Phytopathologia Mediterranea (2018), 57, 3, 363–364
DOI: 10.14601/Phytopathol_Mediterr-24454

LETTERS TO THE EDITORS

First detection of *Xylella fastidiosa* subsp. *multiplex* DNA in Tuscany (Italy)

Guido MARCHI[1], Domenico RIZZO[2], Francesco RANALDI[3], Luisa GHELARDINI[1], Massimo RICCIOLINI[2], Ilaria SCARPELLI[2], Lorenzo DROSERA[2], Emanuele GOTI[4], Paolo CAPRETTI[1] and Giuseppe SURICO[1]

[1] Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, Sezione di Patologia Vegetale ed Entomologia, Università degli Studi, Piazzale delle Cascine 28, 50144 Firenze, Italy
[2] Regione Toscana, Servizio Fitosanitario Regionale e di Vigilanza e Controllo Agroforestale, Via A. Manzoni 16, 50121 Firenze, Italy
[3] Dipartimento di Scienze Biomediche Sperimentali e Cliniche, Sezione Cubo, Università degli Studi, Viale Pieraccini 6, 50139 Firenze, Italy
[4] Centro Interdipartimentale di Servizi per le Biotecnologie di Interesse Agrario, Chimico e Industriale, Università degli Studi, Via Romana 21, 50125 Firenze, Italy

Dear Editor, this letter is to inform you, the members of the Mediterranean Phytopathological Union, as well as the whole phytopathological community, on the finding of *Xylella fastidiosa* (Wells *et al*., 1987) in Tuscany (Italy).

During the execution of the early detection surveillance program, carried out by the Regional Phytosanitary Service and the University of Florence, the DNA of the bacterium was detected in early October 2018 in a plant of *Spartium junceum* growing in the municipality of Monte Argentario (Grosseto). Monte Argentario is a promontory (an island in the past) near the border with Latium, that extends in the Tyrrhenian sea overlooking Corsica (France), which it is approx. 120 km away. Monte Argentario is joined to the mainland by two stretches of land, called "tomboli": tombolo of Feniglia and tombolo of Giannella. The climate is mild temperate with dry and hot summer (Csa according to Köppen and Geiger) and an annual average precipitation of 455 mm.

After the conclusion of the initial screening, nucleic acids extracts as well as plant tissues samples were delivered to both national reference laboratories for confirmation (D.M. 07.12.2016) and demarcated areas were established according to EU Decision 2015/789. Sampling on Monte Argentario was consequently intensified and, by applying two independent Real-time PCR protocols (PM 7/24-3) of: Harper *et al*., (2010, erratum 2013) and Francis *et al*. (2006) the DNA of *X. fastidiosa* was detected in different plant-hosts, including: *Spartium junceum*, *Polygala myrtifolia*, *Cistus* spp., *Rhamnus alaternus*, *Prunus amygdalus* and *Lavandula* spp. To date, in order to limit the risk of accidental spread of the bacterium outside the delimited zone, no attempt of isolating *X. fastidiosa* has been carried out. However, since the aforementioned protocols do not allow to discriminate between *X. fastidiosa* subspecies (PM 7/24-3), amplification and sequencing of a fragment of *X. fastidiosa gyrB* gene (Rodrigues *et al*., 2003) as well as a MLST typing approach (Yuan *et al*., 2010; PM 7/24-3) was attempted using the whole nucleic acids extracted from *S. junceum* (three samples), *Polygala myrtifolia* (one) and *Rhamnus alaternus* (one). BLASTN comparisons of the resulting *gyrB* sequence, showed 100 identity over 390 bp with the homologous sequence of *X. fastidiosa* subsp. *multiplex* ATCC35871 (Schaad *et al*., 2004). Identity searches among the MLST alleleles described in the pubmlst database (http://pubmlst.org/xfastidiosa/), have indicated the presence in each of the five extracts, of alleles: 5

Corresponding author: G. Marchi
E-mail: guido.marchi@unifi.it

(*leuA*), 3 (*petC*), 5 (*malF*), 3 (*cysG*), 3(*holC*) and 3 (*gltT*) of *X. fastidiosa*. Nevertheless, since the allele number of the *nuoL* gene fragment could not be assigned because of only a partial match with the corresponding allele 3 of *X. fastidiosa* (G to A at position 276 of the nucleotide sequence), the resulting Sequence Type (ST) remains to be determined, although the data so far obtained indicate the occurrence in Tuscany of a new MLST allelic profile. Although the procedure of using plant extracted nucleic acids for MLST typing has been previously applied with success (PM 7/24-3), current findings highlight, once and again, the need to compare the results to those obtained from pure cultures of the genotype/s involved in the bacterial outbreak. Concordantly with the *gyrB* sequencing results, the comparison of the 6 MLST alleles we could univocally determine, with those reported in PM 7/24-3 is indicative of the presence of the DNA of subsp. *multiplex* in the 5 plants from Monte Argentario, with alleles 5 (*malF*), 3 (*cysG*) and 3 (*holC*) being the most discriminatory for subspecies assignment. *X. fastidiosa* subsp. *multiplex* is widely spread in North America where may infect both native and non-native plant species, including *Olea europea*, albeit its ability to cause disease on this host is yet to be proven (Nunney *et al*., 2013; Krugner *et al*., 2014). More recently the presence of subsp. *multiplex* has been recorded in Europe, mainly in France and Spain, where 41 host plants have been found to be susceptible (EU 2018). Some of these species are cultivated for commercial purposes, as *Polygala myrtifolia*, *Prunus* sp. and *Olea europea*, others are spontaneous as those that are part of the Mediterranean scrub.

During the continuation of future work, it will be our care to share available information's with local and international colleagues in order to ensure that decisions will always be based on the best available knowledge.

Literature cited

EU, 2018. *Commission database of host plants found to be susceptible to* Xylella fastidiosa *in the Union territory*. http://ec.europa.eu/food/plant/plant_health_biosecurity/legislation/emergency_measures/xylella-fastidiosa/susceptible_en.htm.

EPPO, 2018. PM 7/24-3 *Xylella fastidiosa. EPPO Bulletin* 48, 175–218.

Francis M., H. Lin , J. Cabrera-La Rosa, H. Doddapaneni and E.L. Civerolo, 2006. Genome-based PCR primers for specific and sensitive detection and quantification of *Xylella fastidiosa*. *European Journal of Plant Pathology* 115, 203–213.

Harper S.J., L.I. Ward and G.R.G. Clover, 2010. Development of LAMP and real-time PCR methods for the rapid detection of *Xylella fastidiosa* for quarantine and field applications. *Phytopathology* 100,1282–1288.

Krugner R., M. S. Sisterson, J. Chen, D. C. Stenger and M. W. Johnson, 2014. Evaluation of olive as a host of *Xylella fastidiosa* and associated sharpshooter vectors. *Plant Disease* 98, 1186–1193.

Nunney L., *et al*., 2013. Recent evolutionary radiation and host plant specialization in the *Xylella fastidiosa* subspecies native to the United States. *Applied and Environmental Microbiology* 79, 2189–2200.

Rodrigues J. L., M. E. Silva-Stenico, J. E. Gomes, J. R. S. Lopes and S. M. Tsai, 2003. Detection and diversity assessment of *Xylella fastidiosa* in field-collected plant and insect samples by using 16S rRNA and *gyrB* sequences. *Applied and Environmental Microbiology* 69, 4249–4255.

Schaad N. W., E. Postnikova, G. Lacy, M. B. Fatmi and C. Chung-Jan, 2004. *Xylella fastidiosa* subspecies: *X. fastidiosa* subsp. *piercei*, subsp. nov., *X. fastidiosa* subsp. *multiplex* subsp. nov., and *X. fastidiosa* subsp. *pauca* subsp. nov. *Systematic and Applied Microbiology* 27, 290–300.

Wells J. M., B. C. Raju, H. Y.Hung, W. G. Weisburg, L. Mandelco-Paul and D. J. Brenner, 1987. *Xylella fastidiosa* gen. nov., sp. nov: gram-negative, xylem-limited, fastidious plant bacteria related to *Xanthomonas* spp. *International Journal of Systematic Bacteriology* 37, 136–143.

Yuan X., L. Morano, R. Bromley, S. Spring-Pearson, R. Stouthamer and L. Nunney, 2010. Multilocus sequence typing of Xylella fastidiosa causing Pierce's disease and oleander leaf scorch in the United States. *Phytopathology* 100, 601–661.

Accepted for publication: December 23, 2018