

The current status of the quick decline syndrome of olive in southern Italy

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Abstract The quick decline syndrome of olive (OQDS) is a disease that appeared all of a sudden some years ago in a restricted area near the city of Gallipoli (Ionian coast of the Salento peninsula, southern-east Italy) and began spreading through the heavily olive-grown countryside of lower Salento. *Xylella fastidiosa*, a quarantine pathogen of American origin previously undetected in the European Union territory, except for two unconfirmed records from Kosovo and Turkey, proved to be consistently associated with symptomatic trees. *X. fastidiosa* is a Gram-negative bacterium that invades and multiplies in the xylem vessels of infected hosts, from which it is acquired by xylem-feeding insect vectors (belonging to Auchenorrhyncha, including cicadellids sharpshooter leafhoppers group, Cicadellidae, Cicadellinae), and aphrophorids (cercopids and spittlebugs, Cercopidae) and transferred to other plants. The Salentian strain of *X. fastidiosa*, denoted CoDiRO, was obtained in axenic culture. Its genome, a DNA molecule *ca.* 2.5 million base-pairs in size, was sequenced and identified as a genotype of *X. fastidiosa* subsp. *pauca* molecularly identical to an isolate of the same subspecies from Costa Rica. In nature, strain CoDiRO infects a number of woody and shrubby hosts but not grapevines and citrus and is mainly transmitted by *Philaenus spumarius* (meadow spittlebug), a froghopper quite common in the Salento area where it thrives primarily on olive.

Since OQDS eradication and sanitation of infected olives are unfeasible, strategies have been envisaged for restraining the spread of the pathogen and its vector within the boundaries of the currently infected zone.

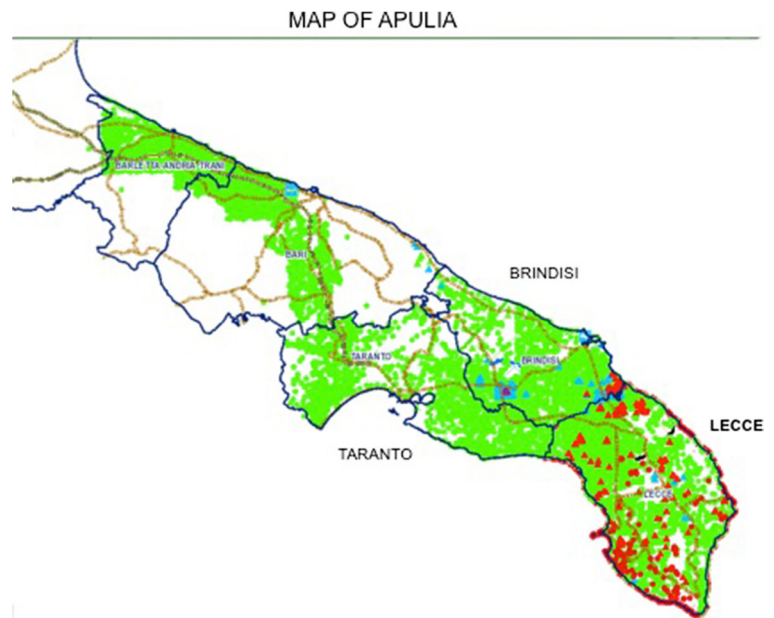
Keywords *Xylella fastidiosa* subspecies *pauca* · leaf scorch · spittlebugs · *Philaenus spumarius* · epidemiology · disease management

Introduction

Towards the end of the first decade of the year 2000, a disease of olive (*Olea europaea* L.) denoted quick decline syndrome (OQDS) appeared suddenly in a restricted area near the city of Gallipoli (Ionian coast of the Salento peninsula, south-east Italy), and began spreading fast. In October 2013, when systematic investigations for determining its aetiology were initiated, the affected acreage was estimated at *ca.* 8,000 ha. Currently (autumn 2015), many of the olive groves in an area of the province of Lecce of *ca.* 2,300km² are diseased. A year ago the infected surface area given over strictly to olive (i.e. the totality of symptomatic groves taken together) was estimated to *ca.* 10,000 ha, comprising about 1,000,000 trees. These already huge figures have grown in the meantime, because many previously scattered infection foci have coalesced, and the disease continues to expand. In fact, OQDS was confined to the province of Lecce up to a few months ago (Fig. 1), when new infection sites appeared in the neighbouring province of Brindisi, one of which some 70 Km north of the

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Fig. 1 Map of Apulia (south-east of Italy) showing that the present distribution of *Xylella fastidiosa* infections (red spots) is still largely limited to the province of Lecce. The green areas are still Xf-free, according to laboratory tests



Lecce's closest foci. This represented the first alarming evidence that long-distance spreading of the disease can take place, likely because of passive transport of infective vectors.

The disease and its putative agent

The outward manifestations of OQDS consist of scattered desiccation of twigs and small branches that show first on the upper part of the crown. These symptoms extend to the rest of the canopy conferring upon it a burned appearance (Fig. 2A). Tissue desiccation starts at the tip of the leaves and progresses towards the petiole, soon extending to the whole blade. Dead leaves remain attached to the twigs and are shed, following winter rains. These symptoms recall those of severe attacks of "brusca" (olive scorch), a recurrent long-known physiological disorder (Sanzani *et al.* 2012) reported since the end of the 18th Century in the same areas where OQDS is expanding (reviewed by Frisullo *et al.* 2014). Thus, "brusca" was included among the causes, i.e. phytotoxicity due to pollution of the ground water, severe attacks of *Colletotrichum* spp. (the agent of olive anthracnose), poor management of the groves, heavy infestations of the lepidopteron *Zeuzera pyrina* (leopard moth), that were tentatively identified by local growers and farm advisors as the possible elicitors of OQDS. None of these, however, kills the plants,

contrary to OQDS whose attacks culminate with the death of the trees within a few years from the onset of symptoms.

The most severely and impressively affected olives are the centuries-old trees of the locally grown highly susceptible cultivars Cellina di Nardò and Ogliarola salentina, which the growers unsuccessfully try to save through a drastic rejuvenation pruning to stimulate new growth. In fact, the new vegetation pushed by these skeletal-looking trees will soon wither and desiccate (Fig. 2B). These trees show also a variously extended browning of the sapwood of branches and trunks associated with the presence of fungal species of the genera *Phaeoacremonium*, *Phaemoniella*, *Pleurostomophora* and *Neofusicoccum* (Nigro *et al.* 2013, 2014), whose penetration is favoured by the leopard moth galleries.

The overall aspect of OQDS and the modality of its spreading recalled very much the outcome of *Xylella fastidiosa* (*Xf*) infections to fruit and shade trees as described in the North American literature. Hence, samples from different plants with leaf scorch (almond, oleander and olive) collected in different sites of the OQDS-affected area were tested with *Xf*-specific serological and molecular reagents. These assays disclosed the presence of the bacterium (Saponari *et al.* 2013).

Xf is a Gram-negative gamma-proteobacterium in the family Xanthomonadaceae whose cells are rod-shaped,

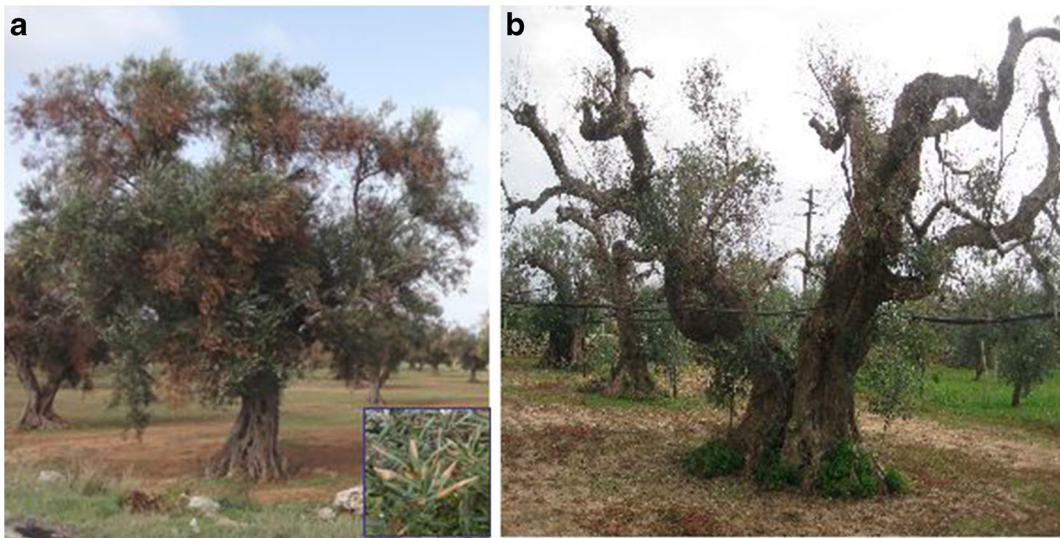


Fig. 2 **A** Quick decline symptoms shown by aged olive tree infected by *Xylella fastidiosa*. Inset shows a detail of leaf scorch. **B** Final stage of quick decline. The new growth pushed by an infected tree following heavy pruning is desiccated

1.0–4.0 x 0.25–0.50 mm in size, show a characteristically rippled cell wall and are deprived of flagella (Wells *et al.* 1987). The bacterium localizes in the xylem vessels of the host plants from where it is acquired by xylem-feeding insects that transfer it to other susceptible plants. Colonization of tracheids results from migration of the bacterial cells from a vessel to another through pit membranes. When the intravascular movement is active *Xf* multiplies, its colonies clog the vessels and impair water uptake, which gives rise to the symptoms (leaf scorch and desiccation of twigs and branches) shown by most of the infected hosts.

Xf, a much feared quarantine pathogen included in the EPPO A1 list (Smith *et al.* 1997), had never been reported in the Old World, except for two unconfirmed records from Kosovo in grapevines (Berisha *et al.* 1998) and Turkey in almond (Güldür *et al.* 2005). More recently, however, epidemics broke in southern Italy, caused by a strain belonging to subsp. *pauca* (Saponari *et al.* 2013), Iran, subsp. *multiplex* (Amanifar *et al.* 2014) and France, subsp. *multiplex* (Corsica and French Riviera) (EPPO 2015; Le Monde, October 13th 2015). In Iran crops affected by subsp. *multiplex* are grapevines and almond. In Corsica, well over 100 different infection foci have already been recorded, with the highest concentration in the south-west of the island (<http://www.corse-du-sud.gouv.fr>). The subsp. *multiplex* hosts are *Polygala myrtifolia*, the most frequently affected species, and a few plants of *Spartium junceum*, *Pelargonium* sp., *Citrus racemosus*,

Acer pseudoplatanus, *Genista ephedroides*, *Hebe* sp., *Lavandula dendata*, *Myrtus communis* and *Rosmarinus officinalis* (<http://www.draaf.paca.agriculture.gouv.fr>). *P. myrtifolia* is the only infected species in the single *Xf* subsp. *multiplex* outbreak detected in continental France.

By contrast, a most valuable agricultural crop like olive is the main subject of *Xf* infection in Italy, a finding that raised much concern, due the heavy damage suffered by infected trees and the risk that the contagion could extend to the rest of the country and elsewhere. Thus, studies were promptly initiated for a better understanding of the role in the OQDS aetiology and epidemiology of the local *Xf* subsp. *pauca* strain, which was denoted CoDiRO (abbreviation from the Italian name "Complesso del disseccamento rapido dell'olivo").

Priority was given to the finalization of sensitive and specific detection methods to be used for large scale surveys of the olive groves of south Salento where OQDS was spreading, and for the identification of alternative hosts that could have a bearing in *Xf* establishment in the environment. The *Xf* detection threshold in olive tissue extracts by enzyme-linked immunosorbent assay (ELISA) and conventional polymerase chain reaction (PCR) was up to a dilution of 10^{-5} , whereas quantitative real-time PCR was 100 fold more sensitive than either method (Loconsole *et al.* 2014a, b). Direct tissue blot immunoassay (DTBIA) and Real time loop-mediated isothermal amplification polymerase chain reaction (LAMP PCR) proved also useful for *Xf* detection

in olive twigs and insect vectors, respectively (Djelouah *et al.* 2014; D'Onghia *et al.* 2014). The extensive use of these techniques and the visual inspection of several thousand olive trees carried out in the last couple of years has allowed a better appraisal of the aetiology of OQDS as they disclosed that: (i) *Xf* is consistently detected in all symptomatic trees, regardless of their age; (ii) the xylem-inhabiting fungi occur almost exclusively in ancient trees, some of which display also leopard moth galleries. This suggests that whereas leopard moth infestations are of little consequence in determining OQDS, the fungi, when present, act as aggravators of the decline condition, whose origin rests primarily on the presence of *Xf*.

To prove this likelihood, artificial infections of olive seedlings and rooted cuttings were performed by prick-inoculating colonies of the CoDiRO strain obtained in axenic culture from several naturally infected hosts (almond, oleander, olive, sweet cherry, *Polygala myrtifolia* and *Wistaria fruticosa* (Cariddi *et al.* 2014; Saponari *et al.* 2014a). These colonies resemble those of *Xf*, look the same regardless of the host of origin, and comprise bacterial cells morphologically comparable to those described in the literature (Fig. 3A,B). All inoculated plants were successfully infected and the bacterium successfully re-isolated. Some of them are reacting with apical leaf scorch (Fig. 3C).

Taxonomic position of the CoDiRO strain

Xf is a single species, within which, based on DNA relatedness and multilocus sequence typing (MLST), four subspecies had been identified, i.e. *Xf* subsp. *fastidiosa*, *Xf multiplex*, *Xf sandyi*, and *Xf pauca* (Almeida *et al.* 2014). The existence of a fifth

subspecies denoted *Xf morus* (a recombinant of *Xf multiplex* and *Xf fastidiosa*) has now been proposed, and an additional unclassified genotype (Taiwanese *Xylella*) has been found in Taiwan (Almeida and Nunney 2015).

Xf subspecies have a diverse geographical origin and a partially different host range (Table 1). Olive hosts two different subspecies, i.e. *multiplex* in California (Krugner *et al.* 2014) and *pauca* in Argentina (Haelterman *et al.* 2015) and Brazil (H. Coletta Filho, personal communication). Symptoms described from the latter countries resemble very much those of the Salentinian OQDS. A MLST (Maiden *et al.* 1998) of bacteria from strain CoDiRO colonies disclosed that this strain is genetically homogeneous and belongs to subspecies *pauca*, of which, however, it represents a variant classified as "sequence type 53" (ST53) (Elbeaino *et al.* 2014; Loconsole *et al.* 2014c), which is molecularly identical to a strain from Costa Rica (Nunney *et al.* 2014). Sequencing of the entire genome of strain CoDiRO, a DNA molecule of 2,460,000 bp (Giampetruzzi *et al.* 2015), confirmed this taxonomic allocation.

Multiple *Xf* introductions into Europe, mostly with ornamental plants, have apparently taken place over time. The recent interception in The Netherlands of a number of coffee plant consignments (*Coffea arabica*) from Costa Rica and Honduras infected by *Xf* (EFSA 2015a) and also the interceptions in France of coffee (*C. arabica*) imported from Ecuador (subsp. *pauca*) and coffee (*C. canephora*) imported from Mexico (subsp. *fastidiosa*). (see EPPO 2015; Legendre *et al.* 2014) supports the notion that Central America is the area from where the bacterium has entered Italy.

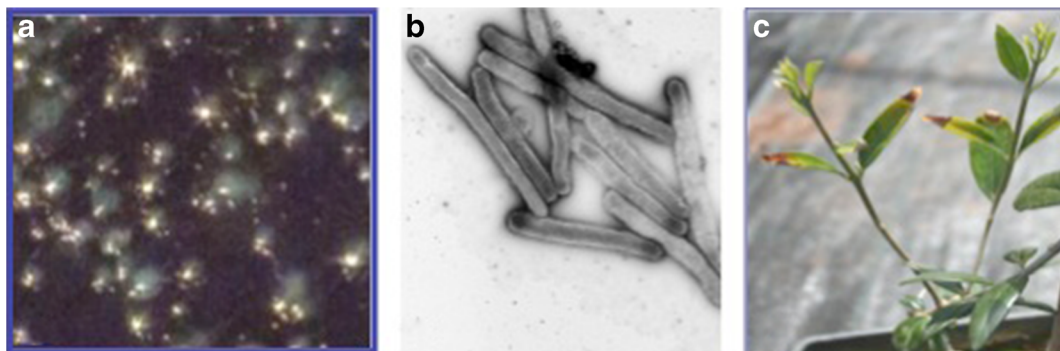


Fig. 3 **A** Colonies of the Salentinian strain of *Xylella fastidiosa* in axenic culture. **B** Bacterial cells from a pure culture. **C** Apical scorching on the leaves of an olive rooted cutting prick-inoculated with a bacterial culture

Table 1 Subspecies of *Xylella fastidiosa*, their origin (see Almeida and Nunney 2015) and main hosts

Subspecies	Origin	Main hosts
<i>Xylella fastidiosa fastidiosa</i>	Central America	Grapevine (North and Central Americas, Peru, Iran, Taiwan), Almond (USA, Argentina)
<i>Xylella fastidiosa multiplex</i>	Temperate and subtropical North America	Oleander, stone fruits, oaks, olive (USA), polygala (France)
<i>Xylella fastidiosa sandyi</i>	Southern regions of the USA	Oleander
<i>Xylella fastidiosa pauca</i>	South America	Citrus, coffee, olive (Italy, Argentina, Brazil), oleander (Italy, Costa Rica)
<i>Xylella fastidiosa morus</i>	Eastern and western USA	Mulberry (<i>Morus rubra</i>)

Epidemiology

Xf has a natural host range comprising 63 families, 193 genera, and species in excess of 300 (EFSA 2015b). Thus, securing information on the natural host range of the strain CoDiRO and on its putative vectors was thought to be of paramount importance if an efficient strategy for controlling the disease and restraining the contagion were to be envisaged. To this effect, extensive surveys were carried out in the last couple of years for identifying members of the native flora susceptible to infection. So far *Xf* infections have been found in symptomatic shrubs (*Nerium oleander*, *Cytisus scoparius*, *Acacia saligna*, *Polygala myrtifolia*, *Westringia fruticosa*, *Rosmarinus officinalis*, *Ramnus alaternus*, *Myrtus communis*, *Lippia citriodora*, *Grevillea juniperina*, *Asparagus acutifolius*, *Dodonaea viscosa*, *Myoporum insulare*) and in three woody plants (*Prunus dulcis*, *P. avium*, *Laurus nobilis*) (Saponari *et al.* 2013, 2014c, and unpublished information). By contrast, virtually none of over 100 species of monocot and dycot weeds proved to host the bacterium (Susca *et al.* 2014), and equally *Xf*-free were samples from different conifers (207), palms (105) and succulent plants (208) both from nurseries and private gardens (Potere *et al.* 2015). Likewise, no symptoms nor infections were detected in surveyed citrus (more than 350 trees of lemon, mandarin and sweet orange) and grapevine stands (over 600 vines of 25 different cultivars) growing next to or within heavily OQDS-infected orchards, and in over 2000 mother stocks and grafted vines for planting from the nurseries of Otranto (province of Lecce). Moreover, prick inoculations with the CoDiRO strain of rooted grapevine cuttings of cv. Cabernet sauvignon showed that after a year post inoculation the bacterium had not moved from the

inoculation site and no symptoms had appeared on the canopy (Saponari *et al.* 2014a; Saponari and Boscia 2015).

Although, as mentioned above, a wide number of agricultural and ornamental plant species grown in southern Salento seem to be *Xf*-free, many representatives of the native flora (perennial shrubs in particular) are infected. Multiple sources of infection that are readily accessible to potential vectors are therefore available, including olives which, as contributors of a massive inoculum load, qualify as the major source responsible for OQDS spread.

The search for potential vectors was initiated late in 2013, resulting in the capture of the xylem feeders *Philaenus spumarius* (the meadow spittlebug and a recognized *Xf* vector) *Neophilaenus campestris*, *Cercopsis sanguinolenta* and *Cicada orni* (Cornara and Porcelli 2014; Cornara *et al.* 2014). When adults of these species were analyzed by PCR for the presence of *Xf*, a positive response was obtained from a few individuals of *N. campestris* and a very high number of *P. spumarius*. This spittlebug is the most abundant among the captured species (e.g. it outnumbered *N. campestris* by nearly 99 to 1) and the one that, more than any other, thrives happily on olive. Hundreds of adults colonize olive trees in spring-late summer and the population carrying *Xf* may reach extremely high values (above 90% in some captures of August 2014). Thus, *P. spumarius* appears to be an excellent candidate vector of the CoDiRO strain, which was experimentally proven by transmission trials to periwinkle (Saponari *et al.* 2014b) and olive (Cornara *et al.* 2014).

P. spumarius produces one generation per year. Eggs are laid in autumn, juveniles hatch and develop within spittles on weeds during winter. Adults emerge in springtime and move from the ground to the olive

canopy in April-May, feed on these plants till July-August, then move to the ground when the first green weeds emerge. As reported by Cornara *et al.* (personal communication), the first *X. fastidiosa*-infective *P. spumarius* were collected in May from olive canopies: all the individuals previously collected on weeds beneath olive trees had tested negative for the bacterium. Scanning electron microscopy (SEM) revealed bacterial cells resembling *X. fastidiosa* in the foreguts of adult *P. spumarius*. Vector transmission experiments demonstrated that *P. spumarius* acquires *X. fastidiosa* from diseased olives and transmits it to healthy olive plants. Moreover, *P. spumarius* acquired *X. fastidiosa* from all the known hosts of the bacterium, except for broom and periwinkle, with the highest acquisition rate from olive, *P. myrtifolia* and *A. saligna*.

To assess natural transmission in the field, groups of bait plants comprising 1-year-old plants of olive, oleander, almond, grapevine and citrus were planted in spring 2014 in an aged heavily infected olive orchard and visually observed throughout the year. By late summer 2015, when a thorough laboratory checking was made, most of the olives and oleanders proved *Xf*-positive. Some oleanders exhibited initial leaf scorch. No symptoms, nor *Xf* were detected in grapevines and citrus (M. Saponari and G. Loconsole, personal communication).

From all the above it ensues that the meadow spittlebug is very active in the field, where it moves readily among hosts, and is the major, if not the only responsible factor for the transmission of strain CoDiRO from olive to olive and other susceptible plants. As such, it qualifies as the main target for restraining *Xf* dispersal through its control.

Control and containment measures

The long-standing American experience reflects that when *Xf* enters a territory endowed with favourable climatic conditions and a receptive flora, the bacterium becomes so firmly established that its eradication is no longer achievable. This is in conflict with the European Community rule (Directive 2000/29/EC) that imposes the eradication of any quarantinable pathogen that is detected in any of its member states. On the other hand, by late spring of 2014 it was clear that the OQDS outbreak had spread so extensively that it could hardly be eradicated. Hence, in agreement with the Italian

National Phytosanitary Service it was decided to face the urgency of restraining the disease within the boundaries of the affected area by implementing actions directed primarily against the vector and its hosts.

To this aim, in summer 2014 a OQDS control plan (XyCP) was devised based on the identification in the south-north direction of three areas across the provinces of Lecce and Brindisi, each several kilometers in depth, termed an eradication area, buffer zone and containment belt, respectively (Fig. 4). The actions to be implemented in A (containment belt) and B (buffer zone) consisted of: (i) verification of the absence of *Xf* infections through an extensive and continuous monitoring of the health status of olive trees and alternative hosts and the presence of infective vectors; (ii) preservation of the health status of the olive groves by controlling spittlebug with chemical treatments and mechanical weeding, and elimination of alternative hosts from highways, canals, green areas, etc. The eradication area (C) was to be the object of spittlebug control and of extensive monitoring for the identification of infection foci (location, number and size) and their elimination, whenever feasible.

Due to the strong opposition of some environmentalist and grower associations that were very much against the felling of olive trees, many of which are majestic and characterize the landscape, the XyCP could only be implemented partially. A new edition of the control plan, however, launched for application in these months, follows the same guidelines of the old one but takes into account the modifications that became necessary in the meantime: (i) widening of the buffer zone in consideration of the new infection foci detected in the province of Brindisi; (ii) adoption of the EU request for drastic eradication actions in these foci, i.e. destruction of all *Xf* hosts, including olives, within a 100-metre radius from infected trees; (iii) compensation to the growers for the voluntary uprooting of olive trees.

From a technical point of view the XyCP, more than controlling *Xf*, for which no effective means are available, aims at restraining the spread of the bacterium by reducing the inoculum potential at the edge of the unaffected area through the elimination of susceptible hosts and combating *P. spumarius* using a two-pronged strategy: (i) at the ground level, by mechanical weeding in winter, so as to mow down the spittlebug larval stages that thrive on the native flora; (ii) at the canopy level, by spraying olives in spring to kill the *P. spumarius* adults that have moved to the crown of the trees, where they feed, acquire *Xf* and transfer it to the neighbouring trees.

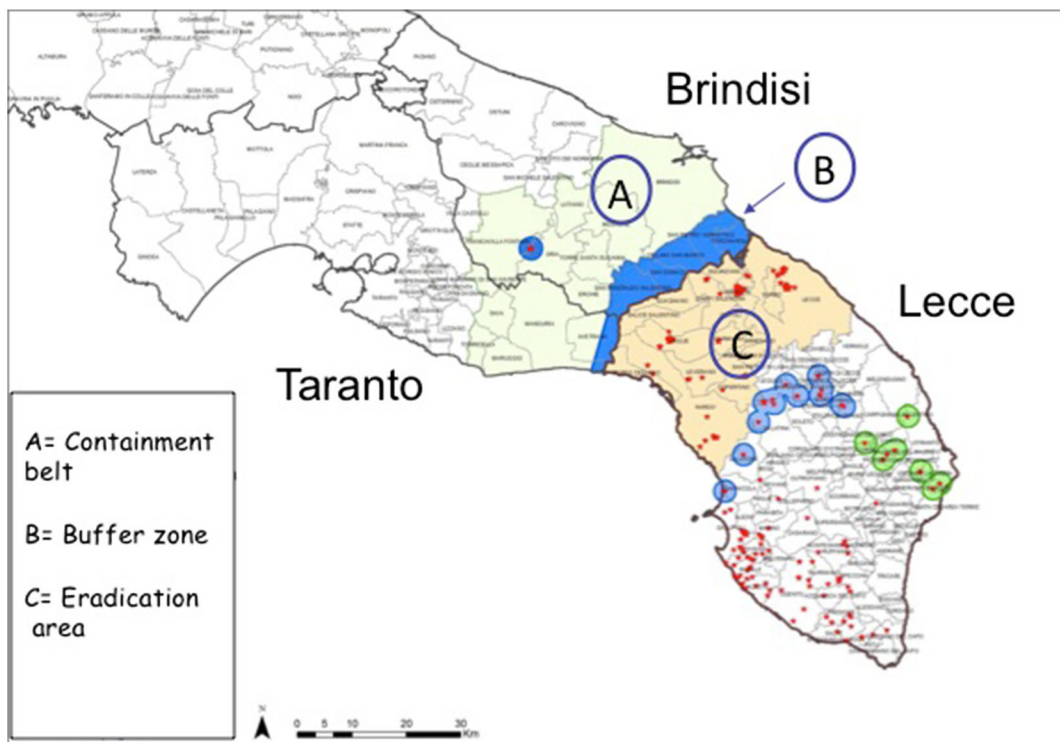


Fig. 4 *Xylella fastidiosa* containment plan 2014. In the three delimited areas of the Salento peninsula (A,B,C), different strategies are being enacted for restraining the spread of the bacterium

It was estimated that the mechanical weeding performed last winter in a fair number of farms has reduced by 70% or more the number of spittlebug nymphs, which would in turn reduce the number of adults. Such a drastic blow to the vector's population is expected to reduce *Xf* infections and slow down their spread.

While restraining, as it is hoped, the spread of OQDS in its current boundaries, what are the options for controlling the disease in the infected area? A number of actions, based primarily on foreign experience could be

through the targeted uprooting of olive trees and alternative hosts and controlling the population of *Philaenus spumarius*, the vector of the Salentinian strain of *Xf*

taken into consideration: (i) induction of symptom remission by fluidifying the bacterial biofilm with N-acetyl cysteine (Muranaka *et al.* 2013); (ii) interfering with the pathogen within the host by regulating its growth with the expression of a *Xf*-induced diffusible signal factor (Lindow *et al.* 2014); (iii) protecting plants with benign bacterial isolates (Hopkins 2014); (iv) biocontrol with bacteriophages (Das *et al.* 2015); (v) introducing into the xylem of infected plants nanoparticle-carrying toxic molecules to the bacterium (G. Ciccarella and

Fig. 5 An infected but vigorously vegetating cv. Leccino plant next to a couple of infected coetaneous trees of cv. Ogliarola salentina



A. De Stradis, personal communication); (vi) search for resistant cultivars.

As mentioned, the Salentinian olive industry is almost exclusively based on the traditional cvs Cellina di Nardò and Ogliarola salentina, both of which are sensitive enough to *Xf* so as to succumb when infected. However, a few other varieties are grown locally, either as scattered trees or as small standings intermingled with the larger orchards of the two leading cultivars. In the course of field surveys, it was repeatedly observed that plants of cv. Leccino growing within or next to olive stands heavily affected by OQDS had a healthy look and a green canopy with very little leaf scorching (Fig. 5). Laboratory investigations for elucidating the reasons for this intriguing differential behaviour have disclosed that: (i) the bacterial titre is much lower in cv. Leccino than in cv. Ogliarola ($1.3.10^4$ CFU.ml⁻¹ tissue extract vs $2.1.10^5$ CFU.ml⁻¹) (M. Saponari and G. Loconsole, personal communication); (ii) a comparative transcriptome analysis of infected trees of both cultivars has shown that a set of receptor-like proteins involved in signal transduction is overexpressed only in cv. Leccino (P. Saldarelli, personal communication). These results, although preliminary, support the notion that the differential response of cv. Leccino to *Xf* infection, rather than being accidental, is due to the expression of an active plant reaction liable of practical exploitation.

Since it is plausible that other olive cultivars behave in the same way as cv. Leccino, if not better, an experimental field has been established in the province of Lecce, in which a wide array of olive types, coming also from the international collections sponsored by the Conseil Olivicole International (COI, Madrid), will be grown under conditions of heavy natural inoculum.

In perspective, the finding of *Xf*-resistant cultivars could allow for the reconversion of the Salentinian olive industry, with undoubted benefits for the economy of the region. These beneficial effects, however, will not extend to the majority of what used to be a landscape embellished by superb centuries-old olive trees that, in the meantime, will no longer exist.

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