variables, our model consistently choses a reduced set of environmental variables, such as water stress and mild winter temperatures, that are known from laboratory studies to enhance the probability of plant infection and the survival of the insect vector. The model is then used to predict the susceptibility of the entire territory, highlighting the potential risk of infection in previously unsuspected zones. Finally, we will also assess and measure the uncertainties associated with our predictions, based on the fact that the model is trained on a rather restricted geographical area (with mostly Mediterranean climate), that may not be representative of the entire national territory.

## The spread of *X. fastidiosa* subsp. *pauca* among the olive orchards of southern Italy (Apulia)

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Abstract: In 2013 an outbreak of Xylella fastidiosa was identified for the first time in Europe, in the extreme South of Italy (Apulia, Salento territory). The locally identified subspecies turned out to be lethal for olive trees, starting an unprecedented phytosanitary emergency for one of the most iconic crops of the Mediterranean area. The Apulian monitoring programme of the epidemic amassed data on several hundreds of thousands of samples laboratory-screened for the presence of the bacterium, jointly with georeferenced sample information. Starting from these data, it is possible to show that Xylella fastidiosa spreads by forming new, tightly clustered groups of infected plants (epidemic hotspots), with 9 8% of the infected trees separated by less than 100 m from another infected tree. Surprisingly, more than three quarters of the newly detected epidemic hotspots are farther than 1 km from any previously known infected plants. Considering this finding, either long-range spreading of the bacterium is underestimated, or the current monitoring strategy must be called into question. In both cases, however, it can be anticipated that, under the current monitoring protocol, yearly epidemic spreading 1 to 15 km far from olive trees currently labelled as infected will be more common than previously thought.

## **Session 5: Vectors II**

## Insights into the transmission dynamics of *Xylella fastidiosa* by *Philaenus spumarius*

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**Abstract:** The establishment and relentless spread of the bacterium *Xylella fastidiosa* in some areas in Europe call for effective containment measures based on sustainable control strategies. However, the development of such strategies requires a thorough characterisation of the reciprocal interactions among the three key factors of the pathosystem, i.e. the bacterium, the vector, and the host plant. One of the major differences between European and American or Taiwanese epidemics refers to the vector species driving bacterium spread. Indeed, while sharpshooters (Hemiptera: Cicadellidae) are the key vectors in all the *X. fastidiosa* outbreaks other than European ones, spittlebugs such as *Philaenus spumarius* seem to play the main role in bacterial spread in Europe. Currently, knowledge about *X. Fastidiosa*—spittlebug interactions and the characterisation of the mode of transmission considerably lags behind the background on sharpshooters. Here we began to fill this knowledge gap by carrying out EPG (Electrical Penetration Graph)-assisted transmission tests of *X. fastidiosa* by *P. spumarius* (acquisition from

infected olive plants and inoculation of healthy olives and oleanders). Furthermore, we conducted comparative observations on the probing and feeding behaviour of infective versus non-infective spittlebugs on healthy olive plants. Spittlebug acquisition rate of X. fastidiosa from olive appeared to be extremely low; bacterial cells binding to the foregut occurred in a time as short as 15 minutes spent by the insect in xylem ingestion or activities interspersed with xylem ingestion (interruption during xylem ingestion and resting). Either in olive or oleander, P. spumarius inoculation of bacterial cells into the xylem was associated with an early (2.5 to 7 minutes after the onset of the first probe) and occasional behaviour, visualised by a specific DC-EPG waveform (Xe), presumably related to egestion of fluids regulated by pre-cibarial valve fluttering following a lack of phagostimulation. Behaviours stereotypically repeated by the insect and commonly performed during most of the probes did not lead to bacterial inoculation to the host plant. Infective spittlebugs compared with non-infective spittlebugs exhibited: i) significantly longer non-probing and shorter xylem ingestion; ii) longer duration of single non-probing events; iii) fewer sustained ingestions (ingestion longer than 10 min) and interruptions of xylem activity (N); iv) longer time required to perform the first absolute probe. These observations suggest difficulties in feeding for infective P. spumarius probably caused by the presence of X. fastidiosa in the foregut. Overall, our findings open new perspectives for research on the X. fastidiosa-spittlebug relationship and for sustainable control strategies based on the disruption of bacterium-vector interaction.

## A barcode database to identity the vectors of *Xylella fastidiosa* in Europe

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**Abstract:** Fast and reliable identification is a critical point for the early detection of biological invasions. The research unit, CBGP (Centre for Biology and Management of Population), develops morphological and molecular tools to characterise pests and beneficial organisms associated with crops, with a strong focus on quarantine pests and invasive arthropods. After the first detection of the bacterium *Xylella fastidiosa* (Xf) in Corsica four years ago, we started to work on a reference barcode database to identify putative vectors of Xf. Our final goal in the framework of the H2020 project XF-Actors, was to decipher the interaction network between plants, vectors and Xf to get a better understanding of how the disease spread in the ecosystems. COI barcodes were generated through a two-step PCR approach followed by Miseq sequencing. Multiple quality controls were implemented including i) a non-destructive DNA extraction that allows vouchering and morphological re-examination by taxonomists; ii) a bioinformatic pipeline that discards non-coding sequences, contaminants and NUMTs through BLAST comparison and phylogenetic inferences.

While a reference library of validated barcodes is mandatory for a reliable identification of the vectors of *Xylella*, our first results reveal taxonomic issues: e.g. i) the closely related species *Philaenus spumarius*/*P. tesselatus* have identical COI sequences; ii) misidentifications are present in international databases.

All barcodes have been implemented in a web-interfaced database (Arthemis DB@se: <a href="http://arthemisdb.supagro.inra.fr/DefaultInfo.aspx?Page=Home">http://arthemisdb.supagro.inra.fr/DefaultInfo.aspx?Page=Home</a>). Currently, the database hosts 376 sequences of putative vectors of *Xylella*: 260 representing 21 European species and 116 representing 15 North American species. Users can identify a query sequence to species through BLAST comparison against our reference library. Metadata associated with all records are available online, as well as biological data on vectors and pictures that illustrate habitus, genitalia and diagnostic characteristics.

This data set is continuously supplemented and updated. Several species are still missing but it already enables the identification of the most frequent vectors of *Xylella*. A call is made to obtain the species not yet included in the database.