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Xylella fastidiosa: the status of the infection and control measures in France

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The xylem-limited bacterium *Xylella fastidiosa* is the causal agent of diseases on a large host range of plants. It has been observed in Americas since the XIXth century causing notably Pierce's disease of grape (*Vitis vinifera*) in North America, citrus variegated chlorosis on orange tree (*Citrus sinensis*) in South America and diseases on fruit trees (*Prunus dulcis*, *P. domestica*, *P. persica*, etc.), ornamentals (*Coffea* spp., *Nerium oleander*, *Platanus occidentalis*, etc.) and forest trees (*Quercus* spp., *Ulmus* spp., etc.). *X. fastidiosa* is a quarantine pest for the European Union and as such is listed on the directive 2000/29/EC and the introduction of many plants into the European Union is regulated. A risk of introduction is still pending due to the high number of non-regulated plant hosts which are imported from contaminated areas and to the presence of asymptomatic contaminated plants.

In 2012, 2014 and 2015 several interceptions of coffee plants (*Coffea arabica* and *C. canephora*) contaminated with *X. fastidiosa* occurred in France, generally on asymptomatic plants. Different subspecies and sequence-types were identified, demonstrating a great diversity: *X. fastidiosa* subsp. *fastidiosa* (ST75), *X. fastidiosa* subsp. *sandyi* (ST72 and ST76) and *X. fastidiosa* subsp. *pauca* (ST53 and ST74) (Jacques *et al.*, 2016; Denancé et al., 2017). In the same time, *Coffea* sp. were intercepted on different occasions in other EU Member States (The Netherlands, Italy, Germany, Switzerland…).

In 2013, the causal agent of the syndrome of quick decline of olive in Puglia was identified as *X. fastidiosa* subsp. *pauca* (Elbeaino *et al.*, 2014).

In France, the first outbreak of a disease caused by *X. fastidiosa* was observed in July, the 22nd 2015 in the island of Corsica on *Polygala myrtifolia*, a popular ornamental shrub which has been widely planted. Since then, *X. fastidiosa* has been detected on twentyseven plant species (21 genera) in Corsica, including ornamentals and native flora. At the beginning of November 2016, the total number of outbreaks reaches 289 in Corsica. Two strains have been identified belonging to the subspecies *X. fastidiosa* subsp. *multiplex* (ST6 and ST7).

On October, the 22th 2015, the first outbreak was discovered in mainland along the south-East Mediterranean coast of France on *Polygala myrtifolia*. Fifteen outbreaks were notified in Provence - French Riviera area at the beginning of November 2016 and two new host plants have been identified (*Spartium junceum* and *Lavandula angustifolia*) infected by. *X. fastidiosa* subsp. *multiplex* (ST6 and ST7). These three hosts are in common with Corsican ones. Recently, in the same area, in Menton, *X. fastidiosa* subsp. *pauca* (ST53) was identified on *Polygala myrtifolia* in

a single outbreak which is now eradicated. In France including Corsica, *X. fastidiosa* has never been detected on olive tree, citrus species, grapevine and oleander.

Since 2012, ANSES has carried out methodology work based on Real-Time PCR and DNA extraction kits on various plant matrices such as coffee (*Coffea* spp.), grapevine (*Vitis vinifera*), peach (*Prunus persica*), orange (*Citrus sinensis*) and other host plants in order to provide efficient tools for early detection and to validate the more performant detection method. After officialisation by the French Ministry of agriculture, a reference method was published in October 2015 by ANSES (MA039 version 1 - https://www.anses.fr/fr/system/files/ANSES_MA039_Xylellafastidiosa_final.pdf). The method is based on Real-Time PCR (Harper *et al*., 2010) after QuickPick™ Plant DNA kit (Bio-Nobile) DNA extraction. In order to assure high throughput DNA extractions, KingFisher™ (Thermo Fisher Scientific) robots are used allowing 15 or 96 samples serial extractions. Each sample is composed of 0.5 to 1 g of petioles, from 5 to 100 petioles according to plant species (e.g. oleander vs olive), or green twigs for very small plants or plants with leaves without petiole (e.g. *Rosmarinus* sp.). The limit of detection (LOD) of this method reaches 10^2 bact./mL on some matrices (i.e. *Citrus sinensis*) but on others, due to the presence of PCR inhibitors as polyphenolic compounds, the LOD aims about 10^5 bact./mL, for example on oaks (*Quercus* spp.) and olive tree (*Olea europaea*). But this protocol remains more sensitive than others such as ELISA or PCR.

Since November 2015, official analyses have been transferred to a network of five laboratories approved by the Ministry of agriculture after training and successful proficiency's assessment.

The Plant Health Laboratory – ANSES performed confirmation analyses on positive samples when concerning new outbreaks or new host plants in the "contaminated" area. The characterization of isolates directly on plant extract or on pure strain isolated from plant material is performed since May 2016 according to a multilocus sequence analysis/typing (MLSA/MLST) (http://pubmlst.org/xfastidiosa/) using partial sequences of seven housekeeping genes (cysG, gltT, holC, leuA, malF, nuoL and petC) following EPPO 2016, Diagnostic PM 7/24 (2) *Xylella fastidiosa*. INRA IRHS – EmerSys team (Beaucouzé - France) is implementing modifications in the amplification protocol (Denancé *et al.*, 2017).

For additional confirmation purpose, isolation is performed on modified PWG medium. Plant Health Laboratory-ANSES set up a collection of 20 strains isolated from 10 different host plants sampled in Corsica, 4 strains from PACA area and 8 strains from intercepted *Coffea* spp.. These strains are transferred to the Biological Resource Center CIRM-Plant Associated Bacteria (CIRM-CFBP) (Beaucouzé-France).

Xylem sap-feeding insects belonging to Hemiptera order and *Auchenorrhynca* sub-order are known to be the main way for *X. fastidiosa* spreading. In consequence, forty seven species belonging to Aphrophoridae, Cercopidae, Cicadellidae and Cicadidae families are potential vectors and present in mainland France and twelve are present in Corsica island.

The Aphrophoridae *Philaenus spumarius*, known as vector in Puglia (Saponari *et al.,* 2014, Cornara *et al*., 2016), is widespread in mainland France and Corsica as in all Europe. More than three hundred *P. spumarius* specimens collected from October to December 2015 on eight Corsican outbreaks were tested individually for the detection of *X. fastidiosa* by Real-Time PCR (Harper *et al*., 2010) in duplex with internal controls 18S (Ioos *et al.*, 2009) after DNA extraction using a commercial kit usable with a robot. The observed contamination rate of individual insect varied, according to the outbreak locations, from 4% to 25% with an average of 8.8% (non-official results – method being in development and validation).

Controls and surveys have been implemented by the French Ministry of agriculture to investigate dispersion of the disease and to eradicate the outbreaks according to the Commission Implementing Decision (EU) 2015/789 of May 18th 2015. The main regulatory measures consist in the delimitation of a demarcated zone around each outbreak comprising an infested zone

with a radius of 100 m and a buffer zone with a radius of 10 km around the outbreak in order to confine the bacteria and to avoid its spreading. Eradication measures are maintained for 5 years after the discovery of the last infected plant of an outbreak. Into the infected zone, insect control/ eradication and removal of host plants and symptomatic plants are processed and specified plants are sampled for analysis. Planting of host plants is prohibited into the infected zone. Visual inspection and sampling of symptomatic plants are performed into the buffer zone and the movement of specified plants is forbidden outside the buffer zone. Outside the demarcated zones, a monitoring plan has been applied since 2015 consisting in inspections of the territory, production sites, retailers and controls at the EU Entry points (ship and port facilities, airports, etc.). From January 1, 2015 to November 07, 2016, more than 15000 samples have been tested. 11090 samples from Corsica were processed, among which 6.91% of positive samples, 2385 samples from Provence-French Riviera area, among which 2.25% of positive and 1630 samples among which 1.08% of positives for other French areas. For the latter case, positives are related to intercepted coffee plants imported from third countries.

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