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EUROPEAN COOPERATION
IN SCIENCE & TECHNOLOGY

SHORT TERM SCIENTIFIC MISSION (STSM) SCIENTIFIC REPORT

This report is submitted for approval by the STSM applicant to the STSM coordinator.

Action number: CA16107
STSM title: PLANT DISEASES CAUSED BY *Xylella fastidiosa*: DETECTION, IDENTIFICATION, MONITORING AND CONTROL.
STSM start and end date: 12/11/2018 to 16/11/2018
Grantee name: Milan Ivanovic

PURPOSE OF THE STSM

Advanced course "PLANT DISEASES CAUSED BY *Xylella fastidiosa*: DETECTION, IDENTIFICATION, MONITORING AND CONTROL, held in the CIHEAM, through the Mediterranean Agronomic Institute of Zaragoza (IAMZ), was jointly organized by the Ministry of Agriculture, Fisheries and Food (MAPA), the EU H2020 funded projects XF-ACTORS "Xylella Fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy" and POnTE "Pests Organisms Threatening Europe", and the EU H2020 MSCA-RISE project CURE-XF "Capacity Building and Raising Awareness in Europe and in Third Countries to Cope with *Xylella fastidiosa*".
Purpose of this STSM was to improve knowledge about *Xylella fastidiosa* – a vector-borne bacterial pathogen that has emerged in Europe in recent 5 years. This bacterium has caused severe socio-economic damages to agricultural crops in Italy and recently the bacterium has been detected in France and Spain. The aim of this STSM was to improve capacity building and raise awareness by providing advanced knowledge on biology and ecology of *X. fastidiosa* in the host plants and vectors, transmission of bacteria in short and long distances, epidemiology of various diseases it causes in most important agricultural plants. Additionally, the purpose was to receive expertise in methods of inspection, sampling and monitoring of *X. fastidiosa*, current situation and the status of *X. fastidiosa* in Europe and worldwide, strategies for *X. fastidiosa* control and EU legislation concerning this pathogen.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSM

The course was held over a period of 1 week, from 12 to 16 November 2018, organized in morning and afternoon sessions at the Mediterranean Agronomic Institute of Zaragoza, Spain. In the opening sessions of the course (1h) the participants were introduced to the genus *Xylella* and the species *X. fastidiosa*: taxonomy and identification; distribution; host range of the bacterium, symptomatology of diseases caused by *X. fastidiosa*; and vectors of this quarantine bacterium.
The Introduction was followed by 1 hour of lecture on main ongoing research programs in the EU concerning *X. fastidiosa*.
Biology and ecology of *X. fastidiosa* in the host plants (2 hours) was presented in sections such as host-bacteria interactions; colonization; plant defence; *X. fastidiosa* genome analyses; and virulence factors.
The next section of the course (6 hours) consisted of lectures on biology and ecology of insect vectors and *X. fastidiosa* transmission through subsections such as: known vectors of *X. fastidiosa* in USA, Brazil and Europe; vector identification; life cycle of main vectors; and insect-bacteria interactions: transmission mechanisms.

Current situation of *X. fastidiosa* worldwide: main diseases and socioeconomic impact was presented during 3 hours through topics such as: the situation in the Americas; PD – Pierce's disease of grapevine; CVC – citrus variegated chlorosis; ALS – almond leaf scorch; other leaf scorches of fruit and landscape trees. The *X. fastidiosa* situation in Europe was elaborated in lectures with a special emphasis of occurrence in Italy: OQDS – olive quick decline syndrome; Corsica and PACA region, France; Balearic Islands and Alicante, Spain; and subsection Interceptions of the *X. fastidiosa* in Europe.
During the next session the participants were introduced to Methods of inspection, sampling and monitoring of *X. fastidiosa* This subject was presented during 5 hours through lectures such as: survey methodology: statistical basis, planning and implementation; IPPC standards, ISPM6 and ISPM31, EU Guidelines, EPPO protocols for inspection; guidelines for sampling and sample preparation; demonstrative field practicals for plant sampling.
Methods for detection and identification of *X. fastidiosa* in plants and vectors (8 hours) were elaborated across lectures in: EPPO protocol for *X. fastidiosa* diagnosis; subspecies and sequence-type identification; molecular methods for on-site detection; and proximal and remote sensing. On-site detection was demonstrated by companies Enbiotech and Agdia during practical work groups during two hours.
The last subject on *X. fastidiosa* detection and identification was: subspecies and sequence-type identification demonstration on MLST and NCBI database consultation.
Epidemiology of *X. fastidiosa* (2 hours) was covered in lectures: modelling; and pest risk assessment.
Strategies for *X. fastidiosa* control (3 hours) were presented during lectures: quarantine, prevention and eradication; containment; sources and search of resistance in host plants; agronomical and chemical tools for controlling vector populations; managing bacterial population in the plant.
Legislation on *X. fastidiosa* in Europe (2 hours) was discussed in lectures: EU Decision 2015/789 and its amendments; implementation in the affected countries; and example of a Contingency Plan: Spain, France and Spain.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

(max. 500 words)

The vector-borne bacterial pathogen *X. fastidiosa*, widely distributed in America, has re-emerged as global threat for agricultural crops, the natural environment and landscape after its recent introduction in Asia and Europe. When entering a new area with adequate ecological conditions, including suitable plant hosts (hundreds of plant species), climate, and native vectors, this pathogen can rapidly become entrenched in the territory, causing severe socio-economic damages and loss of biodiversity.
To date there are no efficacious means to cure infected host plants, therefore efficient monitoring programmes for early detection are necessary to prevent the establishment in new areas. Knowledge of the different components of the pathosystems (the specific interactions strain(s)-host(s)-vector(s)-environment) is relevant for designing containment and management strategies in the areas where the bacterium established itself.
Main results of this advanced course are acquiring knowledge on the biology and ecology of the bacterium *X. fastidiosa* and its interactions with host plants and vectors; understanding vector life cycle and mechanisms of bacterial transmission. Also, during the practical session the participants could gain expertise on on-site detection with commercial available kits from companies such as Enbiotech and Agdia. We were able of acquiring theoretical and practical knowledge and developing skills on sampling and advanced diagnostic procedures, including molecular, remote sensing approaches for the early detection of the infections and subspecies assignation. Also, the participants of this course have improved competence in plant health: principles of the current EU legislation on *X. fastidiosa*, official protocols and guidelines for monitoring and diagnosis of the bacterium.
Serbia is still considered to be a Xylella-free country and this status is continuously being checked by surveying and sampling host plants coming from other countries, especially from countries where *X. fastidiosa* has been detected. During the course I had the opportunity to share our experiences in testing samples of different host plants in detecting *X. fastidiosa* with our fellow colleagues from other phytosanitary laboratories, discuss current monitoring strategies, preventive and quarantine measures for *X. fastidiosa.*
Course participants gained knowledge on the different aspects concerning the epidemics, especially from case study in south Italy in the Apuglia region. At the end of the course the attendants should be competent in performing pest risk assessment and developing management tools adapted to different scenarios as well as control measures for this new and emerging bacterium and the devastating diseases that this bacterium causes.

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FUTURE COLLABORATIONS (if applicable)

The 5-day course at IAMZ provided an opportunity to discuss the latest results on quarantine and containment measures, methods for early specific and sensitive detection of *X. fastidiosa*, having in mind that *X. fastidiosa* has not been detected in Serbia. Serbia is considered a Xylella-free country and the status has been checked continuously during the year with plant material that is being imported from countries with high risk of infection (Italy, France, Spain), but also from other countries. Plant material with high risk of infection with *X. fastidiosa* that is imported in Serbia is being routinely inspected at the border crossings and sent to our laboratory for analysis by using molecular techniques. Since the establishment of *X. fastidiosa* in Italy and later in France and Spain, a number of plant species inspected and sampled for the analysis at the border crossings have increased rapidly. Also, possible emergence of *X. fastidiosa* is controlled in coordination with the extension service across Serbia from July through September by surveying and sampling of potential host plants mainly of the external origin. No pathogen was detected in 173 samples in 2016, 126 samples in 2017. And 130 samples so far in 2018, both from the border crossings and collected through the extension service and analysed in Phytobacteriology laboratory at Faculty of agriculture, University of Belgrade.
Our laboratory conducts *X. fastidiosa* detection in plant samples by conventional PCR according to Minsavage et al. (1994) following the recommendation of the EPPO diagnostic procedure (2016). DNA is extracted from the xylem tissue taken from various parts, depending on the sample material, by using the DNEasy plant mini kit (Qiagen). DNA from *X. fastidiosa* subsp. pauca strain CoDiRo is used as a positive control. Suspected samples (e.g. faint band) are further tested with by qPCR (Harper et al. 2010, erratum 2013). In order to improve sensitivity of *X. fastidiosa* detection as suggested by the EPPO protocol (2016), a real-time PCR (qPCR, Applied Biosystems StepOne™Real-Time PCR System), following the assay designed by Harper et al. (2010, erratum 2013), was validated during first half of 2017 and implemented in the expertise and practice at Laboratory for phytobacteriology, Faculty of Agriculture, University of Belgrade. Our laboratory took part in External Quality Assessment studies for laboratory performance EU-XF-PT-2017-02: Proficiency testing for the evaluation of molecular and serological diagnosis of *X. fastidiosa*. Since Serbia is a Xylella-free country any laboratory experiments with living cells is prohibited. Therefore, future collaboration can only include a cell inactivated DNA for any kind of experiments and trials, or for a new round of proficiency testing in detection of *X. fastidiosa* in plant material. Also, future cooperation will focus on development of protocols for diagnosis of *X. fastidiosa*, including improved methods for sampling, for isolation of *X. fastidiosa* from plant material and for molecular analysis of strains. This course has also been an opportunity to establish contacts, to share experiences among lecturers and participants coming from many countries (26) and consolidate relationships with other experts from various areas of the world with similar agricultural crops and climate conditions.

Read and approved,

Maite Aguinaco

Signed: Maite Aguinaco
Course coordinator

CIHEAM
IAM ZARAGOZA