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

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Highlights

- *Xylella fastidiosa* subsp. *pauca* is associated with the olive quick decline syndrome in a vast area of Salento (Apulia, Italy).
- In the long-term period, a foliar bio-fertilizer that contains zinc (4%), copper (2%) and citric acid has shown to significantly reduce the disease.
- Upon the sprays, interdisciplinary studies revealed a rapid re-programming of tree metabolites towards the healthy status.
- Recently, aggressive *Neofusicoccum* spp. are also found associated with declining olive trees.
- Specific treatments for fungal infections should be also found to manage this disease complex.

Abstract

Xylella fastidiosa subsp. *pauca* (*Xfp*) is associated to the "olive quick decline syndrome", a severe disease affecting olive groves of Salento (Apulia, Italy). Through a series of interdisciplinary studies, it has been possible to assess and to set up an effective management strategy aimed at maintaining the traditional olive germplasm of Salento. To this aim, a systemic bio-fertilizer that contain zinc (4%), copper (2%) and citric acid, namely Dentamet®, is sprayed to the tree canopy, once per month, from spring to early autumn. The strategy also includes a sustainable vector control through agronomical techniques as well as the regular tree pruning and soil fertilization. Quantitative real-time PCR assessments performed in the long-term period showed a significant reduction of *Xfp* concentration in the leaf xylem tissue upon the treatments, thus allowing the olive trees to normally yield. Both ^{1}H-NMRmetabolomic and mass-spectrometry lipidomic analyses of leaf extracts revealed the occurrence of biomarkers linked to the disease or to tree restoration. Mannitol and oleuropein derivatives and 13-oxylipins/DOX-oxylipins and of 9-oxylipins appear related to the remission of the symptoms. Both techniques point to a rapid re-programming of the metabolic tree activity upon the spray treatments toward a healthy status. Multi-scale satellite imagery monitoring through high-resolution Sentinel-2, very high-resolution Pleiades and vegetation indices confirmed the robustness of the strategy through several years in both experimental and productive olive groves. Currently, the strategy is applied in many olive groves of the infected areas of Salento. To note that some aggressive fungal species belonging to *Neofusicoccum* genus have been recently found associated to olive trees that show symptoms like those induce by *Xfp*. Co-infections between the bacterium and fungi have been also observed, this suggests approaching a more in-depth assessment of the olive decline syndrome epidemiology and management.

Key words: Olive quick decline syndrome, zinc, copper, quantitative real-time PCR, ^{1}H-NMR metabolomic, bacterial lipids, satellite imagery, *Neofusicoccum* spp.

Abbreviations

ANOVA, Analysis of variance, BTD, Branch and twig dieback; ARVI, Atmospherically resistant vegetation index; CFU, Colony forming unit; DOX, Dioxygenase; DSF, Diffusible signal factors; EPPO, European and Mediterranean Plant Protection Organization; LOX, Lipoxygenase; MLST, multilocus sequence typing; NDRE, Normalized difference red edge index; NDVI, Normalized Difference Vegetation Index; NIR, Near Infrared Reflectance; NMR, Nuclear magnetic resonance, OQDS, Olive quick decline syndrome; OSAVI, Optimized soil adjusted vegetation index; PCA, Principal component analysis; ST, sequence type; *Xfp*, *Xylella fastidiosa* subsp. *pauca*.

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

Xylella fastidiosa is a long-known pathogenic bacterium that represents an ancient problem in the Americas, despite being recently introduced in Europe. For the European and Mediterranean Plant Protection Organization it is included into the quarantine list of bacterial pathogens. The first report of a disease caused by *X. fastidiosa* dates back to the end of 1800 on grapevines in California, where Pierce's disease significantly impacted on the viability of growing grapes. Viticulture was largely abandoned in the area (Hopkins et al., 2002), and the disease became a limiting factor for grape cultivation (Myers et al., 2007). Crop substitutions and altered land use patterns in response to pathogen pressure have a large impact particularly in areas where agriculture is directly linked to cultural identity, as occurred in Italy with the "Olive Quick Decline Syndrome" (OQDS) (Scortichini, 2020a). The unexpected and impressive quick progression of the *X. fastidiosa* epidemic in Italy not only impacted the agricultural production and landscape, but also on cultural heritage and social dynamics (Scortichini, 2020a, Colella, 2023). Following the Apulia infection, new recoveries of *X. fastidiosa* occurred in Italy (Tuscany and Latium regions), in Europe (France, Spain, Portugal), and in other countries such as Israel, Iran and Taiwan, thus affecting several host species (EPPO, 2023). *X. fastidiosa* is highly polyphagous being capable to survive in 638 host plants, in many cases symptomless (i.e., latent) (EFSA, 2022), and is divided in subspecies based on host range and genetic relationships (Nunney et al., 2014). Recently, the subspecies *pauca, multiplex* and *fastidiosa*, the latter including also the *sandyi* and *morus* populations, have been reclassified on the basis comparative genomic analyses (Marcelletti and Scortichini, 2016a; Denancè et al., 2019).

In addition to the subspecies categorization, *X. fastidiosa* can be subdivided also into sequence types (STs) using a Multilocus Sequence Typing (MLST) approach based on sequencing of seven housekeeping genes (Yuan et al., 2010). The introduction and spread of the different strains took place through mechanisms of recombination and translocation between plant hosts. The MLST study demonstrated that two of the three subspecies of *X. fastidiosa* found in the United States (i.e., *X. f.* subsp. *fastidiosa* and *sandyi* population) are non-native, presumably introduced in 1880 (i.e., the population *sandyi*) and 1890 (i.e., subsp. *fastidiosa*) from 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 of Citrus variegated chlorosis and coffee leaf scorching is that one capable to cause the OQDS in Apulia region, namely the strain "De Donno". This strain is closely related to a strain of *X. f.* subsp. *pauca* (*Xfp*) from Costa Rica, which infect 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* originates from the southeastern United States and was more recently spread in California, Brazil and Europe. Whole-genome sequences analyses of *X. f.* subsp.

multiplex from America in comparison with strains associated with recent outbreaks in southern Europe, indicated multiple introductions of *X. fastidiosa* subspecies *multiplex* into Italy, Spain, and France, with a likely origin in California, USA (Landa et al., 2020).

If a prompt detection of the pathogen is essential in order to monitor its introduction and/or diffusion on the territory, the individuation the subspecies and STs is crucial in case of new outbreak of *X. fastidiosa* in a pest-free area or in association to 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 (4) and the Annex 4 of the EU regulation 2020/1201 lists the methods allowed for official diagnoses. Finally, is worth noting that the ST characterization and, better, the whole genome sequencing (Landa et al., 2020) should indicate the presence of possible recombination among strains that drive the evolution and adaptation of *Xylella fastidiosa* to new plant hosts and provide useful information on the possible origin of the strains.

2. The olive declines in Salento: a complex disease

The epidemic caused by *Xfp* in Salento region represents the most serious phytosanitary event occurred in recent years in Italy with very serious economic, landscape and social consequences. The olive groves of Salento, indeed, represent a remarkable case where an agricultural crop is strictly linked to a territory through a pluri-millennial 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). Great public alarm was created further in 2020 for the danger related to the area of monumental olive trees because of the high value of these millennial trees, which in addition to being protected by regional law, have been proposed for UNESCO World Heritage status (Sportelli, 2020). It should be also added that the local cultivars, traditionally, trained in Salento since ancient times, namely Cellina di Nardò and Ogliarola salentina, are characterized by a relevant content of high value for nutrition and human health (i.e., polyphenols) (Del Coco et al., 2014; Negro et al., 2019).

X. fastidiosa was associated in October 2013 with the declining olive trees (Saponari et al., 2013) occurred in a restricted area of the Apulian region (i.e., Salento, province of Lecce, south-east Italy), and characterized as subspecies *pauca* in 2014 (Cariddi et al., 2014). This bacterium was proposed as the causal agent of OQDS. In Salento, *Xfp* is characterized by a wide polyphagia, with a range of 56 host/plant species (EFSA, 2022), including ornamental species typical of the Mediterranean flora and crop species as olive, cherry and almond. The bacterium is naturally transmitted by insect vectors such as *Philaenus spumarius*, which feed on the xylem sap of host plants (Cornara et al., 2017).

In olive, symptoms of OQDS include leaf scorching, scattered desiccation of twigs and branches starting from the top of the tree canopy and expands to the rest of the crown, delayed growth, desiccation and death of plants. In several species, the pathogen multiplies and colonizes the vascular system inducing alterations by occlusions 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).

It should be said that symptoms resembling OQDS were already reported by olive growers some years before its identification. Sicard et al. (2022), by using a tip-dating approach, estimated that the introduction of *Xfp* in Apulia region most likely occurred in 2008 through infected coffee ornamental plant from Central America. Genomic studies suggest that the genome of the strain identified in Apulia region (namely sequence type ST53) is closely related to that of Costa Rica (Marcelletti and Scortichini 2016; Giampetruzzi et al., 2017). However, the date of 2008 may not be strictly related to when the pathogen was first introduced in Italy or to when it started to be adapted to olive trees as a host plant or when the epidemic really 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 on the latency period of the disease, it can be also postulated that the diffusion of OQDS started from 2002-2003 (Scortichini, 2022).

At the time of its first report in 2013, about 8.000-10.000 hectares were already affected, such an area corresponds to about 1 million olive trees (Martelli, 2016); 12 months later the hectares compromised were more than 20.000 and a few years afterwards the disease was defined endemic (Strona et al., 2017), and considered no longer eradicable. In order to apply the measures for containing quarantine pathogens, the National and European Phytosanitary Authorities propose to move from an “eradication” 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 has allowed the rapid spread of the disease in Salento territory, where the olive grove is present in extensive monoculture of two autochthonous susceptible cultivars, namely Cellina di Nardò and Ogliarola salentina, for many kilometers (Scortichini, 2022). Another significant predisposing factor may have been favorable conditions for vector *P. spumarius*, the predominant xylem-sap feeder in that area, with a crucial role in spreading the pathogen, able to cause repeated inoculations in olive trees canopy by many adults present (Cornara et al., 2017). The widespread presence of spontaneous host plants and ornamental shrubs, improper agronomic practices (i.e., hard pruning), events of adverse climatic conditions (drought, frost and extreme rainy events) (Scortichini et al., 2018) and alteration of the equilibrium of microelements in the soil (Del Coco et al., 2020) have further favored the rapid spread of the bacterium in that region (Scortichini, 2022).

The phytosanitary legislation has demarcated three main areas related to *Xfp* outbreaks in Apulia that can be modifieds according to the disease progression northward: a) the “infected” area, that includes a vast portion of territory further south of Salento (i.e., the whole Lecce and Brindisi provinces and part of Taranto province); b) the “containment” and “buffer” areas subject to the monitoring and laboratory analyses for pointing out 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 has been estimated that moves with an advance front of about 20 km per year (Kottelenberg et al., 2021).

During recent field surveys to perform epidemiological studies on olive decline, it has been noticed twig and branch diebacks that apparently resembled those incited by *Xfp* (Fig. 1). However, by carefully observing the different facets of the disease, some consistent difference with OQDS were revealed. Among these, foliar (Fig. 2) and wood infection allowed to differentiate OQDS from a branch and twig die-back caused by some *Botryosphaeriaceae* fungi; the name "Branch and Twig Dieback" (BTD) was given to the disease (Brunetti et al. 2022; Manetti et al., 2023). To note, that both *Xfp* and *Neofusicoccum mediterraneum* and *N. stellenboschiana* have been frequently isolated from the same olive tree in different olive groves of Salento (Scortichini et al., 2023).

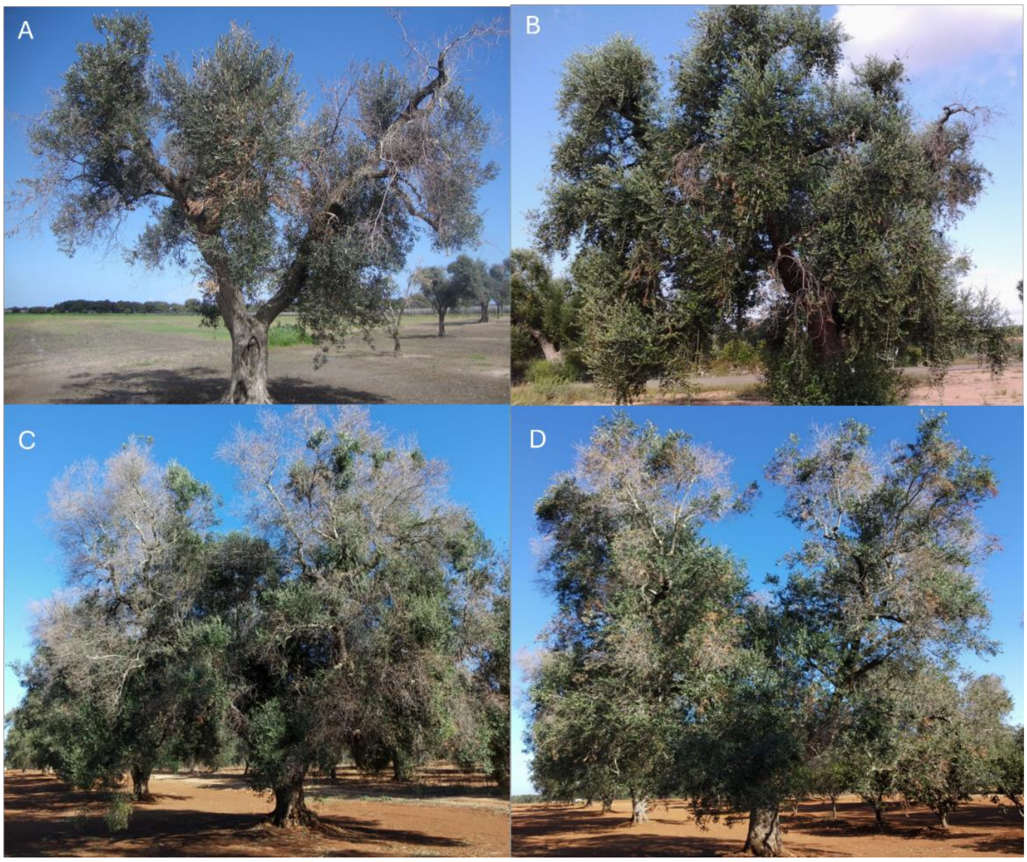

Fig. 1 Symptoms that show twig and branch diebacks incited by *Xylella fastidiosa* subsp. *pauca* (*Xfp)* (A and B) and *Neofusicoccum* spp. (C and D) to olive trees in Salento (Apulia, Italy). Such symptoms can be easily confounded during field surveys. The two phytopathogens were detected (*Xfp*) or isolated (*Neofusicoccum*) from such trees.

Remarkably, apart *Xfp*, also other phytopathogens can cause severe diebacks to olive trees in Salento, and that such diebacks can be easily confounded with those of OQDS attributed to *Xfp*, as repeatedly observed during the monitoring surveys for assessing *Xfp* occurrence in the demarcated areas of Salento (Scortichini and Cesari, 2019; Ciervo and Scortichini, 2024).

Many trees apparently showing OQDS symptoms, indeed, did not host the bacterium upon the molecular analyses, and in one of the last surveys (i.e., 2022) only 3.21% of symptomatic olive trees hosted *Xfp* (Ciervo and Scortichini, 2024).

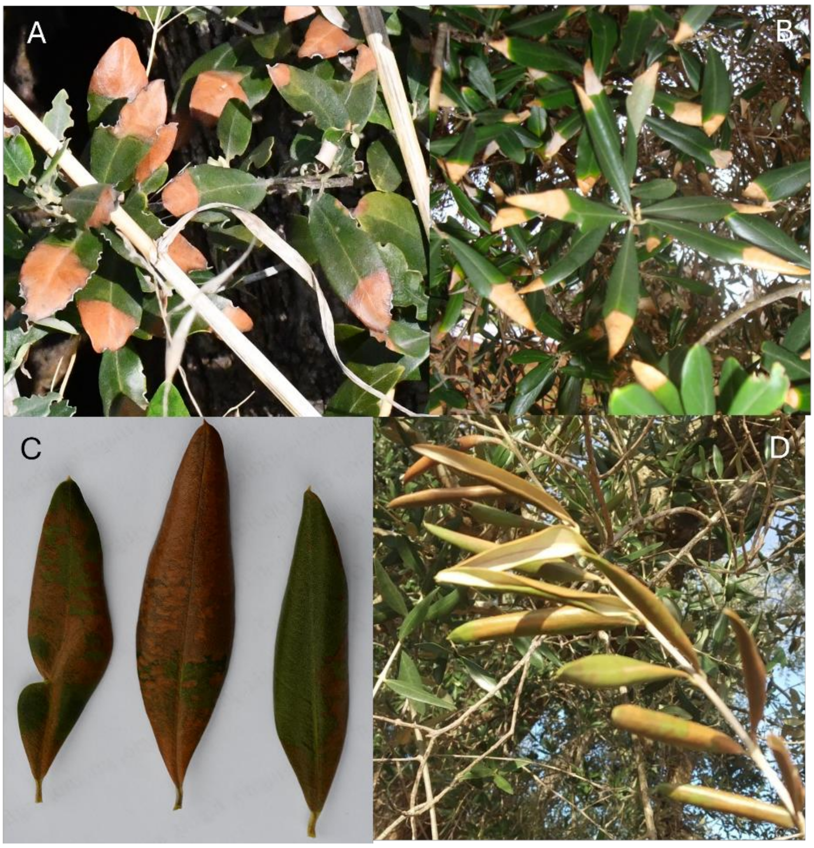

Fig. 2. Leaf tip wilting induced by *Xylella fastidiosa* subsp. *pauca* (A and B), and leaf reddening caused by *Neofusicoccum* spp. (C and D) to olive trees in Salento (Apulia, Italy). The two phytopathogens were detected (*Xfp*) or isolated (*Neofusicoccum*) from such trees.

Some years ago, indeed, *Phaoeoacremonium* spp., *Neofusicoccum parvum*, *Diplodia seriata*, and *Pleurostomophora richardsiae* were found associated with declines of 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 associated with *Xfp* in the same tree (Carlucci et al., 2020). More recently, *Arthrinium marii*, was found to be the causal agent of a severe twig dieback and wood discoloration in very young olive trees in Fasano (Brindisi province) and in Andria (north of Bari) (Gerin et al., 2020). This fungus was considered quite dangerous since in the pathogenicity tests expressed a virulence like that induced in the artificial infections by *N. mediterraneum* an agent of a severe olive dieback in Spain and in California (Moral et al., 2010; 2017; Urbez-Torres et al., 2013). *N. mediterraneum* and *N. stellenboschiana* were recently found in many olive groves of Salento that showed severe twig and branch diebacks resembling those incited by *Xfp*, and both species resulted significantly pathogenic to olive trees upon pathogenicity tests, and especially *N. mediterraneum* showed a relevant aggressiveness in term of bark cankering and wilting capacity. (Brunetti et al., 2022; Manetti et al., 2023). Interestingly, in our ongoing surveys in Salento, we are ascertaining that *Xfp*, *Botryosphaeriaceae* and even other fungal families can co-infect the same olive trees which show significant wood discoloration among the other symptoms (Manetti *et al*., 2023; Scortichini et al., 2023). This would suggest a complex disease for the declines of olive groves of Salento.

We still do not know if 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) since until 2020 it was reported that pathogenic fungi were not found associated with trees infected by *Xfp* in Salento (Carlucci et al., 2020). Within this scenario, we are currently testing different possibility of interaction between *Xfp* and such fungi (Scortichini et al., 2023): a) *Xfp* causes leaf and one–three-year-old twig/branch diebacks. This allows the growth of virulent *Botryosphaeriaceae*, which further aggravate the tree wilting. In addition, also low-virulent *Botryosphaeriaceae* can occur, thus acting as a polyspecies complex; b) *Xfp* incites the whole crown wilting, this affecting the viability of the main trunk. Subsequently, virulent and low-virulent *Botryosphaeriaceae* start the infections; c) *Xfp* colonizes the tree without causing any apparent damage but predisposing it to a subsequent symptomatic infection by virulent *Botryosphaeriaceae* which incite twig and branch diebacks; in this case the equilibrium between the bacterium and the tree is disrupted.

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

According to the principles of plant diseases control illustrated by the American Phytopathological Society, in most cases, when the disease is established in an area, it is not possible to eliminate (i.e., eradicate at zero level) a phytopathogen in a crop but it is possible to reduce its severity and keep the disease progression below an acceptable level that allows the crop to yield year after year (i.e., the field applying of 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 more expensive and sometimes poses doubts due to the economic and/or cultural relevance represented by the crop for a territory. For quarantine pathogens that are ruled by supranational phytosanitary laws aiming at eliminating in a definite territory a dangerous pathogen, introduced for the first time from abroad, it is technically and biologically difficult to achieve an eradication especially when the quarantine microorganism is not detected or isolated soon after its introduction (EFSA, 2015). There were cases where prolonged, accurate and expensive attempts to eradicate *Xanthomonas citri* pv. *citri*, the causal agent of citrus canker, in Florida (U.S.A.) and Brazil failed in the long-term period (Schubert et al., 2001; Behlau et al., 2016). Consequently, in both countries, the coexistence with the pathogen resulted more reliable and this has been reached by applying both preventive and curative integrated strategies that allow the crop to yield in presence of the pathogen (Kumar et al. 2019). Similarly, long-term attempts to eradicate *X.*

fastidiosa from vineyard in U.S.A. failed (EFSA, 2015). Also, the case of *Xfp* in olive groves of Apulia represents an example where a tardy detection of a quarantine bacterium and the large occurrence of diseased trees made the eradication of the pathogen in the infected area no more feasible (Burbank, 2022). Consequently, for a polyphagous bacterium largely spread in the area by a very prolific insect vector such as *P. spumarius*, the attempt of eradication in the area it would have been a failure.

Within quarantine and emerging bacteria, an effective field control has been achieved, also in areas with a large occurrence of the pathogen, for *Erwinia amylovora*, the causal agent of fire blight of pome fruits, and *Pseudomonas syringae* pv. *actinidiae*, the causal agent of kiwifruit bacterial canker. In both cases, an integrated approach, based on the knowledge of the epidemiological cycle of the pathogen and on the accordingly and timely applying of different types of effective compounds, allows to cultivate the crop in an infected area (Stockwell et al., 2010. Farkas et al., 2012; De Jong et al., 2019). By keeping this in mind, we have 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 also in presence of the bacterium within the tree.

4. Studies to reduce *Xylella fastidiosa* impact to crops

It should be noted that until 1987 the most common and well-known disease caused by *X. fastidiosa*, namely the Pierce's disease in the U.S.A., was retained 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 a measure very rarely resulted fully successful for eliminating the pathogen from the field (Hopkins and Purcell, 2002). Later on, the discovery of other novel emerging diseases caused by the bacterium further on induced to enlarge the studies on the assessment of additional control strategies to mitigate the damages caused by *X. fastidiosa* to cultivated and ornamental plant species (Hopkins and Purcell, 2002). Currently, there are under studies both preventive and curative strategies aimed at avoiding and/or reducing the incidence and the severity of the diseases caused by *X. fastidiosa* in the field (Kirkou et al., 2018; Burbank, 2022). Among the curative strategies under study aimed at lowering the concentration of the pathogen within the xylem tissue and manage the disease in the presence of the pathogen, there are bacteriophages, antagonistic bacteria, antibacterial microelements, natural compounds, nanoparticles and synthetic peptides (Table 1). The antibiotics are also studied and employed in some circumstances but, due to their restriction in Europe for the risk posed to the human beings in term of developing of antibiotic resistance, it is not retained a useful option. The cold or environmental therapy, based on the reduction of the bacterium concentration in the xylem vessels upon the occurrence of air temperature close or below 0°C, received some attention in the past (Purcell, 1977; 1980) but it was no longer studied in detail. The obtaining of tolerant or, possibly, resistant cultivars is another important goal to pursuit for facing diseases caused by *X. fastidiosa* (Kirkou et al., 2018; Della Coletta-Filho et al., 2020; Morelli et al., 2021) and, currently, there are several studies aimed at finding out source of resistance within grapevine, citrus and olive germplasm (Della Coletta-Filho et al., 2020; Pavan et al., 2021; Aguero et al., 2022). It should be stressed that the control of the insect vectors, through the timely distribution of insecticides, the possible applying of species-specific vector parasitoids or the mechanical elimination of eggs in the ground and weeds, is the other pillar for the field control of the bacterium (Kirkou et al., 2018; Della Coletta-Filho et al., 2020).

So far, it should be said that most of the studies carried out with the curative strategies regard *in vitro* tests, greenhouse assays performed with potted plants or few plants trained in open field (Kirkou et al., 2018), and that, apart the case of *Xfp* on olive in Italy (Scortichini et al., 2018; Tatulli et al., 2021), there is not a strategy that has been verified in open field for a consistent time lapse. Some promising lines of research are briefly reported herein.Among bacteriophages, some lytic broad host-range strains have incited a reduction in Pierce's disease symptoms in preliminary glasshouse inoculation of potted plants carried out with a cocktail of four phages (Ahern et al., 2014; Das et al., 2015). Additional phages with potential for a reduction of *X. fastidiosa* activity have been recently found in the Mediterranean areas (Clavijo-Coppens et al., 2021).

Endophytic antagonistic bacteria can also represent a potential valid strategy to reduce the activity of *X. fastidiosa* within the xylem tissue. Non-virulent *X. fastidiosa* strains were first used to verify such a possibility that resulted as promising (Hopkins, 2005). In addition, other endophytic species have shown some interesting activity in mitigating *X. fastidiosa* symptoms. A strain of *Parabulkholderia phytofirmans*, namely PsJN, when sprayed on the foliage shows a significant activity in reducing Pierce's disease severity by possibly priming expression of innate disease resistance pathways (Baccari et al., 2019). Its activity, however, was not found on olive infected by *Xfp* (Morelli et al., 2019). An antagonistic activity towards *X. fastidiosa* was also found for *Pseudomonas fluorescens*, obtained from grapevine, and for *Curtobacterium flaccumfaciens*, isolated from citrus (Araujo 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). Also, some endophytic fungi, namely *Aureobasidium* sp. and *Cladosporium* sp., have shown antagonist activity in vitro to *X. f.* subsp. *fastidiosa* (Rolshausen and Loper, 2010).

Among antibacterial microelements, zinc was among the first to be investigated for a possible activity towards *X. fastidiosa*, and the first to be judged as promising for a curative effect. This microelement, indeed, was applied to vineyard exposed to Pierce's disease as zinc sulphate through foliar spray, trunk endotherapy or applied to the soil and showed a 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 upon a certain dose (i.e., 0,25mM) this ion inhibited the biofilm formation by the bacterium (Cobine et al., 2013). In addition, zinc detoxification *in planta* is required to incite a full *X. fastidiosa* virulence, this suggesting that a removal of such microelement 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 defense for the plant that limits the growth of the bacterium, and, consequently, a manipulation of the plant level of zinc could represent a disease management strategy (Navarrete and De La Fuente, 2015). Recently, the possibility that the applying of

Table 1. Main options under study to reduce the impact of *Xylella fastidiosa* to crops.

	Effective tool	**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	Araujo et al., 2002
	Pseudomonas fluorescens	Deyett et al., 2017
	Paraburkholderia phytofirmans	Baccari et al., 2019
Antagonistic fungi	*Aureobasidium, Cladosporium*	Rolshausen abd Roper,
Microelements	Zinc	Kirkpatrick, 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	Fosetil-Al nanocristals	Baldassarri et al., 2020
	Thymol nanoparticles	Baldassarri 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

zinc-based compounds to control *X. fastidiosa* has gained particular attention. A nanoformulation of zinc oxide, indeed, 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 also against *Xfp* either in *in vitro* tests or 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 taken into consideration (Cobine et al., 2013). However, its specific activity as unique microelement to counteract the multiplication *in planta* of the bacterium has been not retained significant (Kirkpatrick et al., 2003; 2004, Ge et al., 2020).

In vitro tests revealed the antibacterial activity towards *X. fastidiosa* of several natural compounds. Among them, radicinin, a phytotoxin obtained from the grapevine endophytic fungus *Cochliobolus* sp., showed to reduce the growth of *X. f.* subsp. *fastidiosa* (Aldrich et al., 2015). Antibacterial activity towards *X. f.* subsp. *fastidiosa* and *pauca* was also found in several phenolic compounds. Flavonoids, stilbenes and coumarins showed antibacterial activity against *X. f.* subsp. *fastidiosa*, with catechol, caffeic acid and resveratrol as the most effective (Maddox et al., 2010), whereas 4-methylcathecol, cathecol, veratric acid, caffeic acid, and oleuropein showed bacteriostatic activity towards *Xfp* (Bleve et al., 2018). Phenolic extract from olive leaves have shown bacteriostatic activity to *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 of *X. f.* subsp. *fastidiosa* population level in potted plants of almond when injected in the trunk through endotherapy (Moll et al., 2022). Nanomaterial compounds (i.e., fosetyl-Al nanocristals, thymol nanoparticles) also showed interesting bactericidal activities towards *X. fastidiosa* subspecies (Baldassarre et al., 2020; 2023). Mixture of plant extracts also allowed a reduction of field symptoms caused by *Xfp* (Bruno et al., 2020).

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

The uniqueness of the olive agroecosystem, joint to the impossibility to achieve an effective eradication of the bacterium, already widespread over about 10.000 ha at the time of the first report, prompted us to study the possibility to coexist with the pathogen through the application of a field management strategy. The overall sustainability of the strategy was also considered. It was our idea to propose, indeed, a low-cost strategy through the utilization of a compound that could be utilized also for organic farming and that do not perturbate the soil ecological equilibrium. Herein we summarize the main results concerning either the principles that has led us to investigate the possibility of mitigating *Xfp* outbreak or the field efficacy and the basic knowledge that has been achieved by the studies that have been performed within this framework.

5.1 The choice of the compound

The choice of a compound capable to reach the xylem tissue of the leaves and, possibly, of twigs and achieve there the significant reduction in a long-term period of *Xfp* concentration in olive trees without causing any phytotoxic effect to the tree and allowing the obtaining of the yield without altering the environment, implies the contemporary occurrence of several characteristics. The most important are: i) to have a bactericidal activity against the pathogen; ii) to be highly systemic in the xylem tissue; iii) to release in the xylem tissue the active elements; iv) to be not present in the oil; v) to be effective in the long-term period. By following the studies of Cobine et al. (2013) and Navarrete and De la Fuente (2015), the choice fell to a patented bio-fertilizer, utilized as a foliar fertilizer also for organic agriculture, namely Dentamet®, that contains zinc (4% w/w) and copper (2% w/w) salts complexed with hydracid of citric acid. The compound is obtained through a process of fermentation similar to that carried out by soil fungi. This bio-fertilizer was previously verified effective for reducing the exudates produced by *Pseudomonas syringae* pv. *actinidiae*, the causal agent of kiwifruit bacterial canker, oozing from twig cankers of kiwifruit (Scortichini, 2016). To note that after the first publication on its efficacy towards *Xfp* (Scortichini et al., 2018), the significant antimicrobial activity of this bio-fertilizer has been further verified in many *in vivo* assays for the pathogenic fungus *Plenodomus tracheiphilus* (i.e., previously known as *Phoma tracheiphila*), causal agent of citrus “mal secco” (Olivieri et al., 2022), for *Xanthomonas euvesicatoria* pv. *perforans*, causal agent of leaf spot and pith necrosis of tomato (Aiello *et al*., 2022) as well as for controlling some important insects, namely *Halyomorpha halys* (i.e., brown marmorated stink bug), and *Bactrocera oleae* (i.e., olive fruit fly), through the suppression of the bacterial symbionts that occur on the egg surface (i.e., symbiotic control of pests) (Gonella et al., 2019; Checchia et al., 2022; Perin et al., 2023). Other points retained important for the choosing of this bio-fertilizer were due to its availability in commerce, its easy in the utilization since it is not required any special license, absence of phytotoxicity, and its affordable cost.

5.2 In *vitro* antibacterial and antibiofilm activity

The possible bactericidal activity of Dentamet® was ascertained through a series of *in vitro* assays (Tatulli et al., 2021). Such tests showed a 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 Apulia 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 from the inoculation of the tubes, this indicating a long last effect of the antibacterial activity. To note that the antibacterial activity towards *X. fastidiosa* subspecies was also observed up to 1:100 dilution of the bio-fertilizer, so that the minimum bactericidal concentration (MBC) for it was 400mg/L and at 200mg l^{-1} for zinc and copper, respectively. Dentamet® also significantly reduced the biofilm formation in all *X. fastidiosa* subspecies tested (Fig. 3). These data indicated that the bio-fertilizer can potentially reduce either the pathogen vessel colonization (planktonic phase) or the biofilm phase, thus justifying further testing in the field (Tatulli et al., 2021).

5.3 The relevant *in planta* systemicity of the bio-fertilizer

To assess the capability of the bio-fertilizer in effectively reaching 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 Dentamet® reached the olive xylem

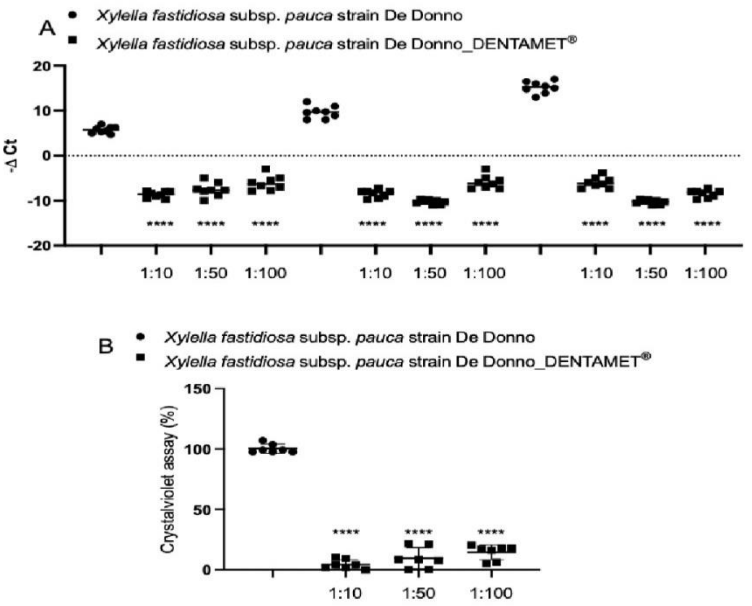

Fig. 3. A) *In vitro* significant efficacy of Dentamet® dilutions (i.e., 1:10, 1:50, 1:100) towards *Xylella fastidiosa* subsp. *pauca* strain "De Donno" (control) planktonic growth, assessed by real-time PCR at 6-, 15- and 30-days post inoculation (from left to right). B) *In vitro* biofilm assay 30 days post inoculation for *X. f.* subsp. *pauca* strain "De Donno" (control) according to different Dentamet® dilutions (i.e., 1:10, 1:50, 1:100). Values are mean ± SD of three biological replicates (n=7). ****: significative upon one-way ANOVA test and Dunnett's test at p<0.0001vs control. -ΔCt: difference for each sample between the real-time Ct value at each time point and the Ct value at time 0.

tissue both after the spraying of the canopy or through endotherapy, thus demonstrating its effective systemicity. Fluorescent staining of leaf, leaf petiole and of two- and five-year-old twigs, xylem tissue clearly showed the migration of the biofertilizer from the entry points up to 80cm 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 the ions were effectively released in the xylem. These data clearly indicated that the bio-fertilizer reaches the xylem tissue and corroborated its potential use in the field (Scortichini et al., 2018).

5.4 Field trials and quantitative real-time PCR for testing the bio-fertilizer efficacy in the long-term period

In parallel to the *in vitro* and *in planta* studies described above, we retained fundamental to verify the effectiveness of the bio-fertilizer towards *Xfp* directly in the open-field conditions. To this aim, we have selected traditional olive groves located in the infected area of Salento (i.e., Lecce province) and trained with the local susceptible cultivars, namely Ogliarola salentina and Cellina di Nardò with an age that varied from 25 to more than 70 years. A first preliminary study was carried out at Veglie, and, subsequently, to verify the effectiveness of the strategy in the mid-term period, at Galatone and Cannole (Scortichini et al., 2018; Tatulli et al., 2021). The olive grove for performing the initial three-year trial study was selected with the aim to represent the mean situation for the olive growing of Salento: local cultivars, adult trees, large space between the rows, traditional agronomical techniques (i.e., no irrigation, occasional soil fertilization, herbicides utilization, not regular pruning, occasional pest, and disease management). Moreover, this olive grove was officially declared infected by *Xfp* by the regional phytosanitary service before the starting of the trial.

In addition to the statistical analysis performed with the treated versus the untreated trees, we also carried out another efficacy test through the performing of quantitative real-time PCR to some trees. To note that at the time of the trial such an additional molecular test usually was not applied in the field tests for evaluating the efficacy of agrochemicals. Quantitative real-time PCR was performed on leaf samples to assess the population level of *Xfp* in treated versus untreated trees. It was carried out by following the official procedures established by the European and Mediterranean Plant Protection Organization (EPPO). In the first study (Scortichini et al., 2018), the spray treatments to the olive crowns, performed during spring and autumn, induced a significant reduction of both field symptoms (i.e., twig wilting) and pathogen concentration within the leaves.

To note that during the study, apart from *Xfp* infection, the trees faced some adverse climatic events such as frost at the beginning January 2017 and a severe heat wave during summer 2017. Frost events, over one week, reduced the *Xfp* cell populations both in the Dentamet®-treated and the untreated trees. Bacterium concentration then increased during the following months. This confirmed the assumption that prolonged cold poses a disadvantage to *X. fastidiosa*, but not to the point of completely curing infected host plants (Purcell, 1977; 1980). No zinc and copper residues were found within the oil obtained from trees that received the bio-fertilizer over three years.

In the mid-term evaluation (i.e., farms that applied the control strategy since three or four years consecutively), Leccino trees were also present in the Galatone farm. Generally, a trend that indicates a reduction of the field symptoms during the year in both farms and for all cultivars was observed, with the number of wilted twigs that resulted higher in March and decreased in July and October. Ogliarola salentina and Cellina di Nardò cultivars were confirmed to be more sensitive to *Xfp* than Leccino. At the end of the season, just before the harvest time, only a few new wilted twigs per tree were recorded for all cultivars in both farms (Tatulli et al., 2021).

The foliar treatments induced a significant reduction of *Xfp* concentration in all cultivars, as observed by the quantitative real-time PCR analyses. A first score of the mean bacterial concentration was performed in March. At Galatone Leccino trees had a lower bacterial concentration (i.e., mean of 9.0 10^2 CFUg^{-1}) when compared with Cellina di Nardò (1.7 $10^4 \mathrm{CFUg}^{-1}$) and Ogliarola salentina (8.7 10^3 CFUg^{-1}). A similar trend was also observed in the Cannole grove that showed a mean *Xfp* concentration that ranged, during spring, summer and autumn, between 1.0 10^2 and 10^4 CFUg^{-1}, in Cellina di Nardò, and between 1.0 10^3 and 10^4 CFUg^{-1} in Ogliarola salentina (Fig. 4). Remarkably, the treatments allowed to obtain a good yield of about 18 to 23Kg of olives per tree recorded in autumn 2019 in the farms of Galatone and Cannole, respectively, whereas the untreated trees resulted completely wilted (Tatulli *et al*., 2021). To the reduction of *Xfp* cell concentration within the foliage corresponded a higher vegetation index as revealed by satellite monitoring of the experimental fields (Table 2). Subsequently, a long-term assessment after seven and eight years of application of the bio-fertilizer was performed with the same olive groves. Also, in this case quantitative real-time PCR confirmed the efficacy of Dentamet® in maintaining the *Xfp* cell concentration to a level that allows the tees to vegetate and yield. In particular, Cellina di Nardò trees in both fields showed a concentration of about 1.0 10^3 CFUg^{-1}, whereas Ogliarola salentina trees showed a *Xfp* concentration of about 1.0 10^2 CFUg^{-1} at Galatone, and of about 5.0 10^3 CFUg^{-1} at Cannole. Leccino trees at Galatone showed a concentration of about 1.0 10^2 CFUg^{-1} (Table 2, Fig. 4). To note that in the last assessment the control trees bordering the treated plots just 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 the olive groves not completely damaged by the bacterium and that a coexistence with the pathogen is possible in the “infected” area of Salento. It is worth noting that the olive groves are in areas severely attacked by the bacterium and are surrounded by completely withered olive trees. Upon the crown treatments, the amount of copper released for one year was about 500g/ha, much less than 4 kg/ha, that represents the current limit for copper amount in soil allowed for organic agriculture.

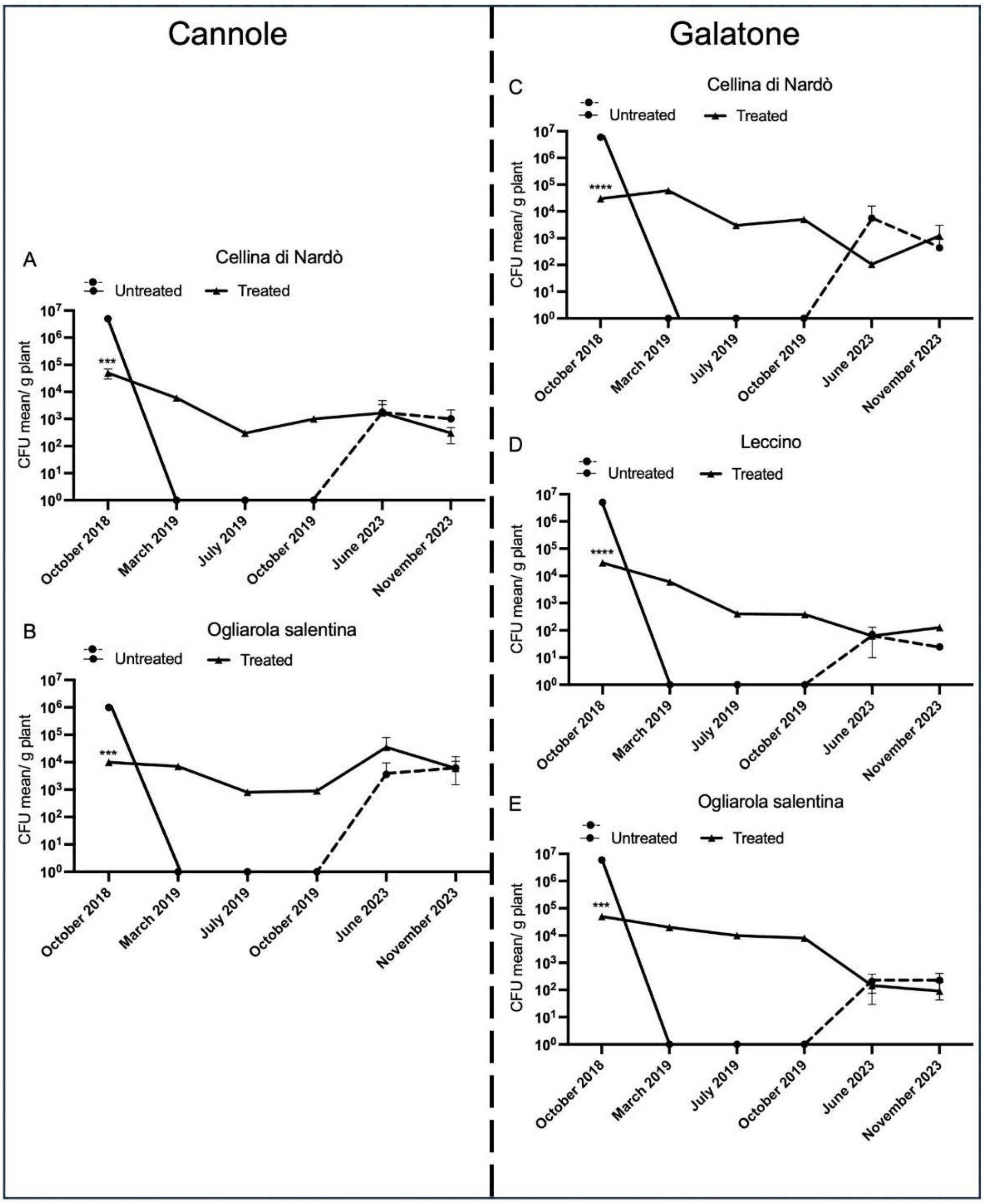

Fig. 4. *Xylella fastidiosa* subsp. *pauca* DNA concentration, expressed in a logarithmic scale of CFU equivalents g^{-1} of leaf as determined for un-treated plant (assessed as time 0 control) and Dentamet®-treated cultivars Cellina di Nardò, Ogliarola salentina, and Leccino at Cannole (A and B) and Galatone (C, D and E) (Lecce province) as assessed during 2018-2019 and during 2023. The untreated control plants dead 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 of Leccino, Cellina di Nardò and Ogliarola salentina at Galatone. Dotted lines represent the bacterium concentration observed in suckers taken as controls during 2023.

Table 2. The efficacy of *Xylella fastidiosa* subsp. *pauca* management in olive groves of Salento (Lecce province) as assessed by average quantitative real-time PCR (Colony Forming Units equivalent/g of leaf) and Normalized Difference Vegetation Index (NDVI). Data that refer to Veglie are obtained upon three years of applying the management strategy; data that refer to Galatone are obtained after three years and seven of applying the management strategy; data that refer to Cannole are obtained after four years and eight of applying the management strategy. For Galatone and Cannole, the data report the overall range values observed during 2019 and 2023; control trees resulted dead. Data that refer to two plots in Nardò refer to three years of measurements and report the NDVI values from 2015 to 2020 as obtained in July and August through satellite imagery for the treated plot over the not treated control plots. See also: Scortichini et al., (2018); Tatulli et al., (2021), and Blonda et al., (2023). CFU: colony forming units.

		CFU eq/g	NDVI 2018	NDVI 2019	NDVI 2020
	Ogliarola	10^2			
Veglie					
	Cellina	10^2			
	Ogliarola	10^3-10^4			
Cannole					
	Cellina	10^2-10^4			
	Ogliarola	10^2-10^4			
Galatone	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

6. **Interdisciplinary studies confirmed the efficacy of the management**

6.1 The re-programming of phenolic compounds and carbohydrates

Through a metabolomic approach, a series of studies were performed for monitoring the trend of some olive tree metabolites in trees naturally infected by *Xfp*, and in trees treated with Dentamet®. To assess the response of naturally infected untreated trees, Ogliarola salentina and Cellina di Nardò in comparison with Dentamet®-treated trees, non-targeted ^{1}H-NMR fingerprinting, in combination with unsupervised principal component analysis (PCA) and supervised pattern recognition techniques were applied by Girelli et al. (2017). Xylematic polyphenols and carbohydrates content changed upon the bio-fertilizer treatments, with Cellina di Nardò trees that showed a higher polyphenols relative content, and Ogliarola salentina that showed a higher relative sugar content.

The metabolic response of *Xfp*-infected-treated and untreated olive trees Ogliarola salentina and Cellina di Nardò was further investigated during the first year of foliar treatments with Dentamet® (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 as discriminating for the untreated trees. Quinic acid, a precursor of lignin, was confirmed as a disease biomarker for the olive trees infected by *Xfp*. Interestingly, the two cultivars showed a distinct response upon the bio-fertilizer treatments: a consistent increase in malic acid was observed for the Ogliarola salentina trees, whereas in the Cellina di Nardò ones, the treatments induced the accumulation of γ–aminobutyrate (GABA), a known stress mitigation molecule (Kinnersley and Turano, 2000).

Then, the ^{1}H-NMR metabolomic approach was also used to analyze the xylematic extracts of the *Xfp*-tolerant cultivar Leccino in comparison with the susceptible cultivars Ogliarola salentina and Cellina di Nardò, following a mid-term period of Dentamet® foliar treatment (i.e., three years of spray treatments) (Girelli et al., 2021). A higher mannitol content was observed in the treated trees. Due to its osmoprotectant and antioxidant ability to protect chloroplast, an intracellular accumulation of mannitol is a strategy to improve tolerance against water deficit. The accumulation of mannitol also suggests an improvement of the physiological performance and photosynthetic capability of olive trees. For the untreated *Xfp*-infected samples, a higher relative content of phenolic compounds was observed. Tyrosol and hydroxytyrosol moieties of oleuropein and its aldehydic forms, and quinic acid were observed for all the analyzed cultivars (Girelli et al., 2021).

The overall results revealed how *Xfp* strongly modifies the metabolism of olive trees, and how the bio-fertilizer spray treatments can induce an early re-programming of the metabolic pathways in the infected trees. The different responses to Dentamet® treatment would seem to be correlated to olive cultivars physiology and/or the pathogen attack levels. An additional confirm of the metabolic re-programming induced by Dentamet® treatments to olive trees was recently obtained by the metabolic assessment of olive trees upon endotherapy. Following the injection of the bio-fertilizer in the short-term period, it has been observed specific variations in the olive leaf content of some specific metabolites in the susceptible cultivars Ogliarola salentina and Cellina di Nardò. In particular, the endotherapy induced a significant decrease of both the disease biomarkers, namely quinic acid and mannitol, with the simultaneous increase of polyphenols and oleuropein-related compounds in the leaves (Girelli et al., 2022). Such results were confirmed when the endotherapy treatments were assessed during a six s period (Hussain et al., 2023). To note that foliar application demonstrated a more specific time related progressive effectiveness with respect to intravascular treatments.

6.2 The re-programming of lipids involved in *X. fastidiosa* subsp. *pauca*-olive interaction

Host–pathogen interactions is determined by different factors that modulate virulence and plant defense. 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 in determining *X. fastidiosa* virulence, cis-2-enoic fatty acids, named diffusible signal 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 *lesA*/*lipA* gene (Rapicavoli et al., 2018), and high DSFs concentration induces adhesion to plant vessels or 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, 18:1) (Lindow et al., 2014; Ionescu et al., 2016). The DSFs induce the expression of adhesins needed for biofilm formation and the formation of the small colonies. Low DSFs concentration induces twitching motility and pit membrane degradation (Chatterjee et al., 2008).

Also, oxylipins, are involved in *X. fastidiosa*-host plant interaction. Plant oxylipins regulate processes related to physiological and pathological events by activating defense-related gene pathways and by interfering with the pathogen growth and reproduction (Siebers et al., 2016; Fernandes and Ghag, 2022). Since most of them have antimicrobial activity, the plant can produce oxylipins also to kill the pathogen (Deboever et al., 2020). Jasmonates are the best characterized plant oxylipins that regulate defense, reproduction, and pathogenesis. Bacteria can employ host oxylipins to augment their virulence (i.e., switching to biofilm stage) (Zheng et al., 2012; Martínez et al., 2019). In *X. fastidiosa*, the oxylipins mediate the autocrine signals within the bacterial cells (i.e., regulation of quorum sensing) and/or paracrine signals in the communication with their hosts or vectors (Ionescu et al., 2016; Martínez et al., 2019; Scala et al., 2020).

In *Xfp*, oxylipins and FAs play are pivotal in shaping bacterial lifestyle. *Xfp* accumulates different lipoxygenase and dioxygenase-derived oxylipins both *in vitro* and during the interaction with different hosts (i.e., tobacco and olive tree) (Scala et al., 2018, Scala et al., 2020). Oxylipins also influence the switch from planktonic growth to biofilm formation, thus indicating that *Xfp* can synthesize and secrete oxylipins. To note that oxylipins emerged as hallmarks of pathogenic invasion by *Xfp* in host tissues: infected plants accumulate more oxylipins (i.e., 7,10-diHOME and 13-HODE) respect to the not infected ones (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 non-infected, (b) belonging to different cultivars, and (c) treated or untreated with Dentamet®. Lipid entities emerging as predictors of the thesis are free fatty acids (C16:1, C18:1, C18:2, C18:3); the LOX-derived oxylipins 9- and 13-HPOD/TrE; the DOX-derived oxylipin 10-HPOME; and diacylglyceride DAG36:4(18:1/18:3). The analysis of dataset of *Xfp*-positive vs *Xfp*-negative highlight as significant compounds 9- 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). These data were supported also by bioinformatic analysis of RNA-seq data files (Scala et al., 2022).

According to these results, it is possible to assume that the oxylipins are differently accumulated in the *Xfp*-susceptible or *Xfp*-tolerant cultivars such 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 field is characterized by an accumulation of oxylipins (i.e., 7,10-diHOME and 13-HODE) that are most probably employed by the bacterium to switch its lifestyle to a virulent phase. Moreover, the olive trees treated with Dentamet® and positive to *Xfp* show the lipid profile with the accumulation of the typically healthy plant lipid signature (i.e., decreasing 13-oxylipins/DOX-oxylipins, and increasing of 9-oxylipins), this resulted different from the lipid signature of olive trees infected by *Xfp* and not treated with the bio-fertilizer.

6.3 The management strategy checked by high-resolution satellite imagery monitoring

A confirmation of the field efficacy of the management strategy applied in the olive groves of Salento to face the *Xfp* epidemic has been obtained through a study performed with satellite imagery data coupled with selected vegetation indices (Blonda et al., 2023). Starting from 2015 until 2020, during July and August, multi-resolution source satellite data obtained by time series High Resolution (HR) Sentinel-2 images (10m), and Very High Resolution (VHR) Pleiades imagery (2 meters) were analysed for field 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 of just five days, when both satellites are operational. The span of 13 spectral bands, from the visible and the near infrared to the shortwave infrared at different spatial resolutions ranging from 10 to 60m takes land monitoring and plant status to an unprecedented level (https://www.eurisy.eu/monitoring-plant-health-from-space/).

Time series of remote sensing imagery and derived vegetation indices, indeed, have been shown particularly useful to characterize land ecosystem dynamics, as they can provide consistent measurements at different spatio-temporal scales. Such measurements result 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 proxy of geophysical variables (https://www.usgs.gov/landsat-missions/landsat-surface-reflectance-derived-spectral-indices). Obtained through selected spectral bands combination, vegetation indices are proxy indicators of plant health (Montero *et al*., 2023).

Among the vegetation indexes, the Normalized Difference Vegetation Index (NDVI) is based on red and Near Infrared Reflectance (NIR) light to identify the amount of chlorophyll in leaves. Initially, it 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. Multi-scale satellite data and techniques have been used to monitor *Xfp* spreading across Salento olive groves and to detect early symptoms of OQDS in the vegetation. In particular, medium resolution data have been exploited to quantify the extension of areas covered by wilting olive trees (Scholten et al., 2019), whereas Sentinel-2 data and hyper-spectral data from airplane campaigns have been used by Hornero et al. (2020) to quantify the extension of areas covered by wilting olive trees. In addition, hyper-spectral data were analyzed to extract spectral features 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 of Salento (Lecce province). The monitoring was carried out at field scale and at tree-scale. At field scale, the trends of four spectral indices, namely NDVI, OSAVI, NDRE and ARVI, from time series of freely available High Resolution (HR) Sentinel-2 images (10 meters), were analyzed and correlated to meteo-events in the years from 2015 to 2020. At tree-scale, the study has evaluated both the recovery status of each olive tree, in the experimental and productive fields, and the response of different cultivars to treatments through Very High Resolution Pléiades data. The data were collected during July and August, when the contribution to the vegetation indices from the background is minimal and the occurrence of *Xfp* symptoms are very evident.

In all treated fields, all spectral indices resulted higher than the untreated ones after Dentamet® treatments at both field and tree scale, from HR and VHR images (Table 2). The correlation between the HR index time series with meteo-events indicated that the treated olive trees were more responsive to rain events than untreated ones (Blonda et al., 2023). At tree scale, VHR indices revealed different cultivar response to treatments and showed Ogliarola salentina more promptly responding to the treatments than Leccino and Cellina di Nardò. Specifically, the finding reported from HR data could be used to evaluate plant conditions at field level after restoration actions, while VHR imagery could be used to optimize treatment doses per cultivar. These findings, in agreement with the effect of the bio-fertilizer of *Xfp* concentration in olive trees (Tatulli et al., 2021) and with metabolomic studies that revealed a re-programming of plant metabolites upon the curative treatments (Girelli et al., 2019; 2021) showed that indices for plant health status were higher in treated fields than in those untreated.

7. **The management strategy for the disease complex**

Since 2016, after the first promising results observed in the experimental field, some farmers of Salento started to introduce the strategy either as preventive or curative practice towards the pathogen. The ease of application as foliar spray and the low cost of the bio-fertilizer allows its application by all farmers, including the part-time and the amateur ones. However, the success of the management strategy, as for other plant diseases, is strongly due to the consistency of the foliar applications over several months, so that the spraying Dentamet® two or three times occasionally does not solve the problem (Scortichini, 2022). The studies performed so far suggest the starting of the application, once per month, from the end of February-early March until end of September-early October. The dose is of 3.9l ha^{-1} (i.e., 280 ml in 100l of water). For centennial trees, some farmers use a bit more concentrated spray solution (i.e., 300-350ml in 100l of water) without scoring any phytotoxicity. It is important to provide a right amount of foliar spray to the tree crown (i.e., 20l of spray solution for an olive tree of 60-70 years; 25-30l of spray solution for the centennial specimens) to allow the penetration of the bio-fertilizer within the foliage. It has been calculated that the yearly cost per tree for six treatment is of about 3.0 Euro that, in case of spraying by means of modern atomizers, goes down to 1.0 Euro per each treated tree.

In parallel, the reduction of the presence of the insect vector in the farm 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-stage of the vector by means of mechanical techniques. The egg reduction should be performed through a light tillage during winter (i.e., from December to February) to eliminate the eggs deposited by the adults in ground slits. Subsequently, from early February to early May, spontaneous weeds should be mowed to eliminate juvenile-stage specimens (i.e., naiades and nymphs). It should be stressed that the abandoned olive groves host the highest population of vectors (Picciotti et al., 2021). So, to be effective, the vector control strategy should be preferably applied to a vast area, and punctiform vector control carried out at single olive grove is not retained fully effective.

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

Soil fertility is also retained very important for a rationale management of the olive grove in relation to *Xfp* infection. A soil depletion of some micronutrients, namely zinc, copper and manganese, indeed, 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* has been found a low bioavailability of copper and boron (Scortichini et al., 2019). Consequently, measures aimed at increasing the soil content of macroelements, microelements as well as 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 soil and within the infected trees, is an indirect confirmation of the effectiveness of the protocol capable to provide such microelements through the foliar applications.

It should be said that, despite the continuous claiming about "there is no cure for *Xylella fastidiosa*" (Colella, 2023) and the discouragement to try the application any field control protocol performed by national and local press as well as by the major farmer organizations (Martella, 2022), a relevant number of 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 practicing the control strategy in the "infected" area, within the provinces of Lecce, Brindisi and Taranto since a relevant time span (i.e., more than six years) (Fig. 5). The tree ages range from 30 to more than 500 years old and the yield range, according to the classical olive trend of yield (i.e., alternate bearing), from 30 to 50 q.li/ha per year. It should be stressed that, in many cases, the olive farms are surrounded by dead or severely damaged trees, this representing "green oasis" in the devastated Salento (Fig. 6). Moreover, there are other farms of the "infected" area that have begun to apply the protocol one or 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 bio-fertilizer during spring and summer. In these cases, also, the farms border areas characterized by a relevant occurrence of OQDS but the applying of the bio-fertilizer promptly incited a relevant sprouting of the infected trees. There are also cases where farmers utilize the protocol as a preventive measure to avoid possible infection by the bacterium. Noteworthy, in some cases, groups of farmers funded local "social promotion associations" (APS) with the aim to save olive trees through the application of the *Xfp* control protocol herein described. To date, more than 1.500 hectares are currently applying this control strategy in Salento, this representing a safeguard for the heritage of that olive agroecosystem.

According to the new evidence that points for a significative role of *Botryosphaeriaceae* fungi in causing diebacks to olive groves together with *Xfp*, an additional effort to find active compounds capable to mitigate the aggressiveness of such fungi is required. Currently, several strategies are under study to face the virulence of *Botryosphaeriaceae* to woody species (Guarnaccia et al., 2022; Aiello et al., 2023). A fundamental approach to reduce the spread of such fungi within and between the olive groves is the disinfection of pruning shears, and the protection of pruning wounds, since pycniodiospores of these fungi, spread by rain splash, can easily infect them after the cutting, as observed in vineyards (Urbez-Torres and Gubler, 2011; Otoya-Martinez et al., 2023).

Fig. 5. Different approaches to face *Xylella fastidiosa* subsp. *pauca* in the "infected" area of Salento. Top: July 2019: productive olive grove of 10 ha at Nardò (Lecce province) (on the left) that has applied the management strategy based on Dentamet® since 2016; abandoned olive groves completely wilted (on the right). Below: June 2022, the olive grove that continues to apply the management strategy is still productive (on the left), whereas the abandoned olive grove was uprooted and planted with tomato (on the right).

Fig. 6. Top: olive farm (on the left) at San Pietro Vernotico (Brindisi province) in June 2022 that applied the management strategy during the last four years compared to a close and abandoned olive grove completely wilted (right); Below: experimental field at Cannole (Lecce province) in June 2022. This olive grove has continuously applied the control strategy since 2016 and it regularly yields. To note the completely wilted olive groves nearby and the "drift effect" in the first olive row bordering the treated ones due to the aerial spray of Dentamet® that partly also reached the neighboring olive trees.

Pruning shears have been verified as a source of inoculum for *Botryospheriaceae* that infect grapevine (Agusti-Brisach et al., 2015). The disinfection of the shears could be obtained by soaking them into a liquid formulation that contains a broad-spectrum fungicide (Diaz and Latorre, 2013). The protection of pruning wounds could be obtained by means of *Thricoderma* spp. (i.e., *T. asperellum* and *T. gamsii*) biocontrol agents (Blundel and Eskalen, 2022), and the pruning should be preferably performed during dry and warm weather (Urbez-Torres and Gubler, 2011). Also very important to reduce the risks posed by the inoculum sources, is the elimination of pruning residues and dead wood (Aiello et al., 2023). A confirmation of the fundamental role played by pruning in the dispersal of *Botryospheriaceae* within and between the olive groves of Salento has been achieved by direct observations of farmers that observed first signs of BTD (i.e., extensive twig diebacks) one or two years after pruning. Currently, in Italy there are not specific, authorized fungicides to control *Botryospheriaceae* in olive groves, and the search for biocontrol agents or ecofriendly compounds capable to reduce the tree colonization and significantly mitigate their aggressiveness is important.

8. General remarks and perspettive

Through an in-depth interdisciplinary approach, we have verified that is now possible to apply a field management strategy capable to cure olive trees infected by *Xfp* in Apulia. Such a cure is not intended at eliminating the bacterium from the xylem tissue of the tree but at reducing its concentration at a level compatible with the obtaining of new vegetation and yield, as commonly obtained for most of the crops infected by plant pathogens. This strategy, associated to the vector control strategy, should be applied every year. The misunderstanding between "cure" and "eradication" of *Xfp* has created either confusion or a relevant negative approach towards the acceptance of the management strategy, this resulting in the following abandon of the olive groves and the subsequent general collapse of the trees in a very vast area of Salento. In the case of OQDS, an integrated approach, indeed, that consider 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 comprehension of the disease itself and to the obtaining of an effective control strategy.

Within this frame, it is worth noting that a fungus-associated syndrome, namely BTD, caused by *Neofusicoccum* spp., has been recently described in the olive groves of Salento, which severely impacts the olive trees and whose symptoms can be confused with symptoms of OQDS. Further studies are ongoing to fully understand the role of the associated fungi. However, it should be outlined that, in woody species declines, polymicrobial complexes can often be involved, and each microbial species may either cause specific symptoms or interact with the others in a temporally regulated succession which ultimately cause the decline symptomatology (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), an integration with measures aimed at restricting the progress of such additional pathogens, could provide an even better control of all the causal agents involved in olive trees diebacks in Salento and Apulia as a whole. Studies are under way to select effective compounds capable to significantly reduce the aggressiveness of *Botryospheriaceae* 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, Stefania Loreti, Valeria Scala, Nicoletta Pucci, Massimo Pilotti, Giuseppe Tatulli, Erica Cesari, Alessia L'Aurora, Massimo Reverberi, Nicola Cristella, Paolo Marangi, Palma Blonda, Cristina Tarantino, Patrizia Adamo, Sabino Maggi, Gianluigi Cesari, Chiara Roberta Girelli, Federica Angilè, Mudassar Hussain, Danilo Migoni, Francesco Paolo Fanizzi. All authors contributed to: data curation, writing, review & editing.

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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