assay 30 days post inoculation for the *X. f.* subsp. *pauca* strain De Donno (control) according to different biocomplex dilutions (i.e., 1:10, 1:50, 1:100). The values are the means ± SDs of three biological replicates (n = 7). ****: significant difference according to one-way ANOVA and Dunnett's test at $p < 0.0001$ vs. the control. -ΔCt: difference for each sample between the real-time Ct value at each time point and the Ct value at time 0. (Redrawn from Tatulli et al., 2021).

showed a mean *Xfp* concentration that ranged between 1.0 10^2 and 10^4 CFU g^{-1} during spring, summer, and autumn; between 1.0 10^2 and 10^4 CFU g^{-1} in Cellina di Nardò; and between 1.0 10^3 and 10^4 CFU g^{-1} in Ogliarola salentina (Fig. 6). Remarkably, the treatments resulted in a good yield of approximately 18 and 23 kg of olives per tree recorded in autumn 2019 on the farms of Galatone and Cannole, respectively, whereas the untreated trees were completely wilted (Tatulli et al., 2021). A reduction in the *Xfp* cell concentration within the foliage corresponded to a greater vegetation index, as revealed by satellite monitoring of the experimental fields (Table 3). Subsequently, long-term assessments after seven and eight years of biocomplex application were performed with the same olive groves. In this case, quantitative real-time PCR also confirmed the efficacy of PRC2022 in maintaining the *Xfp* cell concentration to a level that allows the tees to vegetate and yield fruit.

Cellina di Nardò trees in both fields showed a concentration of approximately 1.0 10^3 CFU g^{-1}, whereas Ogliarola salentina trees showed an *Xfp* concentration of approximately 1.0 10^2 CFU g^{-1} at Galatone and approximately 5.0 10^3 CFU g^{-1} at Cannole. Leccino trees at Galatone had a concentration of approximately 1.0 10^2 CFU g^{-1} (Table 3, Fig. 6). Notably, in the last assessment, the control trees bordering the treated plots showed some suckers scattered within a wilted crown.

These results further supported the conclusions from the first study and clearly showed that *Xfp* can be managed in olive groves not completely damaged by the bacterium and that coexistence with the pathogen is possible in the "infected" area of Salento. Notably, the olive groves are in areas severely attacked by the bacterium and are surrounded by completely withered olive trees. After the crown treatments, the amount of copper released for one year was approximately 500 g/ha, which is much less than the 4 kg/ha that represents the current allowable limit of copper in soil for organic agriculture.

6. Interdisciplinary studies confirmed the efficacy of this management strategy

6.1. Reprogramming of phenolic compound and carbohydrate metabolite production

Through a metabolomic approach, several studies were performed to monitor the trend of some olive tree metabolites in trees naturally infected by *Xfp* and in trees treated with PRC2022. To assess the response of naturally infected untreated Ogliarola salentina and Cellina di Nardò trees in comparison with that of PRC2022-treated trees, non-targeted ^{1}H-NMR fingerprinting in combination with unsupervised principal component analysis (PCA) and supervised pattern recognition techniques was applied by Girelli et al. (2017). Xylematic polyphenol and carbohydrate contents changed in response to the biofertilizer treatments, with Cellina di Nardò trees showing relatively high polyphenol contents and Ogliarola salentina trees showing relatively high sugar contents.

The metabolic response of *Xfp*-infected and untreated Ogliarola

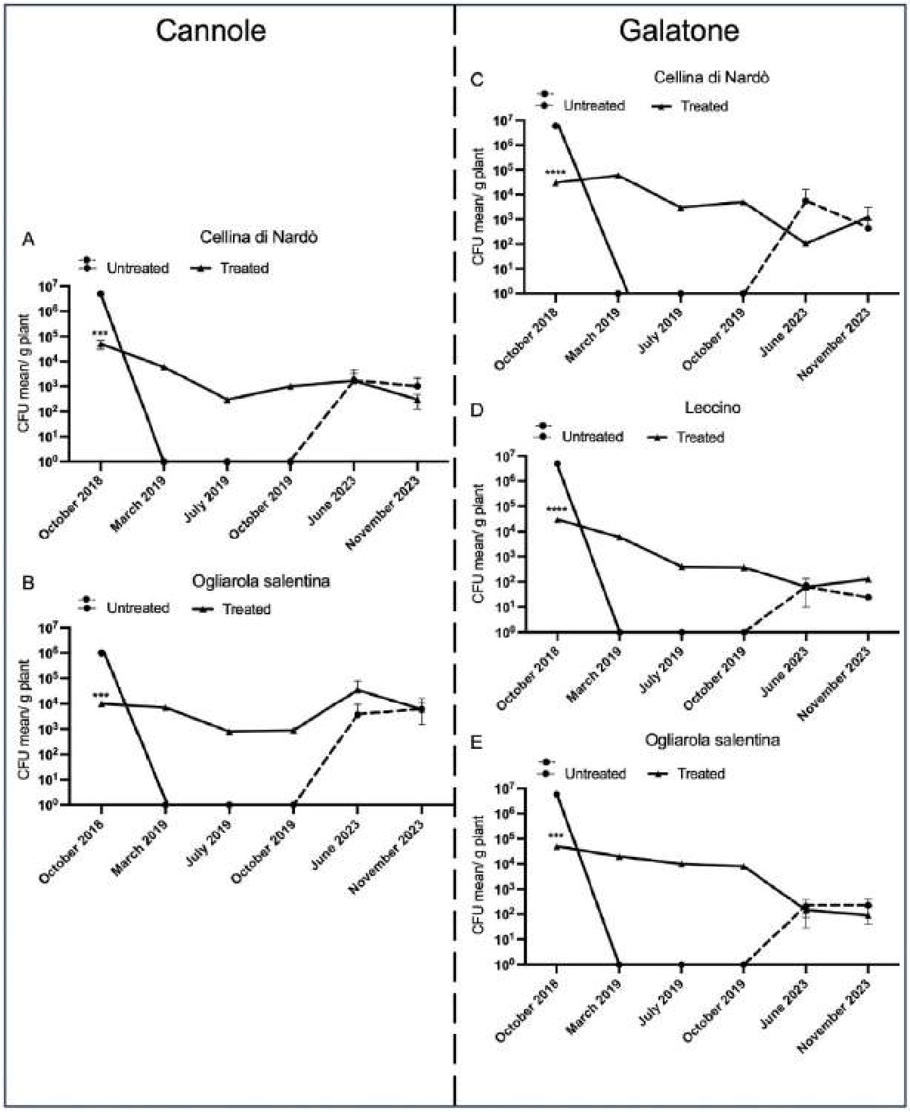

Fig. 6. The *Xylella fastidiosa* subsp. *pauca* DNA concentration, expressed on a logarithmic scale of CFU equivalents g^{-1} leaf as determined for untreated plants (assessed as the time 0 control) and PRC2022-treated cultivars Cellina di Nardò, Ogliarola salentina, and Leccino at Cannole (A and B) and Galatone (C, D and E) (Lecce Province) as assessed during 2018–2019 (medium-term evaluation) (redrawn from: Tatulli et al., 2021) and during 2023 (long-term evaluation). The untreated control plants died at the beginning of 2019, so the absence of concentration data is indicative of plant death. A, B: Graphical representation of the bacterial concentration over time of Cellina di Nardò and Ogliarola salentina at Cannole. C, D, E: Graphical representation of the bacterial concentration over time in Leccino, Cellina di Nardò and Ogliarola salentina trees at Galatone. Dotted lines represent the bacterial concentration observed in new suckers taken from wilted trees as controls during 2023.

salentina and Cellina di Nardò olive trees was further investigated during the first year of foliar treatment with PRC2022 (Girelli et al., 2019). The ^{1}H-NMR metabolomic approach showed that for both cultivars, metabolites such as quinic acid, oleuropein-related compounds, and polyphenols were consistently found to be differentially accumulated between treated and untreated trees. Quinic acid, a precursor of lignin, was confirmed to be a disease biomarker for olive trees infected by *Xfp*. Interestingly, the two cultivars showed a distinct response to the biofertilizer treatments: a consistent increase in malic acid was observed for the Ogliarola salentina trees, whereas in the Cellina di Nardò trees, the treatments induced the accumulation of γ–aminobutyrate (GABA), a known stress mitigation molecule (Kinnersley and Turano, 2000).

The ^{1}H-NMR metabolomic approach was also used to compare the xylematic extracts of the *Xfp*-tolerant cultivar Leccino with those of the susceptible cultivars Ogliarola salentina and Cellina di Nardò following a medium-term period of PRC2022 foliar treatment (i.e., three years of spray treatment) (Girelli et al., 2021). A greater mannitol content was detected in the treated trees. Due to its ability to protect chloroplasts from osmoprotectants and antioxidants, the intracellular accumulation of mannitol is a strategy for improving tolerance to drought. The accumulation of mannitol also suggested an improvement in the physiological performance and photosynthetic capability of olive trees. For the untreated *Xfp*-infected samples, a greater relative content of phenolic compounds was observed. The tyrosol and hydroxytyrosol moieties of oleuropein and its aldehydic forms and of quinic acid were observed for all the analysed cultivars (Girelli et al., 2021).

Overall, the results revealed that *Xfp* strongly modifies the metabolism of olive trees and that biocomplex spray treatment can induce early reprogramming of metabolic pathways in infected trees. The different responses to PRC2022 treatment seem to be correlated with olive cultivar physiology and/or pathogen attack levels. An additional confirmation of the metabolic reprogramming induced by PRC2022 treatments on olive trees was recently obtained by the metabolic assessment of olive trees upon endotherapy. Short-term injection of the

Table 3
The efficacy of *Xylella fastidiosa* subsp. *pauca* management in olive groves of Salento (Lecce Province) was assessed by average quantitative real-time PCR (colony forming unit (CFU) equivalent/g of leaf), and the normalized difference vegetation index (NDVI) through satellite imagery. The NDVI data were obtained in July and August for the treated plots over the untreated control plots. See also: Scortichini et al. (2018); Tatulli et al. (2021), and Blonda et al. (2023).

Municipality	Cultivar	Overall range CFU eq/g *Xylella fastidiosa*	NDVI 2018 Treated/ Untreated	NDVI 2019 Treated/ Untreated	NDVI 2020 Treated/ Untreated
Veglie	Ogliarola	10^2			
	Cellina	10^2			
Cannole	Ogliarola	10^3–10^4			
	Cellina	10^2–10^4			
Galatone	Ogliarola	10^2–10^4			
	Leccino	10^2–10^4			
	Cellina	10^2–10^4			
Nardò A			0,42/0,26	0,33/0,24	0,31/0,16
Nardò B			0,37/0,31	0,34/0,27	0,39/0,23

biofertilizer has been shown to cause specific variations in the contents of some specific metabolites in the leaves of the susceptible olive cultivars Ogliarola salentina and Cellina di Nardò. In particular, endotherapy induced a significant decrease in both disease biomarkers, namely, quinic acid and mannitol, with simultaneous increases in polyphenols and oleuropein-related compounds in the leaves (Girelli et al., 2022). These results were confirmed when endotherapy treatments were assessed during a six-season period (Hussain et al., 2023). Notably, foliar application demonstrated more specific time-related progressive effectiveness than did intravascular treatments.

6.2. The reprogramming of lipids involved in X. fastidiosa subsp. pauca–olive interactions

Host–pathogen interactions are determined by different factors that modulate virulence and plant defence. Among these factors, lipids, in particular free fatty acids (FAs) and oxylipins, are involved at various stages. These molecules are structurally similar among plant and bacterial taxa. Among the lipid factors crucial for determining *X. fastidiosa* virulence, cis-2-enoic fatty acids, known as diffusible signalling factors (DSFs), can participate in communication with plants or insect vectors. These DSFs are produced from plant complex lipids through the action of a bacterial lipase. In *X. fastidiosa*, this lipase is encoded by the *lesA/lipA* gene (Rapicavoli et al., 2018), and high DSF concentrations induce adhesion to plant vessels or the insect foregut (Chatterjee et al., 2008). In addition, the quorum sensing regulation of *X. fastidiosa* is based on a delicate balance of several DSFs (i.e., 12:1, 14:1, 16:1, and 18:1) (Lindow et al., 2014; Ionescu et al., 2016). DSFs induce the expression of adhesins needed for biofilm formation and the formation of small colonies. Low DSF concentrations have been shown to induce twitching motility and pit membrane degradation (Chatterjee et al., 2008).

Additionally, oxylipins are involved in *X. fastidiosa*-host plant interactions. Plant oxylipins regulate processes related to physiological and pathological events by activating defence-related gene pathways and interfering with pathogen growth and reproduction (Siebers et al., 2016; 2022 Fernandes and Ghag). Since most of these compounds have antimicrobial activity, plants can also produce oxylipins to kill pathogens (Deboever et al., 2020). Jasmonates are the best characterized plant oxylipins that regulate defence, reproduction, and pathogenesis. Bacteria can employ host oxylipins to augment their virulence (i.e., switching to the biofilm stage) (Zheng et al., 2012; Martinez et al., 2019). In *X. fastidiosa*, oxylipins mediate autocrine signals within bacterial cells (i.e., regulation of quorum sensing) and/or paracrine signals in communication with their hosts or vectors (Ionescu et al., 2016; Martinez et al., 2019; Scala et al., 2020). In *Xfp*, oxylipins and FAs play pivotal roles in shaping the bacterial lifestyle. *Xfp* accumulates different lipoxygenase- and dioxygenase-derived oxylipins both *in vitro* and during interactions with different hosts (i.e., tobacco and olive trees) (Scala et al., 2018, 2020). Oxylipins also influence the switch from planktonic growth to biofilm formation, indicating that *Xfp* can synthesize and secrete oxylipins. Notably, oxylipins have emerged as hallmarks of pathogenic invasion by *Xfp* in host tissues: infected plants accumulate more oxylipins (i.e., 7,10-diHOME and 13-HODE) than do noninfected plants (Scala et al., 2020).

By using the relative differences in lipid species, it has been possible to discriminate olive tree samples, namely, (a) infected and noninfected samples, (b) those belonging to different cultivars, and (c) those treated or untreated with PRC2022. Lipid entities emerging as predictors of this hypothesis include free fatty acids (C16:1, C18:1, C18:2, and C18:3), LOX-derived oxylipins 9- and 13-HPOD/TrE, DOX-derived oxylipin 10-HPOME, and the diacylglyceride DAG36:4 (18:1/18:3). The analysis of the *Xfp*-positive vs. *Xfp*-negative dataset revealed 9-LOX and 13-LOX oxylipins, FAs (C16:1, C18:1, C18:2, C18:3), and DAG (with two C18:2 or with C18:1/C18:3 or with C18:1/C18:2) as significantly different compounds. These data were also supported by bioinformatic analysis of RNA-seq data files (Scala et al., 2022). According to these results, it is possible that the oxylipins accumulate differently in *Xfp*-susceptible or *Xfp*-tolerant cultivars, as already demonstrated in other pathosystems (i.e., plant-fungi), and it is possible to conclude that the infection of *Xfp* in olive trees grown in open fields is characterized by the accumulation of oxylipins (i.e., 7,10-diHOME and 13-HODE), which are most likely employed by the bacterium to switch its lifestyle to a virulent phase. Moreover, the lipid profile of the olive trees treated with PRC2022 and positive for *Xfp* increased with the accumulation of the typically healthy plant lipid signature (i.e., decreasing 13-oxylipins/-DOX-oxylipins and increasing 9-oxylipins), which differed from the lipid signature of the olive trees infected by *Xfp* and not treated with the biofertilizer.

6.3. Management strategy evaluated by high-resolution satellite imagery monitoring

The field efficacy of the management strategy applied in the olive groves of Salento to face the *Xfp* epidemic was confirmed through a study performed with satellite imagery data coupled with selected vegetation indices (Blonda et al., 2023). From 2015 to 2020, during July and August, multiresolution source satellite data obtained from time series of high-resolution (HR) Sentinel-2 images (10 m) and very high-resolution (VHR) Pleiades imagery (2 m) were analysed for field-scale and tree-scale investigations, respectively. The Sentinel-2 mission includes two operational sensors (Sentinel-2A and Sentinel-2B). (https://www.esa.int/Applications/Observing_the_Earth/Copernicus/Sentinel-2). The mission revisit time was only five days when both satellites were operational. The span of 13 spectral bands, from the visible and the near-infrared regions to the shortwave infrared region at different spatial resolutions ranging from 10 to 60 m, requires land monitoring and plant status determination to an unprecedented extent (https://www.eurisy.eu/monitoring-plant-health-from-space/). Time series of remote sensing imagery and derived vegetation indices have been shown to be particularly useful for characterizing land ecosystem dynamics, as they can provide consistent measurements at different spatiotemporal scales. Such measurements are appropriate for bio and geophysical processes and change events, including natural and anthropogenic disturbances (Verbesselt et al., 2010; Woodcock et al., 2020). Assessing and monitoring the state of ecosystems are essential for biodiversity conservation and ecosystem management (Lhermitte et al., 2011), and vegetation indices are used as proxies for geophysical variables (https://www.usgs.gov/landsat-missions/landsat-surface-reflectance-derived-spectral-indices). Vegetation indices obtained through selected spectral band combinations are proxy indicators of plant health (Montero et al., 2023).

Among the vegetation indices, the normalized difference vegetation index (NDVI) is based on red and near-infrared (NIR) light to determine the amount of chlorophyll in leaves. Initially, this technique was used simply to detect the presence of vegetation, but this spectral index is mostly adopted to quantify photosynthetic capacity, which is a key indicator of plant health. Multiscale satellite data and techniques have been used to monitor the spread of *Xfp* across Salento olive groves and to detect early symptoms of OQDS in the vegetation. Medium-resolution data have been utilized to quantify the extent of areas covered by wilting olive trees (Scholten et al., 2019), whereas Sentinel-2 data and hyperspectral data from airplane campaigns have been used by Hornero et al. (2020) to quantify the extent of areas covered by wilting olive trees. In addition, hyperspectral data were analysed to extract spectral features that are able to specifically identify early symptoms of OQDS (Hornero et al., 2020).

The response to the control strategy treatment was estimated by spectral indices comparing treated and untreated fields at HR and analysing the response to treatments of each different cultivar at VHR. The analyses were performed on both experimental (i.e., Galatone and Cannole) (Tatulli et al., 2021) and productive (i.e., Nardò) olive groves in Salento (Lecce Province). The monitoring was carried out at the field scale and at the tree scale. At the field scale, the trends of four spectral indices, namely, the NDVI, OSAVI, NDRE and ARVI, from time series of freely available HR Sentinel-2 images (10 m), were analysed and correlated with meteorological events from 2015 to 2020. At the tree scale, both the recovery status of each olive tree in the experimental and productive fields and the response of different cultivars to treatments were evaluated through VHR Pléiades data. The data were collected during July and August, when the contribution to the vegetation indices from the background was minimal and the occurrence of *Xfp* symptoms was very evident.

In all treated fields, all spectral indices were greater than those in the untreated fields after PRC2022 treatment at both the field and tree scales according to the HR and VHR images (Table 3). The correlation between the HR index time series and meteo events indicated that the treated olive trees were more responsive to rain events than were the untreated trees (Blonda et al., 2023). At the tree scale, the VHR indices revealed different cultivar responses to the treatments and showed that Ogliarola salentina responded more quickly to the treatments than did Leccino and Cellina di Nardò. Specifically, the findings reported from HR data could be used to evaluate plant conditions at the field level after restoration, while VHR imagery could be used to optimize treatment doses for each cultivar. These findings, in agreement with the effect of the biofertilizer on the *Xfp* concentration in olive trees (Tatulli et al., 2021) and with the findings of metabolomic studies revealing the reprogramming of plant metabolites upon curative treatment (Girelli et al., 2019, 2021), showed that indices of plant health status were greater in treated fields than in untreated fields.

7. The management strategy for the disease complex of olive trees in salento

Since 2016, after the first promising results were observed in the experimental field, some farmers in Salento have started to introduce this strategy either as a preventive or curative practice for pathogen treatment. The ease of application as a foliar spray and the low cost of the biocomplex allow for it to be applied by all farmers, including part-time farmers and amateurs. However, the success of this management strategy, as for other plant diseases, is strongly dependent on the consistency of foliar applications over several months, so spraying PRC2022 two or three times occasionally does not solve this problem (Scortichini, 2022). The studies performed thus far suggest beginning application once per month from the end of February-early March until the end of September-early October. The recommended dose is 3.9 l ha^{-1} (i.e., 280 ml in 100 l of water). For centennial trees, some farmers have used slightly more concentrated spray solution (i.e., 300–350 ml in 100 l of water) without causing any phytotoxicity. It is important to apply the correct amount of foliar spray to the tree crown (i.e., 20 l of spray solution for an olive tree aged 60–70 years; 25–30 l of spray solution for the centennial trees) to allow for the penetration of the biocomplex within the foliage.

In parallel, the reduction in the presence of insect vectors on farms must be considered of fundamental importance. Since the management strategy does not include any chemical treatment for lowering the adult vector population, particular care should be taken to eliminate the eggs and the juvenile vector insects through mechanical techniques. Egg reduction should be performed through light tillage during winter (i.e., from December to February) to eliminate the eggs deposited by adults in ground slits. Subsequently, from early February to early May, spontaneous weeds should be mowed to eliminate juvenile insects (i.e., naiades and nymphs). It should be stressed that abandoned olive groves host the highest population of vectors (Picciotti et al., 2021). Therefore, to be effective, the vector control strategy should preferably be applied to a vast area, and punctiform vector control carried out at a single olive grove is not fully effective.

Regular and rational tree pruning is another pillar for an effective *Xfp* management strategy. In Salento, in recent decades, olive pruning has been carried out on a 4- to 5-year basis, with very large cuttings of the branches being performed (Scortichini, 2020b). Apart from the damage induced to tree physiology and productivity, such a practice, sometimes applied with the intention of eliminating *Xfp* from the tree, has always resulted in its further weakening, and usually, subsequent death is observed in a few months, especially in centennial trees (Scortichini, 2020b; Camposeo et al., 2022). An appropriate time frame for pruning is one or two years (Pannelli and Gucci, 2020), which also allows PRC2022 to enter the foliage more effectively through nebulization.

Soil fertility is also highly important for rational management of olive groves in relation to *Xfp* infection. Indeed, soil depletion of some micronutrients, namely, zinc, copper, and manganese, has been observed in many olive groves of Salento infected by the bacterium (Del Coco et al., 2020). In addition, in olive trees infected by *Xfp*, copper and boron have low bioavailabilities (Scortichini et al., 2019). Consequently, measures aimed at increasing the soil contents of macroelements, microelements and beneficial soil microorganisms or composts should be applied to olive groves to maintain or improve their overall soil fertility.

The depletion of zinc and copper both in the soil and within infected trees indirectly confirmed the effectiveness of the protocol, which can provide such microelements through foliar applications. Despite the continuous claim that "there is no cure for *X. fastidiosa*" (Colella, 2023) and the discouragement of applying any field control protocols performed by national and local presses as well as by major farmer organizations (Martella, 2022), many farmers started to apply the protocol in different areas of Apulia.

Currently, there are farms, mainly planted with the local susceptible cultivars Ogliarola salentina and Cellina di Nardò, that are practising the control strategy in the "infected" area within the provinces of Lecce, Brindisi, and Taranto for more than seven years (Fig. 7). The tree ages range from 30 to more than 500 years, and the yield ranges, according to the classical olive yield trend (i.e., alternate bearing), from 30 to 50 q.li/ha per year. It should be noted that, in many cases, olive farms are surrounded by dead or severely damaged trees, representing "green oases" in devastated Salento (Fig. 8). Moreover, there are other farms in the "infected" area that began to apply the discussed protocol one to two years ago. These farms showed extensive twig and branch diebacks and various dead trees. In all cases, the dead plant parts were removed, and the farmers began to apply the biofertilizer during spring and summer. In these cases, the farms border areas characterized by the occurrence of OQDS, but the application of the biocomplex promptly promoted the

Fig. 7. Different approaches to face *Xylella fastidiosa* subsp. *pauca* in the "infected" area of Salento. Top: July 2019, productive 10 ha olive grove at Nardò (Lecce Province) (on the left) under the management strategy based on PRC2022 since 2016; abandoned olive groves completely wilted (on the right). Below: June 2022, the olive grove that continued to be under the management strategy was still productive (on the left), whereas the abandoned olive grove was uprooted and planted with tomato (on the right).

sprouting of infected trees. Notably, in some cases, groups of farmers funded local "social promotion associations" (APSs) with the aim of saving olive trees through the application of the *Xfp* management protocol described herein. To date, more than 1500 ha have been treated by this control strategy in Salento, representing a safeguard for the heritage of the olive agroecosystem.

According to the new evidence that points to a significant role of *Botryosphaeriaceae* fungi in causing diebacks to olive groves together with *Xfp*, an additional effort to find active compounds capable of mitigating the aggressiveness of such fungi is needed. Currently, several strategies are under study to determine the virulence of *Botryosphaeriaceae* strains to woody species (Guarnaccia et al., 2022; Aiello et al., 2023). A fundamental approach to reduce the spread of such fungi within and between olive groves is the disinfection of pruning shears and the protection of pruning wounds since pycniodiospores of these fungi, spread by rain splashing, can easily infect the wounds after cutting, as observed in vineyards (Urbez-Torres and Gubler, 2011; Otoya-Martinez et al., 2023). Pruning shears have been verified as a source of inoculum for *Botryosphaeriaceae* that infect grapevine (Agusti-Brisach et al., 2015). Shears can be disinfected by soaking them in a liquid formulation that contains a broad-spectrum fungicide (Diaz and Latorre, 2013). The protection of pruning wounds could be achieved by means of *Thricoderma* spp. (i.e., *T. asperellum* and *T. gamsii*) as biocontrol agents (Blundel and Eskalen, 2022), and pruning should preferably be performed during dry and warm weather (Urbez-Torres and Gubler, 2011). Paste formulations containing 0.1% benomyl, 0.5% tebuconazole, and 0.06% iprodione showed efficacy in protecting against pruning wounds (Latorre et al., 2013). The elimination of pruning residues and dead wood is also very important for reducing the risks posed by inoculum sources (Aiello et al., 2023). The fundamental role played by pruning in the dispersal of *Botryosphaeriaceae* within and between the olive groves of Salento has been confirmed by farmers who observed the first signs of BTD (i.e., extensive twig dieback) one or two years after pruning.

Currently, in Italy, there are no specific, authorized fungicides to control *Botryosphaeriaceae* in olive groves, and the search for biocontrol agents or ecofriendly compounds capable of reducing tree colonization and significantly mitigating their aggressiveness is important. Studies performed with nut crops infected by these fungi indicated that a mixture of fluxopirodax and pyraclostrobin was the most effective compound for managing the disease (Moral et al., 2019). The potential use of these molecules to manage infection caused by *Botryosphaeriaceae* is under study. Among these compounds, natamycin, a natural antimicrobial compound obtained from *Streptomyces natalensis*, has been verified to be capable of effectively reducing the *in vitro* growth of *N. parvum* (Gou et al., 2023). The RNA silencing technique has also been applied to reduce the severity of disease caused by *Botryosphaeriaceae*

Fig. 8. Top: olive farm (on the left) at San Pietro Vernotico (Brindisi Province) in June 2022 under the management strategy during the last four years compared to a closed and abandoned olive grove that was completely wilted (right); bottom: experimental field at Cannole (Lecce Province) in June 2022. This olive grove was under continuous control since 2016, and its yield has been relatively normal. The completely wilted olive groves nearby should be noted in addition to the "drift effect" in the first olive row bordering the treated rows due to the aerial spray of PRC2022, which partly reached the adjacent olive trees.

(Nili et al., 2021).

8. General remarks and perspectives

Through an in-depth interdisciplinary approach, we verified that it is now possible to apply a field management strategy capable of curing olive trees infected by *Xfp* in Apulia. Such a cure is not intended to eliminate the bacterium from the xylem tissue of the tree but can reduce its concentration within the tree to a level that does not hinder the development of new vegetation and fruits, which represents the common situation in most crops infected by plant pathogens. It should be noted that also the tolerant olive cultivar, namely Leccino, hosts a bacterium concentration within the canopy very similar to that observed for the cured trees (Giampetruzzi et al., 2016). This strategy, associated with the vector control strategy, should be applied annually. The misunderstanding between "cure" and "eradication" of *Xfp* has created either confusion or a lack of acceptance of the management strategy, resulting in the subsequent abandonment of the olive groves and the general collapse of the trees in a very vast area of Salento. In the case of OQDS, an integrated approach that considers the different facets of the disease (i.e., pathogen(s) life cycle and epidemiology, host–plant susceptibility, vector(s) biology, and predisposing factors of disease) can lead to a better understanding of the disease itself and to the development of an effective control strategy.

Within this context, it is worth noting that a fungus-associated syndrome, namely, BTD, caused by *Neofusicoccum* spp., has been recently described and very frequently isolated in the olive groves of Salento, which severely impacts the olive trees and whose symptoms can be confused with symptoms caused by *Xfp*. Further studies are ongoing to fully understand the role of the associated fungi. We still do not know whether this relevant occurrence of *Botryosphaeriaceae* in olive groves also infected by *Xfp* has been promoted recently by some climatic events (i.e., drought and persistent high temperature during summer), and in 2020, it was reported that pathogenic fungi were not found associated with trees infected by *Xfp* in Salento (Carlucci et al., 2020). Within this context, we are currently testing different possible interactions between *Xfp* and such fungi (Scortichini et al., 2023).

However, polymicrobial complexes can often be involved in woody species declines, and each microbial species may either cause specific symptoms or interact with the others in a temporally regulated succession, which ultimately causes declining symptoms (Manion, 1981; Sinclair and Hudler, 1988; Denman et al., 2018; Griffiths et al., 2020; Hrycan et al., 2020). Since the application of the described management strategy could be less effective in declining trees infected with a complex microbial cohort (*Xfp* plus fungi) than in those infected only by *Xfp*, an integration with measures aimed at restricting the progress of such additional pathogens could provide even better control of all the causal agents involved in olive tree dieback in Salento and Apulia overall. Studies are underway to identify effective compounds capable of significantly reducing the aggressiveness of *Botryosphaeriaceae* towards olive groves.

Ethical approval

This article does not contain any studies with human participants or animal performed by any of the authors.

CRediT authorship contribution statement

Marco Scortichini: Writing – review & editing, Data curation. **Stefania Loreti:** Writing – review & editing, Data curation. **Valeria Scala:** Writing – review & editing, Data curation. **Nicoletta Pucci:** Writing – review & editing, Data curation. **Massimo Pilotti:** Writing – review & editing, Data curation. **Giuseppe Tatulli:** Writing – review & editing, Data curation. **Erica Cesari:** Writing – review & editing, Data curation. **Alessia L'Aurora:** Writing – review & editing, Data curation. **Massimo Reverberi:** Writing – review & editing, Data curation. **Nicola Cristella:** Writing – review & editing, Data curation. **Paolo Marangi:** Writing – review & editing, Data curation. **Palma Blonda:** Writing – review & editing, Data curation. **Cristina Tarantino:** Writing – review & editing, Data curation. **Maria Adamo:** Writing – review & editing, Data curation. **Sabino Maggi:** Writing – review & editing, Data curation. **Gianluigi Cesari:** Writing – review & editing, Data curation. **Chiara Roberta Girelli:** Writing – review & editing, Data curation. **Federica Angilè:** Writing – review & editing, Data curation. **Mudassar Hussain:** Writing – review & editing, Data curation. **Danilo Migoni:** Writing – review & editing, Data curation. **Francesco Paolo Fanizzi:** Writing – review & editing, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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