

Q:1

Xylella fastidiosa in Olive in Apulia: Where We Stand

M. Saponari, A. Giampetruzzi, G. Loconsole, D. Boscia, and P. Saldarelli[†]

First, fourth, and fifth author: Consiglio Nazionale delle Ricerche, Istituto per la Protezione Sostenibile delle Piante, Bari, via Amendola 122/D, Bari, Italy; and second and third author: Dipartimento di Scienze del Suolo della Pianta e degli Alimenti, Università degli Studi di Bari Aldo Moro, via Amendola 165/A, Bari, Italy.

Accepted for publication 24 October 2018.

ABSTRACT

A dramatic outbreak of *Xylella fastidiosa* decimating olive was discovered in 2013 in Apulia, Southern Italy. This pathogen is a quarantine bacterium in the European Union (EU) and created unprecedented turmoil for the local economy and posed critical challenges for its management. With the new emerging threat to susceptible crops in the EU, efforts were devoted to gain basic knowledge on the pathogen biology, host, and environmental interactions (e.g., bacterial strain(s) and pathogenicity, hosts, vector(s), and fundamental drivers of its epidemics) in order to find means to control or mitigate the impacts of the infections. Field surveys, greenhouse tests, and laboratory analyses proved that a single bacterial introduction occurred in the area, with a single genotype, belonging to the subspecies *pauca*, ~~was found~~ associated with the epidemic. Infections caused by isolates of this genotype turned to be extremely aggressive on the local olive cultivars, causing a new disease termed olive quick decline syndrome. Due to the initial extension of the foci and the rapid spread of the infections, eradication measures (i.e., pathogen elimination from the area) were soon replaced by containment measures including intense border surveys of the contaminated area, removal of infected trees, and mandatory vector control. However, implementation of containment measures encountered serious difficulties, including public reluctance to accept control measures, poor stakeholder cooperation, misinformation from some media outlets, and lack of robust responses by some governmental authorities. This scenario delayed and limited containment efforts and allowed the bacterium to continue its rapid dissemination over more areas in the region, as shown by the continuous expansion of the official borders of the infected area. At the research level, the European Commission and regional authorities are now supporting several programs aimed to find effective methods to mitigate and contain the impact of *X. fastidiosa* on olives, the predominant host affected in this epidemic. Preliminary evidence of the presence of resistance in some olive cultivars represents a promising approach currently under investigation for long-term management strategies. The present review describes the current status of the epidemic and major research achievements since 2013.

TIMELINE OF XYLELLA FASTIDIOSA IN OLIVES IN SOUTHERN ITALY

In 2013, while investigating the etiology of a previously unknown olive disease, *Xylella fastidiosa* was detected for the first time in Europe and in the Mediterranean Basin, representing a serious threat to the local agriculture economy and the biodiversity of this region. Bacterial infections were consistently detected in olive trees severely affected by a novel disease, termed olive quick decline syndrome (OQDS), characterized by severe branch desiccation and rapid death of olive trees (Fig. 1; Box 1). OQDS first appeared in olive orchards of the Salento Peninsula in the region of Apulia, one of the main olive-growing area in southern Italy. This was alarming because olive is one of the most important crops in the

Mediterranean Region. Information from interviewing local growers indicated that unusual desiccation symptoms in olive canopies started to appear on a few scattered trees between 2008 and 2010. However, by early 2013, a continuous expansion of these symptoms in olives prompted local Phytosanitary Authorities and researchers to start investigations on the etiology of this disease. Initial field observations had tentatively attributed this novel and rapid-spreading disease to a variety of biotic and abiotic causes: severe attacks of olive anthracnose (*Colletotrichum* spp.); root rot; necrosis of the sapwood associated to fungal species of the genera *Phaeoacremonium*, *Pheoniella*, *Pleurostomophora*, and *Neofusicoccum*; heavy infestations by the leopard moth (*Zeuzera pyrina*); poor management of the olive groves; and pollution of the groundwater and ensuing phytotoxicity (Martelli 2015; Martelli et al. 2016). In late 2013, following a significant increase of the reports of OQDS-affected trees, additional pathogen screening tests revealed the presence of the quarantine pathogen *X. fastidiosa*. This finding was the first confirmed outdoor outbreak of this exotic pathogen, which had never been detected in any European Union (EU) country. After the identification of *X. fastidiosa* in olive trees, surveys were immediately extended to surrounding plant species

[†]Corresponding author: P. Saldarelli; E-mail: pasquale.saldarelli@ipsp.cnr.it

Funding: Support was provided by Horizon 2020 Framework Programme grants 645636 and 727987.

and vegetation, searching primarily for plants showing typical symptoms of leaf scorch. Oleander (*Nerium oleander*) and almond (*Prunus dulcis*) were also found infected in the area, both showing symptoms of leaf scorch and/or, in the case of oleander, shoot dieback. In the framework of the field surveys carried out in these past 5 years the bacterium has been isolated and/or detected in more than 30 host/species (Table 1), including many ornamentals, endemic species of the Mediterranean flora, and three crop species: olive, cherry (*Prunus avium*), and almond.

The finding of *X. fastidiosa* in this new environment and infecting primarily olive stimulated several research programs targeting *X. fastidiosa* and its role in the development of OQDS, the identification of its local insect vector, the taxonomy of the bacterial genotype(s), and the complex factors contributing to the epidemiology of the infections in the infected area in the Salento Peninsula.

Bacterial infections in the initial foci were detected mostly on ancient and century-old olive trees, which also showed severe attacks of leopard moth, variously extended browning of the sapwood of twigs, branches, and trunks associated with the presence of different wood-inhabiting fungal species (Nigro et al. 2013). These consistent associations led to the hypothesis that OQDS could result from the impairment of the xylem tissues caused by the concomitant actions of the leopard moth, wood decay fungi (whose establishment is favored by the leopard moth galleries), and *X. fastidiosa* colonization,

ultimately responsible for the occlusions of the remaining active vessels. As such, the disease was originally named in Italian “Complesso del Disseccamento Rapido dell’Olivo (CoDiRO)” to indicate its association with a complex of causal agents. However, as the infections continued to spread, the extensive surveys and laboratory analyses disclosed that maps of OQDS and *X. fastidiosa* were superimposable, hence, showing high spatial correlation. In contrast, leopard moth attacks and trees colonized by different wood

BOX 1

OLIVE QUICK DECLINE SYNDROME

OQDS is characterized by leaf scorching and scattered desiccation of twigs and branches that usually starts at the top of the tree canopy and expands to the rest of the crown to which it confers a scorched aspect and culminates with the death of the trees (Figs. 1 and 2) within a few years from the onset of symptoms. The most severely and ominously affected olives are the century-old trees of local cultivars Cellina di Nardò and Ogliarola salentina, which are highly susceptible.

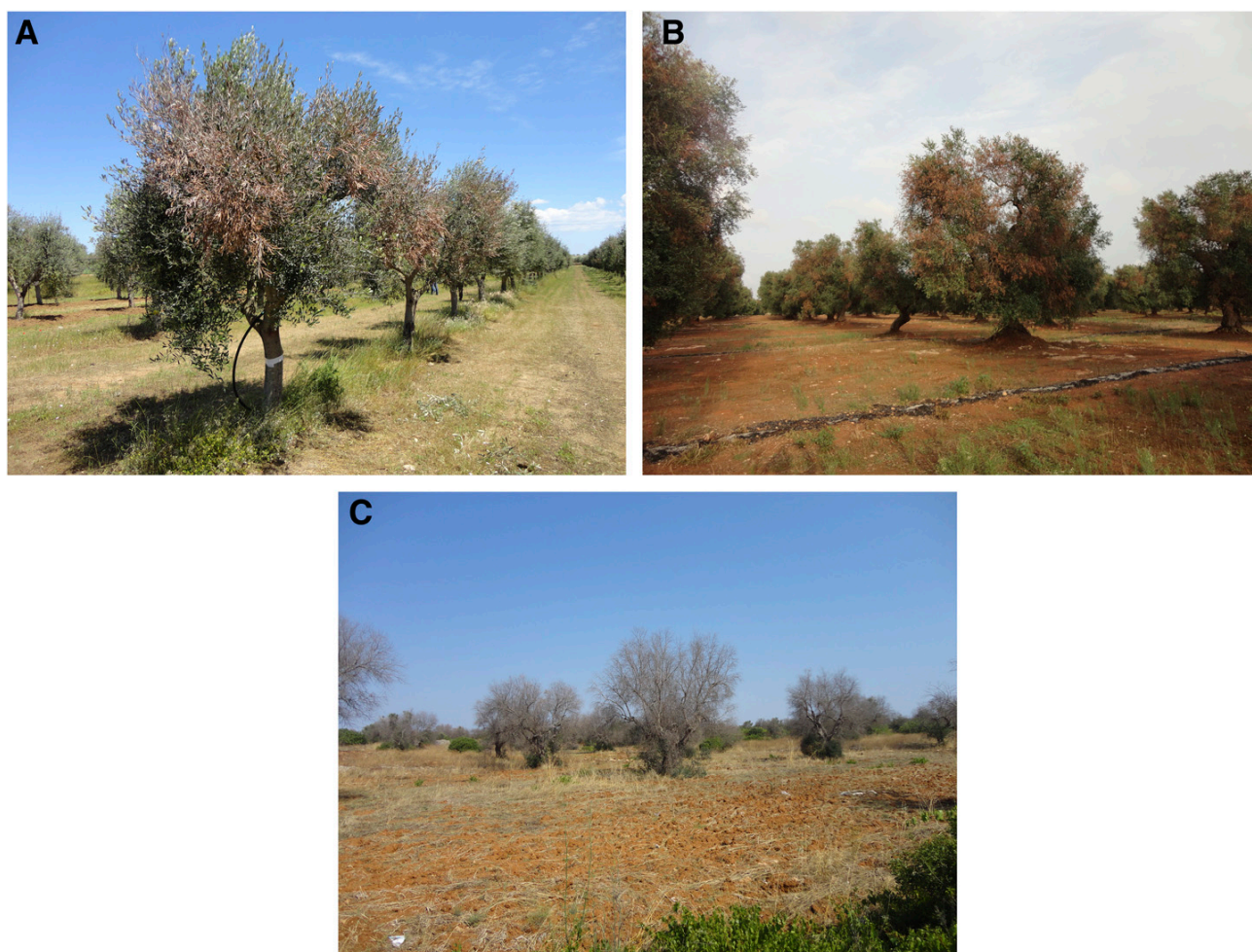


FIGURE 1

A, Young and **B**, centenary olive trees with canopies showing manifest branch desiccations induced by *Xylella fastidiosa* subsp. *pauca* ST53. **C**, Olive orchard at advanced stage of disease with whole trees dead. Several, if not all, of the trees in the orchard show symptoms.

decay fungi were found widespread in olives not showing OQDS symptoms (EFSA, 2015). Indeed, as time progressed, symptoms of OQDS were observed in young olive orchards (Fig. 1A). These symptomatic young trees were positive for *X. fastidiosa* but, in most cases, did not show any evidence of wood discoloration or galleries of leopard moth. This further suggested that *X. fastidiosa*, by itself, was able to cause the severe branch desiccation and dieback. Similarly, field surveys to associate problems such as poor management, phytotoxicity, organic- and nutrient-poor soils, and low-input growing conditions were not fruitful and abandoned as alternative causes of OQDS. As a demonstration, in orchards consisting of olives consociated (in alternate rows) with other fruit trees, symptoms of leaf scorching and desiccation occurred only in olive trees, thus excluding that the soil conditions or poor general management could be responsible for this severe syndrome.

Conclusive evidence on the primary role of *X. fastidiosa* as causal agent of OQDS was obtained with fulfillment of the Koch's

postulates. *X. fastidiosa* was isolated from olives on artificial media and was needle-inoculated to healthy olives and other *Xylella*-susceptible hosts. Shoot dieback and/or leaf scorching symptoms appeared in olives, oleander, and myrtle leaf milkwort (*Polygala myrtifolia*), and the pathogen was successfully reisolated from the inoculated plants (Saponari et al. 2017). The aggressiveness of this genotype was also noted by the impact of infections on other hosts in the infected area. Although, the predominant host so far remains olive, lethal infections have been also observed in oleander, acacia (*Acacia saligna*), and myrtle leaf milkwort (*Polygala myrtifolia*). Notably, multiple year surveys and artificial inoculations showed the inability of this strain to infect grapevines (*Vitis* spp.) and citrus (*Citrus* spp.) (Potere et al. 2015).

As reported in the following paragraph, genotypic analysis of the isolates recovered from infected olives and other hosts revealed that all harbored the same *X. fastidiosa* sequence type (ST), designated as ST53, which clustered in a clade in subspecies *pauca*.

TABLE 1
List of the hosts of *X. fastidiosa* subspecies *pauca* ST53 reported in Apulia

Host species	Notes on symptoms	Method of detection
<i>Acacia saligna</i>	Severe dieback and desiccation	Isolation, qPCR, ELISA
<i>Asparagus acutifolius</i>	Not recorded	qPCR, ELISA
<i>Catharanthus</i> sp.	Not recorded	Isolation, qPCR, ELISA
<i>Chenopodium album</i>	Not recorded	qPCR, ELISA
<i>Cistus creticus</i>	Leaf scorch and desiccation	qPCR, ELISA
<i>Dodonaea viscosa</i>	Not recorded	qPCR, ELISA
<i>Eremophila maculata</i>	Not recorded	qPCR, ELISA
<i>Erigeron sumatrensis</i>	Not recorded	qPCR, ELISA
<i>Erigeron bonariensis</i>	Not recorded	qPCR, ELISA
<i>Euphorbia terracina</i>	Symptomless	qPCR, ELISA
<i>Grevillea juniperina</i>	Leaf scorch	qPCR, ELISA
<i>Hebe</i> sp.	Leaf scorch	qPCR, ELISA
<i>Heliotropium europaeum</i>	Not recorded	qPCR, ELISA
<i>Laurus nobilis</i>	Leaf scorch	Isolation, qPCR, ELISA
<i>Lavandula angustifolia</i>	Desiccation	Isolation, qPCR, ELISA
<i>Lavandula stoechas</i>	Leaf scorch and desiccation	Isolation, qPCR, ELISA
<i>Myrtus communis</i>	Extensive yellowing	Isolation, qPCR, ELISA
<i>Myoporum insulare</i>	Symptomless	qPCR, ELISA
<i>Nerium oleander</i>	Leaf scorch followed by severe dieback and desiccation	Isolation, qPCR, ELISA
<i>Olea europaea</i>	Leaf scorch followed by severe dieback and desiccation	Isolation, qPCR, ELISA
<i>Pelargonium × fragrans</i>	Dieback	qPCR, ELISA
<i>Phillyrea latifolia</i>	Leaf scorch	Isolation, qPCR, ELISA
<i>Polygala myrtifolia</i>	Leaf scorching followed by severe dieback and desiccation	Isolation, qPCR, ELISA
<i>Prunus avium</i>	Leaf scorch	Isolation, qPCR, ELISA
<i>Prunus dulcis</i>	Leaf scorch	Isolation, qPCR, ELISA
<i>Rhamnus alaternus</i>	Symptomless	qPCR, ELISA
<i>Rosmarinus officinalis</i>	Leaf scorch followed by dieback and desiccation	qPCR, ELISA
<i>Spartium junceum</i>	Severe dieback and desiccation	qPCR, ELISA
<i>Vinca</i> sp.	Symptomless	qPCR, ELISA
<i>Westringia fruticosa</i>	Leaf scorch followed by dieback and desiccation	Isolation, qPCR, ELISA
<i>Westringia glabra</i>	Symptomless	qPCR, ELISA

It is now undisputed that the *X. fastidiosa* genotype spreading in the Apulia Region is the causal agent of OQDS, as well as of severe infections in other hosts. This scenario is consistent with reports of severe olive desiccations that have recently been reported in Brazil (Coletta-Filho et al. 2016) and Argentina (Haelterman et al. 2015) where olive is not a major crop, but where epidemics of *X. fastidiosa* subsp. *pauca* are known to have been present for a long time.

As for the specific scenario in Apulia, the landscape and the topology of the olive agroecosystem in the region with the occurrence of contiguous olive orchards, grassy meadows and vegetation for xylem-sap feeding insects, and warm summers allow establishment and spread of *X. fastidiosa* and complicates its control (Strona et al. 2017).

The occurrence of *X. fastidiosa* in southern Italy, prompted for the implementation of a series of phytosanitary measures, enforced by the European Authorities, including but not limited to, intense surveys to delimit the infected area and to exclude the presence of the bacterium in other areas of the Italian peninsula as well as in other Member States. Outcomes from the continuous surveillance program indicate that, although continuously expanding, infections in Italy are still confined to the southern portion of Apulia. However, *X. fastidiosa* outbreaks have now been discovered in two additional EU countries, France and Spain (EFSA PLH Panel et al. 2018). Like in the Salento Peninsula, infections were found in different hosts including species previously unknown as susceptible hosts of the bacterium (e.g., *Polygala myrtifolia*, *Laurus nobilis*, *Myrtus communis*, *Myoporum insulare*, and *Dodonaea viscosa* 'Purpurea').

GENETIC CHARACTERIZATION OF *X. FASTIDIOSA* PRESENT IN THE AREA

Initial investigation on the genetics of *X. fastidiosa* infecting olives in Salento indicated that it harbored a previously undescribed sequence type to which the consequential identification code ST53 was assigned (Elbeaino et al. 2014; Loconsole et al. 2016). Isolates harboring ST53 were concomitantly described in 2014 in Central America by Nunney et al. (2014). ST53 has a well-defined genetic

heritage as subspecies *pauca* as five out of seven multilocus sequence typing alleles were shared among isolates of this subspecies and that the two alleles that differ have a clear *pauca* ancestry. Phylogenetic analysis identified the closest sequence type as ST16, previously identified in Brazil from coffee plants (Nunney et al. 2014). Interestingly, a few years later, ST16 isolates have been found infecting olive in Brazil (Coletta-Filho et al. 2016), with infected trees showing symptoms resembling OQDS, further supporting the evidence that strains of this subspecies are capable of causing severe infections in olives.

Multilocus sequence typing analysis of strains isolated from different hosts and infected olives recovered in geographically distinct foci of the Apulia Region showed that all isolates harbored the same sequence type, ST53 (Loconsole et al. 2016).

Furthermore, high-throughput sequencing allowed to reconstruct the draft genome sequence of an olive-infecting strain (Giampetruzzi et al. 2015), and later on to accomplish the complete genome sequence of the reference strain "De Donno," by a combination of high-throughput (Illumina and PacBio) and conventional Sanger sequencing (Giampetruzzi et al. 2017a). Distinctive traits of this isolate are the presence of 35,723-bp conjugative plasmids showing homologies with a widely distributed pXF-RIV5/pXFAS01 plasmid (Rogers and Stenger, 2012), the existence of nucleotide frameshifts (Fig. 2) in the three annotated hemagglutinin (Hfx) genes (locus tags B9J09_03935, B9J09_10075, and B9J09_11875), and a protein belonging to the family of histidine kinase-like ATPase gene (Giampetruzzi et al. 2017b; A. Giampetruzzi, unpublished data). Of note, mutagenesis inactivation of HfxA and HfxB genes made Temecula hypervirulent in grape (Guillabert and Kirkpatrick, 2005). By analogy, the Hfx frameshift mutations in the De Donno strain may contribute to the severe symptoms elicited in the susceptible olive cultivars to OQDS. The discovery of an exclusive histidine kinase-like ATPase gene in the De Donno strain and *X. fastidiosa* isolates from Costa Rica strongly identify, based on current knowledge, the origin of the Apulian strain in Central America (Giampetruzzi et al. 2017b).

Indeed, the availability of the complete genome of the reference strain De Donno allowed for initiating studies of population

Xylella fastidiosa subsp. *pauca* strain De Donno chromosome, complete genome

GenBank: CP020870.1

[GenBank](#) [FASTA](#)

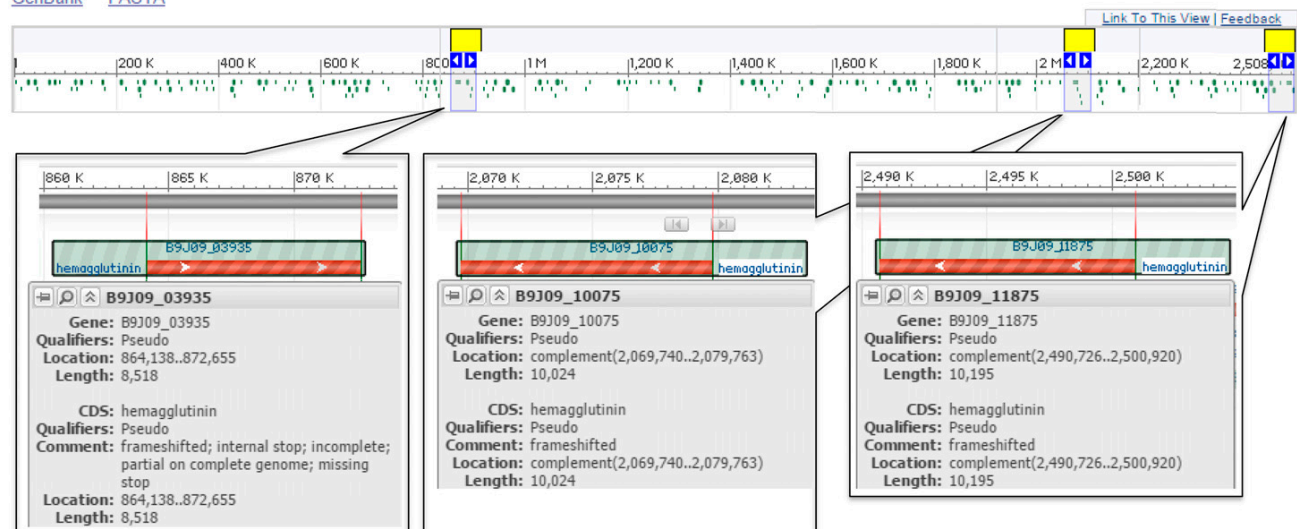


FIGURE 2

Scheme showing the existence of nucleotide frameshifts in three annotated hemagglutinin (Hfx) genes (locus tags B9J09_03935, B9J09_10075, and B9J09_11875) in the complete genome sequence of *Xylella fastidiosa* strain De Donno, modified from <https://www.ncbi.nlm.nih.gov/nuccore/CP020870.1?report=graph>.

genetics through whole-genome single nucleotide polymorphisms (SNPs) analysis. The low number of SNPs detected within a first panel of 50 sequenced olive-infecting isolates supports the hypothesis that the origin of the *X. fastidiosa* outbreak in Apulia is linked to a single introduction of *X. fastidiosa* subsp. *pauca* ST53 (Sicard et al. 2017). Moreover, the limited number of SNPs differentiating the Apulian and Costa Rican ST53 isolates is a strong indication that the introduction in Apulia may have occurred recently (Giampetruzzi et al. 2017b), as suggested by the limited nucleotide changes detected among the bacterial genomes recovered in the two countries.

SPITTLEBUGS AS VECTORS OF *X. FASTIDIOSA* IN OLIVE ORCHARDS

Vector surveys and transmission tests carried out after the discovery of the *X. fastidiosa* outbreak in Apulia led to the identification of the meadow spittlebug, *Philaenus spumarius*, as the first ascertained European vector of *X. fastidiosa* (Saponari et al. 2014). Later surveys for the identification of other insect vector species indicated that *P. spumarius* is the predominant xylem-sap feeder in the area and has a major role in spreading the pathogen (Ben Moussa et al. 2016; Cornara et al. 2017a, b). In fact, the observation of the rapid progression of the disease observed in olive orchards of susceptible varieties reaching nearly 100% (Fig. 1) in 3 to 4 years from the onset of the first infected tree was associated with infestations of abundant *P. spumarius* populations and a high proportion (>70%) of adults testing positive for *X. fastidiosa* during the summer season (Cornara et al. 2017a, b). Although the efficiency of transmission of *P. spumarius* has yet to be experimentally determined, the large number of adults present on the olive canopy for several weeks/months by causing repeated inoculations may explain the dramatic and quick progression of the infections (Daugherty and Almeida 2009). Results of multiyear surveys carried out in selected olive groves showed that secondary spread of the infections (olive to olive) contribute substantially to the recorded aggregate patterns of the disease (Montes-Borrego et al. 2017), supporting the evidence that *P. spumarius* transmits the bacterium from olive to olive. Field experiments using mark-release-recapture methods in meadows and olive groves estimated an active dispersal of approximately 120 m radius from the release points within approximately 15 days (Plazio et al. 2017).

The biology of *P. spumarius* has been investigated to develop targeted control strategies. Dongiovanni et al. (in press) examined the ecology and host preference. Cornara et al. (2017b) examined factors involving population dynamics and fluctuation in olive groves. Surveys in Apulian olive groves indicated that eggs start to hatch in late February/early March, five instar nymphs complete development by late April (preferentially on plant species of the families Asteraceae, Fabaceae, and Apiaceae), and in early May when ground vegetation senesces and desiccates, adult insects disperse to perennial hosts such as olive. Concomitantly, *X. fastidiosa*-positive *P. spumarius* can be identified in infected olive groves, with positive insects detected throughout the entire season until late autumn, when the adults disperse to newly emerged ground vegetation for oviposition and subsequent egg hatch and nymphal development in the next spring cycle (Cornara et al. 2018). Based on these observations, management of ground vegetation, i.e., a correct timing of soil tilling to disrupt nymphal development reduce adult emergence, is a key factor to reduce *P. spumarius* populations and limit spread of the pathogen. Control of the juveniles remains the most effective and sustainable approach to reduce the insect population; conversely, the control of adults requires several applications of insecticides for the entire period the adults feed on olives. Regarding insecticides, pyrethroids and neonicotinoids have proven to be, by far, the most efficacious and persistent chemicals among the different formulations tested (Dongiovanni et al. 2018). However, more research on new

formulations and new testing are still needed to achieve more sustainable and effective vector control.

In a recent report, transmission competence of two additional spittlebug species, *Neophilaenus campestris* and *P. italosignus*, was shown under experimental conditions (Cavalieri et al. 2018). Both species acquired the bacterium from infected olives and transmitted the pathogen to different hosts. However, their role in the current olive epidemic remain marginal considering that in the monitored olive groves *N. campestris* populations are significantly lower than *P. spumarius*; whereas to date, *P. italosignus* has never been recorded in the infected zone (Cavalieri et al. 2018).

PHYTOSANITARY MEASURES AND SURVEILLANCE PROGRAM FOR THE DETECTION OF *X. FASTIDIOSA* IN APULIA

Since 2014, large field inspections and sampling campaigns have been in place under the coordination and responsibility of the Phytosanitary Authorities to support eradication and containment measures. Procedures for monitoring, sampling, and laboratory tests have been set based on a regional pest risk assessment (i.e., proximity to the infected area or to a newly emerged disease foci).

Upon the initial attempts to apply eradication measures (i.e., eliminate the bacterium from the area) (FAO 2016), the results gathered from the surveys carried out in 2014 led to the discovery of several additional foci, and by May 2015 the eradication measures were officially replaced with containment measures. Briefly, the most southern part of the Apulia Region was declared “infected zone,” i.e., the bacterium was considered established and not technically eradicable, whereas actions consisting of intensive surveillance and immediate removal of infected plants were enforced in the north boundary of the infected zone. More precisely, specific actions (inspections, sampling, tree removal, and vector control) are in place in the so called “containment area,” the last 20 km strip of the infected zone, and in the “buffer zone” (10 km width) immediately north of it, both of which crossing the east-west region from the Adriatic to the Ionian coast (Fig. 3). To facilitate inspections, the territories comprised in both areas are subdivided in grids (100 m × 100 m) that are subjected to systematic visual inspections and sampling of suspicious symptomatic plants if any, or asymptomatic hosts. Following the laboratory testing, a plant is declared infected by *X. fastidiosa* if the sample yielded positive results with at least two diagnostic methods. If a positive plant is detected in the containment area, it should be immediately removed, and all host plants known to be susceptible to the Apulian *Xylella* strain located within a 100 m radius have to be individually tested for *X. fastidiosa* and, if found positive, should be removed. Currently, the part of the infected area subjected to mandatory removal of infected trees covers about 1,000 km², corresponding to 14% of the whole infected area, whereas for the remaining 85% (about 6,150 km²) removal of the infected hosts is not mandatory. If a positive plant is detected in the “buffer zone,” it should be immediately removed along with all the host plants located in the 100 m radius, regardless of their infection status.

Indeed, strict requirements are applied and enforced for the movement of plants or for planting known susceptible species out of these demarcated areas, unless they are propagated in vitro or under protected facilities.

Mandatory actions to control vector populations are in place in the demarcated areas. These include the following: (i) at the ground level, mechanical weeding in winter and/or spring to mow down or cultivate native flora and other vegetation where spittlebug nymphs thrive before adult emergence; and (ii) at the canopy level, apply pesticides to olives to kill the adults of *P. spumarius* that feed on suckers and on the crown of trees, where they acquire and transfer the bacterium from an infected tree to the neighboring trees.

Territories included in the demarcated zones are continuously adjusted in relation to the results of the surveys and the finding of new outbreaks. Figure 3 shows an official map of the infected areas in 2013 and 5 years later in 2018. The infected area extended from approximately 8,000 ha in 2013 to approximately 715,000 ha in 2018. Although there are no official registered data regarding the number of infected olive orchards/trees in the infected area, the borders of the territories currently declared “infected” cover almost 36% of the Apulia Region, with approximately 21 million olive trees under the threat of the bacterial infections.

The massive field sampling campaigns are supported by geo-referencing applications for iOS and Android devices to precisely locate sites, to help inspectors to survey the assigned grids and pinpoint sampled plants/trees and record all the key information, ensuring traceability when a positive tree is detected and there is a need to trace back the tree/plant for the subsequent mandatory actions. The system provides a real-time map at the level of the single plant, showing the progress of the inspections, sampling, and associated laboratory results. The map is freely accessible on the Apulia Region official website (www.emergenzaxylella.it/portal/portale_gestione_agricoltura).

Since the first identification of *X. fastidiosa* in olives, research has been devoted to optimizing sensitive detection protocols in olives (Loconsole et al. 2014). When *X. fastidiosa* was first detected in olives, the official diagnostic protocols were those

optimized and validated primarily for grapes and citrus (EPPO 2004; Firrao and Bazzi 1994; Francis et al. 2006; Harper et al. 2010; Li et al. 2013), the main hosts of the bacterium in the American continent. Since *X. fastidiosa* in Apulia was found in a large number of plant and crop species, including olive, *Prunus* spp., many shrubs and ornamental species (http://www.emergenzaxylella.it/portal/portale_gestione_agricoltura/Documenti/Specie), a critical need existed to optimize and validate diagnostic protocols on a much wider array of plant species and tissue matrices inclusive of woody and herbaceous plants. Several interlaboratory validations were conducted and rapid, efficient diagnostic procedures were developed and validated including a panel of serological and molecular protocols (EPPO 2018) for individual samples. Some of these procedures are currently adopted in the ongoing large-scale surveys mentioned above. Among the serological tests, enzyme-linked immunosorbent assay (ELISA) and direct tissue blot immunoassay (DTBIA) proved to be reliable tests for the identification of the bacterium in olive matrices. The ELISA detection threshold in olive tissue extracts was estimated to be in the range of 10^4 CFU/ml, allowing the reliable detection of the bacterium in both susceptible and resistant olive cultivars, in which bacterial concentrations have been estimated to range between 10^6 CFU/ml to 10^4 CFU/ml (Giampetruzzi et al. 2016). For molecular detection, quantitative real-time PCR (qPCR) and loop mediated isothermal amplification

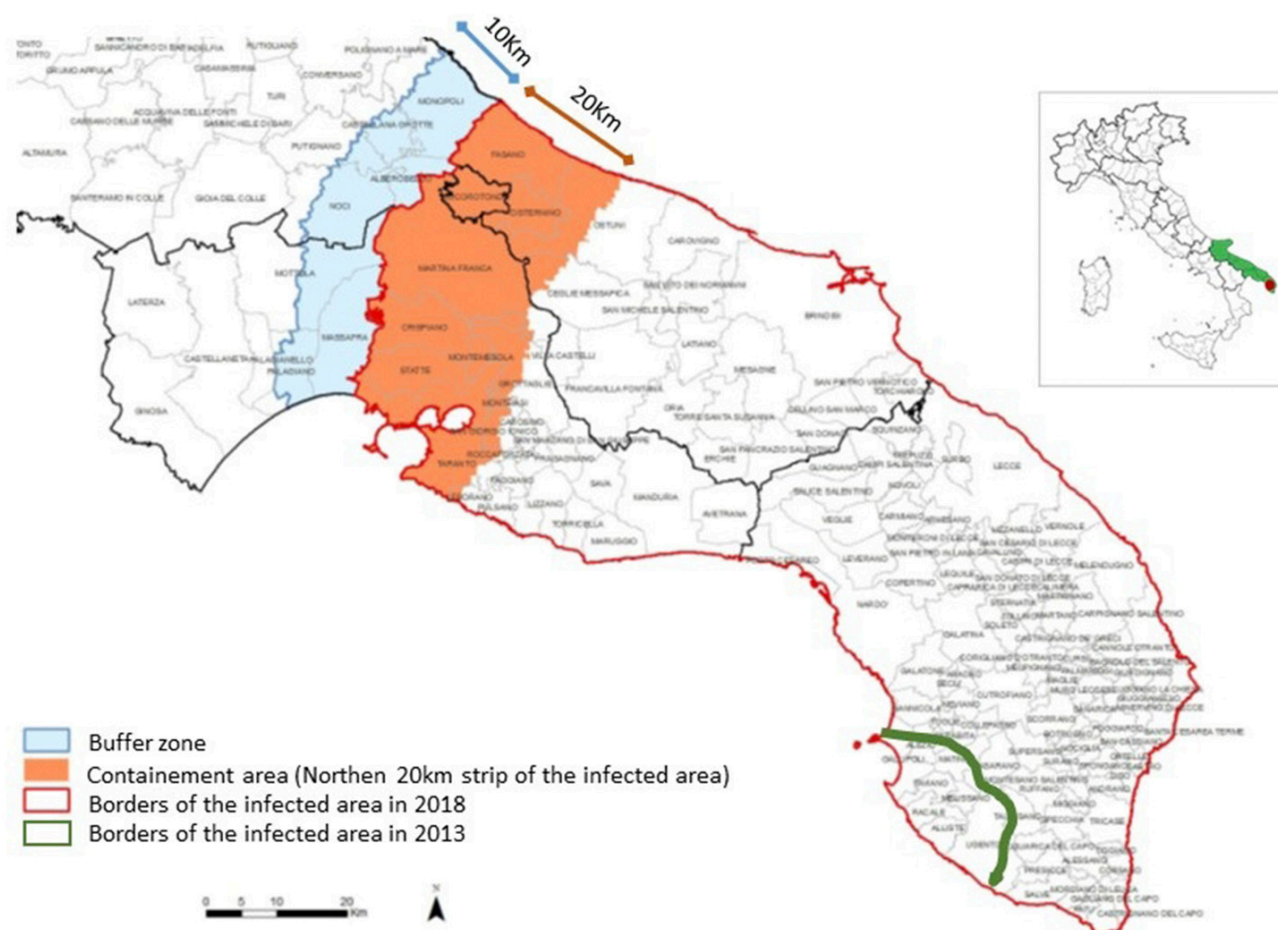


FIGURE 3

Map of the Apulia Region (southern Italy) showing the territory included in each of the current demarcated areas after the last update in August 2018: buffer zone, containment area, and infected area. The approximate delineation of the initial infected area in 2013 is also indicated. The upper right map of Italy shows the Salento Peninsula (red dot) in the Apulia Region (green).

polymerase chain reaction (LAMP) are the most used sensitive approaches used, with qPCR yielding a detection threshold of 10^2 CFU/ml.

The diagnostic workflow officially adopted in Apulia integrates protocols based on both serological and molecular approaches. Diagnostic assays are carried out at a two-tier level of diagnostic assays by independent laboratories. Briefly, all field samples collected undergo an initial ELISA-based screening test using different commercially available serological kits. All samples that yield positive or undetermined results in the first level laboratory test are then sent for confirmation to another laboratory which performs two qPCR assays: a TaqMan-based assay using the primers designed by Harper et al. (2010) and a SYBR green-based assay using the primers designed by Francis et al. (2006). Samples testing positive with the SYBR green-based assay undergo a melting curve analysis, which serves either to confirm that amplicons are specific for *X. fastidiosa* genotype ST53 or to highlight the presence of putative different *X. fastidiosa* strains.

To further authenticate this diagnostic workflow, a percentage of ELISA-negative samples (ranging from 1 to 5%) is subjected to the double qPCR testing assay to ensure that false negatives do not occur. Using this integrated approach, during the last monitoring campaign from October 2017 to April 2018 more than 150,000 samples (the majority olives) were processed by ELISA in four accredited laboratories (designated as first-level laboratories) and more than 10,000 samples were then subjected to qPCR assays by the regional reference laboratory. An estimation of the agreement of the results of the two diagnostic assays (ELISA and qPCR), based on the data produced by the most proficient first-level laboratory, indicate approximately 97% concordance of the correct calls of olive infection status. It should be noted, however, that some ELISA false-positives were encountered, especially with certain plant matrices. To give some context, while the rate for olives was in the range of 1%, false-positive rates reached 8% for asparagus (*Asparagus acutifolius*) and 11% for almond (M. Saponari, unpublished data). Further research efforts are ongoing to overcome the obstacles that limit the use of DTBIA and real-time LAMP for large-scale surveys, i.e., automation of the analysis of the results of the DTBIA assay (capture and interpretation of the signals in the imprints), or increase the sample processing capability of the real-time LAMP platforms.

Meanwhile, promising results were obtained by innovative tools for early detection of bacterial infections using remote sensing-based approaches. A case study was developed in the infected area of Apulia to identify plant stress indicators associated with physiological alterations in olive trees at the early stage of the bacterial infections when symptoms are asymptomatic. Intensive multiyear in-situ inspections of >7,000 trees and a 2-year airborne campaign using high-resolution imaging spectroscopy and thermography demonstrated that alterations in plant functional traits associated with *X. fastidiosa* infections are detectable at previsual stage, with an accuracy of disease detection exceeding 80% (Zarco-Tejada et al. 2018). These results provide evidence that advanced physiology-focused remote sensing methods relying on plant functional traits could provide critical support for large-scale *Xylella* disease surveillance programs in support of management efforts to prevent and manage plant disease epidemics.

Research activities have also included developing disease spread models as tools for risk-based management plan. White et al. (2017) developed a spatially explicit simulation model for *X. fastidiosa* spread in Apulia, showing that increasing buffer zone widths, together with the intensity of surveillance, decrease infection risk beyond the control zone, although long-distance jumps may still contribute to the expansion of the infections, due to long-distance vector dispersal. However, to significantly implement predictive spread models, there are many biological and epidemiological aspects that need to be determined.

OLIVE RESISTANCE TO *X. FASTIDIOSA*

Olive is characterized by the largest genetic and phenotypic variability among the cultivated fruit species, with more than 900 different varieties reported in the Mediterranean countries (Muzzalupo and Perri 2009). In the infected area, the majority of olive groves consists of old plantations (in several cases, century-old trees) of two local cultivars: Ogliarola salentina and Cellina di Nardò; however, few orchards (20 to 50 years old) have been planted with different Italian cultivars. In this regards, even if relatively few olive cultivars are naturally exposed to *Xylella* inoculum pressure, field observations revealed several promising resistance/tolerant phenotypic traits, i.e., mild OQDS symptoms in some cultivars, suggesting differential susceptibility to bacterial infections may exist in the olive germplasm. Encouraged by existence of resistance mechanisms against *X. fastidiosa* in other crop species (Coletta-Filho et al. 2007; Krivanek et al. 2005), more in depth investigations were undertaken in the *X. fastidiosa* infected area by planting and evaluating many different cultivars of olives from Italy and other Mediterranean countries with the hope of finding resistant or tolerant cultivars. While it was evident that the two local cultivars were highly susceptible (i.e., high incidence of infections and severe symptoms), trees of cultivar Leccino showed mild branch dieback or were asymptomatic despite being adjacent to or close to orchards with severe OQDS symptoms in cultivars Ogliarola salentina and Cellina di Nardò. Specifically, the canopy of the OQDS-affected trees of cultivar Ogliarola salentina showed progressive and complete desiccation; whereas *X. fastidiosa* symptoms in Leccino were limited to a few scattered twigs or distal branches. These observations, collected initially in few olive orchards, were confirmed in the framework of a larger survey conducted in 16 different olive orchards located in 11 municipalities of the infected area, with 600 olive trees scored for symptoms and tested by ELISA and qPCR (Boscia et al. 2017b). While 100% of the trees of cultivar Cellina di Nardò and cultivar Ogliarola Salentina tested positive and were highly symptomatic, the infection rate was as low as 35% for the trees of Leccino with trees being mostly asymptomatic or showing only few scattered desiccated branches. In the framework of this survey, laboratory tests also supported the evidence that the infected trees of Leccino harbor a significant lower bacterial population size than the symptomatic trees of Cellina di Nardò and Ogliarola Salentina, 2.9×10^3 CFU/ml versus 1.06×10^6 CFU/ml and 1.3×10^6 CFU/ml, respectively.

Artificial inoculations performed under controlled conditions using potted plants of Cellina di Nardò and Leccino reproduced results similar to those collected in the fields (Saponari et al. 2017). Furthermore, to understand molecular events involved in host-pathogen interactions, a transcriptome profiling of the xylem tissues from twigs of naturally infected plants of both cultivars was conducted (Giampetruzzi et al. 2016) in comparison with those of healthy plants of the same cultivars (two plants/cultivar). A similar gene expression study was performed on complete leaf tissues from the same plants (A. Giampetruzzi, unpublished results) (Box 2).

These studies indicated that (i) infected field trees or potted inoculated plants of cultivar Leccino harbor a lower bacterial titer (4×10^4 CFU/ml) than cultivar Ogliarola salentina or Cellina di Nardò (2×10^6 CFU/ml) (Giampetruzzi et al. 2016; Saponari et al. 2017); (ii) artificially inoculated plants produced the same symptomatic phenotypes observed in the field, i.e., plants of Cellina di Nardò developed severe shoot dieback; whereas limited dieback were recorded on the plants of Leccino; and (iii) transcriptome comparisons of the of field-grown and naturally infected trees of Leccino and Ogliarola salentina identified several differentially expressed genes (DEGs), with genes encoding receptor-like kinases (RLK) and receptor-like proteins (RLP) being upregulated in Leccino; whereas several genes linked to drought stress were upregulated in Ogliarola salentina

Q:2

as shown in grapevine infected by Pierce's disease (Choi et al. 2013). Moreover, both cultivars react with a strong remodeling of cell wall proteins.

These data suggest that plant response to *X. fastidiosa* infection is likely recognized by the susceptible cultivars Ogliarola salentina and Cellina di Nardò similarity to drought stress, as shown by the up-regulation of several genes known to be differentially expressed in other plant species (Choi et al. 2013) during this condition; whereas the lower pathogen concentration in cultivar Leccino suggests that this cultivar may harbor genetic constituents and/or

regulatory elements which effectively limit bacteria replication and counteract infections (Giampetruzzi et al. 2016). The transcriptomes analyzed initially using a self-assembled olive transcripts database, because of the unavailability of a public accessible olive genome, are currently being reanalyzed against a published cultivar Farga olive genome (Cruz et al. 2016). Peculiar traits of this ongoing analysis to date are as follows: (i) Leccino has a lower number of DEGs than Ogliarola salentina either in xylem or in leaf tissues (Fig. 4); (ii) in Leccino, *X. fastidiosa* transcripts perturbation is more pronounced in xylem than in leaf tissues; (iii) Ogliarola salentina transcriptome is more perturbed than Leccino either in xylem and leaf tissues; (iv) the range of fold change of DEGs is wider in Ogliarola s (xylem: $-9.3 < \text{fold change} < 8.8$; leaf: $-8.7 < \text{fold change} < 8.9$) than in Leccino (xylem: $-8 < \text{fold change} < 4.4$; leaf: $-4.6 < \text{fold change} < 2.8$). Most perturbed biological processes in the susceptible Ogliarola salentina are related to cell wall remodeling and defense reactions involving receptor kinases. Conversely, the resistant Leccino, besides perceiving the presence of the bacterium in the xylem tissues, controls the bacterial multiplication without showing a dramatic alteration in gene expression (Giampetruzzi et al. 2016; A. Giampetruzzi, unpublished data).

A set of genes with altered expression is being investigated in greenhouse-infected olives of resistant Leccino and susceptible Cellina di Nardò cultivars. These genes are mainly related to the drought stress imposed by the pathogen, particularly controlling abscisic acid (ABA) and cell wall remodeling (i.e., pectate lyase, polygalacturonase, expansin) biochemical pathways and to the pathogen perception by receptor-like kinases (RLKs). Similar findings have been described in grapevine (Rapicavoli et al. 2018; Zaini et al. 2018) in which the pathogen is perceived, in the early time of infection, as an abiotic stress related to drought. In this scenario, the weakening of the cell wall of xylem parenchyma cells promoted by the altered expression of polygalacturonases and expansins helps to counteract the pathogen-induced water loss although inducing tylose formation (De Souza et al. 2017; Giampetruzzi et al. 2016; Rapicavoli et al. 2018; Rodrigues et al. 2013; Zaini et al. 2018). In addition, a differential expression of

BOX 2

XYLELLA FASTIDIOSA RESISTANCE IN OLIVE

Plant responses to a systemic pathogen infection can result in susceptibility (i.e., high pathogen population in all tissues, severe symptoms), tolerance (i.e., high pathogen population in all tissues, ability to tolerate symptoms), and resistance (low pathogen titer in all tissues, ability to limit symptom expression) (Agrios 2005). In the case of *Vitis* genotypes, *X. fastidiosa* subsp. *fastidiosa* infections in resistant cultivars is characterized by a lower bacterial titer between stems and leaves and a reduced expression of symptoms (Krivanek and Walker 2005; Krivanek et al. 2005). It follows that an indication of resistance is the ability to hamper bacteria replication in infected tissues, an ability that is missing in tolerant genotypes. Extensive field studies, in which plants are exposed to multiple vector-mediated infections, showed that cultivar Leccino hosts a lower bacterial titer compared with susceptible cultivars Ogliarola salentina and Cellina di Nardò. Besides field observations, resistance of cultivar Leccino was also demonstrated by single-inoculated greenhouse-grown plants (Saponari et al. 2017), in which either the titer or bacterium movement was reduced in this cultivar.

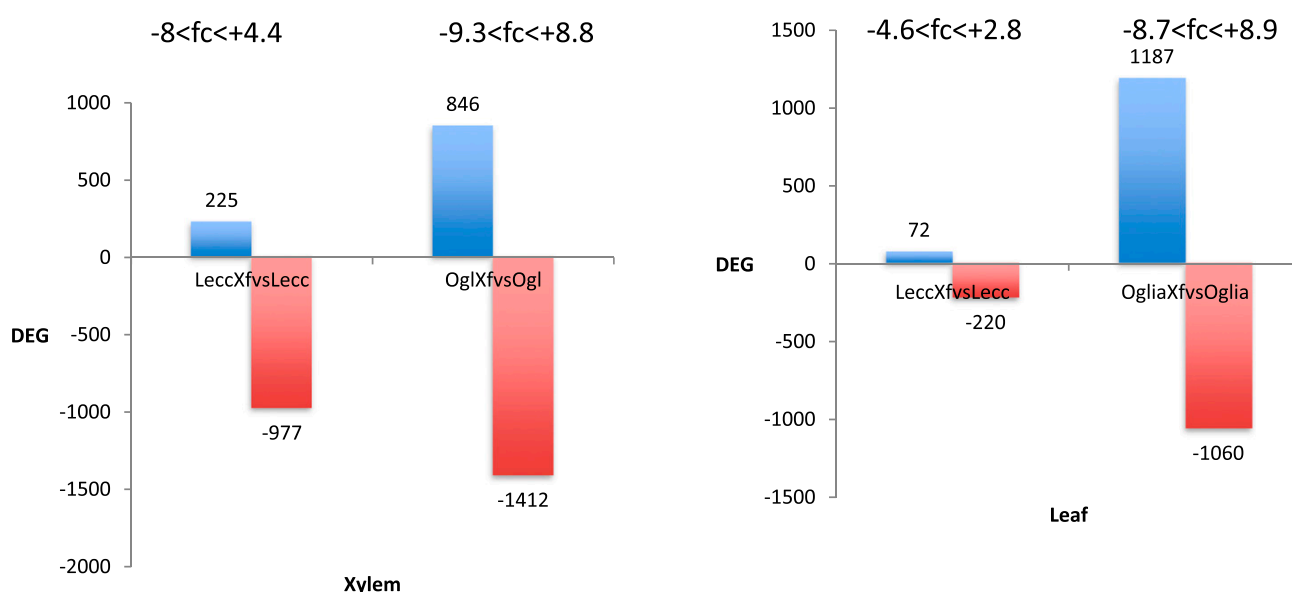


Fig. 4.

Graphs showing the numbers and distribution (up, positive; down, negative) of differentially expressed genes (DEG) in the xylem and leaf tissues. Comparison between infected and healthy plants of the same cultivars are showed. Reads were mapped to the genome of olive cultivar Farga and analyzed as described in Giampetruzzi et al. (2016). Only DEGs whose fold change (fc) is in the range $-2 > \text{fold change} > 2$ are shown. Fold change ranges of each comparison are above the columns.

genes related to calcium metabolism was unraveled between the resistant (Leccino) and susceptible (Ogliarola salentina) following the analysis of the transcriptomes (Giampetruzzi et al. 2016; A. Giampetruzzi, unpublished data). Among these genes, the increased expression of a calcium-dependent protein kinase 1 (CDPK-1) was observed in tissues from symptomatic leaves of the susceptible cultivar Ogliarola salentina, grown in the field as opposed to Leccino, which remains unaltered (D'Attoma et al. 2018). Conversely, the ionome analysis of the same tissues revealed that in cultivar Leccino a 30% increased accumulation of calcium occurs when symptoms are present while the same increase, although observed also in Ogliarola salentina, was not statistically significant. These latter findings, besides the involvement of calcium as a secondary messenger during stress response, are consistent with the observed accumulation of this element in tobacco, blueberry, grapes, and pecan plants infected by *X. fastidiosa* (De La Fuente et al. 2013; Oliver et al. 2014).

The observation that Leccino exhibits traits of resistance to *X. fastidiosa* infection was confirmed by Luvisi et al. (2017) who found that an increase of quinic acid, a lignin precursor, in this cultivar is possibly involved as a defense response. The role of bacterial aggregates in inducing symptoms in susceptible cultivars was suggested by Cardinale et al. (2018) who found that occlusion of petiole vessels has a primary role in pathogenicity. However, De Benedictis et al. (2017) did not find such involvement as their observations were limited to vessels from branches. Conversely, these latter authors found that tyloses and gels mainly occurred in vessels from branches of infected plants and suggested that resistant cultivar Leccino has a better management of this defense response compared with Ogliarola salentina and Cellina di Nardò.

Periodic surveys and monitoring in the epidemic area led to the identification of traits of resistance to *X. fastidiosa* also in the selection FS17 (Boscia et al. 2017a). Like Leccino, lower incidences of infections were detected in FS17 orchards, with infected trees harboring a lower bacterial titer than those of susceptible Ogliarola salentina.

As a long-term management strategy for OQDS, an intense screening program for *X. fastidiosa* resistance was started, combining exposure of the plants to the natural pressure of inoculum in the field, and by artificially inoculating the Apulian strain on potted plants maintained under controlled conditions. Different experimental plots were planted in the infected area to test different olive cultivars and *Olea* spp. originating from different Mediterranean olive-growing areas (<https://www.xfactorsproject.eu/screening-cultivars-resistance-xf/>), with approximately 100 different genotypes currently under evaluation. In parallel, within a grower supported initiative support, more than 400 olive genotypes have been grafted on ancient infected trees of the two local cultivars, with the aim of shortening the incubation period of infection to rapidly determine the relative susceptibility/tolerance in shorter period of time. In addition, this experiment will provide some indications on the possibility to reconstitute the canopy of infected centennial trees by grafting scions of a resistance cultivar. Indeed, surveys are ongoing in the heavily infected area to identify symptomless spontaneous seed-derived plants, which may represent important sources of resistance. All these ongoing experiments require long-term evaluation, although preliminary data confirm that differential susceptibility to *X. fastidiosa* exists in the olive germplasm.

EMERGENCE OF *X. FASTIDIOSA* IN APULIA: MAJOR CHALLENGING CONCERNS

The epidemic of *X. fastidiosa* in Apulia clearly showed that, for this complex pathogen, the prevention strategies relying on quarantine EU legislative provisions and inspections at entry ports, failed to intercept imported infected plants. The subsequent

establishment of field infections in Apulia, somehow follows a predicted scenario that the Mediterranean area is under major risk for *X. fastidiosa* invasion due to a favorable climatic condition (Bosso et al. 2016; Feil and Purcell 2001). In the case of the Apulian outbreak, several factors concurred to the rapid expansion of the infections resulting in the current dramatic epidemic. Among these, the high susceptibility of local olive cultivars to *X. fastidiosa*, the aggressiveness of the Apulian genotype, coupled with the abundant populations of *P. spumarius* and the widespread presence of the most susceptible host, the olives (Strona et al. 2017).

Because the landscape is dominated by centennial olive trees and a significant part of the olive crops is associated with family-based agriculture activities (i.e., small-scale farms), the impact of the epidemic had extreme negative implications not only to olive producers and/or olive industry but has threatened the entire local economy, and the symbolic crop and landscape symbol of this territory. Indeed, the local nursery industry has suffered severe economic impacts as a result of the strict restrictions on the marketing and movement of plants for planting produced in the infected areas. Although, the infections have been found to be confined in the southern part of the Apulian region, with the rest of the national territory declared *X. fastidiosa* free, the whole national nursery industry was affected by the unjustified limitations to importation imposed by some countries concerned about the potential risks of buying plants from Italy. As such, the true economic estimation of the overall damage suffered as a consequence of this *X. fastidiosa* epidemic is overwhelming and difficult to accurately determine.

It is clear that the emergence of *X. fastidiosa* in the region has caused unprecedented turmoil, affecting not only the growers and farmers, but directly or indirectly the local society as a whole. The complex management of the disease needed active involvement of researchers, local authorities, stakeholders, and public, i.e., involvement of specialists/experts or producers but also proactive involvement of citizens, politicians, touristic operators with no specific background in plant pathology or crop production and management.

Currently there is no cure for *X. fastidiosa* infections. The available management strategies aim to limit or eliminate the pathogen by eradication and containment measures, relying on removal of infected hosts to eradicate or reduce the source of inoculum. Historically, eradication programs have been controversial, generating strong public opposition that delay or halt interventions (Vicent and Blasco 2017). The need to remove infected trees in containment and buffer zones, i.e., at the forefront of the infected area, represented one of the main controversial aspect in the management of the Apulian emergence. Olive is a symbolic tree for the region, with emotional and aesthetic values, representing cultural traditions and natural heritage. It is difficult for public opinion to accept extreme action like the removal of century-old trees. The lack of effective communication and educational strategies at the public opinion level led to conspiracy and pseudoscientific theories that questioned the demonstrated role of *X. fastidiosa* as the causal agent of OQDS and the effectiveness of the removal of the infected trees. In this era of social networking, these theories easily gained more consensus than the science-based information, generating strong public opposition that hampered timely interventions and management strategies. Indeed, the area where the outbreak was first discovered is a residential district with high density of housing or touristic residences with private yards, where monitoring and interventions were not easy to carry out.

This scenario was further exacerbated by the lack of cooperation or trust among stakeholders. Although the implementation of any actions for the containment of the epidemic under a crisis scenario complicates a balanced and rational communication among all the parties involved, several critical factors may have contributed to the poor engagement of stakeholders. The lack of a structured plan to

provide farmers with financial support to remove infected trees from their orchards and to provide some financial assistance to counteract the impact of the olive disease was major factor that discouraged their active cooperation. Although it is difficult to monetize the value of an olive trees and, thus, to reach an agreement on an appropriate compensation for the removal of an infected tree, lack of public support for the additional interventions required by the contingency plan (i.e., removal of the weeds in spring for vector control) was another aspect that limited the inclination of the farmers to effectively contribute to the containment plan. Farmers in the infected area where no official monitoring, surveys, and actions are being implemented by the local Phytosanitary Authorities, felt they were left alone to combat this disease, and related social and economic threats. Moreover, the prohibition in the infected area of planting host plants, including plantation of new olive orchards and the absence of any structured plan to reconstruct the agricultural landscape with alternative crops contributed further to dampen farm businesses.

It should be added that difficulties were also encountered in applying containment measures by the public bodies, i.e., control of the vector population in municipalities properties, due to the shortage in public funds. This induced contradictory feelings of local farmers who still received penalties in case of noncompliance with regulatory actions aiming at controlling the vector. In addition, numerous farms in the area are managed under organic regime that makes insecticide use incompatible with organic practices.

On top of all these issues, the long administrative and legal criteria necessary to notifying the grower of the presence of infected trees were somehow conflicting with the need for timely removal of infected sources to limit inoculum and further pathogen spread. On the other hand, olive is by law among the most protected plant species in Italy, implying that removal of the trees requires specific regional administrative authorization.

PARTICIPATORY APPROACHES TO RESEARCH

Credit should be given to several farmers, that regardless of economic and social pressures, significantly contributed to the implementation of the containment program and, most importantly, to strive to search for remedies and to rebuild the agriculture sector in the infected area. One of the most promising research programs relies on the identification of resistant cultivars. A program largely promoted with the efforts, interest, and the forward-looking approaches of some local growers, who teamed up with researchers for developing strategies that may help in the future to reconstruct the olive industry and the landscape in the affected area.

Despite the challenges described in the previous paragraph, international research programs have been effectively set up by the local research institutions. The century-long experience gained by research teams in the Americas on the management of *X. fastidiosa* induced diseases on grapes and citrus has been critical to foster relevant research in the EU, and, in particular, to reach several research milestones on the epidemic of *X. fastidiosa* in Apulia. Although research-based criteria have reduced the uncertainties and helped to devise sound containment measures, these should be further implemented along with long-term research programs that ensure continuity, discovery of sustainable control mechanisms, and transfer these research technologies to field application.

The outbreak in Apulia has had a negative cascade of effects on the entire society. Therefore, active interactions among plant pathologists, epidemiologists, entomologists, economists, and social scientists should be strengthened to improve the efficacy of the containment programs (Vicent and Blasco 2017). On the other side, to tackle such complex scenario the processes for the implementation of the legislative provisions should be contextualized in relation to the fast-evolving circumstances, ensuring priority for the management of the plant health emergency.

The case of the Apulian outbreak and the issues discussed above were recently analyzed elsewhere (Almeida 2018) in which two points were emphasized in terms of implementing an effective disease management plan: (i) to consider and address the concerns and the priorities of diverse stakeholders (farmers, governmental agencies, public, and politicians), so that the social context of epidemics is amalgamated in the process of plant disease management; and (ii) although challenging, scientists should make efforts to communicate science to a broad range of stakeholders outside the scientific communities.

ACKNOWLEDGMENTS

We thank the researchers of the Horizon 2020 consortia “PONTE—Pest Organisms Threatening Europe-635646” and “XF-ACTORS—*Xylella fastidiosa* Active Containment Through a Multidisciplinary-Oriented Research Strategy-727987” for their scientific collaborations; and E. Manni, G. Melcarne, and F. Specchia and the associates of APROL for support in the field experiments.

LITERATURE CITED

- Agrios, G. N. 2005. Plant Pathology, Fifth Edition. Elsevier Academic Press, New York.
- Almeida, R. P. P. 2018. Emerging plant disease epidemics: Biological research is key but not enough. *PLoS Biol.* 16:e2007020.
- Ben Moussa, I. E., Mazzoni, V., Valentini, F., Yaseen, T., Lorusso, D., Speranza, S., Digiario, M., Varvaro, L., Krugner, R., and D'Onghia, A. M. 2016. Seasonal fluctuations of sap-feeding insect species infected by *Xylella fastidiosa* in Apulian olive groves of Southern Italy. *J. Econ. Entomol.* 109:1512-1518.
- Boscia, D., Altamura, G., Ciniero, A., Di Carolo, M., Dongiovanni, C., Fumarola, G., Giampetruzzi, A., Greco, P., La Notte, P., Loconsole, G., Manni, F., Melcarne, G., Montilon, V., Morelli, M., Murrone, N., Palmisano, F., Pollastro, P., Potere, O., Roseti, V., Saldarelli, P., Saponari, A., Saponari, M., Savino, V. N., Silletti, M. R., Specchia, F., Susca, L., Tauro, D., Tavano, D., Venerito, P., Zicca, S., and Martelli, G. P. 2017a. Resistenza a *Xylella fastidiosa* in diverse cultivar di olivo. *L'Informatore Agrario* 11:59-63.
- Boscia, D., Altamura, G., Saponari, M., Tavano, D., Zicca, S., Pollastro, P., Silletti, M. R., Savino V. N., Martelli, G. P., Delle Donne, A., Mazzotta, S., Signore, P. P., Trisi, M., Drazza, P., Conte, P., D'Ostuni, V., Merico S., Perrone, G., Specchia, F., Stanca, A., and Tanieli, M. 2017b. Incidenza di *Xylella* in oliveti con disseccamento rapido. *Inform. Agrario* 27:59-64, 47-50.
- Bosso, L., Di Febraro, M., Cristinzio, G., Zoia, A., and Russo, D. 2016. Shedding light on the effects of climate change on the potential distribution of *Xylella fastidiosa* in the Mediterranean Basin. *Biol. Invasions* 18: 1759-1768.
- Cardinale, M., Luvisi, A., Meyer, J. B., Sabella, E., De Bellis, L., Cruz, A. C., Ampatzidis, Y., and Cherubini, P. 2018. Specific fluorescence *in situ* hybridization (FISH) test to highlight colonization of xylem vessels by *Xylella fastidiosa* in naturally infected olive trees (*Olea europaea* L.). *Front. Plant Sci.* 9:431.
- Cavaliere, V., Dongiovanni, C., Tauro, D., Altamura, G., Di Carolo, M., Fumarola, G., Saponari, M., and Bosco, D. 2018. Transmission of the CODIRO strain of *Xylella fastidiosa* by different insect species. In Proceedings, XI European Congress of Entomology, Naples, Italy.
- Choi, H. K., Iandolino, A., da Silva, F. G., Cook, D. R. 2013. Water deficit modulates the response of *Vitis vinifera* to the Pierce's disease pathogen *Xylella fastidiosa*. *Mol. Plant-Microbe Interact.* 26:643-657.
- Coletta-Filho, H., Francisco, C. S., Lopes, J. R. S., De Oliveira, A. F., and Da Silva, L. F. D. O. 2016. First report of olive leaf scorch in Brazil, associated with *Xylella fastidiosa* subsp. *pauca*. *Phytopathol. Mediterr.* 55: 130-135.
- Coletta-Filho, H., Pereira, E. O., Souza, A. A., Takita, M. A., Cristofani-Yale, M., and Machado, M. A. 2007. Analysis of resistance to *Xylella fastidiosa* within a hybrid population of Pera sweet orange × Murcott tangor. *Plant Pathol.* 56:661-668.
- Cornara, D., Bosco, D., and Fereres, A. 2018. *Philaenus spumarius*: When an old acquaintance becomes a new threat to European agriculture. *J. Pest Sci.* 91:957-972.
- Cornara, D., Cavaliere, V., Dongiovanni, C., Altamura, G., Palmisano, F., Bosco, D., Porcelli, F., Almeida, R. P. P., and Saponari, M. 2017a. Transmission of *Xylella fastidiosa* by naturally infected *Philaenus spumarius*

- (Hemiptera, Aphrophoridae) to different host plants. *J. Appl. Entomol.* 141: 80-87.
- Cornara, D., Saponari, M., Zeilinger, A. R., de Stradis, A., Boscia, D., Loconsole, G., Bosco, D., Martelli, G. P., Almeida, R. P. P., and Porcelli, F. 2017b. Spittlebugs as vectors of *Xylella fastidiosa* in olive orchards in Italy. *J. Pest Sci.* 90:521-530.
- Cruz, F., Julca, I., Gómez-Garrido, J., Loska, D., Marcet-Houben, M., Cano, E., Galán, B., Frias, L., Ribeca, P., Derdak, S., Gut, M., Sánchez-Fernández, M., García, J. L., Gut, I. G., Vargas, P., Alioto, T. S., and Gabaldón, T. 2016. Genome sequence of the olive tree, *Olea europaea*. *Gigascience* 5:29.
- D'Attoma, G., De La Fuente, L., Saldarelli, P., Abou Kubaa, R., Morelli, M., Giampetruzzi, A., Boscia, D., Savino, V. N., and Cobine, P. 2018. Evaluation of the effect of *Xylella fastidiosa* on leaf ionome and calcium-related gene expression profiles of infected olive trees. Abstracts of the 6th Xanthomonas genomics conference and 2nd Annual Euroxanth conference, Halle, Germany.
- Daugherty, M. P., and Almeida, R. P. P. 2009. Estimating *Xylella fastidiosa* transmission parameters: decoupling sharpshooter number and feeding period. *Entomol. Exp. Appl.* 132:84-92.
- De Benedictis, M., De Caroli, M., Baccelli, I., Marchi, G., Bleve, G., Gallo, A., Ranaldi, F., Falco, V., Pasquali, V., Piro, G., Mita, G., and Di Sansebastiano, G. P. 2017. Vessel occlusion in three cultivars of *Olea europaea* naturally exposed to *Xylella fastidiosa* in open field. *J. Phytopathol.* 165:589-594.
- De La Fuente, L., Parker, J. K., Oliver, J. E., Granger, S., Brannen, P. M., van Santen, E., and Cobine, P. A. 2013. The bacterial pathogen *Xylella fastidiosa* affects the leaf ionome of plant hosts during infection. *PLoS One* 8:e62945.
- De Souza, A. A., Takita, M. A., Rodrigues, C. M., Coletta-Filho, H., Giampetruzzi, A., Morelli, M., Saponari, M., Boscia, D., and Saldarelli, P. 2017. Conserved genetic defense response against *X. fastidiosa* subsp. *pauca* in olive and citrus. Page 46 in: Abstract Book, European Conference on Xylella 2017: Finding answers to a global problem, Palma de Mallorca. <https://www.efsa.europa.eu/en/events/event/171113>
- Dongiovanni, C., Altamura, G., Di Carolo, M., Fumarola, G., Saponari, M., and Cavalieri, V. 2018. Evaluation of efficacy of different insecticides against *Philaenus spumarius* L., vector of *Xylella fastidiosa* in olive orchards in Southern Italy, 2015-17. *Arthropod Manage. Tests* 43:1-4.
- Dongiovanni, C., Cavalieri, V., Bodino, N., Taurò, D., Di Carolo, M., Fumarola, G., Altamura, G., Lasorella, C., and Bosco, D. Plant selection and population trend of spittlebug immatures (Hemiptera: Aphrophoridae) in olive groves of the Apulia Region of Italy. *J. Econ. Entomol.* (in press). doi.org/10.1093/jee/toy289
- EFSA (European Food Safety Authority). 2015. Response to scientific and technical information provided by an NGO on *Xylella fastidiosa*. *EFSA J.* 13:4082.
- EFSA PLH Panel (EFSA Panel on Plant Health), Jeger, M., Caffier, D., Candresse, T., Chatzivassiliou, E., Dehnen-Schmutz, K., Gilioli, G., Gregoire, J. C., Jaques Miret, J. A., MacLeod, A., Navajas Navarro, M., Niere, B., Parnell, S., Potting, R., Rafoss, T., Rossi, V., Ürek, G., Van Bruggen, A., Van der Werf, W., West, J., Winter, S., Almeida, R., Bosco, D., Jacques, M. A., Landa, B., Purcell, A., Saponari, M., Czwieneczek, E., Delbianco, A., Stancanelli, G., and Bragard, C. 2018. Scientific opinion on the updated pest categorisation of *Xylella fastidiosa*. *EFSA J.* 16:5357.
- Elbeaino, T., Valentini, F., Abou Kubaa, R., Moubarak, P., Yaseen, T., and Digiaro, M. 2014. Multilocus sequence typing of *Xylella fastidiosa* isolated from olive affected by "olive quick decline syndrome" in Italy. *Phytopathol. Mediterr.* 53:533-542.
- EPPO (European and Mediterranean Plant Protection Organization). 2018. PM 7/24 (3) *Xylella fastidiosa*. *OEPP/EPPO Bulletin* 48:175-218.
- EPPO (European Plant Protection Organization). 2004. PM 7/24 (1) *Xylella fastidiosa*. *OEPP/EPPO Bulletin* 34:187-192.
- FAO. 2016. ISPM 5: Glossary of phytosanitary terms. International Standards for Phytosanitary Measures. http://www.fao.org/fileadmin/user_upload/faoterm/PDF/ISPM_05_2016_En_2017-05-25_PostCPM12_InkAm.pdf
- Feil, H., and Purcell, A. H. 2001. Temperature-dependent growth and survival of *Xylella fastidiosa* in vitro and in potted grapevines. *Plant Dis.* 85:1230-1234.
- Firrao, G., and Bazzi, C. 1994. Specific identification of *Xylella fastidiosa* using the polymerase chain reaction. *Phytopathol. Mediterr.* 33:90-92.
- Francis, M., Lin, H., Cabrera-La Rosa, J., Doddapaneni, H., and Civerolo, E. L. 2006. Genome-based PCR primers for specific and sensitive detection and quantification of *Xylella fastidiosa*. *Eur. J. Plant Pathol.* 115:203-213.
- Giampetruzzi, A., Chiumenti, M., Saponari, M., Donvito, G., Italiano, A., Loconsole, G., Boscia, D., Cariddi, C., Martelli, G. P., and Saldarelli, P. 2015. Draft genome sequence of the *Xylella fastidiosa* CoDiRO strain. *Genome Ann.* 3:e01538-e14.
- Giampetruzzi, A., Morelli, M., Saponari, M., Loconsole, G., Chiumenti, M., Boscia, D., Savino, V., Martelli, G. P., and Saldarelli, P. 2016. Transcriptome profiling of two olive cultivars in response to infection by the CoDiRO strain of *Xylella fastidiosa* subsp. *pauca*. *BMC Genomics* 17:475.
- Giampetruzzi, A., Saponari, M., Almeida, R. P. P., Essakhi, S., Boscia, D., Loconsole, G., and Saldarelli, P. 2017a. Complete genome sequence of the olive-infecting strain *Xylella fastidiosa* subsp. *pauca* De Donno. *Genome Ann.* 5:e00569-e17.
- Giampetruzzi, A., Saponari, M., Loconsole, G., Boscia, D., Savino, V. N., Almeida, R. P. P., Zicca, S., Landa, B. B., Chacón-Díaz, C., and Saldarelli, P. 2017b. Genome-wide analysis provides evidence on the genetic relatedness of the emergent *Xylella fastidiosa* genotype in Italy to isolates from Central America. *Phytopathology* 107:816-827.
- Guilhabert, M. R., and Kirkpatrick, B. C. 2005. Identification of *Xylella fastidiosa* antivirulence genes: Hemagglutinin adhesins contribute a biofilm maturation to *X. fastidiosa* and colonization and attenuate virulence. *Mol. Plant-Microbe Interact.* 18:856-868.
- Haelterman, R. M., Tolocka, P. A., Roca, M. E., Guzman, F. A., Fernandez, F. D., and Otero, M. L. 2015. First presumptive diagnosis of *Xylella fastidiosa* causing olive scorch in Argentina. *J. Plant Pathol.* 97:393.
- Harper, S. J., Ward, L. I., and Clover, G. R. G. 2010. Development of LAMP and real-time PCR methods for the rapid detection of *Xylella fastidiosa* for quarantine and field applications. *Phytopathology* 100:1282-1288.
- Krivanek, A. F., Stevenson, J. F., and Walker, M. A. 2005. Development and comparison of symptom indices for quantifying grapevine resistance to Pierce's disease. *Phytopathology* 95:36-43.
- Krivanek, A. F., and Walker, M. A. 2005. *Vitis* resistance to Pierce's disease is characterized by differential *Xylella fastidiosa* populations in stems and leaves. *Phytopathology* 95:44-52.
- Li, W., Teixeira, D. C., Hartung, J. S., Huang, Q., Duan, Y., Zhou, L., Chen, J., Lin, H., Lopes, S., Ayres, A. J., and Levy, L. 2013. Development and systematic validation of qPCR assays for rapid and reliable differentiation of *Xylella fastidiosa* strains causing citrus variegated chlorosis. *J. Microbiol. Methods* 92:79-89.
- Loconsole, G., Potere, O., Boscia, D., Altamura, G., Djelouah, K., Elbeaino, T., Frasher, D., Lorusso, D., Palmisano, F., Pollastro, P., Silletti, M. R., Trisciuzzi, N., Valentini, F., Savino, V. N., and Saponari, M. 2014. Detection of *Xylella fastidiosa* in olive trees by molecular and serological methods. *Phytopathol. Mediterr.* 53:7-14.
- Loconsole, G., Saponari, M., Boscia, D., D'Attoma, G., Morelli, M., Martelli, G., and Almeida, R. P. P. 2016. Intercepted isolates of *Xylella fastidiosa* in Europe reveal novel genetic diversity. *Eur. J. Plant Pathol.* 146:85-94.
- Luvisi, A., Aprile, A., Sabella, E., Vergine, M., Nicoli, F., Nutricati, E., Miceli, A., Negro, C., and de Bellis, L. 2017. *Xylella fastidiosa* subsp. *pauca* (CoDiRO strain) infection in four olive (*Olea europaea* L.) cultivars: Role of phenolic compounds in leaves and progression of leaf scorch symptoms. *Phytopathol. Mediterr.* 56:259-273.
- Martelli, G. P. 2015. The current status of the quick decline syndrome of olive in southern Italy. *Phytoparasitica* 44:1-10.
- Martelli, G. P., Boscia, D., Porcelli, F., and Saponari, M. 2016. The olive quick decline syndrome in south-east Italy: A threatening phytosanitary emergency. *Eur. J. Plant Pathol.* 144:235-243.
- Montes-Borrego, M., Boscia, D., Landa, B. B., Saponari, M., and Navas-Cortes, J. A. 2017. Spatial and temporal dynamics of olive quick decline syndrome in orchards in Puglia, southern Italy. Page 27 in: Abstract Book, European Conference on Xylella 2017: Finding answers to a global problem, Palma de Mallorca. <https://www.efsa.europa.eu/en/events/event/171113>
- Muzzalupo, I., and Perri, E. 2009. Genetic diversity in olive tree cultivars from Italy and other countries of the Mediterranean Basin as revealed by RAPD and SSR molecular marker. *Adv. Hortic. Sci.* 23:263-275.
- Nigro, F., Boscia, D., Antelmi, L., and Ippolito, A. 2013. Fungal species associated with a severe decline of olive in Southern Italy. *J. Plant Pathol.* 95:668.
- Nunney, L., Ortiz, B., Russell, S. A., Sánchez, R. R., and Stouthamer, R. 2014. The complex biogeography of the plant pathogen *Xylella fastidiosa*: Genetic evidence of introductions and subspecific introgression in Central America. *PLoS One* 9:e112463.
- Oliver, J. E., Sefick, S. A., Parker, J. K., Arnold, T., Cobine, P. A., and De La Fuente, L. 2014. Ionome changes in *Xylella fastidiosa*-infected *Nicotiana tabacum* correlate with virulence and discriminate between subspecies of bacterial isolates. *Mol. Plant-Microbe Interact.* 27:1048-1058.
- Plazio, E., Bodino, N., Cavalieri, V., Dongiovanni, E., Fumarola, G., Ciniero, A., Galetto, L., Saponari, M., and Bosco, D. 2017. Investigations on dispersal capability of *Philaenus spumarius* by marker-release-recapture method. Page 56 in: Abstract Book, European Conference on Xylella 2017: Finding answers to a global problem, Palma de Mallorca. <https://www.efsa.europa.eu/en/events/event/171113>
- Potere, O., Susca, L., Loconsole, G., Saponari, M., Boscia, D., Savino, V. N., and Martelli, G. P. 2015. Survey for the presence of *Xylella fastidiosa*

Q:3

- subsp. *pauca* strain CoDiRO in some forestry and ornamental species in the Salento peninsula. *J. Plant Pathol.* 97:373-376.
- Rapicavoli, J. N., Blanco-Ulate, B., Muszyński, A., Figueroa-Balderas, R., Morales-Cruz, A., Azadi, P., Dobruchowska, J. M., Castro, C., Cantu, D., and Roper, M. C. 2018. Lipopolysaccharide O-antigen delays plant innate immune recognition of *Xylella fastidiosa*. *Nature Comm.* 9:390.
- Rodrigues, C. M., De Souza, A. A., Takita, M. A., Kishi, L. T., and Machado, M. A. 2013. RNA-Seq analysis of *Citrus reticulata* in the early stages of *Xylella fastidiosa* infection reveals auxin-related genes as a defense response. *BMC Genomics* 14:676.
- Rogers, E. E., and Stenger, D. C. 2012. A conjugative 38 kB plasmid is present in multiple subspecies of *Xylella fastidiosa*. *PLoS One* 7:e52131.
- Saponari, M., Boscia, D., Altamura, G., Loconsole, G., Zicca, S., D'Attoma, G., Morelli, M., Palmisano, F., Saponari, A., Tavano, D., Savino, V. N., Dongiovanni, C., and Martelli, G. P. 2017. Isolation and pathogenicity of *Xylella fastidiosa* associated to the olive quick decline syndrome in southern Italy. *Sci. Rep.* 7:17723.
- Saponari, M., Boscia, D., Nigro, F., and Martelli, G. P. 2013. Identification of DNA sequences related to *Xylella fastidiosa* in oleander, almond and olive trees exhibiting leaf scorch symptoms in Apulia (Southern Italy). *Disease Note. J. Plant Pathol.* 94:688.
- Saponari, M., Loconsole, G., Cornara, D., Yokomi, R. K., De Stradis, A., Boscia, D., Bosco, D., Martelli, G. P., Krugner, R., and Porcelli, F. 2014. Infectivity and transmission of *Xylella fastidiosa* by *Philaenus spumarius* (Hemiptera: Aphrophoridae) in Apulia, Italy. *J. Econ. Entomol.* 107:1316-1319.
- Sicard, A., Saponari, M., Vanhove, M., Essakhi, S., Giampetruzzi, A., Saldarelli, P., Boscia, D., and Almeida, R. P. P. 2017. Identifying *Xylella fastidiosa* host adaptation candidate genes: the case of *X. fastidiosa* subsp. *pauca* isolates and olive trees in Italy. Page 43 in: Abstract Book, European Conference on *Xylella* 2017: Finding answers to a global problem, Palma de Mallorca. <https://www.efsa.europa.eu/en/events/event/171113>
- Strona, G., Carstens, C. J., and Beck, P. S. A. 2017. Network analysis reveals why *Xylella fastidiosa* will persist in Europe. *Sci. Rep.* 7:71.
- ~~Tolocka, P. A., Mattio, M. F., Oter, M. L., Paccioretti, M. A., Roca, M. E., Canteros, B. I., Guzmán, F. A., and Hacherman, R. M. 2017. Characterization of *X. fastidiosa* from different hosts in Argentina. Page 44 in: Abstract Book, European Conference on *Xylella* 2017: Finding answers to a global problem, Palma de Mallorca. <https://www.efsa.europa.eu/en/events/event/171113>~~
- Vicent, A., and Blasco, J. 2017. When prevention fails. Towards more efficient strategies for plant disease eradication. *New Phytol.* 214:905-908.
- White, S. M., Bullock, J. M., Hooftman, D. A. P., and Chapman, D. S. 2017. Modelling the spread and control of *Xylella fastidiosa* in the early stages of invasion in Apulia, Italy. *Biol. Invasions* 19:1825-1837.
- Zaini, P. A., Nascimento, R., Gouran, H., Cantu, D., Chakraborty, S., Phu, M., Goulart, L. R., and Dandekar, A. M. 2018. Molecular profiling of Pierce's disease outlines the response circuitry of *Vitis vinifera* to *Xylella fastidiosa*. *Inf. Front. Plant Sci.* 9:771.
- Zarco-Tejada, P. J., Camino, C., Beck, P. S. A., Calderon, R., Hornero, A., Hernández-Clemente, R., Kattenborn, T., Montes-Borrego, M., Susca, L., Morelli, M., Gonzalez-Dugo, V., North, P. R. J., Landa, B. B., Boscia, D., Saponari, M., and Navas-Cortes, J. A. 2018. Previsual symptoms of *Xylella fastidiosa* infection revealed in spectral plant-trait alterations. *Nature Plants* 4:432-439.

Q:4

Q:5

Proof Only