

The olive quick decline syndrome in south-east Italy: a threatening phytosanitary emergency

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Abstract The olive quick decline syndrome (OQDS) is a disease that appeared suddenly a few years ago in the province of Lecce (Salento peninsula, southeastern Italy). Among the factors that may be involved in its aetiology, the most relevant is *Xylella fastidiosa*, a quarantine pathogen of American origin, whose presence in Italy represents its first confirmed record in the European Union. *X. fastidiosa* is a Gram-negative bacterium that invades the xylem of a wide range of hosts, from which it is acquired by xylem-feeding insect vectors and transferred to other plants. The bacterium multiplies within the plant vessels and occludes them, thus impairing water uptake. Besides olive, the Salentian strain of *X. fastidiosa* infects in nature a number of woody (almond, cherry) and shrubby (oleander, broom, *Acacia saligna*, *Polygala myrtifolia*, *Westringia fruticosa*, *Rosmarinus officinalis*, *Rhamnus elaternus*, *Myrtus communis*) hosts, with no evidence for grapevines or citrus being hosts. The bacterium was isolated in culture and identified as a genotype of *X. fastidiosa* subsp. *pauca*, molecularly identical to an isolate from Costa Rica. *Philaenus spumarius* (meadow spittlebug), a froghopper quite common in the Salento area where it thrives on olive, was identified as the main vector.

Disease eradication and sanitation of infected olives are unfeasible. However, strategies are being enacted for restraining the spread of pathogen and vector(s) within the boundaries of the currently infected zone.

Keywords *Xylella fastidiosa* subsp. *pauca* · Disease management · Spittlebugs

Introduction

Xylella fastidiosa (Wells et al. 1987) is a Gram-negative, xylem-limited, slow-growing bacterium, transmitted by a number of xylem-feeding insect vectors. This pathogen is widely distributed in the American continent, where it infects a large number of hosts, i.e. 309 plant species belonging in 193 genera of 63 families (EFSA 2015) and is included in the EPPO A1 list of quarantine pathogens (EPPO/OEPP 1992). Its properties, biology, epidemiology and disease management strategies have been exhaustively described in a number of reviews to which the readers are referred for more detailed information (Hopkins 1989; Purcell and Hopkins 1996; Redak et al. 2004; Chatterjee et al. 2008; Janse and Obradovic 2010; Purcell 2013).

The unexpected arrival of *X. fastidiosa* in the Salento peninsula of Italy, the heel of the boot (Fig. 1), has created unprecedented turmoil. This is because of the dramatic damage suffered by the olive groves where the bacterium has established itself, and the alarm that this finding has raised in Italy whose olive/oil industry is a primary asset, and in the European Union, which is

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Fig. 1 Map of part of southern Italy with the Salento peninsula, the heel of the boot. The yellow line delimits the area of the province of Lecce. Most of which is affected by the Olive quick decline syndrome. The red star indicates the site of initial infection. The yellow stars indicate the north most infection foci



facing the first confirmed record in its territory of this alien and much feared pathogen.

X. fastidiosa is associated with a disease that appeared suddenly a few years ago, likely between 2008 and 2010, in a restricted area of the Ionian coast of the province of Lecce (Fig. 1). The disease had not escaped the attention of the growers and local farm advisors, who tentatively attributed it to a variety of causes, e.g. severe attacks of olive anthracnose (*Colletotrichum* spp.), pollution of the groundwater aquifer and ensuing phytotoxicity, root rot, poor management of the olive groves and heavy infestations by the leopard moth (*Zeuzera pyrina*), a lepidopteron endemic in the area.

When, in the early autumn of 2013, the problem was brought to the attention of the researchers of the University of Bari and of a unit of the Italian Research Council, it had already affected a surface of more than 8000 ha of arable land. This area is currently thought to extend to a good deal of the olive-growing area of the province of Lecce (about 23,000 ha). However, the most recent visual surveys indicate that, by adding up the surface area of the individual infection foci which are scattered in a leopard skin-like fashion, a total of about 10,000 ha strictly given over to olive can roughly be estimated. This would account for about one million infected trees. Hereafter, a brief account is given of the investigations carried out so far for determining the aetiology and epidemiology of the disease and for implementing an integrated control strategy based on

chemical and agronomical measures, in the attempt to restrain spreading of the disease within the boundaries of the currently infected zone.

The disease As shown in Fig. 2, the olive quick decline syndrome is characterized by the presence of leaf scorch and scattered desiccation of twigs and small branches which, in the early stages of the infection, prevail on the upper part of the canopy. As time passes, these symptoms become increasingly severe and extend to the rest of the crown, which acquires a burned look. The more seriously affected plants are heavily pruned by the growers to favour pushing of new growth which, however, is scanty and desiccates in a short while. The skeletal-looking trees are not dead, as shown by the abundant production of suckers from the base and survive for some time, i.e. as long as the roots are viable.

The most severe symptoms are shown by large and aged (centenarian) trees in which the early field observations identified the concomitant presence of three putative agents of damage: (i) extensive galleries drilled by *Z. pyrina* larvae; (ii) necrosis of the sapwood, which is invaded by a set of fungi of different genera, *Phaeoacremonium* and *Phaemoniella* in particular, but also *Pleurostomophora* and *Neofusicoccum* (Nigro et al. 2013, 2014), that take advantage of the moth galleries to penetrate the tissues; and (iii) *X. fastidiosa*, which invades, multiplies and occludes the tracheary elements (Saponari et al. 2013).



Fig. 2 The olive quick decline syndrome: initial (a), intermediate (b) and final (c) stages. Trees in c have been heavily pruned, but the new vegetation is already desiccated

Since these findings suggested that the disease could be induced by the complex of the above causes, it was denoted in Italian “Complesso del disseccamento rapido dell’olivo” (CoDiRO). This abbreviation was retained for identifying the Salentinian strain of *X. fastidiosa* although the disease name was subsequently changed into “Olive quick decline syndrome” (OQDS) due to the more extensive observations carried out in 2014. These showed that the role of the leopard moth is marginal, whereas the fungi may act as aggravators. This likelihood is supported by the outcome of extensive laboratory analyses that disclosed a thorough correlation between the presence of OQDS and *X. fastidiosa*, the map of their territorial distribution being totally superimposable. This was not the case for *Z. pyrina*, nor for most of the fungi, which are also found in *X. fastidiosa*-free areas. It ensues that *X. fastidiosa* appears as the main agent of decline of the Salentinian olives, and is strongly suspected to be able by itself of damaging the plants to the point of killing them. This is the hypothesis under study, which is being pursued with artificial inoculations of olive and other susceptible hosts (Saponari et al. 2014a).

These trials were made possible by the isolation in axenic culture of the CoDiRO strain from different hosts (olive, oleander, almond, sweet cherry, periwinkle, *Polygala myrtifolia* and *Westringia fruticosa*) (Cariddi et al. 2014; Saponari et al. 2014a). All isolates, regardless of the host of origin, have slow-growing colonies with the typical outward appearance of those of *X. fastidiosa* (Fig. 3a) and are made up of bacterial cells (Fig. 3b, d) morphologically in line with literature descriptions.

Diagnosis A clue to the search for *X. fastidiosa* in OQDS-affected olives was given by: (i) the

symptoms, which recalled very much the severe leaf scorching of fruit and shade trees induced by this bacterium, as described in the north American literature; (ii) the modality of disease spreading, which was compatible with that of *X. fastidiosa* infections. The use of a commercial ELISA kit that had been utilized for detecting *X. fastidiosa* in Turkish almonds (Güldür et al. 2005) gave a positive response, which was soon confirmed by molecular assays (Saponari et al. 2013).

This alarming discovery prompted the finalization of efficient diagnostic protocols for field surveys. Upon the request of the regional phytosanitary service, these were developed by the four accredited diagnostic laboratories present in the Apulia region, and validated by a ring-test (Loconsole et al. 2014a, b). The *X. fastidiosa* detection threshold by ELISA and conventional PCR in olive extracts was up to a dilution of 10^{-5} , whereas quantitative PCR proved to be 100-fold more sensitive than either method (Loconsole et al. 2014b). Loop-mediated isothermal amplification and direct tissue blot immunoassay (Djelouah et al. 2014; D’Onghia et al. 2014) are also being tested.

The bacterium Although *X. fastidiosa* is regarded as a single species, it has molecular variants that identify four subspecies with a diverse geographical origin and a partially different host range (Table 1). Two different subspecies have been found in olive plants from Argentina (Haelterman et al. 2015) and California (Krugner et al. 2014), that display a symptomatology resembling that shown by the Salentinian olives. Specifically, a strain of *X. fastidiosa* subsp. *pauca* molecularly different from the salentinian strain has been recently reported in Argentina, whereas *X. fastidiosa*

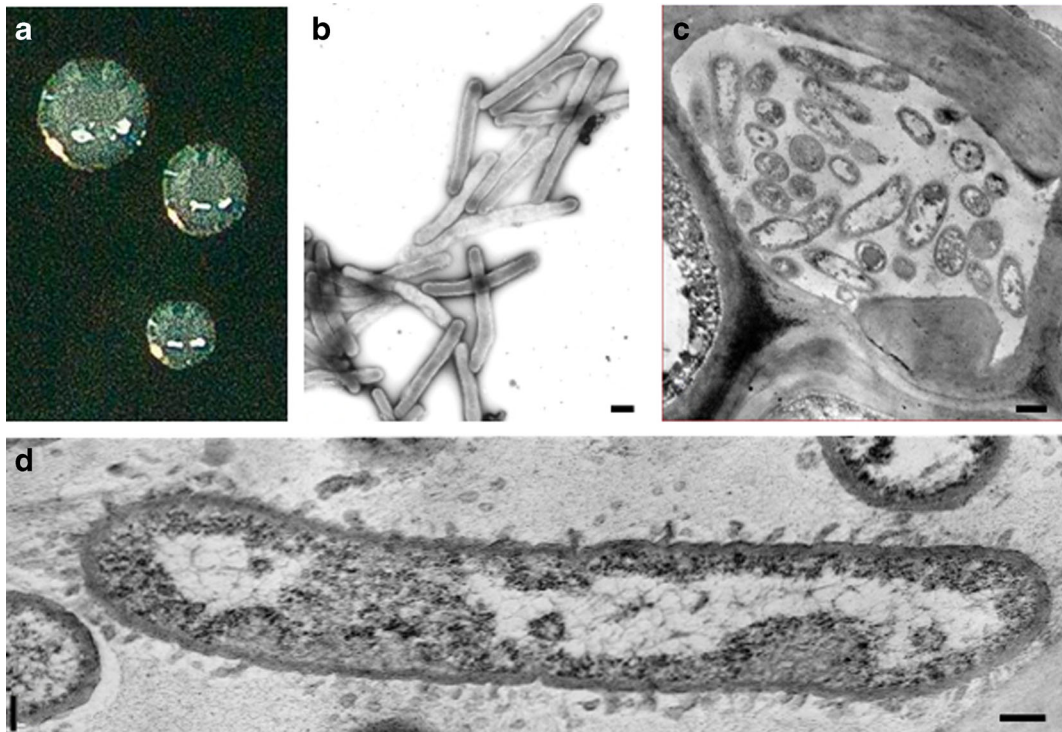


Fig. 3 **a** *Xylella fastidiosa* colonies on PWG medium. **b** Electron micrograph of bacterial cells from one of the colonies (Bar = 250 nm). **c** Cross-sectioned tracheary element from a diseased olive tree showing an accumulation of bacterial cells

(Bar = 200 nm). **d** Close-up of a *X. fastidiosa* cell in a tracheary element (Bar = 100 nm) (from Cariddi et al. 2014. *Journal of Plant Pathology*, 96, 425–429. By permission)

subsp. *multiplex* was isolated from symptomatic olives in California. Multilocus sequence typing (Maiden et al. 1998), applied to bacterial isolates from axenic cultures, determined that the CoDiRO strain is genetically homogeneous, belongs to the subspecies *pauca*, but represents a variant (Fig. 4) apparently identical to a strain from Costa Rica (Loconsole et al. 2014c; Nunney et al. 2014). This taxonomic allocation was confirmed by the sequencing of the CoDiRO genome, a 2,507,614 bp DNA molecule, whose reconstruction has been completed (Giampetruzzi et al. 2015).

From the above, the difference between the CoDiRO and bacterial strains infecting olive in California and Argentina appears clearly, as well as possible origin in

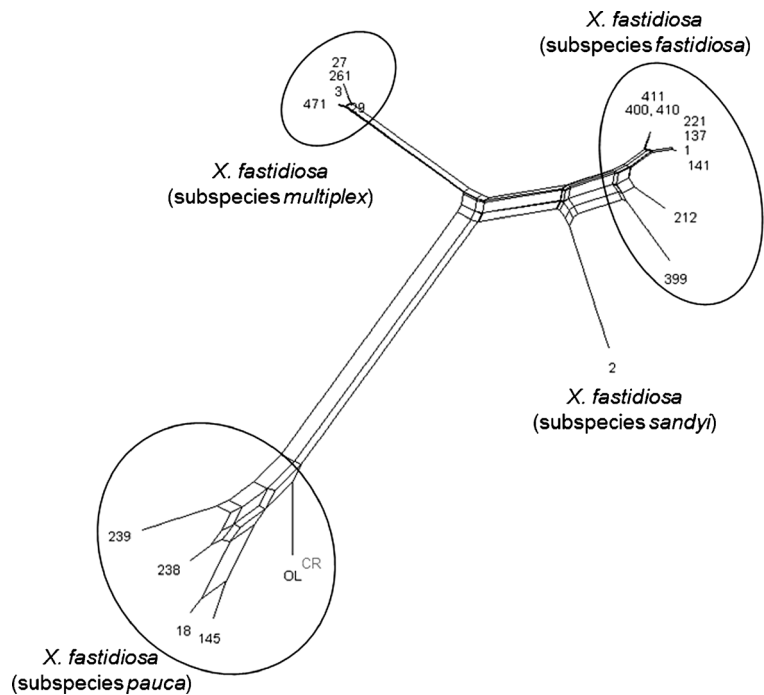
Central America. This supports the notion that it was recently introduced into Apulia with imported plant material (such as ornamentals) or, more unlikely, with a vector present in a plant consignment. The very recent interception in the Netherlands (October 2014, Netherlands Food and Consumer Product Safety Authority) of *X. fastidiosa* in ornamental coffee plants from Costa Rica supports what is stated above.

Epidemiology *X. fastidiosa* has a wide natural host range (309 plant species in 63 families and 193 genera) comprising wild and cultivated herbaceous, shrubby and woody plants (EFSA 2015). This allows the bacterium to firmly establish itself in any favourable environment in

Table 1 Subspecies of *Xylella fastidiosa*, their putative origin and main hosts

Subspecies	Origin	Main hosts
<i>Xylella fastidiosa fastidiosa</i>	Central America	Grapevine
<i>Xylella fastidiosa multiplex</i>	Southern USA	Oleander, stone fruits, oaks, olive (California)
<i>Xylella fastidiosa sandyi</i>	Undetermined	Oleander, magnolia
<i>Xylella fastidiosa pauca</i>	South America	Citrus, coffee, olive (Argentina)

Fig. 4 Phylogenetic tree based on concatenated MLST-gene sequences using the Split tree v4.13.1 program. Numbers refer to the sequence ID of the isolates retrieved from the *X. fastidiosa* MLST Databases (<http://pubmlst.org/xfastidiosa/>). OL and CR are the isolates recovered from a OQDS-affected olive tree and a Costa Rican oleander, respectively



which it is introduced. As mentioned, *X. fastidiosa* invades the xylem vessels of infected plants (Fig. 3c) and is transmitted by insect vectors (Cicadellidae and Aphrophoridae) that acquire the bacterium while feeding, and transmit it to other susceptible hosts. It is common knowledge that *X. fastidiosa* localizes in the foregut of the vectors where it multiplies, producing a carpet of cells that are injected in the host tissues while feeding. Transmission is persistent and very efficient, with no latent period between acquisition and transmission.

Since vectors and natural hosts share an equally important role in *X. fastidiosa* epidemiology (Stancanelli et al. 2014), their identification in any outbreak site is of the utmost importance. Thus, in late autumn 2013 investigations were initiated in the OQDS-affected areas, aiming at:

- (i) *Identifying natural inoculum sources.* Sampling of weeds, initiated in November 2013, is still under way with a monthly collection of 100 samples from a wide range of species vegetating in the infected area. So far, none of the over 100 species of weeds belonging in 40 different monocotyledonous and dicotyledonous families for a total of over 1000 samples, proved to host the CoDiRO strain (Susca

et al. 2014). Equally *X. fastidiosa*-free (Potere et al. 2014) were samples from different conifers (207), palms (105) and succulent plants (208), as well as citrus plants (350) and grapevines (in excess of 300) growing within or next to OQDS-infected orchards, plus nearly 2000 samples collected in the grapevine nurseries of the Otranto district. Notably, monthly observations throughout the 2014 vegetative season, did not disclose the presence of symptoms on any vine or citrus plant. Incidentally, prick inoculations of the CoDiRO strain made on vegetating rooted grapevine cuttings, have shown that 6 months afterwards the bacterium had not moved from the point of inoculation (Saponari et al. 2014a and unpublished information).

X. fastidiosa was found in symptomatic plants of almond, sweet cherry, oleander, broom, *Polygala myrtifolia*, *Westringia fruticosa* and *Acacia saligna* (Boscia 2014; Saponari et al. 2013, c) and, more recently, *Rosmarinus officinalis*, *Rhamnus alaternus*, *Myrtus communis* (unpublished information). All these plants, but oleander, are a likely source of inoculum for olive. In fact, oleander may be a dead-end host of the bacterium (Boscia

et al. 2014) as it does not seem to be to the liking of *Philaenus spumarius* (the meadow spittlebug), the vector of the CoDiRO strain.

- (ii) *Capturing and identifying leafhoppers thriving on the natural flora and analysing them for the presence of X. fastidiosa*. Since the end of 2013, at least four hemipteran species that are potential vectors have been captured: the already mentioned *P. spumarius* (Fig. 5a), *Neophilenus campestris*, *Cercopsis sanguinolenta* and *Cicada orni* (Cornara and Porcelli 2014; Cornara et al. 2014). *P. spumarius* is the most common and widespread species of all, and the one that more than any other thrives on olive. Very high populations (hundreds of adults) of this spittlebug colonize olive trees in spring-late summer, and equally high is the number of its individuals that are *Xylella*-positive (up to nearly 100 % in August 2014). Thus, *P. spumarius* has a tremendous inoculum potential (Fig. 5b) that is discharged on olives, i.e. the trees with which it entertains a preferential relationship.
- (iii) *Performing transmission tests with X. fastidiosa-positive spittlebugs*. Based on the above findings *P. spumarius* became the prime suspect as a putative vector of the CoDiRO strain. Its capability to transmit this strain was experimentally proven by successful transmission to periwinkle (Saponari et al. 2014b) and olive (Cornara et al. 2014).

Disease containment plan The experience gained in the United States, then in Central and South American countries where *X. fastidiosa* is endemic,

has shown that, once penetrated into a territory characterized by favourable climatic conditions, this bacterium establishes itself firmly and, thanks to his wide range of hosts and vectors, it becomes ineradicable. The implementation of an eradication plan in the Salento peninsula was therefore regarded as impracticable, notwithstanding that this is what the European Community rules impose against quarantinable pathogens (Directive 2000/29/EC). On the other hand, infected plants cannot be sanitized and most, if not all of them, seem bound to die. Moreover, hopes to restrain vector spreading in the active foci within the very large OQDS-infected area are very slim. Whereas future actions can address specific interventions aimed at, for example, induction of symptom remission (Muranaka et al. 2013), interference with the intracellular movement of the pathogen (Lindow et al. 2014), protection with benign bacterial isolates (Hopkins 2014), the urgency rests with devising a plan able to restrain pathogen and vector(s) within the boundaries of the OQDS-affected area. That the Salento peninsula would be at high risk in the unfortunate case of *X. fastidiosa* introduction, had been foreseen by Purcell (1997 and http://www.cost873.ch/_uploads/_files/Purcell_Xylella_Full_1.pdf) who had drawn a map (Fig. 6) showing that the potential for the establishment of the *Xylella*-induced Pierce's disease in this area is very high. The risk, however, decreases going northwards. This encouraging prediction and the realization that in the province of Lecce OQDS is moving southwards much faster than northwards, has oriented the efforts towards the enforcement of a set of

Fig. 5 **a** Adults of meadow spittlebug (*Philaenus spumarius*) collected from an infected olive tree. **b** Accumulation of bacterial cells in the foregut of a *X. fastidiosa*-positive spittlebug

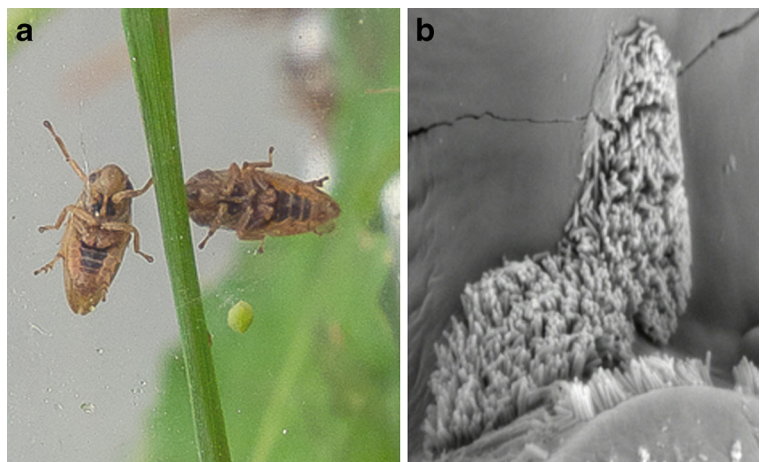
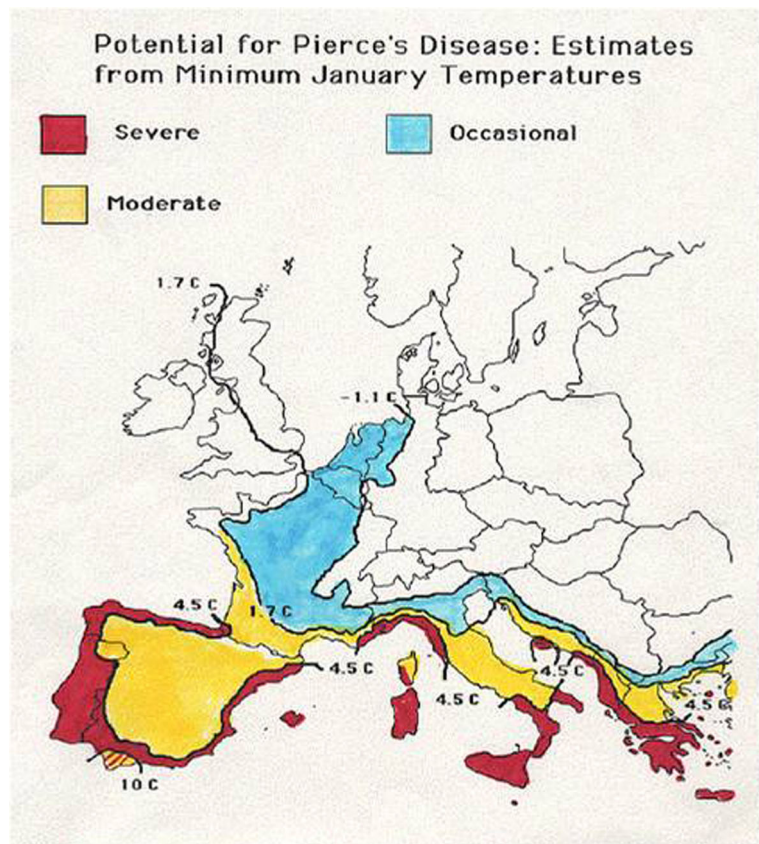


Fig. 6 Map of Europe with the predictive identification of the areas at risk for the establishment of *X. fastidiosa*, based on minimum January temperatures. The Salento peninsula is one of the “hot spots” (courtesy A.H. Purcell)



measures intended to stop the northbound progress of the disease.

As described in a Decree issued on September 9th, 2014 by the Italian Ministry of Agriculture, these measures consist essentially in the delimitation of two areas, each a couple of kilometers wide, which extend across the Salento peninsula, from the Adriatic to the Ionian coast. One of these belts borders the OQDS-infected area, while the other is located about 10 km north of the last infection foci so far identified (Fig. 1). Both areas will be the object of: (i) extensive monitoring of *X. fastidiosa* and vector(s); (ii) chemical treatments against adult vector populations; (iii) elimination of weeds and shrubs on which vector juveniles thrive in autumn-early spring; (iv) elimination of all alternative hosts of the bacterium; and (v) immediate uprooting of newly infected olive trees and those bordering them. Norms are also dictated for olive grove management according to “good cultural practices”, for avoiding the passive transport of vectors and pathogen across the delimited areas, and for the certification of nursery productions of susceptible hosts. A region-wide

information campaign for growers and farm advisors will also be conducted. Whether or not this earnest attempt to restrain OQSD within its current boundaries will succeed, very much depends on the continuous surveillance of the territory and the rigorous enforcement of the envisaged actions.

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Compliance with ethical standards Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the authors.

Conflict of interest The authors declare no conflict of interest.

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