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# Work done and actions taken on *Xylella fastidiosa* in Lebanon

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The importance of the olive tree goes back a long way in human history. It was cultivated by many ancient civilizations that settled in the Mediterranean basin. Olive (*Olea europaea* L.) is cultivated in Lebanon on a surface of ca. 53 600 ha, accounting for 43% of the area given over to perennial crops (Anonymous, 2010).

In autumn 2013, an epidemic outbreak of *Xylella fastidiosa* was reported in the Mediterranean region, namely in Puglia (South-Eastern Italy), where this pathogen is the main cause of the so-called quick decline syndrome (QDS) of olive trees, leading to destructive dieback and wilting of trees. The pathogen also infected oleander and almond, which exhibited typical leaf scorch symptoms (Guario *et al.*, 2013; Saponari *et al.*, 2013) and presently, has extended to include sweet cherry, and several landscape plants (Catalano, 2015). There are concerns that this disease could also reach Lebanon and destroy many economically important crops as well as the culturally important and beautiful centennial olive trees.

In Lebanon, *X. fastidiosa* was recently reported as being associated with leaf scorch, chlorosis and stunting symptoms of oleander growing in the American University of Beirut (AUB) campus (Temsah *et al.*, 2015); ELISA tests for this pest were positive as were in situ observations made with a scanning electron microscope (SEM). Although isolation and culturing on appropriate media are mandatory for a first occurrence of *X. fastidiosa* (EPPO, 2004), and molecular assays are crucial to confirm ELISA-positive samples (Amanifar *et al.*, 2014; Loconsole *et al.*, 2014), neither of these two techniques were performed on these oleander ELISA-positive samples from AUB to confirm the presence of the pathogen in Lebanon.

Since 2013, symptoms of leaf scorch, accompanied in a few cases with severe branch defoliation, have been commonly observed on olive in the main growing areas in Lebanon. Furthermore, despite the outbreak of *X. fastidiosa* in Puglia, Lebanon continued to import olive seedlings and ornamentals from Italy, until the decree 1/161 in 04 March 2015 (Amendment to the list of quarantine pests for Decree Nr. 783/1 dated 26/8/2011) (Annex 1 "Arabic version") was issued by the Lebanese Ministry of Agriculture, to impose the import of plant propagation material only from *X. fastidiosa* pest-free areas. In addition, the Decree Nr. 1068/1 (Annex 2 "English version") for the protection from *Xylella fastidiosa* when importing plant product was issued. Within this decree all consignments of fruit seedling and ornamental plants, including plants, plant parts such as leaves, branches, twigs, roots, flowers (except seeds), must be accompanied by an original phytosanitary certificate and mention in the additional declaration that consignment is free from the bacterium *Xylella fastidiosa* and must be accompanied by an additional declaration certificate as proof that consignment was produced in Pest Free Area Zone free from *Xylella fastidiosa* under the supervision of the NPPO of the country of origin specifying the name of the place of production.

Considering the risk this pathogen presents and the devastating disease it can cause in Lebanon, on both agricultural and landscaping plants, it was crucial to quickly monitor the area in which the occurrence of *X. fastidiosa* is suspected.

A survey on the spread of *X. fastidiosa* in Lebanon was organized by the Department of Plant Protection of the Lebanese Agricultural Research Institute (LARI)-Tal Amara, the Laboratory of Mycology, Department of Plant Protection LARI-Fanar, the Department of Olive and Olive Oil, LARI-Tal Amara and the Department of Plant Protection of the Lebanese Ministry of Agriculture (MoA) in May 2014, starting from the main olive growing areas in which symptoms similar to those described for QDS were observed. Samples (twigs and mature leaves) were collected during three vegetative seasons: spring 2014 and 2015, and autumn 2014. A total of 82 different olive trees from 24 groves were sampled at different heights of the canopy. In addition, 30 grapevine plants expressing symptoms similar to Pierce's disease were also sampled during fall 2014.

Following the report on the occurrence of *X. fastidiosa* on oleander in Lebanon (Temsah *et al.*, 2015), and upon the request from the Phytosanitary service of the Lebanese Ministry of Agriculture, twigs samples were collected from oleander plants at AUB campus (Beirut) to confirm the previous ELISA results by molecular, serological and cultural methods. Four twigs per plant were taken from 15 oleander plants showing leaf scorch and stunting symptoms and located in the same landscape area where the bacterium was previously reported; in addition, 10 asymptomatic plants located in the surroundings were also sampled. Seven samples from symptomatic oleander were also collected from landscape areas in Byblos and Bekaa Valley.

In spring 2015, samples were gathered from nurseries that imported ornamentals and olive seedlings from Italy during 2014–2015. In total, samples were collected randomly from 26 three-year-old olive grafted seedlings and 48 ornamental plants, already reported as hosts of *X. fastidiosa* in Puglia.

All the samples showing symptoms similar to those described on olive, almond, oleander and other ornamentals in Southern Italy were used in attempts to isolate the bacterium on different media, according to the procedures described by Cariddi *et al.* (2014).

For serological detection on symptomatic and asymptomatic samples, tissue extracts obtained from leaf petioles and midveins excised from 8–10 mature leaves and macerated in plastic bags (Loconsole *et al.*, 2014) were tested by Double Antibody Sandwich ELISA using specific antibodies to *X. fastidiosa* (Loewe Biochemica GmdH, Germany) following the manufacturer's instructions. In addition, Direct Tissue Blot Immunoassay technique for the detection of *X. fastidiosa* was carried out as described by Djelouah *et al.* (2014).

For molecular detection on symptomatic and asymptomatic samples, DNA was extracted using CTAB-based extraction procedure (Loconsole *et al.*, 2014) with slight modification. Molecular detection of *X. fastidiosa* in the samples was carried out by PCR using three sets of primers: RST31/RST33, which is mandatory for the detection of quarantine pathogen, according to the EPPO protocol (EPPO, 2004); FXYgyr499/RXYgyr907 and HL5/HL6. Laboratory activities were undertaken at the Department of Plant Protection, Tal Amara at the Lebanese Agricultural Research Institute in collaboration with the Department of Soil Sciences, Plant and Food, University of Bari, Italy.

Results of all tests were negative and unequivocally demonstrated that all the collected samples were free from the pathogen. In addition both serological and molecular tests and attempts at isolating the pathogen demonstrated that oleander samples gathered from American University campus in Beirut, where *X. fastidiosa* was previously reported, were not infected. A scientific article entitled "*Xylella fastidiosa* Does Not Occur in Lebanon" was published in Journal of Phytopathology on the results of this survey (Habib *et al.*, 2016) (Annex 3). Nevertheless, continuous and large monitoring and rigorous control measures of propagative materials are necessary to prevent the introduction of *Xylella fastidiosa* in Lebanon.

Numerous species of xylem fluid-feeding sharpshooters and spittlebugs are known to transmit the bacterium worldwide. The species ascertained as an effective vector for the CoDiRO Italian strain

is the meadow spittlebug, *Philaenus spumarius*, although potential vectoring roles of *Neophilaenus campestris* and the phloem feeder *Euscelis lineolatus* have been reported. *Philaenus spumarius* has not yet been reported in Lebanon, but any xylem fluid-feeding hemipteran should be regarded as a potential vector of the bacterium. To date, in Lebanon, no research has been carried out on insect vectors of *Xylella fastidiosa*. The knowledge of the insect vectors is crucial for well-timed and efficient control strategies, to avoid further spreading of the bacterium. The study of the entomofauna related to the crops of interest is not always easy and often different sampling techniques should be combined, due to the different life cycle of the insects. Moreover the search should not be restricted only to the crop(s), but it should be extended to the surrounding weeds. For this reason, to optimize the search for the vectors in Lebanon, a clear plan and in depth training for LARI and MoA staff is needed in order to choose the best sampling method, to identify the effective/potential vectors based on the transmission trials.

Among the awareness raising and training activities undertaken in the country, LARI research staff organized several meeting with MoA Agricultural Engineers in order to prepare a decree to impose the import of plant propagation material only from *X. fastidiosa* pest-free areas. During the field inspections, LARI staff explained to farmers about the serious damage that this quarantine pathogen could cause if it is spread in olive groves. Two seminars were carried out for farmers to make them aware them about the economic impact of this lethal disease and other olive diseases. Training was given to Dr. Elia Choueiri at IAM Bari during May 2015 where he had the opportunity to discuss and exchange scientific knowledge with Italian experts about the main characteristics of Olive Quick Decline Syndrome. This was part of a Training course on Early Warning System for Integrated Pest Management in the framework of the ClimaSouth Project. In addition, for the other Scientific staff from LARI; Dr. Wassim attended a Workshop “*Xylella fastidiosa* threat on food industry” at IAM-Bari, Italy (2014); Dr. E. Choueiri, Dr W. Habib and Eng. Elvis Gerges participated in the Round Table “*Xylella fastidiosa*: a serious menace to the Mediterranean fruit industry” during the 14th congress of Mediterranean Phytopathological Union, Istanbul, Turkey (2014) and lastly Eng. Farah Baroudy from LARI attended the International symposium on the European outbreak of *Xylella fastidiosa* in olive, Locorotondo, Italy (2014).

Recently, a Regional Technical Cooperation Programme (TCP) on *Xylella fastidiosa* for Lebanon expected to be funded by FAO is upcoming with the following objectives: (i) Building capacities of the technical team from MoA and LARI on monitoring and management of *Xylella fastidiosa*; (ii) Development of a national *Xylella fastidiosa* survey; (iii) Building capacities for the survey team; Awareness raising for farmers; (iv) Building capacities of phytosanitary officers on identification of *Xylella fastidiosa*; (v) Development of a manual/handbook for the identification of *Xylella fastidiosa* at border points; (vi) Development and implementation of regulations to limit the entry of *Xylella fastidiosa*.

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