

FELLOWSHIP REPORT

COVER PAGE

Leonardo De La Fuente

Title: Characterization of environmental calcium content as predictor of disease severity by the emergent plant pathogen *Xylella fastidiosa*

Theme number: 2

Host institution: Institute for Sustainable Agriculture-CSIC (Córdoba, Spain)

Collaborators: Dr. Juan Navas-Cortés, Dr. Blanca Landa

DATES: 09/26/2022-11/18/2022; 4/24/2023-7/21/2023

I consent to my report being posted on the Co-operative Research Programme's website.

1. What were the objectives of the research project? Why is the research project important?

This fellowship focused in understanding the role of the mineral element calcium (Ca) as a predictor of the geographic distribution, potential for establishment, and severity of diseases caused by *Xylella fastidiosa*. *X. fastidiosa* is an emergent plant pathogen historically restricted to the Americas, but in the last years has caused important economical losses in Europe. In 2013, Xf was first reported associated with the disease known as olive quick decline syndrome in the Salento area in the south of Italy. A few years later the devastation caused by this pathogen is alarming, infecting practically all the olive trees in that area, in many cases leading to death of centuries-old trees. Besides Italy, infection foci have been identified in France, Spain, and Portugal among other countries. The situation in Europe has triggered an aggressive implementation by the European Union of new policies geared towards the detection of the bacteria and removal of infected (and surrounding) plant hosts. These policies have been based on very limited scientific information regarding conditions conducive to development of diseases caused by *X. fastidiosa* in Europe. My previous work demonstrated that Ca potentiates virulence traits of this pathogen, and plants infected by *X. fastidiosa* accumulate higher concentration of Ca in leaves and xylem sap. We are modelling the relationship between soil Ca content in Europe and US and *X. fastidiosa* disease occurrence and severity. We will be able to develop risk maps combining available climatic suitability maps with soil mineral content to estimate the potential for the establishment of *X. fastidiosa*, as well as to model and predict the severity of *X. fastidiosa* outbreaks. This CRP involved collaborations with Dr. Navas-Cortés and Dr. Landa from the Institute for Sustainable Agriculture-Spanish National Research Council (IAS- CSIC) (Córdoba, Spain). The objectives of the fellowship were: 1) Development of risk maps for the prediction of the potential establishment and severity of *X. fastidiosa* diseases based on soil mineral content; 2) Determine the effect of Ca on virulence of European strains of *X. fastidiosa*; and 3) Determine the effect of xylem sap on *X. fastidiosa* growth and virulence traits.

2. Were the objectives of the fellowship achieved?

The objectives defined in the fellowship to assess the relationship between the calcium content in the soil and the severity of *X. fastidiosa* infection are still ongoing. During my stay at IAS-CSIC we started a big data analysis to identify factors influencing the severity of *X. fastidiosa* outbreaks. The preliminary analysis showed very promising results, but the analysis is currently being confirmed using different approaches. We anticipate that this joint research and corresponding manuscript will be finalized and submitted during 2024. Besides the original objectives, other various projects were started and/or advanced during my CRP fellowship, to further the collaboration between the two laboratories. One of those projects is a collaboration between students of the two laboratories that are currently conducting some bioinformatic and experimental analyses to determine the distribution of natural competence among *X. fastidiosa* isolates from Europe and the US. Another joint effort was the design and writing of a review article highlighting the challenges that need to be addressed to control *X. fastidiosa* diseases.

3. What were the major achievements of the fellowship?

1. Develop new areas of research as a collaboration between the two labs, that already led to two published manuscripts, two others in preparation, plus a review article that is being finalized.

Published manuscripts:

Velasco-Amo, M.P.*^G, L.F. Arias-Giraldo, M. Roman Ecija*^G, L. De La Fuente, E. Marco-Noales, E. Moralejo, J. Navas-Cortés, and B.B. Landa. 2024. Complete circularized genome resources of seven strains of *Xylella fastidiosa* subsp. *fastidiosa* using hybrid assembly reveals unknown plasmids. *Phytopathology*, 113:1128-1132, doi: 10.1094/PHYTO-10-22-0396-A. *Acknowledgements sections note* "L. De La Fuente was a recipient of a research fellowship awarded by the Organization for Economic

Cooperation and Development, Cooperative Research Programme, during the writing of this manuscript.”

Román Ecija*^G, M., J. Navas-Cortés, M.P. Velasco-Amo*^G, L.F. Arias-Giraldo*^G, L.M. Gomez*^G, L. De La Fuente, and B.B. Landa. 2023. Two *Xylella fastidiosa* subsp. *multiplex* strains isolated from almond in Spain differ in plasmid content and virulence traits. *Phytopathology*, 113:960-974, doi: 10.1094/PHYTO-06-22-0234-R. *Acknowledgements sections note “L. De La Fuente was a recipient of a research fellowship awarded by the Organization for Economic Cooperation and Development, Cooperative Research Programme, during the writing of this manuscript.”*

*^G Graduate student.

2. *Expand scientific network connections through seminar presentations and attendance to scientific meetings.*

Attended the kick-off meeting for the EU project Beyond Xylella, Integrated Management Strategies for Mitigating *Xylella fastidiosa* impact in Europe (BeXyl). Cordoba, Spain, 17-19 October, 2022.

Attended the XX Congreso de la Sociedad Española de Fitopatología (SEF) (XX Meeting of the Spanish Phytopathological Society), Valencia, Spain, 24-26 October, 2022.

Speaker at the Institutional seminar series of the Instituto de Agricultura Sostenible – Consejo Superior de Investigaciones Científicas (IAS-CSIC), Córdoba, Spain. Presentation in Spanish on 11/4/2022. Presentation: “Evolución y adaptación al xilema por parte de la bacteria fitopatógena *Xylella fastidiosa*”.

Invited speaker at the Institutional seminar series of the Ludwig Maximilian University of Munich (LMU Munich Biocenter), SFB924 Hybrid seminar, Munich, Germany. Hybrid presentation in zoom and in person on 11/8/2022. Presentation: “Evolution and adaptation to the xylem environment by the plant pathogen *Xylella fastidiosa*”.

Invited seminar speaker, Centro Nacional de Biotecnología-Consejo Superior de Investigaciones Científicas (National Center for Biotechnology, Spanish National Research Council, CNB-CSIC). Madrid, Spain, 4/28/2023. “Evolution and adaptation to the xylem environment by the plant pathogenic bacteria *Xylella fastidiosa*.”

Moderator and organizer, together with host Blanca Landa (CSIC-IAS) of round table “*Xylella fastidiosa* disease control, surveillance and diagnostics”, held online by Zoom, 5/2/2023. This was part of a seminar series “The Xylella files” that reaches >500 people each week.

3. *Involvement on the Horizon Europe Programme-funded project (BeXyl) that will help continue the collaboration between the host and visiting lab.*

Thanks in part to the interactions with the IAS-CSIC host institution originated from this CRP-OECD fellowship, I am a co-PI of the European-Union funded project Beyond Xylella (BeXyl) (<https://bexylproject.org/>). Dr. Blanca Landa, one of the hosts of my fellowship, is the PI of this project.

4. **Will there be any follow-up work?**

- *Is a publication envisaged? Will this be in a journal or a publication? When will it appear?*

Besides the two manuscripts finalized and published during my fellowship (see above), we will publish three more papers. One is an invited review paper on challenges of dealing with the pathogen *Xylella fastidiosa*, co-authored with the two scientific hosts from IAS-CSIC (Drs. Landa and Navas-Cortés). This review will be submitted before

the end of the year and published in *Phytopathology* in 2024. The other two research papers will need more time since the final analysis and experiments are still ongoing. These two papers are planned to be submitted mid-late 2024.

- *Is your fellowship likely to be the start of collaboration between your home institution and your host?*

Collaboration between the host and guest labs has already started and is moving forward in the framework of the BeXyl project (see above).

- *Is your research likely to result in protected intellectual property, novel products or processes?*

No

5. How might the results of your research project be important for helping develop regional, national or international agro-food, fisheries or forestry policies and, or practices, or be beneficial for society?

The information generated with this study will help define geographical areas and conditions that will predict the likelihood of more severe infections by *X. fastidiosa*. By combining computer-based analysis with experimentation *in planta* and *in vitro*, we are creating a body of knowledge that will contribute to the scientific information on this difficult pathogen. We will determine the relationship between mineral elements and occurrence of *X. fastidiosa* outbreaks, considering soils characteristics. Since there is an ongoing considerable effort in Europe to screen for the presence of this pathogen, we will define areas with higher risk of establishment of the bacterium, infections and or severity of disease.

6. How was this research relevant to:

- *The objectives of the CRP?*
- *The CRP research theme?*

This study was aligned with the objectives of *Theme II Managing Risks in a Connected World*. The outbreak of *X. fastidiosa* in Europe is a clear example of the devastation that introduction of a foreign pathogen can cause in the local agricultural production, having also an impact in industries such as tourism, and socially causing loss of jobs opportunities for local people. Our research will provide risk assessment tools to prevent *X. fastidiosa* infections, in particular focusing on areas with higher risk of devastation. We will contribute knowledge geared towards helping early detection and assessment of a disease with a high impact in an agricultural production. Since we will determine soil properties and xylem sap characteristics that are conducive for severe outbreaks of a plant pathogen, we anticipate that this information will be used in the future for precise nutrition management options.

7. Satisfaction

- *Did your fellowship conform to your expectations?*
Yes
- *Will the OECD Co-operative Research Programme fellowship increase directly or indirectly your career opportunities? Please specify.*

This CRP increased my career opportunities in both ways. Directly, this OECD CRP fellowship allowed me to interact closely to the hosts Drs. Navas-Cortés and Landa at IAS-CSIC, that resulted already in joint publications, project participation and new research ideas to follow in the future. Indirectly, my time in Spain allowed me to

increase my networking opportunities by invitations to give seminars in different institutions in Spain and Germany, as well as attending scientific meetings that were very productive.

○ *Did you encounter any practical problems?*

From the fellowship perspective, no problems at all. The main issue was the bureaucracy, very long processing times and unreasonable demands to obtain a visa from the Spanish consulate. This led to severe delays in starting the fellowship (that was already delayed by the pandemic) and ended up causing the break of my stay into two instances because of the impossibility of staying more than 3 months at once without a visa.

○ *Please suggest any improvements in the Fellowship Programme.*

Increasing the visibility of the program will be useful.

8. Advertising the Co-operative Research Programme

○ *How did you learn about the Co-operative Research Programme?*

Colleague conversation, email.

○ *What would you suggest to make it more “visible”?*

Send several email reminders, expand mailing list, social media. Target specific groups with the corresponding theme.

○ *Are there any issues you would like to record?*

No, it was a great experience, and I am extremely grateful.