CURRENT TOPICS

Has *Xylella fastidiosa* "chosen" olive trees to establish in the Mediterranean basin?

Antonia CARLUCCI[1], Francesco LOPS[1], Guido MARCHI[2], Laura MUGNAI[2] and Giuseppe SURICO[2]

[1] Dipartimento di Scienze agrarie, degli alimenti e dell'ambiente, Università degli Studi di Foggia, Via Napoli, 25, 71121 Foggia, Italy
[2] Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, Sezione Patologia vegetale e Entomologia, Università degli Studi di Firenze, P.le delle Cascine 28, 50144 Firenze, Italy

Summary. Severe decline of olive trees was observed in the Lecce province, Apulia (Italy), and received the name 'complesso del disseccamento rapido dell'olivo' (olive rapid decline complex). Affected plants showed leaf scorch symptoms and dieback of twigs, branches and even of the whole plant. Similar symptoms, unusual for the area, have also been observed in other Apulian localities (Cerignola, Foggia, Canosa di Puglia, and Andria). Three fungal species were associated with the symptoms: *Phaeoacremonium aleophilum*, *Neofusicoccum parvum*, and *Pleurostomophora richardsiae*. The latter is the first report of this fungal species infecting olives. In the Lecce province, the bacterium *Xylella fastidiosa* also was detected from affected olive trees. *Xylella fastidiosa* is a quarantine agent in Europe that had been previously reported in the Mediterranean region, but did not spread probably because of the lack of a vector. Present findings suggest that this fundamental condition has now been met.

Key words: leaf scorch, Apulia, quarantine pathogen.

Introduced plant pathogens often have dramatic effects on agricultural and native plant communities. Examples of damaging pathogens in Europe and the Mediterranean region include the causes of powdery mildew (*Erysiphe necator*), downy mildew (*Plasmopara viticola*), potato blight (*Phytophthora infestans*), Dutch elm disease (*Ophiostoma ulmi*, *O. novo-ulmi*), canker stain of plane tree (*Ceratocystis platani*), cypress canker (*Seiridium* spp.), fire blight (*Erwinia amylovora*), and many others. On the other hand, most exotic plant pathogens are likely to encounter hosts and conditions favoring their development in European and Euro-Mediterranean areas, due to the wide variety of climate conditions and plant populations.

A recent review by Janse (2012) examined the epidemiology, management and main risks of emerging bacterial diseases approaching or already occurring in Europe. Diseases not yet known to occur in Europe included: Huanglongbing of citrus (associated bacteria are: "*Candidatus* Liberibacter asiaticus"/"*Ca.* L. africanus"/ *Ca.* L. americanus"), citrus canker (*Xanthomonas citri* pv. *citri*), black spot of Mango (*X. citri* pv. *mangiferindicae*), bacterial blight of pomegranate (*X. axonopodis* pv. *punicae*), bacterial blight of guava (*Erwinia psidii*), bacterial spot of passion fruit (*X. campestris* pv. *passiflorae*), and also leaf scorch and leaf scald diseases on various fruit and ornamental trees caused by *Xylella fastidiosa*.

Xylella fastidiosa is a xylem-limited bacterium that can not be grown in culture media (Davis *et al*., 1978) and was named by Wells *et al*. (1987). It is transmitted by xylem-fluid feeding leafhoppers (especially members of the subfamily Cicadellinae and the spittlebug family Cercopidae). The first reported host of this pathogen was grapevine, causing Pierce's disease, the initial symptoms of which include leaf scorch and stunted growth, usually followed by vine death in 1–5 years.

Corresponding author: A. Carlucci
Fax: +39 0881 589501
E-mail: antonia.carlucci@unifg.it

www.fupress.com/pm

ISSN (print): 0031-9465
ISSN (online): 1593-2095

Xylella fastidiosa affects several economically important plants including grapevines, almond, pear, peach, coffee, and citrus. The bacterium also infects ornamental plants such as oleander and *Prunus* spp., forestry crops (sycamore, mulberry, red maple, elm, oak) and several weeds and native plants (Schaad *et al*., 2004; Hernandez-Martinez *et al*., 2007). In these last cases the bacterium often does not cause any known visible symptom.

Since plants infected by *X. fastidiosa* mostly grow in parts of the American continent (North, Central and South America) that have mild winters and long growing seasons, disease models have long predicted that the pathogen could establish in Mediterranean countries with similar climatic and environmental conditions (Janse and Obradovic, 2010). For instance, Greece, southern Spain, and Italy have been identified as particularly at risk from *X. fastidiosa* if vectors of the bacterium occur. Indeed in southern California, USA, which has a climate very close to the one found in the Mediterranean region, both the host range and spread of *X. fastidiosa* have expanded further as a consequence of the introduction and spread of the glassy-winged sharpshooter (*Homalodisca vitripennis*) as a new vector of the bacterium.

One of the most recently reported hosts of *X. fastidiosa* has been olive. Wong *et al.* (2004) from the University of California-Riverside, examined over 500 samples from 78 landscape plants showing wilt, dieback or leaf scorch in 2003. Samples were collected from plants located in five cities (Fillmore, San Diego, Redlands, Riverside, and Tustin) in southern California counties. Twenty-six of the 78 plant species sampled tested positive for *X. fastidiosa.* However, in only a few cases it was possible to culture the bacterium successfully on appropriate agar media. *Olea europea* was one of the tree species that tested positive for *X. fastidiosa via* ELISA and PCR tests (Minsavage *et al*., 1994; Pooler *et al*., 1997). The pathogen was later isolated (strain G12) and molecularly identified as a strain belonging to the *multiplex* clade (Hernandez-Martinez *et al*., 2007).

After increasing incidences of olive tree mortality occurring in the Los Angeles area in 2008, surveys were conducted by Krugner *et al.* (2010) to evaluate the association of *X. fastidiosa* with scorch and dieback symptoms in olive trees in southern California and the southern San Joaquin Valley. The survey was financed by the California Olive Committee (COC).

Xylella fastidiosa was isolated from naturally infected olive trees and re-inoculated on healthy olive trees of seven cultivars (Mission, Manzanillo, Sevillano, Arbequina, Arbozano, Koroneiki, Barouni), 30 trees per cultivar. Trees were monitored for symptoms for 1 year. Some of the re-inoculated and control trees showed branch dieback and leaf scorch, the same symptoms as those that were observed in the field. *Xylella fastidiosa* was detected in a few plants that had been inoculated, but was not re-isolated from these trees, and thus Koch's postulates were not fulfilled at the time (the final report was submitted to the COC in 2010). Later RAPD analysis showed that the *X. fastidiosa* populations infecting olive trees belonged to the A genotype group, which is known to cause leaf scorch disease in almond, but does not cause Pierce's disease in grapevine. Krugner *et al*. concluded in the report that "a longer evaluation period was necessary to confirm or rule out *X. fastidiosa* as the causal agent of [the] disease".

In Italy in 2013 there has been "a rapidly spreading decline of old (60–70 years or older) olive trees in a large part of the Salento peninsula (cities of Gallipoli, Taviano, Alliste, Felline, etc., on the Gulf of Taranto) at the south-eastern tip of Apulia" (in Sportelli, 2013) (Figure 1). The main symptoms of the disease were described by the Phytosanitary service of the Regione Puglia (2013), as follows:

Figure 1. Map of the Apulia region of Italy showing the three locations where olive decline symptoms were here mentioned: Canosa di Puglia, Cerignola (Province of Barletta-Andria-Trani) and Foggia; Andria (Province of Barletta-Andria-Trani); Gallipoli (Lecce province).

- dieback of twigs, branches or the entire tree;
- wood browning of the twigs, branches and trunks;
- leaf scorch.
- In these infected trees were also noted:
- severe attacks of a moth, *Zeuzera pyrina*, causing wilting of the twigs and branches;
- trees not pruned regularly, or not at all, soil not well cultivated;
- the tracheomicotic pathogens *Phaeoacremonium parasiticum*, *P. rubrigenum*, *P. aleophilum*, *P. alvesii*, and *Phaeomoniella* spp. were a common occurrence in the olive wood;
- *Xylella fastidiosa* occurred in the infected olive trees.

With regard to the last, polymerase chain reaction (PCR) assays on extracts from the leaf veins and petioles of diseased trees gave positive reactions using *X. fastidiosa* gene-specific primers. Moreover, *X. fastidiosa* was detected in almond and oleander trees exhibiting leaf scorch symptoms and growing next to diseased olive orchards (Saponari *et al*., 2013).

At the same time, a survey of olive groves in the Canosa di Puglia, Cerignola and Foggia areas in the northern part of Apulia detected a disease of olive trees showing symptoms similar to those observed in the Salento peninsula (see Carlucci *et al*., 2013). [A similar syndrome, named Olive twig and branch dieback, has also been recently reported by Úrbez-Torres *et al*., in California (2013).] The symptoms observed in the northern part of Apulia consisted of generalised decline of the trees, beginning with foliar discoloration and leaf fall, wilting of the apical shoots, dieback of twigs and branches, and brown wood streaking of the trunks, branches and twigs. With the exception of the brown wood streaking, all these symptoms resembled *Verticillium dahliae* infections. At a later stage of the disease, necrosis and cankers developed on the bark of affected trees. Morphological and molecular analyses assigned the cause of the disease to *Pleurostomophora richardsiae, Neofusicoccum parvum* and *Phaeoacremonium aleophilum,* which are, in part, the same species found in olive trees of the Salento peninsula (and also in the olives in California affected by twig and branch dieback disease). Moreover, olives with symptoms similar to leaf scorch disease have also been seen in the Bari area (Carlucci, personal communication) (Figure 2). Instead in the observations made in California on olives affected by twig and branch dieback, 18 fungal species were detected including *Neofusicoccum mediterraneum* and *Diplodia mutila* (the most virulent) and also *P. aleophilum*, *Phaeomoniella chlamydospora*, *N. luteum* and *N. vitifusiforme*, etc. In a separate survey, trees showing similar symptoms were screened for presence of *X. fastidiosa*, but a vast majority of samples tested negative for *X. fastidiosa* (Krugner, personal communication).

Figure 2. Leaf scorch symptoms observed in olive trees in northern Apulia (Adria, province of Barletta-Adria-Trani).

At the moment, the findings presented here illustrate a very unknown and complex pathosystem that needs to be elucidated. *Xylella fastidiosa* appears to be established and widely spread in the Apulia region. Pure laboratory colonies of *X. fastidiosa* are needed for further identification and pathogenicity tests, but to our knowledge the bacteria has not yet been cultured. In addition, the mechanism of dispersal (e.g., insect vectors, infected propagative material, contaminated pruning equipment) has not yet been identified. In contrast with previous introductions of *X. fastidiosa* to Europe (Berisha *et al*., 1998; Güldür *et al*., 2005), the rapid spread suggests that an efficient vector is involved. Some other questions remain: why does the bacterium occur especially in aged trees, and why did it so suddenly and so severely attack olive trees over such a large area (approx. 8,000 ha) (in Sportelli, 2013). Can it be hypothesized that the bacterium was already present as non-symptomatic infections? Investigations should now be extended to the whole of Apulia and to other Italian olive-growing regions. A common effort should be made to face this emergency, and to set up national and international networks to protect Mediterranean agriculture by quickly detecting and accurately diagnosing and identifying potential pathogens (and also insects, nematodes, and weeds). Olive trees are emblematic of the entire Mediterranean area, typifying the landscape and the close relationship between nature and man's work in it. What is now happening in Apulia should be an alarm bell for the Mediterranean region and for the whole community of plant pathologists; it must not be ignored. In the meanwhile the Secretariat of the European – and Mediterranean - Plant Protection Organization (EPPO), has already published a warning on the new report of *X. fastidiosa* in the EPPO region on its website (http://www.eppo.int/QUARANTINE/special_topics/Xylella_fastidiosa/Xylella_fastidiosa.htm).

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Accepted for publication: December 3, 2013