

It is a Gram-negative bacterium of the class Gammaproteobacteria, within the family Xanthomonadaceae. The genus *Xylella* nowadays is formed by two species, *X. taiwanensis*, recently described as a pathogen in pear trees, and *X. fastidiosa*, described in 1892 as a vine pathogen causing Pierce's disease. This last one is divided into six subspecies: *fastidiosa*, *multiplex*, *pauca*, *morus*, *sandyi* and *tashke*. Only subsp. *fastidiosa* and subsp. *multiplex* are subspecies validly recognised in taxonomy.

In order to infer the phylogenetic distribution of the different subspecies, all genomes available in the databases, together with different genomes of strains isolated in the Balearic Islands and sequenced in our research group were analysed. Sequence types were determined using the pubMLST website for *X. fastidiosa* (<https://pubmlst.org/xfastidiosa/>). Comparative genomic analysis was carried out using the GET\_Homologues software. *In silico* whole-genome comparison tools (ANIb, TETRA and GGDC) were also used. The results obtained by the different approaches clearly demonstrate a separation into three phylogenomic branches that correspond to the subspecies *fastidiosa*, *multiplex* and *pauca*.

### **Lack of evidence for seed transmission of *Xylella fastidiosa* subsp. *pauca* from infected olive trees and annual host plants**

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**Abstract:** In 2013, *Xylella fastidiosa* emerged in southern Italy threatening mainly olive trees, which upon bacterial infections succumb to severe desiccation and rapid decline. High rates of infected and symptomatic trees are usually recorded in the contaminated olive groves. Such evidence prompted several investigations to assess the pathways of local spread of the infections. Beside graft-/insect vector-mediated transmission, the possibility that the pathogen may be vertically transmitted through infested seeds was also investigated, by testing seeds collected from naturally infected olives and weeds (*Erigeron* spp. and *Chenopodium album*).

Four lots of olive fruits were harvested in January 2014 and 2016 from infected olive trees selected in three different locations in the Apulia region (southern Italy). Seeds were cleaned from the pulp and used either for the diagnostic tests (qPCR assays) or stratified at 4°C for three months followed by germination. For diagnostic tests, 24 seeds for each source were used to test either the excised embryos or the endosperm plus the seed coats.

Upon germination, the number of seedlings recovered varied between 30 and 50 for each lot, with a total of 160 seedlings grown in confined conditions for five years. Diagnostic tests on seedlings were performed one year after the germination and then repeated three (seeds collected in 2016) or five years (seeds collected in 2014) later.

Similarly, for the infected weeds diagnostic tests were performed (i) on groups of seeds (>100 seeds/sample) harvested in 2016 from infected plants, and (ii) on six-month-old plantlets obtained after seed germination.

The results of the qPCR assays on the seeds and on the recovered seedlings (both for olives and weeds) unequivocally indicated lack of positive detections, supporting the evidence of lack of seed-to-seedling transmission of this bacterium as previously shown for other susceptible crops (Della Coletta-Filho et al., 2014).

### **Studies to elucidate the cause of alteration in colony morphotype of *Xylella fastidiosa* subsp. *pauca*, ST53**

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