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in

D'Onghia A.M. (ed.), Brunel S. (ed.), Valentini F. (ed.).
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Bari : CIHEAM

Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 121

2017

pages 25-28

Article available on line / Article disponible en ligne à l'adresse :

<http://om.ciheam.org/article.php?IDPDF=00007202>

To cite this article / Pour citer cet article

Martelli G.P., Nigro F. **The olive quick decline syndrome**. In : D'Onghia A.M. (ed.), Brunel S. (ed.), Valentini F. (ed.). *Xylella fastidiosa & the Olive Quick Decline Syndrome (OQDS). A serious worldwide challenge for the safeguard of olive trees*. Bari : CIHEAM, 2017. p. 25-28 (Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 121)



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The olive quick decline syndrome

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The quick decline syndrome of olive (OQDS) is a disease that appeared suddenly some years ago in a *Olea europaea* grove near the city of Gallipoli, on the Ionian coast of the Salento peninsula (south-east Italy), and began spreading fast in lower Salento. OQDS has been the object of reviews (Martelli *et al.*, 2015; Martelli, 2016) which the readers are referred to for a more exhaustive information. The disease is characterized by the appearance of scattered desiccation of twigs and small branches. Leaves are the first to be affected. Scorching starts at their tip and progresses towards the petiole, extending to the whole blade. Dead leaves remain attached throughout summer to the twigs, which are also desiccating, and are shed in the rainy season. Symptoms are first localized in the upper part of the crown, then they extend to the rest of the canopy. Trees of susceptible cultivars, i.e. 'Cellina di Nardò' and 'Ogliarola salentina', that account for nearly the totality of the Salentine olive industry, decline and die within a few years from the appearance of symptoms. These trees, especially the centuries-old ones, are often pruned heavily, forcing them to push new growth which, eventually, will wither and desiccate. The investigations carried out at Bari by the local University, a phytopathological outfit of the National Research Council of Italy (CNR), the CIHEAM Mediterranean Agronomic Institute of Bari (MAIB) and the Centro di Ricerca, Sperimentazione e Formazione in Agricoltura Basile Caramia at Locorotondo (Bari), disclosed that in addition to other putative disease agents, i.e. wood-inhabiting fungi of the genera *Phaeoacremonium*, *Pseudophaeoniella* gen. nov., *Pleuromastomophora* and *Neofusicoccum*, OQDS-affected trees consistently hosted *Xylella fastidiosa* (*Xf*), a most feared quarantinable pathogen, which had never been detected in any of the European Union (EU) countries. *Xf* is a Gram-negative bacterium of the family Xanthomonadaceae, that enters the xylem vessels of the hosts and is transferred from plant to plant by xylem fluid feeding insects of the family Cicadellidae. Colonization of the xylem vessels results in their clogging by the bacterial biofilm which impairs water uptake. This has a major pathogenic effect. However, a putative lipase/esterase (LesA) secreted by bacterial cells has recently been identified as a virulence factor that initiates marginal scorching of grapevine leaves (Nascimento *et al.*, 2016). Because of its biology, which does not conform to that of bacterial plant pathogens, *Xf* has long been thought to be a virus, until its isolation in axenic culture (Wells *et al.*, 1987). *Xf* is subdivided in subspecies, four of which, *Xf fastidiosa*, *Xf multiplex*, *Xf pauca* and *Xf sandyi*, are currently retained as taxonomically valid. These subspecies have a different geographic distribution in the American continent, the site of origin of *Xf*, and an extremely wide host range: 75 botanical families, 204 genera and 359 plant species (EFSA, 2016a), 22 of which proved to be hosts of the bacterial strain present in Salento, called CoDiRO. When *Xf* enters a new environment with favourable climatic conditions, it becomes entrenched because of its polyphagy and is no longer eradicable. This seems to be the case of Salento, as suggested by the outcome of the studies underway at Bari, whose major results are listed hereafter.

- (i) First identification of *Xylella fastidiosa* in different plants (olive, almond, oleander) showing leaf scorch symptoms in the Salento peninsula (Saponari *et al.*, 2013).
- (ii) Different fungal species colonize the wood of declining olive trees of the Salento peninsula (Nigro *et al.*, 2013; Crous *et al.*, 2015).

- (iii) Finalization of serological (ELISA, DTBIA, immunofluorescence) and molecular (PCR, Real time PCR, LAMP) procedures for the reliable identification of *Xf* in host plants and vector (Loconsole *et al.*, 2014; Djelouah *et al.*, 2014; Yaseen *et al.*, 2014; Cariddi *et al.*, 2014).
- (iv) Isolation in axenic culture of strain CoDiRO from olive and other naturally infected plant species (Cariddi *et al.*, 2014; Elbeaino *et al.*, 2014).
- (v) Identification of CoDiRO as a strain of *Xf pauca*. Molecular evidence of its identity with a bacterial isolate (ST53) of the same subspecies present in Costa Rica, a country from which it may have landed in Salento with an unidentified ornamental plant (Loconsole *et al.*, 2014; Giampetruzzi *et al.*, 2015).
- (vi) Complete sequence of the genome of strain CoDiRO, a DNA molecule of 2.46 MB (Giampetruzzi *et al.*, 2015a).
- (vii) Identification of the spittlebug *Philaenus spumarius* (family Aphrophoridae) as the main if not the only vector of strain CoDiRO, and determination of its biological cycle (Saponari *et al.*, 2014; Cornara *et al.*, 2016).
- (viii) Electron microscopic detection and identification by gold immunolabelling of the bacterium in xylem vessels of infected plants and in the foregut of the spittlebug vector (Cariddi *et al.*, 2014; Cornara *et al.*, 2016).
- (ix) Identification of 22 alternative hosts of strain CoDiRO in the province of Lecce out of more than 600 trees, shrubs and weeds analysed, including grapevines and citrus (Potere *et al.*, 2015; P. La Notte, unpublished information).
- (x) Experimental evidence that upon mechanical inoculation with bacterial cultures, strain CoDiRO does not infect grapevines (cv. Cabernet sauvignon) and citrus (orange Madame Vinous and Navelina, mandarin, grapefruit Duncan, citranges Carrizo, Troyer and C35), whereas it multiplies readily in olive seedlings and in rooted cuttings of cv. Cellina di Nardò and other olive cultivars (Coratina, Frantoio, Leccino), and oleander (Saponari *et al.* 2014, 2016; EFSA, 2015).
- (xi) Complete sequence of the genome of CO33, a coffee-infecting isolate of *Xylella fastidiosa* intercepted in northern Italy, a DNA molecule of 2.68 MB (Giampetruzzi *et al.*, 2015b).
- (xii) Host plants exposed to infective *Philaenus spumarius* in the field are infected at different rates. *Xf* was detected by laboratory assays in still symptomless olive plants as soon as six months after caging with infective vectors (Saponari *et al.*, 2016).
- (xiii) Bait plants. Of the young trees of olive, oleander, citrus, grapevine and almond planted in diseased olive orchards for exposure to infective vectors, only olives and oleanders became infected within 12 months and started to show symptoms 16-18 months after planting (Saponari *et al.*, 2016).
- (xiv) Fulfilment of Koch's postulates upon mechanical inoculation of different hosts (olive, *Polygala myrtifolia*, oleander) with pure cultures of strain CoDiRO (Saponari *et al.*, 2016, as certified by EFSA, 2016b). The Salentinian strain of *Xf pauca* is a primary pathogen causing desiccation and necrosis of inoculated susceptible hosts.
- (xv) A comparative analysis of the transcriptome of infected and healthy plants of cvs Leccino and Ogliarola salentina showed that genes coding for receptor-like kinases (RLK) and receptor-like proteins (RLP) involved in plant defence responses are differentially expressed in the two cultivars. Partial resistance of cv. Leccino to strain CoDiRO seems to be expressed essentially through a remarkable reduction of the bacterial population, i.e. 130.000 CFU/ml of tissue extract in cv. Leccino vs 2,094,000 CFU/ml in cv. Ogliarola salentina (Giampetruzzi *et al.*, 2016).

Being a quarantine pathogen, *Xf* is regulated by EU Directive 2000/29/CE, which must be enforced in all member States, Italy included. This Directive dictates the protective measures to be implemented against the introduction and spreading of such pathogens in the EU territory. Eradication is mandatory or, should this be no longer possible, measures must be adopted for restraining pathogen dissemination. Based on the knowledge acquired with the above-listed investigations, a plan was envisaged by the Italian Ministry of Agriculture and Forestry for confining the contagion within the province of Lecce, its current boundaries, through the control of *P. spumarius*, the OQDS vector: (i) mechanical weeding against the larval stages; (ii) chemical treatments against the adults; (iii) uprooting alternative hosts and infected olive trees in newly identified foci. Stumbling blocks have prevented the enforcement of this plan, thus the disease is moving north, and has reached the neighbouring provinces of Brindisi and Taranto.

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