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State of the art of the research on *X. fastidiosa* in Puglia

Maria Saponari

Istituto per la Protezione Sostenibile delle Piante, CNR Bari - Italy

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The recent emergence of *Xylella fastidiosa* in Europe raised several concerns for the risks and the severe impacts that this plant pathogenic bacterium can determine on the EU agriculture/forestry sectors and natural environment. Effective preventive and containment measures rely on the knowledge of the complex interactions (bacterium-host plant- vector- environment) determining the epidemic spread of this bacterium. Major consequences related to the introduction of this invasive pathogen in the EU territory take into account that: (i) several known hosts of *X. fastidiosa* include many cultivated and wild species growing in Europe and in the Mediterranean Basin; (ii) a wide range of European wild plant species have never been exposed to the bacterium, thus no information is available on their susceptibility; (iii) all xylem fluid-feeding insects in Europe may represent potential vectors; (iv) one of the most susceptible crop species in the EU outbreaks is olive (Martelli *et al*., 2016), a major crop and a relevant species for the Mediterranean landscapes. In response to the first *X. fastidiosa* outbreaks (Saponari *et al*., 2013) in the Puglia region (southern Italy), research programs have been activated (at regional and international level) to develop critical information on the biology and genetics of the Puglia isolate, and on the epidemiology of the infections. International collaborations, with research groups with long-term experience in studying host-pathogen-vector interactions, have been important to capitalise the existing knowledge and to promote the research capability building of the local Institutions. The outcomes of these research programs have significantly contributed to unravel information on the genetic relatedness of the Puglia strain and other intercepted strains, the natural host range, the susceptibility of major crop species, the vector-transmission and the epidemiology of the infections, the impact of the infections. One of the major scientific contributions of the ongoing research investigations refers to the evidence on the pathogenicity and on the role of *X. fastidiosa* strain CoDiRO in the severe olive disease associated to the *Xylella*-outbreak in Puglia. Taken together, the overall experimental results lead toward the implementation of the future scientific knowledge and the reduction of the uncertainties regarding the risk assessment and the development of effective surveillance and management strategies.

Taxonomic position of the Puglia strain

Multilocus sequence typing (MLST) for *X. fastidiosa*, first introduced by Scally *et al.* (2005) and then refined by Yuan *et al.* (2010), has been successfully used to study *X. fastidiosa* diversity at the species/subspecies level, and to infer the phylogenetic placement of newly identified isolates. The MLST data have resulted in a robust taxonomy for the species and for the classification of isolates into sequence types (STs) (unique genotypes based on the 7 loci used in MLST) (Alemida and Nunney, 2015).

MLST analysis of the *X. fastidiosa* isolates recovered from different infected olive trees found in the first outbreak (Gallipoli district) proved that all isolates collected belonged to ST53 (Elbeaino *et al*., 2014; Loconsole *et al*., 2014). Up to now, isolates with the same ST are known to occur only in Costa Rica, associated to *X. fastidiosa* infections on oleander and coffee plants (Nunney *et al*., 2014). The phylogenetic network derived from all MLST data publicly available indicated that ST53 clusters with *X. fastidiosa* subspecies *pauca*. Further molecular investigations assessed that this ST is the only allelic profile associated to the isolates causing infections in olive trees located in geographically distant foci in Puglia and in different naturally infected hosts found in the Puglia infected area (Saponari *et al*., 2014; Loconsole *et al*., 2016).

Host range and pathogenicity tests

Preliminary data on the susceptibility of ornamentals (i.e. oleander and *Polygala myrtifolia*) important tree species (i.e. olive, stone fruit, citrus, grape and holm oak) in the EU to the Puglia strain of *X. fastidiosa* were obtained by conducting small-scale experiments under controlled and field conditions. Needle inoculations with the *X. fastidiosa* strain CoDiRO and exposure of plants to natural infective vector populations have provided critical information that substantiated the field observations made in the last 2 years after the discovery of the first Puglia outbreak. In summary, (i) olives appear to be highly susceptible to infections caused by isolates of the subspecies *pauca*, and in particular to the strain CoDiRO; (ii) olive cultivars display a differential response to *X. fastidiosa* infection, multiplication and movement; (iii) upon systemic infections, symptoms similar to those observed in the affected field (desiccation and dieback) were observed on inoculated olive plants; (iv) amongst the cultivars tested, Cellina di Nardò clearly resulted as the most susceptible to the CoDiRO strain (Saponari *et al.,* 2016).

The field experiments with the exposure of young plants to natural inoculum and to naturally infective specimens of *P. spumarius* resulted in successful infection of the known host plants (olive, oleander and *P. myrtifolia*); conversely no transmission occurred on grapevines, citrus and holm oak. So far no symptoms have been observed on the infected plants under field conditions.

Major research outcomes

- Sequence analyses on the *X. fastidiosa* isolates recovered from different hosts and foci in Puglia showed that all infected samples harbored a single ST, denoted as ST53, with phylogenetic relationships with the subspecies *pauca*. The finding that isolates with the same ST53 have been so far identified only in Costa Rica, provided evidence toward the better understanding of the introduction pathways in the EU, and supports the single introduction hypothesis associated to the Puglia outbreak.
- Artificial inoculations confirmed that olives, oleander and myrtle-leaf milkwort support systemic infections of *X. fastidiosa* strain CoDiRO, and develop symptoms (Fig. 1) resembling those observed in the outbreak area. These results contributed to disclosing the etiology of the olive quick decline syndrome and the role of *X. fastidiosa* strain CoDiRO in this novel olive disease.
- Upon artificial inoculations and the field exposure of citrus, grapevines and holm oak, no successful systemic infections were detected on these plant species, which so far appear to be not susceptible to *X. fastidiosa* strain CoDiRO.

Figure 1. Symptoms recovered on the mechanical inoculated plants with *Xylella fastidiosa* strain CoDiRO. A) Desiccated olive twig; B) Shoot tip dieback on olive inoculated shoot; C) Chlorotic leaf pattern and initial necrosis observed on the mature leaves of oleander; D) Desiccated shoot on *Polygala myrtifolia.*

* Puglia Research Team

Antelmi I., Cariddi C., Cornara D., Giampetruzzi A., Loconsole G., Nigro F., Martelli G.P., Porcelli F., Potere O., Savino V., Susca L.
Dipartimento di Scienze del Suolo della Pianta e degli Alimenti, Università Aldo Moro, Bari

Altamura G., Boscia D., Cavalieri V., Chiumenti M., De Stradis, G. D'Attoma, La Notte P., Minafra A., M. Morelli, Saldarelli P., Saponari M., D. Tavano, S. Zicca
Istituto di Protezione Sostenibile delle Piante del CNR, sezione Virologia, Bari

Dongiovanni V., Palmisano F., M. Di Carolo, G. Fumarola, P. Pollastro, A. Saponari, M.R. Silletti,
Centro di Ricerca, Sperimentazione e Formazione in Agricoltura Basile Caramia, Locorotondo (BA)

Digiaro M., Djelouah K., D'Onghia A.M., Elbeaino T., Frasheri D., S. Gualano, D. Lorusso, F. Santoro, Valentini F., Yaseen T.
CIHEAM - Istituto Agronomico Mediterraneo di Bari

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