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: "XYLELLA FASTIDIOSA: STUDIES ON HOST-PATHOGEN INTERACTIONS"
STSM start and end date: 15/01/2018 to 15/03/2018
Grantee name: Giusy D'Attoma

PURPOSE OF THE STSM

(max. 200 words)

My Ph.D. research program focus on the study of the biology and pathogenicity traits of *Xylella fastidiosa* subsp. pauca 'De Donno' strain, phylotype ST53, and the characterization of the host-pathogen interactions in olive. The general goal of this STSM has been to gain specific skills and to improve my knowledge on this "fastidious" pathogen.
The research activities planned during the STSM stage included the following objectives:

1. Acquisition of basic knowledge on the protocols and procedures for culturing *Xylella fastidiosa*, assess its pathogenicity in experimental conditions and perform its genetic manipulation.
2. The use of *Nicotiana tabacum* SR1 plants as a model system to understand the molecular and physiopathological basis of *X. fastidiosa*-host interactions.
3. Study of the ionome of *Xylella fastidiosa* tolerant and susceptible olive cultivars. The research activities were intended to comprehend if the ions profile may have a role in the plant response to infection, contributing to differentiate highly susceptible cultivars from those that show traits of resistance.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSM

On the basis of the proposed objectives, the three activities have been carried out in parallel:

1. I have become familiar with basic methodologies for the study of this bacterium biology, such as growth curve and biofilm formation assays, and twitching motility assessment, in order to gain skills and methodologies that can be directly transferred to my home laboratory at the University of Bari, for developing investigations on the Apulian strain of *X. fastidiosa*. Particular attention was paid to learning a mutagenesis protocol based on natural competence. Three different strains, namely De Donno (subsp. *pauca*), Temecula1 (subsp. *fastidiosa*) and AlmaEm3 (subsp. *multiplex*) have been tested for their natural competence to acquire exogenous DNA. To this end, an attempt was made to produce a mutant, deficient in the *rpfF* gene responsible for the production of a diffusible signal factor, crucially involved in the bacterial quorum sensing system. The homologous recombination cassette was constructed according to the following steps:

- Design primers to amplify two regions respectively located upstream and downstream of the target gene in *X. fastidiosa* De Donno, and the gene for kanamycin resistance in PUC4k plasmid DNA;
- Perform PCR amplification with Iproof™ HF PCR mix (Bio-rad Laboratories Inc., USA);
- Proceed with a fusion PCR and elute from agarose gel a single PCR-fused product, to yield the homologous recombination cassette;
- Cloning the eluted fragment in a plasmid (CloneJET PCR Cloning, ThermoFisher Scientific, USA);

Once the homologous recombination cassette has been obtained, different attempts of transformation by natural competence, have been tried with the three Xf strains on PW and PD3 plates, according to the protocol in use in the host laboratory.

2. After planting and growing in greenhouse controlled conditions, tobacco plants have been inoculated with three different strains of *X. fastidiosa*, as mentioned before, (De Donno, Temecula1, AlmaEm3) in order to perform a time-course comparison for their ability to cause the appearance of symptoms. Each strain has been inoculated by using two inoculation time points, into ten plants, for a total of thirty plants. In addition, ten plants have been inoculated only with the inoculation buffer, in order to have the negative control of the experiment. The inoculum has been made with 5 days–old bacterial cells (OD_{600}=0.5) grown on PW agar plates, in the three basal leaves, after cutting the top of the plant.

3. Olive leaves of susceptible (Ogliarola salentina and Cellina di Nardò) and tolerant varieties (Leccino and FS-17™), prepared as dried non-infectious extracts at Bari's laboratory, according to protocols agreed with Prof. De La Fuente, have been analyzed by Inductively coupled plasma - optical emission spectrometry (ICP-OES). The analysis included two experimental sets, one concerned symptomatic and asymptomatic leaves of three varieties (Ogliarola salentina, Leccino and FS-17™) collected from three different fields (Sannicola, Alliste and Gallipoli) in Salento area, the other infected and healthy leaves of two varieties (Cellina di Nardò e Leccino) being used in greenhouse trials. Briefly, 10 mg of dry samples have been digested in 200 µl of nitric acid for one hour at 100°C. After cooling to room temperature, the samples have been diluted with water to a total volume of 1 ml and centrifuged to remove undigested tissue residues. The supernatant of each sample has been analyzed for its ion profile.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

(max. 500 words)

- *In vitro* assessment of twitching motility, observed measuring the peripheral fringe widths of bacterial colonies grown on PW and PD3 agar plates, preliminary showed that *X. fastidiosa* De Donno strain has a lower and not always reproducible motility when compared to Temecula1 and AlmaEm3.

- So far, the attempt for mutant production by natural competence has only been successful with *X. fastidiosa* Temecula1 strain on PD3 plates. We assessed the successful inclusion of both De Donno *rpfF* and PUC4k kanamycin resistance genes into the genome of Temecula1, but the same result could not be replicated with the other strains. Nevertheless, having acquired good skill with the technique, this preliminary attempt will be the basis for further experiments.

- The ICP-OES analyses performed on field samples to compare between the partially tolerant (Leccino) and highly susceptible (Ogliarola) varieties revealed changes in ionome. The ionome of the different varieties was, as expected, dependent on the field conditions, therefore we restricted our analysis to within fields. The cv. Leccino had an increase in leaf calcium concentration with symptoms, in contrast the susceptible cv. Ogliarola shows a non-significant trend to increased calcium. In the Sannicola plot the Leccino variety showed a 60% increase in leaf calcium concentration with symptoms, in contrast the susceptible variety Ogliarola shows a non-significant trend to increased calcium. However, in this field, Ogliarola leaves had 44% higher calcium concentration in asymptomatic leaves. In Gallipoli plot, again, the Leccino variety showed a 30%

increase in leaf calcium concentration with symptoms. In contrast the susceptible variety Ogliarola did not show an increase in calcium in symptomatic leaves. In the Alliste plot, in which Ogliarola is the only variety present, the comparison of total leaf calcium showed a trend to increase in symptomatic leaves. These data suggest that calcium variation, in tested varieties, could be affecting or influenced by the disease progression.

- As with regards for the samples collected from plants artificially inoculated with *X. fastidiosa* De Donno strain, and grown in greenhouse controlled conditions, the experiment intended to compare the ionome profile in infected or healthy leaves. Preliminary findings evidenced that calcium concentration appears to be higher in infected tissues of the susceptible (Cellina di Nardo) variety. The tolerant one (Leccino) doesn't show significantly changing in calcium concentration between healthy and infected plants. This finding will require further confirmation, once disease progress will be more evident, and symptoms will start to appear, at least on the susceptible plants.

FUTURE COLLABORATIONS (if applicable)
(max. 500 words)

The work carried out in the host laboratory provides the basis for future collaborations, already started in the recent past, in order to continue to deepen the characterization of the pathosystem involving the *X. fastidiosa* strain, which is responsible for a devastating and highly socio-economically impacting disease in Salento area. This approach opens a perspective of greater knowledge of the pathogen, also thanks to the comparison with different strains belonging to different subspecies, object of long-established studies carried out in the host laboratory, by the research group led by Prof. De La Fuente.
Future collaborations, already planned, and currently under experimental design, will also allow better investigation of the role of macro-micronutrient profiles in host-pathogen interactions, and will offer a relevant contribution to the ongoing screening for resistance traits to be found in olive cultivars.
Having set this collaboration in the framework of the larger scientific community gathered by COST Action CA16107 will allow establishing new contacts and the intention is to present the outcome of the work done and that planned for the future, at the next meetings organized by the EuroXanth working groups. One publication in collaboration between my home institution and the host institution is planned to be submitted before the end of the year, and will be part of my Ph.D. dissertation.